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The c-Jun n-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway in paclitaxel-induced apoptosis of cancer cells

Tzu-Hao Wang

To the Graduate Council:

I am submitting herewith a dissertation written by Tzu-Hao Wang entitled "The c-Jun n-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathway in paclitaxel-induced apoptosis of cancer cells." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.

Jay Wimalasena, Major Professor

We have read this dissertation and recommend its acceptance:

Albert T. Ichiki, Joyce Merryman, Donald S. Torry, Wesley D. Wicks

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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THE C-JUN N-TERMINAL KINASE/STRESS-ACTIVATED PROTEIN KINASE (JNK/SAPK) PATHWAY IN PACLITAXEL-INDUCED APOPTOSIS OF CANCER CELLS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Tzu-Hao Wang
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DEDICATION

This dissertation is dedicated to:
my parents
Mr. Chung-Ling Wang
(王鍾靈)

Mrs. Kuan Cheng Wang
(王鄭玖),

my wife
Mrs. Ching-Ling Wang
(王靜玲),

and my daughters
Shih-Tien (Tien-Tien) Wang
(王師恬)

Shih-Yee (Mimi) Wang
(王師怡).

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ABSTRACT

The essential cellular functions associated with microtubules have led to a wide use of microtubule-interfering agents in cancer chemotherapy with promising results. Although the most well-studied effect of microtubule-interfering agents is an arrest of cells at the G_2/M phase of the cell cycle, other effects may also exist. I have observed that paclitaxel (Taxol), docetaxel (Taxotere), vinblastine, vincristine, nocodazole and colchicine activate the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) signaling pathway in a variety of human cells. Activation of JNK/SAPK by microtubule-interfering agents is dose-dependent and time-dependent and requires interactions with microtubules. Functional activation of the JNKK/SEK1-JNK/SAPK-c Jun cascade was demonstrated by cotransfection with a TPA-response element reporter construct and dominant negative (dn) signal transducers followed by chloramphenicol acetyl-transferase assays. Microtubule-interfering agents also activate both Ras and apoptosis signal-regulating kinase (ASK1), and coexpression of dn Ras and dn ASK1 exerted individual and additive inhibition of JNK/SAPK activation by microtubule-interfering agents. These findings suggest that multiple signal transduction pathways are involved with cellular detection of microtubular disarray and subsequent activation of JNK/SAPK.

To further examine the role of JNK/SAPK signaling cascades in apoptosis resulting from microtubular dysfunction induced by paclitaxel, I have coexpressed dn signaling proteins of the JNK/SAPK pathway (Ras, ASK1, Rac, JNKK, JNK) in human ovarian cancer cells with a selectable marker to analyze the apoptotic characteristics of cells expressing dn-vectors following exposure to paclitaxel. Expression of these dn signaling proteins had no effect on Bcl-2 phosphorylation, yet inhibited apoptotic changes induced by paclitaxel up to 16 h after treatment. Coexpression of these dn-signaling proteins had no protective effect after 48 h of paclitaxel treatment. These data indicate that: (i) activated JNK/SAPK acts upstream of membrane changes and caspase-3 activation in paclitaxel-initiated apoptotic pathways, independently of cell cycle stage, (ii) activated JNK/SAPK is not responsible for paclitaxel-induced phosphorylation of Bcl-2, and (iii) apoptosis resulting from microtubule damage may comprise multiple mechanisms, including a JNK/SAPK-dependent early phase and a JNK/SAPK-independent late phase.

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LIST OF ABBREVIATIONS

7-AAD: 7-amino actinomycin-D.

Ab: antibody,
ACTD: actinomycin-D,
AIF: apoptosis-inducing factor,
AP-1: activating protein-1,
Apaf: apoptosis-associated factor,
ARIA: activated Ras interacting assay,
ASK1: apoptosis signal-regulating kinase,
ATF: activating transcription factor,
AV: annexin V,

BH: bcl-2 homology,
BMP4: bone morphogenic protein 4,
bZIP: basic region leucine zipper,

ced: cell death abnormal gene of *C. elegans*,
ces: cell death specification gene of *C. elegans*,
CAD: caspase-activated Dnase,
CARD: caspase recruiting domain,
CBP: CREB-binding protein,
CAT assay: chloramphenicol acetyl-transferase assay,
CDDP: cis diamminedichloroplatiunm (cisplatin),
CDK: cyclin-dependent kinase,
CKI: CDK inhibitor,
CRE: cAMP response element,
CREB: CRE-binding protien,
CMV: cytomegalovirus,
COL: colchicine,

DAB: deacetylbaccatin,
Daxx: Fas death domain-associated protein,
DcR: decoy receptor,
DD: death domain,
DED: death effector domain,
DFF: DNA fragmentation factors,
DL: death ligand,
DMEM: Dulbecco's modified Eagle's medium,
DMSO: dimethly sulfoxide,
dn: dominant negative,
DR: death receptor,
DTX: docetaxel (Taxotere),

EGF: epidermal growth factor,
EGFP: enhanced green fluorescent protein,
EGFR: EGF receptor,
ELISA: enzyme-linked immunosorbant assay,
ERK: extracellular signal-regulated kinase,

FADD: Fas associated protein containing death domain,
FDA: fluorescein diacetate,
FBS: fetal bovine serum,
FC: flow cytometry or flow cytometric,
FITC: fluorescein isothiocyanate,

GCK: germinal center kinase,
GSH: glutathione,
GST: glutathione S-transferase,

h: hour,
HA: hemagglutinin epitope of influenza virus,
HPK1: hematopoetic progenitor kinase 1,
hsp: heat shock protien,

IAPs: inhibitors of apoptosis,
ICAD: inhibitor of caspase-activated DNase,
ICE: interleukin-1β-converting enzyme,
IP: immunoprecipitate,

JAB1: Jun activation domain binding protein 1,
JIP-1: JNK interacting protein-1,
JNK: c-Jun N-terminal kinase.
JNKK: JNK kinase (=SEK1, MKK4),

kD: kilodalton,

MAP: microtubule-associated protein,
MAPK: mitogen-activated protein kinase,
MAPK-APK: MAPK-activated protein kinase,
MBP: myelin basic protein,
MC540: merocyanine 540,
MEF2: myocyte-enhancer factor 2,
MEKK: MEK kinase,
MEK/MKK: MAP Erk kinase / MAPK kinase,
MIAs: microtubule-interfering agents,
min: minute,
MLK: mixed lineage kinase,
MP1: MEK partner 1,
MT: microtubule,

NBS: newborn bovine serum,
NGF: nerve growth factor,
NOC: nocodazole,

PAK: p21-activated kinase,
PARP: poly (ADP-ribose) polymerase,
PBS: phosphate-buffered saline,
PCD: programmed cell death,
PE: phycoerythrin,
PH: pleckstrin-homology,
PI: propidium iodide,
PKA: protein kinase A (=cAMP-dependent protein kinase),
PKB: protein kinase B (=Akt),
PKC: protein kinase C (=Ca^{2+}- and phospholipid-dependent protein kinase),
cPLA2: cytosolic phospholipase A2,
PS: phosphatidylserine,
PTPC: permeability transition pore complex,
PTX: paclitaxel (Taxol),

RBD: Ras binding domain of Raf,
RIP: receptor interacting protein,
RT: room temperature,

s: second,
SAPK: stress activated protein kinase,
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis,
SEK1: SAPK or ERK kinase-1 (=JNKK, MKK4),
SH: Src homology,
SRE: serum response element,
SRF: serum response factor,

TAK-1: TGF-β -activating kinase-1,
TCF: ternary complex factor,
TGF: transforming growth factor,
TPA: 12-*O*-tetradecanoylphorbol-13-acetate,
TNF: tumor necrosis factor,
TNFR: tumor necrosis factor receptor,
TRAF-2: TNFR-associated factor 2,
TRADD: TNFR-associated death domain protein,
TRE: TPA response element,
TUNEL: terminal deoxynucleotidyl transferase mediated dUTP nick end labeling,

UV: ultraviolet,

VBL: vinblastine (Velban),
VCR: vincristine (Oncovin),

WB: Western blot analysis,
wt: wild type,

PART I.

LITERATURE REVIEW

APOPTOSIS

Cell division and cell death are the ultimate differential decisions made by the cell (410). Cell death occurs throughout the life span of all multicellular organisms as a basic mechanism in tissue kinetics, from the formation of limbs during embryogenesis to the peeling of sunburned skin (539). Apoptosis is the most prevalent type of cell death. The machinery for apoptosis in all cell types of multicellular organisms is genetically encoded and readily expressed. Characteristic apoptotic morphology has been detected even in unicellular organisms: the kinetoplastid parasites *Trypanosoma cruzi* and *Trypanosoma brucei rhodensiense*, the free-living slime mold *Dictyostelium discoideum* (135), and the free-living ciliate *Tetrahymena thermophila* (15). These discoveries indicate apoptotic machinery of the cell may have developed for more than 2 billion years, whereas multicellular animals and plants appeared around 0.7 billion years ago (15).

In contrast to necrosis (**Table 1**), apoptosis is an energy-consuming (ATP-dependent) process (315)(426) and may involve a series of well-regulated synthetic events (261), which earns apoptosis the reputation as a suicidal process (539)(630). During the last two decades, this tightly-confined, cellular self-destruction has been recognized as the major mode of cell death. Till the end of 1998, more than twenty thousand apoptosis-related papers have been compiled in the MEDLINE database.

HISTORICAL REVIEW

In Greek, *apó* means "from" , *ptósis* means "a fall", and ***apoptosis*** is used to describe the "dropping off" or "falling off" of petals from flowers, or leaves from trees (406)(501). This word was first adopted by Kerr *et al.* in 1972 to depict the phenomenon of controlled cell deletion as an antithesis to mitosis in the regulation of cell populations (406). The same authors had previously named this mode of cell death as "**shrinkage necrosis**", describing that cells undergoing apoptosis become condensed and compact (402). Key discoveries on apoptosis are summarized in **Table 2.**

PHYSIOLOGY, PATHOLOGY AND CLINICAL SIGNIFICANCE

Apoptosis has wide-ranging implications in tissue kinetics. All multicellular organisms

Table 1

Comparison between apoptosis and necrosis

Features	Apoptosis	Necrosis
Nature of stimuli	Physiological or pathological	Pathological (usually accidental)
Susceptibility	Tightly regulated	Unregulated or poorly regulated
Energy requirement	ATP-dependent	None
Histology	Chromatin condensation, budding of apoptotic bodies, death of single isolated cells	Cellular swelling, disruption of organelles, death of patches of tissue
Plasma membrane	Intact, but with molecular "eat-me" signals	Lysed
Phagocytosis of dead cells	By neighboring cells	By immigrant phagocytes
Tissue reaction	No inflammation	Inflammation
DNA breakdown pattern	Ladder of fragments in internucleosomal multiples of 185 base pairs	Randomly sized fragments
Activation of caspases	Yes	No
Involvement of mitochondria	Yes	Yes
Mitochondrial changes	No swelling	Swelling

References: (315)(426)(686).

Table 2

Milestone studies of apoptosis

Year	Discovery or Event	Reference
1885	"Chromatolysis" was used by Walther Flemming to describe the disappearing process of nuclear debris.	(501)
1951	A form of "physiological cell death" was described by A. Glucksmann.	(501)
1964	"Programmed cell death" was proposed by R. A. Lockshin.	(484)
1971	"Shrinkage necrosis" was used to differentiate a type of cell death from the classical necrosis.	(402)
1972	"Apoptosis" was used to describe a type of cell death with a characteristic morphology.	(406)
1980	"Ladder" pattern of DNA fragmentation in apoptosis	(839)
1988	Bcl-2 is anti-apoptotic	(779)
1991	*ced (cell death abnormal) genes* in *Caenorhabditis elegans*	(182)
1991	The viral protein BHRF1 was recognized to be anti-apoptotic, resembling Bcl-2.	(264)
1992	Fas/APO-1/CD95	(362)(586)
1993	The role of p53 in regulation of apoptosis	(121)(489)
1993	Fas ligand	(735)
1993	Interleukin-1β concerting enzyme (ICE) is the mammalian homolog of *ced-3*	(873)
1993	IAPs (inhibitors for apoptosis)	(138)(47)
1994	Mitochondria was proposed to be a primary target during apoptosis.	(781)
1996	AIF (apoptosis-inducing factor)	(739)
1996	"Caspase" was coined to represent a group of proteases that are activated during apoptosis.	(10)
1996	Leakage of cytochrome c from mitochondria during apoptosis	(478)
1997	Apafs (apoptosis-associated factors)	(898)(460)
1997	DFF (DNA fragmentation factor)	(480)
1998	CAD (caspase-activated DNase) and ICAD (inhibitor for CAD)	(185)(677)

physiologically use apoptosis and/or programmed cell death (PCD) for development, homeostasis, defense, and aging (780). It has been observed in processes of development, metamorphosis of insects and amphibians, terminal differentiations, immune responses, cellular responses to hormones and growth factors, injuries, and neoplastic diseases (698)(801). The magnitude of apoptosis could be enormous in given organs, for example, more than 95% of thymocytes die in the thymus during maturation (570). Apoptosis is also prevalent in diseases, such as the insufficient apoptosis in tumorigenesis and the excessive apoptosis in neurodegeneration, autoimmunity, heart diseases, and infectious diseases including AIDS (25)(315)(668)(757).

Physiological roles of apoptosis

Apoptosis is a self-destructive process. Why have unicellular organisms capable of this self-destruction survived the selection of evolution (15)(135)? For a group of unicellular organisms, apoptosis allows constant selection for the best-fit cell in a colony and optimal adaptation of cell numbers to the environment (15)(630). In multicellular organisms, the general purposes of apoptosis (or PCD in ontogeny) are summarized in **Table 3.**

Developmental plasticity and selection for the best-fit cells

A common developmental scheme of ontogeny is that more-than-needed number of cells are originally generated, then many original cells die through PCD, so that only a fraction of the total number of original cells make the final organs. For instance, only approximately 50% of the motor neurons produced during chick embryogenesis survive to function prior to birth (283)(593). An intriguing question therefore is "why should these cells die before they have had a chance to function?" (365). The answers could be two fold. First, PCD may be a mechanism providing a developmental plasticity in addressing a wide range of developmental problems by using a limited number of genes. For example, given about 100,000 genes in the human genome and about 10 billion cells in the human brain, it is impossible to assign each individual cell a unique phenotype. A general strategy of neurogenesis is to generate many more cells than will be needed for a particular function, then to trim this group of cells by a common set of instructions regarding differentiation, migration, and target specificity. Only those cells functionally interacting with their natural targets receive a retrograde trophic factor to survive (539). This strategy of "system matching" ensures the functional integrity of the central nervous systems where given patterns of intercellular connection are essential. Second, PCD may be the selecting mechanism in the developmental

Table 3

Physiological roles of apoptosis

Functions	Examples	References
Developmental plasticity and selection for the best-fit cells	Brain development, development of immune system	(593) (301)
Sculpting the body	Disappearance of the human tail, digit formation of limbs,	(195) (235)
Homeostasis	Hematopoietic system, ovary, endometrium	(523) (388) (742)
Protection of an organism from dangers created by its own deleterious cells	Inactivation of active immune cells after an immune response, host defense against tumorigenesis and viral infections	(776) (802)(613)

scheme where developing cells constantly compete for a limited amount of survival signals. Only the best-fit cells, *i.e.*, the most healthy, fastest growing cells toward the correct direction, are able to access the survival signals from their microenvironment (365)(630).

Sculpting the body

Massive loss of cells through PCD and/or apoptosis is the chisel to sculpt the body during embryogenesis, metamorphosis, and in adult life across species. The elimination of surplus cells is necessary for the formation of digits of the limbs (15)(235)(538), hollowing out solid structures to create lumina (365), and disappearance of the human tail (195). The cells that have outlived their usefulness, such as tadpole tails (748) and the human endometrium at the end of each menstrual cycle (596) should be eliminated during metamorphosis and preparation for the next cycle, respectively.

Homeostasis

Homeostasis of cell numbers requires a delicate balance between input (proliferation) and output (apoptosis) processes (96). Hence the sizes of both organs and organisms are maintained by regulation of cell division (629) and cell death (365). Apoptosis is essential for size control, renewal of cell populations, and maintenance of functions. How proliferation and apoptosis are coordinated to achieve a balance within each cell lineage is not completely clear. These two processes might be controlled by independent genetic pathways with regulation dependent on the level of extracellular signals (96). Alternatively, proliferation and apoptosis may be controlled by overlapping genetic pathways and the cell may employ the same mechanistic components to achieve each process.

During fetal development, apoptosis accounts for the regression of Müllerian ducts in males and Wolffian ducts in females (623). Apoptosis is also responsible for both the ovarian follicle development from the fetal period through the reproductive ages (388)(759) and the renewal of endometrium during each menstrual cycle (332)(742). Both the ovary and endometrium requires hormones and growth factors for development and functions, hence, they are the unique systems for examining the hormonal regulation of apoptosis.

Protection of organisms from deleterious cells

PCD and/or apoptosis also play an important role in protecting organisms from deleterious cells as in the "Better dead than wrong" maxim pointed out by Cohen *et al.* (131). For example, elimination of an auto-reactive cells that bear self-reactive T cell receptors to avoid auto-immune response is the purpose of the negative selection of thymocytes (301)(566). Equally importantly, upon encountering adverse insults such as UV or ionizing irradiation, one safeguarding mechanism for the organism against potential

tumorigenesis is to trigger apoptosis of genome-damaged cells, mainly through the p53-dependent pathways (802)(895). In short, organisms employ apoptosis to delete unwanted, injured, or virus-infected cells (613).

Apoptosis-associated pathology

Homeostasis of cell numbers is tightly balanced between proliferation and apoptosis. Either inadequate or excess apoptosis may result in disease **(Table 4)**.

Induction of apoptosis as the end point of antineoplastic therapy

Accumulating evidence indicates that induction of apoptosis is the common result of different types of antineoplastic therapy (206)(254)(317)(780)(802). Both successful chemotherapy and radiation therapy induce apoptosis of tumor cells rather than killing them as the result of a direct insult to DNA (521)(757). The apoptotic response correlates well with the efficacy of antineoplastic treatment, hence the propensity to apoptosis of tumor cells has been proposed to be a candidate predictor for tumor treatment response (537).

One potential advantage of setting therapy-induced apoptosis as a goal of antineoplastic treatment is that critical concentrations of many anticancer agents may induce apoptosis only in tumor cells but not in normal cells (317)(802). This also suggests that the threshold for the onset of cell death can be determined by the relative expression levels of genes which promote or suppress apoptosis (318). The foremost example is the gestational trophoblastic diseases (GTD), a group of neoplasms (hydatidiform mole, invasive mole, choriocarcinoma) originating from the placenta (801). GTD may become fatal, however, proper combined chemotherapy has brought the cure rate up to 94% (496). Both the embryonic origin and the good response to chemotherapy earn GTD the reputation as "God's first cancer, man's first cure." commented by A. T. Hertig (252). Since the placenta functions as a "disposable" organ after delivery, trophoblasts may be destined to cell death at the end of pregnancy. Their propensity to apoptosis may be the fundamental explanation for the good response of GTD to chemotherapy (801).

How to increase the apoptotic response of tumors to anticancer therapy has been enthusiastically studied. In addition to conventional radiation therapy and chemotherapy, newer approaches include enhancement of pro-apoptotic pathways and inhibition of anti-apoptotic signaling cascades in cancer cells.

More than 60% of human cancer contain mutations in the p53 genes (291)(326)(456) and p53

Table 4

Apoptosis-associated diseases

Diseases associated with Inhibition of Apoptosis	Diseases associated with Increased Apoptosis
Autoimmune diseases:	*Acquired immunodeficiency diseases (AIDS)*
Autoimmune diabetes	
Autoimmune lymphoproliferative syndromes (ALPS)	*Chemically induced:*
Immune-mediated glomerulonephritis	Alcohol-related liver disorders
Systemic lupus erythematosus (SLE)	*Decreased blood production:*
	Myelodysplastic syndromes
Cancer:	Aplastic anemia
Bax mutation	
Bcl-2 overexpression	*Heart diseases:*
Hormone-dependent tumors (breast, ovary, prostate)	Idiopathic dilated cardiomyopathy (IDCM)
p53 mutation	*Ischemic injuries:*
pRb mutation	Myocardial infarction
	Reperfusion injury
Endometriosis	Stroke
Viral infections:	*Neurodegenerative disorders:*
Adenovirus	Alzheimer's disease
Epstein-Barr virus	Amyotrophic lateral sclerosis
Herpesvirus	Cerebellar degeneration
Poxvirus	Parkinson's disease
	Retinitis pigmentosa
	Physically induced:
	Sunburn

References: (25)(40)(41)(96)(173)(243)(315)(497)(523)(524)(526)(528)(539)(802)(831)(757)(869).

mutations have been correlated to poor prognoses in a variety of tumor types (1)(455)(521). Both radiation and chemotherapy can induce apoptosis that depends on the function of wt p53, perhaps through both downregulation of Bcl-2 and upregulation of Bax (282)(549)(550)(702)(741). Therefore, restorations of wt p53 function in tumor cells to enhance the efficacy of antineoplastic therapy have been extensively tried with some encouraging results (122)(174)(224)(255)(706)(743)(808)(865)(867).

From the clinical viewpoint, however, induction of p53-independent apoptosis may be a more important strategy to treat cancers with mutant p53 (802). Various chemotherapeutic agents have been demonstrated to induce p53-independent apoptosis, such as cisplatin, camptothecin, etoposide, vincristine, paclitaxel (Taxol®) (58)(714)(802). A study further indicates that loss of wt p53 function confers cell susceptibility to paclitaxel (PTX) by increasing the number of cells arrested at G_2/M and subsequent apoptosis (788).

The advances in apoptosis research also pave the avenue for development of novel strategies for fighting cancers by targeting the apoptotic pathways of tumor cells. The anti-apoptotic Bcl-2 is found at abnormally high levels in more than half of all human cancers (640). Anti-sense technology has been used to reduce the Bcl-2 protein levels in tumor cells with the effects of inducing tumor cell apoptosis and/or increasing sensitivity to chemotherapy (41)(77)(412)(639)(751)(814).

Other potential targets for augmentation of treatment-induced apoptosis may reside in signal transduction pathways mediating apoptosis (32)(302)(457). Many cell types requires the presence of specific growth factors for survival. A proto-oncogene, the serine/threonine protein kinase Akt (also known as protein kinase B, PKB), may play a critical role for the growth factor-dependent cell survival by sensing the survival signals from growth factor receptors and inhibiting apoptosis by inactivating the pro-apoptotic BAD (140)(152)(176)(210)(211)(212)(302)(306)(305). Our understanding of this growth factor-dependent survival pathway reveals a series of potential targets for therapeutic intervention (302).

APOPTOTIC CHARACTERISTICS

Apoptosis can be detected morphologically and biochemically. Two apoptotic characteristics commonly used to document apoptosis are the apoptotic morphology (20)(404) and "ladder" pattern of DNA fragmentation (839)(841). The characteristic apoptotic morphology recognized by electron or light microscopy is consistently similar across cell types and species and is believed to be the most unequivocal

experimental proof of apoptosis (324). On the other hand, DNA fragmentation is not always found together with morphological changes in cells undergoing apoptosis. The characteristic apoptotic morphology has been found in the absence of typical DNA fragmentation (129)(583)(762) and it even could be induced in a nucleus-free cytoplast system (364). Many other biochemical characterizations of apoptosis, such as externalization of phosphatidylserine and activation of caspases, as well as identification of various substrates for caspases, have facilitated advances in apoptosis research. Equally importantly, rapid clearance of apoptotic cells without inflammation is also a unique feature of apoptosis.

Morphology

Morphological changes **(Figure 1)(Table 5)** (802) in apoptotic cells include compaction of nuclear chromatin *(pyknosis)* which is packed against the nuclear membrane (margination of chromatin), condensation of cytoplasm, convolution of nuclear and cell outlines, followed by rupture and fragmentation of nucleus *(karyorrhexis)*, and formation of apoptotic bodies (budding) (20)(404). The descriptive terms budding and blebbing have been used interchangeably to describe the formation of apoptotic bodies and their separation from the original cell (86)(405)(408)(677)(786). The interchangeable usage may be inappropriate, however, because apoptotic bodies contain any type of organelles including nuclear fragments while blebs are fluid-filled structure, typically devoid of organelles (501).

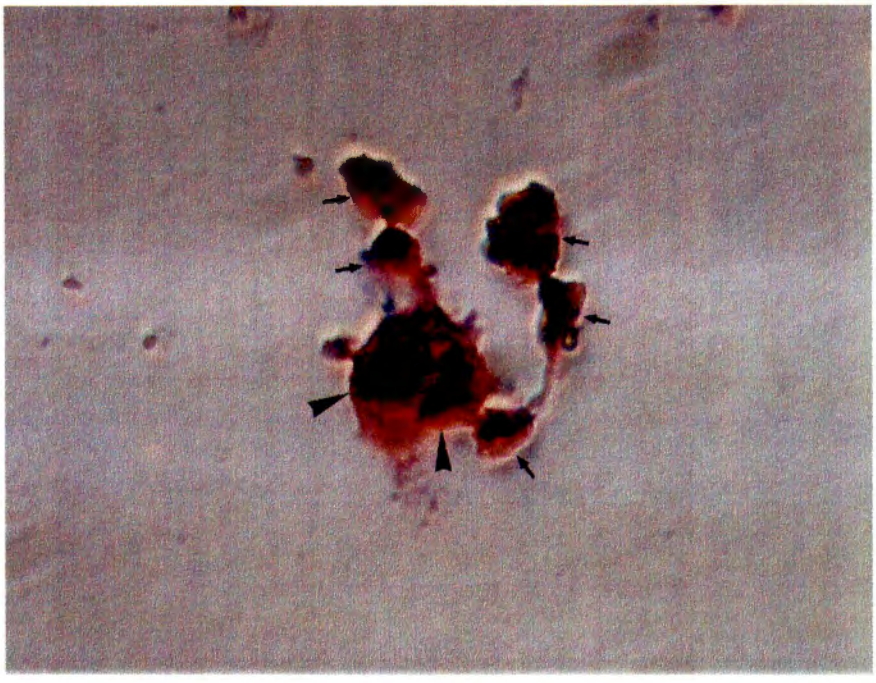
To study effects of particular genes in regulation of apoptosis in transfection experiments, expression vectors for genes of interest and for β-galactosidase (such as pCMV-lacZ) have been simultaneously delivered into cells. Cells coexpressing β-galactosidase become blue when stained with X-gal. Thus occurrence of apoptosis among cells expressing genes of interest can be evaluated by counting the number of cells with apoptotic morphology among the whole population of blue cells (483).

Biochemistry

Biochemical features of apoptosis include externalization of phosphatidylserine exerting high affinity to annexin-V (AV), activation of caspases, cleavage of 112-kD poly ADP-ribose polymerase into a 86-kD species, and DNA fragmentation (128)(509)(786).

Membrane changes of apoptotic cells

Apoptotic cells have to express some "eat-me" signals (686) that can be recognized by



(Figure 1) Characteristic morphology of an apoptotic cell. Apoptosis of ovarian cancer BR cells was induced by treatment with 0.1 μM paclitaxel for 24 h. Compaction of nuclear chromatin (*arrowheads*), convolution of nuclear and cell outlines, formation and budding of apoptotic bodies (*arrows*) are characteristic for apoptosis (*H & E staining, 1,000X*).

Table 5

Morphological characteristics of apoptosis

Pyknosis: compaction of nuclear chromatin which is packed against the nuclear membrane (*margination* of chromatin),
Condensation of cytoplasm,
Convolution of nuclear and cell outlines,
Rupture and fragmentation of nucleus,
Formation of apoptotic bodies (*budding*).

macrophages or neighboring cells, facilitating clearance of apoptotic cells through phagocytosis **(Table 6)**. Teleologically, these "eat-me" signals on the cell membrane should be expressed as early as possible by an apoptotic cell, when its membrane still remains intact, to ensure it to be phagocytosed before noxious cell content in the apoptotic cell leaks and initiates inflammatory reactions. Several "eat-me" signals have been discovered, including anionic thrombospondin-1 binding sites (215)(688)(687), carbohydrate modification (178)(228)(559), and externalization of phosphatidylserine (PS) (68)(193)(783)(878).

PS exposed in apoptotic cells exerts high affinity to AV, hence labeling PS on apoptotic cells with flurochrome-conjugated AV is a sensitive identification for apoptotic cells (417)(509). Furthermore, since apoptotic cells, but not dead cells, are still able to exclude nucleotide intercalating dyes such as propidium iodide (PI) and 7-amino actinomycin D (7AAD), the combined applications with FITC-conjugated AV with PI or PE-conjugated AV with 7-AAD for flow cytometric analyses can quantitatively differentiate dead cells (PI or 7AAD positive) from apoptotic cells (AV positive / PI or 7AAD negative) **(Figure 2:A-C).** To study effects of particular genes in regulation of apoptosis in transfection experiments, expression vectors for genes of interest (either wild type or dominant negative) and for an enhanced green fluorescent protein (pEGFP, Clonetech, Palo Alto, CA) can be simultaneously delivered into the cell. At the time of sufficient expression of transfected genes, apoptosis in transfected cells (coexpressing green fluorescent protein) can be evaluated by staining with PE-conjugated AV (**Figure 2:D-F**). Furthermore, apoptosis and cell death can be differentiated by simultaneous staining with the PE-conjugated AV and 7-AAD and analyzed with flow cytometry. Fluorochromes such as green fluorescent protein expressed by pEGFP, PE-AV, and 7-AAD can be excited by laser tuned to 488 nm and emissions can be detected at 507 nm, 575 nm, and 650 nm, respectively (800).

Activation of caspases results in apoptotic characteristics

In an *in vitro* model of apoptosis, exposure to cytosol extracted from apoptotic cells induces apoptotic characteristics in normal nuclei. The apoptotic features include condensation and margination of chromatin, DNA fragmentation, and proteolysis of lamins, which are the main proteins of nuclear envelope (443)(444). Members of the caspase family have been identified to be responsible for these changes (128)(408)(577)(786) and many apoptosis-related cellular targets for caspases have been identified by using caspase inhibitors (184)(487). Proteolysis of cellular targets for caspases account for most of apoptotic characteristics.

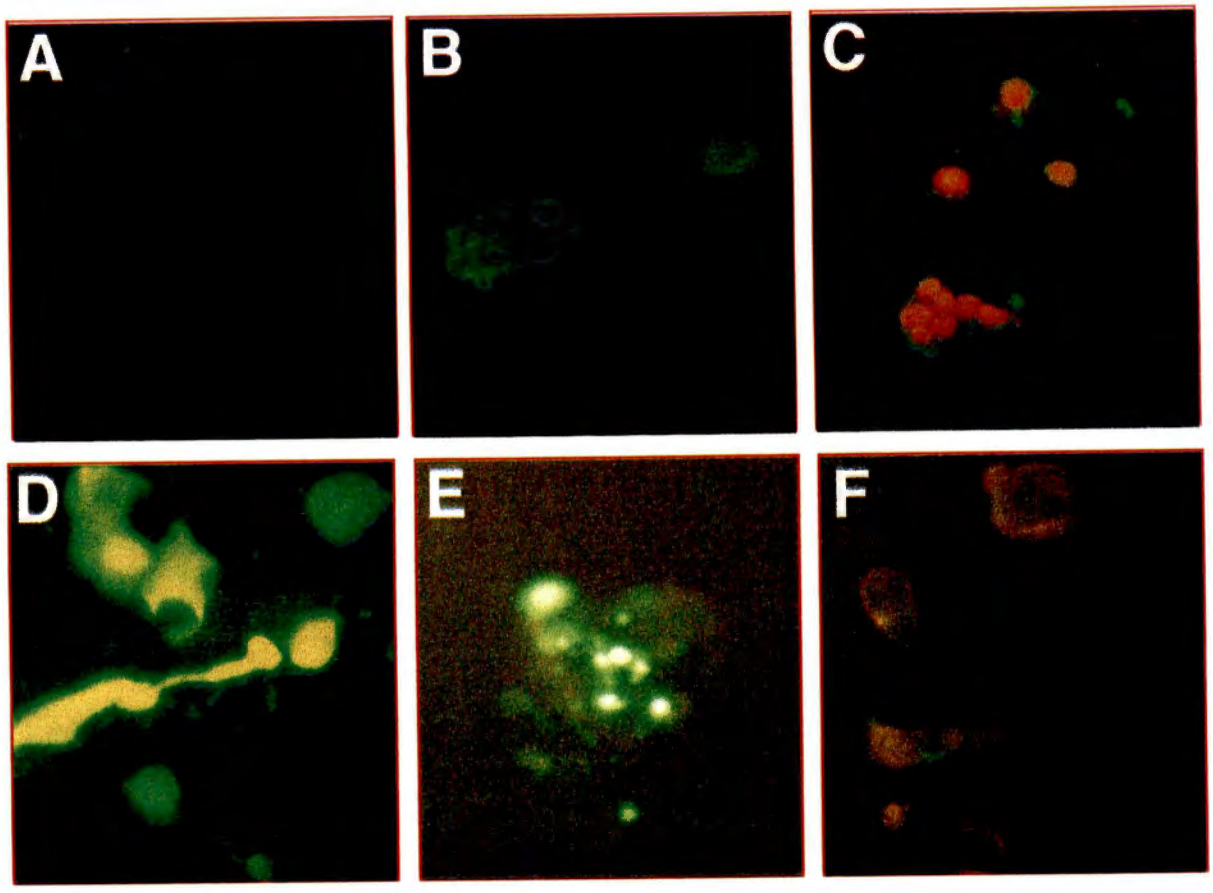
Cellular targets for caspases fall into three categories: (i) procaspases, (ii) cellular proteins that

Table 6

Components associated with elimination of apoptotic cells

"Eat-me" signals on the membrane of apoptotic cells	Membrane components of neighboring cells for interaction with apoptotic cells	Cellular components required for engulfment of apoptotic cells
Anionic thrombospondin 1 binding sites	ATP-binding cassette transporters	CDM (CED-5, DOCK180, MBC) family
Modified carbohydrates	CD14	
Phosphatidylserine	Class A scavenger receptors	
	Lectin	
	Phosphatidylserine receptors	
	Thrombospondin receptors (integrin αvβ5, CD36)	

References: (685)(686).



(Figure 2) Detection of apoptotic cells with fluorochrome-conjugated annexin-V. ***A-C,*** Ovarian cancer BR cells growing on glass slides were treated with 1 μM paclitaxel for 8 h followed by simultaneous staining with FITC-conjugated annexin-V (green) and propidium iodide (red). ***A,*** no staining of either annexin-V or PI were detected in live cells. ***B,*** early apoptotic cells were only stained with annexin-V. ***C,*** dead cells were stained with both FITC-conjugated annexin-V and propidium iodide. ***D-F,*** BR cells were cotransfected with expression vectors for the gene of interest and for the green fluorescent protein (pEGFP), treated with paclitaxel, and stained with phycoerythrin (PE)-conjugated annexin-V (orange color). ***D,*** green fluorescence served as an indicator for expression of transfected genes. ***E,*** apoptosis in cells expressing the gene of interest was detected by identification of PE-annexin-V in the green cells. ***F,*** apoptosis in non-expressing cells was detected by identification of PE-annexin-V in the non-green cells (*fluorescent microscopy, 200X*).

need to be inactivated for cell death to occur, and (iii) cellular proteins whose activation is required for the apoptotic process **(Table 7)**(408). It is noteworthy that some of the target proteins for caspases listed in **Table 7** may not always be cleaved during apoptosis. Furthermore, none of the cleavage events compiled in **Table 7** has been proven to be absolutely required to kill cells (745).

DNA fragmentation

One biochemical hallmark of apoptosis (133) commonly used to document apoptosis is the "ladder" pattern of DNA fragmentation **(Figure 3)**(802), which results from chromatin cleavage at internucleosomal sites at about 180-200 bp intervals by activated DNases (839)(841). Since this internucleosomal DNA cleavage is distinct from the nonspecific DNA degradation present in cell necrosis, such DNA fragmentation has also been used as the basis for several histochemical techniques for identification of apoptotic cells: such as the TdT-mediated dUTP nick end labeling (TUNEL) assay **(Figure 4)**(240)(802)(821). Although the role of DNA destruction during apoptosis is not entirely clear, it has been speculated to play a role in suppressing inflammatory responses (60).

Various DNases, such as DNase I, DNase II, and cyclophilins, have been proposed to be the executor for DNA fragmentation (34)(552)(611). However, whether they are really apoptosis-specific had been controversial, until the human DNA fragmentation factor (DFF) was identified in 1997 (480). DFF is a heterodimer of the 40-kD DNase (DFF40) and the 45-kD inhibitor of DNase (DFF45). DFF45 is cleaved by caspase-3 (CPP32), suggesting that DFF functions downstream of caspases.

Several months later in 1998, a caspase-activated DNase (CAD) and its inhibitors (ICAD) were identified in murine cells (185). In addition to being an inhibitor of CAD, ICADs also functions as chaperon proteins, stabilizing CAD during its synthesis (185). Apoptotic cells from DFF45 knock-out mice lacked DNA fragmentation, however, homozygous DFF45/ICAD null mice were healthy and fertile, indicating that apoptosis required for ontogenic development is intact despite lack of DNA fragmentation (888).

In apoptotic cells, activated caspases (mainly the caspase-3) cleave ICAD/DFF45 and release it from CAD/DFF40, resulting in activation of CAD/DFF40 and internucleosomal DNA degradation (185)(677). The role of caspase-3 in induction of apoptotic DNA fragmentation was supported by the observation of suppression of DNA fragmentation during apoptosis of breast cancer MCF-7 cells, which lose caspase-3 due to a 47-bp deletion within exon 3 of the *CASP-3* gene (367).

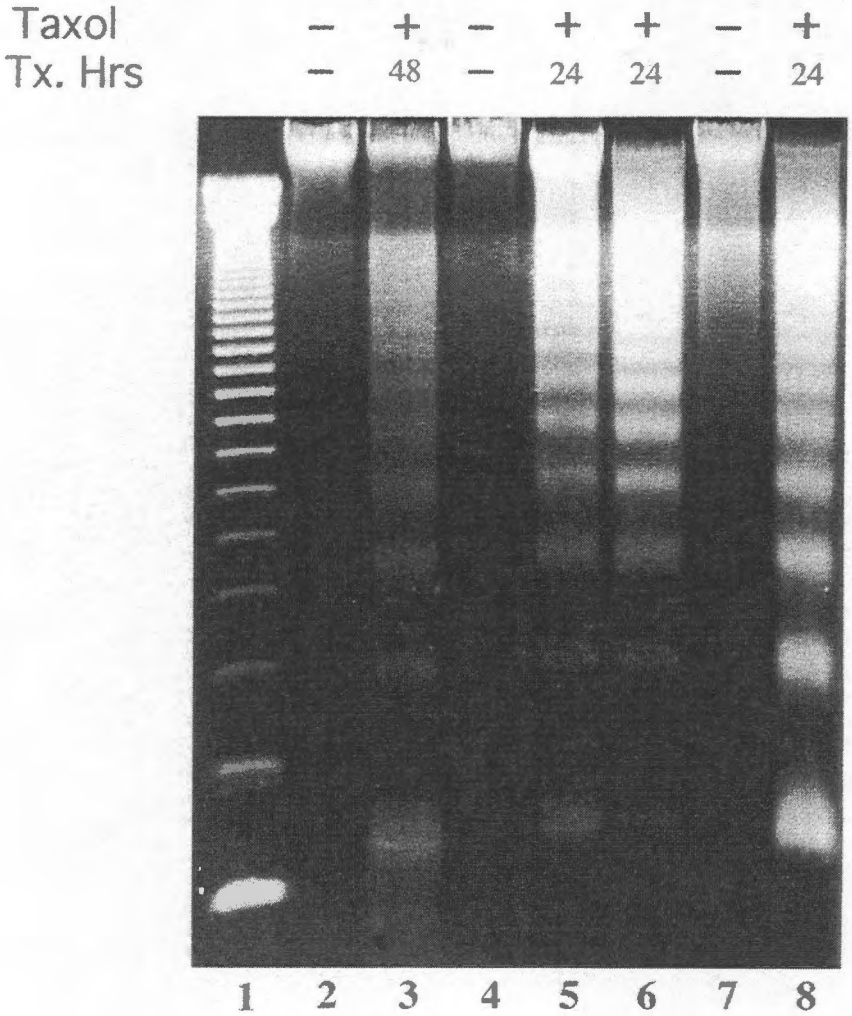
In summary, (i) activation of CAD/DFF40 by caspases is a late event in the apoptotic process,

Table 7

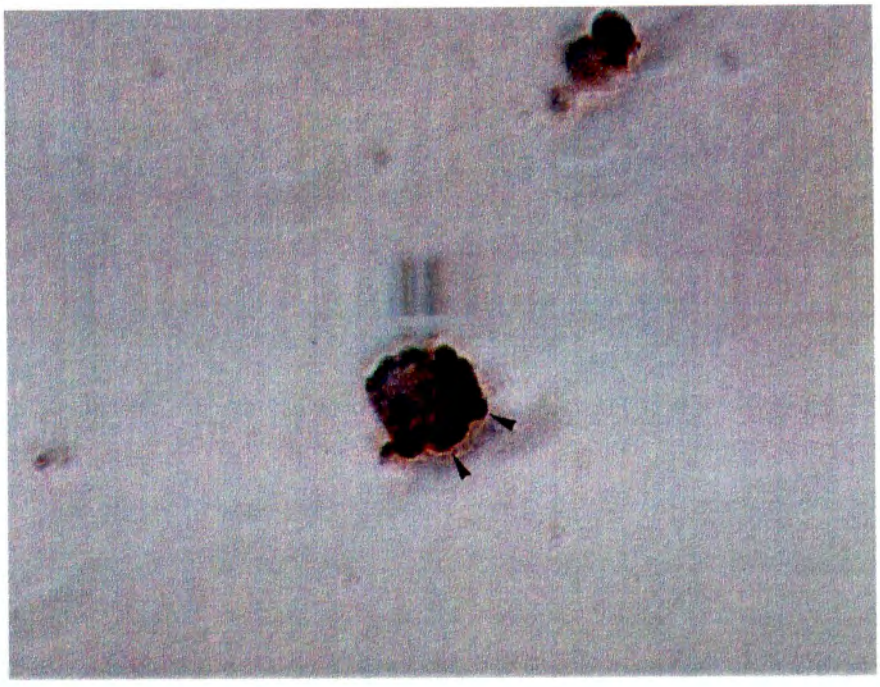
Caspase activation resulting in apoptotic characteristics

	Group	Target Proteins	Functions of Target Proteins
I	Procaspases	Procaspases	Precursor of caspases
II	Proteins needing to be inactivated	Actin	Cytoskeleton components
		Bcl-2, Bcl-X_L	Anti-apoptotic proteins
		CDK27, p21, p27	Inhibitors of some CDKs
		DFF-45kD/ICAD	Inhibitor of caspase-activated DNase
		DNA-PK	DNA repair
		FAK	Protein kinase
		Fodrin	Cytoskeleton components
		GAS2	Cytoskeleton components
		Gelsolin	Actin-severing protein
		Keratin	Intermediate filament components
		Lamins	Nuclear envelope components
		MDM2	Inhibitor of p53
		PARP	DNA repair
		pRB	Assembly of repressor complex
		Rho-GDI	Inhibitor of small G-protein
		70kD-U1-RNP	Pre-mRNA splicing
III	Proteins needing to be activated	MEKK1	Protein kinase
		PAK2	Protein kinase
		PKCδ	Protein kinase
		PKCθ	Protein kinase
		PITSLRE kinase	Protein kinase
		c-PLA2	Phospholipid metabolism
		SREBPs	Cholesterol-regulating factors

References: (106)(123)(128)(408)(458)(480)(677)(745)(898).



(Figure 3) The ladder pattern of DNA fragmentation in apoptotic cells. Apoptosis of four human cancer cell lines was induced by treatment with paclitaxel (0.1 μM) for 24 or 48 h. *Lane 1*: 123 bp ladder DNA marker; *lanes 2 and 3*: breast cancer MCF-7 cells; *lanes 4 and 5*: ovarian cancer BR cells; *lane 6*: ovarian cancer BG1 cells; *lanes 7 and 8*: osteosarcoma SAOS-2 cells.



(Figure 4) Terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP nick end labeling (TUNEL) assay. Human ovarian cancer BR cells were treated with 0.1 μM paclitaxel for 24 h and assay with an in situ DNA fragmentation detection kit, Apoptag (Oncor, Bethesda, MD). Fragmented DNA in condensed chromatin of apoptotic cells is labeled in dark brown (*arrowheads*) (*methyl green counterstaining, 1,000X*).

(ii) DNA fragmentation may be dispensable during apoptosis (771), explaining the observations where DNA fragmentation was not detectable in some cell types exhibiting characteristic apoptotic morphology (583)(762), (iii) the DNase activity of purified DFF40/CAD is stimulated by chromatin-associated histone H1 and high mobility group proteins, and (iv) both DFF40/CAD and DFF45/ICAD are localized in the nucleus, and DFF40/CAD also induces chromatin condensation (479).

Summary (how to document occurrence of apoptosis?)

In addition to morphological identifications, various methods are commonly used to identify the apoptotic process at different stages in cultured cells **(Figure 5)**. The early apoptosis is characterized by externalization of phosphatidylserine on the intact cell membrane that is still able to exclude viability dyes such as PI, 7-AAD, and trypan blue. AV exerts high affinity to phosphatidylserine, therefore, early apoptotic cells can be labeled as AV (+) and viability dye (-). Ongoing apoptosis is characterized by activation of caspases, which can be measured *in vitro* by using colorimetric or fluorometric substrates. Consequences of caspase activation, such as PARP cleavage and DNA fragmentation (detected by the ladder pattern of electrophoretic DNA or the TUNEL assay for cells or tissues), have been used for documentation of apoptotic process. The severity of DNA fragmentation can be quantified by ELISA-based methods, such as the Cell Death Detection ELISA (Boerhinger Mannheim, Indianapolis, IN). At the end stage of apoptosis, dead cells and apoptotic bodies can no longer exclude viability dyes and are stained by DNA-targeting dyes such as PI and 7-AAD.

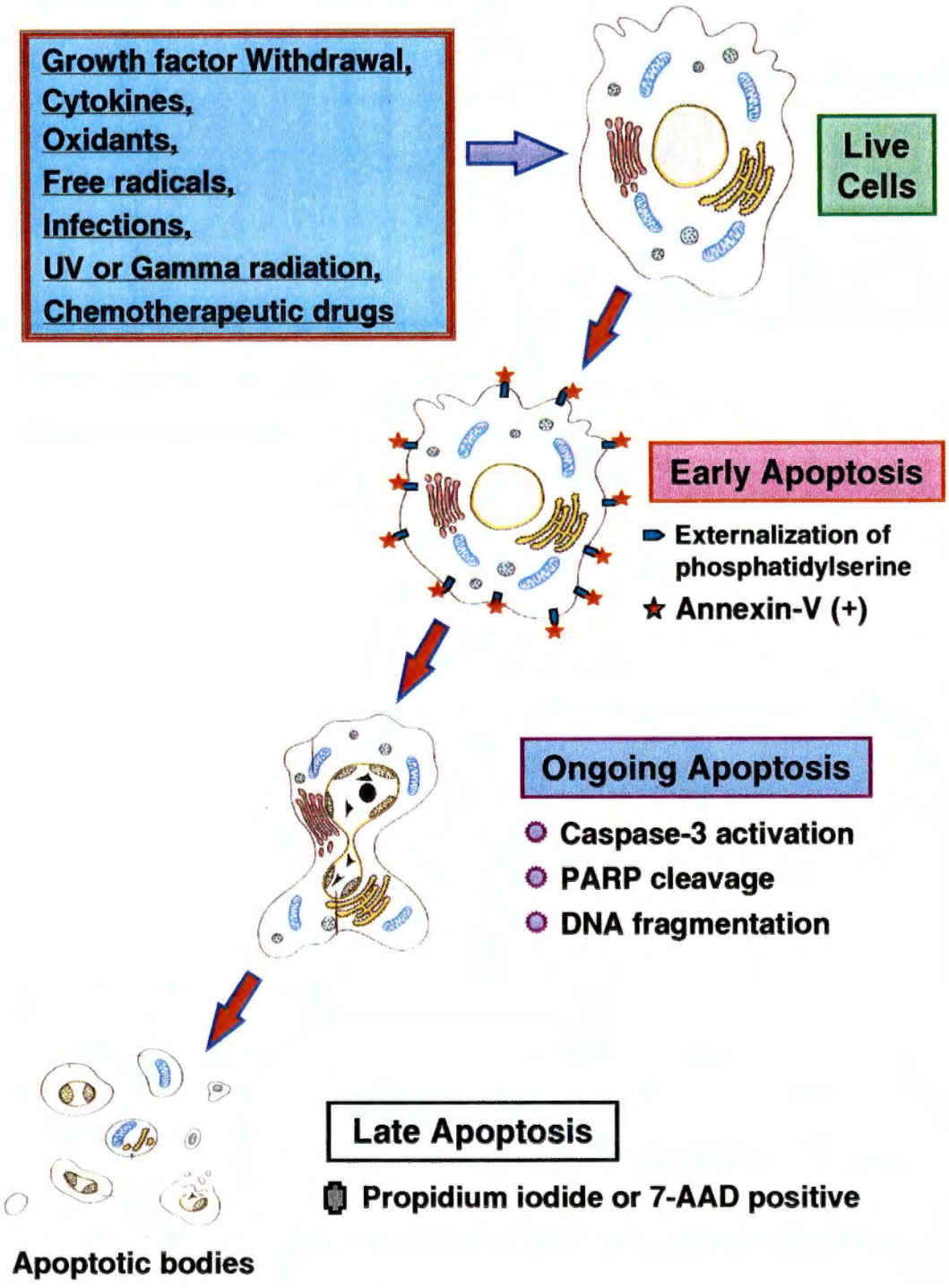
Elimination of apoptotic cells

Elimination without inflammation

The third unique characteristic of apoptosis is the minimal, if any, involvement of inflammation in the rapid elimination of apoptotic cells by phagocytosis (406)(630). The absence of inflammation minimizes tissue injury and scar formation, hence apoptosis is the ideal shaping mechanism during ontogenic development. Two factors may contribute to the absence of inflammation during apoptosis. (i) The membrane integrity of apoptotic cells and apoptotic bodies prevents leakage of cell contents into environment (315)(630). (ii) The apoptotic cell itself may have immunosuppressive effects on phagocytes (787).

Phagocytes require some membrane components to interact with apoptotic cells

A variety of membrane components on phagocytes may be required for recognition and



(Figure 5) Some detection methods for apoptosis. Abbreviations used are: PARP, poly (ADP-ribose) polymerase; and 7-AAD, 7-amino actinomycin D.

phagocytosis of apoptotic cells (**Table 6**). The abundance of phagocyte receptors for apoptotic cells apparently reflects the vital importance of the clearance process, since secondary necrosis and catastrophic damage to tissues may occur in the absence of clearance or when apoptosis is overwhelmed (587).

Cellular components required for engulfment of apoptotic cells

Phagocytes, both the "professional macrophages" and "semi-professional" cells neighboring apoptotic cells, employ various membrane "tethering" components (**Table 6**) to interact with apoptotic cells. The interactions, through yet to be defined mechanisms, trigger transmembrane signaling which leads to activation of the CED-5, DOCK180, MBC (CDM) family members and reorganization of the cytoskeleton, ultimately engulfing the apoptotic cell (685).

At least six *ced* genes: *ced-1, -2, -5, -6, -7,* and *-10*, of the nematode *C. elegans* regulate the engulfment of apoptotic cells (**Table 8**)(182). Mutations of these genes inhibit extension of the membranes of engulfing cells around the dying cells. The amino acid sequence of the human DOCK180 shares 26% homology with CED-5. Both CED-5 and DOCK180 also share significant homology with the protein product of the *Drosophila* gene *mbc* (837). The mutation of *ced-5* in *C. elegans* results in defects of engulfment of cell corpses and migration of the distal tip cells. The latter defect can be rescued by expression of the human DOCK180 (837). DOCK180 is a cytoplasmic protein with an SH3 domain which allows it to interact with various signal transduction pathways (294). Furthermore, the farnesylated DOCK180 drives cell spreading, suggesting its involvement in the regulation of cell movement by tyrosine kinases (685). Collectively, from the rescue of cell migration in *ced-5* mutant and the functions of MBC (Myoblast City) in myoblast fusion, Wu *et al.* suggest a potential role of CDM family members in the cytoskeletal reorganization required for engulfing the apoptotic cell.

Summary

The abundance and redundancy of phagocyte receptors for apoptotic cells apparently reflect the absolute importance for efficient elimination of apoptotic cells (**Table 6**). Possible answers for the question "why so many mechanisms?" are discussed as the followings. (i) Phagocyte receptors may need cooperation to exert optimal phagocytosis. (ii) Some of the cooperative phagocyte receptors may elicit essential immunosuppressive signals to avoid the harmful inflammatory responses from the macrophage. (iii) Redundancy of the phagocyte receptors ensures the critical functions as each receptor backs up the others. (iv) Recognition of apoptotic cells may be only the "part-time" functions of some phagocyte receptors (such as $\alpha v\beta 5$, CD36, class A scavenger receptors).

Table 8

Nematode gene products associated with apoptosis and their mammalian homologs

Stages	Functions	*C. elegans*	mammalian
Decision to die	Pro-apoptotic	*ces-2* *egl-1*	
	Anti-apoptotic	*ces-1*	
Execution of death	Pro-apoptotic	*ced-3* *ced-4* *ced-8*	ICE Apaf-1
	Anti-apoptotic	*ced-9*	Bcl-2
Engulfment	Required for engulfment	*ced-1* *ced-2* *ced-5* *ced-6* *ced-7* *ced-10*	DOCK180
Degradation	Required for degradation of engulfed cells	*nuc-1*	

Reference: (239)

COMPONENTS OF THE APOPTOTIC PATHWAY

Depending on the cell type, a variety of stimuli may initiate apoptosis. The apoptotic process can be arbitrarily divided into three phases: detection and activation, regulation, and execution by effectors (**Table 9**).

STIMULI

Genomic damage

Apoptosis is the major mode for an organism to remove genome-damaged cells. Genomic assaults include: UV irradiation (67)(539)(895), ionizing irradiation (245)(357), and DNA-targeting chemicals including many anticancer chemotherapeutic agents such as *cis*-platinum, 5-fluorouracil, 5-fluorodeoxyuridine, and others (33). Genomic damage initiates apoptosis mainly through p53-dependent pathways (121)(489)(490)(741)(884). Hence, p53 earns the reputation as the "guardian of the genome" (438).

Cells also employ additional pathways to stop or eliminate genome-damaged cells. A comparison of activation of p53, c-Abl, and JNK/SAPK in murine fibroblasts treated with cis-platinum, ionizing radiation, methyl methanesulfonate (MMS), mitomycin C, or UV irradiation indicates that p53 is induced by all of these tested genotoxic stresses; c-Abl is activated by most stimuli except UV; and JNK is only strongly activated by UV and MMS (79)(482).

Withdrawal of survival signals

Survival signals can be provided by: (i) growth factors, such as colony stimulating factor (823) and nerve growth factor (94), (ii) hormones, such as steroid hormones (651), prolactin (791), and ACTH (840), (iii) interactions between integrin system and the extracellular matrix (64)(631), or (iv) the cell shape (669)(98).

Exposure to apoptotic stimuli

Apoptotic stimuli may act systemically or locally. Systemically, glucocorticoids trigger apoptosis in thymocytes (130)(839), eosinophils (398), and leukemia cells (518), whereas thyroid initiates apoptosis

Table 9

Components of apoptosis

Category	Examples
Stimuli	Genomic damage, Withdrawal of survival signals, Exposure to apoptotic stimuli, Viral infections
Detection and activation	Apoptosis-related proto-oncogene and tumor suppressor gene products, Apoptosis-related signal transduction pathways
Regulators	Bcl-2 family Apoptosis-associated factors (Apafs) Inhibitors of apoptosis (IAP) family Mitochondria and ATP status
Effectors	Caspase family Cyclin-dependent kinases (CDKs)

during metamorphosis of the tadpole (403).

Most daily apoptotic events in the body are more likely regulated by locally acting stimuli, such as TNF-α, TGF-β, granzyme B, Fas ligand (FasL) and others. Examples include TNF-α in the regulation of cyclic renewal of endometrium (742), TGF-β in the peri-implantation decidua (562), and bone morphogenetic proteins (BMPs) in interdigital apoptosis (899). Cytotoxic T-lymphocytes (CTL) use multiple local factors to execute their killing: (i) CTL express the ligand (FasL) for Fas/CD95 and thus kill Fas-bearing target cells, and (ii) CTL also produce the granzyme B and deliver it into target cells through the help by another protein, perforin (387)(491)(710). Fas-mediated apoptosis also accounts for maintenance of immune privilege sites such as the testis and the anterior chamber of the eye. Cells of immune privilege tissues naturally produce FasL and kill infiltrating Fas-bearing lymphocytes and inflammatory cells (37)(263)(265)(570). Some tumors may also express FasL and evade immune surveillance by killing the infiltrating CTL of the host (584)(671).

Viral infections

Apoptosis may be a defense mechanism of the host to control infection of viruses (125)(126)(780). Viruses would perish if all infected host cells die before viruses have enough time to replicate. Thus, evolution has selected for those viruses equipped with gene products capable of counteracting apoptotic processes of the host cell (520). Such viral anti-apoptotic proteins include BHRF1 (Epstein Barr virus) (264)(307), crmA (cowpox virus) (227)(636), E1B19k (adenovirus) (148), LML5-HL (African swine fever virus) (7), p35 (baculovirus) (73)(851), and IAPs (baculovirus) (47)(138).

DETECTION AND ACTIVATION OF APOPTOSIS

Apoptosis-related proto-oncogenes and tumor suppressors

Several proto-oncogenes and tumor suppressors are involved in the detection and activation of apoptotic process (410)(729)(802). They fall into three groups: pro-apoptotic (Bax, p53, p73), anti-apoptotic (Akt, Bcl-2, pRb) , and dual-functional (E2F-1, c-Myc) **(Table 10)**.

Akt

Many cell types requires specific growth factors for survival. A proto-oncogene, the serine/threonine protein kinase Akt (36), also known as protein kinase B (PKB) (127)(377), may play a

Table 10

Apoptosis-related proto-oncogenes and tumor suppressors

Functions	Proto-oncogenes	Tumor suppressors
Anti-apoptotic	Akt Bcl-2	pRb
Dual-functional (anti- or pro-apoptotic depending on the cellular context)	c-Myc E2F-1	E2F-1
Pro-apoptotic		Bax p53 p73?

critical role for the growth factor-dependent cell survival (140)(176)(306)(305). Activation of a tyrosine kinase growth factor receptor by binding of growth factor results in receptor tyrosine phosphorylation, which serves as a docking site for the Src-homology 2 (SH2) domains of several proteins, including GRB2 and the p85 subunit of phosphoinositide 3-kinase (PI 3-K). GRB2, through SOS, interacts with Ras that in turn activates the Raf-MEK-ERK cascade which may promote cell proliferation. Additionally, both Ras (647)(648) and the p85 subunit of PI 3-K are capable of activation of p110, the catalytic subunit of PI 3-K. Active PI 3-K at the membrane converts phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) into PI-3,4,5-P_3, which can be further converted into PI-3,4-P_2 by inositol 5'-polyphosphatase (302). The PI-3,4-P_2 recruits Akt to the membrane and activates it (213)(210)(211)(212). Activated Akt may phosphorylate the pro-apoptotic BAD and release BAD from the formation of heterodimers on the mitochondrial membrane with other anti-apoptotic Bcl-2 family members (140). Phosphorylated BAD may be further sequestered in the cytoplasm in a complex with 14-3-3 proteins (152)(302)(306)(305).

Akt also inhibits apoptosis by direct regulation of caspase activity. Overexpression of either active Ras or Akt abolishes cytochrome *c*-induced proteolytic processing of pro-caspase-9. Akt phosphorylates caspase-9 on Ser196 and inhibits its protease activity. Mutant procaspase-9 (Ser196 -Ala) results in apoptosis that is resistant to Akt-mediated phoshorylation and inhibition (83). In short, the Akt may sense the survival signals from activated growth factor receptors and inhibit apoptosis through inactivation of the pro-apoptotic BAD and caspases.

E2F-1

The E2F transcription factor family consists of eight members in two subfamilies: E2F (-1, -2, -3, -4, -5, -6) and DP (-1, -2) (375). All of these eight members share homology in the DNA-binding domain and dimerization domain, but only E2F-1, -2, and -3 contain the N-terminal nuclear localization signals (375). E2F and DP form heterodimers (303)(832) and these heterodimers can further form heterotrimers with members of the RB pocket protein family (pRB, p107, p130) (375)(424).

The functions and regulations of the pRB/E2F/DP complex in the progression of the cell cycle are summarized as: (i) pRB/E2F/DP heterotrimers function as transcription repressors in the G_0 and early G_1 phase; (ii) during the late G_1 and early S phase, cyclin D/CDK4,6 phosphorylate pRB and thus release E2F/DP, which function as transcription activators; and (iii) during the late S and G_2/M phases, E2F/DP are inactivated through phosphorylation of DP by cyclin A/Cdc2 (375)(423).

While the other E2Fs only induce S-phase entry, E2F-1 can both promote S-phase entry and

induce apoptosis (151)(705)(835). E2F-1 has been considered as an oncogene because of *in vitro* evidence that overexpressed E2F-1 induces anchorage-independent growth in immortalized fibroblasts (718)(845) and, when in cooperation with an activated *ras* gene, it leads to oncogenic transformation (700). On the other hand, E2F-1(-/-) knockout mice are viable and fertile, but exhibit defective apoptosis and develop a wide range of tumors at old ages (205)(853)(854), suggesting that E2F-1 functions as a tumor suppressor (314).

Indeed, accumulating evidence has indicated that E2F-1 promotes apoptosis (205)(705)(835). E2F-1-induced apoptosis can be either p53-dependent or p53-independent. (i) E2F-1 can induce accumulation of p53 (319), perhaps through binding to Mdm2 and blocking its downregulating effects on p53 (295)(429)(508). (ii) Induction of p53-independent apoptosis by E2F-1 has been demonstrated in cultured cells (339)(615), in transgenic animals (327), and in tissues after adenoviral delivery of E2F-1 (8). E2F-1 induces apoptosis which cannot be rescued by coexpression of E2F-2 and E2F-3, suggesting that E2F-1 induces apoptosis through induction of an apoptosis-promoting activity rather than the lack of induction of a survival activity (151). Although the exact mechanism remains unclear, E2F-1-mediated CDK activation, may be also responsible for apoptosis, since catastrophic CDK activities have been suggested to be the actual effector of apoptosis (293)(458)(894).

c-Myc

The proto-oncogene product c-Myc is a 64-67 kD protein with a short half-life 30 min. Myc may exist as homodimers, however, it preferentially forms heterodimers with Max that is a 21-22 kD protein with a longer half life (758). Myc/Max heterodimers transactivate many genes including Cdc25A (230), cyclins A and E (370), Cdc2 (57), dihydrofolate reductase (500), ornithine decarboxylase (598), and heat shock protein 70 (384). It also can repress expression of various genes including β-integrin (382) and cyclin D1 (370)(614). The homozygous null mutation of *myc* in mice is embryonically lethal (142).

Myc apparently promotes cell growth (13): it is expressed during proliferation in a variety of cells (314) and it promotes cell progression through G_1/S phases and G_2 phase of the cell cycle (704). The dual mechanisms for Myc to move cells through the cell cycle are believed to be: (i) that Myc transactivates components required for the cell cycle such as Cdc25A, cyclins E and A, and Cdc2, and (ii) that Myc promotes pRb phosphorylation, which is an essential step in the G_1 phase, through the dissociation of an inactive complex formed by cyclins/CDKs/$p27^{Kip1}$ (314). The most intriguing role of Myc, however, is the induction of apoptosis: both decreased and increased levels of Myc have been shown to cause apoptosis (758).

Paradoxically, apoptosis has been shown to be induced by either downregulation of *c-myc* (392)(519)(828) or upregulation of *c-myc* (191)(262)(349)(656)(712). The mechanisms for the dual functions of Myc remain unclear. Myc may be required for transactivation of survival genes and/or for repression of lethal genes, explaining apoptosis induction by downregulation of *c-myc* (758).

On the other hand, upregulated c-Myc may promote apoptosis through two mechanisms described below. (i) Myc activates cyclin-dependent kinases (CDKs) (13) and accumulating evidence suggests that various CDKs may be the common components required for both cell cycle and apoptosis (226)(293)(345)(458)(529)(711)(894). (ii) Myc may sensitize cells to Fas-mediated apoptosis (348), perhaps by inhibition of Saf (suppressor of apoptosis by Fas) (260). The identity of Saf remains unclear but one candidate is FLIP (359). To avoid the fate of apoptosis, cells need survival signals including those from activated Akt (302)(306)(305) and Bcl-2 (50)(96).

p53

The tumor suppressor p53 can regulate both the cell cycle and apoptosis. When the genome is damaged, p53 can either slow down the cell cycle allowing the DNA repairing mechanisms to fix the damage, or trigger the apoptotic process to eliminate the genome-damaged cell (563)(802). How the cell decides to repair its DNA or undergo apoptosis remains unclear. Transactivation of target genes by p53 appears to be important for both slowing down of the cell cycle and triggering apoptosis (594). To become a more efficient transcription factor, p53 may have to form a complex with other gene products, such as p33 encoded by the *ING1* gene (237)(238)(236).

p53 may control the cell cycle at both G_1/S phases and G_2/M phase. (i) Progression through the G_1/S phases can be inhibited by p53 transcriptional activation of the CDK inhibitor $p21^{WAF1/CIP1}$ (179)(267)(290) and GADD45 (396)(885)(883). (ii) The G_2/M phase progression may be blocked by p53 (9)(728) through transactivation of the protein 14-3-3σ that sequesters phosphorylated Cdc25C, preventing it from dephosphorylating Cdc2 (312). Additionally, the p53-dependent G_2/M arrest may result from the as-yet unclear spindle checkpoint control mechanism (139) and centrosome duplication control mechanism (225).

Results from p21 knockout animals and other studies indicate that p21 is not required for induction of apoptosis (24)(153). Nevertheless, both transactivator and non-transactivator functions of p53 have been shown to be involved in apoptotic regulation (288)(563). In a systematic approach toward understanding how increased p53 expression leads to apoptosis, Vogelstein's group infected a human colorectal cancer cell line DLD-1, which has no functional p53, with an adenoviral vector carrying wt

human p53, then analyzed the changes of gene transcription resulting from overexpression of p53 (617). Among more than 7,000 transcripts analyzed by the serial analysis of gene expression (SAGE), 34 transcripts differed in expression by more than ten fold after p53 expression: 14 transcript increased (resulting from p53 transactivation) while 20 decreased (repression by p53) (617)(838). Among the top 14 transactivated transcripts is the CDK inhibitor $p21^{WAF1/CIP1}$, whereas intriguingly, Bax and GADD45 are not on this list. In this p53 restoration study, no additional genome-damaging treatment was administered, which may account for the lack of Bax and GADD45 transactivation.

The promoter of the pro-apoptotic *Bax* gene contains p53-binding sequences (550) and induction of p53 by DNA-damaging agents increases Bax expression (549)(884). The sole upregulation of Bax by p53, however, cannot explain all p53-dependent apoptosis, as shown in studies (i) that the *Bax* transgene in *p53* null mice does not increase DNA damage-induced apoptosis compared with *p53* null thymocytes not carrying Bax transgene (66) and (ii) that transfection of wt *p53* into Saos-2 and H1299 tumor cell lines does not increase Bax protein levels compared with nontransfected controls (657). Therefore, additional factors may be required and/or tissue-specificity may also account for these observations (563). Other transcriptional targets for p53 include Fas/CD95 (597), the insulin growth factor I-binding protein 3 (IGF-BP-3) (72)(216)(492) and the protein p85, a regulator of the PI 3-K (866).

The non-transactivator role of p53 has been shown by studies that inhibition of RNA or protein synthesis cannot inhibit UV-induced, p53-dependent apoptosis (75) and that overexpression of a transactivation-defective mutant of p53 still induces apoptosis (296). To promote apoptosis, p53 functions as a transcriptional repressor in downregulation of *Bcl-2* (282)(548), *IGF-1R* (625)(816), and the microtubule associated protein 4 (*MAP4*) genes (567).

p73

The protein p73 is originally isolated from the chromosomal locus 1p36 that is a region frequently deleted in neuroblastoma (386). p73 shares high homology to p53, especially in the p53 DNA-binding domain; it activates p53-responsive promoters; and it induces apoptosis in tumor cells lacking functional p53; suggesting that p73 is another tumor suppressor related to p53 (166)(381)(385)(386)(622). The putative p53 family also include p40 (769) and p51 (595), both share protein sequence homology with p53 and p73. Unlike p53, however, no mutations of the *p73* gene have been identified in human cancers (385)(737). Thus, it does not conform to the classical Knudson's "two-hit" model. Other results also argue against the tumor suppressor role of p73: (i) mice lacking p73 are viable and do not develop tumors (385), and (ii) activation of a silent allele and overexpression of wild-type p73 are associated with lung

tumorigenesis (499). In short, the role of p73 as a tumor suppressor and its effect on apoptosis regulation remain to be clarified.

pRb

Like p53, the retinoblastoma protein pRB also regulates both the cell cycle and apoptosis. Unphosphorylated pRB arrests cells in the early G_1 phase of the cell cycle; unlike p53 however, pRB protects cells from apoptosis (170)(410)(745).

The anti-apoptotic role of pRB has been extensively demonstrated. (i) The *Rb*(-/-) knockout mutation causes embryonic lethality in the mouse with widespread apoptosis in the peripheral and central nervous systems (120)(363)(445). (ii) Transfection of hepatocytes with *Rb* antisense oligonucleotides results in apoptosis (196). (iii) The human papilloma virus (HPV) E7 binds to and inactivates pRB (798), inducing apoptosis (817)(602). (iv) Co-expression of the wt *Rb* gene inhibits E2F-mediated apoptosis (420).

During the apoptotic process, pRB is both dephosphorylated (171)(554)(795) and cleaved (102)(368)(744). Inactivation and elimination of pRB during apoptosis may result from at least two types of caspase-dependent cleavages: the initial C-terminal cleavage and the latter cleavage and degradation (170)(745). The C-terminal cleavage of pRB at the peptide bond between Asp_{886}-Gly_{887} results in the truncated RB (ΔRB) and a 5-kD fragment (102)(368)(744) whereas the latter cleavage may further produce p68 and p48 fragments (200).

Activation of p53 and E2F (and c-Abl) has been proposed to be the mechanism for pRB to lose anti-apoptotic functions after caspase-dependent cleavage (745). (i) The truncated ΔRB loses the binding to Mdm2 (368) yet remains able to bind E2F and c-Abl tyrosine kinase as wild-type pRB (744). Mdm2 protects the cell from p53-dependent apoptosis (100)(416) but Mdm2 is also a substrate of caspase (188). Thus the release of Mdm2 from pRB may increase the susceptibility of Mdm2 to caspase cleavage (745). (ii) The latter degradation of pRB would release its binding partners E2F (205)(835) and c-Abl (755)(874), two proteins when overexpressed promote apoptosis.

Apoptosis-related signal transduction pathways

The cell constantly responds to its microenvironment through various signal transduction pathways and decides whether to divide, stay in the G_0 phase of the cell cycle, or undergo apoptosis. Some signal transduction pathways important in initiating apoptosis are discussed below.

Ceramide

Ceramide, a sphingosine-based lipid, plays important roles not only in the metabolism of sphingolipids but also as a second messenger molecule (287)(415). Although ceramide can be synthesized de novo from serine and palmitoyl-CoA after a series of reactions catalyzed by ceramide synthase and dihydroceramide reductase (61)(532), the major production of ceramide in response to stimuli is the sphingomyelinase-catalyzed catabolism of sphinomyelin into ceramide (590). Sphingomyelinases, the sphingomyelin-specific forms of phospholipase C, exist in several isoforms that are distinguished by different pH optima (415)(820). Acid sphingomyelinase (A-SMase) prefers pH 4.5-5.0 and is localized in acidic compartments such as lysosome or endosome (118)(476)(696)(820). Neutral sphingomyelinase (N-SMase) may reside in the plasma membrane (476)(820). Activation of A-SMase and N-SMase may respond to stimulus within seconds to minutes whereas activation of ceramide synthase may take hours (415), hence SMase activation is the main response to stress-related stimuli.

Increased levels of ceramide suppress cell growth and promote apoptosis. Ceramide-mediated cell cycle arrest in the G_1 phase is accompanied by dephosphorylation of pRB and cell cycle arrest is not elicited in response to ceramide in pRB-deficient cells (144)(253)(371)(635). Although pRB appears to be critical for ceramide-mediated suppression of cell growth, it is not required for the ceramide-mediated apoptosis (887). The role of ceramide in apoptosis has been demonstrated in many studies (415). (i) Activation of Fas (268) or treatments with TNF-α (582), ionizing radiation, UV radiation, heat shock or oxidative stress (277)(782) induces rapid generation of ceramide, and the ED50 for ceramide formation correlates well with the LD50 for induction of apoptosis. (ii) Specific cell-permeable ceramide analogues induce apoptosis (268)(277)(582)(782). (iii) Cells derived from patients with Niemann-Pick disease, an inherited deficiency of A-SMase, and from A-SMase-deficient mice show a defective apoptotic response (683).

How do these stress-related stimuli activate SMases? In the cells treated with TNF-α, both N-SMase and A-SMase are activated and likely to exert opposite effects toward cell activation and apoptosis, respectively (415). Two motifs in the intracellular regions of the p55 TNF receptor account for the differential activation of SMases: the NSD motif through interaction with a WD-repeat protein FAN activates N-SMase (2); the death domain through interaction with the adaptor proteins TRADD and FADD activates A-SMase (697)(820). Downstream of N-SMase may be the ERK pathways leading to cell growth, whereas downstream of A-SMase may be the JNK/SAPK pathway and mitochondrial regulation leading to apoptosis (415). On the other hand, ionizing radiation, UV-irradiation, and other stresses may

act directly on membranes and activate A-SMase (161)(649).

Like all apoptotic processes mediated by other signaling pathways, regulation of mitochondrial permeability transitions (607)(738) and caspase activation (183)(887) have been shown to be involved in ceramide-mediated apoptosis. The exact role of ceramide in the induction of apoptosis, however, remains unclear due to the lack of mechanistic evidence for the interactions among ceramide, its direct target ceramide-activated protein phosphatase (826), mitochondria, and caspases.

It is noteworthy that ceramide may initiate apoptosis but it does not commit the cell to the death process. Two models have been proposed to explain the role of ceramide in apoptosis. (i) Ceramide may function as a "biostat" that measures cellular stress and initiates either growth arrest or apoptosis (287). All ceramide inputs from de novo synthesis and catabolic pathways contribute to the final ceramide concentration. Hence, the final ceramide levels may reflect the effects of multiple stimuli and the overall amount of stress or injury to which the cell has been exposed. (ii) Another model emphasizes the topology of ceramide generation. In an attempt to explain how increased levels of ceramide may lead to different cellular responses, this model proposes that selective activation of various signaling pathways is determined by its co-localization with a given sphingomyelinase in the same subcellular compartment (415). Both models, however, remain to be tested.

Death receptors

Certain cell types are equipped with "death receptors (DRs)" on their surface to initiate the apoptotic pathway in response to "death ligands (DLs)" (23). Some of the best characterized DRs and DLs are summarized in **Table 11** and the downstream proteins interacting with them are summarized in **Table 12**. DRs are members of the tumor necrosis factor receptor (TNFR) superfamily. In addition to the extracellular cysteine-rich domains shared by all TNFR family members, DRs contain the death domain (DD) in the cytoplasmic region (571)(570). The DDs, which are conserved protein-protein interaction sequences of about 80 amino acids, are also found in various adaptor proteins interacting with death receptors **(Table 12)** (793). Since DD have a propensity to associate with one another perhaps via electrostatic interactions (793), the binding of DLs to DRs leads to clustering of the death receptors' DDs that further interact with the DDs of other downstream interacting proteins (23). Some proteins interacting with DRs also have the death effector domains (DED). The DED functions as the caspase recruiting domain (CARD), which is also found in the caspases with a large prodomain (caspases-2, -8, -9, -10) (23).

Table 11

Death receptors and ligands

Type	Name	Other names	Tissue Distribution	Functions
Receptors	TNFR1	p55, CD120a	Ubiquitous expression	Activation of NF-κB and AP-1; apoptosis when protein synthesis is blocked.
	Fas	Apo1, CD95	Many tissues	Apoptosis
	DR3	Apo3, WSL-1, TRAMP, LARD	Spleen, thymus, peripheral blood, stimuli-activated transcription in T cells	NF-κB activation, apoptosis
	DR4	Apo2, KILLER, TRAIL-R2,	Many tissues	Apoptosis
	DR5	TRICK2	Many tissues	Apoptosis
Ligands	*TNF*	*Apo1L, CD95L*	Activated mϕ and T cells	Binds TNFR1
	FasL		Activated T cells and NK cells, immune-privileged tissues (eye, testis, etc.)	Binds Fas
	Apo3L	*TWEAK*	Many tissues	Binds DR3
	Apo2L	*TRAIL*	Constitutive in many tissues, stimuli-activated transcription in T cells	Binds DR4, DR5, DcR1, and DcR2

Reference: (23)

Table 12

Downstream proteins interacting with death receptors

Proteins	DD*	DED/CARD*	Interact with**:
FADD (Fas-associated death domain)	+	+	Fas (U), TRADD (U) Caspase-8 (D)
RAIDD/CRADD (RIP-associated ICH-1/CED3 homologous protein with a death domain)	+	+	TNFR1 (U) RIP (D), Caspase-2 (D)
TRADD (TNFR-associated death domain protein)	+		TNFR1 (U) FADD (D), RIP (D), TRAF2 (D)?
RIP (Receptor-interacting protein)	+		RAIDD (U), TRADD (U)
Daxx (Fas death domain-associated protein)	-		Fas (U) ASK1 (D)
TRAF2 (TNFR-associated factor 2)			TRADD (U) ASK1 (D), NIK (D)
Caspases-2, -8, -9, -10	-	+	FADD (U) Substrates (D)
cFLIP (FLICE-inhibitory protein)	-	+	FADD (U)?

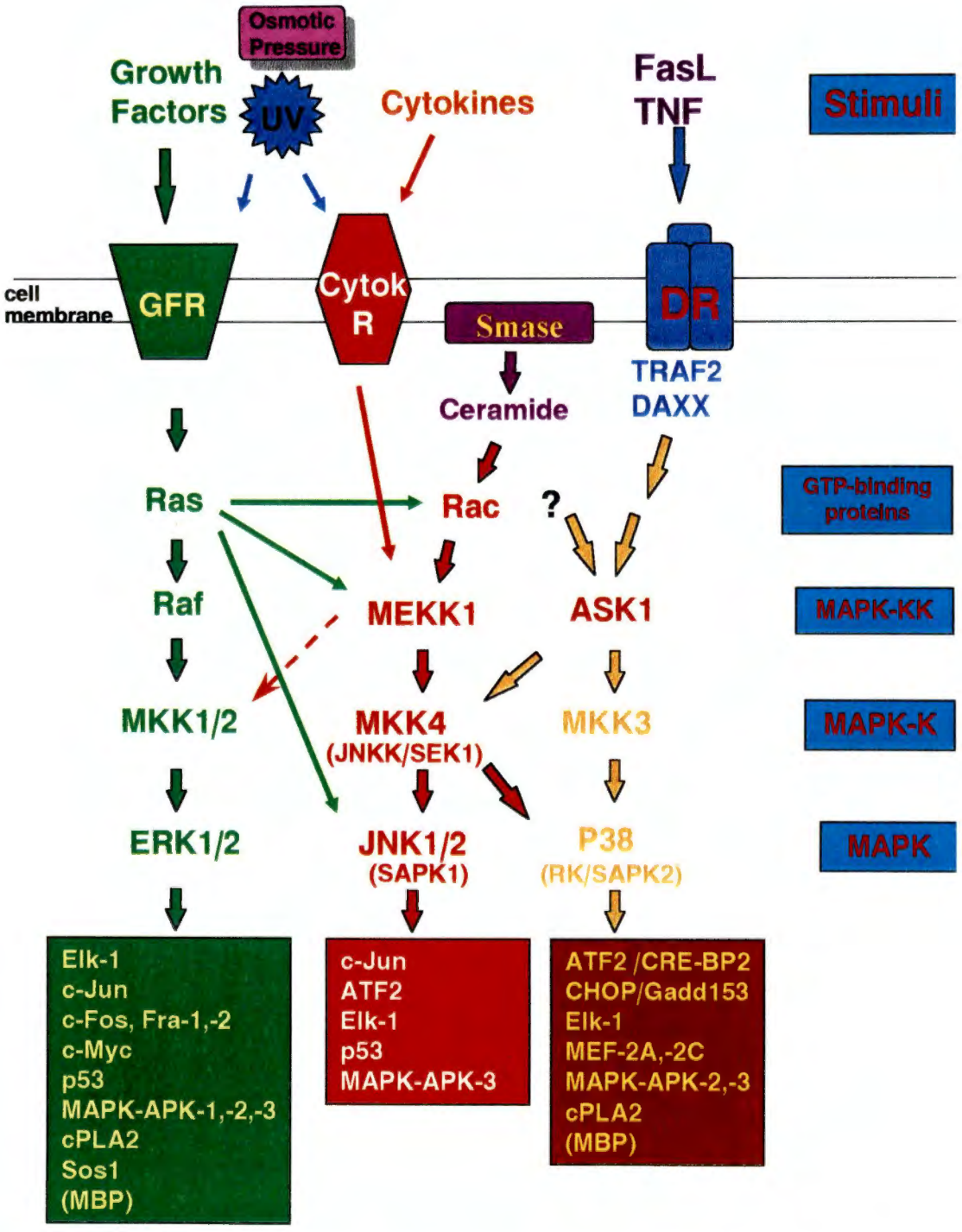
* DD: death domain; DED: death effector domain; CARD: caspase recruitment domain.
** U: upstream; D: downstream.
References: (23)(93)(581)(859)

The binding of FasL, TNF, and Apo3L induces clustering of Fas, TNFR1, and DR3, respectively **(Table 11)**. These clustered DRs interact with downstream adaptor proteins **(Table 12)**, which further recruits caspase-8 through the DED-DED interaction. Upon induction of proximity, caspase-8 may oligomerize and activate one another through self-cleavage (569), initiating apoptosis. Initiation of apoptosis through DRs requires no protein synthesis (793), thus DRs seem to be the express ways leading to apoptosis. Nevertheless, binding of these DLs to DRs not only leads to activation of caspase-8 but also recruits other adaptor proteins and activates some signal transduction pathways, such as the NF-κB and the JNK pathways, exerting additional functions **(Table 12)**. For instances, Daxx (93) and TRAF2 (581) may activate the apoptosis signal-regulating protein (ASK1), which is an upstream regulator of JNK/SAPK (356)(676)(803).

Binding of Apo2L(TRAIL) to DR4 or DR5 leads to apoptosis; however, DR4, DR5 and Apo2L are surprisingly expressed in many tissues. It turns out to be that signaling through DR4 and DR5 is modulated by a unique set of "decoy" receptors (DcRs), DcR1 (TRID/TRAIL-R3/ LIT) and DcR2 (TRAIL-R4/TRUNDD), both efficiently binding Apo2L (601)(708). Since DcR1 lacks the cytoplasmic region and DcR2 has a loss-of-function deletion on its DD, binding of Apo2L to these DcRs transmits no death signals. The genes encoding DcRs, DR4, and DR5 map together to the human chromosome 8p21-22, suggesting they may originate from a common ancestral gene (150)(507). Interestingly however, expression of DcR1 and DcR2 in normal tissues are more frequently than in tumor cells, suggesting the potential use of Apo2L in cancer therapy (23)(274). To prevent spontaneous clustering of DRs and subsequent apoptosis, cells widely express an cytoplasmic protein, silencer of death domain (SODD), to inhibit the interaction between DRs and TRADD (373).

JNK/SAPK

c-Jun N-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPKs), are involved in a signal transduction pathway parallel to that of mitogen-activated protein kinases (MAPKs) **(Figure 6)** (156)(389)(436). This highly conserved cascade is responsive to stress-related stimuli such as UV irradiation, ionizing radiation, ischemia and reperfusion, and inflammatory cytokines, eliciting phosphorylation and activation of JNK/SAPKs (393)(394)(435)(558). Activated JNK/SAPKs phosphorylate a variety of transcription factors including c-Jun, leading to transcriptional activation through interactions with c-Jun responsive DNA elements such as TPA-response element (TRE)(459). In addition to responding to extracellular stimuli, the JNK/SAPK pathway is also activated by intracellular



(Figure 6) A simplified scheme of three mitogen-activated protein kinase (MAPK) pathway. Abbreviations used are: GFR, growth factor receptors; Cytok R, cytokine receptors; Smase, sphingomyelinase; DR, death receptors; ASK1, apoptosis signal-regulating kinase-1.

stresses including inhibition of protein synthesis, treatment with antimetabolites, DNA damage, or microtubule dysfunctions induced by chemotherapeutic agents (105)(393)(435)(803).

Activation of the JNK/SAPK signaling pathways has been mechanistically implicated in regulation of apoptosis (263)(358)(394)(415)(435), however, the roles of JNK/SAPK in promoting (104)(531)(879) or preventing apoptosis (579)(621) differ, depending on both cell type and apoptosis-triggering stimuli (358)(394). Furthermore, in addition to apoptosis, JNK/SAPK activation may be involved in proliferation (63)(720) and oncogenic transformation (646).

JNK/SAPK has been shown to be involved in activation of caspases that are required for execution of the apoptotic process (76)(84)(104)(666)(701). The role of JNK/SAPK in activation of caspases is, however, not straightforward. JNK/SAPK could be either upstream (104)(701) or downstream of (76)(84)(666) caspase activation, depending on the cell type and apoptosis-initiating agents. We have demonstrated that inhibition of the JNK/SAPK cascade transiently inhibits paclitaxel (Taxol®)-induced caspase-3 activation, PARP cleavage, and DNA fragmentation , indicating that JNK/SAPK is upstream of caspase-3 activation in paclitaxel-initiated apoptosis (800). Collectively, it is tempting to speculate that JNK/SAPK may act upstream of caspase activation in chemotherapy-initiated apoptosis.

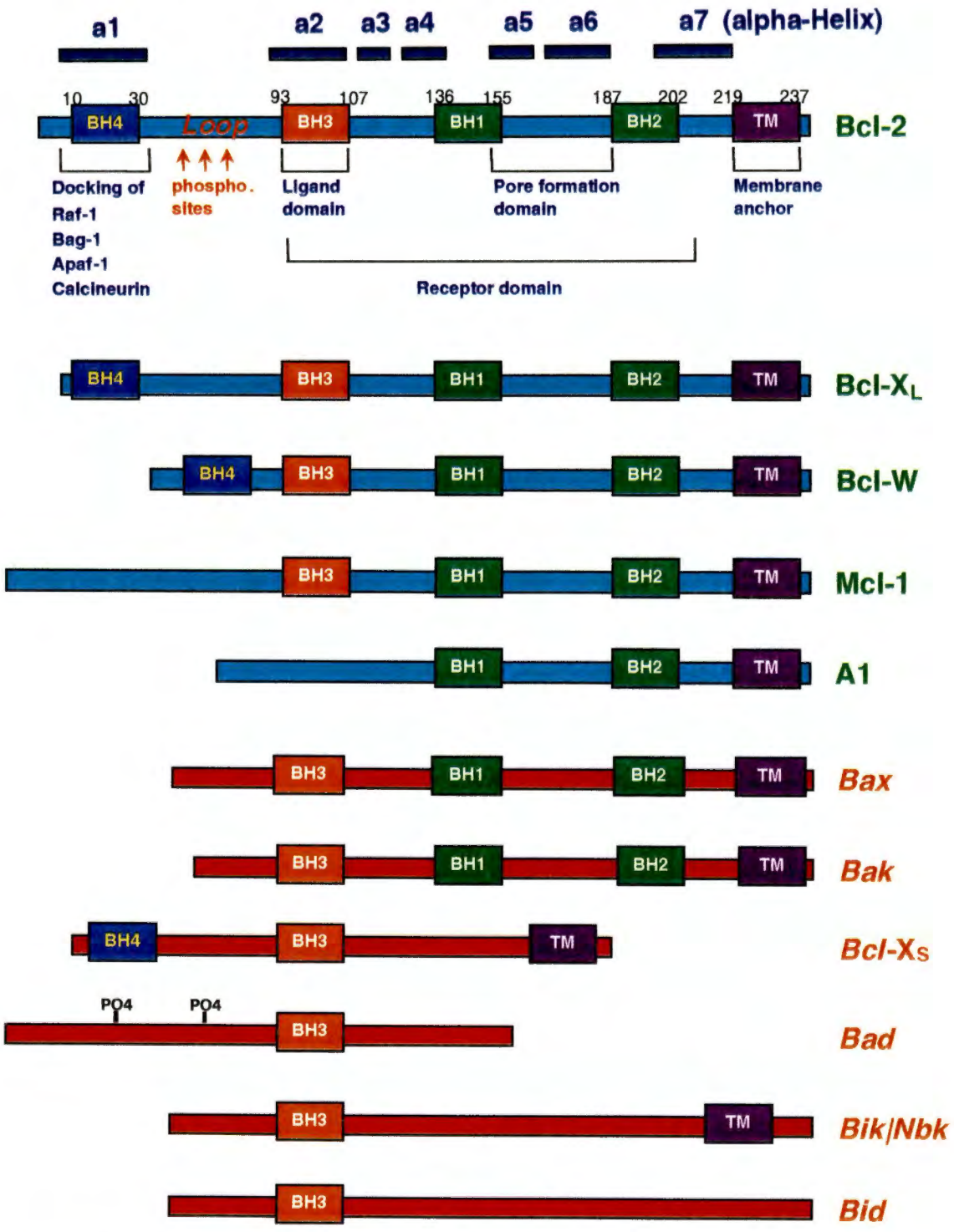
REGULATORS

Apoptosis is regulated by a variety of cellular proteins, including Bcl-2 family members, apoptosis-associated factors (Apafs), and inhibitors of apoptosis (IAPs) family members, perhaps through either changing the permeability transitions of mitochondria, regulation of caspase activation, or both.

Bcl-2 family members

The Bcl-2 family represents a class of proto-oncogenes that block cell death without promoting cell proliferation (418). Furthermore, Bcl-2 overexpression has been shown to delay cell entry to S phase and promote cells back to G_0 phase (773).

Members of Bcl-2 family can be either anti-apoptotic (Bcl-2, Bcl-XL, etc.) or pro-apoptotic (Bax, Bak, Bcl-Xs, etc.) **(Figure 7)** (3)(55)(96)(401)(425)(638). They can form either homodimers or heterodimers and the ratio of Bcl-2 to Bax has been shown to be important in determining the susceptibility to apoptosis (591)(96). Nevertheless, studies of transgenic and knock-out mice indicate that,



(Figure 7) Representative Bcl-2 family members. The anti-apoptotic members are drawn with green bars, while the pro-apoptotic members are with red bars and labeled by italic legends. The numbers represent the number of amino acid residues. Abbreviations used are: BH, Bcl-2 homology; and TM, transmembrane domain.

despite the *in vivo* competition between Bcl-2 and Bax, each is capable of regulating apoptosis independently of the other molecule (414). Bax can directly cause release of cytochrome *c* from mitochondria (383) and cytochrome *c* can initiate apoptotic characteristics *in vivo* and *in vitro* (599)(893). In co-transfection studies, Bcl-2 co-localizes with Bax to mitochondria but does not prevent Bax-induced cytochrome *c* release, yet Bcl-2 inhibits apoptosis initiated by cytochrome *c* release (650). Collectively, these results suggest that (i) dimerization between Bax and Bcl-2 does not necessarily counteract the functions of each molecule, and (ii) Bcl-2 has anti-apoptotic functions beyond the regulation of mitochondrial release of cytochrome *c*, probably through direct inhibition of caspase activation.

Structures and functions

Bcl-2 is a 239-residue protein and contains many structural motifs, which account for three distinct functions: (i) dimerization, (ii) adaptor function, and (iii) channel formation. From the N-terminus to the C-terminus, there are four Bcl-2 homology (BH) domains (BH4, BH3, BH1, BH2) and a transmembrane (TM) domain, which anchors Bcl-2 to the outer mitochondrial membrane, the endoplasmic reticulum, and the nuclear envelope **(Figure 7)** (3)(96)(401)(425)(638). In Bcl-2 and Bcl-X_L, there is a loop domain between BH4 and BH3. This loop is the target site for phosphorylation and caspase-mediated cleavage, hence it is generally considered as a site for negative regulation (i.e., inhibition of protective effects of Bcl-2 and Bcl-X_L) (401). BH1 and BH2 domains are required for dimerization (864) while the BH3 is essential for pro-apoptotic functions (111)(350). Mammalian anti-apoptotic Bcl-2 family members generally harbor at least three BH domains (BH3, BH1, BH2) and a transmembrane domain. On the other hand, the minimum requirement for a pro-apoptotic member is a BH3 domain (401). Some pro-apoptotic members, such as Bik, Blk, Hrk, BNIP3, Bim_L, Bad, and Bid, only have BH3 domain (3). On the other hand, most viral anti-apoptotic Bcl-2 homolog, such as BHRF1, LMW5-HL, ORF16, KS-Bcl-2, and E1B-19K, do not have BH3 domain (3). BH3 is therefore believed to be the death domain of Bcl-2 family members.

The three-dimensional structure of Bcl-X_L indicates that BH3, BH1, and BH2 cooperatively form a hydrophobic groove on the surface of Bcl-X_L, which serves as the "receptor" during dimerization (564). The inserting "ligand" is an amphipathic helix BH3 provided by the dimerizing partner (164)(684). BH3 binds with high affinity to the hydrophobic pocket ("receptor") created by BH3, BH1, and BH2 domains through hydrophobic and electrostatic interactions (684).

The N-terminal BH4 domain is not required for heterodimerization but is likely involved in

protein-protein interactions with other regulatory proteins outside the Bcl-2 family (401). BH4 is the docking site for Raf-1 (796) , Bag-1 (a Bcl-2 binding protein) (797), calcineurin (a calcium-dependent protein phosphatase) (713), and Apaf-1 (apoptosis-associated factor-1) (600). Inactivation of Bad by Raf-1 examplifies how interactions between Bcl-2 and other proteins through BH4 regulate Bcl-2 functions. The pro-apoptotic Bad has no membrane anchoring TM region yet inhibits the cytoprotective functions of Bcl-2 and Bcl-X_L by heterodimerizing with them. Bcl-2 has been shown to target the protein kinase Raf-1 to mitochondria. Once there, Raf-1 phosphorylates Bad and releases it from Bcl-2 (796).

X-ray and NMR data indicate structural similarity between Bcl-X_L and the membrane translocation domain of bacterial toxins, such as diphtheria toxin and the colicins (564). The structural analyses of Bcl-X_L reveal a seven α-helical bundle, at the center of which lies a hairpin comprised of two hydrophobic core helices, α5 and α6. Undergoing profound conformational changes, these two α-helices presumably insert perpendicularly across the lipid bilayer (564). Indeed, Bcl-X_L and Bcl-2 can form ion channels in planar lipid bilayers (546)(692) and the removal of α5 and α6 from Bcl-2 abrogates channel formation *in vitro* and abolishes its anti-apoptotic functions in cells (692). The pro-apoptotic Bax also can form ion channel (695).

The mechanism how ion-conductive pores regulate apoptosis, however, remains unclear. Speculations include: (i) Bcl-2 or Bax may regulate an electrochemical gradient, altering some substrates or products residing in the intermembrane space of the mitochondria, and/or (ii) the Bcl-2 family members may regulate other ion channels or transport other unidentified molecules (96). Alternatively, the Bcl-2 family members may participate in the formation of mitochondrial megachannel (also called permeability transition pore complex, PTPC) that accompanies apoptosis (259). The mitochondrial megachannel functions as a calcium-, voltage-, pH-, and redox-gated channel but with little, if any, ion selectivity (259). Intriguingly, only Bax and Bag-1, but not Bcl-2 or Bcl-X_L, have been co-purified in the megachannel complex (513), although Bcl-2 has been shown to antagonize the channel-forming activity of Bax (18). Bax has recently been shown to cooperate with adenine nucleotide translocator (ANT) within the PTPC to increase mitochondrial membrane permeability and to trigger cell death (512).

Regulation of Bcl-2 by phosphorylation

Phosphorylation is involved in the regulation of Bcl-2 functions (92). The potential phosphorylation sites are multiple serine residues that reside in the loop region between the α1 (BH4) and α2 (BH3) helices (92)(279)(516). The roles of Bcl-2 phosphorylation in promoting (51)(92)(99)(281)(652) or inhibiting apoptosis (334)(360), however, remain controversial.

Phosphorylation of Bcl-2 has been shown in cells treated with microtubule-active agents (paclitaxel, vinblastine, vincristine) and is thought to promote cell death (52)(51)(280). Raf-1 activation has been suggested to be required for Bcl-2 phosphorylation in paclitaxel-treated cells (52)(51), however, opposite observations have been reported (355). Bcl-2 phosphorylation can be induced by a phosphatase inhibitor okadaic acid (279) and cAMP-dependent protein kinase (PKA) has been shown to be required for paclitaxel-induced Bcl-2 phosphorylation (725). When apoptosis is induced by the simultaneous PKC inhibition and v-Ras overexpression, Ras may account for phosphorylation of Bcl-2 (99).

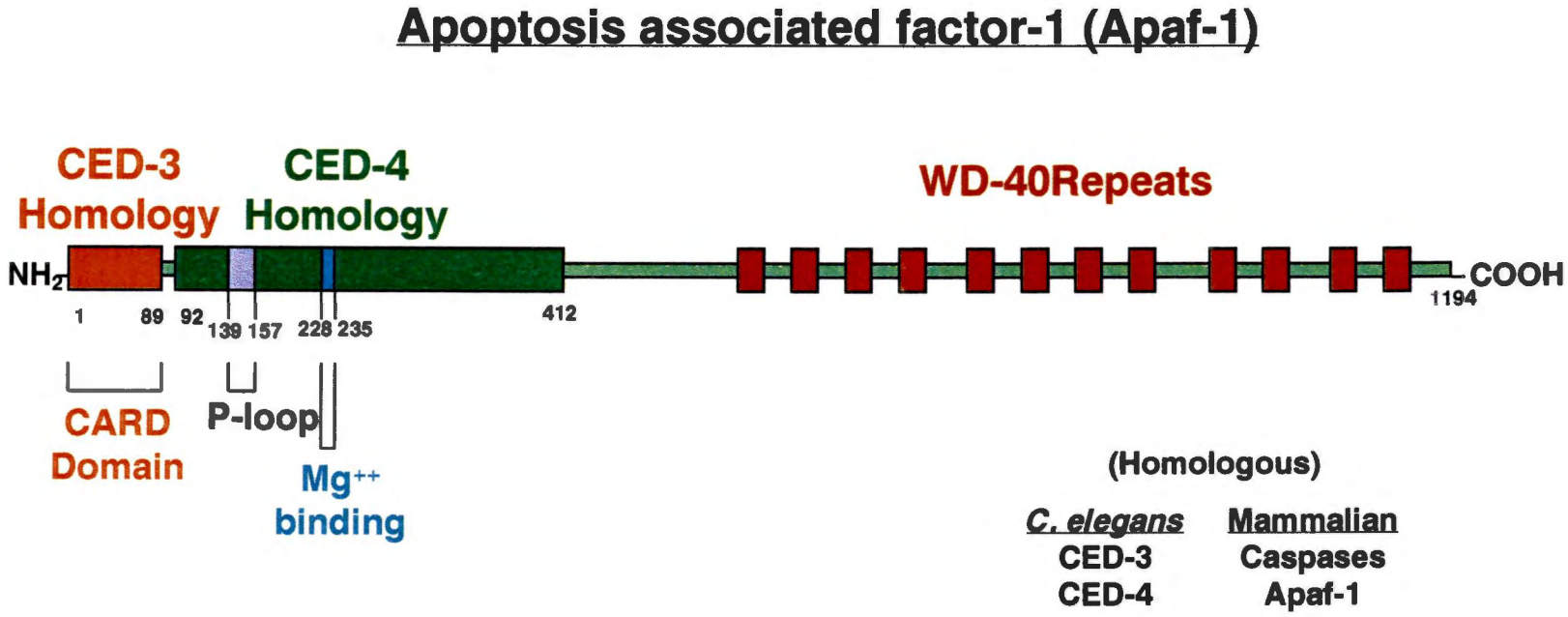
On the other hand, Bcl-2 phosphorylation has been noted to occur only in the cells arrested at the G_2/M phase and Bcl-2 therefore is proposed to be "the guardian of the microtubule integrity" (278). A recent study also indicates that Bcl-2 phosphorylation is a marker of M phase of the cell cycle and argues against its role as a determinant of apoptosis (471).

Cleavage of Bcl-2 and Bcl-X_L to ensure apoptosis

Bcl-2 family members regulate apoptosis by at least two ways: (i) regulation of mitochondrial release of cytochrome *c* and other apoptosis-inducing factors (AIFs) and (ii) direct inhibition of caspase activity (383)(546)(564)(650)(692)(695)(893). Likewise, CED-9 (the *C. elegans* Bcl-2 homolog) (**Table 8**) has been shown to be a bifunctional cell-death inhibitor (850). More intriguingly, CED-9 is also a substrate for CED-3 (the *C. elegans* caspase homolog) (850) and Bcl-2 and Bcl-X_L can be cleaved by caspases (106)(123)(257). Caspases cleave the 26-kD Bcl-2 into the 23-kD C-terminal fragment at the Asp31 and Asp34 residues in the loop region of Bcl-2 (**Figure 7**) (106)(257). The consequent C-terminal fragment of Bcl-2 or Bcl-X_L, which lacks BH4, promotes apoptosis (106)(123)(257). In summary, during the apoptotic process, activated caspases convert the anti-apoptotic Bcl-2 and Bcl-X_L into the pro-apoptotic fragments, ensuring the irreversibility of cell death. This also help to explain the paradoxical observations that higher Bcl-2 expression in breast cancers is correlated with better prognosis (304)(374)(473).

Apoptosis-associated factors (Apafs)

Three identified Apafs are: Apaf-1, a 130-kD cytosolic protein, the human homolog to *C. elegans* CED-4 (**Table 8**); Apaf-2 which turns out to be cytochrome *c* (898); and Apaf-3 which is identical to caspase-9 (460). From the N-terminus to the C-terminus, Apaf-1 contains regions homologous to CED-3 (caspases) and CED-4, and twelve Walker's domain (WD) repeats (**Figure 8**) (600)(898). Apaf-1 is not a



(Figure 8) Apoptosis associated factor-1 (Apaf-1). Abbreviations used are: CARD, caspase recruiting domain; and WD, Walker's domain. The numbers represent the number of amino acid residues.

caspase. Instead, it functions as an adaptor protein through interacting with caspases using the CED-3 homolog as the caspase recruitment domain (CARD) and associating with other proteins through multiple WD repeats that are potential sites for both protein-nucleotide and protein-protein interactions (573)(790). Indeed, Apaf-1 has been shown to associate with caspases-4, -8, and -9 (343). For interaction between Apaf-1 and the Bcl-2 family members, both the CED-4 homologous domain and the WD repeats-rich C-terminal region can bind Bcl-X_L (343).

The discovery of Apafs and subsequent studies again confirm the evolutionary conservation of apoptotic components among species from the nematode *C. elegans* to the human (309). Three key cell death proteins that have been identified in *C. elegans* are CED-4, CED-9, and CED-3; while the mammalian equivalents are Apaf-1, Bcl-2 or Bcl-X_L, and caspase-9 (Apaf-3), respectively (**Table 8**). In *C. elegans*, interaction of CED-4 and proCED-3 is necessary and sufficient for activation of CED-3. The nematode Bcl-2 homolog, CED-9, prevents apoptosis by binding CED-4 in an inactive conformation, thereby preventing CED-4-mediated activation of proCED-3 (309). Likewise, Apaf-1 (the human homolog of CED-4), Bcl-X_L (a human homolog of CED-9), and caspase-9 (Apaf-3, the human homolog of CED-3) have been found to form a ternary complex (600), suggesting that Bcl-X_L regulates caspase-9 through Apaf-1 (343). The complex has been designated as the mammalian "apoptosome" (460)(898).

How does CED-4 (Apaf-1) activate pro-CED3 (pro-caspase-9) and how does CED-9 (Bcl-2) inhibit this activation? A current model is that CED-4 (Apaf-1) may function as the "apoptotic chaperone" which holds multiple inactive pro-CED3 (pro-caspase-9) molecules together to increase their local concentration and may also arrange them into the conformation that promotes their activation (308). Indeed, point mutations that abolish CED-4 oligomerization inhibit its pro-apoptotic activity (858). This model also explain the role of CED-9: CED-9 (Bcl-2) may bind CED-4 (Apaf-1) and interfere with the CED-4-mediated oligomerization of pro-CED-3 (pro-caspase-9), inhibiting the activation of CED-3 (caspase-9). Supporting this model, (i) CED-9-bound CED-4 cannot oligomerize (858), and (ii) deletion of the Apaf-1 WD-40 repeats region (**Figure 8**), which has been shown to bind Bcl-X_L (343), makes the truncated Apaf-1 constitutively active and capable of activating pro-caspase-9 independent of cytochrome *c* (724). Although the "induced proximity" of pro-CED-3 (pro-caspase-4) by CED-4 (Apaf-1) appears to be required for activation of CED-3 (caspase-9), this may not be the only role for CED-4 (Apaf-1). Both the presence of nucleotide-binding domain in CED-4 (Apaf-1) and the requirement of ATP suggest that CED-4 (Apaf-1) should have ATPase activity and likely other functions too (308).

Inhibitors of apoptosis (IAPs) family

IAPs (inhibitors for apoptosis), although unrelated to p35, have also been discovered in the baculovirus (47)(138). Human homologs of IAP include NAIP (neuronal apoptosis inhibitor protein) (474), c-IAP1/hIAP-2/MIHB (mammalian IAP homolog-B), c-IAP2/hIAP-1/MIHC, hILP (human IAP-like protein)/X-IAP (X-linked IAP) /MIHA (653)(772) and survivin (4)(14).

The conserved structures of the IAP family members are the multiple N-terminal BIRs (baculovirus IAP repeats) and a C-terminal RING finger, which is a type of zinc finger (124). The unique BIRs are required for the anti-apoptosis activity of IAPs and contain conserved residues, Cys-XX-Cys and Cys-X_6-His motifs, which suggest zinc-binding ability (124). The human IAPs, c-IAP1 and c-IAP2, use BIRs to interact with the TRAF-N domain of TRAF-2 (TNF receptor associated factor-2) (653). TRAF-2 is an upstream regulator of NF-κB, suggesting IAPs may regulate apoptotic pathways via NF-κB. In summary, the mechanisms of anti-apoptosis by IAPs may be through (i) direct inhibition of caspases-3 and -7 by hILP/X-IAP (162), c-IAP1 and c-IAP2 (664), and (ii) indirect modulation of other signaling proteins, such as NF-κB (727) or JNK1 (682).

EFFECTORS

Caspase family members

Members of the cysteine protease family (**the caspase family**) play the executioner role in apoptosis **(Table 13)** (31)(128)(408)(577)(786). The term "caspase" was coined to highlight the general properties of these protease: the "c" in caspase denotes the presence of cysteine at the active site, a pentapeptide QACR(or Q/G)G, and the "aspase" denotes the principle cleavage activity adjacent to aspartate (10).

Knock-out mice studies have demonstrated the essential roles of caspase in apoptosis. Caspase-3 null mice, born at a frequency lower than expected by Mendelian genetics, are smaller than their litter mates and die at 1-3 weeks. Decreased apoptosis and defective brain development are detected by embryonic day 12 (430), suggesting that each caspase may have tissue-type specific functions. Signal transducer and activator of transcription 1- (STAT1) null mice are resistant to apoptosis by TNF-α and reintroduction of STAT1 restores both TNF-α-induced apoptosis and the expression of caspases-1, -2, and -3 (431), suggesting that STAT1 is required for efficient constitutive expression of these caspases.

Table 13

Summary of Caspases

Caspase	Other names	Recognition site	Inhibitors (Peptide or chemical*)	Viral Inhibitors	Cellular substrates
1	CED-3 ICE	YVAD ↓ G YVPD ↓ S	DEVD YVAD zVAD	CrmA E8-FLIP p35	Actin, CED-9, PARP, PITSLRE, Pro-caspase-1, -2, -3, Pro-IL-1β
2	ICH-1 NEDD-2		zVAD	p35	Pro-caspase-2, PARP
3	Apopain CPP32 Yama	DEVD ↓ G DMQD ↓ N YVPD ↓ S	DEVD-CHO TPCK* zVAD	CrmA p35	Bcl-2, Bcl-X_L, DFF_{45kD}, DNA-PK, Fodrin, GAS2, ICAD, MDM2, PAK2, PARP, PKC, PITSLRE, pRB, Pro-caspase-3, -6, -9, RNP, Rbo-GDI, SREBP
4	ICE-II ICH-2 TX		DEVD YVAD zVAD	p35	PARP, Pro-IL-1β, Pro-caspase-1, -4
5	ICE-III ICH-2 TY			CrmA	
6	Mch2	VEID ↓ NG	TLCK* zVAD	CrmA p35	Lamins, PARP
7	Mch3 ICE-LAP3	DEVD ↓ G	DEVD-CHO EVD	CrmA	PARP, Pro-caspase-6, RNP
8	FLICE1 MACH1 Mch5		DEVD IETD zVAD	CrmA E8-FLIP	Pro-caspase-3, -4, -7, -9
9	ICE-LAP6 Mch6			CrmA	PARP
10	FLICE2 Mch4		DEVD	p35	PARP, Pro-caspase-3, -7

References:(10)(106)(123)(128)(408)(480)(677).

Structure-functional considerations

Caspases are synthesized as procaspases that can be activated through cleavage by proteases, including itself and other caspases. A procaspase contains an N-terminal prodomain structure of variable length and two enzyme subunits. During its activation, the prodomain of the procaspase is removed and two subunits are released but further heterodimerize into an active enzyme **(Figure 9)**. According to the different lengths of prodomains of procaspases and preferences for substrates, caspases are further categorized into two classes: regulatory caspase (e.g., caspase-1, -2, -4, -5, -8, -9, -10) and effector caspases (e.g., caspase-3, 6, 7) (128)(786). Through the long prodomains, regulatory caspases may interact with regulators, such as Apaf-1, FADD, and RAIDD (RIP-associated ICH-1/CED-3-homologous protein with a death domain)/CRADD (caspase and RIP adapter with death domain) (109)(128)(786).

Chemical and peptide inhibitors and substrates

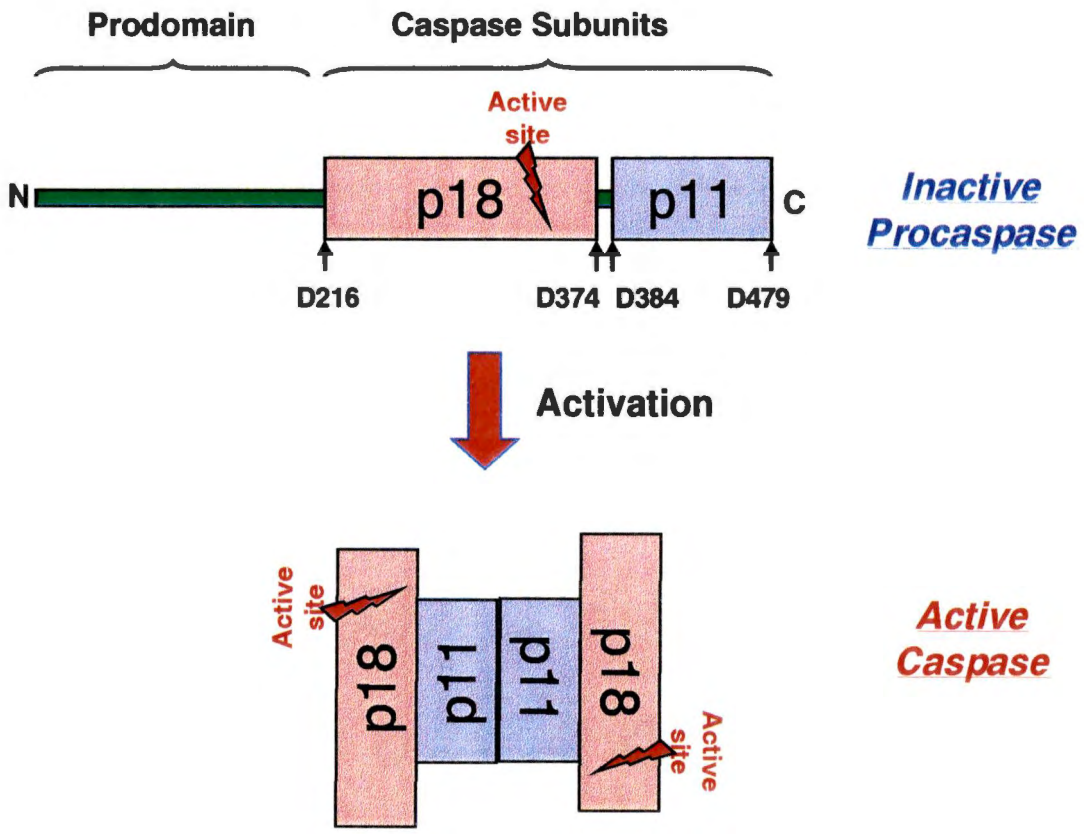
The serine protease inhibitors, N-tosyl-L-phenyl-alanylchloromethyl ketone (TPCK) and N-tosyl-L-lysylchloromethyl ketone (TLCK) (71)(206), and several short peptides of three to four amino acid residues have been used in studies of caspase functions. Synthetic peptide inhibitors include DEVD-fmk (acetyl-Asp-Glu-Val-Asp-fluoromethylketone), VEID-fmk (acetyl-Val-Glu-Ile-Asp-fluoromethylketone), YVAD-fmk (acetyl-Tyr-Val-Ala-Asp-fluoromethylketone), zVAD-fmk (benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone)(**Table 13**)(22)(184)(487). Synthetic substrates include DEVD-AMC (acetyl-ASP-Glu-Val-Asp-aminomethylcoumarin) and YVAD-AMC (acetyl-Tyr-Val-Ala-Asp-aminomethlcoumarin). The hydrolysis of these substrates by caspases produces the fluorescent AMC product (22)(511).

Cellular targets for caspases

Cellular targets for caspases fall into three groups: (i) procaspases, (ii) cellular proteins that need to be inactivated for cell death to occur, and (iii) cellular proteins whose activation is required for the apoptotic process, as summarized in **Table 7.**

Cellular and viral inhibitors for caspases

Apoptotic functions of caspases are regulated at least by the Bcl-2 family members and the inhibitor for apoptosis (IAP) family members. Bcl-2 has been suggested to protect cells from apoptosis through its inhibition on caspase activation (22)(110). Supporting the role of Bcl-2 in inhibition of caspase activation, the *C. elegans* CED-9 (Bcl-2 homolog) has been shown to interact with CED-4 and prevent the ability of CED-4 to activate the CED-3 (the homolog of caspase-1) (833)(834). Likewise in mammalian cells, Bcl-2 or Bcl-X_L inhibits apoptosis through its interaction with the apoptosis-initiating



(Figure 9) Activation of procaspase by cleavage at the aspartate residues. The cleavage sites of the procaspase are labeled as D (aspartate) followed by the number of amino acid residues.

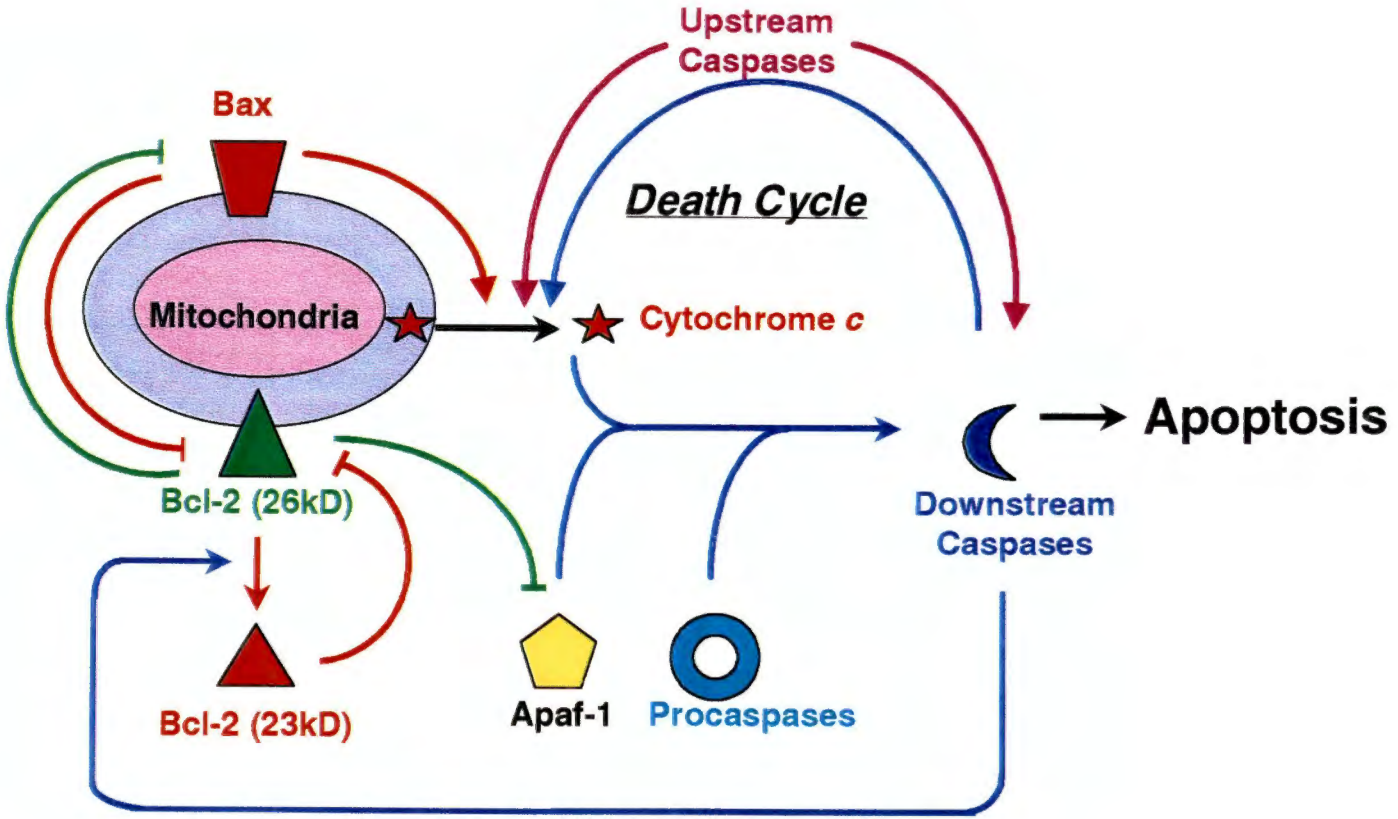
complex apoptosome, which is comprised of Apaf-1 (the mammalian homolog of CED-4), cytochrome *c* (Apaf-2), and caspase-9 (Apaf-3) **(Figures 10, 11)** (343)(460)(599)(650)(898). Among members of the inhibitors for apoptosis (IAP) family, hILP/X-IAP has been shown to directly inhibit caspases-3 and -7 (162).

Various viral proteins have been shown to inhibit caspase activity, including the cowpox virus cytokine response modifier A (CrmA) (227)(636), the baculovirus p35 (73)(851), the baculovirus inhibitors for apoptosis (IAPs) (47)(138), and the viral FLICE-inhibitory proteins (FLIPs) (44)(342)(756). CrmA is a 38-kD serine protease inhibitor (serpin) that preferentially inhibits caspase 1-like proteases by forming a stable complex with the caspase. p35 has a broader target range than CrmA and can inhibit both caspase 1-like and caspase 3-like proteases. The viral IAPs, although unrelated to p35, are also isolated from the baculovirus. FLIPs are viral proteins containing sequences related to the death effector domain (DED), which is found in the intracellular part of Fas and TNFR1 (408). Viral FLIPs have been isolated from the equine herpesvirus type 2, bovine herpesvirus-4, herpesvirus saimiri (HVS), human herpesvirus-8 (HHV-8), and the human molluscum contagiosum virus (MCV). Through interactions between DED-like domain with the prodomain of FLICEs (caspase 8 and 10), FLIPs effectively inhibit apoptosis mediated by FLICEs.

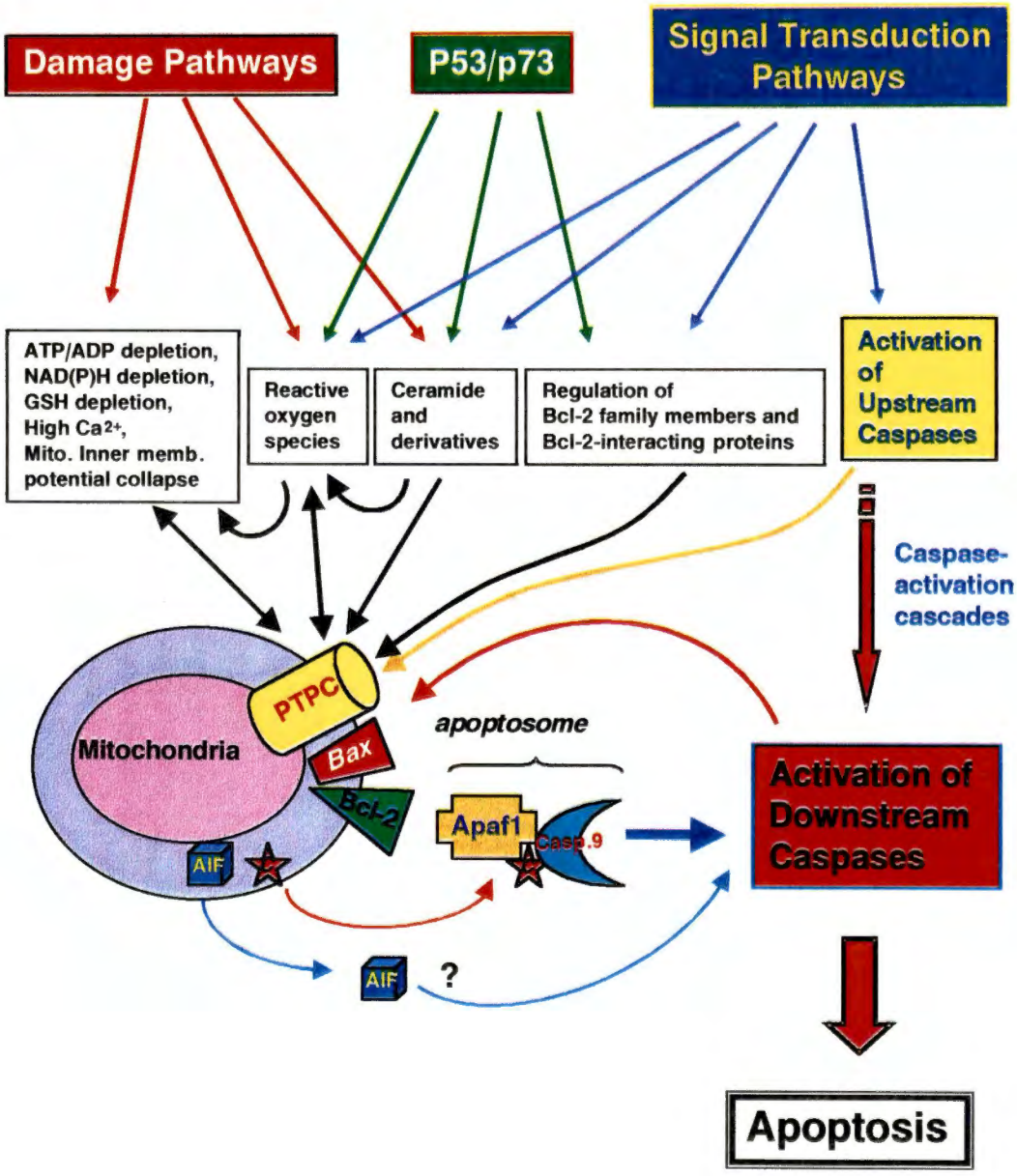
Cyclin-dependent kinases (CDKs)

Functions and regulations

The eukaryotic cell cycle is regulated by the periodic activation of various cyclin-dependent kinases (CDKs). The CDK holoenzymes are heterodimeric complexes consisting of a catalytic subunit (kinase) and an activating subunit (cyclin). A series of different cyclin-CDK complexes are required to catalyze the phosphorylation of various protein substrates important for the cell to progress through the cell cycle. To ensure perfect timing and coordination of cell-cycle events, activities of cyclin-CDK complexes are tightly regulated by the following mechanisms. (i) The monomeric kinase subunit of CDK alone is inactive and requires association with a cyclin subunit and subsequent phosphorylation of a key threonine residue, e.g., T160 in CDK2, to become fully active. (ii) Activity of a cyclin-CDK complex can be reversibly inactivated either by phosphorylation on a tyrosine residue, such as Y15 located in the ATP-binding domain of the CDK2, or by association with cyclin-kinase inhibitory proteins (CKIs). (iii) After completion of the cell-cycle transition, the cyclin-CDK complex is irreversibly inactivated by the



(Figure 10) The death cycle model of apoptosis. In this model, Bcl-2 can act both upstream and downstream of the cytochrome *c* release from the mitochondrion.



(Figure 11) The circular model of apoptosis. In this model, there are multiple points of entry to the mitochondria-caspase activation circle, explaining why this circle is vulnerable to inhibition by either caspase inhibitors or mitochondria-stabilizing agents. Abbreviations used are: GSH, glutathione; Mito, mitochondria; PTPC, permeability transition pore complex (also known as "megachannel"); AIF, apoptosis-inducing factors; and C, cytochrome *c*.

degradation of the cyclin subunit (510).

There are two families of CDK inhibitors (CKIs): the INK4 family (p15, p16, p18, p19) and the CIP/KIP family (p21, p27, p57) (709). Members of the INK4 family specifically bind to CDK4 and CDK6, acting as inhibitors of cyclin Ds. On the other hand, members of the CIP/KIP family form heterotrimeric complexes with the CDK and the cyclin. The CIP/KIP family members bind to most CDK-cyclin complexes to some extent but have the highest affinity for G_1 CDK-cyclin complexes (cyclin D-CDK4, cyclin D-CDK6, cyclin E-CDK2) (510).

CDKs in apoptosis

The discoveries of apoptosis in unicellular organisms (15)(135) indicate that apoptotic machinery may be as ancient as 2 billion year-old, perhaps appearing only a little later than, if not at the same time as the cell replication machinery (15). It also raises a possibility that it would be economical if both cell replication and apoptosis employ the same machinery. Furthermore, the co-existent apoptosis may contribute to the well-being of multicellular organisms in reducing the risk of tumorigenesis accompanying cell proliferation because an additional set of survival signals are required for counteracting apoptosis (260). In favor of this hypothesis, many genes that control the cell cycle and cell differentiation including proto-oncogenes, tumor suppressors, cyclins, and CDKs, have been demonstrated to participate in the control of apoptosis (191)(300)(494)(822).

Recent data have indicated an important role for CDK activation in apoptosis. Premature activation of $p34^{cdc2}$ has been shown to be required for apoptosis (711)(714), and apoptosis has been shown to be inhibited either by expression of dominant negative (dn)-CDKs (529) or by butyrolactone I, a specific inhibitor of Cdc2 (226). Activated CDK7 has also been associated with activation of $p34^{cdc2}$ in paclitaxel-treated cells (345).

Furthermore, some regulators for CDK activity have been shown to be substrates of caspases. For instances, caspase-3 or caspase-3-like enzymes can cleave CDK inhibitors $p21^{Cip1/Waf1}$, $p27^{Kip1}$ (458), as well as Wee1 and CDK27, the inhibitory kinases for Cdc2 and CDK2 (894). Catastrophic CDK activities therefore have been proposed to be the actual effector of cell death (293)(458)(894).

MITOCHONDRIA AND ATP STATUS: CURRENT MODELS

Two fundamental decisions of cell fate are: (i) life versus death, and (ii) apoptosis versus necrosis. What event determines whether the cell passes "the point of no return" toward death has been

long under debate. DNA fragmentation and caspase activation, have turned out to be consequences, but not decision makers of apoptosis. The Bax/Bcl-2 ratio has been proposed as a determinant for life or death (591), however, the observations that caspase inhibitors block Bax-induced apoptosis (650) suggest that, although Bcl-2 family members may regulate apoptosis, they are not the ultimate decision makers.

Increasing amount of data demonstrate the involvement of mitochondrial changes in cell death, both necrosis and apoptosis (426)(428). Disruption of mitochondrial transmembrane potential has been shown to precede nuclear DNA fragmentation (89)(781)(877)(878). Bcl-2 may regulate apoptosis through preservation of mitochondrial functions (89)(505)(739)(857)(877). Activation of caspases and mitochondrial changes represent two distinct yet interacting events, demonstrated by the observations (i) that caspase inhibition may delay and/or prevent mitochondrial dysfunction (62)(323)(738), (ii) that caspases cause mitochondrial dysfunction (513)(738), and (iii) that mitochondrial dysfunctions cause caspase activation (181)(413)(460)(478)(738)(857)(898). Caspases can be classified into the upstream regulatory (caspases-2, -8, -10) and the downstream effector (caspases-3, -6, -7) (259). Activation of the downstream effector caspases often occurs with a several-hours lag phase after activation of upstream regulatory caspases (184)(738), and Bcl-2 can prevent activation of downstream effector caspases without effects on the upstream regulatory caspases (527). Collectively, these studies suggest the existence of a link between the upstream and downstream caspases and the link is likely to be the mitochondria, whereas Bcl-2 may regulate apoptosis through inhibiting mitochondrial dysfunction. A linear model of " upstream caspase activation ➔ mitochondrial dysfunctions ➔ downstream caspase activation", however, cannot explain the recent observations that Bcl-2 protects cells from apoptosis induced by either injected cytochrome *c* (893) or Bax-induced cytochrome *c* release from mitochondria (650).

A "death cycle" and a "circular" model have been independently proposed (259)(310), both characterized by a self-amplifying vicious cycle that eventually leads to a large-scale caspase activation and apoptosis. The death cycle model emphasizes that mitochondria act as the apoptotic amplifier and that Bcl-2 can act both upstream and downstream of cytochrome *c* release from the mitochondria **(Figure 10)** (310). The circular model highlights multiple points of entry to the mitochondria-caspase activation circle, which explains why this circle is vulnerable to the inhibitory effect of caspase inhibitors and of agents that stabilize the mitochondrial membranes **(Figure 11)** (259). Both models indicate the decision-maker role of mitochondria, dysfunctions of which could be the point of no return (259)(310) and after amplification, irreversible mitochondrial disruption commits the cell to die irrespective of subsequent

apoptotic or necrotic processes (259).

The second decision for cell death through either apoptosis or necrosis may be dictated by whether the downstream caspases can be activated. Despite the historical use of necrosis in contrast to apoptosis (**Table 1**) (315)(406)(426), accumulating data argue against the antithesis between apoptosis and necrosis (338)(361)(427)(441)(587). Since functions of the "apoptosome" (the complex of Apaf-1, cytochrome *c*, caspase-9, Bcl-2 or Bcl-X_L) require ATP, abundant cytosolic levels of ATP would allow cells undergo apoptosis, while cells with depleted ATP levels would die of necrosis (259)(426). This model is supported by the observations that ATP depletion (451) or caspase inhibition (145)(323)(843) cause cells to die through necrosis in response to classical apoptotic stimuli.

JNK/SAPK PATHWAY

OVERVIEW OF KINASES

The cell uses a complex signal transduction network not only to respond to the changes in its microenvironment but also to monitor and integrate all intracellular signals. One important part of the signaling network is composed of a variety of protein kinases. The protein kinase superfamily is the largest protein family of the cell: they are encoded by approximately 1,000 protein kinase genes, about 1% of the whole mammalian genome (289).

The basic function of a protein kinase is to transfer the γ-phosphate of ATP (or GTP) to the protein alcohol groups on serine (Ser) and threonine (Thr) and/or to the protein phenolic groups on tyrosine (Tyr). To counteract the cellular functions of protein kinases, members of the phosphatase superfamily catalyze hydrolysis of phosphoesters, removing phosphate from the Ser, Thr, and Tyr residues. The Ser/Thr protein phosphatases (PPs) specifically remove phosphates from Ser and Thr; the protein tyrosine phosphates (PTPs) are phosphotyrosine-specific; while the dual specificity PTPs can hydrolyze phosphates from both Tyr and Ser/Thr **(Table 14)** (30)(155)(201)(351)(691)(730)(763)(897).

Structure-functional considerations of kinase

Multiple sequence alignment of kinases has revealed that all protein kinases contain a highly conserved "kinase domain" which consists of 250-300 amino acid residues (289). Taken from the type α

Table 14

Phosphatase superfamily

Class	Family	Example members
Ser/Thr protein phosphatases (PPs)	PPP	PP1 PP2A PP2B (calcineurin)
	PPM	PP2C Pyruvate dehydrogenase phosphatase
Protein tyrosine phosphatase (PTPs)	Receptor PTP (RPTP)	CD45 PTPα PTPβ PTPμ
	Intracellular PTP (Non-receptor PTP)	SHP-1 (SH2-containing tyrosine phosphatase-1) SHP-2 FAP-1 (Fas-associated phosphatase-1)
	Dual specificity PTP	Cdc25 MAPKP (=MKP-1)

*References: (30)(155)(201)(351)(691)(730)(763)(897).

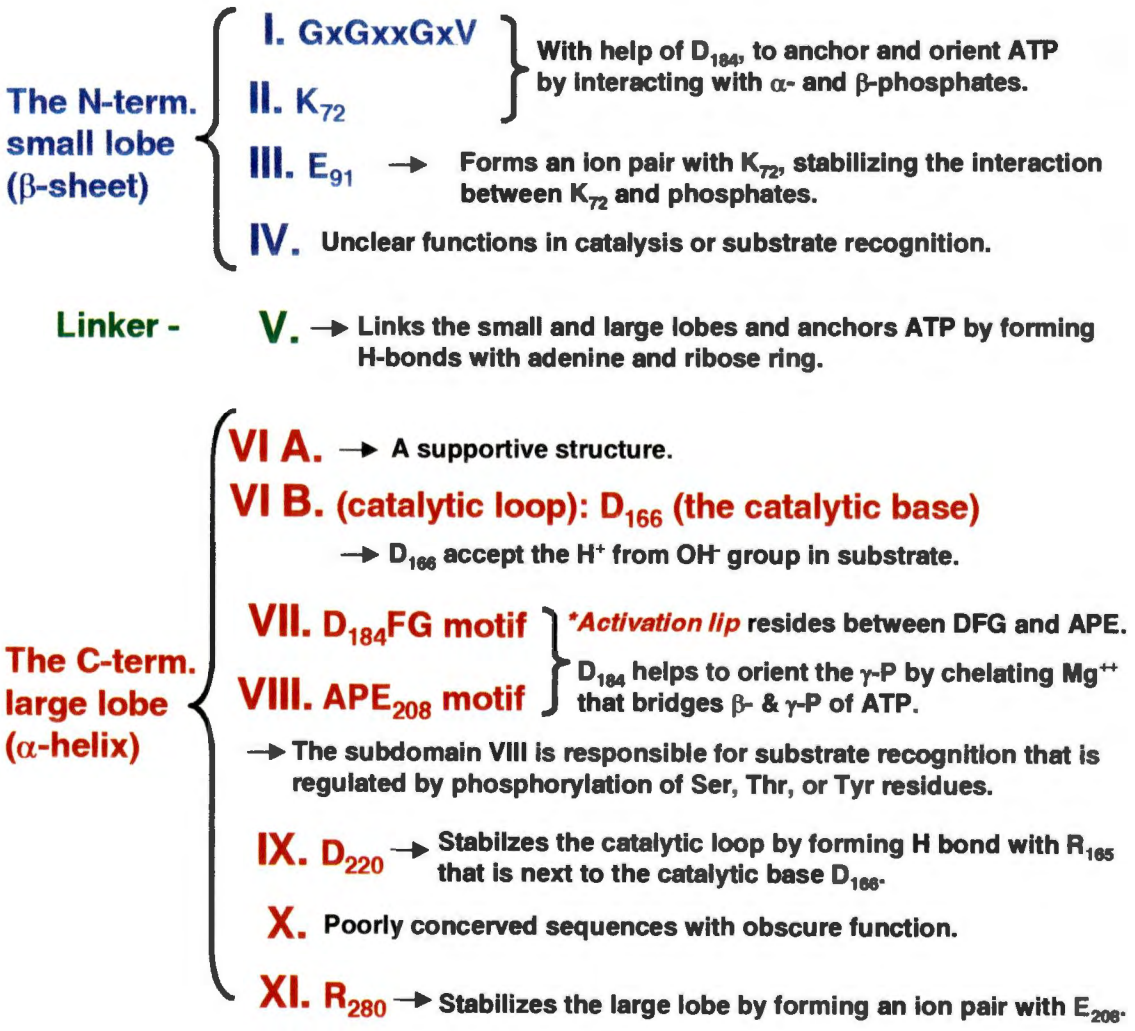
catalytic subunit of protein kinase A (PKA-Cα) as an example, conserved features and essential roles of the 12 subdomain regions are summarized in **(Figure 12)**. From the N-terminus to C-terminus of the kinase domain, there are the small lobe (subdomains I to IV) that consists of almost entirely β-sheets, the linker (subdomain V), and the large lobe (subdomains VI to XI) that is dominated by α-helix. In other parts of the kinase domain, different protein kinases exhibit variability such as additional domains, subunits, or both.

Three steps of catalysis by a kinase are: (i) binding and orientation of the ATP (or GTP) phosphate donor as a complex with a divalent cation (usually Mg^{++} or Mn^{++}), (ii) binding and orientation of the protein substrate, and (iii) transfer of the γ-phosphate from ATP (GTP) to the acceptor hydroxyl residues (Ser, Thr, Tyr) of the substrate. Both (i) and (iii) are achieved by the highly conserved kinase domain. Taking residues of PKA-Cα as an example **(Figure 13)**: D_{184} chelates Mg^{++} that bridges the β- and γ-phosphates of ATP; K_{72} and E_{91} collaboratively orient ATP by interacting with α- and β-phosphates of ATP; and the linker further stabilizes ATP via formation of hydrogen bonds with adenine or ribose of ATP (289). Subdomain VIB is the catalytic loop that contains D_{166} as the catalytic base that accepts proton from the attacking substrate hydroxyl group during a phosphotransfer mechanism (868).

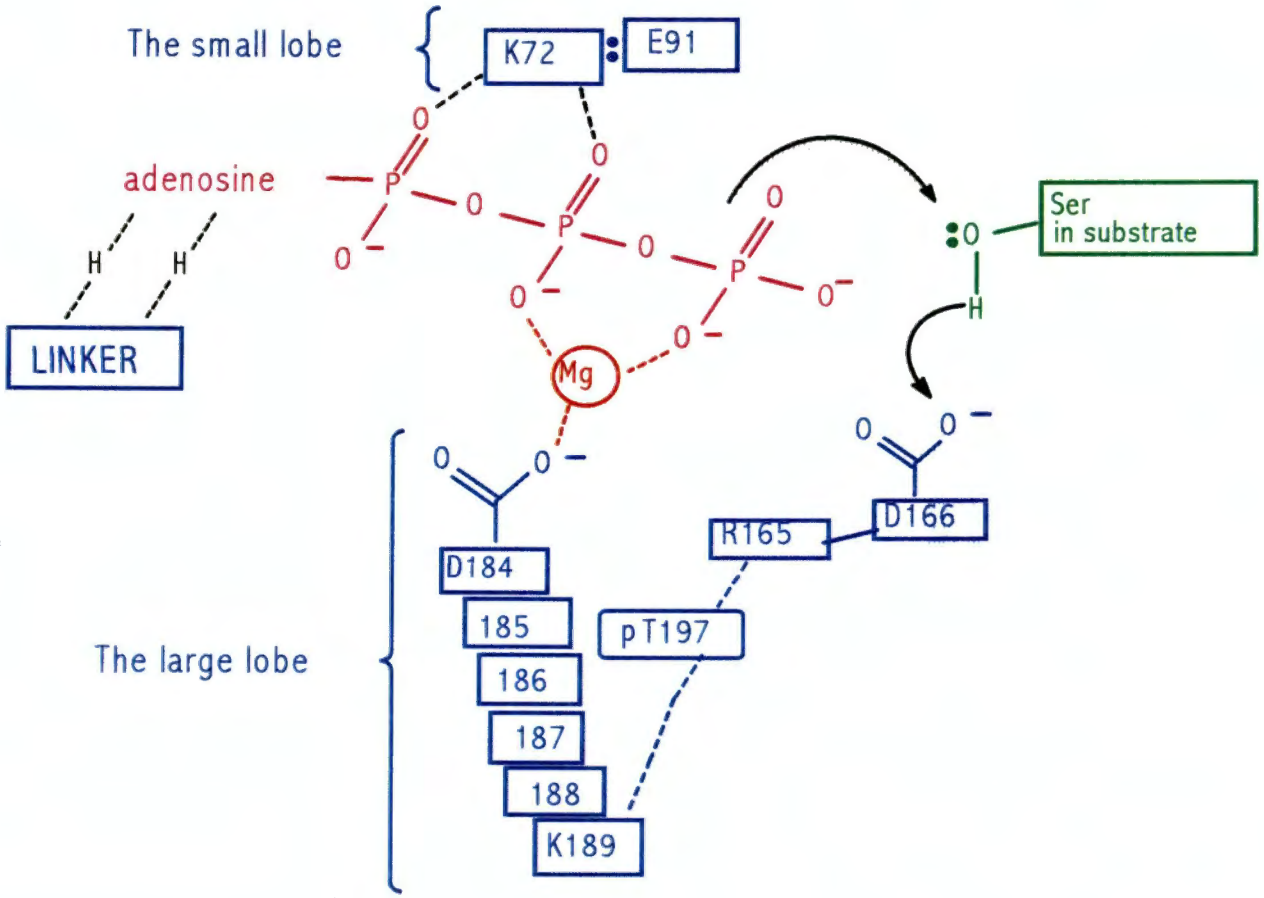
Recognition, binding and orientation of the substrate protein rely on the activation lip (segment) residing in subdomains VII and VIII. Many protein kinases may be activated by phosphorylation of residues in the activation lip. Taking PKA-Cα as an example, residing in the activation lip between D_{184}FG and APE_{208} motif is Thr_{197} that has to be phosphorylated during activation of PKA **(Figure 12)**. The phosphate oxygen of phospho-T_{197} form hydrogen bonds with the charged side-chains of R_{165} and K_{189} as well as the hydroxyl group of T_{195}, hence stabilizing the subdomain VIII loop in the active conformation that permits proper orientation of the substrate peptide (289). The activation lip varies among kinases. The variability in sequences of activation lip allows the kinase to be constitutively active, to be controlled by autophosphorylation, or to be controlled by phosphorylation directed by other kinases.

General mechanisms regulating the functions of protein kinases

The activity of protein kinases may be controlled by: (i) additional subunits that may function in response to second messengers (such as the cAMP binding to the regulatory subunit of PKA); (ii) additional subunits whose expression levels vary depending on the functional state of the cell (such as cyclin and cyclin-dependent kinase); (iii) additional domains (such as SH2 and SH3 of Src) that target the



(Figure 12) Subdomains of the kinase domain in protein kinase A. Abbreviations used are: G, glycine; x, any amino acid residure; K, lysine; E, glutamic acid; D, aspartic acid; F, phenylalanine; a, alanine; P, proline; R, arginine.



(Figure 13) Kinase-catalyzed phosphorylation. Protein kinase A is taken as an example: kinase is in blue, ATP is in purple, substrate is in green, and the Mg ion is in red. Only some key residues are shown (D, aspartic acid; E, glutamic acid; K, lysine; R, arginine; T, threonine).

kinase to different molecules or subcellular localizations; (iv) additional domains that inhibit the kinase activity by an autoregulatory process (such as in myosin light chain kinase); or (v) phosphorylation and dephosphorylation by other kinases and phosphatases, respectively.

OVERVIEW OF MAPK PATHWAYS

Actions of various signals in the cellular signal transduction network converge at the mitogen-activated protein kinase (MAPK) cascades, which lead to and activate transcription factors in the nucleus and other effectors throughout the cell (180)(810). At least twelve MAPKs **(Table 15)** and seven MKKs (MAPKK) **(Table 16)** have been identified in the mammalian cells (459). Each MAPK is activated by its cognate MKKs through a common mechanism involving phosphorylation of Thr and Tyr residues in the regulatory triplet, Thr-X-Tyr, located in the activation lip between subdomains VII and VIII. MKKs can be activated by various MAPK kinase kinases (MAPKKKs), including Raf family members, MOS, TPL2, TGF-β-activated kinase (TAK1), MEKK family members, mixed lineage kinases (MLKs), germinal center kinases (GCKs) and apoptosis signal-regulating kinase (ASK1). Thus, multiple signals from cell receptors mainly converge at the level of MKK activation.

Comparisons among MAPK pathways

ERK (extracellular signal regulated protein kinase) was first described as a protein kinase that phosphorylates microtubule-associated proteins 1 and 2 (MAP1, MAP2) (336)(637). Likewise, JNK/SAPK was first discovered as a cycloheximide-activated MAP2 kinase (434). It was also identified as a protein kinase which copurified with and phosphorylated c-Jun (6)(316). p38 MAPK was first characterized as a protein phosphorylated upon treatment with lipopolysaccharide (286) and as a "reactivating kinase (RK)" that phosphorylates and activates phosphatase-treated MAPK-APK2 (655).

ERKs, JNK/SAPK, and p38 share 40-45% overall sequence identity. Generally speaking among MAPK pathways, the ERK pathway is often stimulated by growth and differentiation factors (459), whereas the JNK and the p38 MAPK pathways are mainly activated by stress-related stimuli, such as UV or gamma irradiation, ischemia and reperfusion, and inflammatory cytokines **(Figure 6)** (393)(394)(435)(558). Nevertheless, opposing effects of JNK and p38 have been reported: for example, p38 mediates, but JNK suppresses, the development of myocyte hypertrophy (575).

Table 15

MAPK family

Subfamily	*Phosphorylation motif	Kinase	Alternative names
ERK	T-E-Y (Thr-Glu-Tyr)	ERK1	p44MAPK
		ERK2	p42MAPK
JNK	T-P-Y (Thr-Pro-Tyr)	JNK1	SAPKγ
		JNK2	SAPKα
		JNK3	SAPKβ
p38	T-G-Y (Thr-Gly-Tyr)	p38α	SAPK2a, RK (reactivating kinase), CSBP1 (**CSAIDs-binding protein 1)
		p38β	SAPK2b, CSBP2
		p38γ	SAPK3, ERK6
		p38δ	SAPK4
Others		ERK3	
		ERK4	
		ERK5	SAPK5, BMK1 (big MAP-kinase 1)

* Phosphorylation motif resides between the DFG (Asp-Phe-Gly) motif of kinase subdomain VII and the APE (Ala-Pro-Glu) motif of kinase subdomain VIII.
** CSAIDs: cytokine-suppressive anti-inflammatory drugs.
***References: (132)(459).

Table 16

MAPKK family

MAPK kinase	Alternative names	Substrates
MKK1	MEK1 (MAPK or ERK kinase-1)	ERK1, ERK2
MKK2	MEK2 (MAPK or ERK kinase-2)	ERK1, ERK2
MKK4	SEK1 (SAPK or ERK kinase-1), JNKK (JNK kinase)	JNK1, JNK2, JNK3, p38α, p38β
MKK7		JNK1, JNK2, JNK3
MKK3		p38α, p38β, p38γ, p38δ
MKK6	MEK6 (MAPK or ERK kinase-6)	p38α, p38β, p38γ, p38δ
MKK5	MEK5 (MAPK or ERK kinase-5)	ERK5

* References: (132)(459).

ERK pathways

ERK1(42 kD) and ERK2 (44 kD) are expressed ubiquitously in mammalian cells at micromolar levels (344), with some tissue type-specific variations (555). Both enzymes are activated by MKK1/2 through phosphorylation within their activation lip at Thr-Glu-Tyr (T-E-Y) in a reaction where phosphorylation of Tyr precedes that of Thr **(Table 15)**(299).

MKK1 and MKK2, both about 44 kD in mass, are activated by various upstream kinases through phosphorylation of Ser residues at the sequence Ser_{218}-Met-Ala-Asn-Ser_{222} (numbered as in human MKK1) within the activation lip (892)(891). The upstream kinases for MKKs include: Raf family members (Raf-1, B-Raf) (836), Mos (620), Tpl-2 (678), and MEKK-1 (544). In vitro activation of MKKs through phosphorylation of Ser_{218} and Ser_{222} residues is followed by intramolecular autophosphorylation at Thr_{23}, Ser_{298}, and Tyr_{300} (642).

Substrates for ERKs include various transcription factors (Elk-1, c-Jun, c-Fos family members, c-Myc, p53) and signaling proteins (MAPK-APKs, cPLA2, Sos1). Myelin basic protein (MBP) is commonly used as substrate for ERKs in *in vitro* assays **(Figure 6)**.

Elk-1, also known as p62 ternary complex factor ($p62^{TCF}$), can be phosphorylated by ERK (369)(504), JNK (250)(819)(896), and p38 (624), regulating serum response element (SRE) transcription through its interaction with serum response factor (SRF) (256). ERK can phosphorylate c-Jun both at Ser_{63} and Ser_{73} within its activation domain (627) and at residues at the C-terminus of c-Jun (545) that may lead to negative regulation through inhibition of DNA binding (12)(112). ERK also phosphorylates c-Fos, Fra-1, and Fra-2 *in vitro* and *in vivo* (101)(589). In cell extracts, ERK binds to the N-terminus of c-Myc (273) and phosphorylates its Ser_{62} (12)(143). The tight interaction between ERK and c-Myc is disrupted by phosphorylation of c-Myc, suggesting functional interactions between enzyme and substrate (459). ERK also phosphorylates the tumor suppressor gene product p53 (540).

Activated ERK may phosphorylate MAPK-activated protein kinases (MAPK-APK)-1 (207)(266)(734)(740), MAPK-APK-2 (39), and MAPK-APK-3 (493)(525). Substrates for MAPK-APK-1 include transcription factors such as cAMP-response element-binding protein (CREB), CREB-binding protein (CBP), c-Fos, Nur77, and serum response factor, suggesting that ERK may regulate gene expression indirectly through activation of MAPK-APK-1 (459). Both MAPK-APK-2 and MAPK-APK-3 phosphorylate heat shock protein 27 (hsp27), which may act as an actin capping protein (186)(525). Thus, ERK may regulate cytoskeletal assembly through MAPK-APKs. Cytosolic phospholipase A2 (cPLA2),

upon phosphorylation by ERK (469)(574), becomes activated and is translocated to the plasma membrane (177)(670). ERK also phosphorylates the Ras guanine nucleotide exchange factor, Sos1 (107)(134). Phosphorylation of Sos1 leads to destabilization of interactions among Sos1-EGFR, Sos1-Grb2, and Sos1-Shc, downregulating Ras-Raf activation (665). Thus ERK may negatively regulate its own activity through phosphorylation of Sos1.

JNK/SAPK pathways

Ten JNK isoforms encoded by three genes (*jnk1, 2, 3*) have been identified (271)(358). Stress-related stimuli do not alter expression levels of JNKs but rather activate existing JNK through dual phosphorylation of Thr and Tyr in the Thr-Pro-Tyr (T-P-Y) motif (156)(436). Two direct upstream kinases for JNKs are: MKK4 (also known as SEK1, JNKK1) that also activates p38 (157)(467)(680) and MKK7 (JNKK2) that has been shown to exclusively activate JNK (765). Activated JNK/SAPKs phosphorylate a variety of transcription factors including c-Jun, leading to transcriptional activation through interactions with c-Jun responsive DNA elements such as TPA-response element (TRE) (459). In addition to responding to extracellular stimuli, the JNK/SAPK pathway is also activated by intracellular stresses including inhibition of protein synthesis, treatment with antimetabolites, DNA damage (105)(393)(435), or microtubule dysfunction induced by chemotherapeutic agents (803).

p38 pathways

Four forms of p38 MAPK (p38α, p38β, p38γ, p38δ) have been identified **(Table 15)** (132)(251)(372). Mxi2, a C-terminal truncation of p38α resulting from alternative splicing of p38α, was discovered by its interaction with Myc and Max (882). p38 MAPKs are activated by MKK3, MKK4 (JNKK/SEK1), and MKK6 through phosphorylation of Thr and Tyr on the Thr-Gly-Tyr (T-G-Y) motif **(Table 15)(Table 16)**.

Substrates for p38 include transcription factors (ATF2/CRE-BP2, CHOP/Gadd153, Elk-1, MEF2s) and signaling proteins (MAPK-APK-2, MAPK-APK-3, cPLA2) and MBP has been used as substrate in immunocomplex kinase assay for p38. Both p38 and JNK (54)(272) phosphorylate the bZIP transcription factor ATF2, enhancing its transcriptional activity. The C/EBP family member CHOP/Gadd153, upon phosphorylation by p38, inhibits adipocyte differentiation through heterodimerization with other C/EBP family members (806). Members of the myocyte-enhancer factor 2 (MEF2) transcription factor, such as MEF-2A (890) and MEF-2C (285), are also substrates for p38. p38 also phosphorylates Elk-1 (624), MAPK-APK-2 (187), MAPK-APK-3 (493), and cPLA2 (59)(422).

Activation of p38 is frequently associated with stress-related stimuli and apoptosis, however, conflicting data exist depending on cell types and stimuli. A pro-apoptotic role of p38 has been demonstrated in HeLa cells upon inhibition of basal ERK activity (43), neurons upon withdrawal of trophic factors (335), cultured neutrophils undergoing spontaneous apoptosis (214), cardiomyocytes (807), serum-starved fibroblast and NGF-deprived PC12 pheochromocytoma cells (432)(842), glutamate-induced apoptosis in cerebellar granule cells (400), fibroblasts treated with sodium salicylate (699), and IgM-induced apoptosis of B-lymphocytes (258). A p38-dependent reorganization of actin is further proposed to be required for membrane blebbing during apoptosis (352). On the other hand, p38 has been shown to be required for B-cell proliferation (137), protect cells from apoptosis (576)(654), or not have any effect on apoptosis (679)(804).

Scaffolding proteins maintaining pathway specificity

On the stage of MAPK signaling pathways, the players include at least twelve MAPKs **(Table 15)**, seven MAPKKs /MKKs **(Table 16)**, and about a dozen of MAPKKKs (Raf, MOS, MEKKs 1-5, TPL2, TAK1, ASK1, MLKs, DLK/MUK) (459). Each MAPK may have multiple, sometimes even overlapping, substrates as exemplified in **Figure 6**, further creating problems of potential cross-regulation between different sets of kinases. To achieve exquisite specificity for an extracellular stimulus reliably activating the proper target, the cells have developed some "custom-designed MAPK cascade regulatory proteins" (180), such as JNK interacting protein-1 (JIP-1) (818), MEK partner 1 (MP1) (690), and potentially MEKK-1 (180)(846).

These scaffolding proteins may form multi-enzyme complexes with a kinase in a signaling cascade, selectively enhancing activation of this pathway and excluding cross-talk with others (180). JIP-1, first characterized as a cytoplasmic inhibitor of the JNK pathway when overexpressed (165), was recently demonstrated to bind JNK, MKK7, and MLK/DLK, channeling signals through the specific set of kinases leading to JNK activation (818). MP1, which has no enzymatic activity, has been shown to scaffold only MKK1 and ERK1, facilitating ERK activation (690). The small, 13.5-kD MP1 may bind MKK1 and ERK1 through a common site and then dimerize (180). The 195-kD MEKK1 phosphorylates MKK1, MKK2, and MKK4 *in vitro*, however, it preferentially activates JNK through MKK4 over ERK activation through MKK1 and MKK2 (544)(847)(855)(880). More interestingly, although MKK4 activates both JNK and p38, MEKK1 is a poor activator of p38 *in vivo*, suggesting a specifically

regulatory role of MEKK1 for JNK (880), perhaps through complex formation with MKK4 and JNK.

SPECIFIC CONSIDERATIONS OF THE JNK/SAPK PATHWAY

Genes, isoforms, and tissue distribution

Three genes (*jnk1, jnk2, jnk3*) encode at least ten JNK/SAPK isoforms (156)(436). Four isoforms of JNK1 (α1, α2, β1, β2), four isoforms of JNK2 (α1, α2, β1, β2), and two isoforms of JNK3 (α1, α2) result from alternative mRNA splicing **(Table 17)** (271). Two types of alternative splicing of JNKs have been described (271). Firstly, all three genes can be expressed as 46-kD and 55-kD proteins due to differential processing of the 3' coding region of the corresponding mRNA. Therefore, the JNK isoforms with molecular weight of 46 kD and 55 kD detected by in-gel kinase assays (316) represent mixtures of JNK isoforms: JNK1-α1, JNK1-β1, JNK2-α1, JNK2-β1, and JNK3-α1 may migrate together in the 46-kD band; while JNK1-α2, JNK1-β2, JNK2-α2, JNK2-β2, and JNK3-α2 may cluster in the 55-kD band (271). No functional differences between these 46-kD and 55-kD isoforms have been reported (358). The second type of alternative splicing of mRNA of *jnk1* and *jnk2* (but not found in *jnk3*) have functional consequences. This type of alternative splicing involves the mutually exclusive utilization of two exons encoding the kinase subdomains IX and X, and the resulting JNK1α vs JNK1β (likewise, JNK2α vs JNK2β) exhibit different substrate specificity *in vitro* **(Table 17)** (271)(358). Expression of *jnk1* and *jnk2* is ubiquitous in many tissues whereas *jnk3* expression is found only in brain, heart, and testis (156)(358)(436)(856).

Regulators of JNK activation

Two direct activators of JNK **(Table 16)** are MKK4/JNKK/SEK1 (157)(467)(680) and MKK7 (325)(557)(765)(860).

The first characterized MAPKKK is a MEKK family member: MEKK1 was originally identified as a truncated 73-kD protein (439) and later cloned to be a full-length 195-kD protein (848). MEKK1 phosphorylates MKK1, MKK2, and MKK4 *in vitro*, however, it preferentially activates JNK through MKK4 *in vivo* (544)(847)(855)(880). MKK4 also can be activated by MEKK2 (53), MEKK4 (246), and MEKK5/MAPKKK5 (805), but not by MEKK3 (53).

Other MAPKKKs activating MKK4 and subsequently JNK include TPL-2 (678), TAK1 (556)(852), ASK1 (356), and some MLK family members (320)(321). TPL-2 was discovered as an

Table 17

Human JNK isoforms

Genes	Isoform	M. W.	Accession numbers* for nucleotide sequence	Preference for substrate binding
jnk1	JNK1-α1	46 kD	L26318	c-Jun = ATF2
	JNK1-α2	55 kD	U34822	
	JNK1-β1	46 kD	U35004	c-Jun > ATF2
	JNK1-β2	55 kD	U35005	
jnk2	JNK2-α1	46 kD	U34821	c-Jun > ATF2
	JNK2-α2	55 kD	L31951	
	JNK2-β1	46 kD	U35002	c-Jun < ATF2
	JNK2-β2	55 kD	U35003	
jnk3	JNK3-α1	>46 kD	U34820	c-Jun > ATF2
	JNK3-α2	>55 kD	U34819	

Reference: (271).
* Numbers in the EMBL GenBank and DDBJ Nucleotide Sequence database.

oncogene in rat T-cell lymphoma (609)(610). TGF-β-activating kinase 1 (TAK1) was identified as a kinase upregulated in response to TGF-β and bone morphogenic protein 4 (MBP4) (852). Apoptosis signal-regulating kinase 1 (ASK1), which shares sequence similarity to MEKKs, activates both MKK3 and MKK4, leading to activation of p38 and JNK (356). Through interactions with TRAF2 (581) and with DAXX (93), ASK1 conveys signals from various membrane receptors to the MKK4-JNK signaling cascade. Among the mixed lineage kinase (MLK) family members, MLK2 (168)(321), MLK3 (231)(633) and DLK/MUK (320)(330) activate MKK4.

Further upstream regulators that lead to JNK activation include the Rho family of small GTPases (Rac1, Rac2, Cdc42, RhoA) (136)(543)(753), p21 Ras-activated kinases (PAKs) (26)(218)(889), germinal center kinase (GCK) (618), and hematopoetic progenitor kinase 1 (HPK1) (341)(409). Rac and Cdc42 may interact with PAKs (PAK1, PAK2, PAK3) to activate the JNK pathway (26)(218)(889), whereas JNK activation by RhoA does not involve PAK (753). Instead, RhoA may regulate the JNK pathway through interaction with protein kinase N (PKN) (565). MKK4 also can be activated by some members of the GCK family, such as GCK (618) and HPK1 (341)(409).

Effects of JNK activation

Activation of the JNK pathway has been shown to regulate gene expression, stability of target proteins, and other signal transduction pathways.

Transcriptional activation

Many downstream effectors of JNK are transcription factors including c-Jun, ATF2, ATFa, and Elk-1. Activating protein-1 (AP-1) was originally identified as a DNA binding activity recognizing a DNA element (TGACTCA) found in the enhancer region of the human metallothionein IIA gene and SV40 (208)(449). AP-1 now becomes a collective term for the dimeric transcription factors composed of bZIP (basic region leucine zipper) subunits that bind to a common DNA element, the AP-1 binding site (394). The bZIP proteins (419) include members of the Jun family (c-Jun, JunB, JunD) (154), Fos family (c-Fos, FosB, Fra1, Fra2) (515)(881), ATF family (ATF2, ATF3/LRF1, B-ATF) (169)(340)(394), and many others (C/EBP, CHOP, CREB).

Jun-Fos and Jun-Jun dimers preferentially bind to the DNA sequence TCACTCA that is known as the phorbol 12-O-tetradecanoate-13-acetate-responsive element (TRE), whereas Jun-ATF and ATF-ATF bind to the DNA sequence TGACGTCA that is the cAMP-responsive element (CRE)

(276)(394)(719). Both c-Jun (6)(316) and ATF2 (54)(272)(775) are substrates for JNK, thus JNK regulates AP-1 activity. Studies using *jnk3* -/- mice further provide *in vivo* evidence that the JNK signaling pathway regulates AP-1 activity (358)(856). Transcriptional activation by AP-1 is further regulated through interactions with transcriptional coactivators, such as CREB-binding protein (CBP) (21)(28)(390)(433) and Jun activation domain binding protein (JAB1) (119). When phosphorylated by JNK, c-Jun gains enhanced affinity for CBP (28). JAB1 selectively interacts with c-Jun and Jun D but not with JunB, providing a potentially differential transcription activation for distinct genes (119). In summary, AP-1 regulates various biological functions through: (i) different combinations of dimerization forming different AP-1 factors, (ii) phosphoregulation of subunits of AP-1 by specific protein kinases, and (iii) further interactions between AP-1 factors and transcriptional coactivators (393)(394).

Regulation of protein stability

Direct associations between members of phosphorylation/dephosphorylation cascade of the JNK/SAPK pathway and regulation of target protein stability through ubiquitination have been recently established (222). Depending on its activation status, JNK may act oppositely to affect c-Jun stability. One unique feature of JNK is the tight binding of non-activated JNK to its substrates c-Jun, ATF2, p53, and to the nonsubstrate JunB (222). For instance, both the delta region and proline- and glutamine-rich (P-Q) regions collaborate to tether non-activated JNK to c-Jun (517). Binding of JNK, even in the form of a phosphorylation mutant on residues 183 and 185 (221), targets c-Jun to ubiquitination (767). Activated JNK, however, can stimulate c-Jun phosphorylation which prevents its ubiquitination (221)(767).

Inactive JNK also targets ubiquitination of ATF2 and JunB (223). Phosphorylation of ATF2 on Thr_{69} and Thr_{71}, either by JNK (272) or p38 (632), prevents its ubiquitination. On the other hand, Elk1 is phosphorylated by JNK (90)(819), but JNK neither associates with nor targets Elk1 for ubiquitination (223).

Inactive JNK is able to bind to p53, targeting its ubiquitination (219)(222), whereas activated JNK can phosphorylate p53 and induce conformational changes and dissociation from JNK (5)(219)(541). Ubiquitination of p53 is also regulated by mdm2 (295)(331)(429). JNK-p53 complexes are mainly found in G_0/G_1, whereas mdm2-p53 complexes are identified in S and G_2/M phase of the cell cycle (219). A proposed hypothesis includes that: (i) in nonstressed cells, JNK targets p53 ubiquitination of G_0/G_1, whereas mdm2 targets p53 ubiquitination of S and G_2/M populations; and (ii) when cells are bombarded with a stress such as UV, activated JNK (as well as DNA-PK) phosphorylates p53 and changes its

conformation, which is no longer recognized by mdm2 and cannot be targeted for ubiquitination (222)(220).

Regulation of other signaling proteins

JNK/SAPK phosphorylates MAPK-APK-3 (MAPK-activated protein kinase-3) (493). A substrate for MAPK-APK-3 is heat shock protein 27 (hsp27), which may act as an actin-capping protein (186)(525). Thus, JNK may involve with regulation of cytoskeletal assembly.

APOPTOSIS INDUCTION AND OTHER FUNCTIONS OF JNK/SAPK

Activation of the JNK/SAPK pathway has been frequently demonstrated to be involved in the apoptotic process **(Table 18)**. However, the role of JNK activation in apoptosis appear to be cell type-specific and stimulus-specific, since substantial amounts of evidence indicate no role of JNK activation in certain cases of apoptosis **(Table 19)**. Furthermore, instead of induction of apoptosis, JNK activation may promote cell survival, cell proliferation, or tissue regeneration **(Table 20)**.

JNK/SAPK activation associated with apoptosis

Apoptosis in neuronal cells

The most established pro-apoptotic role of JNK activation is in neuronal apoptosis (11)(85)(108)(203)(292)(311)(322)(391)(453)(481)(495)(715)(842)(856). During the ontogenic development of nervous system, more-than-needed number of cells are originally generated and many original cells die through apoptosis (283)(593). Among original cells, only those which reach out to perfectly connect with appropriate target cells can access sufficient trophic factors to survive, making the final nervous system. It is not surprising to observe that withdrawal of nerve growth factor (NGF) is a consistent stimulus for apoptosis induction in pheochromocytoma PC12 cells and other neuronal cells **(Table 18)**. JNK activation is also involved in some receptor-mediated apoptosis after treatment with kainic acid (856) or dopamine (391)(495). On the other hand, acute transient JNK activation may have functions, instead of apoptosis induction, which help in dealing with some non-lethal environmental stresses, such as new environment for mice (849) **(Table 19)**.

Apoptosis induced by stress-related stimuli

As the term stress-activated protein kinase (SAPK) indicates, JNK/SAPK responds to various

Table 18

Positive correlations between JNK activation and apoptosis

Cell types	Induction of apoptosis	Year	References
Ovarian cancer BR cells	Paclitaxel	1999	(800)
Cerebellar granular neuron	Suppression of PI3-K activity	1999	(715)
PC12 pheochromocytoma	Withdrawal of trophic factors	1999	(453)
Neonatal sympathetic neurons	NGF withdrawal	1998	(11)
Developing brain neurons	Transient hypoxia	1998	(108)
Bovine aortic endothelial cells	Mannitol (300 mOsm)	1998	(502)
PC12 cells	Dopamine	1998	(391)
Immature thymocytes	Initiation of negative selection	1998	(643)
Rat hippocampal neurons	Polyglutamine-huntingtin	1998	(481)
Cos-7 cells	Overexpression of Gα12 & Gα13	1998	(42)
H9C2 cardiac muscle cells	Oxidative stress (menadione, H_2O_2)	1998	(770)
PC 12 cells	Manganese	1998	(322)
Breast cancer MDA-MB435	Vitamin E succinate	1998	(872)
Jurkat (T-cells)	Dominant active MEKK1	1998	(199)
Prostate cancer DU145	Radiation	1998	(65)
Bladder cancer MGH-U1	Intracellular acidification	1998	(879)
Jurkat cells	Etoposide, teniposide, UV	1998	(395)
Cervical cancer HeLa cells	H_2O_2	1998	(804)
Adult mammalian brain	Ischemia, axotomy	1998	(311)
PC12 cells	Ceramide	1998	(292)
Multiple myeloma cells	IL-6 withdrawal	1998	(844)
Remote myocardium	Induced myocardial infarction	1998	(461)
Rat mesangial cells	TNF-α	1998	(270)
HL-60	Anisomycin	1998	(726)
HL-60, U937, Jurkat	Edelfosine (ET-18-OCH3)	1998	(229)
Jurkat cells	UV, γ-irradiation, anisomycin	1998	(198)
Jurkat cells	Fas ligation	1998	(667)
U937, 293 kidney cells	proteasome inhibitors	1998	(531)
Rat mesangial cells	TNF-α with cycloheximide or act-D	1998	(269)
Rat striatal neuron cells	Dopamine	1998	(495)
U937 leukemia cells	Bufalin	1998	(811)
Mouse keratinocyte PAM212	Cisplatin	1998	(681)
Jurkat, HeLa, 293 cells	Isothiocyanates (PMITC, PEITC)	1998	(104)
Hippocampal neurons	Kainic acid (glutamate-R agonist)	1997	(856)
Jurkat cells	Dominant active GTPase Cdc42	1997	(117)
PEER (T-cells)	Heat	1997	(560)
Jurkat cells	Fas ligation, synthetic C6-ceramide	1997	(69)
Heart, kidney	Ischemia-reperfusion	1997	(863)
Madin-Darby canine kidney	Loss of integrin-ECM contacts	1997	(84)
HeLa, 293, L929 cells	Overexpression of DAXX	1997	(859)
Cultured synovial cells	Fas ligation with anti-Fas	1997	(588)
Murine mast cells	Bruton's tyrosine kinase-ind. apopt.	1997	(399)
Developing rat brain	Injection of botenic acid	1997	(203)
Small cell lung cancer cells	UV radiation	1997	(74)
293, Jurkat, Mv1Lu cells	Overexprssion of ASK1	1997	(356)
Prostate cancer cells	Androgen ablation	1997	(498)
Jurkat, 293 cells	UV, γ-radiation, anti-Fas	1996	(105)(103)
Epithelial cells	Detachment from matrix	1996	(217)
T-cells, B-cells	Fas ligation	1996	(76)
Oligodendrocytes	NGF binding to its receptor p75	1996	(85)
H9 T-cell leukemia line	Adriamycin	1996	(871)
T-lymphocytes	Fas ligation	1996	(824)(442)
U937 (monoblastic leukemia), BAE (bovine aortic EC)	Ceramide	1996	(782)
PC-12 cells	NGF withdrawal	1995	(842)

Table 19

No correlation between JNK activation and apoptosis

Cell Types	Apoptotic stimuli or Diseases	Year	References
LNCap prostate cancer	Protease inhibitors	1998	(313)
Breast cancer MCF7 cells	Ceramide pathway in TNF-mediated apoptosis	1998	(16)
Mouse Hepa1C hepatoma cells	Benzo(a)pyrene	1998	(450)
Rat cardiac myocytes	Staurosporine	1998	(875)
Madin-Darby canine kidney (MDCK) cells	Detachment of epithelial cells (anoikis)	1997	(407)
Motor neurons	Amyotropic lateral sclerosis (ALS)	1997	(533)
JNK activation in mouse brain	Environmental stimuli that do not induce apoptosis	1997	(849)
Multiple myeloma cells	Dexamethasone	1997	(97)
Syk-, Lyn-deficient chicken B-cell line	Osmotic stress	1997	(628)
293 (human kidney) cells	TNF-R1-mediated apoptosis	1997	(572)
Jurkat cells	Fas ligation-induced apoptosis	1997	(452)
MCF7, HeLa, 293 cells	TNF-R1-mediated apoptosis	1996	(483)

Table 20

Functions of JNK activation other than induction of apoptosis

Cell Types	Observed functions of JNK	Year	References
Mouse fibroblasts	Protect cells from UV-induced apoptosis	1999	(825)
Bone marrow-derived mast (BMMC) cells	Rac1/JNK pathway is required for Kit ligand-induced proliferation of mast cells	1998	(760)
Adult rat hepatocytes	Required for liver regeneration after partial hepatectomy	1998	(70)
T-cells	Required for T-cell stimulation	1998	(580)
L929, KYM-1 cells	Early transient JNK activation protects cells from TNF-induced apoptosis	1998	(654)
NIH3T3 fibroblasts	Constitutive activation of JNK is required for cell transformation by the constitutively active EGFR variant typeIII	1998	(19)
BAF3 pre-B cells (IL-3-dependent)	Required for cell proliferation of BAF3 cells	1997	(720)
TRAF2-/- cells	Deficiency of JNK activation is correlated with enhanced TNF-induced apoptosis (JNK promotes survival)	1997	(861)
T-lymphocytes	TRAF2/JNK pathway promotes survival	1997	(448)
T-lymphocytes	SEK1/JNK pathway protects T-cells from Fas- and CD3-mediated apoptosis	1997	(579)

stresses and may lead to apoptosis (65)(74)(198)(395)(531)(726). These stresses include: UV, γ-irradiation (65)(105), heat (560), oxidative stresses (H_2O_2, menadione) (770)(804), osmotic stress (502), hypoxia (108), ischemia-reperfusion (461)(311)(863), intracellular acidification (879), and antimetabolites (anisomycin, proteosome inhibitors). Involvement of JNK in osmotic stress-indued apoptosis may be cell type-specific. For example, JNK activation was observed in the apoptosis of bovine aortic endothelial cells induced by hyperosmotic mannitol (300 mOsm) (502); whereas the osmotic stress-induced apoptosis in chicken B cell lines (DT40 cells) was noted to be independent of JNK activation (628).

Apoptosis induced by lack of survival signals

In addition to the most extensively demonstrated example of neuronal apoptosis induced by withdrawal of NGF (11)(453)(842), JNK activation is involved in apoptosis induced by withdrawal of trophic factors in many other systems. For instance, androgen ablation induces apoptosis in prostate cancer cells (498) and IL-6 withdrawal causes apoptosis of multiple myeloma cells (844). Survival signals can also be provided by contacts between integrins and extracellular matrix (ECM). Loss of contacts between integrins and ECM may result in "anoikis", a type of apoptosis in epithelial cells (84)(217). JNK activation has been implicated in these two reports (84)(217), although a conflicting report exists. Using the same Madin-Darby canine kidney (MDCK) cells as Cardone *et al* did (84), Khwaja and Downward observed that (i) dn JNKK inhibited JNK activation without protection from anoikis, and (ii) caspase inhibitors prevented anoikis without affecting JNK activation induced by detachment of MDCK cells (407).

Apoptosis induced by cancer therapies

JNK activation has been involved in apoptosis of cancer cells induced by treatments such as adriamycin (871), γ-radiation (65)(105)(198), isothiocyanates (104), cisplatin (681), bufalin (811), edelfosin (ET-18-OCH3) (229), etoposide and teniposide (395), vitamin E succinate (872), and paclitaxel (Taxol®) (800).

Fas-mediated apoptosis

Involvement of JNK in Fas-mediated apoptosis of lymphocytes and cultured synovial cells has been indicated in several studies (69)(76)(103)(442)(588)(667)(824). However, JNK activation may be a result, rather than a cause, of Fas-mediated apoptosis (452). JNK activation following Fas ligation requires activated caspases (76)(766) and caspase-dependent cleavages of p21-activated kinase (PAK)

(667)(794) and MEKK-1 (84)(147), two regulators of the JNK pathway, have been demonstrated in Fas-mediated apoptosis. In summary, the early activation of caspases in the Fas-initiated apoptotic pathway can account for both apoptotic characteristics and JNK activation.

While JNK activation appears to be a consequence in Fas-mediated apoptosis, in other circumstances, activated MEKK-1 and/or JNK may upregulate expression of Fas ligand (FasL), resulting in apoptosis (198)(199)(311)(395)(453). Upregulation of FasL expression in apoptosis induced by DNA damaging agents was noted to require activation of AP-1 (395) and one AP-1 complex (ATF2 and c-Jun heterodimer) has been demonstrated to bind to a novel MEKK-1-regulated response element in the FasL promoter (199). Thus, MEKK-1 and the transcription factors regulated by JNK, at least partly, promote apoptosis through upregulation of FasL.

Other functions of JNK/SAPK activation (Table 20)

Depending on the types of cells and stimuli, activation of the JNK pathway may be associated with: (i) protection of cells from apoptosis (448)(579)(654)(825)(861), (ii) cell proliferation (720)(760), (iii) T-cell stimulation (580), (iv) cell transformation (19), and (v) liver regeneration after partial hepatectomy (70).

PACLITAXEL-INDUCED APOPTOSIS

DEVELOPMENT AND PRODUCTION OF PACLITAXEL (TAXOL®)

Historical developments of paclitaxel (PTX) discovery are summarized in **Table 21**. Despite the demonstrations of PTX extraction from cultured plant tissues in 1992 (204) and the total synthesis of PTX in 1994 (578) **(Table 21)**, neither is yet economically practical for PTX production at a commercial level. On the other hand, the yield of PTX extracted from the Pacific yew bark is extremely low in the range of 0.014-0.017% (784). An alternative, semisynthetic production of PTX from the more abundant precursor taxanes present in yews has been developed (608).

A complex mixture of taxanes (PTX, 10-deacetyltaxol, cephalomannine, baccatin III, 10-deacetylbaccatin III, taxol C) at various levels (608) can be extracted from many species of Taxus plants all over the world **(Table 22)**. The most valuable precursor materials for semisynthesis are the taxane

Table 21

Historical development of paclitaxel discovery

Year	Key development
1955	NCI started planning for the Cancer Chemotherapy National Service Center to facilitate discovery of new anti-cancer drugs.
1962	Specimens of the Pacific yew (*Taxus brevifolia*) were collected by a USDA team from the Gifford Pinchot National Forest of Washington state.
1966	Paclitaxel, the substance with anti-tumor activity against nasopharyngeal KB cells, was purified from the bark of *T. brevifolia.*
1971	The molecular formula for paclitaxel was determined to be $C_{47}H_{51}NO_{14}$.
1974	Intraperitoneal (i.p.) injection with paclitaxel increased life span of mice i.p. implanted with melanoma B16 cells.
1976	NCI developed the human tumor xenograft model in the nude mice for screening of anti-tumor drugs. Paclitaxel caused regression of breast cancer xenograft and growth inhibition of lung and colon cancers.
1980	Paclitaxel was demonstrated to interfere with microtubule function through binding to microtubule polymer.
1980	Paclitaxel was reported to be present in other yews: *T. baccata* and *T. cuspidata.*
1982	Investigational New Drug (IND) application of paclitaxel was filed to the Food and Drug Administration (FDA).
1984	FDA approved paclitaxel for clinical trials.
1989	The Johns Hopkins group reported that paclitaxel was effective in treatment of refractory ovarian cancer.
1991	Bristol-Myers Squibb Company won the Cooperative Research and Development Agreement (CRADA) and was responsible for increasing the production of paclitaxel from bark and for developing an alternative supply of paclitaxel.
1992	The first report of paclitaxel production from plant cell cultures of *T. cuspidata* and *T. canadensis*.
1992	Taxol® (paclitaxel) was approved for marketing for the treatment of refractory ovarian cancer.
1994	The first report of total synthesis of paclitaxel.

References: (204)(522)(578)(608)(694)(693)(809)

Table 22

Paclitaxel-producing yews

Common name	Scientific name	Geographic distribution
Pacific yew	*Taxus brevifolia*	Western parts of Canada and the United States.
Mexican yew	*Taxus globosa*	Mexico, El Salvador, Honduras, and Guatemala.
Florida yew	*Taxus floridana*	Northern Florida.
Canadian yew	*Taxus canadensis*	Canada: from Newfoundland to Manitoba; United States: from Iowa to North Carolina.
English yew	*Taxus baccata*	Europe and north Africa.
Japanese yew	*Taxus cuspidata*	Eastern Asia (China, Japan, Korea, and parts of Russia)
Himalayan yew	*Taxus wallichiana*	From eastern Afghanistan to Tibet and China.

Reference: (608)

"nucleus" component of baccatin III and 10-deacetylbaccatin-III (10-DAB) **(Figure 14)**. Three enzymes, C-7 xylosidase, C-10 deacetylase, and C-13 taxolase, are discovered to be valuable in enrichment of 10-DAB concentrations in the mixture of taxanes (578)(608) and the semisynthetic production of PTX is currently used to supply an increasing demand for PTX (658).

MICROTUBULE FUNCTIONS AND BINDING OF PACLITAXEL

Structure-functional considerations of microtubules

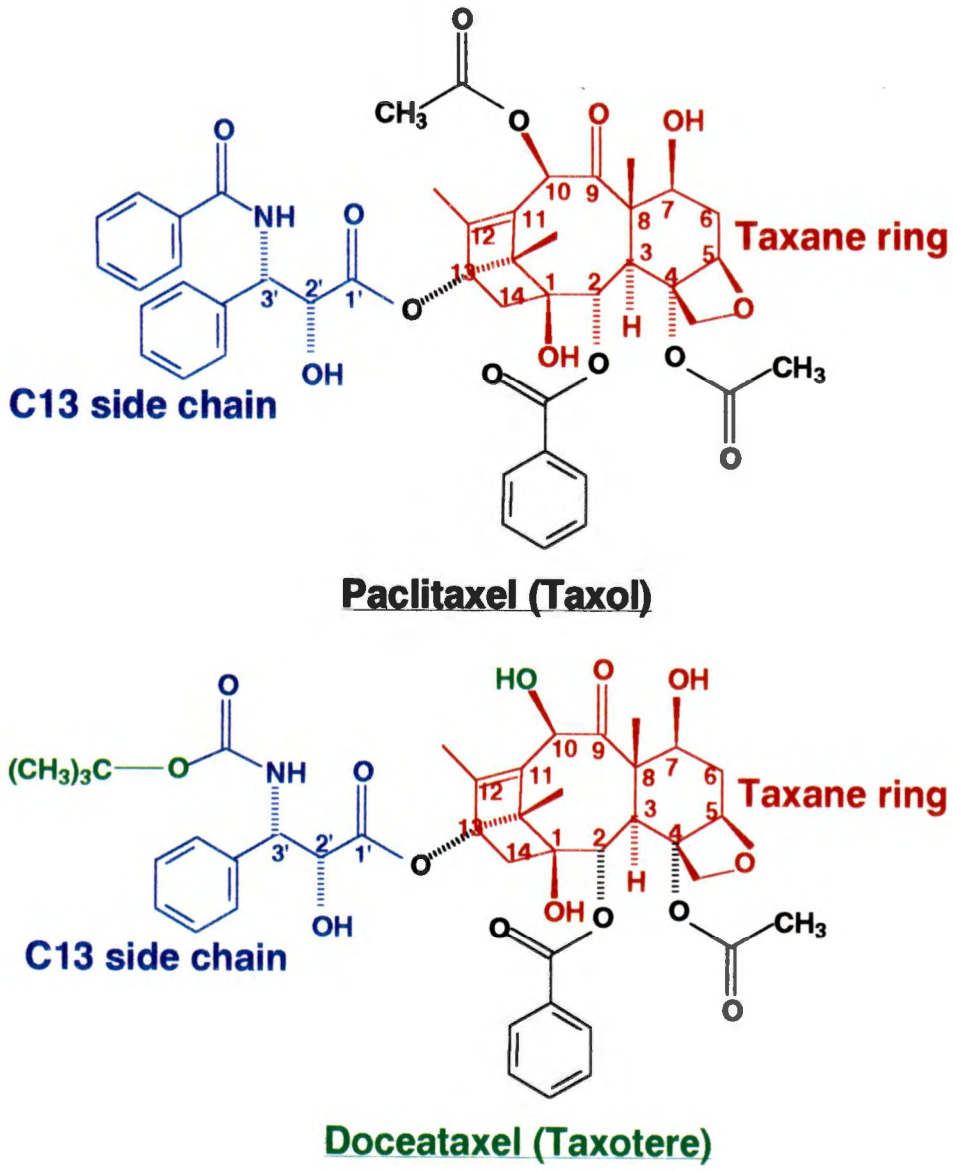
MTs are made of α- and β-tubulin heterodimers of about 110 kD. Both types of tubulin at an 1:1 ratio are arrayed head-to-tail like beads on a string, which is called a protofilament (774). Thirteen protofilaments line up side by side to form a sheet that wraps around its longitudinal axis and form the cylinder-like MT (172). MT can grow by the reversible, non-covalent addition of tubulin at both ends; however, its polarity is created by the alternate stacking of two types of tubulin. The fast-growing "plus" end is ringed by α-tubulin and the slow-growing "minus" end by β-tubulin **(Figure 15)** (812).

GTP binds to both types of tubulin: at the non-exchangeable site (N-site) of α-tubulin and at the exchangeable (E-site) of β-tubulin (159). A tubulin heterodimer can be added to a MT when both α- and β-tubulin have bound GTP. After a tubulin heterodimer is incorporated into a MT, the GTP at the E-site of β-tubulin is hydrolyzed irreversibly to GDP. The bulk of a MT therefore consists mainly of GDP-bound β-tubulin, except both its ends are still capped with GTP-bound subunits. The GTP-bound tubulin caps are believed to be critical for stability of the MT ends **(Figure 15)** (774)(485)(159). Hydrolysis of GTP removes the constraints of a simple equilibrium from MT assembly (812), as shown in the formula:

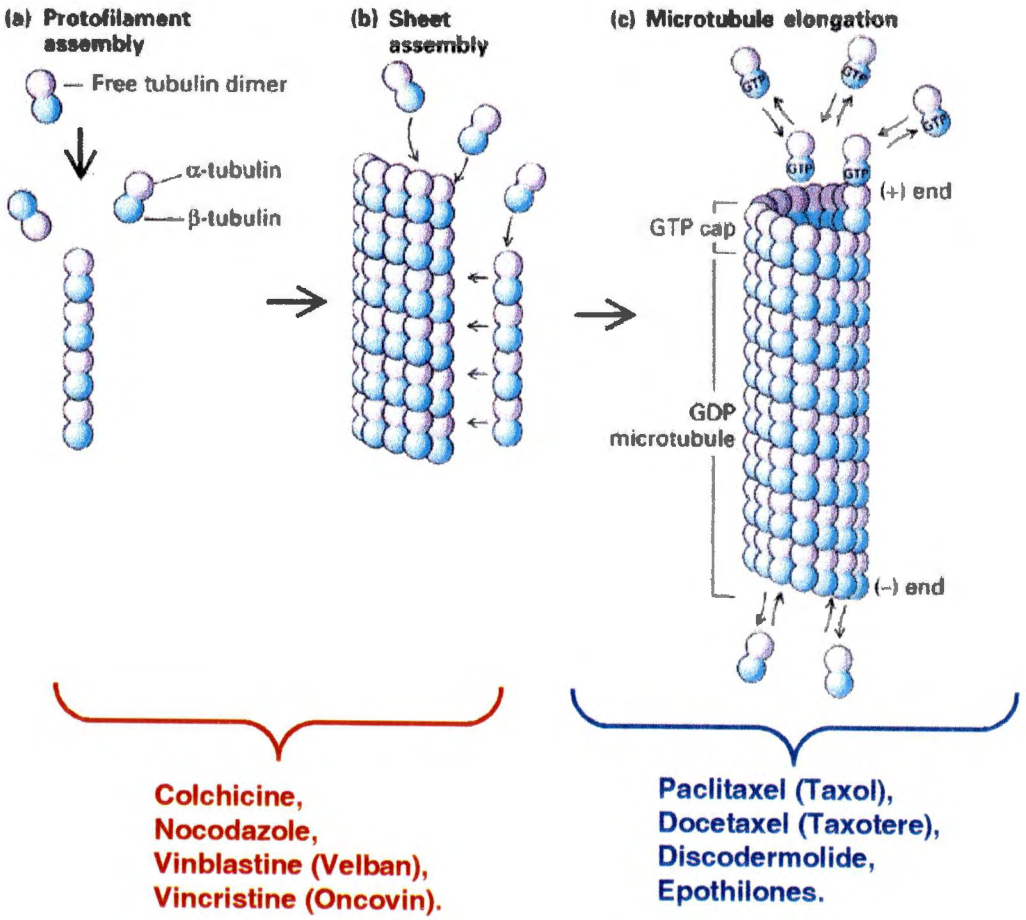
$$\underbrace{\text{GTP} + \text{tubulin} \rightleftharpoons \text{GTP-tubulin}}_{\text{cytosolic}} \rightarrow \underbrace{\text{GDP-tubulin} + \text{Pi}}_{\text{in MT}}$$

The direct coupling of MT assembly dynamics to GTP hydrolysis further provides the potential for polymerization and depolymerization, allowing the unique assembly behaviors such as "dynamic instability" and "treadmilling" (159).

"Dynamic instability" was proposed in 1984 based on an analysis of the length distributions of fixed MTs (547). According to this model, each MT persists in prolonged states of polymerization and depolymerization that interconvert infrequently, although the whole population of MTs may exhibit a bulk



(Figure 14) Chemical structures of two taxanes. Both the taxane ring and the C13 side chain are required for anti-tumor activity of taxanes. Structures in docetaxel that are different from those in paclitaxel are labeled in green.



(Figure 15) Microtubule assembly is targeted by various microtubule-active agents. (a) Tubulin heterodimers form protofilament. **(b)** Thirteen protofilaments line up side by side to form a sheet that wraps into the cylinder-like microtubule. **(c)** Microtubule can elongate or shorten at both ends, however, both polymerization and depolymerization are faster at the (+) end than at the (-) end. At saturating concentrations, the microtubule-active agents that preferentially bind to tubulin are labeled in red color, whereas those only bind to microtubules are labeled in blue.

steady state. Several real-time, dark field and Normarski (differential interference contrast) video microscopy studies confirmed the existence of dynamic instability (333)(792). Increasing amount of *in vitro* and *in vivo* evidence has convinced researchers that dynamic instability is the predominant mechanism governing MT assembly (88)(244)(189)(830).

Dynamic instability is characterized by four parameters: polymerization rate, depolymerization rate, the frequency of catastrophe (the transition from polymerization to depolymerization), and the frequency of rescue (transition from depolymerization to polymerization) (159). MT polymerization is dependent on the free tubulin concentration, whereas depolymerization is independent of it. Thus, increasing the tubulin concentration increases the polymerization rate, which may result in a decreased catastrophe frequency. The relationship between polymerization rate and catastrophe frequency, however, is not supported by every study. The catastrophe frequency has been demonstrated to be independent of polymerization rate (585).

Intuitively, dynamic instability seems to require an energy source, which is likely to be GTP hydrolysis by β-tubulin. Studies using nonhydrolyzable GTP analogues, however, revealed that polymerization does not require GTP hydrolysis and that the MT lattice is more stable with a GTP analogue-bound β-tubulin than with GDP-bound subunits (411)(530). Therefore, the primary role of GTP hydrolysis is likely to destabilize the MT lattice through weakened contacts among GDP-bound subunits (80). On the other hand, soluble tubulin undergoes GTP hydrolysis only very slowly; whereas the rate of GTP hydrolysis highly increases when tubulin subunits are incorporated into MTs (141)(82). In this assembly-driven GTP hydrolysis, the formation of inter-subunit contacts during polymerization may enhance tubulins function as a GTPase activating protein (GAP), accelerating GTP hydrolysis by itself (159). Thus, tubulin can be considered as a *cis*-acting factor in the regulation of MT dynamics. At the end of a MT, the ring of newly incorporated tubulins which have not undergone hydrolysis remain stable, acting as a stabilizing cap of MT (547). The GTP-cap hypothesis has been supported by microscopic studies using nonhydrolyzable GTP analogues (81)(175).

MT dynamics are also regulated by several *trans*-acting factors that regulate the catastrophe frequency and the polymerization rate (722). Two catastrophe-promoting factors are Op18/stathmin (17)(38)(488)(764) and XKCM1 (Xenopus kinesin central motor 1) (158)(789). Op18/stathmin has been shown to bind to free tubulin and sequester it into a pool where it cannot polymerize into MTs (38). XKCM1 is a motor protein that binds to an assembled MT and alters its dynamics (789). Both

Op18/stathmin and XKCM1 may also be involved in the regulation of increased MT dynamics during mitosis (353). On the other hand, MT-associated proteins (MAPs) bind to the MT lattice and thereby promote polymerization (503). Phosphorylation of MAPs, which increases during mitosis, reduces their affinity to MTs, perhaps also accounting for increased MT dynamics during mitosis (514)(592).

MTs function both as constitutive cytoskeletal components in the interphase cell and as spindle fibers for chromosome segregation during mitosis. Dynamic instability allows MTs to move through and fill the newly formed regions of cytoplasm, facilitating recruitment of the membrane system to use MT motor proteins (746). Dynamic instability is also the mechanism for a MT to "search and capture" its target (*e.g.* duplicated chromatids) in the three-dimensional space more effectively than equilibrium polymerization (298)(329)(159).

The "treadmilling" model of MT assembly was proposed in 1978 based on the observation of continuous incorporation of tubulin into MT at a steady state when tubulin subunits are constantly added at one end with a balanced loss of subunits at the other end (506). Treadmilling of MTs in a plus-to-minus direction, *i.e.*, tubulin subunits adding to the plus end and dropping from the minus end, was visualized using dark-field microscopy in 1988 (337). Observations of treadmilling have been made both in mitotic cells (813) and in interphase cells (645)(644). By definition, the rates of both assembly and disassembly of MTs are much higher at the plus end than at the minus end; thus, how can a state of treadmilling exist? A "biased dynamic instability" mechanism has been proposed that some factors including MAPs may regulate dynamic instability in an end-specific manner, controlling the rates of assembly and disassembly, thus catastrophe is suppressed at the plus end and rescue is suppressed at the minus end (812).

The most dramatic function of MTs is to equally separate duplicated chromatids into two daughter cells during mitosis. Indeed, MT dynamics are 20- to 100-fold higher in mitotic cells than in interphase cells (380). When cells enter mitosis, the cytoskeletal MT network is dismantled and spindle MTs are oriented with their minus ends at the spindle pole and their plus ends pointing to the midplane of the cells, reaching the kinetochores of chromosomes (35). The source of force required to capture and pull chromosomes is believed to be generated by MT polymerization and depolymerization (87)(160). Nevertheless, forces and movements required for mitosis may also be provided by members of the MT-associated motor family, such as the plus-end-directed kinesins and the minus-end-directed dyneins (35) (722).

Kinetic suppression of microtubule dynamics by microtubule-active agents

Various chemical agents, mostly derived from natural products, bind to tubulin or MTs and inhibit cell division at the metaphase-anaphase transition of mitosis through interference with mitotic spindle functions (380). Many of this group of "MT-active" or "anti-mitotic" agents become valuable chemotherapeutic drugs against cancers. They may fall into one of two groups according to their preferred targets being tubulin (colchicine, vinblastine=Velban®, vincristine=Oncovin®, nocodazole) or MTs (paclitaxel=Taxol®, docetaxel=Taxotere®, discodermolide, epothilones) **(Figure 15)**(48)(380)(421)(694)(752).

Although at high concentrations, these anti-mitotic agents exhibit differential affinities to either tubulin or MTs; studies using lower concentrations revealed that both groups of agents cause a common kinetic suppression of MT dynamics (380). At high concentrations, colchicine (603), vinblastine (163)(604), and nocodazole (778) destabilize MTs; whereas at low concentrations, they suppress MT dynamics without accompanying MT depolymerization. In summary, the kinetic suppression (stabilization) of MT dynamics is a common mechanism for these MT-active agents (380).

Paclitaxel binds to microtubule at β-tubulin

Both the taxane ring and the C-13 side chain are essential for the anti-tumor activity of PTX **(Figure 14)** (809) and docetaxel (Taxotere®) (49), both derived from Taxus. Epothilones A and B, natural products from bacterium *Sorangium cellulosum* (56), despite no structural similarity with taxanes, also bind to MTs and induce the formation of hyperstable tubulin polymers (421). This structural differences may explain that epothilones remain cytotoxic on PTX-resistant cells which express multidrug resistance (*mdr*) gene (421).

Six α-tubulin isotypes and six β-tubulin isotypes have been identified in human cells. Expression of these isotypes differs in a tissue-, cell- and tumor-specific manner (380). Differences in the content of β-tubulin isotypes in MTs particularly affect the dynamics and drug binding of MTs (397)(437). For instance, βIII and βIV isotypes are associated with PTX resistance, explaining overexpression of these isotypes in taxol-resistant ovarian cancer tissues (397) as well as in cultured leukemia, prostate cancer, and lung cancer cells (366)(397)(634).

Four regions of β-tubulin are important for binding of PTX: (i) a.a. residues 1-36 at the N-terminus and (ii) residues 217-231 at central region (27)(284), both are the binding sites for PTX; (iii) a

surface-exposed, drug-sensitive loop from residues 270 to 290, and (iv) an amphipathic helical region from residues 370-390. Exposure of the 270-290 loop to chymotrypsin cleavage is enhanced by binding of colchicine and vinblastine (672) but is suppressed by PTX (674). Unfolding of the 370-390 helical regions to chymotrypsin cleavage is enhanced by binding of colchicine (673) but is suppressed by vinblastine (672). Sequence analyses of β-tubulin in PTX-resistant ovarian cancer PTX-10 and PTX-22 cells revealed that two a.a. residues Phe_{270} and Ala_{364} are important modulators for PTX's interaction with tubulin, perhaps through regulations of the 270-290 loop sequence and the 370-390 amphipathic helical region (247). Single amino acid mutations of either Phe_{270} or Ala_{364} blocks PTX binding to MTs, resulting in PTX-resistance.

Our further understanding of structural interactions between tubulin and MT-active agents may have clinical importance. For example, combinations of anti-mitotic agents that bind to different sites on tubulin or MTs have been demonstrated to exert additive or synergistic effects against cell growth (234)(605)(616)(723).

CLINICAL USES OF PACLITAXEL

Taxanes (paclitaxel, docetaxel) may have the broadest anti-tumor spectrum among all classes of anticancer agents (658). Both agents have been demonstrated effective as the second-line treatment for patients with advanced stages of refractory cancers. Furthermore, taxanes may have greater activity in early stage diseases, making a greater impact on survival and cure of cancer patients. Current clinical trials are focusing on development of strategies using taxanes as first-line agents in cancer patients.

Paclitaxel-responsive tumor types

PTX (721) is currently approved in the United States as a second-line drug to treat: (i) ovarian cancer after the failure of previous platinum (cisplatin, carboplatin) treatment (78)(275)(522)(660) and (ii) breast cancer after failed treatment with anthracyclines (doxorubicin, epirubicin, daunorubicin, idarubicin) (328)(454)(641). PTX is also active in treatment of lung cancer (91)(192)(568), head and neck cancers (209), bladder cancer, and esophageal cancer (660)(658). On the contrary, it may not be beneficial in treatment of colorectal, renal, prostate, pancreatic, gastric, and brain cancers (658).

Since PTX has been shown to enhance the effects of ionizing radiation in murine mammary

carcinoma (535), HL60 cells (561), laryngeal carcinoma (626), its combined use with radiotherapy in treatment of locally advanced lung cancer (113)(114)(116) may be useful. However, the radiosensitizing effect of PTX appears to be cell type-specific, because it only exhibits minimal effect in cervical cancer cell lines (190).

Adverse effects of paclitaxel

Common adverse effects associated with PTX are (i) major hypersensitivity reactions (HSR), manifested by dyspnea with bronchospasm, urticaria, and hypotension (815)(663)(661), (ii) neutropenia (663)(661), (iii) neurotoxicity, including peripheral sensory loss as well as motor and autonomic dysfunctions (659), and (iv) cardiac effects such as bradycardia, myocardial ischemia, atrial arrhythmia, and ventricular tachycardia (662)(661)(248). Almost major HSR occur within the first 10 min after treatment with the first or second dose of PTX. To prevent HSR, premedication with dexamethasone, diphenhydramine, and one of histamine H_2-antagonists (cimetidine, famotidine, ranitidine) before treatment is recommended (658). The onset of neutropenia is usually during the days 8-10 after administration of PTX and typically recovers by day 15-21 (658). Other side effects include nausea, vomiting, diarrhea, and reversible hair loss (661)(660).

MECHANISMS OF PACLITAXEL-INDUCED CYTOTOXICITY

Potential advantages of using PTX in cancer therapy include: (i) killing cancer cells by PTX does not rely on a functional p53 (149)(197)(777)(829), (ii) PTX even kills cancer cells with p53 mutations more efficiently than those with wt p53 (788), and (iii) it preferentially induces G_2/M arrest and apoptosis in transformed cells but rather induce reversible G_1 arrest in non-transformed cells (768). All of these features of PTX suggest that it may differentially kill cancer cells but spare normal cells.

Apoptosis accounts for paclitaxel-induced apoptosis

Apoptosis was first shown to be a mechanism of PTX-induced cytotoxicity in human lymphoid leukemia cells in 1993 (46). That observation has been supported by numerous similar reports in other leukemia cells (462), ovarian cancer cells (297), gastric cancer cells (95), head and neck tumors (232), prostate tumors (862), adrenocortical carcinoma cells (194), and human glioma cell lines (754). A positive

correlation of cytotoxicity and apoptosis induced by PTX has been confirmed by studies *in vitro* (233) (249) and *in vivo* (542).

Status of p53 and paclitaxel-induced apoptosis

One unique feature of PTX anti-tumor actions is that they do not rely on p53-dependent pathways to initiate apoptosis in treated cancer cells (149)(197)(777)(829). The p53-independent mechanism of Taxol cytotoxicity was clinically demonstrated by the fact that response rate to the treatment with PTX and radiation in cancer patients with p53 mutant was as good as in cancer patients with wt p53 (675). More importantly, loss of normal p53 function, which is found in more than 60% of human cancers, has been shown to confer sensitization to PTX (612)(785)(788)(876).

Several explanations have been proposed for the association between p53 mutations and increased sensitivity to PTX. (i) Lack of p53-dependent G_1 arrest promotes the progression of cells to the G_2/M phase, which will become of the target of mitotic arrest (788). (ii) Upregulation of $p21^{WAF1/CIP1}$ by p53 may facilitate exit from mitotic arrest (29). (iii) MT-associated protein 4 (MAP4), which is downregulated by activation of wt p53 (567), may play a role in MT stabilization (886). The levels of MAP4 may increase in the absence of functional p53, stabilizing MTs and sensitizing cells to PTX treatment.

Bcl-2 family members and paclitaxel-induced apoptosis

Like apoptosis initiated by other stimuli, PTX-induced apoptosis is also regulated by members of the Bcl-2 family (**Figure 7**). Apoptosis induced by PTX has been shown to be inhibited by overexpression of Bcl-2 (747)(346)(241) or Bcl-X_L (475), and to be enhanced by Bcl-X_s (736), Bak (717), Bad (733), or Bax (732)(731).

PTX treatment itself may be able to modulate expression of the Bcl-2 family members and may also cause post-translational modification of Bcl-2 molecules. PTX has been shown to downregulate Bcl-X_L expression or upregulate Bak, depending on the cell type (477). Upregulation of proapoptotic Bcl-2 family members by PTX has been demonstrated in Bak (376) and Bax (725). PTX has been shown to induce Bcl-2 phosphorylation, which has been proposed to promote PTX-initiated apoptosis (51)(281) (279)(652). Raf-1 has been proposed to be required for the PTX-induced Bcl-2 phosphorylation (51)(52), however, contrary data exist (355). On the other hand, recent reports demonstrate that phosphorylation of

Bcl-2 occurs only in cells blocked at the G_2/M phase after PTX treatment (279)(689), arguing that Bcl-2 phosphorylation is a mere consequence of mitotic arrest. Furthermore, since the role of Bcl-2 phosphorylation in promoting (51)(92)(99)(281)(652) or inhibiting apoptosis (334)(360) remains controversial; its role in regulating apoptosis initiated by PTX remains to be clarified.

Apoptosis following the cell cycle arrest

Mitotic arrest of PTX-treated cells has been associated with apoptosis (379)(486)(829), however, the biochemical events downstream of kinetic stabilization of MT dynamics which lead to apoptosis remain largely unclear (380). Furthermore, substantial evidence indicates that G_2/M arrest may not be the only mechanism to induce apoptosis (440)(534)(542)(553); additional phosphoregulatory pathways may be involved in inducing apoptosis (51)(281)(619)(827). Interestingly, PTX has been shown to activate ERK1/2 and this may, in turn, accelerate progression of the cell cycle, further facilitating the eventual G_2/M arrest (466).

PTX-induced apoptosis can occur either directly from mitotic arrest (829) or following aberrant mitotic exit into a G_1-like "multinucleate state" (379)(468)(829). In addition to cell type specificity (768), the fate of cells following PTX treatment depends on: (i) the concentration of PTX used (378)(465) and (ii) the duration of exposure (465). For instance, a short (1 h) exposure of HL-60 cells to a low concentration (20 nM) of PTX may induce a reversible mitotic block without apoptosis, whereas either 60 nM PTX for 1 h or 10 nM for 12 h induces apoptosis (465).

The spindle assembly checkpoint is also involved in regulation of PTX-initiated apoptosis (722). Several mammalian gene products have been identified to function as the spindle assembly checkpoint, such as *hs*MAD2 (463)(464)(722), BUB1 (750), BUB3 (722)(749) (these three are kinetochore-localized), PP2A, and MAPK (722). Protracted activation of these gene products may be involved in apoptosis followed by mitotic arrest. For instance, overexpression of AV BUB1 proteins in HeLa cells reduces the severity of nocodazole-induced apoptosis (722)(750). The mechanism by which the spindle assembly checkpoint initiates apoptosis, however, remains unclear.

Apoptosis by aberrant activation of cyclin-dependent kinases (CDKs)

PTX-initiated apoptosis has been associated with activation of $p34^{cdc2}$ (167)(345)(354)(707), Cdc2-like kinase (689), and other CDKs (226)(529)(619). Recent studies suggest that catastrophically

activated Cdks may be terminal effectors in the apoptotic pathway (293)(458), further strengthening the close relationships between PTX-induced $p34^{cdc2}$ activation and apoptosis. However, whether the activated Cdc2 is the cause (870) or the consequence (471) of mitotic arrest remains unclear. Alternatively, both Cdc2 activation and mitotic arrest may be two parallel consequences of MT dynamic suppression (167) (470).

Activated cyclin B1/Cdc2 has also been suggested to be responsible for Bcl-2 phosphorylation of mitotic cells (471). The p53-upregulated $p21^{WAF1/CIP1}$ has been shown to associate with Cdc2 and to inhibit its activity (29)(870), thereby helping cells survive PTX-induced apoptosis.

Paclitaxel may activate the release of cytotoxic cytokines

Treatment with a high concentration (30 μM) of PTX has been shown to stimulate local release of TNF-α, a cytokine frequently associated with apoptosis, from murine macrophage (440). Despite its unlikely clinical relevance due to the supra-pharmacological concentration of PTX required, this observation still strengthens the notion that PTX has multiple effects on the cell. PTX has also been shown to transcriptionally activate IL-8 expression in ovarian cancer cells (447) and the AP-1 as well as NF-κB sites of the IL-8 promoter are required for activation by PTX (446).

Other mechanisms of paclitaxel-induced apoptosis

PTX has been shown to regulate apoptosis at the transcriptional level, such as upregulation of c-Mos (472), cyclin B1 (472)(470), and the pro-apoptotic Bak (477)(376) and Bax (725) and downregulation of the anti-apoptotic Bcl-X_L (477). It upregulates the gene *krox-24*, whose gene product exhibits tumor suppressor activity, and several enzymes (2'5'-oligoadenylate synthase, cyclooxygenase-2, and an IκB kinase termed chuk) that is involved in regulation of apoptosis (553).

RESISTANCE AND ENHANCEMENT OF PACLITAXEL-INDUCED APOPTOSIS

Mechanisms of resistance to paclitaxel treatment

In vitro studies have demonstrated that cancer cells become resistant to PTX through a variety of mechanisms: (i) increased drug efflux resulting from upregulation of either *mdr-1* gene and the subsequent increased expression of phosphoglycoproteins (45)(346) or some other membrane transporter

(731); (ii) amino acid mutation in β-tubulin that abolishes PTX binding (247); (iii) differential expression of various tubulin isotypes (397); (iv) the presence of defective caspases, such as in prostate cancer PC-3 cells (606) and breast cancer MCF-7 cells (367); and (v) upregulation of the anti-apoptotic Bcl-2 family members such as Bcl-2 (241) and Bcl-X_L (475), and/or downregulation of the pro-apoptotic Bax (347).

Enhancement of paclitaxel-induced apoptosis by adjuvant treatments

Various adjuvant treatments have been tested for enhancement of the taxanes-triggered apoptosis in cancer cells. (i) Verapamil, a membrane channel blocker, is able to reverse PTX resistance due to overexpression of the *mdr-1* gene (45). (ii) Tamoxifen has been demonstrated to act synergistically with docetaxel against breast cancer cells (202). (iii) An inhibitor of cytoplasmic phospholipase A2, quinacrine, has been shown to exhibit a synergistic effect with PTX against some hormone-refractory prostate cancer cells (146). (iv) TNF-α has been shown to potentiate the anti-tumor effects of PTX in nude mice bearing human breast, ovarian, and liver tumors (703). (v) Farnesyl transferase inhibitors (FTI) have been shown to act synergistically with PTX to inhibit cell growth. FTI causes increased sensitivity to induction of metaphase block by MT-active agents, suggesting that a farnesylated protein may regulate the mitotic check point (551). (vi) A macrocyclic lactone protein kinase C (PKC) activator, bryostatin 1, has been observed to enhance apoptosis of leukemia cells induced by PTX (799). (vii) An inhibitor of phosphatidylinositol synthesis, inostamycin, reduces the dosage of PTX required for apoptosis induction in small cell lung cancer cells (716). (viii) Expression of the pro-apoptotic BAD has been shown to reverse PTX resistance in ovarian cancer cells, suggesting that small molecules which mimic the effects of BAD may become potential adjuvant drugs for PTX treatment (733).

PTX has been found to be a potential radiosensitizing agent that augments the efficiency of radiotherapy (761)(242). For patients with locally advanced unresectable non-small cell lung cancer, the combined treatment with PTX and radiation has demonstrated an overall response rate of 77% (115). Multiple mechanisms may be involved in the radiosensitizing effects of PTX, including G_2/M arrest of the cell cycle (761) and tumor reoxygenation (535)(536). Cells in the G_2 and M phases are known to be the most sensitive to ionizing radiation (761), explaining the radiosensitization of cancer cells by PTX. On the other hand, the greatest augmentation of the radiation response was noted to not be at the time of highest mitotic arrest but at one day after PTX treatment (535), suggesting involvement of other mechanisms such as reoxygenation of hypoxic tumor cells (536).

LITERATURE CITED IN PART I.

1. **Aas, T., A. L. Borresen, S. Geisler, B. Smith-Sorensen, H. Johnsen, J. E. Varhaug, L. A. Akslen, and P. E. Lonning.** 1996. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nat. Med. **2:**811-4.
2. **Adam-Klages, S., D. Adam, K. Wiegmann, S. Struve, W. Kolanus, J. Schneider-Mergener, and M. Kronke.** 1996. FAN, a novel WD-repeat protein, couples the p55 TNF-receptor to neutral sphingomyelinase. Cell **86:**937-47.
3. **Adams, J. M., and S. Cory.** 1998. The Bcl-2 protein family: arbiters of cell survival. Science **281:**1322-6.
4. **Adida, C., D. Berrebi, M. Peuchmaur, M. Reyes-Mugica, and D. C. Altieri.** 1998. Anti-apoptosis gene, survivin, and prognosis of neuroblastoma. Lancet **351:**882-3.
5. **Adler, V., M. R. Pincus, T. Minamoto, S. Y. Fuchs, M. J. Bluth, P. W. Brandt-Rauf, F. K. Friedman, R. C. Robinson, J. M. Chen, X. W. Wang, C. C. Harris, and Z. Ronai.** 1997. Conformation-dependent phosphorylation of p53. Proc. Natl. Acad. Sci. U S A **94:**1686-91.
6. **Adler, V., A. Polotskaya, F. Wagner, and A. S. Kraft.** 1992. Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. J. Biol. Chem. **267:**17001-5.
7. **Afonso, C. L., J. G. Neilan, G. F. Kutish, and D. L. Rock.** 1996. An African swine fever virus Bcl-2 homolog, 5-HL, suppresses apoptotic cell death. J. Virol. **70:**4858-63.
8. **Agah, R., L. A. Kirshenbaum, M. Abdellatif, L. D. Truong, S. Chakraborty, L. H. Michael, and M. D. Schneider.** 1997. Adenoviral delivery of E2F-1 directs cell cycle reentry and p53- independent apoptosis in postmitotic adult myocardium in vivo. J. Clin. Invest. **100:**2722-8.
9. **Agarwal, M. L., A. Agarwal, W. R. Taylor, and G. R. Stark.** 1995. p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. Proc. Natl. Acad. Sci. U S A **92:**8493-7.
10. **Alnemri, E. S., D. J. Livingston, D. W. Nicholson, G. Salvesen, N. A. Thornberry, W. W. Wong, and J. Yuan.** 1996. Human ICE/CED-3 protease nomenclature. Cell **87:**171.
11. **Aloyz, R. S., S. X. Bamji, C. D. Pozniak, J. G. Toma, J. Atwal, D. R. Kaplan, and F. D. Miller.** 1998. P53 is essential for developmental neuron death as regulated by the TrkA and p75 neurotrophin receptors. J. Cell Biol. **143:**1691-703.
12. **Alvarez, E., I. C. Northwood, F. A. Gonzalez, D. A. Latour, A. Seth, C. Abate, T. Curran, and R. J. Davis.** 1991. Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c- myc and c-jun proteins by an epidermal growth factor receptor threonine 669 protein kinase. J. Biol. Chem. **266:**15277-85.
13. **Amati, B., K. Alevizopoulos, and J. Vlach.** 1998. Myc and the cell cycle. Front. Biosci. **3:**D250-68.
14. **Ambrosini, G., C. Adida, G. Sirugo, and D. C. Altieri.** 1998. Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. J. Biol. Chem. **273:**11177-82.
15. **Ameisen, J. C.** 1996. The origin of programmed cell death. Science **272:**1278-9.
16. **Ameyar, M., A. Atfi, Z. Cai, R. Stancou, V. Shatrov, A. Bettaieb, and S. Chouaib.** 1998. Analysis of human breast adenocarcinoma MCF7 resistance to tumor necrosis factor-induced cell death. Lack Of correlation between jnk activation and ceramide pathway [In Process Citation]. J. Biol. Chem. **273:**29002-8.
17. **Andersen, S. S., A. J. Ashford, R. Tournebize, O. Gavet, A. Sobel, A. A. Hyman, and E. Karsenti.** 1997. Mitotic chromatin regulates phosphorylation of Stathmin/Op18. Nature **389:**640-3.
18. **Antonsson, B., F. Conti, A. Ciavatta, S. Montessuit, S. Lewis, I. Martinou, L. Bernasconi, A. Bernard, J. J. Mermod, G. Mazzei, K. Manndrell, F. Gambale, R. Sadoul, and J. C. Martinou.** 1997. Inhibition of Bax channel-forming activity by Bcl-2. Science **277:**370-2.
19. **Antonyak, M. A., D. K. Moscatello, and A. J. Wong.** 1998. Constitutive activation of c-Jun N-terminal kinase by a mutant epidermal growth factor receptor. J. Biol. Chem. **273:**2817-22.
20. **Arends, M. J., and A. H. Wyllie.** 1991. Apoptosis: mechanisms and roles in pathology. Int. Rev. Exp. Pathol. **32:**223-254.
21. **Arias, J., A. S. Alberts, P. Brindle, F. X. Claret, T. Smeal, M. Karin, J. Feramisco, and M. Montminy.** 1994. Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature **370:**226-229.
22. **Armstrong, R. C., T. Aja, J. Xiang, S. Gaur, J. F. Krebs, K. Hoang, X. Bai, S. J. Korsmeyer, D. S. Karanewsky, L. C. Fritz, and K. J. Tomaselli.** 1996. Fas-induced activation of the cell death-related protease CPP32 Is inhibited by Bcl-2 and by ICE family protease inhibitors. J. Biol. Chem. **271:**16850-5.
23. **Ashkenazi, A., and V. M. Dixit.** 1998. Death receptors: signaling and modulation. Science **281:**1305-8.
24. **Attardi, L. D., S. W. Lowe, J. Brugarolas, and T. Jacks.** 1996. Transcriptional activation by p53, but not induction of the p21 gene, is essential for oncogene-mediated apoptosis. EMBO. J. **15:**3693-701.
25. **Aupeix, K., B. Hugel, T. Martin, P. Bischoff, H. Lill, J. L. Pasquali, and J. M. Freyssinet.** 1997. The significance of shed membrane particles during programmed cell death in vitro, and in vivo, in HIV-1 infection. J. Clin. Invest. **99:**1546-54.
26. **Bagrodia, S., B. Derijard, R. J. Davis, and R. A. Cerione.** 1995. Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. J. Biol. Chem. **270:**27995-8.
27. **Bai, R. L., G. R. Pettit, and E. Hamel.** 1990. Binding of dolastatin 10 to tubulin at a distinct site for peptide antimitotic agents near the exchangeable nucleotide and vinca alkaloid sites. J. Biol. Chem. **265:**17141-9.
28. **Bannister, A. J., T. Oehler, D. Wilhelm, P. Angel, and T. Kouzarides.** 1995. Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. Oncogene **11:**2509-14.
29. **Barboule, N., P. Chadebech, V. Baldin, S. Vidal, and A. Valette.** 1997. Involvement of p21 in mitotic exit after paclitaxel treatment in MCF-7 breast adenocarcinoma cell line. Oncogene **15:**2867-75.
30. **Barford, D.** 1996. Molecular mechanisms of the protein serine/threonine phosphatases. Trends Biochem. Sci. **21:**407-412.
31. **Barinaga, M.** 1998. Death by dozens of cuts. Science **280:**32-4.
32. **Barrington, R. E., M. A. Subler, E. Rands, C. A. Omer, P. J. Miller, J. E. Hundley, S. K. Koester, D. A. Troyer, D. J.**

Bearss, M. W. Conner, J. B. Gibbs, K. Hamilton, K. S. Koblan, S. D. Mosser, T. J. O'Neill, M. D. Schaber, E. T. Senderak, J. J. Windle, A. Oliff, and N. E. Kohl. 1998. A farnesyltransferase inhibitor induces tumor regression in transgenic mice harboring multiple oncogenic mutations by mediating alterations in both cell cycle control and apoptosis. Mol. Cell. Biol. **18**:85-92.

33. **Barry, M. A., C. A. Behnke, and A. Eastman.** 1990. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. Biochem. Pharmacol. **40**:2353-62.
34. **Barry, M. A., and A. Eastman.** 1993. Identification of deoxyribonuclease II as an endonuclease involved in apoptosis. Arch. Biochem. Biophys. **300**:440-50.
35. **Barton, N. R., and L. S. Goldstein.** 1996. Going mobile: microtubule motors and chromosome segregation. Proc. Natl. Acad. Sci. U S A **93**:1735-42.
36. **Bellacosa, A., J. R. Testa, S. P. Staal, and P. N. Tsichlis.** 1991. A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science **254**:274-7.
37. **Bellgrau, D., D. Gold, H. Selawry, J. Moore, A. Franzusoff, and R. C. Duke.** 1995. A role for CD95 ligand in preventing graft rejection. Nature **377**:630-2.
38. **Belmont, L. D., and T. J. Mitchison.** 1996. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell **84**:623-631.
39. **Ben-Levy, R., I. A. Leighton, Y. N. Doza, P. Attwood, N. Morrice, C. J. Marshall, and P. Cohen.** 1995. Identification of novel phosphorylation sites required for activation of MAPKAP kinase-2. EMBO. J. **14**:5920-30.
40. **Benoist, C., and D. Mathis.** 1997. Cell death mediators in autoimmune diabetes--no shortage of suspects. Cell **89**:1-3.
41. **Berchem, G. J., M. Bosseler, L. Y. Sugars, H. J. Voeller, S. Zeitlin, and E. P. Gelmann.** 1995. Androgens induce resistance to bcl-2-mediated apoptosis in LNCaP prostate cancer cells. Cancer Res. **55**:735-8.
42. **Berestetskaya, Y. V., M. P. Faure, H. Ichijo, and T. A. Voyno-Yasenetskaya.** 1998. Regulation of apoptosis by alpha-subunits of G12 and G13 proteins via apoptosis signal-regulating kinase-1. J. Biol. Chem. **273**:27816-23.
43. **Berra, E., M. T. Diaz-Meco, and J. Moscat.** 1998. The activation of p38 and apoptosis by the inhibition of Erk is antagonized by the phosphoinositide 3-Kinase/Akt pathway. J. Biol. Chem. **273**:10792-7.
44. **Bertin, J., R. C. Armstrong, S. Ottilie, D. A. Martin, Y. Wang, S. Banks, G. H. Wang, T. G. Senkevich, E. S. Alnemri, B. Moss, M. J. Lenardo, K. J. Tomaselli, and J. I. Cohen.** 1997. Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. Proc. Natl. Acad. Sci. U S A **94**:1172-6.
45. **Bhalla, K., Y. Huang, C. Tang, S. Self, S. Ray, M. E. Mahoney, V. Ponnathpur, E. Tourkina, A. M. Ibrado, G. Bullock, and et al.** 1994. Characterization of a human myeloid leukemia cell line highly resistant to taxol. Leukemia **8**:465-75.
46. **Bhalla, K., A. M. Ibrado, E. Tourkina, C. Tang, M. E. Mahoney, and Y. Huang.** 1993. Taxol induces internucleosomal DNA fragmentation associated with programmed cell death in human myeloid leukemia cells. Leukemia **7**:563-8.
47. **Birnbaum, M. J., R. J. Clem, and L. K. Miller.** 1994. An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J. Virol. **68**:2521-8.
48. **Bissery, M. C., D. Guenard, F. Gueritte-Voegelein, and F. Lavelle.** 1991. Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. Cancer Res. **51**:4845-52.
49. **Bissery, M. C., G. Nohynek, G. J. Sanderink, and F. Lavelle.** 1995. Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: Preclinical experience. Anticancer Drugs **6**:339-55, 363-8.
50. **Bissonnette, R. P., F. Echeverri, A. Mahboubi, and D. R. Green.** 1992. Apoptotic cell death induced by c-myc is inhibited by bcl-2. Nature **359**:552-4.
51. **Blagosklonny, M. V., P. Giannakakou, W. S. el-Deiry, D. G. Kingston, P. I. Higgs, L. Neckers, and T. Fojo.** 1997. Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res. **57**:130-5.
52. **Blagosklonny, M. V., T. Schulte, P. Nguyen, J. Trepel, and L. M. Neckers.** 1996. Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. Cancer Res. **56**:1851-4.
53. **Blank, J. L., P. Gerwins, E. M. Elliott, S. Sather, and G. L. Johnson.** 1996. Molecular cloning of mitogen-activated protein/ERK kinase kinases (MEKK) 2 and 3. Regulation of sequential phosphorylation pathways involving mitogen-activated protein kinase and c-Jun kinase. J. Biol. Chem. **271**:5361-8.
54. **Bocco, J. L., A. Bahr, J. Goetz, C. Hauss, T. Kallunki, C. Kedinger, and B. Chatton.** 1996. In vivo association of ATFa with JNK/SAP kinase activities. Oncogene **12**:1971-80.
55. **Boise, L. H., M. Gonzalez-Garcia, C. E. Postema, L. Ding, T. Lindsten, L. A. Turka, X. Mao, G. Nunez, and C. B. Thompson.** 1993. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell **74**:597-608.
56. **Bollag, D. M., P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, and C. M. Woods.** 1995. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. Cancer Res. **55**:2325-33.
57. **Born, T. L., J. A. Frost, A. Schonthal, G. C. Prendergast, and J. R. Feramisco.** 1994. c-Myc cooperates with activated Ras to induce the cdc2 promoter. Mol. Cell. Biol. **14**:5710-8.
58. **Borner, M. M., C. E. Myers, O. Sartor, Y. Sei, T. Toko, J. B. Trepel, and E. Schneider.** 1995. Drug-induced apoptosis is not necessarily dependent on macromolecular synthesis or proliferation in the p53-negative human prostate cancer cell line PC-3. Cancer Res. **55**:2122-8.
59. **Borsch-Haubold, A. G., F. Bartoli, J. Asselin, T. Dudler, R. M. Kramer, R. Apitz-Castro, S. P. Watson, and M. H. Gelb.** 1998. Identification of the phosphorylation sites of cytosolic phospholipase A2 in agonist-stimulated human platelets and HeLa cells. J. Biol. Chem. **273**:4449-58.
60. **Bortner, C. D., B. E. Nicklas, O. Cidlowski, and J. A. Cidlowski.** 1995. The role of DNA fragmentation in apoptosis. Trends Cell Biol. **5**:21-26.
61. **Bose, R., M. Verheij, A. Haimovitz-Friedman, K. Scotto, Z. Fuks, and R. Kolesnick.** 1995. Ceramide synthase mediates

daunorubicin-induced apoptosis: an alternative mechanism for generating death signals. Cell **82:**405-14.

62. **Bossy-Wetzel, E., D. D. Newmeyer, and D. R. Green.** 1998. Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD- specific caspase activation and independently of mitochondrial transmembrane depolarization. EMBO. J. **17:**37-49.
63. **Bost, F., R. McKay, N. Dean, and D. Mercola.** 1997. The Jun Kinase/stress-activated protein kinase pathway is required for epidermal growth factor stimulation of growth of human A549 lung carcinoma cells. J. Biol. Chem. **272:**33422-33429.
64. **Boudreau, N., C. J. Sympson, Z. Werb, and M. J. Bissell.** 1995. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science **267:**891-3.
65. **Bowen, C., S. Spiegel, and E. P. Gelmann.** 1998. Radiation-induced apoptosis mediated by retinoblastoma protein. Cancer Res **58:**3275-81.
66. **Brady, H. J., G. S. Salomons, R. C. Bobeldijk, and A. J. Berns.** 1996. T cells from baxalpha transgenic mice show accelerated apoptosis in response to stimuli but do not show restored DNA damage-induced cell death in the absence of p53. gene product in. EMBO. J. **15:**1221-30.
67. **Brash, D. E., J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin, and J. Ponten.** 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. U S A **88:**10124-8.
68. **Bratton, D. L., V. A. Fadok, D. A. Richter, J. M. Kailey, L. A. Guthrie, and P. M. Henson.** 1997. Appearance of phosphatidylserine on apoptotic cells requires calcium- mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. J. Biol. Chem. **272:**26159-65.
69. **Brenner, B., U. Koppenhoefer, C. Weinstock, O. Linderkamp, F. Lang, and E. Gulbins.** 1997. Fas- or ceramide-induced apoptosis is mediated by a Rac1-regulated activation of Jun N-terminal kinase/p38 kinases and GADD153. J. Biol. Chem. **272:**22173-81.
70. **Brenner, D. A.** 1998. Signal transduction during liver regeneration. J. Gastroenterol. Hepatol. **13 Suppl:**S93-5.
71. **Bruno, S., G. Del Bino, P. Lassota, W. Giaretti, and Z. Darzynkiewicz.** 1992. Inhibitors of proteases prevent endonucleolysis accompanying apoptotic death of HL-60 leukemic cells and normal thymocytes. Leukemia **6:**1113-20.
72. **Buckbinder, L., R. Talbott, S. Velasco-Miguel, I. Takenaka, B. Faha, B. R. Seizinger, and N. Kley.** 1995. Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature **377:**646-9.
73. **Bump, N. J., M. Hackett, M. Hugunin, S. Seshagiri, K. Brady, P. Chen, C. Ferenz, S. Franklin, T. Ghayur, P. Li, and et al.** 1995. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. Science **269:**1885-8.
74. **Butterfield, L., B. Storey, L. Maas, and L. E. Heasley.** 1997. c-Jun NH2-terminal kinase regulation of the apoptotic response of small cell lung cancer cells to ultraviolet radiation. J. Biol. Chem. **272:**10110-6.
75. **Caelles, C., A. Helmberg, and M. Karin.** 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. Nature **370:**220-3.
76. **Cahill, M. A., M. E. Peter, F. C. Kischkel, A. M. Chinnaiyan, V. M. Dixit, P. H. Krammer, and A. Nordheim.** 1996. CD95 (APO-1/Fas) induces activation of SAP kinases downstream of ICE- like proteases. Oncogene **13:**2087-96.
77. **Campos, L., O. Sabido, J. P. Rouault, and D. Guyotat.** 1994. Effects of BCL-2 antisense oligodeoxynucleotides on in vitro proliferation and survival of normal marrow progenitors and leukemic cells. Blood **84:**595-600.
78. **Canal, P.** 1998. Chapter 16. Platinum compounds: pharmacokinetics and pharmacodynamics, p. 345-373. *In* L. B. Grochow, and M. M. Ames (ed.), A clinician's guide to chemotherapy pharmacokinetics and pharmacodynamics. Williams & Wilkins, Baltimore.
79. **Canman, C. E., and M. B. Kastan.** 1996. Signal transduction. Three paths to stress relief. Nature **384:**213-4.
80. **Caplow, M., R. L. Ruhlen, and J. Shanks.** 1994. The free energy for hydrolysis of a microtubule-bound nucleotide triphosphate is near zero: all of the free energy for hydrolysis is stored in the microtubule lattice. J. Cell Biol. **127:**779-88.
81. **Caplow, M., and J. Shanks.** 1996. Evidence that a single monolayer tubulin-GTP cap is both necessary and sufficient to stabilize microtubules. Mol. Biol. Cell **7:**663-75.
82. **Caplow, M., and J. Shanks.** 1990. Mechanism for oscillatory assembly of microtubules. J. Biol. Chem. **265:**1414-8.
83. **Cardone, M. H., N. Roy, H. R. Stennicke, G. S. Salvesen, T. F. Franke, E. Stanbridge, S. Frisch, and J. C. Reed.** 1998. Regulation of cell death protease caspase-9 by phosphorylation. Science **282:**1318-21.
84. **Cardone, M. H., G. S. Salvesen, C. Widmann, G. Johnson, and S. M. Frisch.** 1997. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell **90:**315-23.
85. **Casaccia-Bonnefil, P., B. D. Carter, R. T. Dobrowsky, and M. V. Chao.** 1996. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. Nature **383:**716-9.
86. **Casciola-Rosen, L., A. Rosen, M. Petri, and M. Schlissel.** 1996. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus. Proc. Natl. Acad. Sci. U S A **93:**1624-9.
87. **Cassimeris, L., C. L. Rieder, and E. D. Salmon.** 1994. Microtubule assembly and kinetochore directional instability in vertebrate monopolar spindles: implications for the mechanism of chromosome congression. J. Cell Sci. **107:**285-97.
88. **Cassimeris, L. U., R. A. Walker, N. K. Pryer, and E. D. Salmon.** 1987. Dynamic instability of microtubules. Bioessays **7:**149-54.
89. **Castedo, M., T. Hirsch, S. A. Susin, N. Zamzami, P. Marchetti, A. Macho, and G. Kroemer.** 1996. Sequential acquisition of mitochondrial and plasma membrane alterations during early lymphocyte apoptosis. J. Immunol. **157:**512-21.
90. **Cavigelli, M., F. Dolfi, F. X. Claret, and M. Karin.** 1995. Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation. EMBO. J. **14:**5957-64.
91. **Chang, A. Y., K. Kim, J. Glick, T. Anderson, D. Karp, and D. Johnson.** 1993. Phase II study of taxol, merbarone, and piroxantrone in stage IV non- small-cell lung cancer: The Eastern Cooperative Oncology Group Results. J. Natl. Cancer Inst. **85:**388-94.
92. **Chang, B. S., A. J. Minn, S. W. Muchmore, S. W. Fesik, and C. B. Thompson.** 1997. Identification of a novel regulatory

domain in Bcl-X(L) and Bcl-2. EMBO. J. **16**:968-77.

93. **Chang, H. Y., H. Nishitoh, X. Yang, H. Ichijo, and D. Baltimore.** 1998. Activation of apoptosis signal- regulating kinase 1 (ASK1) by the adapter protein Daxx. Science **281**:1860-3.
94. **Chang, J. Y., D. P. Martin, and E. M. Johnson, Jr.** 1990. Interferon suppresses sympathetic neuronal cell death caused by nerve growth factor deprivation. J. Neurochem. **55**:436-45.
95. **Chang, Y. F., L. L. Li, C. W. Wu, T. Y. Liu, W. Y. Lui, P. e. FK, and C. W. Chi.** 1996. Paclitaxel-induced apoptosis in human gastric carcinoma cell lines. Cancer 77:14-8.
96. **Chao, D. T., and S. J. Korsmeyer.** 1998. BCL-2 family: regulators of cell death. Annu. Rev. Immunol. **16**:395-419.
97. **Chauhan, D., P. Pandey, A. Ogata, G. Teoh, S. Treon, M. Urashima, S. Kharbanda, and K. C. Anderson.** 1997. Dexamethasone induces apoptosis of multiple myeloma cells in a JNK/SAP kinase independent mechanism. Oncogene **15**:837-43.
98. **Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber.** 1997. Geometric control of cell life and death. Science **276**:1425-8.
99. **Chen, C. Y., and D. V. Faller.** 1996. Phosphorylation of Bcl-2 protein and association with p21Ras in Ras- induced apoptosis. J. Biol. Chem. **271**:2376-9.
100. **Chen, J., X. Wu, J. Lin, and A. J. Levine.** 1996. mdm-2 inhibits the G1 arrest and apoptosis functions of the p53 tumor suppressor protein. Mol. Cell. Biol. **16**:2445-52.
101. **Chen, R. H., C. Abate, and J. Blenis.** 1993. Phosphorylation of the c-Fos transrepression domain by mitogen- activated protein kinase and 90-kDa ribosomal S6 kinase. Proc. Natl. Acad. Sci. U S A **90**:10952-6.
102. **Chen, W. D., G. A. Otterson, S. Lipkowitz, S. N. Khleif, A. B. Coxon, and F. J. Kaye.** 1997. Apoptosis is associated with cleavage of a 5 kDa fragment from RB which mimics dephosphorylation and modulates E2F binding. Oncogene **14**:1243-8.
103. **Chen, Y. R., C. F. Meyer, and T. H. Tan.** 1996. Persistent activation of c-Jun N-terminal kinase 1 (JNK1) in gamma radiation-induced apoptosis. J. Biol. Chem. **271**:631-4.
104. **Chen, Y. R., W. Wang, A. N. Kong, and T. H. Tan.** 1998. Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. J. Biol. Chem. **273**:1769-75.
105. **Chen, Y. R., X. Wang, D. Templeton, R. J. Davis, and T. H. Tan.** 1996. The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation. J. Biol. Chem. **271**:31929-36.
106. **Cheng, E. H., D. G. Kirsch, R. J. Clem, R. Ravi, M. B. Kastan, A. Bedi, K. Ueno, and J. M. Hardwick.** 1997. Conversion of Bcl-2 to a Bax-like death effector by caspases. Science **278**:1966-8.
107. **Cherniack, A. D., J. K. Klarlund, and M. P. Czech.** 1994. Phosphorylation of the Ras nucleotide exchange factor son of sevenless by mitogen-activated protein kinase. J. Biol. Chem. **269**:4717-20.
108. **Chihab, R., C. Ferry, V. Koziel, P. Monin, and D. Jean-Luc.** 1998. Sequential activation of activator protein-1-related transcription factors and JNK protein kinases may contribute to apoptotic death induced by transient hypoxia in developing brain neurons. Brain Res. Mol. Brain Res. **63**:105-20.
109. **Chinnaiyan, A. M., O. R. K, M. Tewari, and V. M. Dixit.** 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell **81**:505-12.
110. **Chinnaiyan, A. M., K. Orth, O. R. K, H. Duan, G. G. Poirier, and V. M. Dixit.** 1996. Molecular ordering of the cell death pathway. Bcl-2 and Bcl-xL function upstream of the CED-3-like apoptotic proteases. J. Biol. Chem. **271**:4573-6.
111. **Chittenden, T., C. Flemington, A. B. Houghton, R. G. Ebb, G. J. Gallo, B. Elangovan, G. Chinnadurai, and R. J. Lutz.** 1995. A conserved domain in Bak, distinct from BH1 and BH2, mediates cell death and protein binding functions. EMBO. J. **14**:5589-96.
112. **Chou, S. Y., V. Baichwal, and J. E. Ferrell, Jr.** 1992. Inhibition of c-Jun DNA binding by mitogen-activated protein kinase. Mol. Biol. Cell **3**:1117-30.
113. **Choy, H., W. Akerley, H. Safran, J. Clark, V. Rege, A. Papa, M. Glantz, Y. Puthawala, C. Soderberg, and L. Leone.** 1994. Phase I trial of outpatient weekly paclitaxel and concurrent radiation therapy for advanced non-small-cell lung cancer. J. Clin. Oncol. **12**:2682-6.
114. **Choy, H., W. Akerley, H. Safran, S. Graziano, C. Chung, T. Williams, B. Cole, and T. Kennedy.** 1998. Multiinstitutional phase II trial of paclitaxel, carboplatin, and concurrent radiation therapy for locally advanced non-small-cell lung cancer. J. Clin. Oncol. **16**:3316-22.
115. **Choy, H., R. F. DeVore, 3rd, K. R. Hande, L. L. Porter, P. Rosenblatt, F. Yunus, L. Schlabach, C. Smith, Y. Shyr, K. LaPorte, and D. H. Johnson.** 1997. Preliminary analysis of a phase II study of paclitaxel, carboplatin, and hyperfractionated radiation therapy for locally advanced inoperable non-small cell lung cancer. Semin. Oncol. **24**:S12-21-S12-26.
116. **Choy, H., H. Safran, W. Akerley, S. L. Graziano, J. A. Bogart, and B. F. Cole.** 1998. Phase II trial of weekly paclitaxel and concurrent radiation therapy for locally advanced non-small cell lung cancer. Clin. Cancer Res. **4**:1931-6.
117. **Chuang, T. H., K. M. Hahn, J. D. Lee, D. E. Danley, and G. M. Bokoch.** 1997. The small GTPase Cdc42 initiates an apoptotic signaling pathway in Jurkat T lymphocytes. Mol. Biol. Cell **8**:1687-98.
118. **Cifone, M. G., P. Roncaioli, R. De Maria, G. Camarda, A. Santoni, G. Ruberti, and R. Testi.** 1995. Multiple pathways originate at the Fas/APO-1 (CD95) receptor: sequential involvement of phosphatidylcholine-specific phospholipase C and acidic sphingomyelinase in the propagation of the apoptotic signal. EMBO. J. **14**:5859-68.
119. **Claret, F. X., M. Hibi, S. Dhut, T. Toda, and M. Karin.** 1996. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature **383**:453-7.
120. **Clarke, A. R., E. R. Maandag, M. van Roon, N. M. van der Lugt, M. van der Valk, M. L. Hooper, A. Berns, and H. te Riele.** 1992. Requirement for a functional Rb-1 gene in murine development. Nature **359**:328-30.
121. **Clarke, A. R., C. A. Purdie, D. J. Harrison, R. G. Morris, C. C. Bird, M. L. Hooper, and A. H. Wyllie.** 1993. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature **362**:849-52.

122. **Clayman, G. L., A. K. el-Naggar, J. A. Roth, W. W. Zhang, H. Goepfert, D. L. Taylor, and T. J. Liu.** 1995. In vivo molecular therapy with p53 adenovirus for microscopic residual head and neck squamous carcinoma. Cancer Res. **55:**1-6.
123. **Clem, R. J., E. H. Cheng, C. L. Karp, D. G. Kirsch, K. Ueno, A. Takahashi, M. B. Kastan, D. E. Griffin, W. C. Earnshaw, M. A. Veliuona, and J. M. Hardwick.** 1998. Modulation of cell death by Bcl-XL through caspase interaction. Proc. Natl. Acad. Sci. U S A **95:**554-9.
124. **Clem, R. J., and C. S. Duckett.** 1997. The iap genes: unique arbitrators of cell death. Trends Cell Biol. **7:**337-339.
125. **Clem, R. J., and L. K. Miller.** 1993. Apoptosis reduces both the in vitro replication and the in vivo infectivity of a baculovirus. J. Virol. **67:**3730-8.
126. **Clouston, W. M., and J. F. Kerr.** 1985. Apoptosis, lymphocytotoxicity and the containment of viral infections. Med. Hypotheses **18:**399-404.
127. **Coffer, P. J., and J. R. Woodgett.** 1991. Molecular cloning and characterisation of a novel putative protein- serine kinase related to the cAMP-dependent and protein kinase C families. Eur. J. Biochem. **201:**475-81.
128. **Cohen, G. M.** 1997. Caspases: the executioners of apoptosis. Biochem. J. **326:**1-16.
129. **Cohen, G. M., X. M. Sun, R. T. Snowden, and e. al.** 1992. Key morphological features of apoptosis may occur in the absence of internucleosomal DNA fragmentation. Biochem. J. **286:**331-334.
130. **Cohen, J. J., and R. C. Duke.** 1984. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. J. Immunol. **132:**38-42.
131. **Cohen, J. J., R. C. Duke, V. A. Fadok, and K. S. Sellins.** 1992. Apoptosis and programmed cell death in immunity. Annu. Rev. Immunol. **10:**267-93.
132. **Cohen, P.** 1997. The search for physiological substrates of MAP and SAP kinases in mammalian cells. Trends Cell Biol. **7:**353-361.
133. **Compton, M. M.** 1992. A biochemical hallmark of apoptosis: internucleosomal degradation of the genome. Cancer Metastasis Rev. **11:**105-119.
134. **Corbalan-Garcia, S., S. S. Yang, K. R. Degenhardt, and D. Bar-Sagi.** 1996. Identification of the mitogen-activated protein kinase phosphorylation sites on human Sos1 that regulate interaction with Grb2. Mol. Cell. Biol. **16:**5674-82.
135. **Cornillon, S., C. Foa, J. Davoust, N. Buonavista, J. D. Gross, and P. Golstein.** 1994. Programmed cell death in Dictyostelium. J. Cell Sci. **107:**2691-704.
136. **Coso, O. A., M. Chiariello, J. C. Yu, H. Teramoto, P. Crespo, N. Xu, T. Miki, and J. S. Gutkind.** 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell **81:**1137-46.
137. **Craxton, A., G. Shu, J. D. Graves, J. Saklatvala, E. G. Krebs, and E. A. Clark.** 1998. p38 Mapk is required for Cd40-induced gene expression and proliferation in B lymphocytes. J Immunol **161:**3225-36.
138. **Crook, N. E., R. J. Clem, and L. K. Miller.** 1993. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. J. Virol. **67:**2168-74.
139. **Cross, S. M., C. A. Sanchez, C. A. Morgan, M. K. Schimke, S. Ramel, R. L. Idzerda, W. H. Raskind, and B. J. Reid.** 1995. A p53-dependent mouse spindle checkpoint. Science **267:**1353-6.
140. **Datta, S. R., H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, and M. E. Greenberg.** 1997. Akt phosphorylation of BAD couples survival signals to the cell- intrinsic death machinery. Cell **91:**231-41.
141. **David-Pfeuty, T., H. P. Erickson, and D. Pantaloni.** 1977. Guanosinetriphosphatase activity of tubulin associated with microtubule assembly. Proc. Natl. Acad. Sci. U S A **74:**5372-6.
142. **Davis, A. C., M. Wims, G. D. Spotts, S. R. Hann, and A. Bradley.** 1993. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. Genes Dev. **7:**671-82.
143. **Davis, R. J.** 1993. The mitogen-activated protein kinase signal transduction pathway. J. Biol. Chem. **268:**14553-6.
144. **Dbaibo, G. S., M. Y. Pushkareva, S. Jayadev, J. K. Schwarz, J. M. Horowitz, L. M. Obeid, and Y. A. Hannun.** 1995. Retinoblastoma gene product as a downstream target for a ceramide- dependent pathway of growth arrest. Proc. Natl. Acad. Sci. U S A **92:**1347-51.
145. **De Maria, R., L. Lenti, F. Malisan, F. d'Agostino, B. Tomassini, A. Zeuner, M. R. Rippo, and R. Testi.** 1997. Requirement for GD3 ganglioside in CD95- and ceramide-induced apoptosis. Science **277:**1652-5.
146. **de Souza, P. L., M. Castillo, and C. E. Myers.** 1997. Enhancement of paclitaxel activity against hormone-refractory prostate cancer cells in vitro and in vivo by quinacrine. Br. J. Cancer **75:**1593-600.
147. **Deak, J. C., J. V. Cross, M. Lewis, Y. Qian, L. A. Parrott, C. W. Distelhorst, and D. J. Templeton.** 1998. Fas-induced proteolytic activation and intracellular redistribution of the stress-signaling kinase MEKK1. Proc. Natl. Acad. Sci. U S A **95:**5595-600.
148. **Debbas, M., and E. White.** 1993. Wild-type p53 mediates apoptosis by E1A, which is inhibited by E1B. Genes Dev. **7:**546-54.
149. **Debernardis, D., E. G. Sire, P. De Feudis, F. Vikhanskaya, M. Valenti, P. Russo, S. Parodi, D. I. M, and M. Broggini.** 1997. p53 status does not affect sensitivity of human ovarian cancer cell lines to paclitaxel. Cancer Res. **57:**870-4.
150. **Degli-Esposti, M. A., W. C. Dougall, P. J. Smolak, J. Y. Waugh, C. A. Smith, and R. G. Goodwin.** 1997. The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. Immunity **7:**813-20.
151. **DeGregori, J., G. Leone, A. Miron, L. Jakoi, and J. R. Nevins.** 1997. Distinct roles for E2F proteins in cell growth control and apoptosis. Proc. Natl. Acad. Sci. U S A **94:**7245-50.
152. **del Peso, L., M. Gonzalez-Garcia, C. Page, R. Herrera, and G. Nunez.** 1997. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science **278:**687-9.
153. **Deng, C., P. Zhang, J. W. Harper, S. J. Elledge, and P. Leder.** 1995. Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. Cell **82:**675-84.
154. **Deng, T., and M. Karin.** 1993. JunB differs from c-Jun in its DNA-binding and dimerization domains, and represses c-Jun by formation of inactive heterodimers. Genes Dev. **7:**479-90.

155. **Denu, J. M., J. A. Stuckey, M. A. Saper, and J. E. Dixon.** 1996. Form and function in protein dephosphorylation. Cell **87**:361-364.
156. **Derijard, B., M. Hibi, I.-H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R. J. Davis.** 1994. JNK1: a protein kinase situmulated by UV light and Ha-ras that binds and phosphorylates the c-Jun activation domain. Cell **76**:1025-1037.
157. **Derijard, B., J. Raingeaud, T. Barrett, I. H. Wu, J. Han, R. J. Ulevitch, and R. J. Davis.** 1995. Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. Science **267**:682-5.
158. **Desai, A., H. W. Deacon, C. E. Walczak, and T. J. Mitchison.** 1997. A method that allows the assembly of kinetochore components onto chromosomes condensed in clarified Xenopus egg extracts. Proc. Natl. Acad. Sci. U S A **94**:12378-83.
159. **Desai, A., and T. J. Mitchison.** 1997. Microtubule polymerization dynamics. Annu. Rev.Cell. Dev. Biol. **13**:83-117.
160. **Desai, A., and T. J. Mitchison.** 1995. A new role for motor proteins as couplers to depolymerizing microtubules. J. Cell Biol. **128**:1-4.
161. **Devary, Y., R. A. Gottlieb, T. Smeal, and M. Karin.** 1992. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell **71**:1081-91.
162. **Deveraux, Q. L., N. Roy, H. R. Stennicke, T. Van Arsdale, Q. Zhou, S. M. Srinivasula, E. S. Alnemri, G. S. Salvesen, and J. C. Reed.** 1998. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. EMBO. J. **17**:2215-23.
163. **Dhamodharan, R., M. A. Jordan, D. Thrower, L. Wilson, and P. Wadsworth.** 1995. Vinblastine suppresses dynamics of individual microtubules in living interphase cells. Mol. Biol. Cell **6**:1215-29.
164. **Diaz, J. L., T. Oltersdorf, W. Horne, M. McConnell, G. Wilson, S. Weeks, T. Garcia, and L. C. Fritz.** 1997. A common binding site mediates heterodimerization and homodimerization of Bcl-2 family members. J. Biol. Chem. **272**:11350-5.
165. **Dickens, M., J. S. Rogers, J. Cavanagh, A. Raitano, Z. Xia, J. R. Halpern, M. E. Greenberg, C. L. Sawyers, and R. J. Davis.** 1997. A cytoplasmic inhibitor of the JNK signal transduction pathway. Science **277**:693-6.
166. **Dickman, S.** 1997. First p53 relative may be a new tumor suppressor. Science **277**:1605-6.
167. **Donaldson, K. L., G. L. Goolsby, P. A. Kiener, and A. F. Wahl.** 1994. Activation of p34cdc2 coincident with taxol-induced apoptosis. Cell Growth Differ. **5**:1041-50.
168. **Dorow, D. S., L. Devereux, E. Dietzsch, and T. De Kretser.** 1993. Identification of a new family of human epithelial protein kinases containing two leucine/isoleucine-zipper domains. Eur. J. Biochem. **213**:701-10.
169. **Dorsey, M. J., H. J. Tae, K. G. Sollenberger, N. T. Mascarenhas, L. M. Johansen, and E. J. Taparowsky.** 1995. B-ATF: a novel human bZIP protein that associates with members of the AP-1 transcription factor family. Oncogene **11**:2255-65.
170. **Dou, Q. P., and B. An.** 1998. RB and apoptotic cell death. Front. Biosci. **3**:d419-30.
171. **Dou, Q. P., B. An, and P. L. Will.** 1995. Induction of a retinoblastoma phosphatase activity by anticancer drugs accompanies p53-independent G1 arrest and apoptosis. Proc. Natl. Acad. Sci. U S A **92**:9019-23.
172. **Downing, K. H., and E. Nogales.** 1998. Tubulin and microtubule structure. Curr. Opin. Cell Biol. **10**:16-22.
173. **Drappa, J., A. K. Vaishnaw, K. E. Sullivan, J. L. Chu, and K. B. Elkon.** 1996. Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. N. Engl. J. Med. **335**:1643-9.
174. **Drazan, K. E., X. D. Shen, M. E. Csete, W. W. Zhang, J. A. Roth, R. W. Busuttil, and A. Shaked.** 1994. In vivo adenoviral-mediated human p53 tumor suppressor gene transfer and expression in rat liver after resection. Surgery **116**:197-203.
175. **Drechsel, D. N., and M. W. Kirschner.** 1994. The minimum GTP cap required to stabilize microtubules. Curr. Biol. **4**:1053-61.
176. **Dudek, H., S. R. Datta, T. F. Franke, M. J. Birnbaum, R. Yao, G. M. Cooper, R. A. Segal, D. R. Kaplan, and M. E. Greenberg.** 1997. Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science **275**:661-5.
177. **Durstin, M., S. Durstin, T. F. Molski, E. L. Becker, and R. I. Sha'afi.** 1994. Cytoplasmic phospholipase A2 translocates to membrane fraction in human neutrophils activated by stimuli that phosphorylate mitogen-activated protein kinase. Proc. Natl. Acad. Sci. U S A **91**:3142-6.
178. **Duvall, E., A. H. Wyllie, and R. G. Morris.** 1985. Macrophage recognition of cells undergoing programmed cell death (apoptosis). Immunology **56**:351-8.
179. **el-Deiry, W. S., T. Tokino, V. E. Velculescu, D. B. Levy, R. Parsons, J. M. Trent, D. Lin, W. E. Mercer, K. W. Kinzler, and B. Vogelstein.** 1993. WAF1, a potential mediator of p53 tumor suppression. Cell **75**:817-25.
180. **Elion, E. A.** 1998. Routing MAP kinase cascades. Science **281**:1625-6.
181. **Ellerby, H. M., S. J. Martin, L. M. Ellerby, S. S. Naiem, S. Rabizadeh, G. S. Salvesen, C. A. Casiano, N. R. Cashman, D. R. Green, and D. E. Bredesen.** 1997. Establishment of a cell-free system of neuronal apoptosis: comparison of premitochondrial, mitochondrial, and postmitochondrial phases. J. Neurosci. **17**:6165-78.
182. **Ellis, R. E., J. Y. Yuan, and H. R. Horvitz.** 1991. Mechanisms and functions of cell death. Annu. Rev. Cell Biol. **7**:663-98.
183. **Emoto, Y., Y. Manome, G. Meinhardt, H. Kisaki, S. Kharbanda, M. Robertson, T. Ghayur, W. W. Wong, R. Kamen, R. Weichselbaum, and et al.** 1995. Proteolytic activation of protein kinase C delta by an ICE-like protease in apoptotic cells. EMBO. J. **14**:6148-56.
184. **Enari, M., A. Hase, and S. Nagata.** 1995. Apoptosis by a cytosolic extract from Fas-activated cells. EMBO. J. **14**:5201-8.
185. **Enari, M., H. Sakahira, H. Yokoyama, K. Okawa, A. Iwamatsu, and S. Nagata.** 1998. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. Nature **391**:43-50.
186. **Engel, K., A. Ahlers, M. A. Brach, F. Herrmann, and M. Gaestel.** 1995. MAPKAP kinase 2 is activated by heat shock and TNF-alpha: in vivo phosphorylation of small heat shock protein results from stimulation of the MAP kinase cascade. J. Cell. Biochem. **57**:321-30.
187. **Engel, K., H. Schultz, F. Martin, A. Kotlyarov, K. Plath, M. Hahn, U. Heinemann, and M. Gaestel.** 1995. Constitutive activation of mitogen-activated protein kinase-activated protein kinase 2 by mutation of phosphorylation sites and an A-helix motif. J. Biol. Chem. **270**:27213-21.
188. **Erhardt, P., K. J. Tomaselli, and G. M. Cooper.** 1997. Identification of the MDM2 oncoprotein as a substrate for CPP32-like

apoptotic proteases. J. Biol. Chem. **272:**15049-52.

189. **Erickson, H. P., and O. B. ET.** 1992. Microtubule dynamic instability and GTP hydrolysis. Annu. Rev. Biophys. Biomol. Struct. **21:**145-66.
190. **Erlich, E., A. R. McCall, R. K. Potkul, S. Walter, and A. Vaughan.** 1996. Paclitaxel is only a weak radiosensitizer of human cervical carcinoma cell lines. Gynecol. Oncol. **60:**251-4.
191. **Evan, G. I., A. H. Wyllie, C. S. Gilbert, T. D. Littlewood, H. Land, M. Brooks, C. M. Waters, L. Z. Penn, and D. C. Hancock.** 1992. Induction of apoptosis in fibroblasts by c-myc protein. Cell **69:**119-28.
192. **Faber, L. P.** 1991. Chapter 14. Lung cancer, p. 194-212. *In* A. I. Holleb, D. J. Fink, and G. P. Murphy (ed.), American Cancer Society textbook of clinical oncology. American Cancer Society, Atlanta.
193. **Fadok, V. A., D. R. Voelker, P. A. Campbell, J. J. Cohen, D. L. Bratton, and P. M. Henson.** 1992. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J. Immunol. **148:**2207-16.
194. **Fallo, F., C. Pilon, L. Barzon, M. Pistorello, U. Pagotto, G. Altavilla, M. Boscaro, and N. Sonino.** 1996. Effects of taxol on the human NCI-H295 adrenocortical carcinoma cell line. Endocr. Res. **22:**709-15.
195. **Fallon, J. F., and B. K. Simandl.** 1978. Evidence of a role for cell death in the disappearance of the embryonic human tail. Am. J. Anat. **152:**111-29.
196. **Fan, G., X. Ma, B. T. Kren, and C. J. Steer.** 1996. The retinoblastoma gene product inhibits TGF-beta1 induced apoptosis in primary rat hepatocytes and human HuH-7 hepatoma cells. Oncogene **12:**1909-19.
197. **Fan, S., B. Cherney, W. Reinhold, K. Rucker, and O. C. PM.** 1998. Disruption of p53 function in immortalized human cells does not affect survival or apoptosis after taxol or vincristine treatment. Clin. Cancer Res. **4:**1047-54.
198. **Faris, M., N. Kokot, K. Latinis, S. Kasibhatla, D. R. Green, G. A. Koretzky, and A. Nel.** 1998. The c-Jun N-terminal kinase cascade plays a role in stress-induced apoptosis in Jurkat cells by up-regulating Fas ligand expression. J. Immunol. **160:**134-44.
199. **Faris, M., K. M. Latinis, S. J. Kempiak, G. A. Koretzky, and A. Nel.** 1998. Stress-induced Fas ligand expression in T cells is mediated through a MEK kinase 1-regulated response element in the Fas ligand promoter. Mol Cell Biol **18:**5414-24.
200. **Fattman, C. L., B. An, and Q. P. Dou.** 1997. Characterization of interior cleavage of retinoblastoma protein in apoptosis. J. Cell. Biochem. **67:**399-408.
201. **Fauman, E. B., and M. A. Saper.** 1996. Structure and function of the protein tyrosine phosphatases. Trends Biochem. Sci. **21:**413-417.
202. **Ferlini, C., G. Scambia, M. Distefano, P. Filippini, G. Isola, A. Riva, E. Bombardelli, A. Fattorossi, P. Benedetti Panici, and S. Mancuso.** 1997. Synergistic antiproliferative activity of tamoxifen and docetaxel on three oestrogen receptor-negative cancer cell lines is mediated by the induction of apoptosis. Br. J. Cancer **75:**884-91.
203. **Ferrer, I., E. Pozas, J. Ballabriga, and A. M. Planas.** 1997. Strong c-Jun/AP-1 immunoreactivity is restricted to apoptotic cells following intracerebral ibotenic acid injection in developing rats. Neurosci. Res. **28:**21-31.
204. **Fett-Neto, A. G., F. DiCosmo, W. F. Reynolds, and K. Sakata.** 1992. Cell culture of Taxus as a source of the antineoplastic drug taxol and related taxanes. Biotechnology (N Y) **10:**1572-5.
205. **Field, S. J., F. Y. Tsai, F. Kuo, A. M. Zubiaga, W. G. Kaelin, Jr., D. M. Livingston, S. H. Orkin, and M. E. Greenberg.** 1996. E2F-1 functions in mice to promote apoptosis and suppress proliferation. Cell **85:**549-61.
206. **Fisher, D. E.** 1994. Apoptosis in cancer therapy: crossing the threshold. Cell **78:**539-42.
207. **Fisher, T. L., and J. Blenis.** 1996. Evidence for two catalytically active kinase domains in pp90rsk. Mol. Cell. Biol. **16:**1212-9.
208. **Foletta, V. C., D. H. Segal, and D. R. Cohen.** 1998. Transcriptional regulation in the immune system: all roads lead to AP-1. J. Leukocyte Biol. .
209. **Forastiere, A. A., D. Shank, D. Neuberg, S. G. t. Taylor, R. C. DeConti, and G. Adams.** 1998. Final report of a phase II evaluation of paclitaxel in patients with advanced squamous cell carcinoma of the head and neck: an Eastern Cooperative Oncology Group trial (PA390). Cancer **82:**2270-4.
210. **Franke, T. F., and L. C. Cantley.** 1997. Apoptosis. A Bad kinase makes good. Nature **390:**116-7.
211. **Franke, T. F., D. R. Kaplan, and L. C. Cantley.** 1997. PI3K: downstream AKTion blocks apoptosis. Cell **88:**435-7.
212. **Franke, T. F., D. R. Kaplan, L. C. Cantley, and A. Toker.** 1997. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science **275:**665-8.
213. **Franke, T. F., S. I. Yang, T. O. Chan, K. Datta, A. Kazlauskas, D. K. Morrison, D. R. Kaplan, and P. N. Tsichlis.** 1995. The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell **81:**727-36.
214. **Frasch, S. C., J. A. Nick, V. A. Fadok, D. L. Bratton, G. S. Worthen, and P. M. Henson.** 1998. p38 mitogen-activated protein kinase-dependent and -independent intracellular signal transduction pathways leading to apoptosis in human neutrophils. J. Biol. Chem. **273:**8389-97.
215. **Frazier, W. A.** 1987. Thrombospondin: a modular adhesive glycoprotein of platelets and nucleated cells. J. Cell Biol. **105:**625-32.
216. **Friedlander, P., Y. Haupt, C. Prives, and M. Oren.** 1996. A mutant p53 that discriminates between p53-responsive genes cannot induce apoptosis. Mol. Cell. Biol. **16:**4961-71.
217. **Frisch, S. M., K. Vuori, D. Kelaita, and S. Sicks.** 1996. A role for Jun-N-terminal kinase in anoikis; suppression by bcl-2 and crmA. J. Cell. Biol. **135:**1377-82.
218. **Frost, J. A., S. Xu, M. R. Hutchison, S. Marcus, and M. H. Cobb.** 1996. Actions of Rho family small G proteins and p21-activated protein kinases on mitogen-activated protein kinase family members. Mol. Cell. Biol. **16:**3707-13.
219. **Fuchs, S. Y., V. Adler, T. Buschmann, Z. Yin, X. Wu, S. N. Jones, and Z. Ronai.** 1998. JNK targets p53 ubiquitination and degradation in nonstressed cells. Genes Dev. **12:**2658-63.

220. **Fuchs, S. Y., V. Adler, M. R. Pincus, and Z. Ronai.** 1998. MEKK1/JNK signaling stabilizes and activates p53. Proc. Natl. Acad. Sci. U S A **95**:10541-6.
221. **Fuchs, S. Y., L. Dolan, R. J. Davis, and Z. Ronai.** 1996. Phosphorylation-dependent targeting of c-Jun ubiquitination by Jun N-kinase. Oncogene **13**:1531-5.
222. **Fuchs, S. Y., V. A. Fried, and Z. Ronai.** 1998. Stress-activated kinases regulate protein stability. Oncogene **17**:1483-90.
223. **Fuchs, S. Y., B. Xie, V. Adler, V. A. Fried, R. J. Davis, and Z. Ronai.** 1997. c-Jun NH2-terminal kinases target the ubiquitination of their associated transcription factors. J. Biol. Chem. **272**:32163-8.
224. **Fujiwara, T., D. W. Cai, R. N. Georges, T. Mukhopadhyay, E. A. Grimm, and J. A. Roth.** 1994. Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. J. Natl. Cancer Inst. **86**:1458-62.
225. **Fukasawa, K., T. Choi, R. Kuriyama, S. Rulong, and G. F. Vande Woude.** 1996. Abnormal centrosome amplification in the absence of p53. Science **271**:1744-7.
226. **Furukawa, Y., S. Iwase, Y. Terui, J. Kikuchi, T. Sakai, M. Nakamura, S. Kitagawa, and M. Kitagawa.** 1996. Transcriptional activation of the cdc2 gene is associated with Fas- induced apoptosis of human hematopoietic cells. J. Biol. Chem. **271**:28469-77.
227. **Gagliardini, V., P. A. Fernandez, R. K. Lee, H. C. Drexler, R. J. Rotello, M. C. Fishman, and J. Yuan.** 1994. Prevention of vertebrate neuronal death by the crmA gene. Science **263**:826-8.
228. **Gahmberg, C. G., and M. Tolvanen.** 1996. Why mammalian cell surface proteins are glycoproteins. Trends Biochem. Sci. **21**:308-11.
229. **Gajate, C., A. Santos-Beneit, M. Modolell, and F. Mollinedo.** 1998. Involvement of c-Jun NH2-terminal kinase activation and c-Jun in the induction of apoptosis by the ether phospholipid 1-O-octadecyl-2-O- methyl-rac-glycero-3-phosphocholine. Mol. Pharmacol. **53**:602-12.
230. **Galaktionov, K., X. Chen, and D. Beach.** 1996. Cdc25 cell-cycle phosphatase as a target of c-myc. Nature **382**:511-7.
231. **Gallo, K. A., M. R. Mark, D. T. Scadden, Z. Wang, Q. Gu, and P. J. Godowski.** 1994. Identification and characterization of SPRK, a novel src-homology 3 domain-containing proline-rich kinase with serine/threonine kinase activity. J. Biol. Chem. **269**:15092-100.
232. **Gan, Y., M. G. Wientjes, D. E. Schuller, and J. L. Au.** 1996. Pharmacodynamics of taxol in human head and neck tumors. Cancer Res. **56**:2086-93.
233. **Gangemi, R. M., M. Tiso, C. Marchetti, A. B. Severi, and M. Fabbi.** 1995. Taxol cytotoxicity on human leukemia cell lines is a function of their susceptibility to programmed cell death. Cancer Chemother. Pharmacol. **36**:385-92.
234. **Garcia, P., D. Braguer, G. Carles, and C. Briand.** 1995. Simultaneous combination of microtubule depolymerizing and stabilizing agents acts at low doses. Anticancer Drugs **6**:533-44.
235. **Garcia-Martinez, V., D. Macias, Y. Ganan, J. M. Garcia-Lobo, M. V. Francia, M. A. Fernandez-Teran, and J. M. Hurle.** 1993. Internucleosomal DNA fragmentation and programmed cell death (apoptosis) in the interdigital tissue of the embryonic chick leg bud. J. Cell Sci. **106**:201-8.
236. **Garkavtsev, I., I. A. Grigorian, V. S. Ossovskaya, M. V. Chernov, P. M. Chumakov, and A. V. Gudkov.** 1998. The candidate tumour suppressor p33ING1 cooperates with p53 in cell growth control. Nature **391**:295-8.
237. **Garkavtsev, I., A. Kazarov, A. Gudkov, and K. Riabowol.** 1996. Suppression of the novel growth inhibitor p33ING1 promotes neoplastic transformation. Nat. Genet. **14**:415-20.
238. **Garkavtsev, I., and K. Riabowol.** 1997. Extension of the replicative life span of human diploid fibroblasts by inhibition of the p33ING1 candidate tumor suppressor. Mol. Cell. Biol. **17**:2014-9.
239. **Gartner, A., and M. O. Hengartner.** 1998. Genetic approaches to programmed cell death in *C. elegans*, p. 131-146. *In* R. A. Lockshin, Z. Zakeri, and J. L. Tilly (ed.), When cells die. A comprehensive evaluation of apoptosis and programmed cell death. Wiley-Liss, New York.
240. **Gavrieli, Y., Y. Sherman, and S. A. Ben-Sasson.** 1992. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J. Cell Biol. **119**:493-501.
241. **Gazitt, Y., M. L. Rothenberg, S. G. Hilsenbeck, V. Fey, C. Thomas, and W. Montegomrey.** 1998. Bcl-2 overexpression is associated with resistance to paclitaxel, but not gemcitabine, in multiple myeloma cells. Int. J. Oncol. **13**:839-48.
242. **Geard, C. R., J. M. Jones, and P. B. Schiff.** 1993. Taxol and radiation. J. Natl. Cancer Inst. Monogr. :89-94.
243. **Gebel, H. M., D. P. Braun, A. Tambur, D. Frame, N. Rana, and W. P. Dmowski.** 1998. Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. Fertil. Steril. **69**:1042-7.
244. **Gelfand, V. I., and A. D. Bershadsky.** 1991. Microtubule dynamics: mechanism, regulation, and function. Annu. Rev. Cell Biol. **7**:93-116.
245. **Geng, L., and C. S. Potten.** 1990. Changes after irradiation in the number of mitotic cells and apoptotic fragments in growing mouse hair follicles and in the width of their hairs. Radiat. Res. **123**:75-81.
246. **Gerwins, P., J. L. Blank, and G. L. Johnson.** 1997. Cloning of a novel mitogen-activated protein kinase kinase kinase, MEKK4, that selectively regulates the c-Jun amino terminal kinase pathway. J. Biol. Chem. **272**:8288-95.
247. **Giannakakou, P., D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, T. Fojo, and M. S. Poruchynsky.** 1997. Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J. Biol. Chem. **272**:17118-17125.
248. **Gianni, L., E. Munzone, G. Capri, F. Fulfaro, E. Tarenzi, F. Villani, C. Spreafico, A. Laffranchi, A. Caraceni, C. Martini, and et al.** 1995. Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. J. Clin. Oncol. **13**:2688-99.
249. **Gibb, R. K., D. D. Taylor, T. Wan, O. C. DM, D. L. Doering, and C. Gercel-Taylor.** 1997. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. Gynecol. Oncol. **65**:13-22.
250. **Gille, H., T. Strahl, and P. E. Shaw.** 1995. Activation of ternary complex factor Elk-1 by stress-activated protein kinases. Curr.

Biol. 5:1191-200.
251. **Goedert, M., A. Cuenda, M. Craxton, R. Jakes, and P. Cohen.** 1997. Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. EMBO. J. **16**:3563-71.
252. **Goldstein, D. P.** 1991. Gestational trophoblastic neoplasia in the 1990s. Yale J. Biol. Med. **64**:639-51.
253. **Gomez-Munoz, A., D. W. Waggoner, O. B. L, and D. N. Brindley.** 1995. Interaction of ceramides, sphingosine, and sphingosine 1-phosphate in regulating DNA synthesis and phospholipase D activity. J. Biol. Chem. **270**:26318-25.
254. **Gottesman, M. M.** 1994. Report of a meeting: molecular basis of cancer therapy. J. Natl. Cancer Inst. **86**:1277-85.
255. **Goyette, M. C., K. Cho, C. L. Fasching, D. B. Levy, K. W. Kinzler, C. Paraskeva, B. Vogelstein, and E. J. Stanbridge.** 1992. Progression of colorectal cancer is associated with multiple tumor suppressor gene defects but inhibition of tumorigenicity is accomplished by correction of any single defect via chromosome transfer. Mol. Cell. Biol. **12**:1387-95.
256. **Graham, R., and M. Gilman.** 1991. Distinct protein targets for signals acting at the c-fos serum response element. Science **251**:189-92.
257. **Grandgirard, D., E. Studer, L. Monney, T. Belser, I. Fellay, C. Borner, and M. R. Michel.** 1998. Alphaviruses induce apoptosis in Bcl-2-overexpressing cells: evidence for a caspase-mediated, proteolytic inactivation of Bcl-2. EMBO. J. **17**:1268-78.
258. **Graves, J. D., K. E. Draves, A. Craxton, J. Saklatvala, E. G. Krebs, and E. A. Clark.** 1996. Involvement of stress-activated protein kinase and p38 mitogen-activated protein kinase in mIgM-induced apoptosis of human B lymphocytes. Proc. Natl. Acad. Sci.U S A **93**:13814-8.
259. **Green, D., and G. Kroemer.** 1998. The central executioners of apoptosis: caspases or mitochondria? Trends Cell Biol. **8**:267-71.
260. **Green, D. R.** 1997. A Myc-induced apoptosis pathway surfaces. Science **278**:1246-7.
261. **Green, D. R., and T. G. Cotter.** 1993. Macromolecular synthesis, c-myc, and apoptosis, p. 153-166. *In* M. Lavin, and D. Watters (ed.), Programmed cell death- the cellular and molecular biology of apoptosis. Harwood academic publishers GmbH, Chur, Switzerland.
262. **Green, D. R., A. Mahboubi, W. Nishioka, S. Oja, F. Echeverri, Y. Shi, J. Glynn, Y. Yang, J. Ashwell, and R. Bissonnette.** 1994. Promotion and inhibition of activation-induced apoptosis in T-cell hybridomas by oncogenes and related signals. Immunol. Rev. **142**:321-42.
263. **Green, D. R., and C. F. Ware.** 1997. Fas-ligand: privilege and peril. Proc. Natl. Acad. Sci. USA. **94**:5986-5990.
264. **Gregory, C. D., C. Dive, S. Henderson, C. A. Smith, G. T. Williams, J. Gordon, and A. B. Rickinson.** 1991. Activation of Epstein-Barr virus latent genes protects human B cells from death by apoptosis. Nature **349**:612-4.
265. **Griffith, T. S., T. Brunner, S. M. Fletcher, D. R. Green, and T. A. Ferguson.** 1995. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science **270**:1189-92.
266. **Grove, J. R., D. J. Price, P. Banerjee, A. Balasubramanyam, M. F. Ahmad, and J. Avruch.** 1993. Regulation of an epitope-tagged recombinant Rsk-1 S6 kinase by phorbol ester and erk/MAP kinase. Biochemistry **32**:7727-38.
267. **Gu, Y., C. W. Turck, and D. O. Morgan.** 1993. Inhibition of CDK2 activity in vivo by an associated 20K regulatory subunit. Nature **366**:707-10.
268. **Gulbins, E., R. Bissonnette, A. Mahboubi, S. Martin, W. Nishioka, T. Brunner, G. Baier, G. Baier-Bitterlich, C. Byrd, F. Lang, and et al.** 1995. FAS-induced apoptosis is mediated via a ceramide-initiated RAS signaling pathway. Immunity **2**:341-51.
269. **Guo, Y. L., K. Baysal, B. Kang, L. J. Yang, and J. R. Williamson.** 1998. Correlation between sustained c-Jun N-terminal protein kinase activation and apoptosis induced by tumor necrosis factor-alpha in rat mesangial cells. J. Biol. Chem. **273**:4027-34.
270. **Guo, Y. L., B. Kang, and J. R. Williamson.** 1998. Inhibition of the expression of mitogen-activated protein phosphatase-1 potentiates apoptosis induced by tumor necrosis factor-alpha in rat mesangial cells. J. Biol. Chem. **273**:10362-6.
271. **Gupta, S., T. Barrett, A. J. Whitmarsh, J. Cavanagh, H. K. Sluss, B. Derijard, and R. J. Davis.** 1996. Selective interaction of JNK protein kinase isoforms with transcription factors. EMBO. J. **15**:2760-70.
272. **Gupta, S., D. Campbell, B. Derijard, and R. J. Davis.** 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science **267**:389-93.
273. **Gupta, S., and R. J. Davis.** 1994. MAP kinase binds to the NH2-terminal activation domain of c-Myc. FEBS Lett. **353**:281-5.
274. **Gura, T.** 1997. How TRAIL kills cancer cells, but not normal cells. Science **277**:768.
275. **Gusberg, S. B., and C. D. Runowicz.** 1991. Chapter 33. Gynecologic cancers, p. 481-497. *In* A. I. Holleb, D. J. Fink, and G. P. Mruphy (ed.), American Cancer Society textbook of clinical oncology. American Cancer Society, Atlanta.
276. **Hai, T., and T. Curran.** 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. U S A **88**:3720-4.
277. **Haimovitz-Friedman, A., C. C. Kan, D. Ehleiter, R. S. Persaud, M. McLoughlin, Z. Fuks, and R. N. Kolesnick.** 1994. Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. J. Exp. Med. **180**:525-35.
278. **Haldar, S., A. Basu, and C. M. Croce.** 1997. Bcl2 is the guardian of microtubule integrity. Cancer Res. **57**:229-33.
279. **Haldar, S., A. Basu, and C. M. Croce.** 1998. Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. Cancer Res. **58**:1609-15.
280. **Haldar, S., J. Chintapalli, and C. M. Croce.** 1996. Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res. **56**:1253-5.
281. **Haldar, S., N. Jena, and C. M. Croce.** 1995. Inactivation of Bcl-2 by phosphorylation. Proc. Natl. Acad. Sci. USA **92**:4507-11.
282. **Haldar, S., M. Negrini, M. Monne, S. Sabbioni, and C. M. Croce.** 1994. Down-regulation of bcl-2 by p53 in breast cancer cells. Cancer Res. **54**:2095-7.

283. **Hamburger, V., and R. Levi-Montalcini.** 1949. Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions. J. Exp. Zool. **111:**457-502.
284. **Hamel, E.** 1992. Natural products which interact with tubulin in the vinca domain: maytansine, rhizoxin, phomopsin A, dolastatins 10 and 15 and halichondrin B. Pharmacol. Ther. **55:**31-51.
285. **Han, J., Y. Jiang, Z. Li, V. V. Kravchenko, and R. J. Ulevitch.** 1997. Activation of the transcription factor MEF2C by the MAP kinase p38 in inflammation. Nature **386:**296-9.
286. **Han, J., J. D. Lee, L. Bibbs, and R. J. Ulevitch.** 1994. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science **265:**808-11.
287. **Hannun, Y. A.** 1996. Functions of ceramide in coordinating cellular responses to stress. Science **274:**1855-9.
288. **Hansen, R. S., and A. W. Braithwaite.** 1996. The growth-inhibitory function of p53 is separable from transactivation, apoptosis and suppression of transformation by E1a and Ras. Oncogene **13:**995-1007.
289. **Hardie, G., and S. Hanks (ed.).** 1995. The protein kinase Facts Book. Academic Press, Harcourt Brace & Company, London.
290. **Harper, J. W., G. R. Adami, N. Wei, K. Keyomarsi, and S. J. Elledge.** 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin- dependent kinases. Cell **75:**805-16.
291. **Harris, C. C.** 1993. p53: at the crossroads of molecular carcinogenesis and risk assessment. Science **262:**1980-1.
292. **Hartfield, P. J., A. J. Bilney, and A. W. Murray.** 1998. Neurotrophic factors prevent ceramide-induced apoptosis downstream of c- Jun N-terminal kinase activation in PC12 cells. J. Neurochem. **71:**161-9.
293. **Harvey, K. J., J. F. Blomquist, and D. S. Ucker.** 1998. Commitment and effector phases of the physiological cell death pathway elucidated with respect to Bcl-2 caspase, and cyclin-dependent kinase activities. Mol. Cell. Biol. **18:**2912-22.
294. **Hasegawa, H., E. Kiyokawa, S. Tanaka, K. Nagashima, N. Gotoh, M. Shibuya, T. Kurata, and M. Matsuda.** 1996. DOCK180, a major CRK-binding protein, alters cell morphology upon translocation to the cell membrane. Mol. Cell. Biol. **16:**1770-6.
295. **Haupt, Y., R. Maya, A. Kazaz, and M. Oren.** 1997. Mdm2 promotes the rapid degradation of p53. Nature **387:**296-9.
296. **Hanpt, Y., S. Rowan, E. Shaulian, K. H. Vousden, and M. Oren.** 1995. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. Genes Dev. **9:**2170-83.
297. **Havrilesky, L. J., A. Elbendary, J. A. Hurteau, R. S. Whitaker, G. C. Rodriguez, and A. Berchuck.** 1995. Chemotherapy-induced apoptosis in epithelial ovarian cancers. Obstet. Gynecol. **85:**1007-10.
298. **Hayden, J. H., S. S. Bowser, and C. L. Rieder.** 1990. Kinetochores capture astral microtubules during chromosome attachment to the mitotic spindle: direct visualization in live newt lung cells. J. Cell Biol. **111:**1039-45.
299. **Haystead, T. A., P. Dent, J. Wu, C. M. Haystead, and T. W. Sturgill.** 1992. Ordered phosphorylation of p42mapk by MAP kinase kinase. FEBS Lett **306:**17-22.
300. **Heald, R., M. McLoughlin, and F. McKeon.** 1993. Human wee1 maintains mitotic timing by protecting the nucleus from cytoplasmically activated Cdc2 kinase. Cell **74:**463-74.
301. **Healy, J. I., and C. C. Goodnow.** 1998. Positive versus negative signaling by lymphocyte antigen receptors. Annu. Rev. Immunol. **16:**645-70.
302. **Heimbrook, D. C., and A. Oliff.** 1998. Therapeutic intervention and signaling. Curr. Opin. Cell. Biol. **10:**284-8.
303. **Helin, K., C. L. Wu, A. R. Fattaey, J. A. Lees, B. D. Dynlacht, C. Ngwu, and E. Harlow.** 1993. Heterodimerization of the transcription factors E2F-1 and DP-1 leads to cooperative trans-activation. Genes Dev. **7:**1850-61.
304. **Hellemans, P., P. A. van Dam, J. Weyler, A. T. van Oosterom, P. Buytaert, and E. Van Marck.** 1995. Prognostic value of bcl-2 expression in invasive breast cancer. Br. J. Cancer **72:**354-60.
305. **Hemmings, B. A.** 1997. Akt signaling: linking membrane events to life and death decisions. Science **275:**628-30.
306. **Hemmings, B. A.** 1997. PtdIns(3,4,5)P3 gets its message across. Science **277:**534.
307. **Henderson, S., D. Huen, M. Rowe, C. Dawson, G. Johnson, and A. Rickinson.** 1993. Epstein-Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2, protects human B cells from programmed cell death. Proc. Natl. Acad. Sci. U S A **90:**8479-83.
308. **Hengartner, M.** 1998. Apoptosis. Death by crowd control. Science **281:**1298-9.
309. **Hengartner, M. O.** 1997. Apoptosis. CED-4 is a stranger no more. Nature **388:**714-5.
310. **Hengartner, M. O.** 1998. Death cycle and Swiss army knives. Nature **391:**441-442.
311. **Herdegen, T., F. X. Claret, T. Kallunki, A. Martin-Villalba, C. Winter, T. Hunter, and M. Karin.** 1998. Lasting N-terminal phosphorylation of c-Jun and activation of c-Jun N- terminal kinases after neuronal injury. J Neurosci **18:**5124-35.
312. **Hermeking, H., C. Lengauer, K. Polyak, T. C. He, L. Zhang, S. Thiagalingam, K. W. Kinzler, and B. Vogelstein.** 1997. 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. Mol. Cell **1:**3-11.
313. **Herrmann, J. L., F. Briones, Jr., S. Brisbay, C. J. Logothetis, and T. J. McDonnell.** 1998. Prostate carcinoma cell death resulting from inhibition of proteasome activity is independent of functional Bcl-2 and p53. Oncogene **17:**2889-99.
314. **Hesketh, R.** 1997. The oncogene and tumour suppressor gene, 2nd ed. Academic Press, Harcourt Brace & Company, San Diego.
315. **Hetts, S. W.** 1998. To die or not to die: an overview of apoptosis and its role in diseases. J. Am. Med. Assoc. **279:**300-307.
316. **Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin.** 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. **7:**2135-48.
317. **Hickman, J. A.** 1992. Apoptosis induced by anticancer drugs. Cancer Metastasis Rev. **11:**121-39.
318. **Hickman, J. A., and C. C. Boyle.** 1997. Apoptosis and cytotoxins. Br. Med. Bull. **53:**632-43.
319. **Hiebert, S. W., G. Packham, D. K. Strom, R. Haffner, M. Oren, G. Zambetti, and J. L. Cleveland.** 1995. E2F-1:DP-1 induces p53 and overrides survival factors to trigger apoptosis. Mol. Cell. Biol. **15:**6864-74.
320. **Hirai, S., M. Izawa, S. Osada, G. Spyrou, and S. Ohno.** 1996. Activation of the JNK pathway by distantly related protein kinases, MEKK and MUK. Oncogene **12:**641-50.
321. **Hirai, S., M. Katoh, M. Terada, J. M. Kyriakis, L. I. Zon, A. Rana, J. Avruch, and S. Ohno.** 1997. MST/MLK2, a

member of the mixed lineage kinase family, directly phosphorylates and activates SEK1, an activator of c-Jun N-terminal kinase/stress-activated protein kinase. J. Biol. Chem. **272:**15167-73.

322. **Hirata, Y., K. Adachi, and K. Kiuchi.** 1998. Activation of JNK pathway and induction of apoptosis by manganese in PC12 cells. J Neurochem **71:**1607-15.
323. **Hirsch, T., P. Marchetti, S. A. Susin, B. Dallaporta, N. Zamzami, I. Marzo, M. Geuskens, and G. Kroemer.** 1997. The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. Oncogene **15:**1573-81.
324. **Hockenbery, D.** 1995. Defining apoptosis. Am. J. Pathol. **146:**16-9.
325. **Holland, P. M., M. Suzanne, J. S. Campbell, S. Noselli, and J. A. Cooper.** 1997. MKK7 is A stress-activated mitogen-activated protein kinase kinase functionally related to hemipterous. J. Biol. Chem. **272:**24994-8.
326. **Hollstein, M., D. Sidransky, B. Vogelstein, and C. C. Harris.** 1991. p53 mutations in human cancers. Science **253:**49-53.
327. **Holmberg, C., K. Helin, M. Sehested, and O. Karlstrom.** 1998. E2F-1-induced p53-independent apoptosis in transgenic mice. Oncogene **17:**143-55.
328. **Holmes, F. A., R. S. Walters, R. L. Theriault, A. D. Forman, L. K. Newton, M. N. Raber, A. U. Buzdar, D. K. Frye, and G. N. Hortobagyi.** 1991. Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. J. Natl. Cancer Inst. **83:**1797-805.
329. **Holy, T. E., and S. Leibler.** 1994. Dynamic instability of microtubules as an efficient way to search in space. Proc. Natl. Acad. Sci. U S A **91:**5682-5.
330. **Holzman, L. B., S. E. Merritt, and G. Fan.** 1994. Identification, molecular cloning, and characterization of dual leucine zipper bearing kinase. A novel serine/threonine protein kinase that defines a second subfamily of mixed lineage kinases. J. Biol. Chem. **269:**30808-17.
331. **Honda, R., H. Tanaka, and H. Yasuda.** 1997. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. FEBS. Lett. **420:**25-7.
332. **Hopwood, D., and D. A. Levison.** 1976. Atrophy and apoptosis in the cyclical human endometrium. J. Pathol. **119:**159-66.
333. **Horio, T., and H. Hotani.** 1986. Visualization of the dynamic instability of individual microtubules by dark-field microscopy. Nature **321:**605-7.
334. **Horiuchi, M., W. Hayashida, T. Kambe, T. Yamada, and V. J. Dzau.** 1997. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. J. Biol. Chem. **272:**19022-6.
335. **Horstmann, S., P. J. Kahle, and G. D. Borasio.** 1998. Inhibitors of p38 mitogen-activated protein kinase promote neuronal survival in vitro. J Neurosci Res **52:**483-90.
336. **Hoshi, M., E. Nishida, and H. Sakai.** 1988. Activation of a Ca2+-inhibitable protein kinase that phosphorylates microtubule-associated protein 2 in vitro by growth factors, phorbol esters, and serum in quiescent cultured human fibroblasts. J. Biol. Chem. **263:**5396-401.
337. **Hotani, H., and T. Horio.** 1988. Dynamics of microtubules visualized by darkfield microscopy: treadmilling and dynamic instability. Cell Motil. Cytoskeleton **10:**229-36.
338. **Hotz, M. A., F. Traganos, and Z. Darzynkiewicz.** 1992. Changes in nuclear chromatin related to apoptosis or necrosis induced by the DNA topoisomerase II inhibitor fostriecin in MOLT-4 and HL-60 cells are revealed by altered DNA sensitivity to denaturation. Exp. Cell Res. **201:**184-91.
339. **Hsieh, J. K., S. Fredersdorf, T. Kouzarides, K. Martin, and X. Lu.** 1997. E2F1-induced apoptosis requires DNA binding but not transactivation and is inhibited by the retinoblastoma protein through direct interaction. Genes Dev. **11:**1840-52.
340. **Hsu, J. C., T. Laz, K. L. Mohn, and R. Taub.** 1991. Identification of LRF-1, a leucine-zipper protein that is rapidly and highly induced in regenerating liver. Proc. Natl. Acad. Sci. U S A **88:**3511-5.
341. **Hu, M. C., W. R. Qiu, X. Wang, C. F. Meyer, and T. H. Tan.** 1996. Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. Genes Dev. **10:**2251-64.
342. **Hu, S., C. Vincenz, J. Ni, R. Gentz, and V. M. Dixit.** 1997. I-FLICE, a novel inhibitor of tumor necrosis factor receptor-1- and CD- 95-induced apoptosis. J. Biol. Chem. **272:**17255-7.
343. **Hu, Y., M. A. Benedict, D. Wu, N. Inohara, and G. Nunez.** 1998. Bcl-XL interacts with Apaf-1 and inhibits Apaf-1-dependent caspase-9 activation. Proc. Natl. Acad. Sci. U S A **95:**4386-91.
344. **Huang, C. Y., and J. E. Ferrell, Jr.** 1996. Ultrasensitivity in the mitogen-activated protein kinase cascade. Proc. Natl. Acad. Sci. U S A **93:**10078-83.
345. **Huang, T. S., C. H. Shu, W. K. Yang, and J. Whang-Peng.** 1997. Activation of CDC 25 phosphatase and CDC 2 kinase involved in GL331- induced apoptosis. Cancer Res. **57:**2974-8.
346. **Huang, Y., A. M. Ibrado, J. C. Reed, G. Bullock, S. Ray, C. Tang, and K. Bhalla.** 1997. Co-expression of several molecular mechanisms of multidrug resistance and their significance for paclitaxel cytotoxicity in human AML HL-60 cells. Leukemia **11:**253-7.
347. **Huang, Y., S. Ray, J. C. Reed, A. M. Ibrado, C. Tang, A. Nawabi, and K. Bhalla.** 1997. Estrogen increases intracellular p26Bcl-2 to p21Bax ratios and inhibits taxol-induced apoptosis of human breast cancer MCF-7 cells. Breast Cancer Res. Treat. **42:**73-81.
348. **Hueber, A. O., M. Zornig, D. Lyon, T. Suda, S. Nagata, and G. I. Evan.** 1997. Requirement for the CD95 receptor-ligand pathway in c-Myc-induced apoptosis. Science **278:**1305-9.
349. **Hundley, J. E., S. K. Koester, D. A. Troyer, S. G. Hilsenbeck, R. E. Barrington, and J. J. Windle.** 1997. Differential regulation of cell cycle characteristics and apoptosis in MMTV-myc and MMTV-ras mouse mammary tumors. Cancer Res. **57:**600-3.
350. **Hunter, J. J., and T. G. Parslow.** 1996. A peptide sequence from Bax that converts Bcl-2 into an activator of apoptosis. J. Biol. Chem. **271:**8521-4.
351. **Hunter, T.** 1995. Protein kinases and phosphatases: the Yin and Yang of protein phosphorylation and signaling. Cell **80:**225-

236.
352. **Huot, J., F. Houle, S. Rousseau, R. G. Deschesnes, G. M. Shah, and J. Landry.** 1998. SAPK2/p38-dependent F-actin reorganization regulates early membrane blebbing during stress-induced apoptosis [In Process Citation]. J Cell Biol **143:**1361-73.
353. **Hyman, A. A., and E. Karsenti.** 1996. Morphogenetic properties of microtubules and mitotic spindle assembly. Cell **84:**401-10.
354. **Ibrado, A. M., C. N. Kim, and K. Bhalla.** 1998. Temporal relationship of CDK1 activation and mitotic arrest to cytosolic accumulation of cytochrome C and caspase-3 activity during Taxol-induced apoptosis of human AML HL-60 cells. Leukemia **12:**1930-6.
355. **Ibrado, A. M., L. Liu, and K. Bhalla.** 1997. Bcl-xL overexpression inhibits progression of molecular events leading to paclitaxel-induced apoptosis of human acute myeloid leukemia HL-60 cells. Cancer Res. **57:**1109-15.
356. **Ichijo, H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, and Y. Gotoh.** 1997. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science **275:**90-4.
357. **Ijiri, K.** 1989. Apoptosis (cell death) induced in mouse bowel by 1,2-dimethylhydrazine, methylazoxymethanol acetate, and gamma-rays. Cancer Res. **49:**6342-6.
358. **Ip, Y. T., and R. J. Davis.** 1998. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. Curr. Opin. Cell Biol. **10:**205-19.
359. **Irmler, M., M. Thome, M. Hahne, P. Schneider, K. Hofmann, V. Steiner, J. L. Bodmer, M. Schroter, K. Burns, C. Mattmann, D. Rimoldi, L. E. French, and J. Tschopp.** 1997. Inhibition of death receptor signals by cellular FLIP. Nature **388:**190-5.
360. **Ito, T., X. Deng, B. Carr, and W. S. May.** 1997. Bcl-2 phosphorylation required for anti-apoptosis function. J. Biol. Chem. **272:**11671-3.
361. **Itoh, G., J. Tamura, M. Suzuki, Y. Suzuki, H. Ikeda, M. Koike, M. Nomura, T. Jie, and K. Ito.** 1995. DNA fragmentation of human infarcted myocardial cells demonstrated by the nick end labeling method and DNA agarose gel electrophoresis. Am. J. Pathol. **146:**1325-31.
362. **Itoh, N., S. Yonehara, A. Ishii, M. Yonehara, S. Mizushima, M. Sameshima, A. Hase, Y. Seto, and S. Nagata.** 1991. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell **66:**233-43.
363. **Jacks, T., A. Fazeli, E. M. Schmitt, R. T. Bronson, M. A. Goodell, and R. A. Weinberg.** 1992. Effects of an Rb mutation in the mouse. Nature **359:**295-300.
364. **Jacobson, M. D., J. F. Burne, and M. C. Raff.** 1994. Programmed cell death and Bcl-2 protection in the absence of a nucleus. EMBO. J. **13:**1899-1910.
365. **Jacobson, M. D., M. Weil, and M. C. Raff.** 1997. Programmed cell death in animal development. Cell **88:**347-54.
366. **Jaffrezou, J. P., C. Dumontet, W. B. Derry, G. Duran, G. Chen, E. Tsuchiya, L. Wilsou, M. A. Jordan, and B. I. Sikic.** 1995. Novel mechanism of resistance to paclitaxel (Taxol) in human K562 leukemia cells by combined selection with PSC 833. Oncol. Res. **7:**517-27.
367. **Janicke, R. U., M. L. Sprengart, M. R. Wati, and A. G. Porter.** 1998. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J. Biol. Chem. **273:**9357-60.
368. **Janicke, R. U., P. A. Walker, X. Y. Lin, and A. G. Porter.** 1996. Specific cleavage of the retinoblastoma protein by an ICE-like protease in apoptosis. EMBO. J. **15:**6969-78.
369. **Janknecht, R., W. H. Ernst, V. Pingoud, and A. Nordheim.** 1993. Activation of ternary complex factor Elk-1 by MAP kinases. EMBO. J. **12:**5097-104.
370. **Jansen-Durr, P., A. Meichle, P. Steiner, M. Pagano, K. Finke, J. Botz, J. Wessbecher, G. Draetta, and M. Eilers.** 1993. Differential modulation of cyclin gene expression by MYC. Proc. Natl. Acad. Sci. U S A **90:**3685-9.
371. **Jayadev, S., B. Liu, A. E. Bielawska, J. Y. Lee, F. Nazaire, M. Pushkareva, L. M. Obeid, and Y. A. Hannun.** 1995. Role for ceramide in cell cycle arrest. J. Biol. Chem. **270:**2047-52.
372. **Jiang, Y., C. Chen, Z. Li, W. Guo, J. A. Gegner, S. Lin, and J. Han.** 1996. Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta). J. Biol. Chem. **271:**17920-6.
373. **Jiang, Y., J. D. Woronicz, W. Liu, and D. V. Goeddel.** 1999. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. Science **283:**543-6.
374. **Joensuu, H., L. Pylkkanen, and S. Toikkanen.** 1994. Bcl-2 protein expression and long-term survival in breast cancer. Am. J. Pathol. **145:**1191-8.
375. **Johnson, D. G., and R. Schneider-Broussard.** 1998. Role of E2F in cell cycle control and cancer. Front. Biosci. **3:**d447-8.
376. **Jones, N. A., J. Turner, A. J. McIlwrath, R. Brown, and C. Dive.** 1998. Cisplatin- and paclitaxel-induced apoptosis of ovarian carcinoma cells and the relationship between bax and bak up-regulation and the functional status of p53. Mol Pharmacol **53:**819-26.
377. **Jones, P. F., T. Jakubowicz, F. J. Pitossi, F. Maurer, and B. A. Hemmings.** 1991. Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proc. Natl. Acad. Sci. U S A **88:**4171-5.
378. **Jordan, M. A., R. J. Toso, D. Thrower, and L. Wilson.** 1993. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc. Natl. Acad. Sci. U S A **90:**9552-6.
379. **Jordan, M. A., K. Wendell, S. Gardiner, W. B. Derry, H. Copp, and L. Wilson.** 1996. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res. **56:**816-25.
380. **Jordan, M. A., and L. Wilson.** 1998. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr. Opin. Cell Biol. **10:**123-30.
381. **Jost, C. A., M. C. Marin, and W. G. Kaelin, Jr.** 1997. p73 is a human p53-related protein that can induce apoptosis. Nature **389:**191-4.

56:1303-8.
448. **Lee, S. Y., A. Reichlin, A. Santana, K. A. Sokol, M. C. Nussenzweig, and Y. Choi.** 1997. TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. Immunity 7:703-13.
449. **Lee, W., P. Mitchell, and R. Tjian.** 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. Cell 49:741-52.
450. **Lei, W., R. Yu, S. Mandlekar, and A. N. Kong.** 1998. Induction of apoptosis and activation of interleukin 1beta-converting enzyme/Ced-3 protease (caspase-3) and c-Jun NH2-terminal kinase 1 by benzo(a)pyrene. Cancer Res 58:2102-6.
451. **Leist, M., B. Single, A. F. Castoldi, S. Kuhnle, and P. Nicotera.** 1997. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J. Exp. Med. 185:1481-6.
452. **Lenczowski, J. M., L. Dominguez, A. M. Eder, L. B. King, C. M. Zacharchuk, and J. D. Ashwell.** 1997. Lack of a role for Jun kinase and AP-1 in Fas-induced apoptosis. Mol. Cell. Biol. 17:170-81.
453. **Le-Niculescu, H., E. Bonfoco, Y. Kasuya, F. X. Claret, D. R. Green, and M. Karin.** 1999. Withdrawal of Survival Factors Results in Activation of the JNK Pathway in Neuronal Cells Leading to Fas Ligand Induction and Cell Death. Mol. Cell. Biol. 19:751-763.
454. **Leveque, D., F. Jehl, and H. Monteil.** 1998. Chapter 22. Vinblastine, vincristine, and vinorelbine, p. 459-470. *In* L. B. Grochow, and M. M. Ames (ed.), A clinician's guide to chemotherapy pharmacokinetics and pharmacodynamics. Williams & Wilkins, Baltimore.
455. **Levine, A. J.** 1997. p53, the cellular gatekeeper for growth and division. Cell 88:323-31.
456. **Levine, A. J., M. E. Perry, A. Chang, A. Silver, D. Dittmer, M. Wu, and D. Welsh.** 1994. The 1993 Walter Hubert Lecture: the role of the p53 tumour-suppressor gene in tumorigenesis. Br. J. Cancer 69:409-16.
457. **Levitzki, A.** 1996. Targeting signal transduction for disease therapy. Curr. Opin. Cell Biol. 8:239-44.
458. **Levkau, B., H. Koyama, E. W. Raines, B. E. Clurman, B. Herren, K. Orth, J. M. Roberts, and R. Ross.** 1998. Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Mol. Cell 1:553-63.
459. **Lewis, T. S., P. S. Shapiro, and N. G. Ahn.** 1998. Signal Transduction through MAP kinase cascades. Adv. Cancer Res. 74:47-139.
460. **Li, P., D. Nijhawan, I. Budihardjo, S. M. Srinivasula, M. Ahmad, E. S. Alnemri, and X. Wang.** 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479-89.
461. **Li, W. G., A. Zaheer, L. Coppey, and H. J. Oskarsson.** 1998. Activation of JNK in the remote myocardium after large myocardial infarction in rats. Biochem Biophys Res Commun 246:816-20.
462. **Li, X., J. Gong, E. Feldman, K. Seiter, F. Traganos, and Z. Darzynkiewicz.** 1994. Apoptotic cell death during treatment of leukemias. Leukemia & Lymphoma 13:65-70.
463. **Li, Y., and R. Benezra.** 1996. Identification of a human mitotic checkpoint gene: hsMAD2. Science 274:246-8.
464. **Li, Y., C. Gorbea, D. Mahaffey, M. Rechsteiner, and R. Benezra.** 1997. MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. Proc. Natl. Acad. Sci. U S A 94:12431-6.
465. **Lieu, C. H., Y. N. Chang, and Y. K. Lai.** 1997. Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. Biochem. Pharmacol. 53:1587-96.
466. **Lieu, C. H., C. C. Liu, T. H. Yu, K. D. Chen, Y. N. Chang, and Y. K. Lai.** 1998. Role of mitogen-activated protein kinase in taxol-induced apoptosis in human leukemic U937 cells. Cell Growth Differ. 9:767-76.
467. **Lin, A., A. Minden, H. Martinetto, F. X. Claret, C. Lange-Carter, F. Mercurio, G. L. Johnson, and M. Karin.** 1995. Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. Science 268:286-90.
468. **Lin, H. L., Y. F. Chang, T. Y. Liu, C. W. Wu, and C. W. Chi.** 1998. Submicromolar paclitaxel induces apoptosis in human gastric cancer cells at early G1 phase. Anticancer Res. 18:3443-9.
469. **Lin, L. L., M. Wartmann, A. Y. Lin, J. L. Knopf, A. Seth, and R. J. Davis.** 1993. cPla2 is phosphorylated and activated by Map kinase. Cell 72:269-78.
470. **Ling, Y. H., U. Consoli, C. Tornos, M. Andreeff, and R. Perez-Soler.** 1998. Accumulation of cyclin B1, activation of cyclin B1-dependent kinase and induction of programmed cell death in human epidermoid carcinoma KB cells treated with taxol. Int. J. Cancer 75:925-32.
471. **Ling, Y. H., C. Tornos, and R. Perez-Soler.** 1998. Phosphorylation of bcl-2 is a marker of M phase events and not a determinant of apoptosis. J. Biol. Chem. 273:18984-91.
472. **Ling, Y. H., Y. Yang, C. Tornos, B. Singh, and R. Perez-Soler.** 1998. Paclitaxel-induced apoptosis is associated with expression and activation of c-Mos gene product in human ovarian carcinoma SKOV3 cells. Cancer Res 58:3633-40.
473. **Lipponen, P., T. Pietilainen, V. M. Kosma, S. Aaltomaa, M. Eskelinen, and K. Syrjanen.** 1995. Apoptosis suppressing protein bcl-2 is expressed in well-differentiated breast carcinomas with favourable prognosis. J. Pathol. 177:49-55.
474. **Liston, P., N. Roy, K. Tamai, C. Lefebvre, S. Baird, G. Cherton-Horvat, R. Farahani, M. McLean, J. E. Ikeda, A. MacKenzie, and R. G. Korneluk.** 1996. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 379:349-53.
475. **Liu, J. R., B. Fletcher, C. Page, C. Hu, G. Nunez, and V. Baker.** 1998. Bcl-xL is expressed in ovarian carcinoma and modulates chemotherapy- induced apoptosis. Gynecol. Oncol. 70:398-403.
476. **Liu, P., and R. G. Anderson.** 1995. Compartmentalized production of ceramide at the cell surface. J. Biol. Chem. 270:27179-85.
477. **Liu, Q. Y., and C. A. Stein.** 1997. Taxol and estramustine-induced modulation of human prostate cancer cell apoptosis via alteration in bcl-xL and bak expression. Clin. Cancer Res. 3:2039-2046.
478. **Liu, X., C. N. Kim, J. Yang, R. Jemmerson, and X. Wang.** 1996. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 86:147-157.
479. **Liu, X., P. Li, P. Widlak, H. Zou, X. Luo, W. T. Garrard, and X. Wang.** 1998. The 40-kDa subunit of DNA fragmentation

511. **Martins, L. M., T. Kottke, P. W. Mesner, G. S. Basi, S. Sinha, N. Frigon, Jr., E. Tatar, J. S. Tung, K. Bryant, A. Takahashi, P. A. Svingen, B. J. Madden, D. J. McCormick, W. C. Earnshaw, and S. H. Kaufmann.** 1997. Activation of multiple interleukin-1beta converting enzyme homologues in cytosol and nuclei of HL-60 cells during etoposide-induced apoptosis. J. Biol. Chem. **272:**7421-30.

512. **Marzo, I., C. Brenner, N. Zamzami, J. M. Jurgensmeier, S. A. Susin, H. L. Vieira, M. C. Prevost, Z. Xie, S. Matsuyama, J. C. Reed, and G. Kroemer.** 1998. Bax and adenine nucleotide translocator cooperate in the mitochondrial control of apoptosis. Science **281:**2027-31.

513. **Marzo, I., C. Brenner, N. Zamzami, S. A. Susin, G. Beutner, D. Brdiczka, R. Remy, Z. H. Xie, J. C. Reed, and G. Kroemer.** 1998. The permeability transition pore complex: a target for apoptosis regulation by caspases and bcl-2-related proteins. J. Exp. Med. **187:**1261-71.

514. **Masson, D., and T. E. Kreis.** 1995. Binding of E-MAP-115 to microtubules is regulated by cell cycle-dependent phosphorylation. J. Cell Biol. **131:**1015-24.

515. **Matsui, M., M. Tokuhara, Y. Konuma, N. Nomura, and R. Ishizaki.** 1990. Isolation of human fos-related genes and their expression during monocyte-macrophage differentiation. Oncogene **5:**249-55.

516. **Maundrell, K., B. Antonsson, E. Magnenat, M. Camps, M. Muda, C. Chabert, C. Gillieron, U. Boschert, E. Vial-Knecht, J.-C. Martinou, and S. Arkinstall.** 1997. Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J. Biol. Chem. **272:**25238-25242.

517. **May, G. H. W., K. E. Allen, W. Clark, M. Funk, and D. A. F. Gillespie.** 1998. Analysis of the interaction between c-Jun and c-Jun N-terminal kinase in vivo. J. Biol. Chem. **273:**33429-35.

518. **McConkey, D. J., M. Aguilar-Santelises, P. Hartzell, L Eriksson, H. Mellstedt, S. Orrenius, and M. Jondal.** 1991. Induction of DNA fragmentation in chronic B-lymphocytic leukemia cells. J. Immunol. **146:**1072-6.

519. **McCormack, J. E., V. H. Pepe, R. B. Kent, M. Dean, A. Marshak-Rothstein, and G. E. Sonenshein.** 1984. Specific regulation of c-myc oncogene expression in a murine B-cell lymphoma. Proc. Natl. Acad. Sci. U S A **81:**5546-50.

520. **McFadden, G.** 1998. Even viruses can learn to cope with stress. Science **279:**40-1.

521. **McGill, G.** 1997. Apoptosis In Tumorigenesis And Cancer Therapy. Front. Biosci. **2:**d353-79.

522. **McGuire, W. P., E. K. Rowinsky, N. B. Rosenshein, F. C. Grumbine, D. S. Ettinger, D. K. Armstrong, and R. C. Donehower.** 1989. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Intern. Med. **111:**273-279.

523. **McKenna, S. L., and T. G. Cotter.** 1997. Functional aspects of apoptosis in hematopoiesis and consequences of failure. Adv. Cancer Res. **71:**121-64.

524. **McLaren, J., G. Dealtry, A. Prentice, D. S. Charnock-Jones, and S. K. Smith.** 1997. Decreased levels of the potent regulator of monocyte/macrophage activation, interleukin-13, in the peritoneal fluid of patients with endometriosis. Hum. Reprod. **12:**1307-10.

525. **McLaughlin, M. M., S. Kumar, P. C. McDonnell, S. Van Horn, J. C. Lee, G. P. Livi, and P. R. Young.** 1996. Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase. J. Biol. Chem. **271:**8488-92.

526. **McNally, J., D. H. Yoo, J. Drappa, J. L. Chu, H. Yagita, S. M. Friedman, and K. B. Elkon.** 1997. Fas ligand expression and function in systemic lupus erythematosus. J. Immunol. **159:**4628-36.

527. **Medema, J. P., C. Scaffidi, P. H. Krammer, and M. E. Peter.** 1998. Bcl-xL acts downstream of caspase-8 activation by the CD95 death- inducing signaling complex. J. Biol. Chem. **273:**3388-93.

528. **Meijerink, J. P., E. J. Mensink, K. Wang, T. W. Sedlak, A. W. Sloetjes, T. de Witte, G. Waksman, and S. J. Korsmeyer.** 1998. Hematopoietic malignancies demonstrate loss-of-function mutations of BAX. Blood **91:**2991-7.

529. **Meikrantz, W., and R. Schlegel.** 1996. Suppression of apoptosis by dominant negative mutants of cyclin- dependent protein kinases. J. Biol. Chem. **271:**10205-9.

530. **Mejillano, M. R., J. S. Barton, and R. H. Himes.** 1990. Stabilization of microtubules by GTP analogues. Biochem. Biophys. Res. Commun. **166:**653-60.

531. **Meriin, A. B., V. L. Gabai, J. Yaglom, V. I. Shifrin, and M. Y. Sherman.** 1998. Proteasome inhibitors activate stress kinases and induce Hsp72. Diverse effects on apoptosis. J. Biol. Chem. **273:**6373-9.

532. **Merrill, A. H., Jr., and D. D. Jones.** 1990. An update of the enzymology and regulation of sphingomyelin metabolism. Biochim. Biophys. Acta. **1044:**1-12.

533. **Migheli, A., R. Piva, C. Atzori, D. Troost, and D. Schiffer.** 1997. c-Jun, Jnk/Sapk kinases and transcription factor Nf-kappa B are selectively activated in astrocytes, but not motor neurons, in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. **56:**1314-22.

534. **Milas, L., N. R. Hunter, B. Kurdoglu, K. A. Mason, R. E. Meyn, L. C. Stephens, and L. J. Peters.** 1995. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. Cancer Chemother. Pharmacol. **35:**297-303.

535. **Milas, L., N. R. Hunter, K. A. Mason, B. Kurdoglu, and L. J. Peters.** 1994. Enhancement of tumor radioresponse of a murine mammary carcinoma by paclitaxel. Cancer Res. **54:**3506-10.

536. **Milas, L., N. R. Hunter, K. A. Mason, C. G. Milross, Y. Saito, and L. J. Peters.** 1995. Role of reoxygenation in induction of enhancement of tumor radioresponse by paclitaxel. Cancer Res. **55:**3564-8.

537. **Milas, L., L. C. Stephens, and R. E. Meyn.** 1994. Relation of apoptosis to cancer therapy. In Vivo **8:**665-73.

538. **Milligan, C. E., D. Prevette, H. Yaginnma, S. Homma, C. Cardwell, L. C. Fritz, K. J. Tomaselli, R. W. Oppenheim, and L. M. Schwartz.** 1995. Peptide inhibitors of the ICE protease family arrest programmed cell death of motoneurons in vivo and in vitro. Neuron **15:**385-93.

539. **Milligan, C. E., and L. M. Schwartz.** 1997. Programmed cell death during animal development. Br. Med. Bull. **53:**570-90.

540. **Milne, D. M., D. G. Campbell, F. B. Caudwell, and D. W. Meek.** 1994. Phosphorylation of the tumor suppressor protein p53 by mitogen- activated protein kinases. J. Biol. Chem. **269:**9253-60.

541. **Milne, D. M., L. E. Campbell, D. G. Campbell, and D. W. Meek.** 1995. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation- induced protein kinase characteristic of the c-Jun kinase, JNK1. J. Biol. Chem. **270:**5511-8.

542. **Milross, C. G., K. A. Mason, N. R. Hunter, W. K. Chung, L. J. Peters, and L. Milas.** 1996. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. J. Natl. Cancer Inst. **88:**1308-14.

543. **Minden, A., A. Lin, F. X. Claret, A. Abo, and M. Karin.** 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81:**1147-57.

544. **Minden, A., A. Lin, M. McMahon, C. Lange-Carter, B. Derijard, R. J. Davis, G. L. Johnson, and M. Karin.** 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. Science **266:**1719-23.

545. **Minden, A., A. Lin, T. Smeal, B. Derijard, M. Cobb, R. Davis, and M. Karin.** 1994. c-Jun N-terminal phosphorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases. Mol. Cell. Biol. **14:**6683-8.

546. **Minn, A. J., P. Velez, S. L. Schendel, H. Liang, S. W. Muchmore, S. W. Fesik, M. Fill, and C. B. Thompson.** 1997. Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature **385:**353-7.

547. **Mitchison, T. J., and M. Kirschner.** 1984. Dynamic instability of microtubule growth. Nature **312:**237-242.

548. **Miyashita, T., M. Harigai, M. Hanada, and J. C. Reed.** 1994. Identification of a p53-dependent negative response element in the bcl- 2 gene. Cancer Res. **54:**3131-5.

549. **Miyashita, T., S. Krajewski, M. Krajewska, H. G. Wang, H. K. Lin, D. A. Liebermann, B. Hoffman, and J. C. Reed.** 1994. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene **9:**1799-805.

550. **Miyashita, T., and J. C. Reed.** 1995. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell **80:**293-9.

551. **Moasser, M. M., L. Sepp-Lorenzino, N. E. Kohl, A. Oliff, A. Balog, D. S. Su, S. J. Danishefsky, and N. Rosen.** 1998. Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones. Proc. Natl. Acad. Sci . U S A **95:**1369-74.

552. **Montague, J. W., F. M. Hughes, Jr., and J. A. Cidlowski.** 1997. Native recombinant cyclophilins A, B, and C degrade DNA independently of peptidylprolyl cis-trans-isomerase activity. Potential roles of cyclophilins in apoptosis. J. Biol. Chem. **272:**6677-84.

553. **Moos, P. J., and F. A. Fitzpatrick.** 1998. Taxane-mediated gene induction is independent of microtubule stabilization: induction of transcription regulators and enzymes that modulate inflammation and apoptosis. Proc. Natl. Acad. Sci. U S A **95:**3896-901.

554. **Morana, S. J., C. M. Wolf, J. Li, J. E. Reynolds, M. K. Brown, and A. Eastman.** 1996. The involvement of protein phosphatases in the activation of ICE/CED-3 protease, intracellular acidification, DNA digestion, and apoptosis. J. Biol. Chem. **271:**18263-71.

555. **Moriguchi, T., Y. Gotoh, and E. Nishida.** 1995. Activation of two isoforms of mitogen-activated protein kinase kinase in response to epidermal growth factor and nerve growth factor. Eur. J. Biochem. **234:**32-8.

556. **Moriguchi, T., N. Kuroyanagi, K. Yamaguchi, Y. Gotoh, K. Irie, T. Kano, K. Shirakabe, Y. Muro, H. Shibuya, K. Matsumoto, E. Nishida, and M. Hagiwara.** 1996. A novel kinase cascade mediated by mitogen-activated protein kinase kinase 6 and MKK3. J. Biol. Chem. **271:**13675-9.

557. **Moriguchi, T., F. Toyoshima, N. Masuyama, H. Hanafusa, Y. Gotoh, and E. Nishida.** 1997. A novel SAPK/JNK kinase, MKK7, stimulated by TNFalpha and cellular stresses. EMBO. J. **16:**7045-53.

558. **Morooka, H., J. V. Bonventre, C. M. Pombo, J. M. Kyriakis, and T. Force.** 1995. Ischemia and reperfusion enhance ATF-2 and c-Jun binding to cAMP response elements and to an AP-1 binding site from the c-jun promoter. J. Biol. Chem. **270:**30084-92.

559. **Morris, R. G., A. D. Hargreaves, E. Duvall, and A. H. Wyllie.** 1984. Hormone-induced cell death. 2. Surface changes in thymocytes undergoing apoptosis. Am. J. Pathol. **115:**426-36.

560. **Mosser, D. D., A. W. Caron, L. Bourget, C. Denis-Larose, and B. Massie.** 1997. Role of the human heat shock protein hsp70 in protection against stress- induced apoptosis. Mol. Cell. Biol. **17:**5317-27.

561. **Mote, P. A., M. W. Davey, R. A. Davey, and L. Oliver.** 1996. Paclitaxel sensitizes multidrug resistant cells to radiation. Anticancer Drugs **7:**182-8.

562. **Moulton, B. C.** 1994. Transforming growth factor-beta stimulates endometrial stromal apoptosis in vitro. Endocrinology **134:**1055-60.

563. **Mowat, M. R.** 1998. p53 in tumor progression: life, death, and everything. Adv. Cancer Res. **74:**25-48.

564. **Muchmore, S. W., M. Sattler, H. Liang, R. P. Meadows, J. E. Harlan, H. S. Yoon, D. Nettesheim, B. S. Chang, C. B. Thompson, S. L. Wong, S. L. Ng, and S. W. Fesik.** 1996. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature **381:**335-41.

565. **Mukai, H., M. Miyahara, H. Sunakawa, H. Shibata, M. Toshimori, M. Kitagawa, M. Shimakawa, H. Takanaga, and Y. Ono.** 1996. Translocation of PKN from the cytosol to the nucleus induced by stresses. Proc. Natl. Acad. Sci. U S A **93:**10195-9.

566. **Murphy, K. M., A. B. Heimberger, and D. Y. Loh.** 1990. Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRlo thymocytes in vivo. Science **250:**1720-3.

567. **Murphy, M., A. Hinman, and A. J. Levine.** 1996. Wild-type p53 negatively regulates the expression of a microtubule-associated protein. Genes Dev. **10:**2971-80.

568. **Murphy, W. K., F. V. Fossella, R. J. Winn, D. M. Shin, H. E. Hynes, H. M. Gross, E. Davilla, J. Leimert, H. Dhingra, M. N. Raber, and et al.** 1993. Phase II study of taxol in patients with untreated advanced non-small- cell lung cancer. J. Natl. Cancer Inst. **85:**384-8.

569. **Muzio, M., B. R. Stockwell, H. R. Stennicke, G. S. Salvesen, and V. M. Dixit.** 1998. An induced proximity model for

caspase-8 activation. J. Biol. Chem. **273:**2926-30.
570. **Nagata, S.** 1997. Apoptosis by death factor. Cell **88:**355-65.
571. **Nagata, S., and P. Golstein.** 1995. The Fas death factor. Science **267:**1449-56.
572. **Natoli, G., A. Costanzo, A. Ianni, D. J. Templeton, J. R. Woodgett, C. Balsano, and M. Levrero.** 1997. Activation of SAPK/JNK by TNF receptor 1 through a noncytotoxic TRAF2-dependent pathway. Science **275:**200-3.
573. **Neer, E. J., C. J. Schmidt, R. Nambudripad, and T. F. Smith.** 1994. The ancient regulatory-protein family of WD-repeat proteins. Nature **371:**297-300.
574. **Nemenoff, R. A., S. Winitz, N. X. Qian, V. Van Putten, G. L. Johnson, and L. E. Heasley.** 1993. Phosphorylation and activation of a high molecular weight form of phospholipase A2 by p42 microtubule-associated protein 2 kinase and protein kinase C. J. Biol. Chem. **268:**1960-4.
575. **Nemoto, S., Z. Sheng, and A. Lin.** 1998. Opposing effects of Jun kinase and p38 mitogen-activated protein kinases on cardiomyocyte hypertrophy. Mol. Cell. Biol. **18:**3518-26.
576. **Nemoto, S., J. Xiang, S. Huang, and A. Lin.** 1998. Induction of apoptosis by SB202190 through inhibition of p38beta mitogen-activated protein kinase. J. Biol. Chem. **273:**16415-20.
577. **Nicholson, D. W., and N. A. Thornberry.** 1997. Caspases: killer proteases. Trends Biochem. Sci. **22:**299-306.
578. **Nicolaou, K. C., Z. Yang, J. J. Liu, H. Ueno, P. G. Nantermet, R. K. Guy, C. F. Claiborne, J. Renaud, E. A. Couladouros, K. Paulvannan, and et al.** 1994. Total synthesis of taxol. Nature **367:**630-4.
579. **Nishina, H., K. D. Fischer, L. Radvanyi, A. Shahinian, R. Hakem, E. A. Rubie, A. Bernstein, T. W. Mak, J. R. Woodgett, and J. M. Penninger.** 1997. Stress-signalling kinase SEK1 protects thymocytes from apoptosis mediated by CD95 and CD3. Nature **385:**350-3.
580. **Nishina, H., L. Radvanyi, K. Raju, T. Sasaki, I. Kozieradzki, and J. M. Penninger.** 1998. Impaired TCR-mediated apoptosis and Bcl-XL expression in T cells lacking the stress kinase activator SEK1/MKK4. J. Immunol. **161:**3416-20.
581. **Nishitoh, H., M. Saitoh, Y. Mochida, K. Takeda, H. Nakano, M. Rothe, K. Miyazono, and H. Ichijo.** 1998. ASK1 is essential for JNK/SAPK activation by TRAF2. Mol. Cell **2:**389-95.
582. **Obeid, L. M., C. M. Linardic, L. A. Karolak, and Y. A. Hannun.** 1993. Programmed cell death induced by ceramide. Science **259:**1769-71.
583. **Oberhammer, F. A., M. Pavelka, S. Sharma, and e. al.** 1992. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta-1. Proc. Natl. Acad. Sci. USA. **89:**5408-5412.
584. **O'Connell, J., G. C. O'Sullivan, J. K. Collins, and F. Shanahan.** 1996. The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand. J. Exp. Med. **184:**1075-82.
585. **Odde, D. J., L. Cassimeris, and H. M. Buettner.** 1995. Kinetics of microtubule catastrophe assessed by probabilistic analysis. Biophys. J. **69:**796-802.
586. **Oehm, A., I. Behrmann, W. Falk, M. Pawlita, G. Maier, C. Klas, M. Li-Weber, S. Richards, J. Dhein, B. C. Trauth, and et al.** 1992. Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily. Sequence identity with the Fas antigen. J. Biol. Chem. **267:**10709-15.
587. **Ogasawara, J., R. Watanabe-Fukunaga, M. Adachi, A. Matsuzawa, T. Kasugai, Y. Kitamura, N. Itoh, T. Suda, and S. Nagata.** 1993. Lethal effect of the anti-Fas antibody in mice. Nature **364:**806-9.
588. **Okamoto, K., K. Fujisawa, T. Hasunuma, T. Kobata, T. Sumida, and K. Nishioka.** 1997. Selective activation of the JNK/AP-1 pathway in Fas-mediated apoptosis of rheumatoid arthritis synoviocytes. Arthritis Rheum. **40:**919-26.
589. **Okazaki, K., and N. Sagata.** 1995. The Mos/MAP kinase pathway stabilizes c-Fos by phosphorylation and augments its transforming activity in NIH 3T3 cells. EMBO. J. **14:**5048-59.
590. **Okazaki, T., A. Bielawska, N. Domae, R. M. Bell, and Y. A. Hannun.** 1994. Characteristics and partial purification of a novel cytosolic, magnesium-independent, neutral sphingomyelinase activated in the early signal transduction of 1 alpha,25-dihydroxyvitamin D3-induced HL-60 cell differentiation. J. Biol. Chem. **269:**4070-7.
591. **Oltvai, Z. N., C. L. Milliman, and S. J. Korsmeyer.** 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell **74:**609-19.
592. **Ookata, K., S. Hisanaga, J. C. Bulinski, H. Murofushi, H. Aizawa, T. J. Itoh, H. Hotani, E. Okumura, K. Tachibana, and T. Kishimoto.** 1995. Cyclin B interaction with microtubule-associated protein 4 (MAP4) targets p34cdc2 kinase to microtubules and is a potential regulator of M-phase microtubule dynamics. J. Cell Biol. **128:**849-62.
593. **Oppenheim, R. W.** 1991. Cell death during development of the nervous system. Annu. Rev. Neurosci. **14:**453-501.
594. **Oren, M.** 1998. Tumor suppressors. Teaming up to restrain cancer. Nature **391:**233-4.
595. **Osada, M., M. Ohba, C. Kawahara, C. Ishioka, R. Kanamaru, I. Katoh, Y. Ikawa, Y. Nimura, A. Nakagawara, M. Obinata, and S. Ikawa.** 1998. Cloning and functional analysis of human p51, which structurally and functionally resembles p53. Nat. Med. **4:**839-43.
596. **Otsuki, Y., O. Misaki, O. Sugimoto, Y. Ito, Y. Tsujimoto, and Y. Akao.** 1994. Cyclic bcl-2 gene expression in human uterine endometrium during menstrual cycle. Lancet **344:**28-9.
597. **Owen-Schaub, L. B., W. Zhang, J. C. Cusack, L. S. Angelo, S. M. Santee, T. Fujiwara, J. A. Roth, A. B. Deisseroth, W. W. Zhang, E. Kruzel, and et al.** 1995. Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol. Cell. Biol. **15:**3032-40.
598. **Packham, G., and J. L. Cleveland.** 1994. Ornithine decarboxylase is a mediator of c-Myc-induced apoptosis. Mol. Cell. Biol. **14:**5741-7.
599. **Pan, G., E. W. Humke, and V. M. Dixit.** 1998. Activation of caspases triggered by cytochrome c in vitro. FEBS. Lett. **426:**151-4.
600. **Pan, G., O. R. K, and V. M. Dixit.** 1998. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. J. Biol. Chem. **273:**5841-5.
601. **Pan, G., J. Ni, Y. F. Wei, G. Yu, R. Gentz, and V. M. Dixit.** 1997. An antagonist decoy receptor and a death domain-containing receptor for TRAIL [see comments]. Science **277:**815-8.

602. **Pan, H., and A. E. Griep.** 1994. Altered cell cycle regulation in the lens of HPV-16 E6 or E7 transgenic mice: implications for tumor suppressor gene function in development. Genes Dev. **8**:1285-99.
603. **Panda, D., J. E. Daijo, M. A. Jordan, and L. Wilson.** 1995. Kinetic stabilization of microtubule dynamics at steady state in vitro by substoichiometric concentrations of tubulin-colchicine complex. Biochemistry **34**:9921-9.
604. **Panda, D., M. A. Jordan, K. C. Chu, and L. Wilson.** 1996. Differential effects of vinblastine on polymerization and dynamics at opposite microtubule ends. J. Biol. Chem. **271**:29807-12.
605. **Panda, D., H. P. Miller, K. Islam, and L. Wilson.** 1997. Stabilization of microtubule dynamics by estramustine by binding to a novel site in tubulin: a possible mechanistic basis for its antitumor action. Proc. Natl. Acad. Sci. U S A **94**:10560-4.
606. **Panvichian, R., K. Orth, M. L. Day, K. C. Day, M. J. Pilat, and K. J. Pienta.** 1998. Paclitaxel-associated multimininucleation is permitted by the inhibition of caspase activation: a potential early step in drug resistance. Cancer Res. **58**:4667-72.
607. **Pastorino, J. G., G. Simbula, K. Yamamoto, P. A. Glascott, Jr., R. J. Rothman, and J. L. Farber.** 1996. The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. J. Biol. Chem. **271**:29792-8.
608. **Patel, R. N.** 1998. Tour de paclitaxel: biocatalysis for semisynthesis. Annu. Rev. Microbiol. **52**:361-95.
609. **Patriotis, C., A. Makris, S. E. Bear, and P. N. Tsichlis.** 1993. Tumor progression locus 2 (Tpl-2) encodes a protein kinase involved in the progression of rodent T-cell lymphomas and in T-cell activation. Proc. Natl. Acad. Sci. U S A **90**:2251-5.
610. **Patriotis, C., A. Makris, J. Chernoff, and P. N. Tsichlis.** 1994. Tpl-2 acts in concert with Ras and Raf-1 to activate mitogen-activated protein kinase. Proc. Natl. Acad. Sci. U S A **91**:9755-9.
611. **Peitsch, M. C., B. Polzar, H. Stephan, T. Crompton, H. R. MacDonald, H. G. Mannherz, and J. Tschopp.** 1993. Characterization of the endogenous deoxyribonuclease involved in nuclear DNA degradation during apoptosis (programmed cell death). EMBO. J. **12**:371-7.
612. **Perego, P., S. Romanelli, N. Carenini, I. Magnani, R. Leone, A. Bonetti, A. Paolicchi, and F. Zunino.** 1998. Ovarian cancer cisplatin-resistant cell lines: multiple changes including collateral sensitivity to Taxol. Ann. Oncol. **9**:423-30.
613. **Peter, M. E., A. E. Heufelder, and M. O. Hengartner.** 1997. Advances in apoptosis research. Proc. Natl. Acad. Sci. U S A **94**:12736-7.
614. **Philipp, A., A. Schneider, I. Vasrik, K. Finke, Y. Xiong, D. Beach, K. Alitalo, and M. Eilers.** 1994. Repression of cyclin D1: a novel function of MYC. Mol. Cell. Biol. **14**:4032-43.
615. **Phillips, A. C., S. Bates, K. M. Ryan, K. Helin, and K. H. Vousden.** 1997. Induction of DNA synthesis and apoptosis are separable functions of E2F- 1. Genes Dev. **11**:1853-63.
616. **Photiou, A., P. Shah, L. K. Leong, J. Moss, and S. Retsas.** 1997. In vitro synergy of paclitaxel (Taxol) and vinorelbine (navelbine) against human melanoma cell lines. Eur. J. Cancer **33**:463-70.
617. **Polyak, K., Y. Xia, J. L. Zweier, K. W. Kinzler, and B. Vogelstein.** 1997. A model for p53-induced apoptosis. Nature **389**:300-5.
618. **Pombo, C. M., J. H. Kehrl, I. Sanchez, P. Katz, J. Avruch, L. I. Zon, J. R. Woodgett, T. Force, and J. M. Kyriakis.** 1995. Activation of the SAPK pathway by the human STE20 homologue germinal centre kinase. Nature **377**:750-4.
619. **Ponnathpur, V., A. M. Ibrado, J. C. Reed, S. Ray, Y. Huang, S. Self, G. Bullock, A. Nawabi, and K. Bhalla.** 1995. Effects of modulators of protein kinases on Taxol-induced apoptosis of human leukemic cells possessing disparate levels of p26BCL-2 protein. Clin. Cancer Res. **1**:1399-1406.
620. **Posada, J., N. Yew, N. G. Ahn, G. F. Vande Woude, and J. A. Cooper.** 1993. Mos stimulates MAP kinase in Xenopus oocytes and activates a MAP kinase kinase in vitro. Mol. Cell. Biol. **13**:2546-53.
621. **Potapova, O., A. Haghighi, F. Bost, C. Liu, M. J. Birrer, R. Gjerset, and D. Mercola.** 1997. The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. J. Biol. Chem. **272**:14041-4.
622. **Prabhu, N. S., K. Somasundaram, K. Satyamoorthy, M. Herlyn, and W. S. El-Deiry.** 1998. p73beta, unlike p53, suppresses growth and induces apoptosis of human papillomavirus E6-expressing cancer cells. Int. J. Oncol. **13**:5-9.
623. **Price, J. M., P. K. Donahoe, Y. Ito, and W. H. d. Hendren.** 1977. Programmed cell death in the Mullerian duct induced by Mullerian inhibiting substance. Am. J. Anat. **149**:353-75.
624. **Price, M. A., F. H. Cruzalegui, and R. Treisman.** 1996. The p38 and ERK MAP kinase pathways cooperate to activate Ternary Complex Factors and c-fos transcription in response to UV light. EMBO. J. **15**:6552-63.
625. **Prisco, M., A. Hongo, M. G. Rizzo, A. Sacchi, and R. Baserga.** 1997. The insulin-like growth factor I receptor as a physiologically relevant target of p53 in apoptosis caused by interleukin-3 withdrawal. Mol. Cell. Biol. **17**:1084-92.
626. **Pulkkinen, J. O., K. Pekkola-Heino, and R. Grenman.** 1996. Paclitaxel and irradiation induce apoptosis in squamous cell carcinoma cell lines in an additive way. Anticancer Res. **16**:2923-9.
627. **Pulverer, B. J., J. M. Kyriakis, J. Avruch, E. Nikolakaki, and J. R. Woodgett.** 1991. Phosphorylation of c-jun mediated by MAP kinases. Nature **353**:670-4.
628. **Qin, S., Y. Minami, T. Kurosaki, and H. Yamamura.** 1997. Distinctive functions of Syk and Lyn in mediating osmotic stress- and ultraviolet C irradiation-induced apoptosis in chicken B cells. J. Biol. Chem. **272**:17994-9.
629. **Raff, M. C.** 1996. Size control: the regulation of cell numbers in animal development. .
630. **Raff, M. C.** 1992. Social controls on cell survival and cell death. Nature **356**:397-400.
631. **Raff, M. C., B. A. Barres, J. F. Burne, H. S. Coles, Y. Ishizaki, and M. D. Jacobson.** 1993. Programmed cell death and the control of cell survival: lessons from the nervous system. Science **262**:695-700.
632. **Raingeaud, J., A. J. Whitmarsh, T. Barrett, B. Derijard, and R. J. Davis.** 1996. MKK3- and MKK6-regulated gene expression is mediated by the p38 mitogen-activated protein kinase signal transduction pathway. Mol. Cell. Biol. **16**:1247-55.
633. **Rana, A., K. Gallo, P. Godowski, S. Hirai, S. Ohno, L. Zon, J. M. Kyriakis, and J. Avruch.** 1996. The mixed lineage kinase SPRK phosphorylates and activates the stress-activated protein kinase activator, SEK-1. J. Biol. Chem. **271**:19025-8.
634. **Ranganathan, S., D. W. Dexter, C. A. Benetatos, A. E. Chapman, K. D. Tew, and G. R. Hudes.** 1996. Increase of beta(III)-

and beta(IVa)-tubulin isotopes in human prostate carcinoma cells as a result of estramustine resistance. Cancer Res. **56:**2584-9.

635. **Rani, C. S., A. Abe, Y. Chang, N. Rosenzweig, A. R. Saltiel, N. S. Radin, and J. A. Shayman.** 1995. Cell cycle arrest induced by an inhibitor of glucosylceramide synthase. Correlation with cyclin-dependent kinases. J. Biol. Chem. **270:**2859-67.
636. **Ray, C. A., R. A. Black, S. R. Kronheim, T. A. Greenstreet, P. R. Sleath, G. S. Salvesen, and D. J. Pickup.** 1992. Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme. Cell **69:**597-604.
637. **Ray, L. B., and T. W. Sturgill.** 1987. Rapid stimulation by insulin of a serine/threonine kinase in 3T3-L1 adipocytes that phosphorylates microtubule-associated protein 2 in vitro. Proc. Natl. Acad. Sci. U S A **84:**1502-6.
638. **Reed, J. C.** 1997. Double identity for proteins of the Bcl-2 family. Nature **387:**773-776.
639. **Reed, J. C.** 1997. Promise and problems of Bcl-2 antisense therapy. J. Natl. Cancer Inst. **89:**988-90.
640. **Reed, J. C.** 1995. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. Curr. Opin. Oncol. 7:541-6.
641. **Reichman, B. S., A. D. Seidman, J. P. Crown, R. Heelan, T. B. Hakes, D. E. Lebwohl, T. A. Gilewski, A. Surbone, V. Currie, C. A. Hudis, and et al.** 1993. Paclitaxel and recombinant human granulocyte colony-stimulating factor as initial chemotherapy for metastatic breast cancer. J. Clin. Oncol. **11:**1943-51.
642. **Resing, K. A., S. J. Mansour, A. S. Hermann, R. S. Johnson, J. M. Candia, K. Fukasawa, G. F. Vande Woude, and N. G. Ahn.** 1995. Determination of v-Mos-catalyzed phosphorylation sites and autophosphorylation sites on MAP kinase kinase by ESI/MS. Biochemistry **34:**2610-20.
643. **Rincon, M., A. Whitmarsh, D. D. Yang, L. Weiss, D. r. B, P. Jayaraj, R. J. Davis, and R. A. Flavell.** 1998. The JNK Pathway Regulates the In Vivo Deletion of Immature CD4(+)CD8(+) Thymocytes. J. Exp. Med. **188:**1817-1830.
644. **Rodionov, V. I., and G. G. Borisy.** 1997. Microtubule treadmilling in vivo. Science **275:**215-8.
645. **Rodionov, V. I., and G. G. Borisy.** 1997. Self-centring activity of cytoplasm. Nature **386:**170-3.
646. **Rodrigues, G. A., M. Park, and J. Schlessinger.** 1997. Activation of the JNK pathway is essential for transformation by the Met oncogene. EMBO. J. **16:**2634-45.
647. **Rodriguez-Viciana, P., P. H. Warne, R. Dhand, B. Vanhaesebroeck, I. Gout, M. J. Fry, M. D. Waterfield, and J. Downward.** 1994. Phosphatidylinositol-3-OH kinase as a direct target of Ras. Nature **370:**527-32.
648. **Rodriguez-Viciana, P., P. H. Warne, B. Vanhaesebroeck, M. D. Waterfield, and J. Downward.** 1996. Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation. EMBO. J. **15:**2442-51.
649. **Rosette, C., and M. Karin.** 1996. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science **274:**1194-7.
650. **Rosse, T., R. Olivier, L. Monney, M. Rager, S. Conus, I. Fellay, B. Jansen, and C. Borner.** 1998. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. Nature **391:**496-9.
651. **Rotello, R. J., R. C. Lieberman, A. F. Purchio, and L. E. Gerschenson.** 1991. Coordinated regulation of apoptosis and cell proliferation by transforming growth factor beta 1 in cultured uterine epithelial cells. Proc. Natl. Acad. Sci. U S A **88:**3412-5.
652. **Roth, W., B. Wagenknecht, C. Grimmel, J. Dichgans, and M. Weller.** 1998. Taxol-mediated augmentation of CD95 ligand-induced apoptosis of human malignant glioma cells: association with bcl-2 phosphorylation but neither activation of p53 nor G2/M cell cycle arrest. Br. J. Cancer 77:404-11.
653. **Rothe, M., M. G. Pan, W. J. Henzel, T. M. Ayres, and D. V. Goeddel.** 1995. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell **83:**1243-52.
654. **Roulston, A., C. Reinhard, P. Amiri, and L. T. Williams.** 1998. Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor alpha. J. Biol. Chem. **273:**10232-9.
655. **Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, and A. R. Nebreda.** 1994. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell **78:**1027-37.
656. **Roussel, M. F., J. L. Cleveland, S. A. Shurtleff, and C. J. Sherr.** 1991. Myc rescue of a mutant CSF-1 receptor impaired in mitogenic signalling. Nature **353:**361-3.
657. **Rowan, S., R. L. Ludwig, Y. Haupt, S. Bates, X. Lu, M. Oren, and K. H. Vousden.** 1996. Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. EMBO. J. **15:**827-38.
658. **Rowinsky, E. K.** 1997. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annu. Rev. Med. **48:**353-74.
659. **Rowinsky, E. K., V. Chaudhry, D. R. Cornblath, and R. C. Donehower.** 1993. Neurotoxicity of Taxol. J. Natl. Cancer Inst. Monogr. :107-15.
660. **Rowinsky, E. K., and R. C. Donehower.** 1995. Paclitaxel (taxol). N. Engl. J. Med. **332:**1004-14.
661. **Rowinsky, E. K., E. A. Eisenhauer, V. Chaudhry, S. G. Arbuck, and R. C. Donehower.** 1993. Clinical toxicities encountered with paclitaxel (Taxol). Semin. Oncol. **20:**1-15.
662. **Rowinsky, E. K., W. P. McGuire, T. Guarnieri, J. S. Fisherman, M. C. Christian, and R. C. Donehower.** 1991. Cardiac disturbances during the administration of taxol. J. Clin. Oncol. **9:**1704-12.
663. **Rowinsky, E. K., N. Onetto, R. M. Canetta, and S. G. Arbuck.** 1992. Taxol: the first of the taxanes, an important new class of antitumor agents. Semin. Oncol. **19:**646-62.
664. **Roy, N., Q. L. Deveraux, R. Takahashi, G. S. Salvesen, and J. C. Reed.** 1997. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO. J. **16:**6914-25.
665. **Rozakis-Adcock, M., P. van der Geer, G. Mbamalu, and T. Pawson.** 1995. MAP kinase phosphorylation of mSos1 promotes dissociation of mSos1-Shc and mSos1-EGF receptor complexes. Oncogene **11:**1417-26.
666. **Rudel, T., and G. M. Bokoch.** 1997. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science **276:**1571-4.
667. **Rudel, T., F. T. Zenke, T. H. Chuang, and G. M. Bokoch.** 1998. p21-activated kinase (Pak) is required for Fas-induced JNK activation in Jurkat cells. J. Immunol. **160:**7-11.

668. **Rudin, C. M., and C. B. Thompson.** 1997. Apoptosis and diseases: regulation and clinical relevance of programmed cell death. Annu. Rev. Med. **48:**267-281.
669. **Ruoslahti, E.** 1997. Stretching is good for a cell. Science **276:**1345-6.
670. **Sa, G., G. Murugesan, M. Jaye, Y. Ivashchenko, and P. L. Fox.** 1995. Activation of cytosolic phospholipase A2 by basic fibroblast growth factor via a p42 mitogen-activated protein kinase-dependent phosphorylation pathway in endothelial cells. J. Biol. Chem. **270:**2360-6.
671. **Saas, P., P. R. Walker, M. Hahne, A. L. Quiquerez, V. Schnuriger, G. Perrin, L. French, E. G. Van Meir, N. de Tribolet, J. Tschopp, and P. Y. Dietrich.** 1997. Fas ligand expression by astrocytoma in vivo: maintaining immune privilege in the brain? J. Clin. Invest. **99:**1173-8.
672. **Sackett, D. L.** 1995. Vinca site agents induce structural changes in tubulin different from and antagonistic to changes induced by colchicine site agents. Biochemistry **34:**7010-9.
673. **Sackett, D. L., and J. K. Varma.** 1993. Molecular mechanism of colchicine action: induced local unfolding of beta-tubulin. Biochemistry **32:**13560-5.
674. **Sackett, D. L., and J. Wolff.** 1986. Proteolysis of tubulin and the substructure of the tubulin dimer. J. Biol. Chem. **261:**9070-6.
675. **Safran, H., T. King, H. Choy, A. Gollerkeri, H. Kwakwa, F. Lopez, B. Cole, J. Myers, J. Tarpey, and A. Rosmarin.** 1996. p53 mutations do not predict response to paclitaxel/radiation for nonsmall cell lung carcinoma. Cancer **78:**1203-10.
676. **Saitoh, M., H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, and H. Ichijo.** 1998. Mammalian thioredoxin is a direct inhibitor of apoptosis signal- regulating kinase (ASK) 1. EMBO. J. **17:**2596-606.
677. **Sakahira, H., M. Enari, and S. Nagata.** 1998. Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature **391:**96-9.
678. **Salmeron, A., T. B. Ahmad, G. W. Carlile, D. Pappin, R. P. Narsimhan, and S. C. Ley.** 1996. Activation of MEK-1 and SEK-1 by Tpl-2 proto-oncoprotein, a novel MAP kinase kinase kinase. EMBO. J. **15:**817-26.
679. **Salmon, R, A., I. N. Foltz, P. R. Young, and J. W. Schrader.** 1997. The p38 mitogen-activated protein kinase is activated by ligation of the T or B lymphocyte antigen receptors, Fas or CD40, but suppression of kinase activity does not inhibit apoptosis induced by antigen receptors. J. Immunol. **159:**5309-17.
680. **Sanchez, I., R. T. Hughes, B. J. Mayer, K. Yee, J. R. Woodgett, J. Avruch, J. M. Kyriakis, and L. I. Zon.** 1994. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-Jun. Nature **372:**794-8.
681. **Sanchez-Perez, I., J. R. Murguia, and R. Perona.** 1998. Cisplatin induces a persistent activation of JNK that is related to cell death. Oncogene **16:**533-40.
682. **Sanna, M. G., C. S. Duckett, B. W. Richter, C. B. Thompson, and R. J. Ulevitch.** 1998. Selective activation of JNK1 is necessary for the anti-apoptotic activity of hILP. Proc. Natl. Acad. Sci. U S A **95:**6015-20.
683. **Santana, P., L. A. Pena, A. Haimovitz-Friedman, S. Martin, D. Green, M. McLoughlin, C. Cordon-Cardo, E. H. Schuchman, Z. Fuks, and R. Kolesnick.** 1996. Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. Cell **86:**189-99.
684. **Sattler, M., H. Liang, D. Nettesheim, R. P. Meadows, J. E. Harlan, M. Eberstadt, H. S. Yoon, S. B. Shuker, B. S. Chang, A. J. Minn, C. B. Thompson, and S. W. Fesik.** 1997. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science **275:**983-6.
685. **Savill, J.** 1998. Apoptosis. Phagocytic docking without shocking. Nature **392:**442-3.
686. **Savill, J.** 1997. Recognition and phagocytosis of cells undergoing apoptosis. Br. Med. Bull. **53:**491-508.
687. **Savill, J., N. Hogg, Y. Ren, and C. Haslett.** 1992. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. J. Clin. Invest. **90:**1513-22.
688. **Savill, J. S,, P. M. Henson, and C. Haslett.** 1989. Phagocytosis of aged human neutrophils by macrophages is mediated by a novel "charge-sensitive" recognition mechanism. J. Clin. Invest. **84:**1518-27.
689. **Scatena, C, D., Z. A. Stewart, D. Mays, L. J. Tang, C. J. Keefer, S. D. Leach, and J. A. Pietenpol.** 1998. Mitotic phosphorylation of Bcl-2 during normal cell cycle progression and Taxol-induced growth arrest. J. Biol. Chem. **273:**30777-30784.
690. **Schaeffer, H. J., A. D. Catling, S. T. Eblen, L. S. Collier, A. Krauss, and M. J. Weber.** 1998. MP1: a MEK binding partner that enhances enzymatic activation of the MAP kinase cascade. Science **281:**1668-71.
691. **Scharenberg, A. M., and J.-P. Kinet.** 1996. The emerging field of receptor-mediated inhibitory signaling: SHP or SHIP? Cell **87:**961-964.
692. **Schendel, S. L., Z. Xie, M. O. Montal, S. Matsuyama, M. Montal, and J. C. Reed.** 1997. Channel formation by antiapoptotic protein Bcl-2. Proc. Natl. Acad. Sci. U S A **94:**5113-8.
693. **Schiff, P. B., and S. B. Horwitz.** 1981. Taxol assembles tubulin in the absence of exogenous guanosine 5'- triphosphate or microtubule-associated proteins. Biochemistry **20:**3247-52.
694. **Schiff, P. B., and S. B. Horwitz.** 1980. Taxol stabilizes microtubules in mouse fibroblast cells. Proc. Natl. Acad. Sci. U S A **77:**1561-5.
695. **Schlesinger, P. H., A. Gross, X. M. Yin, K. Yamamoto, M. Saito, G. Waksman, and S. J. Korsmeyer.** 1997. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc. Natl. Acad. Sci. U S A **94:**11357-62.
696. **Schutze, S., K. Potthoff, T. Machleidt, D. Berkovic, K. Wiegmann, and M. Kronke.** 1992. TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced "acidic" sphingomyelin breakdown. Cell **71:**765-76.
697. **Schwandner, R., K. Wiegmann, K. Bernardo, D. Kreder, and M. Kronke.** 1998. TNF receptor death domain-associated proteins TRADD and FADD signal activation of acid sphingomyelinase. J. Biol. Chem. **273:**5916-22.
698. **Schwartzman, R. A., and J. A. Cidlowski.** 1993. Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocr. Rev. **14:**133-151.
699. **Schwenger, P., P. Bellosta, I. Vietor, C. Basilico, E. Y. Skolnik, and J. Vilcek.** 1997. Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated

protein kinase activation. Proc. Natl. Acad. Sci. U S A **94:**2869-73.

700. **Sears, R., K. Ohtani, and J. R. Nevins.** 1997. Identification of positively and negatively acting elements regulating expression of the E2F2 gene in response to cell growth signals. Mol. Cell. Biol. **17:**5227-35.

701. **Seimiya, H., T. Mashima, M. Toho, and T. Tsuruo.** 1997. c-Jun NH2-terminal kinase-mediated activation of interleukin-1beta converting enzyme/Ced-3-like protease during anticancer drug-induced apoptosis. J.Biol. Chem. **272:**4631-6.

702. **Selvakumaran, M., H. K. Lin, T. Miyashita, H. G. Wang, S. Krajewski, J. C. Reed, B. Hoffman, and D. Liebermann.** 1994. Immediate early up-regulation of bax expression by p53 but not TGF beta 1: a paradigm for distinct apoptotic pathways. Oncogene **9:**1791-8.

703. **Seong, J., C. G. Milross, N. R. Hunter, H. C. Shin, and L. Milas.** 1997. Potentiation of antitumor efficacy of paclitaxel by recombinant tumor necrosis factor-alpha. Anticancer Drugs **8:**80-7.

704. **Seth, A., S. Gupta, and R. J. Davis.** 1993. Cell cycle regulation of the c-Myc transcriptional activation domain. Mol. Cell. Biol. **13:**4125-36.

705. **Shan, B., and W. H. Lee.** 1994. Deregulated expression of E2F-1 induces S-phase entry and leads to apoptosis. Mol. Cell. Biol. **14:**8166-73.

706. **Shaw, P., R. Bovey, S. Tardy, R. Sahli, B. Sordat, and J. Costa.** 1992. Induction of apoptosis by wild-type p53 in a human colon tumor-derived cell line. Proc. Natl. Acad. Sci. U S A **89:**4495-9.

707. **Shen, S. C., T. S. Huang, S. H. Jee, and M. L. Kuo.** 1998. Taxol-induced p34cdc2 kinase activation and apoptosis inhibited by 12-O- tetradecanoylphorbol-13-acetate in human breast MCF-7 carcinoma cells. Cell Growth Differ. **9:**23-9.

708. **Sheridan, J. P., S. A. Marsters, R. M. Pitti, A. Gurney, M. Skubatch, D. Baldwin, L. Ramakrishnan, C. L. Gray, K. Baker, W. I. Wood, A. D. Goddard, P. Godowski, and A. Ashkenazi.** 1997. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors [see comments]. Science **277:**818-21.

709. **Sherr, C. J., and J. M. Roberts.** 1995. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. **9:**1149-63.

710. **Shi, L., C. M. Kam, J. C. Powers, R. Aebersold, and A. H. Greenberg.** 1992. Purification of three cytotoxic lymphocyte granule serine proteases that induce apoptosis through distinct substrate and target cell interactions. J. Exp. Med. **176:**1521-9.

711. **Shi, L., W. K. Nishioka, J. Th'ng, E. M. Bradbury, D. W. Litchfield, and A. H. Greenberg.** 1994. Premature p34cdc2 activation required for apoptosis. Science **263:**1143-5.

712. **Shi, Y., J. M. Glynn, L. J. Guilbert, T. G. Cotter, R. P. Bissonnette, and D. R. Green.** 1992. Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas. Science **257:**212-4.

713. **Shibasaki, F., E. Kondo, T. Akagi, and F. McKeon.** 1997. Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2. Nature **386:**728-31.

714. **Shimizu, T., O. C. PM, K. W. Kohn, and Y. Pommier.** 1995. Unscheduled activation of cyclin B1/Cdc2 kinase in human promyelocytic leukemia cell line HL60 cells undergoing apoptosis induced by DNA damage. Cancer Res. **55:**228-31.

715. **Shimoke, K., S. Yamagishi, M. Yamada, T. Ikeuchi, and H. Hatanaka.** 1999. Inhibition of phosphatidylinositol 3-kinase activity elevates c-Jun N- terminal kinase activity in apoptosis of cultured cerebellar granule neurons. Brain Res. Dev. Brain Res. **112:**245-253.

716. **Simizu, S., K. Tanabe, E. Tashiro, M. Takada, K. Umezawa, and M. Imoto.** 1998. Potentiation of paclitaxel cytotoxicity by inostamycin in human small cell lung carcinoma, Ms-1 cells. Jpn. J. Cancer Res. **89:**970-6.

717. **Simonian, P. L., D. A. Grillot, and G. Nunez.** 1997. Bak can accelerate chemotherapy-induced cell death independently of its heterodimerization with Bcl-XL and Bcl-2. Oncogene **15:**1871-5.

718. **Singh, P., S. H. Wong, and W. Hong.** 1994. Overexpression of E2F-1 in rat embryo fibroblasts leads to neoplastic transformation. EMBO. J. **13:**3329-38.

719. **Smeal, T., P. Angel, J. Meek, and M. Karin.** 1989. Different requirements for formation of Jun: Jun and Jun: Fos complexes. Genes Dev. **3:**2091-100.

720. **Smith, A., F. Ramos-Morales, A. Ashworth, and M. Collins.** 1997. A role for JNK/SAPK in proliferation, but not apoptosis, of IL-3- dependent cells. Curr. Biol. **7:**893-6.

721. **Sonnichsen, D. S., and M. V. Relling.** 1998. Chapter 17. Paclitaxel and docetaxel, p. 375-394. *In* L. B. Grochow, and M. M. Ames (ed.), A clinician's guide to chemotherapy pharmacokinetics and pharmacodynamics. Williams & Wilkins, Baltimore.

722. **Sorger, P. K., M. Dobles, R. Tournebize, and A. A. Hyman.** 1997. Coupling cell division and cell death to microtubule dynamics. Curr. Opin. Cell Biol. **9:**807-14.

723. **Speicher, L. A., L. Barone, and K. D. Tew.** 1992. Combined antimicrotubule activity of estramustine and taxol in human prostatic carcinoma cell lines. Cancer Res. **52:**4433-40.

724. **Srinivasula, S. M., M. Ahmad, T. Fernandes-Alnemri, and E. S. Alnemri.** 1998. Autoactivation of procaspase-9 by Apaf-1-mediated oligomerization. Mol. Cell **1:**949-57.

725. **Srivastava, R. K., A. R. Srivastava, S. J. Korsmeyer, M. Nesterova, Y. S. Cho-Chung, and D. L. Longo.** 1998. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **18:**3509-17.

726. **Stadheim, T. A., and G. L. Kucera.** 1998. Extracellular signal-regulated kinase (ERK) activity is required for TPA-mediated inhibition of drug-induced apoptosis. Biochem. Biophys. Res. Commun. **245:**266-71.

727. **Stehlik, C., R. de Martin, I. Kumabashiri, J. A. Schmid, B. R. Binder, and J. Lipp.** 1998. Nuclear factor (NF)-kappaB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha- induced apoptosis. J. Exp. Med. **188:**211-6.

728. **Stewart, N., G. G. Hicks, F. Paraskevas, and M. Mowat.** 1995. Evidence for a second cell cycle block at G2/M by p53. Oncogene **10:**109-15.

729. **Story, M., and R. Kodym.** 1998. Signal transduction during apoptosis; implications for cancer therapy. Front. Biosci. **3:**d365-375.

730. **Streuli, M.** 1996. Protein tyrosine phosphatases in signaling. Curr. Opin. Cell Biol. **8:**182-188.

731. **Strobel, T., S. K. Kraeft, L. B. Chen, and S. A. Cannistra.** 1998. BAX expression is associated with enhanced intracellular accumulation of paclitaxel: a novel role for BAX during chemotherapy-induced cell death. Cancer Res. **58:**4776-81.
732. **Strobel, T., L. Swanson, S. Korsmeyer, and S. A. Cannistra.** 1996. BAX enhances paclitaxel-induced apoptosis through a p53-independent pathway. Proc. Natl. Acad. Sci. U S A **93:**14094-9.
733. **Strobel, T., Y. T. Tai, S. Korsmeyer, and S. A. Cannistra.** 1998. BAD partly reverses paclitaxel resistance in human ovarian cancer cells. Oncogene 17:2419-27.
734. **Sturgill, T. W., L. B. Ray, E. Erikson, and J. L. Maller.** 1988. Insulin-stimulated MAP-2 kinase phosphorylates and activates ribosomal protein S6 kinase II. Nature **334:**715-8.
735. **Suda, T., T. Takahashi, P. Golstein, and S. Nagata.** 1993. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. Cell **75:**1169-78.
736. **Sumantran, V. N., M. W. Ealovega, G. Nunez, M. F. Clarke, and M. S. Wicha.** 1995. Overexpression of Bcl-XS sensitizes MCF-7 cells to chemotherapy-induced apoptosis. Cancer Res. **55:**2507-10.
737. **Sunahara, M., S. Ichimiya, Y. Nimura, N. Takada, S. Sakiyama, Y. Sato, S. Todo, W. Adachi, J. Amano, and A. Nakagawara.** 1998. Mutational analysis of the p73 gene localized at chromosome 1p36.3 in colorectal carcinomas. Int. J. Oncol. **13:**319-23.
738. **Susin, S. A., N. Zamzami, M. Castedo, E. Daugas, H. G. Wang, S. Geley, F. Fassy, J. C. Reed, and G. Kroemer.** 1997. The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide- induced apoptosis. J. Exp. Med. **186:**25-37.
739. **Susin, S. A., N. Zamzami, M. Castedo, T. Hirsch, P. Marchetti, A. Macho, E. Daugas, M. Geuskens, and G. Kroemer.** 1996. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J. Exp. Med. **184:**1331-41.
740. **Sutherland, C., J. Alterio, D. G. Campbell, B. Le Bourdelles, J. Mallet, J. Haavik, and P. Cohen.** 1993. Phosphorylation and activation of human tyrosine hydroxylase in vitro by mitogen-activated protein (MAP) kinase and MAP-kinase-activated kinases 1 and 2. Eur. J. Biochem. **217:**715-22.
741. **Symonds, H., L. Krall, L. Remington, M. Saenz-Robles, S. Lowe, T. Jacks, and T. Van Dyke.** 1994. p53-dependent apoptosis suppresses tumor growth and progression in vivo. Cell **78:**703-11.
742. **Tabibzadeh, S.** 1996. The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling. Mol. Hum. Reprod. **2:**77-92.
743. **Takahashi, T., D. Carbone, T. Takahashi, M. M. Nau, T. Hida, I. Linnoila, R. Ueda, and J. D. Minna.** 1992. Wild-type but not mutant p53 suppresses the growth of human lung cancer cells bearing multiple genetic lesions. Cancer Res. **52:**2340-3.
744. **Tan, X., S. J. Martin, D. R. Green, and J. Y. J. Wang.** 1997. Degradation of retinoblastoma protein in tumor necrosis factor- and CD95-induced cell death. J. Biol. Chem. **272:**9613-6.
745. **Tan, X., and J. Y. Wang.** 1998. The caspase-RB connection in cell death. Trends Cell Biol. **8:**116-20.
746. **Tanaka, E. M., and M. W. Kirschner.** 1991. Microtubule behavior in the growth cones of living neurons during axon elongation. J. Cell Biol. **115:**345-63.
747. **Tang, C., M. C. Willingham, J. C. Reed, T. Miyashita, S. Ray, V. Ponnathpur, Y. Huang, M. E. Mahoney, G. Bullock, and K. Bhalla.** 1994. High levels of p26BCL-2 oncoprotein retard taxol-induced apoptosis in human pre-B leukemia cells. Leukemia **8:**1960-9.
748. **Tata, J. R.** 1966. Requirement for RNA and protein synthesis for induced regression of the tadpole tail in organ culture. Dev. Biol. **13:**77-94.
749. **Taylor, S. S., E. Ha, and F. McKeon.** 1998. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. J. Cell Biol. **142:**1-11.
750. **Taylor, S. S., and F. McKeon.** 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell **89:**727-35.
751. **Teixeira, C., J. C. Reed, and M. A. Pratt.** 1995. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. Cancer Res. **55:**3902-7.
752. **ter Haar, E., R. J. Kowalski, E. Hamel, C. M. Lin, R. E. Longley, S. P. Gunasekera, H. S. Rosenkranz, and B. W. Day.** 1996. Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol. Biochemistry **35:**243-50.
753. **Teramoto, H., P. Crespo, O. A. Coso, T. Igishi, N. Xu, and J. S. Gutkind.** 1996. The small GTP-binding protein rho activates c-Jun N-terminal kinases/stress-activated protein kinases in human kidney 293T cells. Evidence for a Pak-independent signaling pathway. J. Biol. Chem. **271:**25731-4.
754. **Terzis, A. J., F. Thorsen, O. Heese, T. Visted, R. Bjerkvig, O. Dahl, H. Arnold, and G. Gundersen.** 1997. Proliferation, migration and invasion of human glioma cells exposed to paclitaxel (Taxol) in vitro. Br J Cancer **75:**1744-52.
755. **Theis, S., and K. Roemer.** 1998. c-Abl tyrosine kinase can mediate tumor cell apoptosis independently of the Rb and p53 tumor suppressors. Oncogene **17:**557-64.
756. **Thome, M., P. Schneider, K. Hofmann, H. Fickenscher, E. Meinl, F. Neipel, C. Mattmann, K. Burns, J. L. Bodmer, M. Schroter, C. Scaffidi, P. H. Krammer, M. E. Peter, and J. Tschopp.** 1997. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature **386:**517-21.
757. **Thompson, C. B.** 1995. Apoptosis in the pathogenesis and treatment of disease. Science **267:**1456-62.
758. **Thompson, E. B.** 1998. The many roles of c-Myc in apoptosis. Annu. Rev. Physiol. **60:**575-600.
759. **Tilly, J. L.** 1996. The molecular basis of ovarian cell death during germ cell attrition, follicular atresia, and luteolysis. Front. Biosci. **1:**d1-d11.
760. **Timokhina, I., H. Kissel, G. Stella, and P. Besmer.** 1998. Kit signaling through PI 3-kinase and Src kinase pathways: an essential role for Rac1 and JNK activation in mast cell proliferation. Embo J **17:**6250-6262.
761. **Tishler, R. B., C. R. Geard, E. J. Hall, and P. B. Schiff.** 1992. Taxol sensitizes human astrocytoma cells to radiation. Cancer Res. **52:**3495-7.
762. **Tomei, L. D., J. P. Shapiro, and F. O. Cope.** 1993. Apoptosis in C3H/10T1/2 mouse embryonic cells: evidence for

internucleosomal DNA modification in the absence of double-strand cleavage. Proc. Natl. Acad. Sci. U S A **90:**853-7.
763. **Tonks, N. K., and B. G. Neel.** 1996. From form to function: signaling by protein tyrosine phosphatases. Cell **87:**365-368.
764. **Tournebize, R., S. S. Andersen, F. Verde, M. Doree, E. Karsenti, and A. A. Hyman.** 1997. Distinct roles of PP1 and PP2A-like phosphatases in control of microtubule dynamics during mitosis. EMBO J. **16:**5537-49.
765. **Tournier, C., A. J. Whitmarsh, J. Cavanagh, T. Barrett, and R. J. Davis.** 1997. Mitogen-activated protein kinase kinase 7 is an activator of the c-Jun NH2-terminal kinase. Proc. Natl.Acad. Sci. U S A **94:**7337-42.
766. **Toyoshima, F., T. Moriguchi, and E. Nishida.** 1997. Fas induces cytoplasmic apoptotic responses and activation of the MKK7- JNK/SAPK and MKK6-p38 pathways independent of CPP32-like proteases. J. Cell Biol. **139:**1005-15.
767. **Treier, M., L. M. Staszewski, and D. Bohmann.** 1994. Ubiquitin-dependent c-Jun degradation in vivo is mediated by the delta domain. Cell **78:**787-98.
768. **Trielli, M. O., P. R. Andreassen, F. B. Lacroix, and R. L. Margolis.** 1996. Differential Taxol-dependent arrest of transformed and nontransformed cells in the G1 phase of the cell cycle, and specific-related mortality of transformed cells. J. Cell Biol. **135:**689-700.
769. **Trink, B., K. Okami, L. Wu, V. Sriuranpong, J. Jen, and D. Sidransky.** 1998. A new human p53 homologue. Nat. Med. **4:**747-8.
770. **Turner, N. A., F. Xia, G. Azhar, X. Zhang, L. Liu, and J. Y. Wei.** 1998. Oxidative stress induces DNA fragmentation and caspase activation via the c-Jun NH2-terminal kinase pathway in H9c2 cardiac muscle cells. J. Mol. Cell. Cardiol. **30:**1789-801.
771. **Ucker, D. S., P. S. Obermiller, W. Eckhart, J. R. Apgar, N. A. Berger, and J. Meyers.** 1992. Genome digestion is a dispensable consequence of physiological cell death mediated by cytotoxic T lymphocytes. Mol. Cell. Biol. **12:**3060-9.
772. **Uren, A. G., M. Pakusch, C. J. Hawkins, K. L. Puls, and D. L. Vaux.** 1996. Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. Proc. Natl. Acad. Sci. U S A **93:**4974-8.
773. **Vairo, G., K. M. Innes, and J. M. Adams.** 1996. Bcl-2 has a cell cycle inhibitory function separable from its enhancement of cell survival. Oncogene **13:**1511-9.
774. **van Buskirk, R., and U. K. Liyanage.** 1997. Cytoskeleton and cell movement, An electronic companion to Molecular Cell Biology. Cogito Learning Media, Inc., New York.
775. **van Dam, H., D. Wilhelm, I. Herr, A. Steffen, P. Herrlich, and P. Angel.** 1995. ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. EMBO. J. **14:**1798-811.
776. **Van Parijs, L., and A. K. Abbas.** 1998. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. Science **280:**243-8.
777. **Vasey, P. A., N. A. Jones, S. Jenkins, C. Dive, and R. Brown.** 1996. Cisplatin, camptothecin, and taxol sensitivities of cells with p53- associated multidrug resistance. Mol. Pharmacol. **50:**1536-40.
778. **Vasquez, R. J., B. Howell, A. M. Yvon, P. Wadsworth, and L. Cassimeris.** 1997. Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. Mol. Biol. Cell **8:**973-85.
779. **Vaux, D. L., S. Cory, and J. M. Adams.** 1988. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c- myc to immortalize pre-B cells. Nature **335:**440-2.
780. **Vaux, D. L., and A. Strasser.** 1996. The molecular biology of apoptosis. Proc. Natl. Acad. Sci. USA **93:**2239-2244.
781. **Vayssiere, J. L., P. X. Petit, Y. Risler, and B. Mignotte.** 1994. Commitment to apoptosis is associated with changes in mitochondrial biogenesis and activity in cell lines conditionally immortalized with simian virus 40. Proc. Nat. Acad. Sci. U S A **91:**11752-6.
782. **Verheij, M., R. Bose, X. H. Lin, B. Yao, W. D. Jarvis, S. Grant, M. J. Birrer, E. Szabo, L. I. Zon, J. M. Kyriakis, A. Haimovitz-Friedman, Z. Fuks, and R. N. Kolesnick.** 1996. Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. Nature **380:**75-9.
783. **Verhoven, B., R. A. Schlegel, and P. Williamson.** 1995. Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. J. Exp. Med. **182:**1597-601.
784. **Vidensek, N., P. Lim, A. Campbell, and C. Carlson.** 1990. Taxol content in bark, wood, root, leaf, twig, and seedling from several Taxus species. J. Nat. Prod. **53:**1609-10.
785. **Vikhanskaya, F., S. Vignati, P. Beccaglia, C. Ottoboni, P. Russo, D. I. M, and M. Broggini.** 1998. Inactivation of p53 in a human ovarian cancer cell line increases the sensitivity to paclitaxel by inducing G2/M arrest and apoptosis. Exp. Cell. Res. **241:**96-101.
786. **Villa, P., S. H. Kaufmann, and W. C. Earnshaw.** 1997. Caspases and caspase inhibitors. Trends Biochem. Sci. **22:**388-93.
787. **Voll, R. E., M. Herrmann, E. A. Roth, C. Stach, J. R. Kalden, and I. Girkontaite.** 1997. Immunosuppressive effects of apoptotic cells. Nature **390:**350-1.
788. **Wahl, A. F., K. L. Donaldson, C. Fairchild, F. Y. Lee, S. A. Foster, G. W. Demers, and D. A. Galloway.** 1996. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nat. Med. **2:**72-9.
789. **Walczak, C. E., T. J. Mitchison, and A. Desai.** 1996. XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell **84:**37-47.
790. **Walker, J. E., M. Saraste, M. J. Runswick, and N. J. Gay.** 1982. Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO. J. **1:**945-51.
791. **Walker, N. I., R. E. Bennett, and J. F. Kerr.** 1989. Cell death by apoptosis during involution of the lactating breast in mice and rats. Am. J. Anat. **185:**19-32.
792. **Walker, R. A., E. T. O'Brien, N. K. Pryer, M. F. Soboeiro, W. A. Voter, H. P. Erickson, and E. D. Salmon.** 1988. Dynamic instability of individual microtubules analyzed by video light microscopy: rate constants and transition frequencies. J Cell Biol **107:**1437-48.
793. **Wallach, D.** 1997. Cell death induction by TNF: a matter of self control. Trends Biochem. Sci. **22:**107-109.

794. **Walter, B. N., Z. Huang, R. Jakobi, P. T. Tuazon, E. S. Alnemri, G. Litwack, and J. A. Traugh.** 1998. Cleavage and activation of p21-activated protein kinase gamma-PAK by CPP32 (caspase 3). Effects of autophosphorylation on activity. J. Biol. Chem. **273:**28733-9.
795. **Wang, H., R. J. Grand, A. E. Milner, R. J. Armitage, J. Gordon, and C. D. Gregory.** 1996. Repression of apoptosis in human B-lymphoma cells by CD40-ligand and Bcl-2: relationship to the cell-cycle and role of the retinoblastoma protein. Oncogene **13:**373-9.
796. **Wang, H. G., U. R. Rapp, and J. C. Reed.** 1996. Bcl-2 targets the protein kinase Raf-1 to mitochondria. Cell **87:**629-38.
797. **Wang, H. G., S. Takayama, U. R. Rapp, and J. C. Reed.** 1996. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf- 1. Proc. Natl. Acad. Sci. U S A **93:**7063-8.
798. **Wang, J. Y., E. S. Knudsen, and P. J. Welch.** 1994. The retinoblastoma tumor suppressor protein. Adv. Cancer Res. **64:**25-85.
799. **Wang, S., C. Y. Guo, A. Castillo, P. Dent, and S. Grant.** 1998. Effect of bryostatin 1 on taxol-induced apoptosis and cytotoxicity in human leukemia cells (U937). Biochem. Pharmacol. **56:**635-44.
800. **Wang, T. H., D. M. Popp, H. S. Wang, M. Saitoh, J. G. Mural, D. C. Henley, H. Ichijo, and J. Wimalasena.** 1999. Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells. J. Biol. Chem. :in press.
801. **Wang, T. H., and H. S. Wang.** 1995. Gestational trophoblastic diseases: current trends and perspectives. J. Formos. Med. Assoc. **94:**449-457.
802. **Wang, T. H., and H. S. Wang.** 1996. p53, apoptosis and human cancers. J. Formos. Med. Assoc. **95:**509-522.
803. **Wang, T. H., H. S. Wang, H. Ichijo, P. Giannakakou, J. S. Foster, T. Fojo, and J. Wimalasena.** 1998. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress- activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J. Biol. Chem. **273:**4928-36.
804. **Wang, X., J. L. Martindale, Y. Liu, and N. J. Holbrook.** 1998. The cellular response to oxidative stress: influences of mitogen- activated protein kinase signalling pathways on cell survival. Biochem. J. **333:**291-300.
805. **Wang, X. S., K. Diener, D. Jannuzzi, D. Trollinger, T. H. Tan, H. Lichenstein, M. Zukowski, and Z. Yao.** 1996. Molecular cloning and characterization of a novel protein kinase with a catalytic domain homologous to mitogen-activated protein kinase kinase kinase. J. Biol. Chem. **271:**31607-11.
806. **Wang, X. Z., and D. Ron.** 1996. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP Kinase. Science **272:**1347-9.
807. **Wang, Y., S. Huang, V. P. Sah, J. Ross, Jr., J. H. Brown, J. Han, and K. R. Chien.** 1998. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J. Biol.Chem. **273:**2161-8.
808. **Wang, Y., T. Ramqvist, L. Szekely, H. Axelson, G. Klein, and K. G. Wiman.** 1993. Reconstitution of wild-type p53 expression triggers apoptosis in a p53- negative v-myc retrovirus-induced T-cell lymphoma line. Cell Growth Differ. **4:**467-73.
809. **Wani, M. C., H. L. Taylor, M. E. Wall, P. Coggon, and A. T. McPhail.** 1971. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J. Am. Chem. Soc. **93:**2325-7.
810. **Waskiewicz, A. J., and J. A. Cooper.** 1995. Mitogen and stress response pathways: MAP kinase cascades and phosphatase regulation in mammals and yeast. Curr. Opin.Cell Biol. **7:**798-805.
811. **Watabe, M., K. Ito, Y. Masuda, S. Nakajo, and K. Nakaya.** 1998. Activation of AP-1 is required for bufalin-induced apoptosis in human leukemia U937 cells. Oncogene **16:**779-87.
812. **Waterman-Storer, C. M., and E. D. Salmon.** 1997. Microtubule dynamics: treadmilling comes around again. Curr. Biol. **7:**R369-72.
813. **Waters, J. C., and E. Salmon.** 1997. Pathways of spindle assembly. Curr. Opin. Cell Biol. **9:**37-43.
814. **Webb, A., D. Cunningham, F. Cotter, P. A. Clarke, F. di Stefano, P. Ross, M. Corbo, and Z. Dziewanowska.** 1997. BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. Lancet **349:**1137-41.
815. **Weiss, R. B., R. C. Donehower, P. H. Wiernik, T. Ohnuma, R. J. Gralla, D. L. Trump, J. R. Baker, Jr., D. A. Van Echo, D. D. Von Hoff, and B. Leyland-Jones.** 1990. Hypersensitivity reactions from taxol. J. Clin. Oncol. **8:**1263-8.
816. **Werner, H., E. Karnieli, F. J. Rauscher, and D. LeRoith.** 1996. Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene. Proc. Natl. Acad. Sci. U S A **93:**8318-23.
817. **White, A. E., E. M. Livanos, and T. D. Tlsty.** 1994. Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. Genes Dev. **8:**666-77.
818. **Whitmarsh, A. J., J. Cavanagh, C. Tournier, J. Yasuda, and R. J. Davis.** 1998. A mammalian scaffold complex that selectively mediates MAP kinase activation. Science **281:**1671-4.
819. **Whitmarsh, A. J., P. Shore, A. D. Sharrocks, and R. J. Davis.** 1995. Integration of MAP kinase signal transduction pathways at the serum response element. Science **269:**403-7.
820. **Wiegmann, K., S. Schutze, T. Machleidt, D. Witte, and M. Kronke.** 1994. Functional dichotomy of neutral and acidic sphingomyelinases in tumor necrosis factor signaling. Cell **78:**1005-15.
821. **Wijsman, J. H., R. R. Jonker, R. Keijzer, and e. al.** 1993. A new method to detect apoptosis in paraffin section: in situ end-labelling of fragmented DNA. J. Histochem. Cytochem. **41:**7-21.
822. **Williams, G. T.** 1991. Programmed cell death: apoptosis and oncogenesis. Cell **65:**1097-8.
823. **Williams, G. T., C. A. Smith, E. Spooncer, T. M. Dexter, and D. R. Taylor.** 1990. Haemopoietic colony stimulating factors promote cell survival by suppressing apoptosis. Nature **343:**76-9.
824. **Wilson, D. J., K. A. Fortner, D. H. Lynch, R. R. Mattingly, L. G. Macara, J. A. Posada, and R. C. Budd.** 1996. JNK, but not MAPK, activation is associated with Fas-mediated apoptosis in human T cells. Eur. J. Immunol. **26:**989-94.
825. **Wisdom, R., R. S. Johnson, and C. Moore.** 1999. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. EMBO. J. **18:**188-197.
826. **Wolff, R. A., R. T. Dobrowsky, A. Bielawska, L. M. Obeid, and Y. A. Hannun.** 1994. Role of ceramide-activated protein

phosphatase in ceramide-mediated signal transduction. J. Biol. Chem. **269:**19605-9.

827. **Wolfson, M., C. P. Yang, and S. B. Horwitz.** 1997. Taxol induces tyrosine phosphorylation of Shc and its association with Grb2 in murine RAW 264.7 cells. Int. J. Cancer. **70:**248-52.

828. **Wood, A. C., C. M. Waters, A. Garner, and J. A. Hickman.** 1994. Changes in c-myc expression and the kinetics of dexamethasone-induced programmed cell death (apoptosis) in human lymphoid leukaemia cells. Br. J. Cancer **69:**663-9.

829. **Woods, C. M., J. Zhu, P. A. McQueney, D. Bollag, and E. Lazarides.** 1995. Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. Mol. Med. **1:**506-26.

830. **Wordeman, L., and T. J. Mitchison.** 1994. Dynamics of microtubule assembly in vivo., p. 287-301. *In* J. S. Hyams, and C. W. Lloyd (ed.), Microtubules, vol. 13. John Wiley and Sons, Inc.,, New York.

831. **Wright, S. C., J. Zhong, and J. W. Larrick.** 1994. Inhibition of apoptosis as a mechanism of tumor promotion. FASEB. J. **8:**654-60.

832. **Wu, C. L., L. R. Zukerberg, C. Ngwu, E. Harlow, and J. A. Lees.** 1995. In vivo association of E2F and DP family proteins. Mol. Cell. Biol. **15:**2536-46.

833. **Wu, D., H. D. Wallen, N. Inohara, and G. Nunez.** 1997. Interaction and regulation of the Caenorhabditis elegans death protease CED-3 by CED-4 and CED-9. J. Biol. Chem. **272:**21449-54.

834. **Wu, D., H. D. Wallen, and G. Nunez.** 1997. Interaction and regulation of subcellular localization of CED-4 by CED- 9. Science **275:**1126-9.

835. **Wu, X., and A. J. Levine.** 1994. p53 and E2f-1 cooperate to mediate apoptosis. Proc. Natl. Acad. Sci. U S A **91:**3602-6.

836. **Wu, X., S. J. Noh, G. Zhou, J. E. Dixon, and K. L. Guan.** 1996. Selective activation of MEK1 but not MEK2 by A-Raf from epidermal growth factor-stimulated Hela cells. J. Biol. Chem. **271:**3265-71.

837. **Wu, Y. C., and H. R. Horvitz.** 1998. C. elegans phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. Nature **392:**501-4.

838. **Wyllie, A.** 1997. Apoptosis. Clues in the p53 murder mystery. Nature **389:**237-8.

839. **Wyllie, A. H.** 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature **284:**555-6.

840. **Wyllie, A. H., J. F. Kerr, I. A. Macaskill, and A. R. Currie.** 1973. Adrenocortical cell deletion: the role of ACTH. J. Pathol. **111:**85-94.

841. **Wyllie, A. H., R. G. Morris, A. L. Smith, and e. al.** 1984. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J. Pathol. **142:**67-77.

842. **Xia, Z., M. Dickens, J. Raingeaud, R. J. Davis, and M. E. Greenberg.** 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science **270:**1326-31.

843. **Xiang, J., D. T. Chao, and S. J. Korsmeyer.** 1996. BAX-induced cell death may not require interleukin 1 beta-converting enzyme-like proteases. Proc. Natl. Acad. Sci. U S A **93:**14559-63.

844. **Xu, F. H., S. Sharma, A. Gardner, Y. Tu, A. Raitano, C. Sawyers, and A. Lichtenstein.** 1998. Interleukin-6-induced inhibition of multiple myeloma cell apoptosis: support for the hypothesis that protection is mediated via inhibition of the JNK/SAPK pathway. Blood **92:**241-51.

845. **Xu, G., D. M. Livingston, and W. Krek.** 1995. Multiple members of the E2F transcription factor family are the products of oncogenes. Proc. Natl. Acad. Sci. U S A **92:**1357-61.

846. **Xu, S., and M. H. Cobb.** 1997. MEKK1 binds directly to the c-Jun N-terminal kinases/stress-activated protein kinases. J. Biol. Chem. **272:**32056-60.

847. **Xu, S., D. Robbins, J. Frost, A. Dang, C. Lange-Carter, and M. H. Cobb.** 1995. MEKK1 phosphorylates MEK1 and MEK2 but does not cause activation of mitogen-activated protein kinase. Proc. Natl. Acad. Sci. U S A **92:**6808-12.

848. **Xu, S., D. J. Robbins, L. B. Christerson, J. M. English, C. A. Vanderbilt, and M. H. Cobb.** 1996. Cloning of rat MEK kinase 1 cDNA reveals an endogenous membrane- associated 195-kDa protein with a large regulatory domain. Proc. Natl. Acad. Sci. U S A **93:**5291-5.

849. **Xu, X., J. Raber, D. Yang, B. Su, and L. Mucke.** 1997. Dynamic regulation of c-Jun N-terminal kinase activity in mouse brain by environmental stimuli. Proc. Natl. Acad. Sci. U S A **94:**12655-60.

850. **Xue, D., and H. R. Horvitz.** 1997. Caenorhabditis elegans CED-9 protein is a bifunctional cell-death inhibitor. Nature **390:**305-8.

851. **Xue, D., and H. R. Horvitz.** 1995. Inhibition of the Caenorhabditis elegans cell-death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. Nature **377:**248-51.

852. **Yamaguchi, K., K. Shirakabe, H. Shibuya, K. Irie, I. Oishi, N. Ueno, T. Taniguchi, E. Nishida, and K. Matsumoto.** 1995. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. Science **270:**2008-11.

853. **Yamasaki, L., R. Bronson, B. O. Williams, N. J. Dyson, E. Harlow, and T. Jacks.** 1998. Loss of E2F-1 reduces tumorigenesis and extends the lifespan of Rb1(+/-)mice. Nat. Genet. **18:**360-4.

854. **Yamasaki, L., T. Jacks, R. Bronson, E. Goillot, E. Harlow, and N. J. Dyson.** 1996. Tumor induction and tissue atrophy in mice lacking E2F-1. Cell **85:**537-48.

855. **Yan, M., T. Dai, J. C. Deak, J. M. Kyriakis, L. I. Zon, J. R. Woodgett, and D. J. Templeton.** 1994. Activation of stress-activated protein kinase by MEKK1 phosphorylation of its activator SEK1. Nature **372:**798-800.

856. **Yang, D. D., C. Y. Kuan, A. J. Whitmarsh, M. Rincon, T. S. Zheng, R. J. Davis, P. Rakic, and R. A. Flavell.** 1997. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the JNK3 gene. Nature **389:**865-70.

857. **Yang, J., X. Liu, K. Bhalla, C. N. Kim, A. M. Ibrado, J. Cai, T. I. Peng, D. P. Jones, and X. Wang.** 1997. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science **275:**1129-32.

858. **Yang, X., H. Y. Chang, and D. Baltimore.** 1998. Essential role of CED-4 oligomerization in CED-3 activation and apoptosis. Science **281:**1355-7.

859. **Yang, X., R. Khosravi-Far, H. Y. Chang, and D. Baltimore.** 1997. Daxx, a novel Fas-binding protein that activates JNK and apoptosis. Cell **89:**1067-76.

860. **Yao, Z., K. Diener, X. S. Wang, M. Zukowski, G. Matsumoto, G. Zhou, R. Mo, T. Sasaki, H. Nishina, C. C. Hui, T. H. Tan, J. P. Woodgett, and J. M. Penninger.** 1997. Activation of stress-activated protein kinases/c-Jun N-terminal protein kinases (SAPKs/JNKs) by a novel mitogen-activated protein kinase kinase. J. Biol. Chem. **272:**32378-83.

861. **Yeh, W. C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J. L. de la Pompa, D. Ferrick, B. Hum, N. Iscove, P. Ohashi, M. Rothe, D. V. Goeddel, and T. W. Mak.** 1997. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. Immunity **7:**715-25.

862. **Yen, W. C., M. G. Wientjes, and J. L. Au.** 1996. Differential effect of taxol in rat primary and metastatic prostate tumors: site-dependent pharmacodynamics. Pharm. Res. **13:**1305-12.

863. **Yin, T., G. Sandhu, C. D. Wolfgang, A. Burrier, R. L. Webb, D. F. Rigel, T. Hai, and J. Whelan.** 1997. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. J. Biol. Chem. **272:**19943-50.

864. **Yin, X. M., Z. N. Oltvai, and S. J. Korsmeyer.** 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. Nature **369:**321-3.

865. **Yin, Y., M. A. Tainsky, F. Z. Bischoff, L. C. Strong, and G. M. Wahl.** 1992. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell **70:**937-48.

866. **Yin, Y., Y. Terauchi, G. G. Solomon, S. Aizawa, P. N. Rangarajan, Y. Yazaki, T. Kadowaki, and J. C. Barrett.** 1998. Involvement of p85 in p53-dependent apoptotic response to oxidative stress. Nature **391:**707-10.

867. **Yonish-Rouach, E., D. Resnitzky, J. Lotem, L. Sachs, A. Kimchi, and M. Oren.** 1991. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature **352:**345-7.

868. **Yoon, M. Y., and P. F. Cook.** 1987. Chemical mechanism of the adenosine cyclic 3',5'-monophosphate dependent protein kinase from pH studies. Biochemistry **26:**4118-25.

869. **Young, L. S., C. W. Dawson, and A. G. Eliopoulos.** 1997. Viruses and apoptosis. Br. Med. Bull. **53:**509-21.

870. **Yu, D., T. Jing, B. Liu, J. Yao, M. Tan, T. J. McDonnell, and M. C. Hung.** 1998. Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of p21Cip1, which inhibits p34Cdc2 kinase. Mol. Cell **2:**581-91.

871. **Yu, R., A. A. Shtil, T. H. Tan, I. B. Roninson, and A. N. Kong.** 1996. Adriamycin activates c-jun N-terminal kinase in human leukemia cells: a relevance to apoptosis. Cancer Lett. **107:**73-81.

872. **Yu, W., M. Simmons-Menchaca, H. You, P. Brown, M. J. Birrer, B. G. Sanders, and K. Kline.** 1998. RRR-alpha-tocopheryl succinate induction of prolonged activation of c- jun amino-terminal kinase and c-jun during induction of apoptosis in human MDA-MB-435 breast cancer cells. Mol. Carcinog. **22:**247-57.

873. **Yuan, J., S. Shaham, S. Ledoux, H. M. Ellis, and H. R. Horvitz.** 1993. The C. elegans cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. Cell **75:**641-52.

874. **Yuan, Z. M., Y. Huang, T. Ishiko, S. Kharbanda, R. Weichselbaum, and D. Kufe.** 1997. Regulation of DNA damage-induced apoptosis by the c-Abl tyrosine kinase. Proc. Natl. Acad. Sci. U S A **94:**1437-40.

875. **Yue, T. L., C. Wang, A. M. Romanic, K. Kikly, P. Keller, W. E. DeWolf, Jr., T. K. Hart, H. C. Thomas, B. Storer, J. L. Gu, X. Wang, and G. Z. Feuerstein.** 1998. Staurosporine-induced apoptosis in cardiomyocytes: A potential role of caspase-3. J. Mol. Cell. Cardiol. **30:**495-507.

876. **Zaffaroni, N., R. Silvestrini, L. Orlandi, A. Bearzatto, D. Gornati, and R. Villa.** 1998. Induction of apoptosis by taxol and cisplatin and effect on cell cycle- related proteins in cisplatin-sensitive and -resistant human ovarian cells. Br. J. Cancer **77:**1378-85.

877. **Zamzami, N., P. Marchetti, M. Castedo, D. Decaudin, A. Macho, T. Hirsch, S. A. Susin, P. X. Petit, B. Mignotte, and G. Kroemer.** 1995. Sequential reduction of mitochondrial transmembrane potential and generation of reactive oxygen species in early programmed cell death. J. Exp. Med. **182:**367-77.

878. **Zamzami, N., S. A. Susin, P. Marchetti, T. Hirsch, I. Gomez-Monterrey, M. Castedo, and G. Kroemer.** 1996. Mitochondrial control of nuclear apoptosis. J. Exp. Med. **183:**1533-44.

879. **Zanke, B. W., C. Lee, S. Arab, and I. F. Tannock.** 1998. Death of tumor cells after intracellular acidification is dependent on stress-activated protein kinases (SAPK/JNK) pathway activation and cannot be inhibited by Bcl-2 expression or interleukin 1beta-converting enzyme inhibition. Cancer Res **58:**2801-8.

880. **Zanke, B. W., E. A. Rubie, E. Winnett, J. Chan, S. Randall, M. Parsons, K. Boudreau, M. McInnis, M. Yan, D. J. Templeton, and J. R. Woodgett.** 1996. Mammalian mitogen-activated protein kinase pathways are regulated through formation of specific kinase-activator complexes. J. Biol. Chem. **271:**29876-81.

881. **Zerial, M., L. Toschi, R. P. Ryseck, M. Schuermann, R. Muller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, fos B, interacts with JUN proteins enhancing their DNA binding activity. EMBO. J. **8:**805-13.

882. **Zervos, A. S., L. Faccio, J. P. Gatto, J. M. Kyriakis, and R. Brent.** 1995. Mxi2, a mitogen-activated protein kinase that recognizes and phosphorylates Max protein. Proc. Natl. Acad. Sci. U S A **92:**10531-4.

883. **Zhan, Q., I. T. Chen, M. J. Antinore, and A. J. Fornace, Jr.** 1998. Tumor suppressor p53 can participate in transcriptional induction of the GADD45 promoter in the absence of direct DNA binding. Mol. Cell. Biol. **18:**2768-78.

884. **Zhan, Q., S. Fan, I. Bae, C. Guillouf, D. A. Liebermann, O. C. PM, and A. J. Fornace, Jr.** 1994. Induction of bax by genotoxic stress in human cells correlates with normal p53 status and apoptosis. Oncogene **9:**3743-51.

885. **Zhan, Q., K. A. Lord, I. Alamo, Jr., M. C. Hollander, F. Carrier, D. Ron, K. W. Kohn, B. Hoffman, D. A. Liebermann, and A. J. Fornace, Jr.** 1994. The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. Mol. Cell. Biol. **14:**2361-71.

886. **Zhang, C. C., J. M. Yang, E. White, M. Murphy, A. Levine, and W. N. Hait.** 1998. The role of MAP4 expression in the sensitivity to paclitaxel and resistance to vinca alkaloids in p53 mutant cells. Oncogene **16:**1617-24.

887. **Zhang, J., N. Alter, J. C. Reed, C. Borner, L. M. Obeid, and Y. A. Hannun.** 1996. Bcl-2 interrupts the ceramide-mediated pathway of cell death. Proc. Natl. Acad. Sci. U S A **93:**5325-8.

888. **Zhang, J., X. Liu, D. C. Scherer, L. van Kaer, X. Wang, and M. Xu.** 1998. Resistance to DNA fragmentation and chromatin condensation in mice lacking the DNA fragmentation factor 45. Proc. Natl. Acad. Sci. U S A **95:**12480-5.
889. **Zhang, S., J. Han, M. A. Sells, J. Chernoff, U. G. Knaus, R. J. Ulevitch, and G. M. Bokoch.** 1995. Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. J. Biol. Chem. **270:**23934-6.
890. **Zhao, M., L. New, V. V. Kravchenko, Y. Kato, H. Gram, F. di Padova, E. N. Olson, R. J. Ulevitch, and J. Han.** 1999. Regulation of the MEF2 Family of Transcription Factors by p38. Mol. Cell. Biol. **19:**21-30.
891. **Zheng, C. F., and K. L. Guan.** 1994. Activation of MEK family kinases requires phosphorylation of two conserved Ser/Thr residues. EMBO J. **13:**1123-31.
892. **Zheng, C. F., and K. L. Guan.** 1993. Cloning and characterization of two distinct human extracellular signal- regulated kinase activator kinases, MEK1 and MEK2. J. Biol. Chem. **268:**11435-9.
893. **Zhivotovsky, B., S. Orrenius, O. T. Brustugun, and S. O. Doskeland.** 1998. Injected cytochrome c induces apoptosis. Nature **391:**449-450.
894. **Zhou, B. B., H. Li, J. Yuan, and M. W. Kirschner.** 1998. Caspase-dependent activation of cyclin-dependent kinases during Fas- induced apoptosis in Jurkat cells. Proc. Natl. Acad. Sci. U S A **95:**6785-90.
895. **Ziegler, A., A. S. Jonason, D. J. Leffell, J. A. Simon, H. W. Sharma, J. Kimmelman, L. Remington, T. Jacks, and D. E. Brash.** 1994. Sunburn and p53 in the onset of skin cancer. Nature **372:**773-6.
896. **Zinck, R., M. A. Cahill, M. Kracht, C. Sachsenmaier, R. A. Hipskind, and A. Nordheim.** 1995. Protein synthesis inhibitors reveal differential regulation of mitogen-activated protein kinase and stress-activated protein kinase pathways that converge on Elk-1. Mol. Cell. Biol. **15:**4930-8.
897. **Zolnierowicz, S., and B. A. Hemmings.** 1996. Protein phosphatases on the piste. Trends Cell Biol. **6:**359-362.
898. **Zou, H., W. J. Henzel, X. Liu, A. Lutschg, and X. Wang.** 1997. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell **90:**405-13.
899. **Zou, H., and L. Niswander.** 1996. Requirement for BMP signaling in interdigital apoptosis and scale formation. Science **272:**738-41.

PART II.

RATIONALE AND OBJECTIVES

RATIONALE

1. Paclitaxel is a promising chemotherapeutic drug against both advanced stage ovarian and breast cancers. Further understanding the mechanisms by which it kills cancer cells should help us develop more effective strategies to treat patients with these two gynecological cancers.
2. The most extensively studied target of paclitaxel is the microtubule and suppression of microtubule dynamics is believed to be the mechanism by which paclitaxel exerts its effects on the cell. For instance, through disruption of functional microtubule spindles, paclitaxel arrests mitotic cells at the transition from metaphase to anaphase.
3. In addition to serving as a mechanical device to separate duplicated chromatids in mitotic cells and as a major cytoskeletal component in interphase cells, microtubules may have other physiological functions that require such complex exercises as dynamic instability and treadmilling.
4. Due to suppression of microtubule dynamics, paclitaxel-stabilized microtubules become rigid and dysfunctional. Through its binding to microtubules, paclitaxel may initiate a unique type of intracellular stress.
5. Indeed, I have observed that paclitaxel activates JNK/SAPK, which is a recently identified stress-related kinase. However, it remains unknown: (i) how paclitaxel activates the JNK pathway, and (ii) what is/are the function(s) of paclitaxel-activated JNK.

OBJECTIVES

1. I would like to characterize the pattern of paclitaxel-induced JNK activation, such as the cell type specificity, the dose-effect relationship, and the time course.
2. I want to determine whether activation of JNK is a consequence of paclitaxel binding to microtubules or is independent of microtubular interactions.
3. The signal transduction pathway(s) utilized by the paclitaxel-treated cells leading to JNK activation need to be identified.
4. Since paclitaxel kills cells through apoptosis and JNK may be involved in apoptosis, I would like to elucidate the role of JNK activation in the paclitaxel-induced apoptosis.

PART III.

MICROTUBULE-INTERFERING AGENTS ACTIVATE C-JUN N-TERMINAL KINASE/STRESS-ACTIVATED PROTEIN KINASE (JNK/SAPK) THROUGH BOTH RAS AND APOPTOSIS SIGNAL-REGULATING KINASE (ASK1) PATHWAYS

ABSTRACT

The essential cellular functions associated with microtubules (MTs) have led to a wide use of MT-interfering agents in cancer chemotherapy with promising results. Although the most well-studied effect of MT-interfering agents is an arrest of cells at the G_2/M phase of the cell cycle, other effects may also exist. I have observed that paclitaxel (Taxol), docetaxel (Taxotere), vinblastine, vincristine, nocodazole and colchicine activate the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) signaling pathway in a variety of human cells. Activation of JNK/SAPK by MT-interfering agents is dose-dependent and time-dependent and requires interactions with MTs. Functional activation of the JNKK/SEK1-JNK/SAPK-c Jun cascade was demonstrated by cotransfection with a TPA-response element (TRE) reporter construct and dominant negative signal transducers followed by chloramphenicol acetyl-transferase (CAT) assays. MT-interfering agents also activate both Ras and apoptosis signal-regulating kinase (ASK1), and coexpression of dn Ras and dn ASK1 exerted individual and additive inhibition of JNK/SAPK activation by MT-interfering agents. These findings suggest that multiple signal transduction pathways are involved with cellular detection of microtubular disarray and subsequent activation of JNK/SAPK.

INTRODUCTION

c-Jun N-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPKs), are involved in a signal transduction pathway parallel to that of mitogen-activated protein kinases (MAPKs) (15)(16)(28)(31)(38)(66). This highly conserved cascade is responsive to stress-related stimuli such as UV irradiation, ionizing radiation, ischemia and reperfusion, and inflammatory cytokines, eliciting phosphorylation and activation of JNK/SAPKs (32)(33)(37)(45)(51)(65). Activated JNK/SAPKs phosphorylate a variety of transcription factors including c-Jun, leading to transcriptional activation through interactions with c-Jun responsive DNA elements such as TPA-response element (TRE). In addition to responding to extracellular stimuli (10), the JNK/SAPK pathway is also activated by intracellular stress including inhibition of protein synthesis, treatment with antimetabolites, or DNA damage (9)(32)(37). No association has been shown, however, between MT disruption and JNK/SAPK activation.

MT-interfering agents (MIAs) utilized in the present study include paclitaxel (PTX), docetaxel, vinblastine (VBL), vincristine, nocodazole and colchicine. Through differential binding to MT polymers (PTX, docetaxel) or tubulin monomer and dimers (VBL, vincristine, nocodazole, colchicine), MIAs interfere with the dynamic process of MT assembly (63). Effects of MIAs include an arrest of cells at the G_2/M phase of the cell cycle and initiation of apoptosis (4)(25)(34)(41)(60)(61). It has been proposed, however, that G_2/M arrest may not be sufficient to induce apoptosis; other phosphoregulatory pathways may also be required (3)(22)(60). On the other hand, evidence is also accumulating to indicate that JNK/SAPK activation may regulate the cell cycle (51)(56) and apoptosis (13)(33)(55)(67).

Apoptosis signal-regulating kinase (ASK1) is a recently characterized mitogen-activated protein kinase kinase kinase (MAPK-KK) (26). Overexpression of ASK1 induced apoptosis in mink lung epithelial cells, and ASK1 was activated in cells treated with tumor necrosis factor-α (TNF-α) (26). These findings suggest that ASK1 may have a role in stress- and cytokine-induced apoptosis. Here I report that MT-interfering agents activate JNK/SAPK through signal transduction by both Ras and ASK1, indicating that multiple signal transduction pathways may be required for this type of cellular stress response. These results, for the first time, demonstrate the roles of Ras, ASK1, and JNK/SAPK in signal transduction pathways initiated by microtubular disarray.

EXPERIMENTAL PROCEDURES

CELL CULTURE

Human fibroblasts (CRL1502), breast cancer cells MCF-7 and T47D, choriocarcinoma JEG-3, and osteosarcoma SAOS-2 were obtained from ATCC (Rockville, MD). Epithelial ovarian cancer cells BR and 67R (7)(64), ovarian cancer cells 1A9 and its tubulin-mutant, PTX-resistant derived cells, PTX10 and PTX22 (2)(18), and isolation of primary trophoblast from term placentae (57) were described previously. All cell lines were cultured in DMEM/F12 (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin except that primary trophoblasts were cultured in DMEM-HG (Sigma) supplemented with 20% FBS. Both PTX10 and PTX22 cells were maintained in 15 ng/ml PTX and 5 μg/ml verapamil continuously but were cultured in drug-free medium for 5 days prior to each experiment.

CHEMICALS AND CELL TREATMENT

Unless noted, all chemicals were purchased from Sigma. Docetaxel (Taxotere) was kindly provided by S.A. Coughlin (Rhone-Poulenc Pharmaceutic Inc., Collegeville, PA), and lovastatin was a gift from W.L. Henckler (Merck and Co., Rahway, NJ). All stock solutions of MIAs were prepared with DMSO at a concentration of 10 mM except that colchicine (10 mM) was dissolved in absolute ethanol and bacterial lipopolysaccharide (2 mg/ml) was dissolved in water. Lovastatin (10 mM) was prepared with 10% ethanol (36). Cell treatments were performed in serum-containing culture medium when cells were approximately 80% confluent. As reviewed by Rowinsky (53), peak plasma concentrations of PTX in patients are 0.21 to 13.0 μM . Thus, we treated cells with 1 μM PTX in most experiments. For comparison, other MIAs were used at similar concentrations. UV irradiation was performed by exposing cells to a germicidal ultraviolet lamp (254 nm, 38W, 76 cm distance between plates and the UV lamp) in a tissue culture hood for 2 min. The UV dose was approximately 40 J/m^2 (20). Cells were then incubated at 37°C in 5% CO_2 for 1 h before preparing cell lysates.

IMMUNOCOMPLEX KINASE ACTIVITY ASSAYS

Anti-JNK1 or anti-MAPK (ERK 2) antibodies, purified GST-c-Jun (1-79 a.a.), and protein A-agarose beads were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The procedure for the immunocomplex kinase assay of JNK was modified from Derijard *et al.* (16).About 100 μg of lysate prepared with lysis buffer (20 mM Tris pH 7.4, 200 mM NaCl, 0.1 % NP-40, 1 mM phenylmethylsulfonylfluoride, 1 mM Na_3VO_4, and 10 mM NaF) was immunoprecipitated with antibody excess and protein A-agarose beads at 4°C overnight, and agarose beads were washed with lysis buffer and kinase buffer (25 mM HEPES pH 7.5, 25 mM of $MgCl_2$, and 25 mM β-glycerophosphate). The kinase reactions for JNK/SAPK were performed by incubating immunoprecipitated proteins with kinase mixture (1 mM dithiothreitol, 0.1 mM Na_3VO_4, 10 μM ATP, 5 μCi ^{32}P-γATP, and 0.2 μg of GST-c-Jun in kinase buffer) at room temperature for 30 min. The procedure for the immunocomplex MAPK assay was identical to the JNK/SAPK assay except an anti-ERK 2 Ab was used for immunoprecipitation and myelin basic protein (Upstate Biotechnology, Lake Placid, NY) was the substrate. Laemmli's loading buffer was added to stop the reaction and samples were resolved on SDS-PAGE. GST-c-Jun or MBP bands on

autoradiograms were analyzed with a video densitometer Lynx4000 (Applied Imaging, Santa Clara, CA).

WESTERN BLOTTING ANALYSIS

Aliquots of cell lysates resolved on SDS-PAGE, were transferred to nitrocellulose membranes, and probed with antibodies as specified followed by second antibody conjugated with horseradish peroxidase (Santa Cruz Biotechnology). After washing, proteins were detected by enhanced chemiluminescence (Pierce, Rockville, IL).

PLASMID CONSTRUCTS AND TRANSFECTION

A reporter construct for the TPA-response element, p(TRE)x5-TK-CAT (12) was from Z. Culig. An expression vector for β-galactosidase (pCMV-lacZ), hemagglutinin (HA)-epitope tagged expression vectors pSRα-HA-JNK1 and pSRα-HA-ERK2 (39), and dominant-negative (dn) expression vectors pSRα-dn Ras (17N) and pSRα-dn Rac (17N) (39) were from M. Karin. An expression vector for dn c-Jun (pCMV-TAM67) was from M. Birrer (49). Dominant-negative expression vectors for JNK/SAPK (pSRα-APF) and for JNKK/SEK1 (pSRα-K116R) were from G.L. Johnson (29). Expression vectors for wild type ASK1 (pcDNA3-ASK1-HA) and dn ASK1 (pcDNA3-dn ASK1-HA) were described recently (26). Liposome-mediated transfections were performed by using LipofectAmine (GibcoBRL, Gaithersburg, MD) on MCF-7 cells, and using Tfx-50 (Promega, Madison, WI) on BR cells.

CHLORAMPHENICOL ACETYL-TRANSFERASE (CAT) ASSAY AND STATISTICAL ANALYSES

Cells in six-well plates were cotransfected with p(TRE)x5-TK-CAT (1.5 μg/well for MCF-7 and 2.5 μg/well for BR), 0.5 μg/well of pCMV-lacZ, and 0.5 μg/well of either control DNA or dn expression vectors. At 24 h after transfection, cells were treated with 1 μM MIA for 16 h. The liquid scintillation CAT assay was modified from a standard protocol (30). After transfected cells were treated with MIAs for 16 to 22 h, cells were scraped from plates, washed with 1 ml of washing buffer (40 mM Tris pH 7.4, 1 mM EDTA, 150 mM NaCl), resuspended in 100 μl of 0.25 M Tris (pH 8.0), and subjected to three cycles

of freeze-thawing. Resultant cell lysates were heated at 60°C for 10 min to inactivate endogenous CAT inhibitors and clarified by centrifuging at 16,000 x *g*, 4°C, for 5 min. Each sample in the CAT assay was 108 µl in volume, containing 0.25 mg/ml of butyryl Co-A and 0.15 µCi of [^{14}C]-chloramphenicol (ICN Biomedicals, Irvine, CA) in 0.25 M Tris pH 8.0. After incubation at 37°C for 3 h, 300 µl of Pristane/Xylene (ratio=2:1) was added into each sample, vortexed for 30 s, and centrifuged at 15,000 x *g* for 3 min. The organic phase was re-extracted with 100 µl of 0.25 M Tris buffer (pH 8.0). Two hundred µl of each sample was counted in scintillation cocktail (Biofluor, NEN Research, Boston, MA). Expression of β-galactosidase was measured with a kit purchased from Promega (Madison, WI). Data of CAT activities were normalized with levels of β-galactosidase. Statistical analysis of CAT assay values was performed by *ANOVA* and *Student's t-test*.

ACTIVATED RAS INTERACTION ASSAY (A.R.I.A.)

Activated Ras (Ras-GTP) is precipitated by the Ras-binding domain (RBD) of Raf-1 in a GST-RBD fusion protein immobilized to glutathione beads. Affinity-precipitated Ras is detected by Western blot with anti-Ras antibodies (14)(59). The bacterial expression vector for GST-RBD, pGEX-RBD which encodes residues 1-149 of c-Raf1 (RBD), was from D. Shalloway (59).

Preparation of GST-RBD: Transformed JM109 *E. coli* was grow in 50 ml of LB (or super broth) containing ampicillin overnight. In the next morning, 450 ml of LB was added and incubated for 5 h (or till O.D. is about 0.6-0.8). GST-RBD expression was induced by 1 mM IPTG (isopropyl-1-thio-β-D-galactoside) for 2 h. After centrifugation, bacteria pellet was rinsed once with ice-cold PBS and was freeze at -70°C until use. Bacteria pellet was resuspended in 10 ml bacterial lysis buffer (BLB: 20 mM HEPES, pH=7.5, 120 mM NaCl, 10 % glycerol, 2 mM EDTA, 10 µg/ml leupeptin and 10 µg/ml aprotinin) and lysed by sonication. After centrifuged at 15,000 rpm, supernatant of sonicated lysate was collected and to which 10% NP-40 was added to a 0.5% final concentration. GST-RBD was immobilized onto 300-500 µl of 1:1 slurry GSH agarose (Sigma) during incubation with rocking at 4°C for 30 min, purified by washing agarose 6-8 times with BLB with 0.5% NP-40. Presence of GST-RBD can be detected on Coomassie blue stained gels at about 42 kD. About 30-50 µl of these GSH agarose suspension was used for each sample lysate.

Affinity precipitation of active Ras: BR cells growing to 90% confluence in 10-cm dishes were

serum-starved (0.1% FCS) for 48 h, then treated with 1 μM of PTX or VBL for 30 or 120 min. Cells treated with 50 ng/ml of epidermal growth factor (EGF) for 10 min were used as positive controls. Treated cells were rinsed with ice-cold PBS twice and lysed with 0.4 ml/dish of Mg-containing lysis buffer (MLB: 25 mM HEPES, pH 7.5, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM sodium vanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Activated Ras was precipitated by GST-RBD in excess. After three washes with MLB, activated Ras was eluted from beads by boiling in Laemmli's loading buffer, subjected to 14% SDS-PAGE, transferred to nitrocellulose membrane, and detected using anti-Ras antibody (Santa Cruz #SC-035).

IMMUNOCOMPLEX KINASE ASSAYS OF HA-JNK1, HA-ERK2 AND ASK1-HA

Since efficiencies of transient transfection in both BR and MCF-7 cells were limited (20% and 15%, respectively), we were not able to accurately evaluate the effects of transfected expression vectors on regulation of JNK/SAPK by direct measurement of endogenous JNK/SAPK activities in the whole population of cells. Therefore, we cotransfected expression vectors for HA-JNK1 with vectors expressing its potential upstream regulators, then assayed activities of epitope-tagged HA-JNK1. Since we had determined in pilot experiments that transfected HA-JNK1 was optimally activated by 4 h-treatment with MIAs in BR cells, we treated with MIAs for 4 h before measuring HA-JNK1 activity.

BR cells in 60-mm petri dishes were cotransfected with 4 μg of pSRα-HA-JNK1 and 2 μg of each expression vector. Total amount of DNA per dish was brought to 8 μg by adding control vectors (pSRα or pcDNA3). At 24 h after transfection, cells were treated with 1 μM of MIAs, cell lysates were prepared and subjected to immunocomplex kinase assay using excess anti-HA monoclonal antibody, clone 12CA5 (Boehringer-Mannheim, Indianapolis, IN), to immunoprecipitate expressed HA-JNK1. The same amounts of lysates were probed with either anti-HA or anti-JNK1 monoclonal antibodies (PharMingen, San Diego, CA). Since coexpression of some dominant negative upstream regulators inhibited HA-JNK1 expression, HA-JNK1 activities were normalized by the levels of HA-JNK1 measured in Western blot (39). Identical procedures were performed for HA-ERK2 except that MBP was the substrate and an anti-ERK antibody (Santa Cruz) was used in Western blots.

When pcDNA3-ASK1-HA was cotransfected with other expression vectors into cells, expressed ASK1-HA was immunoprecipitated with excess anti-HA antibody, extensively washed, and subjected to

identical kinase reaction as that in JNK/SAPK assay. Equivalent increase in autophosphorylation of ASK1 was observed in parallel to increased phosphorylation of ATF2 in the coupled-kinase assay for ASK1 using GST-MKK6, GST-p38 and ATF2 as sequential substrates. Therefore, in the present study, activities of ASK1 were measured by levels of *in vitro* autophosphorylation in ASK1-HA bands that migrated in SDS-PAGE at approximately 160-kD.

RESULTS

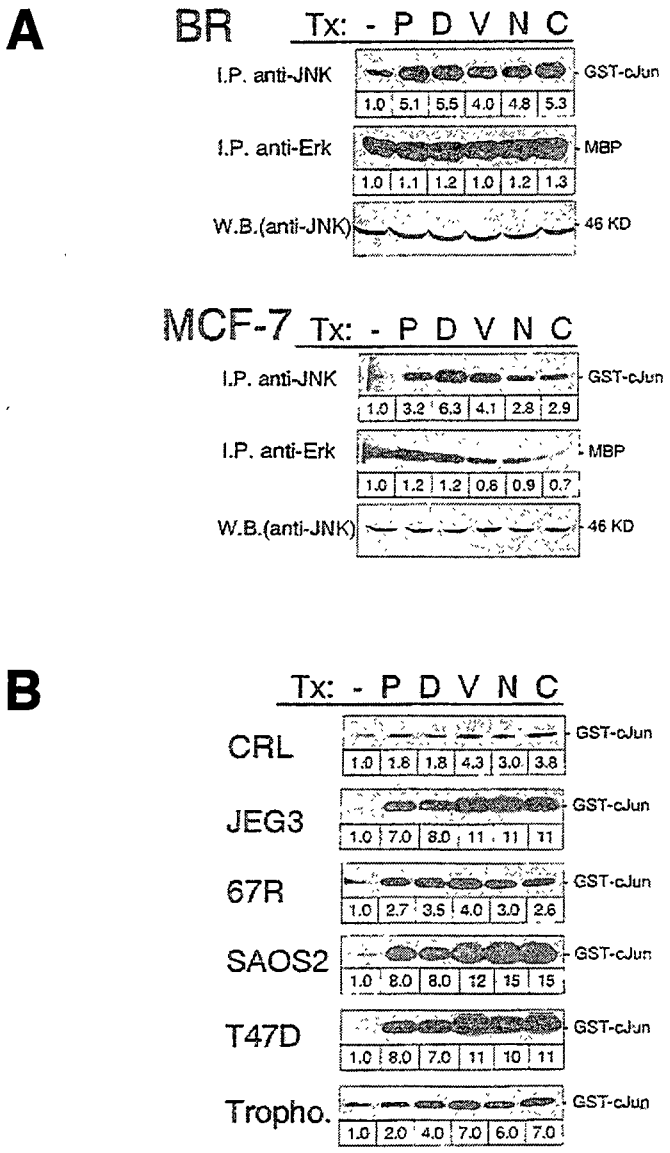
MICROTUBULE-INTERFERING AGENTS (MIAS) ACTIVATE JNK/SAPK IN A VARIETY OF HUMAN CELLS

Activities of JNK/SAPK and MAPK in MIA-treated cells were measured by immunocomplex kinase assays using GST-c-Jun and myelin basic protein (MBP) as substrates, respectively. Treatment with 1 μM PTX, docetaxel, VBL, nocodazole, or colchicine for 2 h activated JNK/SAPK in BR ovarian cancer cells and in MCF-7 breast cancer cells. Activation of JNK/SAPK was not accompanied by alterations in JNK/SAPK protein levels as measured by Western blotting of whole cell extracts (**Figure 1A**). In contrast, treatment with MIAs did not significantly activate MAPK/ERK activities. Since BR cells had higher basal MAPK activities which could obscure modest MAPK activation by MIAs, HA-ERK2 activity was measured following 1 μM PTX or VBL, but only a less than 2 fold activation was detected.

To test whether activation of JNK/SAPK by MIA is a general response, we measured JNK/SAPK activities in other cell lines and primary cells. MIAs activated JNK/SAPK in all tested cell types (**Figure 1B**); the varying magnitude (1.8 to 15 fold) in different cell types suggests sensitivity to MIAs may be cell type-specific. It is noteworthy that MIAs activated JNK/SAPK in both proliferating cancer cell lines and non-proliferating trophoblasts (57), suggesting that activation is independent of cell cycle progression.

MIA-INDUCED ACTIVATION OF JNK/SAPK IS DOSE-DEPENDENT AND TIME-DEPENDENT

In both BR and MCF-7 cells, MIAs activation of JNK/SAPK was dose-dependent over a range of 0.01 to 10 μM (**Table 1**). MIAs activated JNK/SAPK within 30 min of treatment and the JNK/SAPK



(Figure 1) Microtubule-interfering agents activate JNK/SAPK in a variety of human cells. *A*, ovarian cancer BR cells and breast cancer MCF-7 cells were treated with 1 μM paclitaxel (*P*), docetaxel (*D*), vinblastine (*V*), nocodazole (*N*), colchicine (*C*), or DMSO (-) alone for 2 h before JNK/SAPK and ERK2 activities were analyzed by immunocomplex kinase assays. GST-c-Jun and MBP were used as substrates for JNK and ERK2, respectively. The kinase levels in the whole cell lysates were analyzed by Western blot (*W.B.*). *B*, human fibroblast (*CRL*), choriocarcinoma (*JEG3*), ovarian cancer cells (*67R*), osteosarcoma (*SAOS2*), breast cancer cells (*T47D*), and primary trophoblast (*Tropho.*) were treated with 1 μM MIA (*P*, *D*, *V*, *N*, and *C*) or DMSO (-) for 2 h before JNK/SAPK activities were analyzed. Numbers under the corresponding bands indicate the -fold activation of kinase activity as based on video densitometry.

TABLE I

Dose-dependent activation of JNK/SAPK in BR and MCF-7 cells by microtubule-interfering agents

Cells were treated with MIAs at designated concentrations for 2 h. JNK/SAPK activities were assayed by immunocomplex kinase assays and quantitated by analyzing autoradiograms with a video densitometer. Fold activation shown represent the mean ± S.E.M. from 2 or 3 independent experiments as indicated in parentheses.

a. BR cells

Treatment	(no. of exp.)	0.01 μM	0.1 μM	1 μM	10 μM
Paclitaxel	(3)	1.4 ± 0.3	3.2 ± 0.9	6.7 ± 0.7	7.8 ± 1.1
Docetaxel	(2)	1.5 ± 0.5	3.9 ± 1.9	4.4 ± 1.6	15.0 ± 4.9
Vinblastine	(3)	2.6 ± 1.1	2.6 ± 0.8	3.4 ± 0.8	5.2 ± 0.5
Vincristine	(2)	1.7 ± 0.6	2.7 ± 1.3	3.4 ± 1.6	5.5 ± 0.6
Nocodazole	(3)	2.3 ± 1.0	5.0 ± 1.7	6.9 ± 1.2	10.9 ± 1.4
Colchicine	(3)	1.2 ± 0.1	2.7 ± 0.6	3.9 ± 1.0	6.6 ± 1.0

b. MCF-7 cells

Treatment	(no. of exp.)	0.01 μM	0.1 μM	1 μM	10 μM
Paclitaxel	(2)	1.3 ± 0.2	3.0 ± 1.0	3.4 ± 0.4	5.7 ± 2.3
Docetaxel	(2)	1.6 ± 0.5	5.2 ± 0.8	10.3 ± 4.2	12.0 ± 6.0
Vinblastine	(2)	2.0 ± 0.1	2.2 ± 0.2	4.0 ± 0.8	3.5 ± 0.3
Vincristine	(2)	1.8 ± 0.2	2.2 ± 0.2	3.3 ± 0.3	4.5 ± 0.5
Nocodazole	(2)	1.5 ± 0.3	2.7 ± 0.3	3.5 ± 1.5	5.4 ± 0.6
Colchicine	(2)	1.8 ± 0.2	1.8 ± 0.2	2.3 ± 1.3	4.6 ± 0.4

response peaked between 2 to 8 h declining to basal levels by 12 h (**Figure 2**). BR cells (**Figure 2A**) appeared to respond more rapidly than MCF-7 cells (**Figure 2B**), suggesting cell type-specific differences.

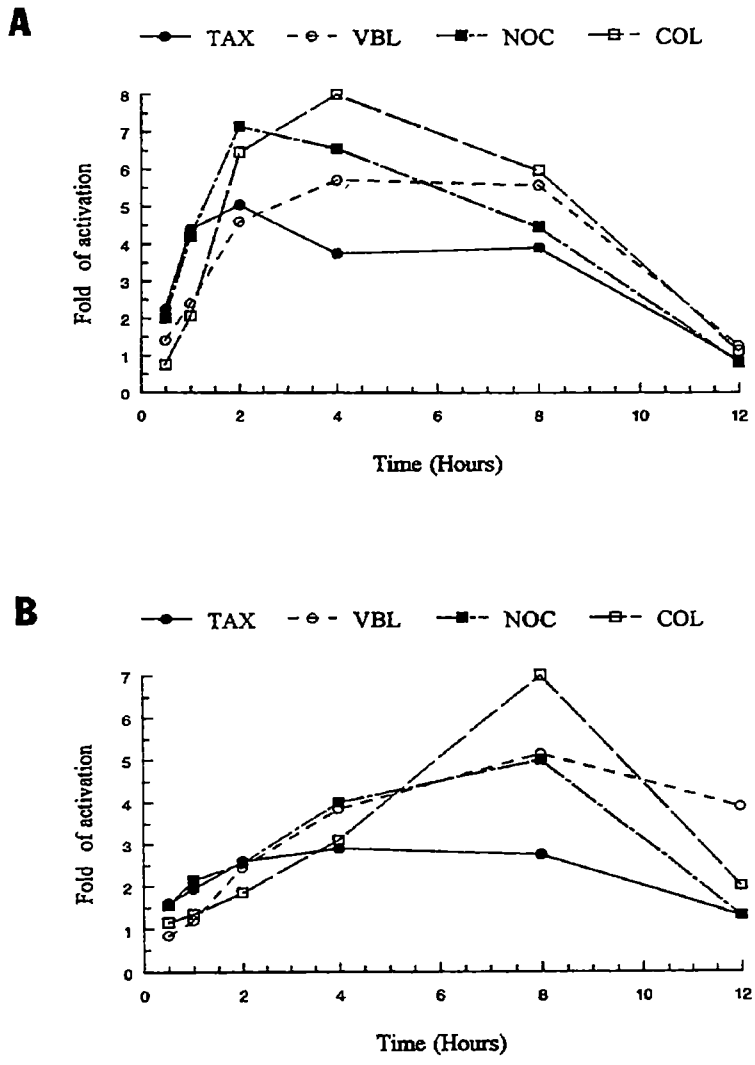
INTERACTIONS WITH MICROTUBULES ARE REQUIRED FOR ACTIVATION OF JNK/SAPK BY MIAS

To elucidate whether interactions between MIAs and tubulin/ MTs are required for activation of JNK/SAPK, we measured JNK/SAPK activities in BR and MCF-7 cells treated with an inactive precursor of PTX, 10-deacetylbaccatin III, or an inactive form of colchicine, β-lumicolchicine. Either agent at concentrations up to 10 μM failed to activate JNK/SAPK (**Figure 3A**). Since PTX exerts lipopolysaccharide (LPS)-like effects (58) and activates JNK/SAPK in macrophages and monocytes (24), we assayed JNK/SAPK activities in BR and MCF-7 cells treated with purified bacterial LPS. No significant activation of JNK/SAPK was observed in BR cells, while in MCF-7 cells, JNK/SAPK activities fell 10-70 % below basal activities in two independent experiments (**Figure 3A**). These data do not suggest a role for LPS-like activity of PTX in JNK/SAPK activation in cancer cells.

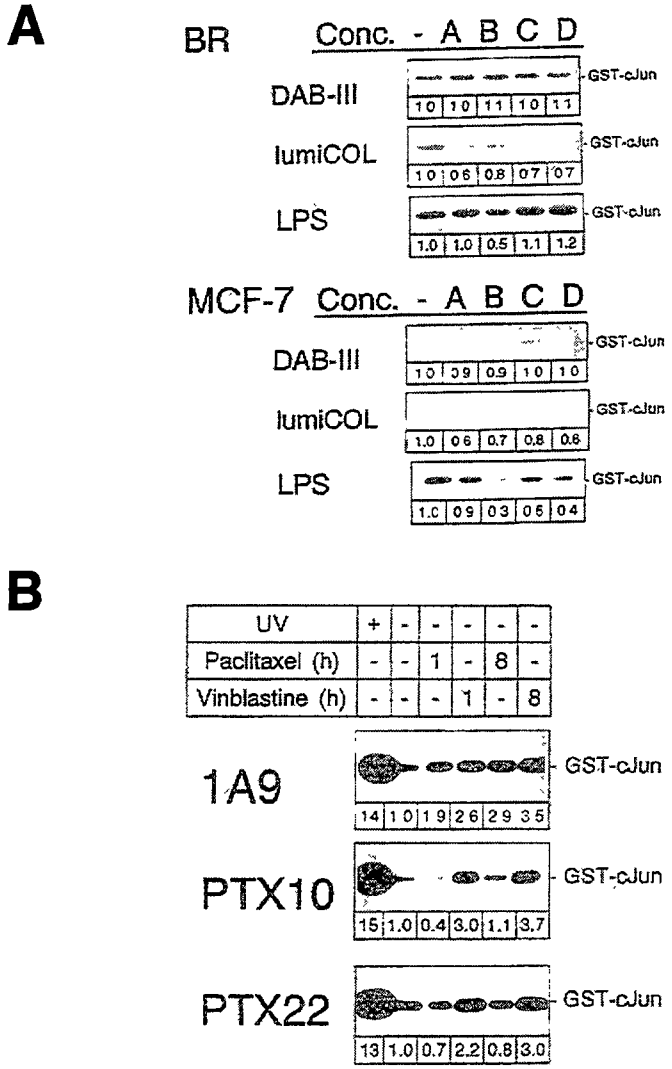
To further confirm the requirement of microtubular interactions with MIAs in the activation of JNK/SAPK, we compared JNK/SAPK activation by PTX in two PTX-resistant cell lines, PTX10 and PTX22 (which express mutant β-tubulins), with that in parental 1A9 cells (2). Both PTX and VBL activated JNK/SAPK in parental 1A9 cells but only VBL was able to activate JNK/SAPK in the PTX-resistant cell lines (**Figure 3B**). Furthermore, UV irradiation activated JNK/SAPK equally in all three cell lines, demonstrating a functional JNK/SAPK signaling cascade in these cells and, indicating the lack of JNK/SAPK activation in PTX-treated PTX10 and PTX22 cells is a result of the failure of PTX to bind tubulin (18)(48).

THE MIA-ACTIVATED, JNKK/SEK1-JNK/SAPK SIGNALING CASCADE ACTIVATES TRANSCRIPTION THROUGH C-JUN

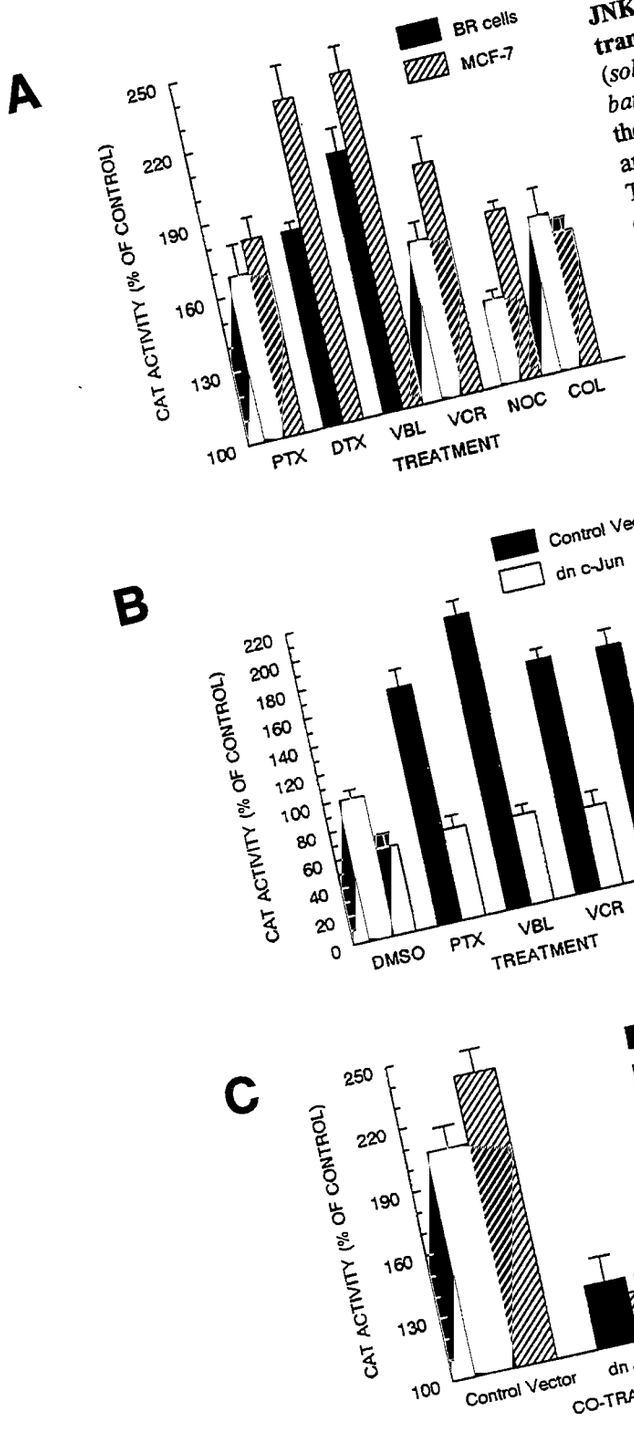
To identify downstream effectors of MIA-activated JNK/SAPK, we transfected BR and MCF-7 cells with a p(TRE)x5-TK-CAT reporter construct for 24 h, treated with MIAs for 16 h, then evaluated CAT activity as a measure of AP-1 transcription factor activity. **Figure 4A** shows statistically significant



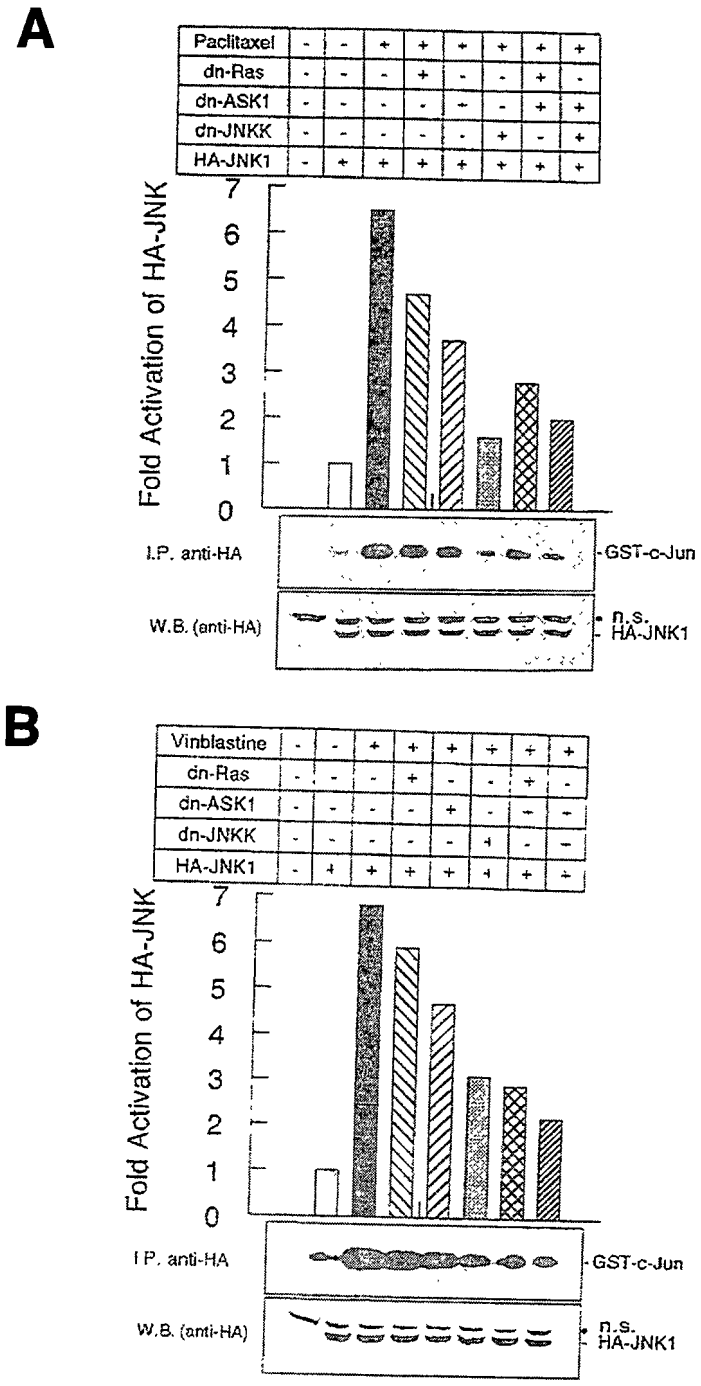
(Figure 2) Time course of JNK/SAPK activation in BR and MCF-7 cells by microtubule-interfering agents. BR cells (*panel A*) and MCF-7 cells (*panel B*) were treated with 1 μM paclitaxel (●, *PTX*), vinblastine (○, *VBL*), nocodazole (■, *NOC*), or colchicine (□, *COL*) for 30 min, 1 h, 2 h, 4 h, 8 h, and 12 h, then JNK/SAPK activities were analyzed by immunocomplex kinase assays. The fold activation of JNK/SAPK was calculated by comparison to basal JNK/SAPK activities. Data shown represent the means from two independent experiments. Average S.E. for each time point were about 24% and 22% of mean values of BR and MCF-7, respectively (S.E. bars not shown for clear presentation of all points).



(Figure 3) Interactions with microtubules are required for activation of JNK/SAPK by MIAs. *A,* ovarian cancer BR cells and breast cancer MCV-7 cells were treated for 2 h with DMSO (-), 10-deacetylbaccatin-III (*DAB-III*), β-lumicolchicine (*lumiCOL*), or purified bacterial LPS at concentrations of 0.01, 0.1, 1, or 10 μM (*DAB-III* and *lumiCOL*) or μg/ml (*LPS*) (*concentrations A-D*, respectively), then JNK/SAPK activities were measured by immunocomplex kinase assay using GST-c-Jun as substrate. ***B,*** paclitaxel-sensitive ovarian cancer 1A9 cells and the paclitaxel-resistant derivatives PTX10 and PTX22 cells were treated with 40 J/m^2 UV, 1 μM paclitaxel or vinblastine for 1 or 8 h, after which JNK activities were measured. The numbers under the corresponding bands indicate -fold activation of JNK as determined by densitometry.



(Figure 4) The MIA-activated JNKK-JNK signaling cascade activates transcription through c-Jun. ***A,*** BR cells (*solid bars*) and MCF-7 cells (*shaded bars*) were transiently cotransfected with the p(TRE)x5-TK-CAT reporter construct and pCMV-lacZ using liposomes. Twenty-four hours after transfection, cells were treated with 1 μM paclitaxel (*PTX*), docetaxel (*DTX*), vinblastine (*VBL*), vincristine (*VCR*), nocodazole (*NOC*), or colchicine (*COL*) for 16 h. CAT activity was measured by liquid scintillation counting and normalized by levels of β-galactosidase. The data given are percent activation of CAT activities compared with DMSO-treated controls (100 ± 6 % for BR and 100 ± 5 % for MCF-7, not shown in the graph). ***B,*** BR cells were cotransfected with p(TRE)x5-TK-CAT and control vector (*gray bars*) or dn c-Jun expression vector, pCMV-TAM67 (*empty bars*), then treated with 1 μM microtubule-active agents or DMSO vehicle control as indicated. ***C,*** activation of transiently transfected p(TRE)x5-TK-CAT by vinblastine was measures in BR cells that were cotransfected with control (*solid bars*) and MCF-7 (*shaded bars*) pSRα vectors (*Control Vector*), dn JNKK/SEK1 expression vector (*dn JNKK*), or dn JNK/SAPK expression vector (*dn JNK*). Data of BR cells shown are the means ± S.E. of four reading from duplicate samples in two independent experiments. Data of MCF-7 cells shown are the means ± S.E. of triplicate samples in a representative experiment that was repeated twice with comparable results.



(Figure 6) Activation of transiently transfected HA-JNK1 by paclitaxel or vinblastine was inhibited by coexpression of dn Ras, dn ASK1, or dn JNKK/SEK1. BR cells in 60-mm dishes were transiently transfected with 4 μg of pSRα-HA-JNK1 and 2 μg of each dominant negative expression vector (or combinations as indicated) for 24 h, then treated with 1 μM paclitaxel (*A*) or vinblastine (*B*) for 4 h. HA-JNK1 activities were measured by immunocomplex kinase assay using an anti-HA antibody (12CA5) to immunoprecipitate HA-JNK1 from cell lysates and GST-c-Jun as substrate. Activities of HA-JNK1 were normalized to levels of HA-JNK1 protein. A nonspecific band recognized by the 12CA5 antibody is labeled with an *asterisk* (*, *n.s.*). Autoradiograms and Western blot shown are from a representative experiment, which was repeated three times with comparable results.

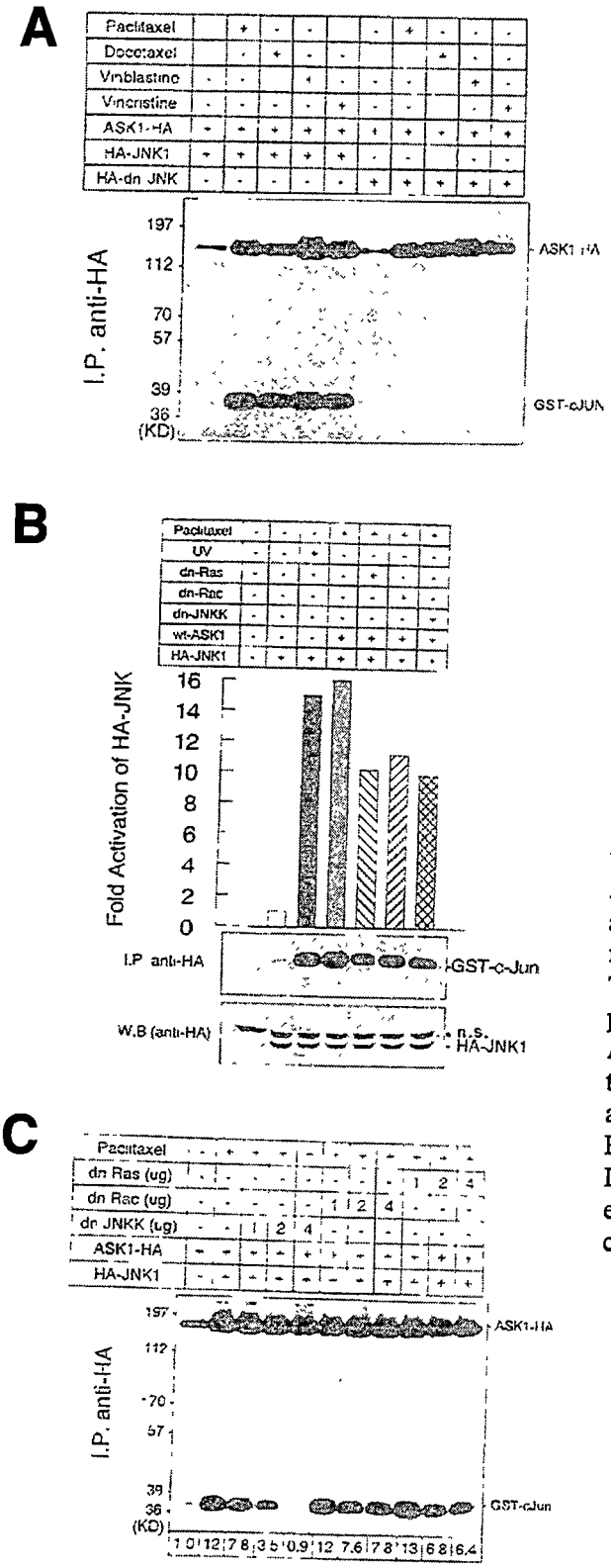
activation, although neither as efficiently as dn JNKK/SEK1. However, coexpression of dn Ras and dn ASK1 exerted additive inhibition on HA-JNK1 activation by either PTX or VBL. In contrast, coexpression of dn ASK1 and dn JNKK/SEK1 did not inhibit HA-JNK1 activity more than dn JNKK/SEK1 alone. These results suggest that ASK1 and JNKK/SEK1 are in the same signal transduction pathway, in agreement with studies indicating JNKK/SEK1 is a downstream effector of ASK1 (26).

Treatment with MIAs also activated transfected ASK1-HA, as shown by autophosphorylation of ASK1-HA and activation of cotransfected HA-JNK1 (**Figure 7A**). Similarly, in cells transfected with ASK1-HA alone, treatment with MIAs induced comparable levels of autophosphorylation of ASK1-HA without phosphorylation of GST-c-Jun, which was present in the kinase reaction mixture. Furthermore, coexpressed HA-dn JNK did not phosphorylate GST-c-Jun and did not interfere with autophosphorylation of ASK1-HA (**Figure 7A**). Taken together, these results suggest that: (i) ASK1-HA does not itself phosphorylate GST-c-Jun, and (ii) the co-immunoprecipitated HA-JNK1 is unlikely to phosphorylate ASK1-HA.

Overexpression of ASK1-HA enhanced PTX-activation of HA-JNK1 16 fold over control levels, comparable to the 15-fold activation induced by UV irradiation (**Figure 7B**). The augmentation of PTX-induced HA-JNK1 activation by ASK1-HA was inhibited by coexpression of dn Ras, dn Rac, or dn JNKK/SEK1. As shown in **Figure 7B**, a 1:1 ratio of the expression vectors for dn JNKK/SEK1 and wt ASK1 did not completely inhibit the ASK1-enhanced activation of HA-JNK1. However, increasing levels of dn JNKK/SEK1 completely blocked the enhanced HA-JNK1 activation by overexpressed ASK1 without decreasing the levels of MIA-induced activation of ASK1-HA (**Figure 7C**). In agreement with the partial inhibition of HA-JNK1 by dn Ras of dn Rac (**Figure 6**), overexpression of either dn Ras or dn Rac partially inhibited ASK1-augmented activation of HA-JNK1 (**Figure 7C**).

DISCUSSION

MTs serve as an intracellular scaffold and their unique polymerization dynamics are critical for many cellular functions (42)(54)(63). It is conceivable that cytoskeletal dysfunction, manifested as either a disrupted MT network or a stabilized, "rigid" MT cytoskeleton, could bear intracellular stress. In the present study we report that disruption of the equilibrium between tubulin monomer/dimers and MT polymers with MT stabilizing (PTX, docetaxel) or destabilizing (VBL, vincristine, nocodazole,



(Figure 7) MIAs activate the ASK1-JNKK-JNK signaling cascade. *A,* BR cells in 60-mm dishes were transiently cotransfected with 4 μg of pcDNA3-ASK1-HA and 4 μg of either pSRα-HA-dn JNK for 24 h, then treated with 1 μM paclitaxel, docetaxel, vinblastine, or vincristine for 4 h. ASK1-HA and HA-JNK1 or HA-dn JNK were co-immunoprecipitated by anti-HA antibody and used in a kinase reaction containing GST-c-Jun. Activities of ASK1-HA and HA-JNK1 were measured by levels of autophosphorylation of ASK1-HA and phosphorylation of GST-c-Jun, respectively. *B,* BR cells were cotransfected with 4 μg of HA-JNK1, 2 μg of ASK1-HA, and 2 μg of dominant negative expression vectors as indicated, then treated with 1 μM paclitaxel for 4 h. For comparison, BR cells that were cotransfected with 4 μg of HA-JNK1 and 4 μg of control vectors were treated with UV irradiation. Activities of HA-JNK1 measured by immunocomplex kinase assay were normalized to levels of HA-JNK1. A non-specific band recognized by the 12CA5 Ab is labeled with an *asterisk* (*, *n.s.*). *C,* BR cells were cotransfected for 24 h with 3 μg of HA-JNK1, 1 μg of ASK1-HA, and increasing amounts (1, 2, and 4 μg) of each dominant negative expression vector as indicated. Transfected cells were then treated with 1 μM paclitaxel for 4 h and assayed for activities of ASK1-HA and HA-JNK1. The *numbers* under the corresponding bands of GST-c-Jun in the autoradiogram indicate the -fold activation of HA-JNK1 as based on video densitometry. Data shown are from a representative experiment, which was repeated twice with comparable results.

colchicine) agents activated the stress-activated protein kinase (JNK/SAPK) signaling cascade. In both BR and MCF-7 cells, JNK/SAPK remained activated for up to 8 h after treatment with MIAs (**Figure 2**). Since induction of JNK/SAPK in T-cell activation and apoptosis can occur in a transient or persistent pattern, respectively (9), the sustained activation of JNK/SAPK following MIA treatment may reflect the apoptosis-inducing nature of these drugs.

For the MIAs used in this study, no membrane-associated receptor or target has been identified (58)(63). Our data demonstrate the requirement of binding to tubulin and/or MTs for MIA activation of JNK/SAPK, because inactive structural derivatives of some MIAs (10-deacetylbaccatin III and β-lumicolchicine) which do not bind tubulin/MTs did not activate JNK/SAPK (**Figure 3A**); and activation did not occur in the PTX-resistant cell lines, PTX10 (F270V) and PTX22 (A364T), which have single amino acid mutation in β-tubulin which abolish binding of PTX to MT and account for PTX resistance (18)(48). The notion that microtubular interactions are required for MIA-activated JNK/SAPK is further strengthened by the observation that while both PTX and VBL activated JNK/SAPK in the parental 1A9 cells, only VBL, but not PTX, activated JNK/SAPK in the PTX resistant PTX10 and PTX22 cell lines (**Figure 3B**).

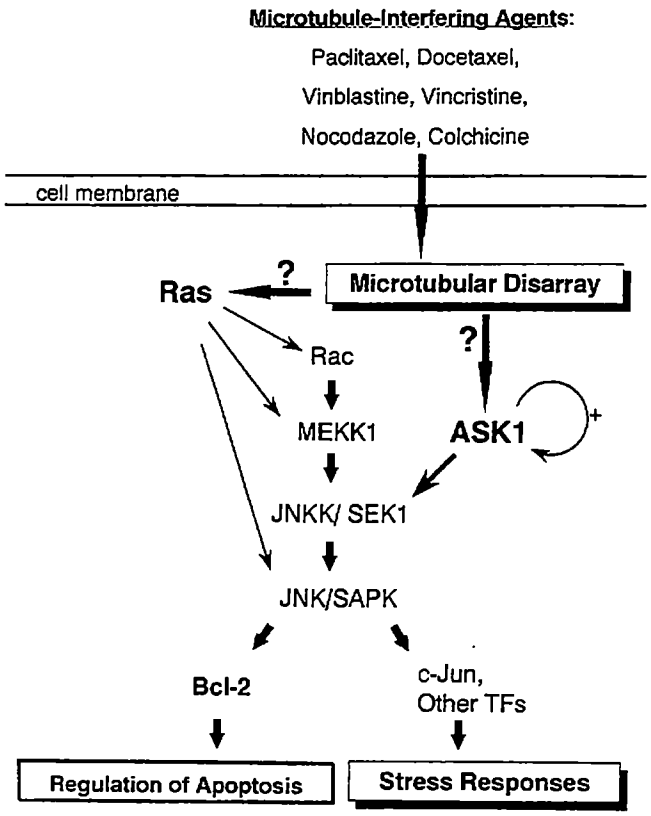
We have verified that the JNKK/SEK1-JNK/SAPK-c Jun signaling cascade was activated by MIAs with the following evidence. Firstly, MIA treatment activated transcription from a TRE-CAT reporter construct and this activation was inhibited by coexpressed dn c-Jun (**Figures 4A & 4B**), indicating c-Jun was a downstream effector responsive to treatment with MIAs. Secondly, coexpression of dn JNK/SAPK or dn JNKK/SEK1 inhibited MIA-induced TRE reporter activity (**Figure 4C**). These results suggest that MIA-activated JNK/SAPK may regulate transcription by activation of c-Jun and formation of functional c-Jun/c-Fos heterodimers (AP-1).

Multiple signal transduction pathways are required for activation of the JNK/SAPK cascade when a cell is perturbed by physical stress (52) and activation of the JNK/SAPK pathway by environmental stress can occur via Ras-dependent or Ras-independent pathways (35). Farnesylation and geranylgeranylation, the major posttranslational modifications of Ras and Rac, respectively, are essential for membrane anchoring and physiological functions (23). Our observations that lovastatin at concentrations which block farnesylation and geranylgeranylation (17) was unable to completely block JNK/SAPK activation by MIAs suggest involvement of other pathways, independent of the Ras-Rac cascade.

Indeed, we demonstrated a requirement for both Ras and ASK1 signaling for full activation of JNK/SAPK by MIAs. Firstly, treatment with PTX or VBL activated Ras (**Figure 5B**). Secondly, dn Ras and dn ASK1 exerted individual and additive inhibition of HA-JNK1 activation by MIAs (**Figure 6**). Thirdly, MIAs activated ASK1-HA with corresponding activation of HA-JNK1 (**Figure 7A**). Lastly, overexpression of ASK1-HA augmented MIA-induced activation of HA-JNK1 (**Figure 7B**) and this augmentation could be completely blocked by high levels of dn JNKK/SEK1, but not by dn Ras or dn Rac (**Figure 7C**). Collectively, these data suggest that both Ras and ASK1 are involved in optimal activation of JNK/SAPK after microtubular disruption and that both may regulate JNK/SAPK activity through the same downstream transducer, JNKK/SEK1.

Unlike treatment with EGF which activates Ras with an amplified activation of MAPK, treatment with MIAs induced a more sustained activation of Ras (**Figure 5B**) with negligible activation of MAPK (**Figure 1A**). Explanations for this discrepancy might be twofold. First, the effects of MIAs on the cell cycle might dissociate the sequential activation in the Ras-Raf1-MEK-MAPK cascade. This is supported by a report that, during progression of the cell cycle, there is a temporal dissociation between Ras and MAPK activation, suggesting Ras may target alternate effector pathways (59). Second, in addition to activation of Ras and ASK1, microtubular disarray might also activate phosphatase(s) which may attenuate MAPK activation. Several phosphatases which might target MAPK have been identified (5)(8)(19)(47)(46).

Based on these results, we propose that activation of the stress-related JNK/SAPK pathway may be a cellular response to intracellular stress caused by interference with MT dynamics (**Figure 8**). In this model, MT-interfering agents enter the cell and disrupt the dynamics of MT assembly. Through a yet-to-be defined mechanism, microtubular disarray activates both Ras and ASK1. Activated Ras may activate the JNK/SAPK through activation of Rac (43)(50), activation of MEKK1 (44), or through direct activation of JNK/SAPK by formation of the Ras-JNK complex (1). On the other hand, the signal from activated ASK1 may involve autophosphorylation followed by sequential activation of JNKK/SEK1 and JNK/SAPK. JNK/SAPK in turn activates downstream effectors, including c-Jun and other transcription factors, mediating cellular responses to this stress. Furthermore, disruption of MT integrity results in phosphorylation of an anti-apoptosis regulator, Bcl-2 (2)(21), and Bcl-2 can be phosphorylated by JNK/SAPK in the presence of Rac1 (40). Since the protective effects of Bcl-2 may be regulated by its phosphorylation status (27), these studies collectively suggest a potential role of activated JNK/SAPK in



(Figure 8) Interaction of signal transduction pathways activated by microtubule-interfering agents. Intracellular stress caused by microtubule-interfering agents activates both Ras and ASK1 signaling cascades, resulting in activation of JNK/SAPK. Activated JNK/SAPK in turn activates transcription factors, including AP-1, to mediate cellular responses to the stress. Activated JNK/SAPK may also regulate apoptosis through phosphorylation of Bcl-2.

apoptotic regulation of cancer cells after chemotherapy with MIAs.

It is intriguing that MIAs with stabilizing or destabilizing effects on MTs elicit similar activation of JNK/SAPK. These observations suggest a surveillance mechanism exists which signals the functional integrity of MTs to nuclear transcription factors. Interestingly, tubulin itself exhibits GTPase activity and acts as a nucleotide-binding protein (6), implying that tubulin may function in a fashion similar to Cdc42/Rac in the JNK/SAPK signal transduction pathway (11)(37). The mechanism(s) by which microtubular disarray activates both Ras and ASK1 remains to be elucidated.

APPENDIX

Additional experiments and results, which were not included in the paper (62), are described in this section.

CELL CYCLE ARREST

To investigate the role of mitotic arrest in PTX-initiated apoptosis, one experimental approach is to study PTX actions in G_1-arrested cells. If mitotic arrest is the sole mechanism for PTX-induced apoptosis, inhibition of cell cycle progression into the G_2/M phase by blocking cells at the G_1 phase should protect these cells from PTX-initiated apoptosis. Therefore, I have tried several methods in attempt to block ovarian cancer cells at the G_1 phase.

In the first set of experiments, ovarian cancer BR or BG-1cells growing on 6-well plates to 50% confluence were subjected to serum starvation (0% FBS) for 16 to 48 h, followed by treatments with or without 1 μM PTX, and analyzed for cell cycle profiles with flow cytometry. In the second set of experiments, cells were treated with 40 μM lovastatin for 16 to 48 h followed by PTX treatment and analyzed for the cell cycle profiles.

Either serum starvation or lovastatin treatment, at best arrested 80% of BR cells at G_1 phase, (*i.e.*, at least 20% cells were still progressing through the cell cycle). Thus, after treatment with PTX, most of serum-starved cells or lovastatin-treated cells still progressed to and were blocked at the G_2/M phase, with cell cycle profiles similar to those in cells that were not serum starved nor lovastatin treated. Since both of these treatments induced cell death of BG-1 cells, they are not suitable for this purpose

either.

My third approach was the use of adenoviral vectors for CDK inhibitors (CKIs), p16 and p27. Ovarian cancer BR and BG cells were cultured with DMEM/F12 medium supplemented with 5% NBS in 6-well plates to 50% confluence. After removal of medium, each of adenoviral vectors (control, p16, p27), which was adjusted to m.o.i. = 50, in 0.5 ml of 5% NBS-DMEM/F12 was added into each well. After incubation at 37°C for 1 h, 1.5 ml of 5% NBS-DMEM/F12 was added into each well and cells were cultured for 24 h. Adenovirus-infected cells were treated with 1 μM PTX or DMSO (vehicle control) for 16 h, trypsinized, and analyzed for the cell cycle profile with flow cytometry.

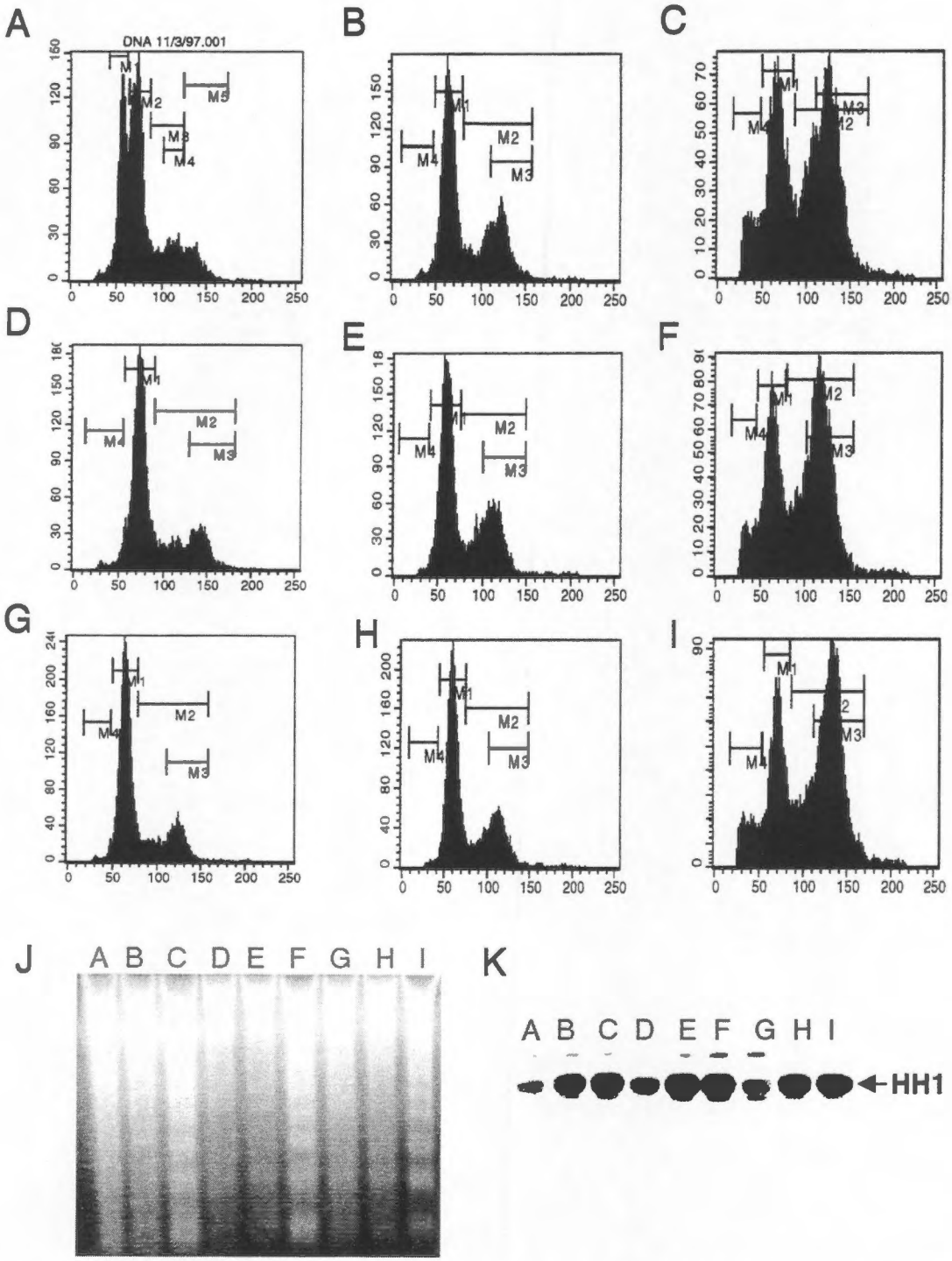
In BR cells, G_1 arrest as results of these adenoviral CKI infections could not be maintained after PTX treatment for either 6 h or 22 h (**Figure 9A-I**). Adenoviral CKI infections did not suppress DNA fragmentation or Cdc2 activation by PTX treatment (**Figure 9J & K**). Therefore, these adenoviral vectors are not suitable for this purpose in BR cells.

On the other hand, both adenovirus p16 (**Figure 10C**) and p27 (**Figure 10E**) infected BG-1 cells exhibited obvious G_1 arrest, when compared with control adenovirus-infected cells (**Figure 10A**). More importantly, after PTX treatment for 16 h, p16-arrested BG-1 cells largely remained in the G_1 phase (**Figure 10D**); and to a lesser extent, p27-arrested BG-1 cells also exhibited a predominant G_1 profiles (**Figure 10F**), when compared with control adenovirus-infected cells (**Figure 10B**).

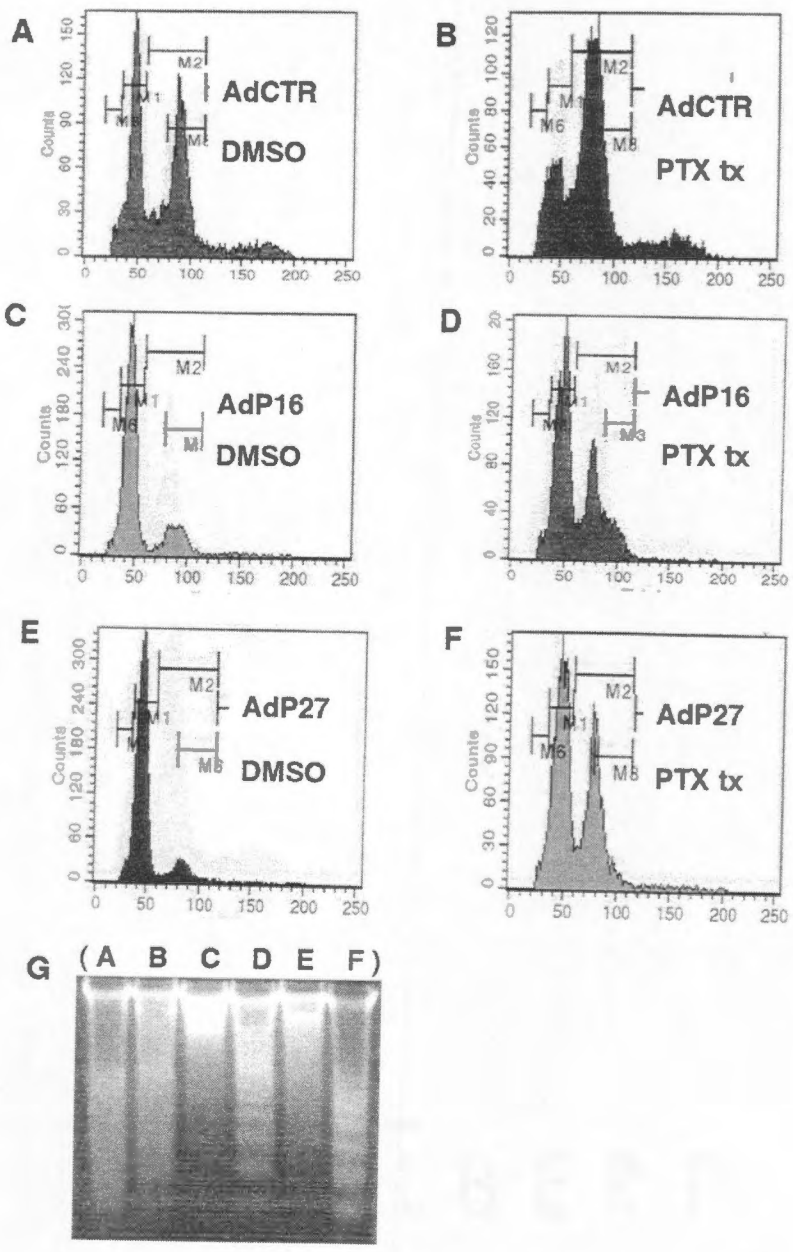
A set of parallel experiment were carried out in 60-mm dishes under the identical condition for detection of the ladder pattern of DNA fragmentation. Surprisingly, sustained G_1 arrest (**Figure 10D & F**) by p16 or p27 did not protect BG-1 cells from DNA fragmentation induced by PTX (**Figure 10G**). These results suggest that additional factor(s), other than mitotic arrest, is(are) involved in the PTX-initiated apoptotic process.

VALIDATION OF THE IMMUNOCOMPLEX JNK ASSAY

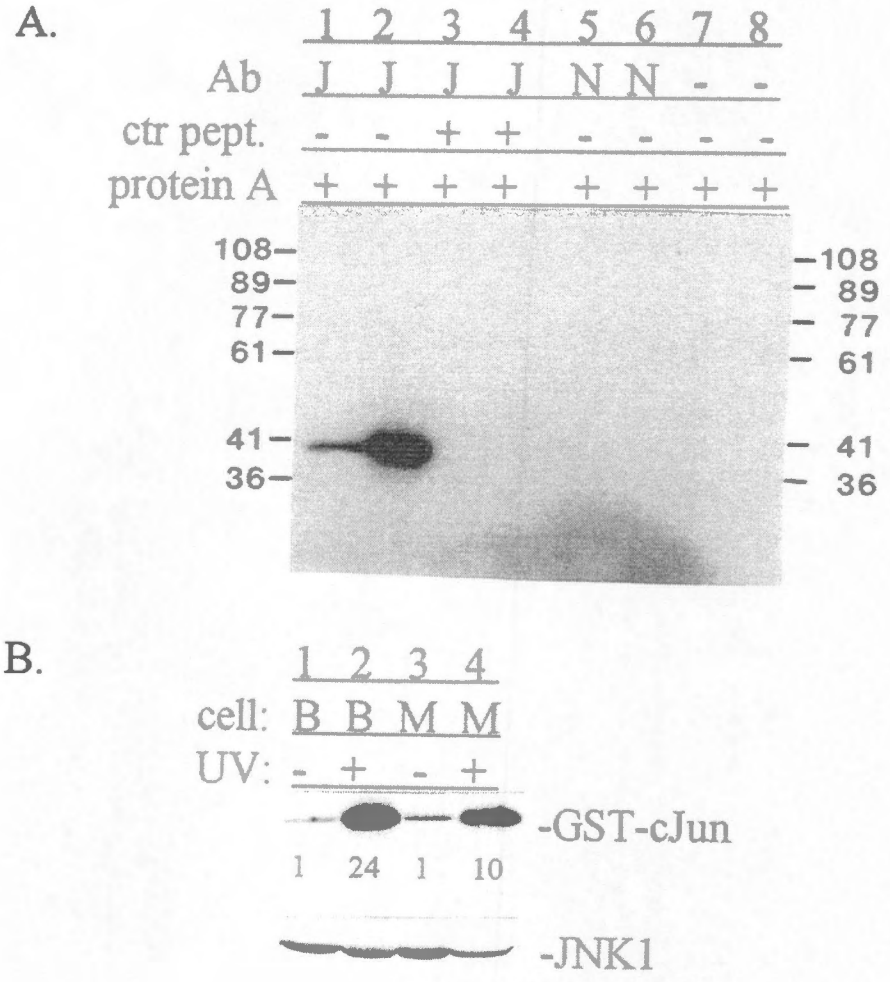
To validate the immunocomplex kinase assay for JNK, I immunoprecipitated lysates of control or UV-irradiated cells either with an affinity-purified, rabbit polyclonal Ab recognizing human JNK1 (a.a. 368-384) or with a comparable amount of non-immune rabbit IgG. To verify the specificity of the anti-JNK Ab, I used a 20-fold excess of a control peptide corresponding to the immunizing epitope of human JNK1 to block the anti-JNK Ab. **Figure 11A** shows that the anti-JNK Ab (lanes 1 and 2), but not the



(Figure 9) BR cells infected with adenoviral-p16 or -p27. ***A-C,*** AdControl; ***D-F,*** Adp16; ***G-I,***Adp27. (***A, D, G***), no PTX; (***B, E, H***), PTX 6 h; (***C, F, I***), PTX 22 h. ***J,*** Ladder pattern of DNA fragmentation. ***K,*** Immunocomplex Cdc2 assay.



(Figure 10) Cell cycle profiles and paclitaxel-induced apoptosis of adenoviral p16- and p27-treated cells. Ovarian cancer BG1 cells were cultred with DMEM/F12 medium supplemented with 5% calf serum (CS) in 6-well plates to 50% confluence. After removal of medium, each of adenoviral vectors (control, p16, p27), which was adjusted to m.o.i. = 50, in 0.5 ml of 5% CS-DMEM/F12 was added into each well. After incubation at 37°C for 1 h, 1.5 ml of 5% CS-DMEM/F12 was added into each well and cells were cultured for 24 h. Adenovirus-infected BG cells were treated with 1 uM paclitaxel or DMSO control for 16 h, and analyzed for the cell cycle profiles with flow cytometry **(panels A-F).** A set of parallel experiments were carried out in 60-mm dishes under the identical condition for assays on apoptotic DNA fragmentation **(panel G).**



(Figure 11) Validation of the immunocomplex JNK assay.
Abbreviations used are: *J*, anti-JNK Ab; *N*, non-immune rabbit IgG; *ctr pept.*, control peptide; *B*, BR cell lysates; *M*, MCF-7 cell lysates.

control rabbit IgG (lanes 5 and 6), efficiently immunoprecipitated JNK from lysates. Excess amounts of the control peptide specifically blocked JNK immunoprecipitation by this Ab (lanes 3 and 4). Non-specific binding of JNK to protein A agarose beads alone was not detected (lanes 7 and 8). **Figure 11B** compares an autoradiogram of the immunocomplex JNK assay to Western blots of the same amount of whole cell lysate from cells with and without UV irradiation. UV irradiation at dose 40 J/m^2 (described above) activated JNK 24 fold in BR cells and 10 fold in MCF-7 cells (upper panel) without detectably increasing JNK protein levels (lower panel). Recognition of a 46 kD band, consistent with the mobility of JNK1, validated the anti-JNK Ab used in this study.

P38 KINASE ASSAY

The procedure of p38 assay was identical to JNK assay except that anti-p38 antibody (Santa Cruz #SC-535) was used for immunoprecipitation and GST-ATF2 (Santa Cruz #SC-4114) was used as substrate. Activation of p38 by PTX treatment was not observed. However, the results were inconclusive because of the anti-p38 antibody did not recognize bands at 38 kD in Western blot analyses. The use of more reliable anti-p38 antibodies should be able to verify these data.

IN-GEL KINASE ASSAY

In an attempt to identify upstream regulator of JNK in PTX-treated cells, I have tried in-gel kinase assay using GST-JNK as substrate mixed in the agarose gel. The protocol for in-gel kinase assay was provided by Dr. J. Merryman.

Preparation of GST-JNK: The procedure of GST-JNK production in JM109 *E. coli* is identical to that of GST-RBD (described in the ARIA section). Bound GST-JNK on glutathione agarose was eluted twice by adding small volume (100-200 µl) of eluting buffer (20 mM reduced glutathione, 50 mM Tris, pH=8.0) and spinning in compact reaction columns (CRC, Cat. #13927, United States Biochemical Corp., Cleveland, OH), which were inserted with CRC filters (pore size: 10 µM, USB #13924). Eluents were pooled.

In-gel kinase assay:

1. Cell lysates were resolved in an 8% GST-JNK-containing polyacrylamide minigel (0.4 M Tris,

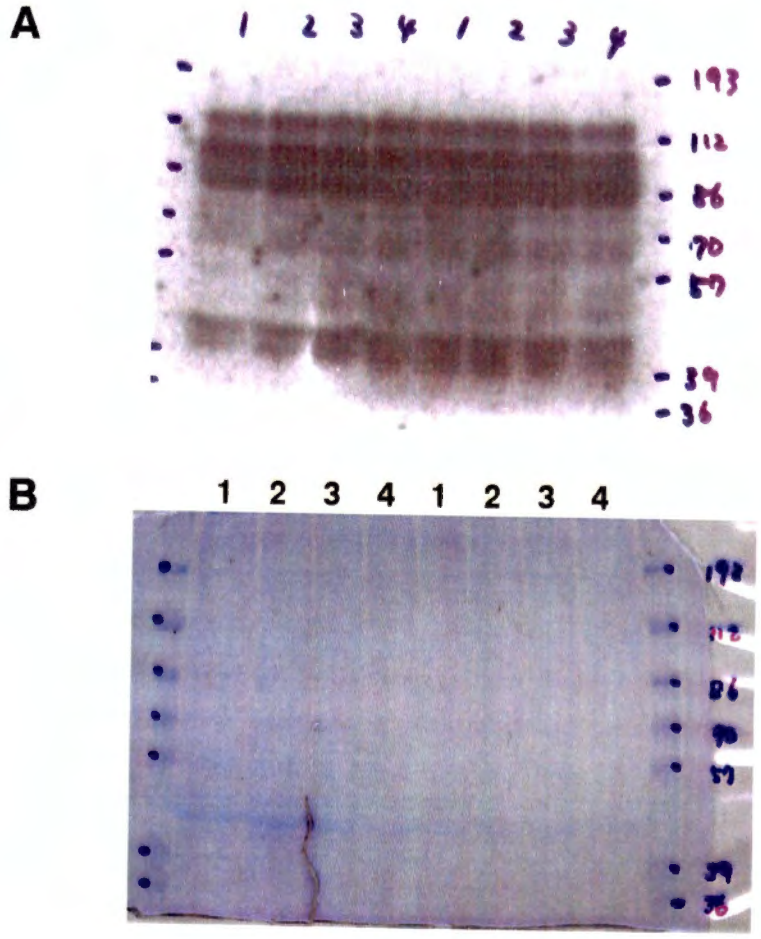
pH=8.8, 0.17% SDS, GST-JNK 200μg/ml) at constant voltage (50-75 V).

2. The gel was fixed in 50 mM Tris (pH=8.0) containing 20% isopropanol on an orbital shaker for 2 h at RT, with four changes in solution.
3. The gel was washed with 50 mM Tris (pH=8.0) containing 5 mM 2-mercaptoethanol for 2 h at RT, with four changes in solution.
4. Proteins were denatured by incubating the gel in 6 M guanidine hydrochloride for 2 h at RT, and renatured by 10 washes of 20 min each in 50 mM Tris (pH=8.0) containing 0.04% Tween-40 and 5 mM 2-mercaptoethanol.
5. The gel was pre-incubated in 40 mM HEPES (pH=8.0) containing 2 mM 2-mercaptoethanol and 10 mM $MgCl_2$ for 1h.
6. The gel was incubated in 6 ml kinase reaction buffer (40 mM HEPES, pH=8.0, 0.5 mM EGTA, 40 μM ATP, 10 mM $MgCl_2$, 25 μCi γ-^{32}P ATP) on an orbital shaker at RT.
7. The gel was washed with 5% TCA/1% sodium pyrophosphate for several hours until background radiation levels were decreased to that of an unused area of the gel.
8. The gel was stained in Coomassie blue, destained, and dried onto blotting paper.
9. The gel can be exposed to phosphoimaging cassette for 4 h and scanned at high resolution (optional).
10. Dried gel was exposed to X-ray film with enhanced screen at -70°C for 5-7 days.

Results: Compared with control lysates, no different pattern of bands were detected in UV-, PTX-, or VBL-treated cell lysates in my in-gel kinase assay (**Figure 12**). In addition to potential technical errors, the cause for the unsatisfactory assay was likely to be an insufficient GST-JNK amount added to the gel. Furthermore, autophosphorylation of preparations of GST-JNKK from the pGEX-JNKK, which I obtained from Anning Lin (University of Alabama, Birmingham), was detected in the following coupled kinase assays. The autophosphorylation of GST-JNKK makes it unsuitable as substrate in in-gel kinase assay.

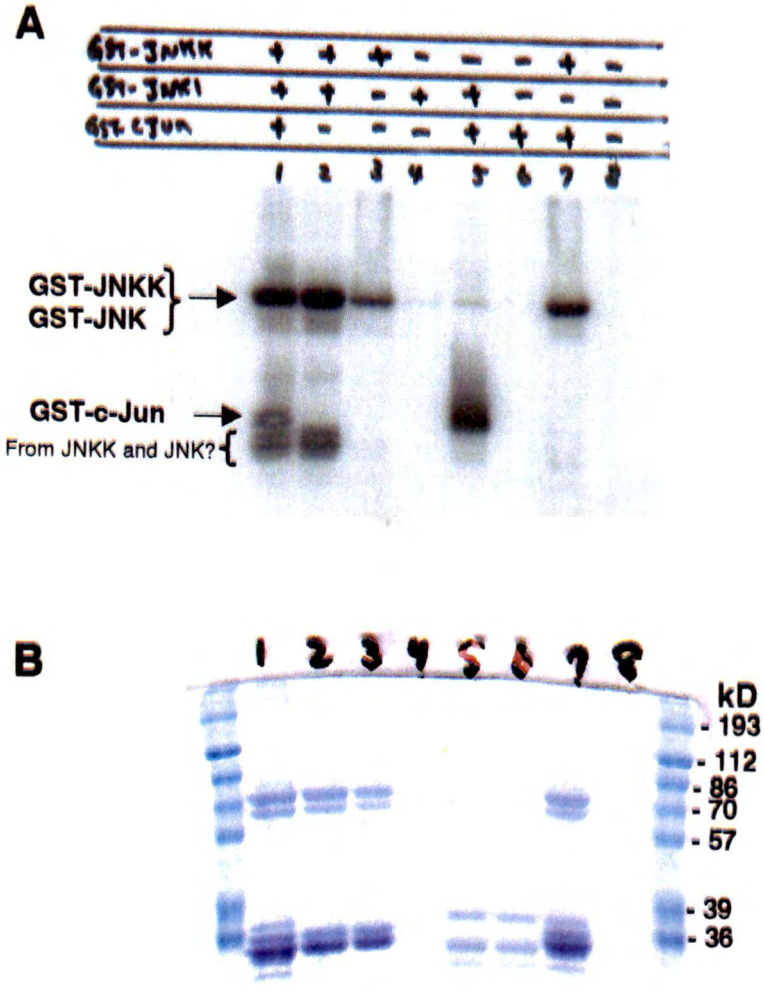
COUPLED KINASE ASSAYS

Preparation of GST-JNKK, GST-JNK, and GST-c-Jun from bacterial expression vectors: pGEX-JNKK, pGEX-JNK (both from Dr. Anning Lin), and pGEX-c-Jun (from Dr. Joyce Merryman),



(Figure 12) In-gel kinase assay using GST-JNK as substrate. MCF-7 cells were treated with control (lane 1), UV (lane 2), PTX (lane 3), or VBL (lane4). For each lane, 100 μg of total cell lysates were loaded and duplicated samples were loaded into this gel. ***A,*** An autoradiograph was derived from exposure of the dried gel between cellulose membranes to X-ray film for 5 days. ***B,*** The same gel was stained with Coomassie blue before drying.

respectively, are described above. Equal amount of GST-protein-bound GSH agarose beads were mixed, washed with ice-cold PBS three times and with kinase buffer once. Kinase reactions were performed in 20 µl kinase buffer in the presence of radioactive ATP, as described in the Immunocomplex kinase assay. Alternatively, and perhaps a better way, is to use equal amount of purified GST-proteins for these coupled kinase assays. In the experiment shown in **Figure 13**, GST-JNKK exhibited autophosphorylation (lanes 3 and 7). In that experiment, SDS-PAGE did not sufficiently separated GST-JNK from GST-JNKK, therefore, both resulted in broad phosphorylated bands in lanes 1 and 2.



(Figure 13) Coupled kinase assays. Ten µl each of suspended GST-protein-bound GSH agarose beads were mixed as designated in *A*, washed three times with ice-cold PBS and once with kinase buffer. Kinase reactions were done in 20 µl kinase buffer in the presence of radioactive ATP, as described in the immunocomplex kinase assay. *A*, An autoradiograph was derived from 15 h exposure of the dried gel to X-ray film. *B*, A parallel set of mixed GST-proteins were resolved by SDS-PAGE and stained with Coomassie blue. *Note* the amount of proteins in *lane 4* of *B* was not accurate due to a spillage during loading.

LITERATURE CITED

1. **Adler, V., M. R. Pincus, P. W. Brandt-Rauf, and Z. Ronai.** 1995. Complexes of p21RAS with c-Jun N-terminal kinase and Jun proteins. Proc. Natl. Acad. Sci. USA **92:**10585-9.
2. **Blagosklonny, M. V., P. Giannakakou, W. S. el-Deiry, D. G. Kingston, P. I. Higgs, L. Neckers, and T. Fojo.** 1997. Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res. **57:**130-5.
3. **Blagosklonny, M. V., T. W. Schulte, P. Nguyen, E. G. Mimnaugh, J. Trepel, and L. Neckers.** 1995. Taxol induction of p21 Wafl and p53 requires c-Raf-1. Cancer Res. **55.**
4. **Bonfoco, E., S. Ceccatelli, L. Manzo, and P. Nicotera.** 1995. Colchicine induces apoptosis in cerebellar granule cells. Exp. Cell Res. **218:**189-200.
5. **Brondello, J. M., A. Brunet, J. Pouyssegur, and F. R. McKenzie.** 1997. The dual specificity mitogen-activated protein kinase phosphatase-1 and -2 are induced by the p42/p44MAPK cascade. J. Biol. Chem. **272:**1368-76.
6. **Burns, R. G., and C. D. Surridge.** 1994. Tubulin: conservation and structure., p. 3-31. *In* J. S. Hyams, and C. W. Lloyd (ed.), Microtubules, vol. 13. John Wiley and Sons, Inc.,, New York.
7. **Cameron, M. R., M. R. Caudle, W. R. Sullivan, Jr., J. J. Peluso, and J. Wimalasena.** 1995. The steroidogenic and morphological effects of paclitaxel on cultured ovarian cancer cells. Oncol. Res. **7:**145-56.
8. **Chajry, N., P. M. Martin, C. Cochet, and Y. Berthois.** 1996. Regulation of p42 mitogen-activated-protein kinase activity by protein phosphatase 2A under conditions of growth inhibition by epidermal growth factor in A431 cells. Eur. J. Biochem. **235:**97-102.
9. **Chen, Y. R., C. F. Meyer, and T. H. Tan.** 1996. Persistent activation of c-Jun N-terminal kinase 1 (JNK1) in gamma radiation-induced apoptosis. J. Biol. Chem. **271:**631-4.
10. **Coso, O. A., M. Chiariello, G. Kalinec, J. M. Kyriakis, J. Woodgett, and J. S. Gutkind.** 1995. Transforming G protein-coupled receptors potently activate JNK (SAPK). Evidence for a divergence from the tyrosine kinase signaling pathway. J. Biol. Chem. **270:**5620-4.
11. **Coso, O. A., M. Chiariello, J. C. Yu, H. Teramoto, P. Crespo, N. Xu, T. Miki, and J. S. Gutkind.** 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell **81:**1137-46.
12. **Culig, Z., A. Hobisch, A. Hittmair, M. Widschwendter, C. Radmayr, G. Bartsch, and H. Klocker.** 1996. Alterations of the transcription factor activating protein-1-mediated response in the DU-145 prostatic carcinoma cells., p. 233. Eighty-seventh annual meeting of the American Association for Cancer Research, Washington, DC.
13. **Cuvillier, O., G. Pirianov, B. Kleuser, P. G. Vanek, O. A. Coso, S. Gutkind, and S. Spiegel.** 1996. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. Nature **381:**800-3.
14. **de Rooij, J., and J. L. Bos.** 1997. Minimal Ras-binding domain of Raf1 can be used as an activation-specific probe for Ras. Oncogene **14:**623-625.
15. **Dent, P., Y. H. Chow, D. K. Morrison, R. Jove, and T. W. Sturgill.** 1994. Expression, purification and characterization of recombinant mitogen-activated protein kinase kinases. Biochem. J. **303:**105-112.
16. **Derijard, B., M. Hibi, I.-H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R. J. Davis.** 1994. JNK1: a protein kinase situmulated by UV light and Ha-ras that binds and phosphorylates the c-Jun activation domain. Cell **76:**1025-1037.
17. **Garcia, A. M., C. Rowell, K. Ackermann, J. J. Kowalczyk, and M. D. Lewis.** 1993. Peptidomimetic inhibitors of Ras farnesylation and function in whole cells. J. Biol. Chem. **268:**18415-18418.
18. **Giannakakou, P., D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, T. Fojo, and M. S. Poruchynsky.** 1997. Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J. Biol. Chem. **272:**17118-17125.
19. **Groom, L. A., A. A. Sneddon, D. R. Alessi, S. Dowd, and S. M. Keyse.** 1996. Differential regulation of the MAP, SAP and RK/p38 kinases by Pyst1, a novel cytosolic dual-specificity phosphatase. EMBO. J. **15:**3621-32.
20. **Gujuluva, C. N., J.-H. Baek, K.-H. Shin, H. M. Cherrick, and N.-H. Park.** 1994. Effect of UV-irradiation on cell cycle, viability and the expression of p53, gadd153, gadd45 genes in normal and HPV-immortalized human oral keratinocytes. Oncogene **9:**1819-1827.
21. **Haldar, S., A. Basu, and C. M. Croce.** 1997. Bcl2 is the guardian of microtubule integrity. Cancer Res. **57:**229-33.
22. **Haldar, S., J. Chintapalli, and C. M. Croce.** 1996. Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res. **56:**1253-5.
23. **Hall, A., and M. Zerial.** 1995. General introduction, p. 3-11. *In* M. Zerial, and L. A. Huber (ed.), Guidebook to the small GTPases. Oxford University Press, Oxford,.
24. **Hambleton, J., S. L. Weinstein, L. Lem, and A. L. DeFranco.** 1996. Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages. Proc. Natl. Acad. Sci. USA **93:**2774-8.
25. **Huschtscha, L. I., W. A. Bartier, C. E. Ross, and M. H. Tattersall.** 1996. Characteristics of cancer cell death after exposure to cytotoxic drugs in vitro. Br. J. Cancer **73:**54-60.
26. **Ichijo, H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, and Y. Gotoh.** 1997. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science **275:**90-4.
27. **Jacobson, M. D.** 1997. Apoptosis: Bcl-2-related proteins get connected. Curr. Biol. **7:**R277-81.
28. **Jelinek, T., P. Dent, T. W. Sturgill, and M. J. Weber.** 1996. Ras-induced activation of Raf-1 is dependent on tyrosine phosphorylation. Mol. Cell. Biol. **16:**1027-1034.
29. **Johnson, N. L., A. M. Gardner, K. M. Diener, C. A. Lange-Carter, J. Gleavy, M. B. Jarpe, A. Minden, M. Karin, L. I.**

Zon, and G. L. Johnson. 1996. Signal transduction pathways regulated by mitogen-activated/extracellular response kinase kinase kinase induce cell death. J. Biol. Chem. **271**:3229-37.

30. **Kahana, J. A., and P. A. Silver.** 1995. Isotopic assays for reporter gene activity., p. 9.7.1-9.7.28. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidmain, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley and Sons, Inc.,, New York, NY.
31. **Kallunki, T., B. Su, I. Tsigelny, H. K. Sluss, B. Derijard, G. Moore, R. Davis, and M. Karin.** 1994. JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. Genes Dev. **8**:2996-3007.
32. **Karin, M.** 1996. The regulation of AP-1 activity by mitogen-activated protein kinases. Philos. Trans. R. Soc. Lond. B Biol. Sci. **351**:127-34.
33. **Karin, M., Z. Liu, and E. Zandi.** 1997. AP-1 function and regulation. Curr. Opin. Cell Biol. 9:240-6.
34. **Kawamura, K. I., D. Grabowski, K. Weizer, R. Bukowski, and R. Ganapathi.** 1996. Modulation of vinblastine cytotoxicity by dilantin (phenytoin) or the protein phosphatase inhibitor okadaic acid involves the potentiation of anti-mitotic effects and induction of apoptosis in human tumour cells. Br. J. Cancer **73**:183-8.
35. **Kawasaki, H., T. Moriguchi, S. Matsuda, H. Z. Li, S. Nakamura, S. Shimohama, J. Kimura, Y. Gotoh, and E. Nishida.** 1996. Ras-dependent and Ras-independent activation pathways for the stress-activated-protein-kinase cascade. Eur. J. Biochem. **241**:315-21.
36. **Keyomarsi, K., L. Sandoval, V. Band, and A. B. Pardee.** 1991. Synchronization of tumor and normal cells from G1 to multiple cell cycles by lovastatin. Cancer Res. **51**:3602-3609.
37. **Kyriakis, J. M., and J. Avruch.** 1996. Sounding the alarm: protein kinase cascades activated by stress and inflammation. J. Biol. Chem. **271**:24313-6.
38. **Kyriakis, J. M., P. Banerjee, E. Nikolakaki, T. Dai, E. A. Rubie, M. F. Ahmad, J. Avruch, and J. R. Woodgett.** 1994. The stress-activated protein kinase subfamily of c-Jun kinases. Nature **369**:156-60.
39. **Lin, Z. G., H. Hsu, D. V. Goeddel, and M. Karin.** 1996. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell **87**:565-76.
40. **Maundrell, K., B. Antonsson, E. Magnenat, M. Camps, M. Muda, C. Chabert, C. Gillieron, U. Boschert, E. Vial-Knecht, J.-C. Martinou, and S. Arkinstall.** 1997. Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J. Biol. Chem. **272**:25238-25242.
41. **McGuire, M. P., W. J. Hoskins, M. F. Brady, P. R. Kucera, E. E. Partridge, K. Y. Look, D. L. Clarke-Pearson, and M. Davidson.** 1996. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. N. Engl. J. Med. **334**:1-6.
42. **McNally, F. J.** 1996. Modulation of microtubule dynamics during the cell cycle. Curr. Opin. Cell Biol. **8**:23-29.
43. **Minden, A., A. Lin, F. X. Claret, A. Abo, and M. Karin.** 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81**:1147-57.
44. **Minden, A., A. Lin, M. McMahon, C. Lange-Carter, B. Derijard, R. J. Davis, G. L. Johnson, and M. Karin.** 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. Science **266**:1719-23.
45. **Morooka, H., J. V. Bonventre, C. M. Pombo, J. M. Kyriakis, and T. Force.** 1995. Ischemia and reperfusion enhance ATF-2 and c-Jun binding to cAMP response elements and to an AP-1 binding site from the c-jun promoter. J. Biol. Chem. **270**:30084-92.
46. **Muda, M., U. Boschert, A. Smith, B. Antonsson, C. Gillieron, C. Chabert, M. Camps, I. Martinou, A. Ashworth, and S. Arkinstall.** 1997. Molecular cloning and functional characterization of a novel mitogen-activated protein kinase phosphatase, MKP-4. J. Biol. Chem. **272**:5141-51.
47. **Muda, M., A. Theodosiou, N. Rodrigues, U. Boschert, M. Camps, C. Gillieron, K. Davies, A. Ashworth, and S. Arkinstall.** 1996. The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. J. Biol. Chem. **271**:27205-8.
48. **Nicolaou, K. C., N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, and E. Hamel.** 1997. Synthesis of epothilones A and B in solid and solution phase. Nautre **387**:268-272.
49. **Rapp, U. R., J. Troppmair, T. Beck, and M. J. Birrer.** 1994. Transfromation by Raf and other oncogenes renders cells differentially sensitive to growth inhibition by a dominant negative c-Jun mutant. Oncogene **9**:3493-3498.
50. **Rausch, O., and C. J. Marshall.** 1997. Tyrosine 763 of the murine granulocyte colony-stimulating factor receptor mediates Ras-dependent activation of the JNK/SAPK mitogen-activated protein kinase pathway. Mol. Cell. Biol. **17**:1170-9.
51. **Robinson, M. J., and M. H. Cobb.** 1997. Mitogen-activated protein kinase pathways. Curr. Opin. Cell Biol. **9**:180-186.
52. **Rosette, C., and M. Karin.** 1996. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science **274**:1194-7.
53. **Rowinsky, E. K.** 1993. Clinical pharmacology of Taxol. Monogr. Natl. Cancer Inst. **15**:25-37.
54. **Saunders, C., and L. E. Limbird.** 1997. Disruption of microtubules reveals two independent apical targeting mechanisms for G-protein-coupled receptors in polarized renal epithelial cells. J. Biol. Chem. **272**:19035-19045.
55. **Seimiya, H., T. Mashima, M. Toho, and T. Tsuruo.** 1997. c-Jun NH2-terminal kinase-mediated activation of interleukin-1beta converting enzyme/Ced-3-like protease during anticancer drug-induced apoptosis. J.Biol. Chem. **272**:4631-6.
56. **Shim, J., H. Lee, J. Park, H. Kim, and E. J. Choi.** 1996. A non-enzymatic p21 protein inhibitor of stress-activated protein kinases. Nature **381**:804-6.
57. **Shore, V. H., T.-H. Wang, C.-L. Wang, R. J. Torry, M. R. Caudle, and D. S. Torry.** 1997. Characterization of vascular endothelial growth factor, placental growth factor and their receptors in isolated human trophoblast. Placenta .
58. **Suffness, M.** 1994. Is taxol a surrogate for a universal regulator of mitosis? in vivo **8**:867-878.
59. **Taylor, S. J., and D. Shalloway.** 1996. Cell cycle-dependent activation of Ras. Curr. Biol. **6**:1621-1627.
60. **Tishler, R. B., D. M. Lamppu, S. Park, and D. P. Brendan.** 1995. Microtubule-active drugs taxol, vinblastine, and

nocodazole increase the levels of transcriptionally active p53. Cancer Res. 55:6021-6025.

61. **Wahl, A. F., K. L. Donaldson, C. Fairchild, F. Y. Lee, S. A. Foster, G. W. Demers, and D. A. Galloway.** 1996. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nat. Med. 2:72-9.

62. **Wang, T. H., H. S. Wang, H. Ichijo, P. Giannakakou, J. S. Foster, T. Fojo, and J. Wimalasena.** 1998. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress- activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J. Biol. Chem. 273:4928-36.

63. **Wilson, L., and M. A. Jordan.** 1994. Pharmacological probes of microtubule function., p. 59-83. *In* J. S. Hyams, and C. W. Lloyd (ed.), Microtubules, vol. 13. John Wiley and Sons, Inc.,, New York.

64. **Wimalasena, J., C. Cavallo, and D. Meehan.** 1991. Human epithelial ovarian cancer cell steroid secretion and its control by gonadotropins. Gynecol. Oncol. 41:56-63.

65. **Woodgett, J. R., J. M. Kyriakis, J. Avruch, L. I. Zon, B. Zanke, and D. J. Templeton.** 1996. Reconstitution of novel signalling cascades responding to cellular stresses. Philos. Trans. R. Soc. Lond. B Biol. Sci. 351:135-41.

66. **Wu, X., S. J. Noh, G. Zhou, J. E. Dixon, and K. L. Guan.** 1996. Selective activation of MEK1 but not MEK2 by A-Raf from epidermal growth factor-stimulated Hela cells. J. Biol. Chem. 271:3265-71.

67. **Xia, Z., M. Dickens, J. Raingeaud, R. J. Davis, and M. E. Greenberg.** 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326-31.

PART IV.

MICROTUBULE DYSFUNCTION INDUCED BY PACLITAXEL INITIATES APOPTOSIS THROUGH BOTH C-JUN N-TERMINAL KINASE (JNK)-DEPENDENT AND -INDEPENDENT PATHWAYS IN OVARIAN CANCER CELLS

ABSTRACT

The antineoplastic agent paclitaxel (Taxol®), a microtubule (MT) stabilizing agent, is known to arrest cells at the G_2/M phase of the cell cycle and induce apoptosis. We and others have recently demonstrated that paclitaxel (PTX) also activates the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) signal transduction pathway in various human cell types, however, no clear role has been established for JNK/SAPK in PTX-induced apoptosis. To further examine the role of JNK/SAPK signaling cascades in apoptosis resulting from microtubular dysfunction induced by PTX, I have coexpressed dominant negative (dn) mutants of signaling proteins of the JNK/SAPK pathway (Ras, ASK1, Rac, JNKK, JNK) in human ovarian cancer cells with a selectable marker to analyze the apoptotic characteristics of cells expressing dn vectors following exposure to PTX. Expression of these dn signaling proteins had no effect on Bcl-2 phosphorylation, yet inhibited apoptotic changes induced by PTX up to 16 h after treatment. Coexpression of these dn signaling proteins had no protective effect after 48 h of PTX treatment. These data indicate that: (i) activated JNK/SAPK acts upstream of membrane changes and caspase-3 activation in PTX-initiated apoptotic pathways, independently of cell cycle stage, (ii) activated JNK/SAPK is not responsible for PTX-induced phosphorylation of Bcl-2, and (iii) apoptosis resulting from MT damage may comprise multiple mechanisms, including a JNK/SAPK-dependent early phase and a JNK/SAPK-independent late phase.

INTRODUCTION

Paclitaxel (Taxol®) is an antineoplastic agent specifically targeting MTs (26)(46) and extensive studies indicate that PTX arrests cells at the G_2/M phase of the cell cycle (34). While mitotic arrest of PTX-treated cells has been observed to initiate apoptosis (33)(43)(77), the biochemical events downstream of kinetic stabilization of MT dynamics which lead to apoptosis remain largely unclear (34). Furthermore, substantial evidence indicates that the G_2/M arrest of the cell cycle may not be the only mechanism to induce apoptosis (39)(49)(50)(53); additional phosphoregulatory pathways may be involved in inducing apoptosis (6)(21)(76).

We and others have recently demonstrated that PTX activates the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) pathways in a variety of human cells through microtubular

interactions (1)(73). The JNK/SAPK signaling pathways respond to various stress-related stimuli and are involved in initiation of apoptosis in many cell types (19)(30)(35)(38)(36). Whether JNK/SAPK activation is required for PTX-induced apoptosis has remained unclear, however; it is known that PTX induces phosphorylation of Bcl-2 (6)(21) and that Bcl-2 can be phosphorylated by activated JNK/SAPK (45).

The purpose of this study was to examine whether inhibition of the JNK/SAPK signaling pathway protects cells from PTX-induced apoptosis and/or abrogates PTX-induced phosphorylation of Bcl-2. These results demonstrate that expression of dn ASK1 (apoptosis signal-regulating kinase 1), dn Rac, dn JNKK, or dn JNK, while exerting no effects on phosphorylation of Bcl-2, inhibits apoptosis induced by PTX treatment up to 16 h. The present study clearly indicates that activation of the JNK/SAPK signaling cascade promotes early phases of PTX-induced apoptosis, independently of cell cycle stage or Bcl-2 phosphorylation.

EXPERIMENTAL PROCEDURES

CELL CULTURE, TRANSFECTION, AND PACLITAXEL TREATMENT

Ovarian carcinoma cells BR (9)(75) were cultured in DMEM/F12 (Sigma, St. Louis, MO) supplemented with 10% NBS, penicillin, and streptomycin and incubated at 37°C in 5% CO_2. Control vectors pCMV-lacZ and pSRα empty vector as well as expression vectors for wild type (wt) JNK1 (pSRα-JNK), dominant-negative (dn) Ras (pSRα-dn Ras), and dn Rac (pSRα-dn Rac) (42) were from Z.-G. Liu and M. Karin (University of California at San Diego). A Myc-epitope tagged expression vector for wt Rac, pEXV3-Rac, was from A. Hall (University College London, UK)(22). Dominant-negative expression vectors for JNK/SAPK (pSRα-APF) and JNKK/SEK1 (pSRα-K116R) were from G.L. Johnson (National Jewish Center for Immunology and Respiratory Medicine, Denver, CO)(32). Expression vectors for wt and dn ASK1 (pcDNA3-ASK1-HA, pcDNA3-dn ASK1-HA) were described previously (12)(29)(55)(63). In addition to pSRα and pcDNA3 (Invitrogen, Carlsbad, CA) empty vectors, expression vectors used for control purposes include: pCMV-CD20 that was from E. Harlow (Massachusetts General Hospital, Boston)(69) and pcDNA3-kinase dead $p70^{S6kinase}$(K100R) that was generated by PCR-mutagenesis from the $p70^{S6kinase}$ cDNA, which was originally from J. Avruch (Massachusetts General Hospital). Liposome-mediated transfections of BR cells using Tfx-50 (Promega, Madison, WI) were performed as described in

Part III (73). A CMV promoter-driven enhanced green fluorescent protein construct, pEGFP (Clontech, Palo Alto, CA), was cotransfected as a selectable marker for transfected cells. Stock solutions of PTX, actinomycin-D, and cisplatin (all from Sigma) were prepared with DMSO at concentrations of 10 mM, 1 mM, and 50 mM, respectively. In this study, 1 μM PTX was used to treat cultured cells.

ANNEXIN-V BINDING, FLOW CYTOMETRIC ANALYSES AND SORTING

Twenty-four h after cotransfection of BR cells with pEGFP and wt or dn expression vectors, 10^5 trypsinized cells were incubated at room temperature for 15 min with 5 μl phycoerythrin (PE)-conjugated annexin-V (AV) (Pharmingen, San Diego, CA) and 0.125 μg/ml of 7-amino actinomycin D (7-AAD) (Sigma) in binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and analyzed by flow cytometry with a FACStar^{Plus} (Becton Dickinson, San Jose, CA). Fluorochromes such as green fluorescent protein expressed by pEGFP, PE-annexin V, and 7-AAD were excited by laser tuned to 488 nm and emissions were detected at 507 nm, 575 nm, and 650 nm, respectively. Data of 10,000 cells from each sample were analyzed with the CellQuest3 software (Becton Dickinson). To compare other apoptotic characteristics in cells with or without expression of dn vectors, the green fluorescent protein positive and negative cells were sorted using a FACStar^{Plus} flow cytometer and analyzed separately.

WESTERN BLOT ANALYSES

Aliquots of cell lysate containing equal protein mass were resolved on SDS-PAGE, transferred to nitrocellulose membranes, and probed with anti-HA epitope (Boehringer Mannheim, Indianapolis, ID), anti-Myc (Calbiochem, San Diego, CA), anti-JNK (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PARP (Upstate Biotechnology, Lake Placid, NY), or anti-Bcl-2 (DAKO, Carpinteria, CA) antibodies followed by relevant second antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology). After washing, proteins were detected by chemiluminescence (ECL, Amersham, Arlington Heights, IL) as previously described (73).

IMMUNOCOMPLEX JNK ASSAY

Activity of the JNK/SAPK was measured as described in **Part III** (73). Endogenous JNKs were immunoprecipitated from cell lysate using an anti-JNK Ab conjugated to agarose beads (Santa Cruz Biotechnology) and the JNK activity was assayed by levels of incorporated [γ-^{32}P]ATP into its substrate GST-cJun (amino acids 1-79) (Santa Cruz).

CELL CYCLE ANALYSES

Green cells expressing control vector or dn expression vectors were isolated by flow cytometric sorting, fixed with 70% ethanol, treated with 0.1 mg/ml RNase (Sigma), stained with 20 μg/ml propidium iodide (PI), and analyzed with flow cytometry. PI-stained DNA content of each cell was used as the parameter of cell cycle profile.

CASPASE-3 ASSAY

We used the colorimetric substrate Ac-DEVD-*p*NA (Calbiochem) for caspase-3 assays in a procedure modified from the manufacturer's protocol. Briefly, aliquots of sonicated cell lysate were prepared in lysis buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM DTT, 0.1 mM EDTA), incubated with 200 μM Ac-DEVD-*p*NA in assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM DTT, 0.1 mM EDTA, 10% glycerol) in 96-well plates at 37°C for 24 h. Absorbance of the cleaved product was measured at 405 nm in a BioKinetic EL340 microplate Reader (Bio-Tek Instruments, Winooski, VT).

DNA FRAGMENTATION ASSAYS

Identification of the ladder pattern of DNA fragmentation was done according to a previously reported method (59). Treated cells from 6-well plates, including both floaters and attached cells, were collected by scraping in their culture media, rinsed once with ice-cold PBS, and spun at 1000 x *g* for 5 min. Cell pellets were resuspended in 0.5 ml digestion buffer (10 mM EDTA, 50 mM Tris pH=8, 0.5 % sodium lauryl sarcosine, 0.5 mg/ml proteinase K) and incubated at 55°C for 3 h. RNase A (final concentration at 0.1 mg/ml) was added and incubated for another 1 h. After extraction with an equal

volume of phenol-chloroform twice and by chloroform once, sodium acetate (0.3 M final concentration) and 2 to 2.5 volumes of pure ethanol were added into the aqueous phase of solution, and the resultant mixture was kept at -70°C for 20 min. DNA was precipitated by spinning at 14,000 rpm, 4°C for 10 min and dried with a Speed Vac (Savant Inc.) for 10 min. Dry DNA pellets were resuspended with 20-50 µl TE buffer (10 mM Tris pH=7.4, 1 mM EDTA) at 4°C overnight. Ten µg per lane DNA was load to 1.6 % agarose gel containing a minimal amount of ethidium bromide, resolved with 110 V electrical current, and visualized with UV.

To quantify fragmented DNA in apoptotic cells, transfected cells isolated by flow cytometry were treated with PTX or vehicle (DMSO) and levels of fragmented DNA resulting from apoptosis were measured with a Cell Death ELISA kit according to the manufacturer's protocol (Boehringer Mannheim).

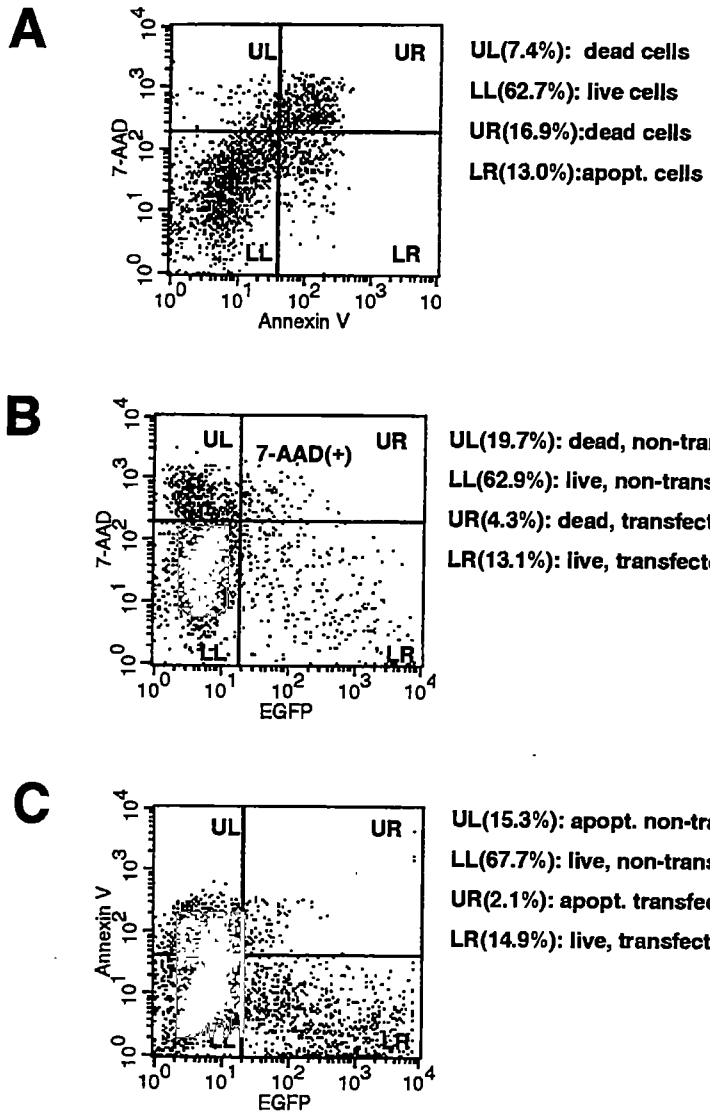
STATISTICS

Analysis of variance (ANOVA) and Scheffe test for *post-hoc* comparisons were used for statistical analyses with the STATISTICA software (Statsoft Inc., Tulsa, OK). The *p*-values equal to or greater than 0.05 are considered to be not significant.

RESULTS

EXPRESSION OF DN ASK1, DN RAC, DN JNKK, OR DN JNK TRANSIENTLY ALLEVIATES CYTOTOXICITY INDUCED BY PACLITAXEL TREATMENT FOR 16 H.

To determine whether inhibition of the JNK/SAPK signaling cascade abolishes apoptosis in cells treated with PTX, we analyzed PTX-induced apoptosis among ovarian cancer BR cells transfected with dn Ras, dn ASK1, dn Rac, dn JNKK, or dn JNK, along with pEGFP to allow selection of transfected cells. The efficacies of these dn expression vectors for inhibition of the JNK/SAPK have been demonstrated previously (73). Following PTX treatment for the indicated times, transfected cells were stained with both PE-conjugated AV (binding to both apoptotic cells and dead cells) and the viability dye 7-AAD (staining dead cells but not early apoptotic cells) to differentiate early apoptotic cells (AV positive / 7-AAD negative) from dead cells (7-AAD positive)(**Figure 1**).



(Figure 1) Three-color analyses for apoptosis and cell death among transfected cells by flow cytometry. Ovarian cancer BR cells were cotransfected with pEGFP and an expression vector for dn Ras for 24 h, treated with 1 μM paclitaxel for 16 h, stained PE-conjugated annexin-V and 7-AAD, and analyzed by flow cytometry. *A*, early apoptotic cells (annexin-V positive/7-AAD negative) in LR region were differentiated from dead cells (7-AAD positive) in UR region. The UL and LL regions contain dead cells (7-AAD positive) and live cells (annexin-V positive) and live cells (annexin-V negative/7-AAD negative), respectively. *B* and *C*, to analyze early apoptotic cells among transfected cells, we first excluded dead cells (7-AAD positive) from the whole population shown in *B* and only analyzed live cells by profiles of EGFP and PE-conjugated annexin-V. Percent of early apoptotic cells among transfected (green) cells were calculated by: 100% x UR/(UR + RL) shown in *C*.

Percentages of early apoptotic cells among transfected cells treated with PTX for 16 h or 48 h are summarized in **Table I**. Data presented as the (% in PTX-treated cells / % in DMSO-treated cells) ratios among cells expressing different dn signaling proteins are compared in **Figure 2**. In transfected cells without PTX treatment, expression of dn vectors decreased the percentage of early apoptotic cells. These decreases in basal levels of apoptosis were specific for expression of these dn expression vectors because expression of irrelevant genes in pCMV-lacZ, pcDNA3-kinase dead-$p70^{S6kinase}$, or pCMV-CD20 did not decrease basal apoptosis (data not shown). Treatment with PTX for 16 h significantly increased apoptosis in both control vector-transfected and dn Ras-transfected cells ($p< 0.05$), but the induction of apoptosis by PTX was decreased to statistically insignificant levels (*n.s.*) in cells expressing dn ASK1, dn Rac, dn JNKK, or dn JNK (**Table I & Figure 2A**). In contrast to 16-h treatment with PTX, 48 h of treatment significantly ($p< 0.05$) increased early apoptotic cells in all transfected cells irrespective of expression of dn vector types (**Table I & Figure 2B**).

To verify that expression of dn signaling proteins efficiently inhibited endogenous JNK/SAPK activity, we cotransfected BR cells with pEGFP and dn expression vectors, isolated transfected cells by flow cytometry using expression of pEGFP (green fluorescence) as a selectable marker, treated with PTX, and analyzed by immunocomplex JNK assay. We isolated only live (7-AAD negative) green cells expressing transfected vectors (**Figure 3A**) and verified expression of dn signaling proteins by Western blot analyses (**Figures 3B & 3C**).

The inability of dn vectors to protect cells from apoptosis following 48-h treatment with PTX did not result from decreased expression of dn signaling proteins. We have confirmed substantial expression of EGFP by fluorescent microscopy (data not shown) and expression of dn signaling proteins by Western blot analyses up to 48 h of PTX treatment (**Figures 3B & 3C**). At 48h of treatment, protein levels of dn JNK or dn ASK1 in DMSO-treated cells remained as high as those at earlier time points; whereas those in PTX-treated cells slightly decreased, perhaps resulting from a general protein degradation during apoptosis (**Figures 3B & 3C**). Despite of its slight decrease in later time points of PTX treatment, expression of these dn signaling proteins efficiently suppressed PTX-induced JNK activation through all the time points (**Figures 3B & 3C**).

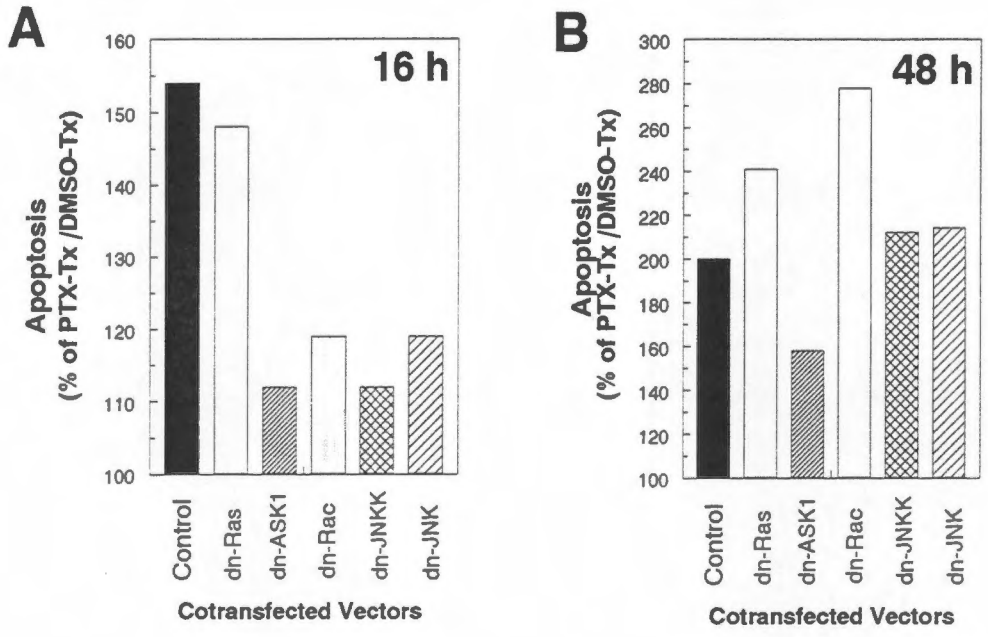
Collectively, these results suggest that expression of dn ASK1, dn Rac, dn JNKK, or dn JNK transiently protects ovarian cancer cells from PTX-induced apoptosis up to 16 h. When treated with PTX for a longer time, such as 48 h, cells may undergo apoptosis through additional, JNK/SAPK-independent

TABLE I

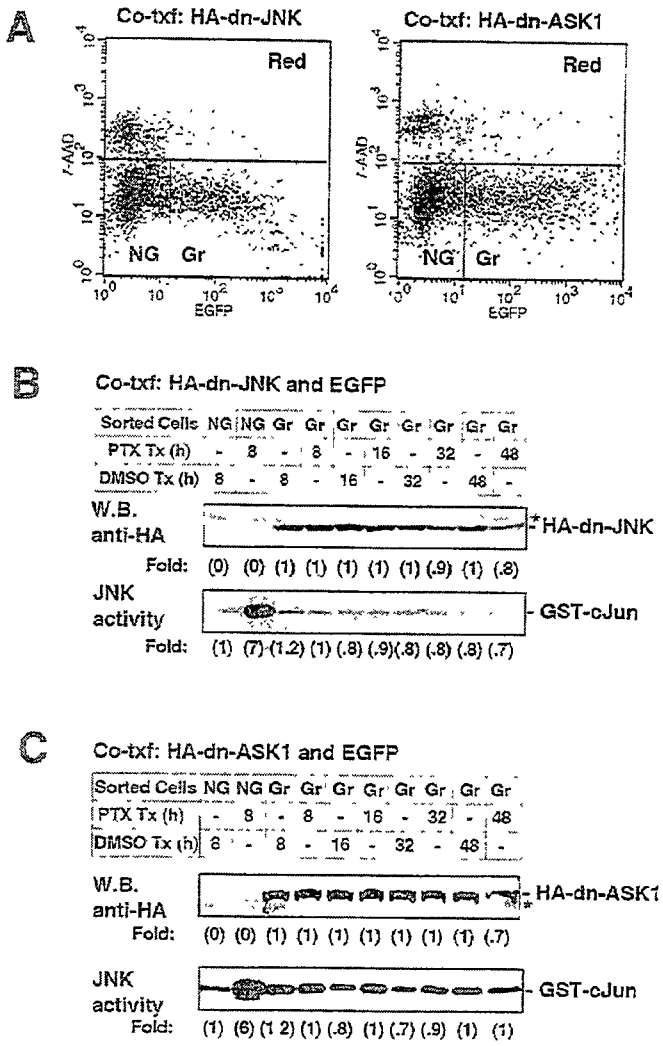
Apoptotic effects of paclitaxel treatment on transfected cells

Human ovarian cancer BR cells were cotransfected with pEGFP and empty (Control) or dominant negative (dn) expression vectors for 24 h, treated with 1 μM paclitaxel for 16 h or 48 h, and stained with phycoerythrin-conjugated annexin-V and 7-amino actinomycin D (7-AAD) followed by flow cytometric analyses. Percentages of early apoptotic cells (annexin-V positive / 7-AAD negative) among green transfected populations, which were set as 100%, are shown as the mean ± S.E. (n = 6) of duplicates from three independent experiments. Statistical data in the right column are comparisons between DMSO- and paclitaxel-treatment (horizontal comparisons). The symbols (*,‡) indicate significant differences ($p < 0.05$) between cells expressing dn-vectors and cells expressing control vector *(italicized values)* within each treatment group (vertical comparisons).

Tx time	Expressed Vectors	DMSO	Paclitaxel	Statistics
16 h				
	Control	*11.5 ± 0.3*	*17.7 ± 0.2*	$p < 0.05$
	dn Ras	6.5 ± 0.2*	9.6 ± 0.8‡	$p < 0.05$
	dn ASK1	8.5 ± 0.5	9.5 ± 0.4‡	*n.s.*
	dn Rac	5.0 ± 0.4*	5.9 ± 0.3‡	*n.s.*
	dn JNKK	8.1 ± 0.3	9.5 ± 1.1‡	*n.s.*
	dn JNK	7.3 ± 0.5*	8.7 ± 1.0‡	*n.s.*
48 h				
	Control	*16.5 ± 1.5*	*33.1 ± 1.3*	$p < 0.05$
	dn Ras	13.9 ± 0.8	33.8 ± 0.7	$p < 0.05$
	dn ASK1	17.8 ± 1.0	28.1 ± 1.2	$p < 0.05$
	dn Rac	13.6 ± 0.9	37.8 ± 1.4	$p < 0.05$
	dn JNKK	14.9 ± 0.6	31.6 ± 0.8	$p < 0.05$
	dn JNK	14.3 ± 1.1	32.1 ± 1.3	$p < 0.05$



(Figure 2) Expression of dn-ASK1, -Rac, -JNKK, or -JNK significantly inhibits apoptosis induced by paclitaxel treatment for 16 h. Apoptosis among transfected BR cells were analyzed by staining profiles of PE-conjugated annexin-V and 7-AAD. *A*, Comparisons of paclitaxel (*PTX*)-induced apoptosis among cells expressing control vector or dn-vectors after paclitaxel treatment for 16 h. *B*, Comparisons of apoptosis among cells expressing different vectors after 48-h treatment with paclitaxel. *Bars* in figures are calculated from the data in **Table I** using the formula: 100% x (% in paclitaxel-treated cells / % in *DMSO*-treated cells).



(Figure 3) Isolation of transfected cells coexpressing green fluorescent protein and verification of expression of dn-signaling proteins in these cells during the period of treatment up to 48 h. *A,* Twenty-four h after cotransfection with pEGFP and dn-expression vectors, transfected cells were isolated by flow cytometric sorting using expression of green fluorescent protein as a selectable marker. Examples of isolating cells transfected with dn-JNK and dn-ASK1 are shown. Three regions, Gr (green), NG (Non-green), and Red (7-AAD positive) were selected to represent live transfected cells (coexpressing pEGFP), live non-transfected cells (EGFP negative), and dead cells, respectively. *B & C,* Western blot analyses and immunocomplex kinase assays were used to measure transfected protein levels and endogenous JNK activity, respectively. Expression of HA-dn-JNK or dn-ASK1-HA was not detected in non-green cells (NG) but was substantial in green transfected cells (Gr) and those protein levels remained largely intact up to 48 h of treatment. Efficient suppression of endogenous JNK activity by expression of dn-JNK or dn-ASK1 were demonstrated by the kinase assay using GST-cJun as substrate for JNK. Data shown are from a representative experiment, which was repeated twice with comparable results. Nonspecific bands also recognized by the 12CA5 antibody are labeled with asterisks (*).

mechanisms.

EXPRESSION OF DN SIGNALING PROTEINS OF THE JNK/SAPK PATHWAY DOES NOT ALTER CELL CYCLE PROFILES OF PACLITAXEL-TREATED CELLS.

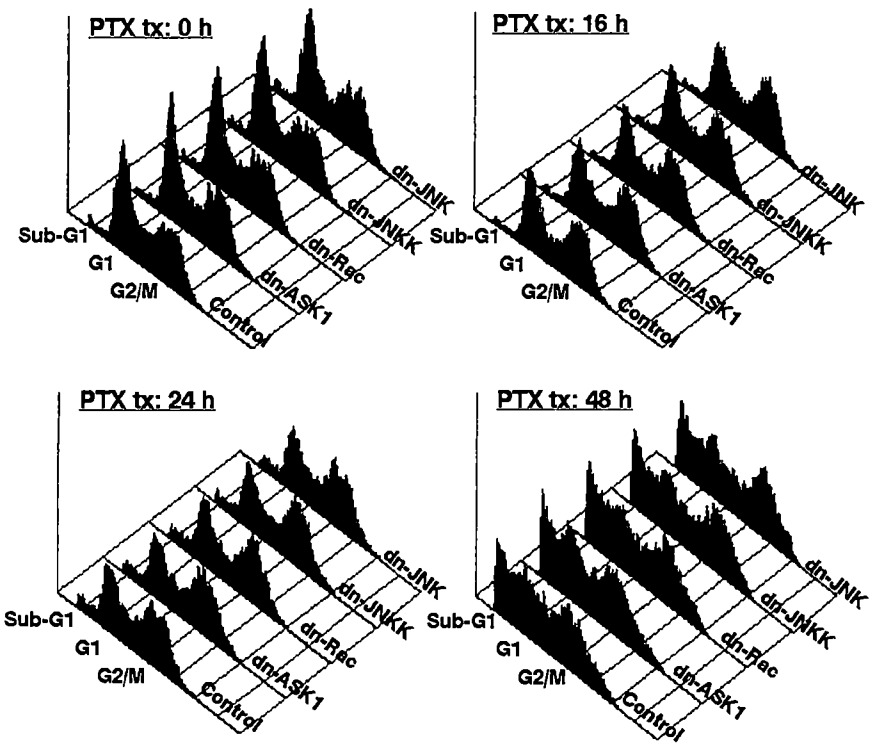
To investigate whether these dn signaling proteins inhibited PTX-induced apoptosis through regulation on cell cycle progression, we analyzed cell cycle profiles of cells expressing dn signaling proteins at 16, 24, and 48 h of PTX treatment. Compared with cells expressing control vector, expression of dn ASK1, dn Rac, dn JNKK, or dn JNK did not change cell cycle profiles, nor interfere with mitotic arrest of cells after PTX treatment (**Figure 4**).

APOPTOTIC CHARACTERISTICS IN PACLITAXEL-TREATED CELLS ARE ABROGATED BY BLOCKAGE OF THE JNK/SAPK PATHWAYS.

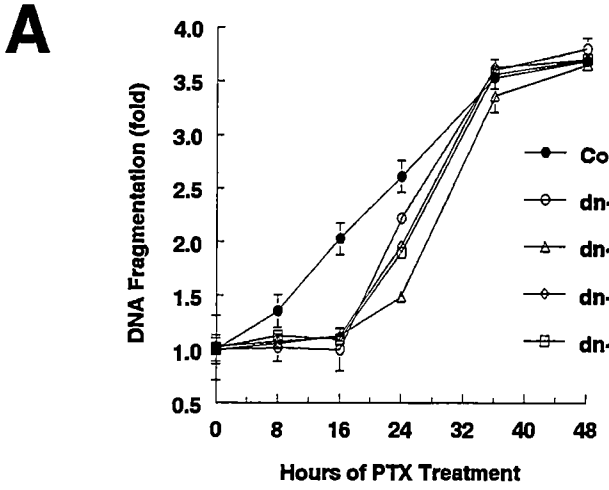
To examine the time course of inhibition of apoptosis by dn signaling proteins, we treated isolated cells expressing control vector or dn signaling proteins with PTX and quantified DNA fragmentation with ELISA. Expression of dn ASK1, dn Rac, dn JNKK, or dn JNK significantly inhibited PTX-induced DNA fragmentation up to 16 h, whereas the inhibition declined at 24 h and completely disappeared at 36 h of treatment (**Figure 5A**). In transfected cells treated with PTX for 16 h, expression of dn ASK1, dn Rac, dn JNKK, or dn JNK significantly inhibited caspase-3 activation by PTX (**Figure 5B**) and suppressed PTX-induced PARP cleavage (**Figure 5C**). These results are consistent with data given above for AV binding (**Table I and Figure 2**), again indicating that inhibition of the JNK/SAPK signaling pathways transiently protects ovarian cancer cells from PTX-induced apoptosis.

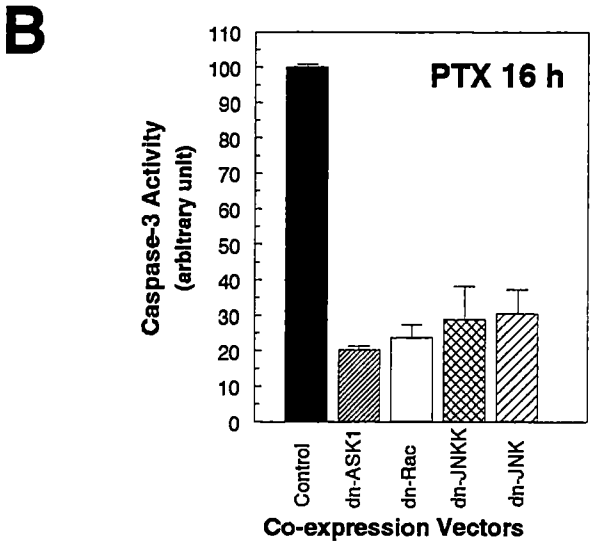
OVEREXPRESSION OF WT ASK1, WT RAC, OR WT JNK PROMOTES APOPTOSIS.

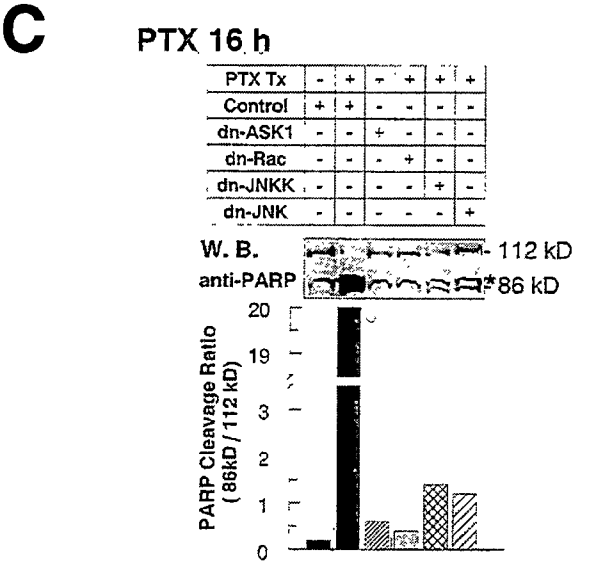
Our data would predict that overexpression of wt signaling proteins of the JNK/SAPK pathways may promote PTX-induced apoptosis. Flow cytometric analyses on AV binding confirmed that overexpression of wt ASK1, wt Rac, of wt JNK significantly ($p< 0.05$) promoted apoptosis in both DMSO-treated and 16 h-PTX treated BR cells (**Figure 6A**). Consistent with flow cytometric data,



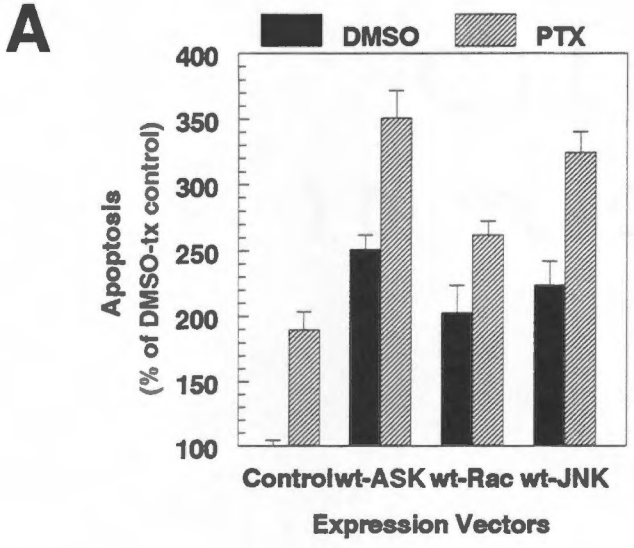
(Figure 4) Expression of dn-signaling proteins does not alter the cell cycle profiles nor prevent the G2/M arrest of paclitaxel-treated cells. Twenty-four h after transfection, cells expressing control or dn-signaling proteins were isolated by flow cytometric sorting, treated with paclitaxel for 16, 24, or 48 h, and analyzed by flow cytometry using propidium iodide-stained DNA content of each cell as parameter for the cell cycle profiles.

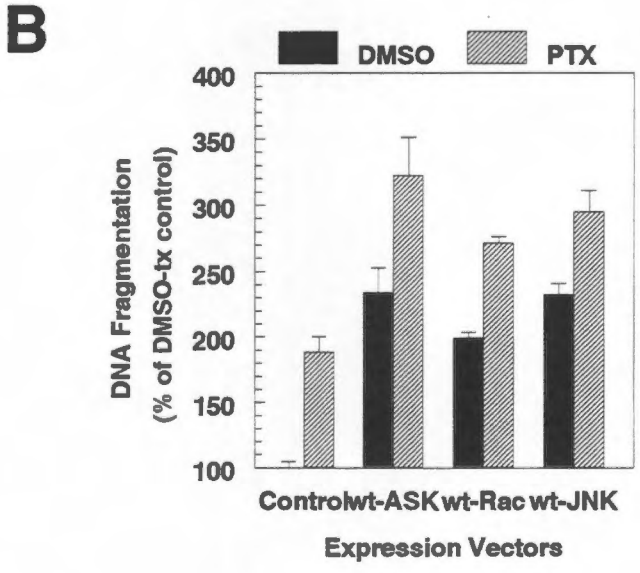




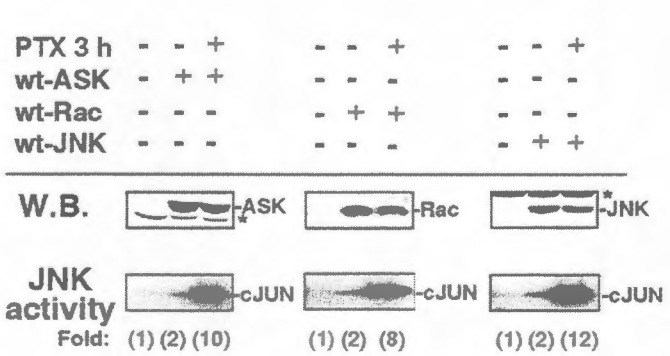


(Figure 5) Expression of dn-ASK1, -Rac, -JNKK, or -JNK transiently inhibits paclitaxel-induced DNA fragmentation, caspase-3 activation, and cleavage of PARP. Twenty-four h after transfection, sorted cells expressing different vectors were treated with paclitaxel and apoptotic features were analyzed. *A,* Time course of paclitaxel-induced DNA fragmentation measured by the Cell Death Detection ELISA. Data shown are mean ± S.E. (n = 6) of triplicates from two independent experiments. *B,* After treated with paclitaxel for 16 h, caspase-3 activity in cells expressing control vector or dn-vectors was measured using Ac-DEVD-pNA as a colorimetric substrate. Levels of caspase-3 activity are calculated by subtracting the mean of OD405 readings of DMSO-treated, control-transfected cells from each reading of paclitaxel-treated cells, then the resultant levels of paclitaxel-treated, control-transfected cells are set as 100 arbitrary units. Data shown are mean ± S.E. (n = 6) of triplicates from two independent experiments. *C,* After treated with paclitaxel (*PTX*) for 16 h, cleavage of PARP among transfected cells was analyzed by Western blot. The ratios between 112-kD (intact) and 86-kD (cleaved) species of PARP were compared in the bar graph. Data shown are from a representative experiment, which was repeated three times with comparable results. A nonspecific band also recognized by this anti-PARP antibody is labeled by an *asterisk* (*).





(Figure 6) Overexpression of wt-ASK1, wt-Rac or wt-JNK1 promotes apoptosis. *A*, BR cells were cotransfected with pEGFP and expression vectors for control, wt-ASK1, wt-Rac, or wt-JNK1 for 24 h, treated with DMSO (*solid bars*) or 1 μM paclitaxel (*hatched bars*) for 16 h, stained with PE-conjugated annexin-V and 7-AAD, and analyzed by flow cytometry as described in Fig. 1. *B*, BR cells overexpressing wt-ASK1, wt-Rac, or wt-JNK1 were isolated by flow cytometric sorting, treated with DMSO (*solid bars*) or 1 μM paclitaxel (*hatched bars*) for 16 h, and DNA fragmentation was quantified by ELISA. OD405 readings of control-transfected cells treated with DMSO are set as 100 arbitrary unit. Data shown are mean ± S.E. (n = 8 or 4) from several independent experiments. *C*, Isolated BR cells expressing control vector, HA-wt-ASK1, HA-wt-JNK1, or Myc-wt-Rac were treated with DMSO or 1 μM paclitaxel (*PTX*) for 3 h and expression of theses epitope-tagged, wt-signaling proteins were confirmed by Western blot analyses with an anti-HA (12CA5) antibody or an anti-Myc (9E10) antibody. Endogenous JNK activities in these lysates were measured by immunocomplex kinase assay using an anti-JNK antibody for immunoprecipitation and GST-c-Jun as substrate. Non-specific bands also recognized by the 12CA5 antibody are labeled by *asterisks* (*).



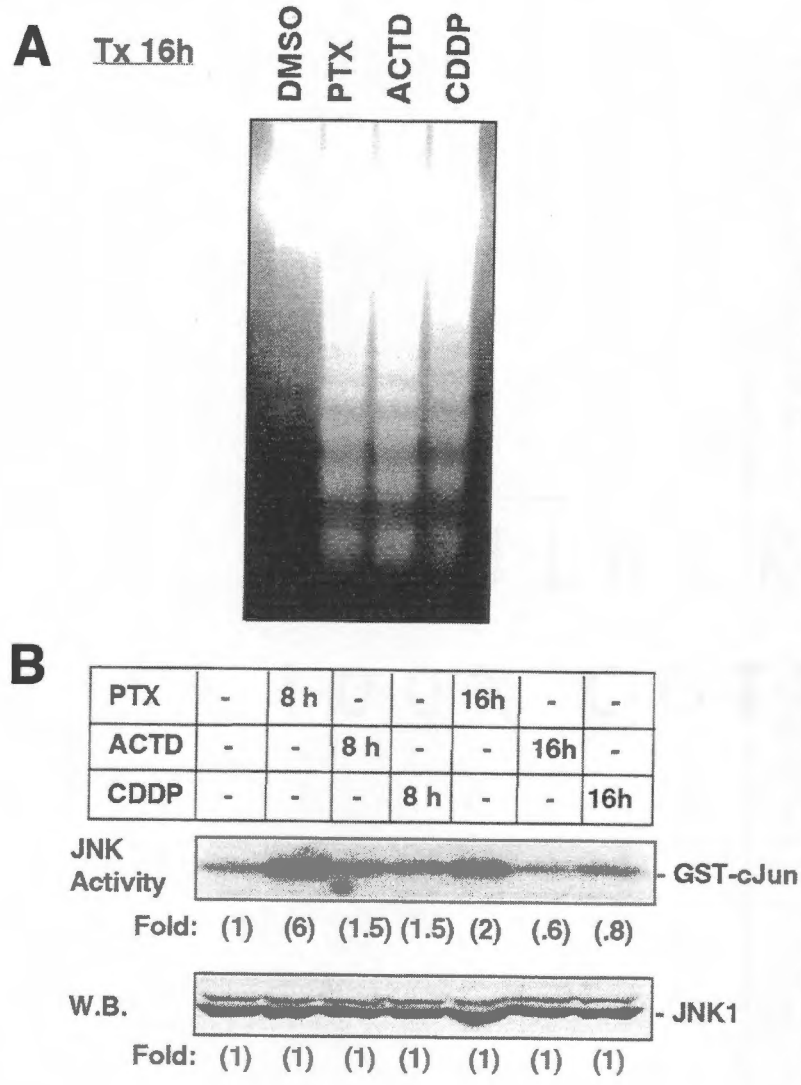
overexpression of wt ASK1, wt Rac, or wt JNK also significantly ($p< 0.05$) increased basal and PTX-induced DNA fragmentation (**Figure 6B**) and the increases in apoptosis measured by two independent methods are very similar. Western blot analyses with an anti-HA epitope antibody (12CA5) and an anti-Myc epitope antibody (9E10) confirmed expression of HA-wt ASK1, HA-wt JNK, and Myc-wt Rac (**Figure 6C**, upper panel) and immunocomplex kinase assays verified the augmentation of PTX-induced JNK activation by overexpressed signaling proteins (**Figure 6C**, lower panel). The increases in the apoptosis induced by PTX above those due to overexpression of wt signaling proteins were the same as that in PTX-treated, control-transfected cells (**Figures 6A & 6B**). Therefore, the effects of PTX and overexpression of wt signaling proteins appear to be additive. However, among cells overexpressing these wt signaling proteins, the 8-12 fold JNK activation induced by PTX (**Figure 6C**) was not proportional to the 2.5-3.5 fold increase of apoptosis (**Figures 6A & 6B**), suggesting that activation of JNK is not linearly related to apoptosis.

SUPPRESSION OF JNK/SAPK ACTIVATION SPECIFICALLY INHIBITS APOPTOSIS INDUCED BY 16 H-PACLITAXEL TREATMENT BUT NOT THAT INDUCED BY ACTINOMYCIN-D OR CISPLATIN.

To examine whether the JNK/SAPK signaling cascade plays a specific role in apoptosis resulting from microtubular dysfunction, we compared JNK activations and cytotoxicities in ovarian cancer cells treated with PTX or treated with two DNA-targeting agents, actinomycin-D (ACTD) and cisplatin (CDDP). Treatment with 5 μM actinomycin-D or 50 μM cisplatin for 16 h induced comparable ladder pattern of DNA fragmentation with that induced by PTX treatment (**Figure 7A**), whereas only PTX significantly activated JNK (**Figure 7B**). Moreover, expression of dn ASK1 or dn JNK only inhibited DNA fragmentation induced by 16 h-PTX treatment but not that induced by actinomycin-D or cisplatin (**Figure 7C**).

SUPPRESSION OF THE JNK/SAPK SIGNALING CASCADE DOES NOT INHIBIT PHOSPHORYLATION OF BCL-2 INDUCED BY PACLITAXEL.

To determine whether PTX-activated JNK/SAPK is required for phosphorylation of Bcl-2 in



(Figure 7) Expression of dn-ASK1 or dn-JNK specifically inhibits DNA fragmentation induced by paclitaxel but not that induced by actinomycin-D or cisplatin. *A*, Treatment with 1 μM paclitaxel (*PTX*), 5 μM actinomycin-D (*ACTD*), or 50 μM cisplatin (*CDDP*) for 16 h induced the ladder pattern of DNA fragmentation resolved in 1.6 % agarose gel. *B*, BR cells were treated with paclitaxel, actinomycin-D, or cisplatin for 8 or 16 h, and JNK activities and JNK protein levels were measured by immunocomplex kinase assay and Western blot analysis, respectively. Relative intensities of bands are shown as fold (shown in *parentheses*) of DMSO-treated control. *C*, Twenty-four h after transfection, sorted cells expressing control vector, dn-ASK1, or dn-JNK were treated with paclitaxel (*solid bars*), actinomycin-D (*hatched bars*), or cisplatin (*checked bars*) for 16 h. DNA fragmentation were quantified by ELISA. Data shown are mean ± S.E. (n=3) from a representative experiment.

PTX-treated cells, we cotransfected BR cells with pEGFP and dn expression vectors and treated cells with 1 μM PTX for 16 h. Following treatment, green fluorescent positive (transfected) and negative (non-transfected) cells were analyzed separately for phosphorylation of Bcl-2. Phosphorylated Bcl-2 bands exhibiting slower mobility on Western blot were identified from both transfected and non-transfected cells, irrespective of which dn vectors were overexpressed (**Figure 8A**). To confirm these results by an alternative approach, we cotransfected cells with pEGFP and dn vectors, isolated transfected cells by flow cytometric sorting, then treated control- or dn vector-expressing cells with PTX or DMSO for 16 h and analyzed the status of Bcl-2 phosphorylation. Consistent with previous experiments, treatment with PTX induced phosphorylation of Bcl-2 in all transfected cells, including those expressing control vector or vectors for dn ASK1, dn Rac, dn JNKK, or dn JNK (**Figure 8B**). Collectively, these results indicated that inhibition of the JNK/SAPK signaling cascade did not influence PTX-induced phosphorylation of Bcl-2.

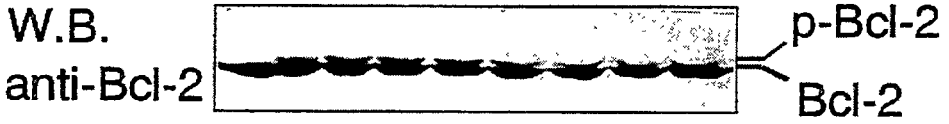
DISCUSSION

Activation of the JNK/SAPK signaling pathways has been mechanistically implicated in regulation of apoptosis (19)(30)(35)(36)(38), however, the roles of JNK/SAPK in promoting (14)(48)(78) or preventing apoptosis (54)(58) differ, depending on both cell type and apoptosis-triggering stimuli (30)(35). Furthermore, in addition to apoptosis, JNK/SAPK activation may be involved in proliferation (7)(67) and oncogenic transformation (60). On the other hand, apoptosis itself can be considered as a form of stress, hence, JNK/SAPK activation may be a stress response secondary to apoptosis, rather than a primary mediator in apoptotic pathways (30). We have previously demonstrated that in BR ovarian cancer cells treated with PTX, activation of JNK/SAPK reaches a peak at 2 h when apoptosis is still minimal (73), suggesting that JNK/SAPK activation is not a secondary response to PTX-induced apoptosis.

One of the early changes in apoptotic cell membranes is externalization of phosphatidylserine, which exerts high affinity to AV. Therefore, increased binding of AV is a sensitive indicator for apoptosis (44). Apoptotic cells are also characterized by increased activities of caspases, cleavage of 112-kD poly ADP-ribose polymerase (PARP) into a 86-kD species, and DNA fragmentation (15)(70). We have previously demonstrated that PTX induces characteristic apoptotic morphology (9)(72) and DNA fragmentation assayed by both gel electrophoresis and the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method in ovarian cancer cells (72). By analyzing these

A

dn-ASK1	-	NG	-	-	-	Gr	-	-	-
dn-Rac	-	-	NG	-	-	-	Gr	-	-
dn-JNKK	-	-	-	NG	-	-	-	Gr	-
dn-JNK	-	-	-	-	NG	-	-	-	Gr
PTX16h	-	+	+	+	+	+	+	+	+



B

dn-ASK1	-	-	+	+	-	-	-	-	-	-
dn-Rac	-	-	-	-	+	+	-	-	-	-
dn-JNKK	-	-	-	-	-	-	+	+	-	-
dn-JNK	-	-	-	-	-	-	-	-	+	+
PTX16h	-	+	-	+	-	+	-	+	-	+

(Figure 8) Blockage of the JNK/SAPK signaling cascade does not interfere with paclitaxel-induced phosphorylation of Bcl-2. ***A,*** BR cells were cotransfected with pEGFP and dn-expression vectors for 24 h, treated with paclitaxel for 16 h, then transfected (green, *Gr*) and non-transfected (non-green, *NG*) populations were separately isolated by flow cytometric sorting. By Western blot analyses, phosphorylated Bcl-2 (*p-Bcl-2*) was identified as an additional band exhibiting slower mobility on SDS-PAGE when it was compared with Bcl-2 in DMSO-treated control. ***B,*** Cotransfected BR cells with pEGFP and different dn-expression vectors were isolated by flow cytometric sorting, treated with either paclitaxel (*PTX*) or DMSO for 16 h, and analyzed for the status of Bcl-2 phosphorylation by Western blots. Data shown are from a representative experiment, which was repeated twice with comparable results.

biochemical apoptotic characteristics in PTX-treated cells overexpressing wt or dn signaling proteins of the JNK/SAPK pathways, our results herein demonstrate an apoptosis-promoting role for JNK/SAPK in ovarian cancer cells treated with PTX (**Figures 2, 5, & 6**). Since suppression of the JNK/SAPK signaling cascade only protects cells from PTX-induced apoptosis but not from apoptosis induced by DNA targeting agents, actinomycin-D or cisplatin (**Figure 7**), the present study further suggests an apoptosis-promoting role of the JNK/SAPK cascade specifically in apoptotic process resulting from microtubular dysfunction.

JNK/SAPK has been shown to be involved in activation of caspases that are required for execution of the apoptotic process (8)(10)(14)(62)(65) . The role of JNK/SAPK in activation of caspases is, however, not straightforward. JNK/SAPK could be either upstream (14)(65) or downstream of (8)(10)(62) caspase activation, depending on cell type and apoptosis-initiating agents. Since PTX has been shown to activate caspases (28)(66), we analyzed the role of JNK/SAPK in caspase-3 activation of PTX-treated cells. Our data show that inhibition of the JNK/SAPK cascade prevented PTX-induced caspase-3 activation, PARP cleavage, and DNA fragmentation (**Figure 5**), indicating that JNK/SAPK is upstream of caspase-3 activation in PTX-initiated apoptosis. These and other studies, where anticancer agents were used to induce apoptosis (14)(65), suggest that JNK/SAPK acts upstream of caspase activation in chemotherapy-initiated apoptosis.

Intriguingly, while expression of dn Ras decreased basal levels of apoptosis (**Table I**), it did not significantly abrogate PTX-induced apoptosis (**Table I & Figure 2**). Although we previously reported that both Ras and ASK1 are required for optimal activation of JNK/SAPK by PTX (73), results of the present study failed to clarify a pro- or anti-apoptosis role for Ras in the apoptotic process initiated by PTX. In addition to activation of MAPK and JNK/SAPK pathways, Ras may also provide a survival signal that is mediated by the phosphoinositide 3'-OH kinase (PI 3-K)-dependent activation of the protein kinase B/Akt (24). Furthermore, inhibition of Ras activity induces apoptosis (3) and exerts synergistic inhibition of cell growth with PTX (52). These studies further collaborate the complex and multifunctional roles of Ras in both cell growth and apoptosis.

In contrast to Ras, it is clear that the related small G-protein, Rac, has a critical role in PTX-induced apoptosis at 16 h, probably via activation of JNK/SAPK. Previous studies have demonstrated that Rac activate the JNK/SAPK pathway (2)(16)(51)(56)(74) and our studies clearly indicate that MT damage activates Rac (**Figure 6C**) and that Rac is at least partially responsible for JNK activation and the resulted apoptosis (**Figures 2, 5, & 6**). It is interesting that among the upstream signaling proteins employed in

this study, the wt ASK1 appeared to have the highest apoptosis-promoting effect (**Figure 6**). Possible explanations for these observations include that ASK1, when overexpressed, may also activate other apoptosis-related kinase such as p38 (12)(29)(55).

Since expression of dn signaling proteins remained largely intact at 48 h after DMSO treatment (**Figure 3**), the disappearance of protection from basal apoptosis can not be explained by a decrease of dn signaling proteins. Forty-eight hours of treatment with DMSO (vehicle control) at a 0.1% final concentration did not activate JNK/SAPK (73) or initiate apoptosis. However, liposome-mediated transfection with control DNA into BR cells was observed to exert mild cytotoxicity and caspase-3 activation, hence it is possible that transfection by itself may initiate an apoptotic process that eventually overrides the transient protection by dn signaling proteins of the JNK/SAPK pathway. Nevertheless, treatment with PTX for 48 h caused a further significant increase in apoptosis above the relatively high basal levels (**Figures 2B & 5A, Table I**), suggesting coexistence of multiple apoptotic pathways. This suggestion is supported by the ability of dn signaling proteins to inhibit apoptosis in both control and PTX-treated cells at 16 h- but not 48 h-treatment. Therefore, the pathways leading to apoptosis after the 16 h- versus 48 h-treatment appear to be fundamentally different.

Multiple mechanisms have been suggested to be involved in PTX-induced apoptosis, such as: abortive mitosis after PTX-induced G_2/M block (33)(43)(77), activation of $p34^{cdc2}$ (17)(27)(66) and other CDKs (18)(47), activation and local release of an apoptosis-inducing cytokine (39), and induction of transcription regulators and enzymes that modulate apoptosis (53). Whereas overexpression of wt ASK1, wt Rac, or wt JNK promotes apoptosis in BR cells (**Figure 6**), expression of dn ASK1, dn Rac, dn JNKK, or dn JNK only transiently inhibits PTX-induced apoptosis (**Table I & Figure 2**). Protection from PTX-induced DNA fragmentation by expression of these dn signaling proteins disappeared when cells were treated for 24 to 48 h (**Figure 5A**). These results are in agreement with data described in **Part III** that PTX-induces JNK/SAPK activation in BR cells is transient with a peak at 2-4 h and declines afterward (73). Since suppression of the JNK/SAPK signaling cascade does not alter cell cycle profiles nor interfere with the PTX-induced mitotic arrest that peaks at 24 h of PTX treatment (**Figure 4**), G_2/M block of the cell cycle may mainly account for the later phase of PTX-induced apoptosis. Our results further suggest that the late phase of PTX-induced apoptosis is independent of JNK/SAPK activity. Several recent studies have suggested that catastrophic activity of CDKs may be a terminal effector in apoptotic pathway (23)(41).

Results in this study do not support the hypothesis that activated JNK/SAPK is required for phosphorylation of Bcl-2 in PTX-treated cells (45). Bcl-2 is known to protect cells from apoptosis (37) and PTX has been shown to induce both phosphorylation of Bcl-2 and apoptosis (6)(21)(20)(61). However, the roles of Bcl-2 phosphorylation in promoting (6)(11)(13)(21)(61) or inhibiting apoptosis (25)(31) remain controversial.

Some reports suggest that Bcl-2 may act upstream of JNK/SAPK (40)(57). In BR cells, however, JNK/SAPK activation peaked at 2 h (73), while Bcl-2 phosphorylation and the G_2/M arrest of the cell cycle required 12 to 16 h of PTX treatment. These temporal differences suggest that phosphorylated Bcl-2 is unlikely to act upstream of JNK/SAPK. The observations that inhibition of JNK/SAPK did not interfere with PTX-induced G_2/M arrest and Bcl-2 phosphorylation (**Figures 4 & 8**) suggest that PTX-activated JNK/SAPK is independent of Bcl-2 phosphorylation occurring in PTX-treated cells. Recent reports further demonstrate that phosphorylation of Bcl-2 occurs only in cells blocked at G_2/M phase after PTX treatment (20)(64). Therefore, JNK/SAPK activation and Bcl-2 phosphorylation may reside in distinct, independent pathways. Instead of the JNK/SAPK cascade, PKA activation has been suggested to account for Bcl-2 phosphorylation in cells with MT damages (68). The role(s) of Bcl-2 phosphorylation in regulating apoptosis in general and in PTX-induced apoptosis in particular apparently requires further clarification.

Our results, for the first time, identify a Bcl-2 phosphorylation-independent role of JNK/SAPK in promoting PTX-induced apoptosis and demonstrate that multiple mechanisms are involved in apoptosis resulting from MT damage, including a JNK/SAPK-dependent early phase and a JNK/SAPK-independent late phase.

APPENDIX

Several experiments and results that were not included in the paper (71) are described in this section.

FLUORESCENT MICROSCOPY

To analyze early apoptosis and cell death, BR cells growing to 50 % confluence on chambered coverglass (Lab-Tek, Nunc Inc, Naperville, IL) were treated with 1 μM PTX for 8 h, stained with FITC-

conjugated AV (Pharmingen) and PI for 10 min according to the manufacturer's protocol, and observed at the 200X magnification under the fluorescent microscope (Olympus, Japan). To detect apoptosis in transfected cells, BR cells were transfected with pEGFP, treated with PTX or DMSO control for 8 h, stained with PE-conjugated AV (Pharmingen) for 10 min, and examined with fluorescent microscopy. Results are shown in **Part I, Figure 2.**

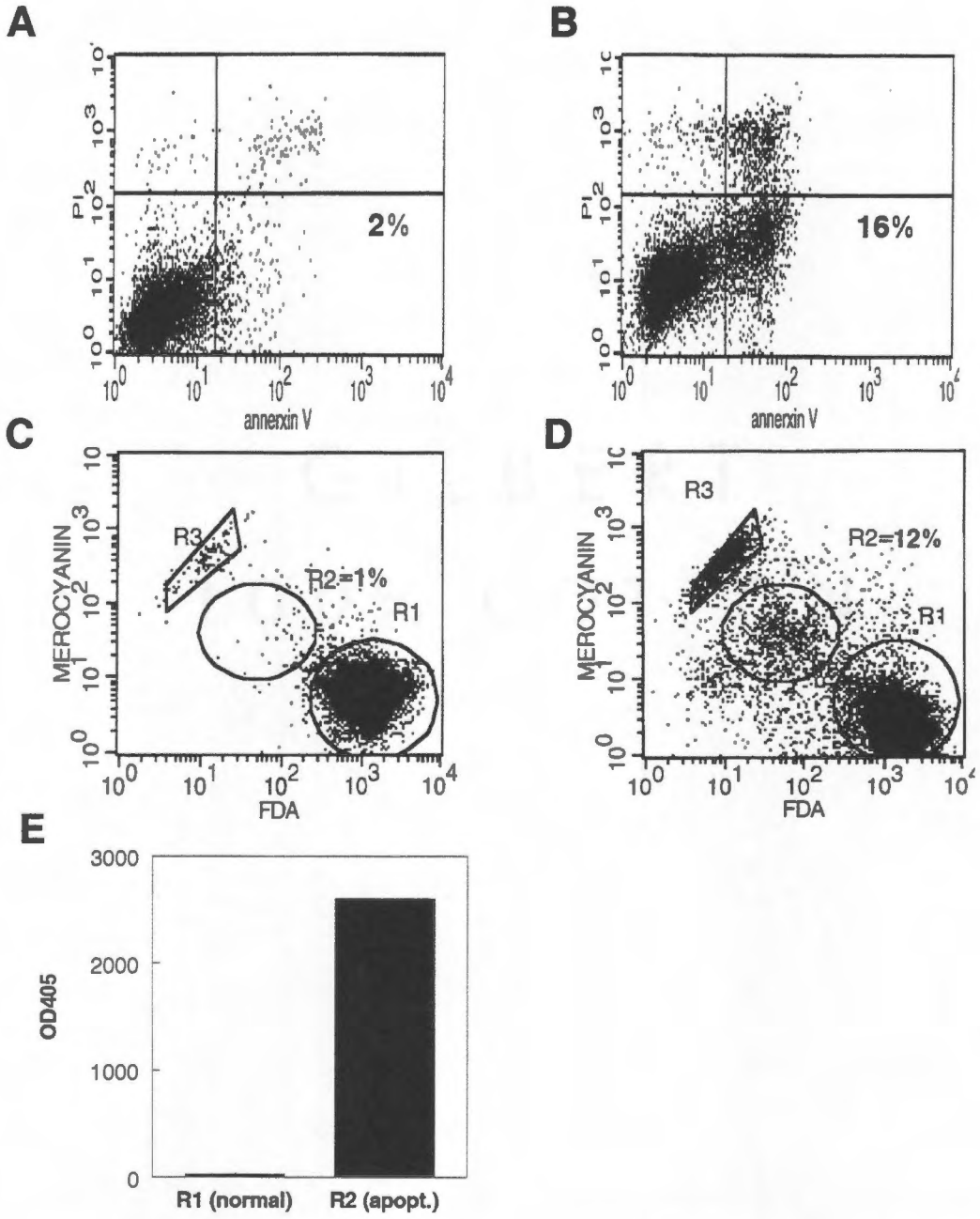
FLOW CYTOMETRIC SORTING USING OTHER MARKERS

In an attempt to isolate apoptotic cells by flow cytometric sorting, I have used either FITC-conjugated AV/PI or FDA/MC540 (both from Sigma) as markers for apoptotic cells. Both methods were able to differentiate apoptotic cell populations from dead or normal cells (**Figure 9**). However, FITC-conjugated AV is not economically practical as an apoptotic marker for sorting because of its high cost.

The procedures for the combined uses of FDA and MC540 are described here. PTX -treated cells were trypsinized, resuspended in DMEM/F12 with 10% NBS, incubated with both 0.02 ng/ml FDA and 1μM MC540 at 37°C for 30 min, then cells were put in ice before flow cytometric analyses and/or sorting were performed. Three populations were identified: R1 (high FDA/low MC540), R2 (intermediate FDA/intermediate MC540), and R3 (low FDA/high MC540) represented normal, apoptotic, and dead cells, respectively (**Figures 9C & D**). Cells in R1 and R2 regions were separately collected and the apoptotic characteristics were verified by a DNA fragmentation ELISA (**Figure 9E**). The combined use of FDA/MC540 is an economical and specific (**Figure 9E**) method to isolate a large number of apoptotic cells, from which we can study biochemical changes, and even discover novel factors, specifically associated with apoptosis.

CELL CYCLE ANALYSES OF SORTED APOPTOTIC CELLS

At different time intervals of 1 μM PTX treatment, apoptotic BR cells were isolated from the total PTX-treated cells by flow cytometric sorting according to their FDA/MC540 profiles as described in **Figure 9**. Some left-over total PTX-treated cells were used as the non-sorted control. Both isolated apoptotic cells and total cells (non-sorted) were fixed with 70% ethanol overnight, and analyzed for cell cycle profiles by flow cytometry.



(Figure 9) Apoptotic analyses and sorting. Apoptotis in BR cells treated with DMSO control (*A, C*) or paclitaxel (*B, D*) were analyzed by staining profiles of AV/PI (*A, B*) or FDA/MC (*C, D*). *D*, Regions R1 and R2 which represent normal and apoptotic cell populations, respectively, were collected separately and analyzed with a DNA fragmentation ELISA (*E*).

Patterns of cell cycle profiles derived from PI staining in apoptotic cells were shifted to the left side (empty areas in **Figure 10**), when compared with that of non-sorted cells (filled areas in **Figure 10**), indicating a lower DNA content in these apoptotic cells. One possible explanation for the observation is that PTX treatment changes cell cycle profiles of apoptotic cells into mainly sub-$G_1$and S-phases, which represent apoptotic bodies and cells with aberrantly activated CDKs, respectively. Further biochemical analyses on CDK activities and levels of cell cycle-related proteins should be able to verify this hypothesis. If so, these data would support a role of CDK in execution of apoptosis.

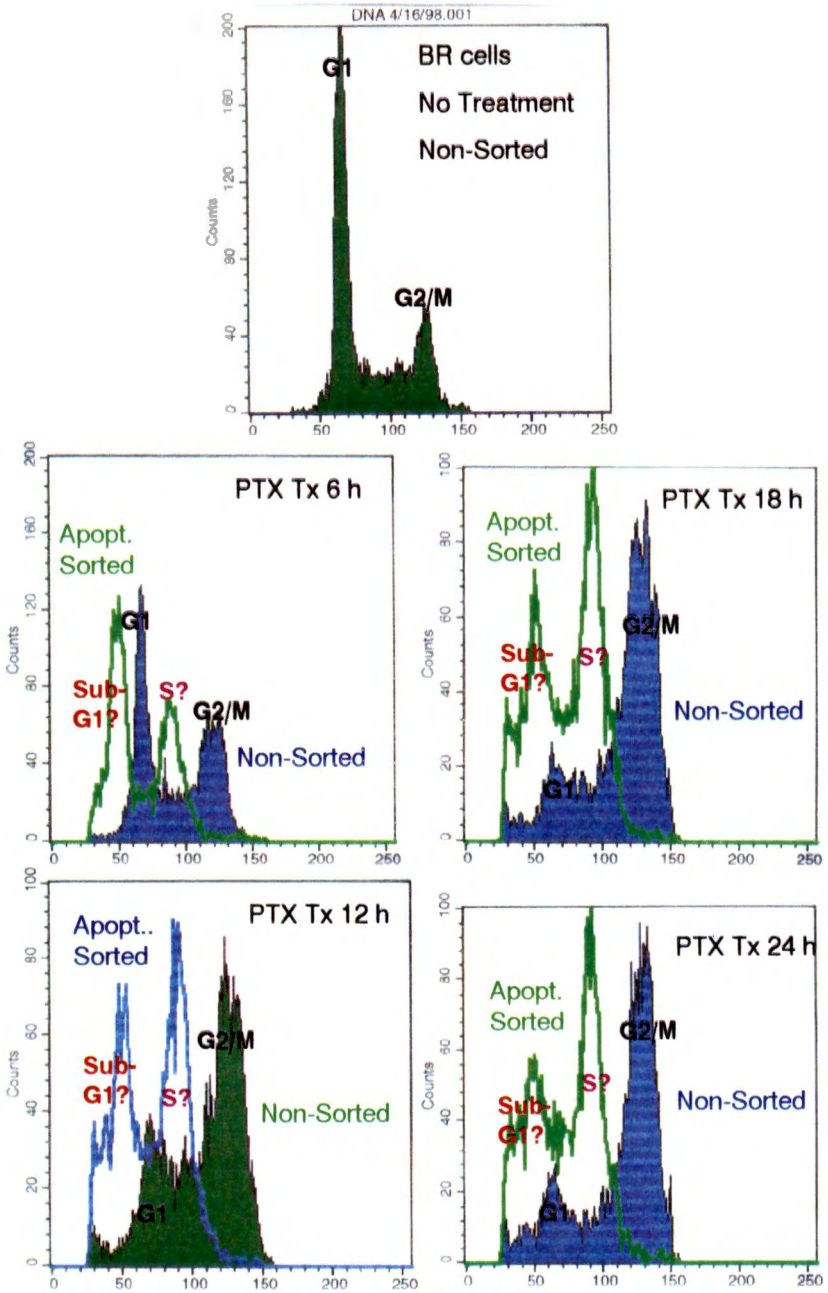
An alternative explanation for this observation is that chromatin becomes more compact in apoptotic cells, restricting the access of PI to bind DNA and resulting in decreased intensities of PI staining (the Y axis of these figures).

SAPONIN PERMEABILIZATION AND CELL CYCLE ANALYSES

In an attempt to isolate PTX-treated cells at different cell cycle phases for further analyses on their kinase activities and various protein levels, I tried to permeabilize live cells using 0.1% saponin (Sigma) in trypsinized cell suspensions, then stained DNA with propidium iodide and analyzed cell cycle profiles. Treatment with saponin sufficiently permeabilized the cell membrane and allowed entry of propidium iodide, resulting in a satisfactory cell cycle analysis and sorting. However, protein quantitation on cell lysates prepared from those sorted cells indicated that most cytoplasmic proteins leaked from saponin-treated cells. Therefore, this method is not suitable for isolating cells according to their cell cycle profiles for further biochemical analyses. Centrifugal elutriation appears to be a better choice for this purpose.

CENTRIFUGAL ELUTRIATION

Centrifugal elutriation was performed according a previously reported method (4)(5). Ten T150 flask of ovarian cancer BR cells growing to 80% confluence (total ~ 4.3 x 10^8 cells) were trypsinized and resuspended with 30 ml of 5% NBS-DMEM/F12 and kept in ice before centrifugal elutriation was done with a Beckman elutriation centrifuge (model J2-21) equipped with the JE-6B elutriator rotor. A speed-constant (rotor speed set to 2,000 rpm) centrifugal elutriation was performed with the initial flow rate of



(Figure 10) Cell cycle profiles of paclitaxel-induced apoptotic cells. After treatment with paclitaxel for designated hours, apoptotic cells (*Apopt. Sorted*), which were cells in R2 region as described in Figure 9, were isolated with flow cytometric sorting using FDA/MC540 profiles as selectable markers, fixed with 70% ethanol, and analyzed for cell cycle profiles. *Non-sorted* cells were mixed populations of cells in regions R1, R2, and R3 as described in Figure 9.

normal saline set to 10 ml/min which was gradually increased to 45 ml/min. The total of 12 fractions (40 ml per fraction) of elutriated cell suspension were collected. Cells from each fraction were collected by centrifuge at 600 x g for 10 min, resuspended with 1 ml 5% NBS-DMEM/F12, kept in ice before analyzed for the cell cycle profiles and Western blot analyses.

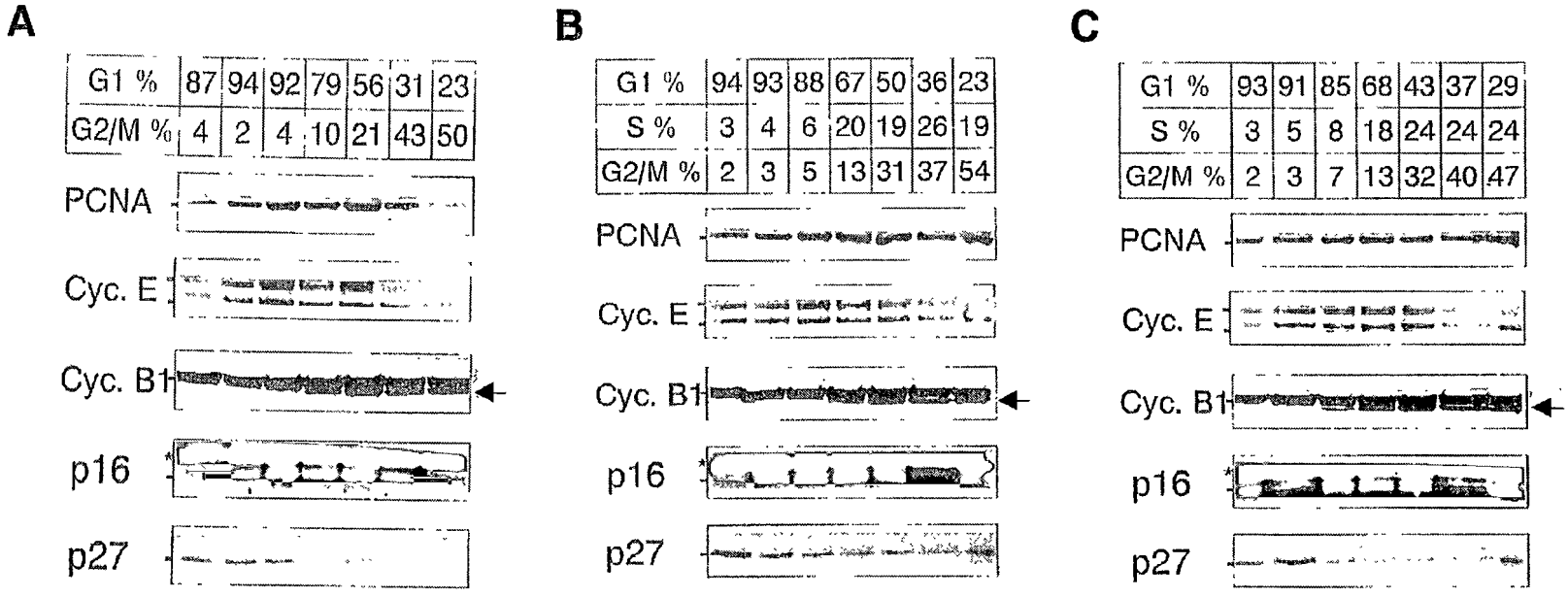
Results of three centrifugal elutriations (**Figure 11**) demonstrate that centrifugal elutriation is a practical method to separate a large number of cells (in the 10^8 range) by the different sizes of cells through the cell cycle. Correlations between cell cycle profiles and cell cycle-related proteins (PCNA, cyclins B1 and E, two CKIs, p16 and p27) also confirm a sufficient fractionation of cells at different phases of the cell cycle by this method.

In addition to the advantage of harvesting a large number of cells in less than 1 h, separation of G_1 from G_2/M phase cells by centrifugal elutriation does not rely on arrest of the cell cycle by chemical treatments or metabolic manipulations, thereby avoiding undesired effects potentially caused by these additional treatments. Nevertheless, since it separates cells by sizes and densities, and cycling cells from one cell line to another may change sizes at different ratios, the rotor speed and the change of flow rates have to be optimized for separation of each cell line.

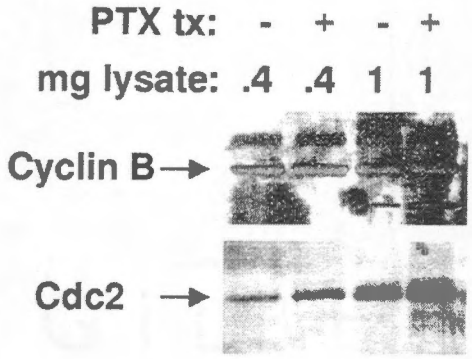
GST-FUSION PROTEIN PULL DOWN ASSAYS

Preparations for purified GST-JNK or GST-JNKK were identical for that for GST-RBD as described in the **Part III, ARIA** section.

Ovarian cancer BR cells were treated with 1 μM PTX or DMSO control for 16 h and cell lysates were prepared with magnesium-containing lysis buffer (MLB: 25 mM HEPES, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM sodium vanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Equal amount of cell lysates (400 and 1,000 μg in parallel experiments) were incubated with 30 μl of GSH beads bound by excess GST-JNK or GST-JNKK on a rocker at 4°C for 1 h. After three washes with MLB, GSH beads with proteins interacting with JNK or JNKK were spun down, boiled in Laemmli's loading buffer, subjected to SDS-PAGE, transferred to nitrocellulose membrane, and detected by anti-Cdc2 (Santa Cruz #54) or anti-cyclin B1(Santa Cruz #SC-245) Abs in Western blot. Results shown in **Figure 12** suggest an *in vitro* complex formation among GST-JNK and cyclin B1 and Cdc2.



(Figure 11) Western blot analyses for cell cycle-related proteins in cells fractionated by centrifugal elutriation. Three sets of fractions of paclitaxel-treated BR cells (*A-C*), which were separated by sizes through centrifugal elutriation, were analyzed for cell cycle profiles and cell cycle-related proteins. Specific bands recognized by anti-cyclin B1 Ab in Western blot analyses are labeled with arrows. Non-specific bands also recognized by anti-p16 Ab are marked with asterisks. Abbreviations: *PCNA*, proliferating cell nuclear antigen; *Cyc.*, cyclin.



(Figure 12) GST-protein pull down experiments. Ovarian cancer BR cells were treated with 1 uM PTX or DMSO control for 16h and cell lysates were prepared with magnesium-containing lysis buffer. Cell lysates (0.4 and 1 mg in parallel experiments) were incubated with GST-JNK bound to GSH agarose at 4°C for 1 h. After three washes, proteins interacting with GST-JNK thus bound to agarose beads were analyzed with Western blot using anti-Cdc2 and anti-cyclin B Ab (Santa Cruz).

LITERATURE CITED

1. **Amato, S. F., J. M. Swart, M. Berg, H. J. Wanebo, S. R. Mehta, and T. C. Chiles.** 1998. Transient stimulation of the c-Jun-NH2-terminal kinase/activator protein 1 pathway and inhibition of extracellular signal-regulated kinase are early effects in paclitaxel-mediated apoptosis in human B lymphoblasts. Cancer Res. **58:**241-7.
2. **Bagrodia, S., B. Derijard, R. J. Davis, and R. A. Cerione.** 1995. Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. J. Biol. Chem. **270:**27995-8.
3. **Barrington, R. E., M. A. Subler, E. Rands, C. A. Omer, P. J. Miller, J. E. Hundley, S. K. Koester, D. A. Troyer, D. J. Bearss, M. W. Conner, J. B. Gibbs, K. Hamilton, K. S. Koblan, S. D. Mosser, T. J. O'Neill, M. D. Schaber, E. T. Senderak, J. J. Windle, A. Oliff, and N. E. Kohl.** 1998. A farnesyltransferase inhibitor induces tumor regression in transgenic mice harboring multiple oncogenic mutations by mediating alterations in both cell cycle control and apoptosis. Mol. Cell. Biol. **18:**85-92.
4. **Bird, R. C.** 1998. Cell separation by centrifugal elutriation, p. 205-208. *In* J. E. Celis (ed.), Cell Biology: a laboratory handbook, 2nd ed, vol. 1. Academic Press, San Diego.
5. **Bird, R. C.** 1998. Synchronous populations of cells in specific phases of the cell cycle prepared by centrifugal elutriation, p. 209-217. *In* J. E. Celis (ed.), Cell Biology: a laboratory handbook, 2nd ed, vol. 1. Academic Press, San Diego.
6. **Blagosklonny, M. V., P. Giannakakou, W. S. el-Deiry, D. G. Kingston, P. L Higgs, L. Neckers, and T. Fojo.** 1997. Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res. **57:**130-5.
7. **Bost, F., R. McKay, N. Dean, and D. Mercola.** 1997. The Jun Kinase/stress-activated protein kinase pathway is required for epidermal growth factor stimulation of growth of human A549 lung carcinoma cells. J. Biol. Chem. **272:**33422-33429.
8. **Cahill, M. A., M. E. Peter, F. C. Kischkel, A. M. Chinnaiyan, V. M. Dixit, P. H. Krammer, and A. Nordheim.** 1996. CD95 (APO-1/Fas) induces activation of SAP kinases downstream of ICE- like proteases. Oncogene **13:**2087-96.
9. **Cameron, M. R., M. R. Caudle, W. R. Sullivan, Jr., J. J. Peluso, and J. Wimalasena.** 1995. The steroidogenic and morphological effects of paclitaxel on cultured ovarian cancer cells. Oncol. Res. **7:**145-56.
10. **Cardone, M. H., G. S. Salvesen, C. Widmann, G. Johnson, and S. M. Frisch.** 1997. The regulation of anoikis: MEKK-1 activation requires cleavage by caspases. Cell **90:**315-23.
11. **Chang, B. S., A. J. Minn, S. W. Muchmore, S. W. Fesik, and C. B. Thompson.** 1997. Identification of a novel regulatory domain in Bcl-X(L) and Bcl-2. EMBO. J. **16:**968-77.
12. **Chang, H. Y., H. Nishitoh, X. Yang, H. Ichijo, and D. Baltimore.** 1998. Activation of apoptosis signal- regulating kinase 1 (ASK1) by the adapter protein Daxx. Science **281:**1860-3.
13. **Chen, C. Y., and D. V. Faller.** 1996. Phosphorylation of Bcl-2 protein and association with p21Ras in Ras- induced apoptosis. J. Biol. Chem. **271:**2376-9.
14. **Chen, Y. R., W. Wang, A. N. Kong, and T. H. Tan.** 1998. Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. J. Biol. Chem. **273:**1769-75.
15. **Cohen, G. M.** 1997. Caspases: the executioners of apoptosis. Biochem. J. **326:**1-16.
16. **Coso, O. A., M. Chiariello, J. C. Yu, H. Teramoto, P. Crespo, N. Xu, T. Miki, and J. S. Gutkind.** 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell **81:**1137-46.
17. **Donaldson, K. L., G. L. Goolsby, P. A. Kiener, and A. F. Wahl.** 1994. Activation of p34cdc2 coincident with taxol-induced apoptosis. Cell Growth Differ. **5:**1041-50.
18. **Furukawa, Y., S. Iwase, Y. Terui, J. Kikuchi, T. Sakai, M. Nakamura, S. Kitagawa, and M. Kitagawa.** 1996. Transcriptional activation of the cdc2 gene is associated with Fas- induced apoptosis of human hematopoietic cells. J. Biol. Chem. **271:**28469-77.
19. **Green, D. R., and C. F. Ware.** 1997. Fas-ligand: privilege and peril. Proc. Natl. Acad. Sci. USA. **94:**5986-5990.
20. **Haldar, S., A. Basu, and C. M. Croce.** 1998. Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. Cancer Res. **58:**1609-15.
21. **Haldar, S., N. Jena, and C. M. Croce.** 1995. Inactivation of Bcl-2 by phosphorylation. Proc. Natl. Acad. Sci. USA **92:**4507-11.
22. **Hall, A.** 1998. Rho GTPases and the actin cytoskeleton. Science **279:**509-14.
23. **Harvey, K. J., J. F. Blomquist, and D. S. Ucker.** 1998. Commitment and effector phases of the physiological cell death pathway elucidated with respect to Bcl-2 caspase, and cyclin-dependent kinase activities. Mol. Cell. Biol. **18:**2912-22.
24. **Heimbrook, D. C., and A. Oliff.** 1998. Therapeutic intervention and signaling. Curr. Opin. Cell. Biol. **10:**284-8.
25. **Horinchi, M., W. Hayashida, T. Kambe, T. Yamada, and V. J. Dzau.** 1997. Angiotensin type 2 receptor dephosphorylates Bcl-2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. J. Biol. Chem. **272:**19022-6.
26. **Horwitz, S. B.** 1994. Taxol (paclitaxel): mechanisms of action. Ann. Oncol. **5:**S3-6.
27. **Huang, T. S., C. H. Shu, W. K. Yang, and J. Whang-Peng.** 1997. Activation of CDC 25 phosphatase and CDC 2 kinase involved in GL331- induced apoptosis. Cancer Res. **57:**2974-8.
28. **Ibrado, A. M., Y. Huang, G. Fang, and K. Bhalla.** 1996. Bcl-xL overexpression inhibits taxol-induced Yama protease activity and apoptosis. Cell Growth Differ. **7:**1087-94.
29. **Ichijo, H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, and Y. Gotoh.** 1997. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. Science **275:**90-4.
30. **Ip, Y. T., and R. J. Davis.** 1998. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. Curr. Opin. Cell Biol. **10:**205-19.

31. **Ito, T., X. Deng, B. Carr, and W. S. May.** 1997. Bcl-2 phosphorylation required for anti-apoptosis function. J. Biol. Chem. **272:**11671-3.
32. **Johnson, N. L., A. M. Gardner, K. M. Diener, C. A. Lange-Carter, J. Gleavy, M. B. Jarpe, A. Minden, M. Karin, L. I. Zon, and G. L. Johnson.** 1996. Signal transduction pathways regulated by mitogen-activated/extracellular response kinase kinase kinase induce cell death. J. Biol. Chem. **271:**3229-37.
33. **Jordan, M. A., K. Wendell, S. Gardiner, W. B. Derry, H. Copp, and L. Wilson.** 1996. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res. **56:**816-25.
34. **Jordan, M. A., and L. Wilson.** 1998. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr. Opin. Cell Biol. **10:**123-30.
35. **Karin, M., Z. Liu, and E. Zandi.** 1997. AP-1 function and regulation. Curr. Opin. Cell Biol. 9:240-6.
36. **Kolesnick, R. N., and M. Kronke.** 1998. Regulation of ceramide production and apoptosis. Annu. Rev. Physiol. **60:**643-65.
37. **Kroemer, G.** 1997. The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nat. Med. **3:**614-620.
38. **Kyriakis, J. M., and J. Avruch.** 1996. Sounding the alarm: protein kinase cascades activated by stress and inflammation. J. Biol. Chem. **271:**24313-6.
39. **Lanni, J. S., S. W. Lowe, E. J. Licitra, J. O. Liu, and T. Jacks.** 1997. p53-independent apoptosis induced by paclitaxel through an indirect mechanism. Proc. Natl. Acad. Sci. U S A **94:**9679-83.
40. **Lee, Y. J., and P. M. Corry.** 1998. Metabolic Oxidative Stress-induced HSP70 Gene Expression Is Mediated through SAPK Pathway. Role of bcl-2 and c-jun nh2-terminal kinase. J Biol Chem **273:**29857-29863.
41. **Levkau, B., H. Koyama, E. W. Raines, B. E. Clurman, B. Herren, K. Orth, J. M. Roberts, and R. Ross.** 1998. Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Mol. Cell **1:**553-63.
42. **Liu, Z. G., R. Baskaran, E. T. Lea-Chou, L. D. Wood, Y. Chen, M. Karin, and J. Y. Wang.** 1996. Three distinct signalling responses by murine fibroblasts to genotoxic stress. Nature **384:**273-6.
43. **Long, B. H., and C. R. Fairchild.** 1994. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. Cancer Res. **54:**4355-61.
44. **Martin, S. J., C. P. Reutelingsperger, A. J. McGahon, J. A. Rader, R. C. van Schie, D. M. LaFace, and D. R. Green.** 1995. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J. Exp. Med. **182:**1545-56.
45. **Maundrell, K., B. Antonsson, E. Magnenat, M. Camps, M. Muda, C. Chabert, C. Gillieron, U. Boschert, E. Vial-Knecht, J.-C. Martinou, and S. Arkinstall.** 1997. Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J. Biol. Chem. **272:**25238-25242.
46. **McGuire, W. P., E. K. Rowinsky, N. B. Rosenshein, F. C. Grumbine, D. S. Ettinger, D. K. Armstrong, and R. C. Donehower.** 1989. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Intern. Med. **111:**273-279.
47. **Meikrantz, W., and R. Schlegel.** 1996. Suppression of apoptosis by dominant negative mutants of cyclin- dependent protein kinases. J. Biol. Chem. **271:**10205-9.
48. **Meriin, A. B., V. L. Gabai, J. Yaglom, V. I. Shifrin, and M. Y. Sherman.** 1998. Proteasome inhibitors activate stress kinases and induce Hsp72. Diverse effects on apoptosis. J. Biol. Chem. **273:**6373-9.
49. **Milas, L., N. R. Hunter, B. Kurdoglu, K. A. Mason, R. E. Meyn, L. C. Stephens, and L. J. Peters.** 1995. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. Cancer Chemother. Pharmacol. **35:**297-303.
50. **Milross, C. G., K. A. Mason, N. R. Hunter, W. K. Chung, L. J. Peters, and L. Milas.** 1996. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. J. Natl. Cancer Inst. **88:**1308-14.
51. **Minden, A., A. Lin, F. X. Claret, A. Abo, and M. Karin.** 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell **81:**1147-57.
52. **Moasser, M. M., L. Sepp-Lorenzino, N. E. Kohl, A. Oliff, A. Balog, D. S. Su, S. J. Danishefsky, and N. Rosen.** 1998. Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones. Proc. Natl. Acad. Sci . U S A **95:**1369-74.
53. **Moos, P. J., and F. A. Fitzpatrick.** 1998. Taxane-mediated gene induction is independent of microtubule stabilization: induction of transcription regulators and enzymes that modulate inflammation and apoptosis. Proc. Natl. Acad. Sci. U S A **95:**3896-901.
54. **Nishina, H., K. D. Fischer, L. Radvanyi, A. Shahinian, R. Hakem, E. A. Rubie, A. Bernstein, T. W. Mak, J. R. Woodgett, and J. M. Penninger.** 1997. Stress-signalling kinase SEK1 protects thymocytes from apoptosis mediated by CD95 and CD3. Nature **385:**350-3.
55. **Nishitoh, H., M. Saitoh, Y. Mochida, K. Takeda, H. Nakano, M. Rothe, K. Miyazono, and H. Ichijo.** 1998. ASK1 is essential for JNK/SAPK activation by TRAF2. Mol. Cell **2:**389-95.
56. **Olson, M. F., A. Ashworth, and A. Hall.** 1995. An essential role for Rho, Rac, and Cdc42 GTPases in cell cycle progression through G1. Science **269:**1270-2.
57. **Park, D. S., L. Stefanis, C. Y. I. Yan, S. E. Farinelli, and L. A. Greene.** 1996. Ordering the cell death pathway. Differential effects of BCL2, an interleukin-1-converting enzyme family protease inhibitor, and other survival agents on JNK activation in serum/nerve growth factor-deprived PC12 cells. J. Biol. Chem. **271:**21898-905.
58. **Potapova, O., A. Haghighi, F. Bost, C. Liu, M. J. Birrer, R. Gjerset, and D. Mercola.** 1997. The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. J. Biol. Chem. **272:**14041-4.
59. **Preston, G. A., J. E. Lang, R. R. Maronpot, and J. C. Barrett.** 1994. Regulation of apoptosis by low serum in cells of

different stages of neoplastic progression: enhanced susceptibility after loss of a senescence gene and decreased susceptibility after loss of a tumor suppressor gene. Cancer Res. **54:**4214-23.

60. **Rodrigues, G. A., M. Park, and J. Schlessinger.** 1997. Activation of the JNK pathway is essential for transformation by the Met oncogene. EMBO. J. **16:**2634-45.
61. **Roth, W., B. Wagenknecht, C. Grimmel, J. Dichgans, and M. Weller.** 1998. Taxol-mediated augmentation of CD95 ligand-induced apoptosis of human malignant glioma cells: association with bcl-2 phosphorylation but neither activation of p53 nor G2/M cell cycle arrest. Br. J. Cancer **77:**404-11.
62. **Rudel, T., and G. M. Bokoch.** 1997. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science **276:**1571-4.
63. **Saitoh, M., H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, and H. Ichijo.** 1998. Mammalian thioredoxin is a direct inhibitor of apoptosis signal- regulating kinase (ASK) 1. EMBO. J. **17:**2596-606.
64. **Scatena, C. D., Z. A. Stewart, D. Mays, L. J. Tang, C. J. Keefer, S. D. Leach, and J. A. Pietenpol.** 1998. Mitotic phosphorylation of Bcl-2 during normal cell cycle progression and Taxol-induced growth arrest. J. Biol. Chem. **273:**30777-30784.
65. **Seimiya, H., T. Mashima, M. Toho, and T. Tsuruo.** 1997. c-Jun NH2-terminal kinase-mediated activation of interleukin-1beta converting enzyme/Ced-3-like protease during anticancer drug-induced apoptosis. J.Biol. Chem. **272:**4631-6.
66. **Shen, S. C., T. S. Huang, S. H. Jee, and M. L. Kuo.** 1998. Taxol-induced p34cdc2 kinase activation and apoptosis inhibited by 12-O- tetradecanoylphorbol-13-acetate in human breast MCF-7 carcinoma cells. Cell Growth Differ. **9:**23-9.
67. **Smith, A., F. Ramos-Morales, A. Ashworth, and M. Collins.** 1997. A role for JNK/SAPK in proliferation, but not apoptosis, of IL-3- dependent cells. Curr. Biol. **7:**893-6.
68. **Srivastava, R. K., A. R. Srivastava, S. J. Korsmeyer, M. Nesterova, Y. S. Cho-Chung, and D. L. Longo.** 1998. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **18:**3509-17.
69. **van den Heuvel, S., and E. Harlow.** 1993. Distinct roles for cyclin-dependent kinases in cell cycle control. Science **262:**2050-2054.
70. **Villa, P., S. H. Kaufmann, and W. C. Earnshaw.** 1997. Caspases and caspase inhibitors. Trends Biochem. Sci. **22:**388-93.
71. **Wang, T. H., D. M. Popp, H. S. Wang, M. Saitoh, J. G. Mural, D. C. Henley, H. Ichijo, and J. Wimalasena.** 1999. Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells. J. Biol. Chem. :in press.
72. **Wang, T. H., and H. S. Wang.** 1996. p53, apoptosis and human cancers. J. Formos. Med. Assoc. **95:**509-522.
73. **Wang, T. H., H. S. Wang, H. Ichijo, P. Giannakakou, J. S. Foster, T. Fojo, and J. Wimalasena.** 1998. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress- activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J. Biol. Chem. **273:**4928-36.
74. **Westwick, J. K., Q. T. Lambert, G. J. Clark, M. Symons, L. Van Aelst, R. G. Pestell, and C. J. Der.** 1997. Rac regulation of transformation, gene expression, and actin organization by multiple, PAK-independent pathways. Mol Cell Biol **17:**1324-35.
75. **Wimalasena, J., C. Cavallo, and D. Meehan.** 1991. Human epithelial ovarian cancer cell steroid secretion and its control by gonadotropins. Gynecol. Oncol. **41:**56-63.
76. **Wolfson, M., C. P. Yang, and S. B. Horwitz.** 1997. Taxol induces tyrosine phosphorylation of Shc and its association with Grb2 in murine RAW 264.7 cells. Int. J. Cancer. **70:**248-52.
77. **Woods, C. M., J. Zhu, P. A. McQueney, D. Bollag, and E. Lazarides.** 1995. Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. Mol. Med. **1:**506-26.
78. **Zanke, B. W., C. Lee, S. Arab, and I. F. Tannock.** 1998. Death of tumor cells after intracellular acidification is dependent on stress-activated protein kinases (SAPK/JNK) pathway activation and cannot be inhibited by Bcl-2 expression or interleukin 1beta-converting enzyme inhibition. Cancer Res **58:**2801-8.

PART V.

GENERAL DISCUSSION

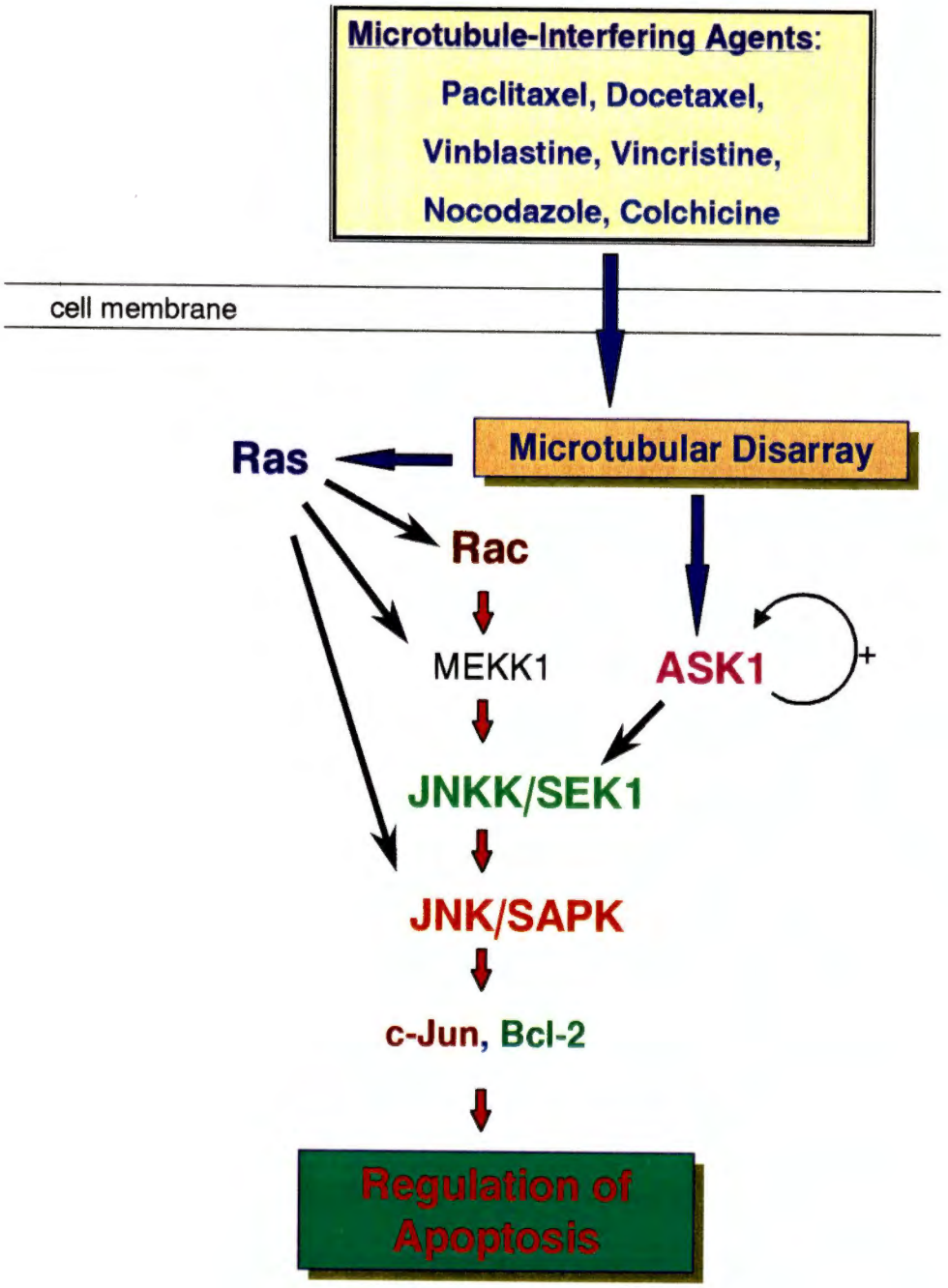
Paclitaxel (Taxol®) is a very useful chemotherapeutic agent in the treatment of ovarian cancer and breast cancer. It has become clear that paclitaxel (PTX)-initiated apoptosis accounts for its anti-tumor effect. Although many studies have associated mitotic arrest of PTX-treated cells with its cytotoxicity against cancer cells (41)(60)(103), the biochemical events downstream of kinetic stabilization of microtubule (MT) dynamics which lead to apoptosis of cancer cells remain largely unclear (42).

Besides the inhibition of mitotic spindle formation, PTX has been demonstrated to have multiple functions, such as: (i) upregulation of aromatase and steroidogenesis (11), (ii) activation of $p34^{cdc2}$ (19)(36)(38)(78), Cdc2-like kinase (77), and other CDKs (73), (iii) stimulation of the local release of TNF-α (46) and of IL-8 expression (49) (47), (iv) transcriptional activation of c-Mos (56), cyclin B1 (56)(55), and the pro-apoptotic Bak (57)(39), Bax (88), the gene *krox-24*, and several enzymes (2'5'-oligoadenylate synthase, cyclooxygenase-2, and an IκB kinase termed chuk) that may regulate apoptosis (69), and (v) downregulation of the anti-apoptotic Bcl-X_L (57).

Furthermore, substantial evidence indicates that the G_2/M arrest of the cell cycle may not be the only mechanism to induce apoptosis (46)(65)(67)(69); additional phosphoregulatory pathways may be involved in inducing apoptosis (8)(30)(73)(102). In an attempt to discover biochemical effects of PTX on cancer cells, I examined JNK/SAPK that is frequently associated with apoptosis. The initial detection of JNK activation in a variety of human cells treated with PTX (**Part III, Figure 1**) encouraged me to further characterize the upstream regulators involved in the PTX-activated JNK and the downstream functions of activated JNK in the PTX-initiated apoptosis.

MULTIPLE UPSTREAM REGULATORS OF THE PACLITAXEL-INDUCED JNK ACTIVATION

Treatment with PTX activates JNK in a variety of human cells (**Part III, Figure 1**). PTX-activated JNK may exert its transactivation function through c-Jun, as demonstrated by activation of a TRE-CAT reporter that measures AP-1 activity (**Part III, Figure 4**). By using expression vectors for a series of dominant negative (dn) signaling proteins of the JNK pathway and the activated Ras interaction assay (A.R.I.A), as well as the observation of autophosphorylation of wild type (wt) ASK1, I have identified that activation of Ras, Rac, ASK1, JNKK are required for PTX-induced JNK activation (**Figure 1**).



(Figure 1) Multiple upstream regulators of the paclitaxel-induced JNK activation.

Based on the observations that (i) inhibitions by dn Ras and dn ASK are additive (**Part III, Figure 6**), and (ii) activation of JNK by wt ASK1 can be overridden only by dn JNKK, but not by dn Ras or dn Rac, I have concluded that the optimal activation of JNK requires activation of both Ras and ASK1 and that both activate the common downstream JNKK, leading to JNK activation (**Figure 1**). Since JNK activation has been frequently associated with apoptosis (**Part I, Table 17**) and JNK may indirectly modulate apoptosis through phosphorylation of Bcl-2 (63), I would predict that activation of JNK as a consequence of chemotherapy-induced MT dysfunction may play a role in regulation of apoptosis (**Figure 1**).

ACTIVATED JNK IS REQUIRED FOR THE EARLY PHASE OF PACLITAXEL-INDUCED APOPTOSIS THAT MAY OCCUR IN CELLS AT ANY PHASE OF THE CELL CYCLE

To further elucidate the role of activated JNK in the PTX-initiated apoptosis, I have examined the effects of a series of dn signaling proteins of the JNK pathway on modulation of PTX-initiated apoptosis. Parameters that were used to evaluate apoptosis include: (i) an increase of annexin-V binding, (ii) DNA fragmentation assays by both electrophoretic "ladder" pattern and a quantitative ELISA-based method, (iii) caspase assay, and (iv) cleavage of poly (ADP-ribose) polymerase (PARP).

Without alternation of the cell cycle profiles (**Part IV, Figure 4**), expression of each of dn signaling proteins, including dn ASK1, dn Rac, dn JNKK, and dn JNK, transiently protects ovarian cancer BR cells from PTX-induced apoptosis up to 16 h (**Part IV, Figures 2 and 5, Table I**). The protective effects of these dn signaling proteins decline at 24 h and completely disappear by 36 h of PTX treatment (**Part IV, Figure 5A**). Since expression of dn signaling proteins remains sufficient to completely inhibit endogenous JNK activities at 48-h treatment of PTX (**Part IV, Figure 3**), the disappearance of protection at the later time points does not result from insufficient JNK inhibition. Instead, the later phase of the PTX action may be independent of the JNK pathway.

Since dn signaling proteins had no effects on Bcl-2 phosphorylation, which were confirmed by two experimental procedures (**Part IV, Figure 8**), I have withdrawn my original hypothesis that activated JNK may account for Bcl-2 phosphorylation in PTX-treated cells, a hypothesis based on a report where JNK induced Bcl-2 phosphorylation (63).

In summary, activation of the JNK pathway independently of Bcl-2 phosphorylation is required for the early phase of PTX-induced apoptosis (**Figure 2**), whereas JNK activation may be dispensable for the late phase. Since blockage of the JNK pathway does not interfere with cell cycle profiles and only protects cells at early time points of PTX treatment where mitotic arrest has not become predominant, I would predict that the early phase of PTX-induced, JNK-dependent apoptosis may occur in cells at any phase of the cell cycle, or at least independently of mitotic arrest.

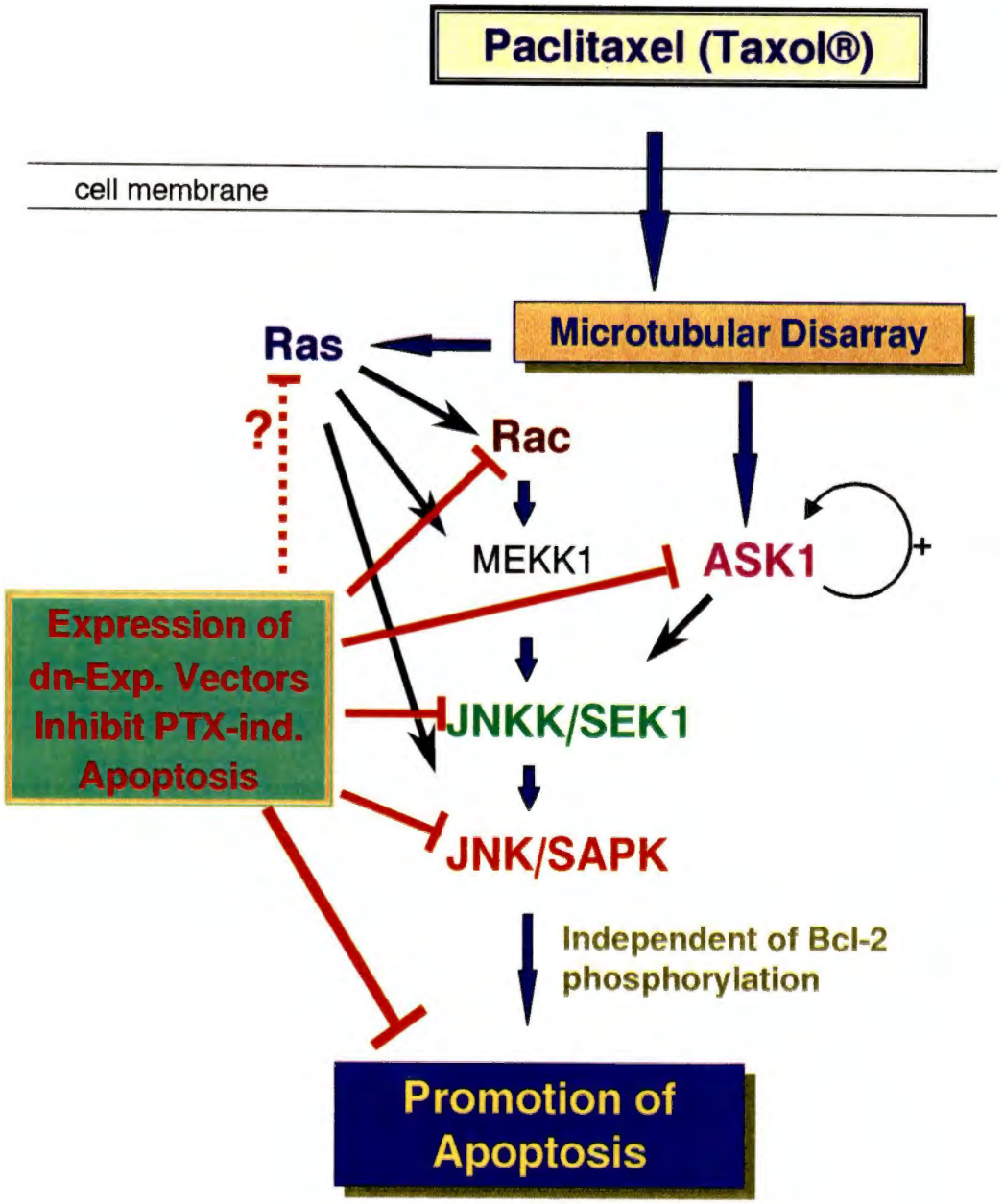
POTENTIAL MECHANISMS FOR ACTIVATION OF THE JNK PATHWAY BY MICROTUBULE DYSFUNCTION

MT dysfunction as a consequence of PTX treatment appears to be required for the JNK activation because that (i) other MT-interfering agents (docetaxel, vinblastine, vincristine, nocodazole, colchicine) also activate JNK (**Part III, Figure 1**), (ii) structural analogs of PTX (10-deacetylbaccatin-III) and of colchicine (lumicolchicine), which do not cause MT dysfunction, cannot activate JNK (**Part III, Figure 3A**), and (iii) PTX cannot activate JNK in the ovarian cancer PTX10 and PTX22 cells containing mutant β-tubulin that abolishes PTX binding (**Part III, Figure 3B**).

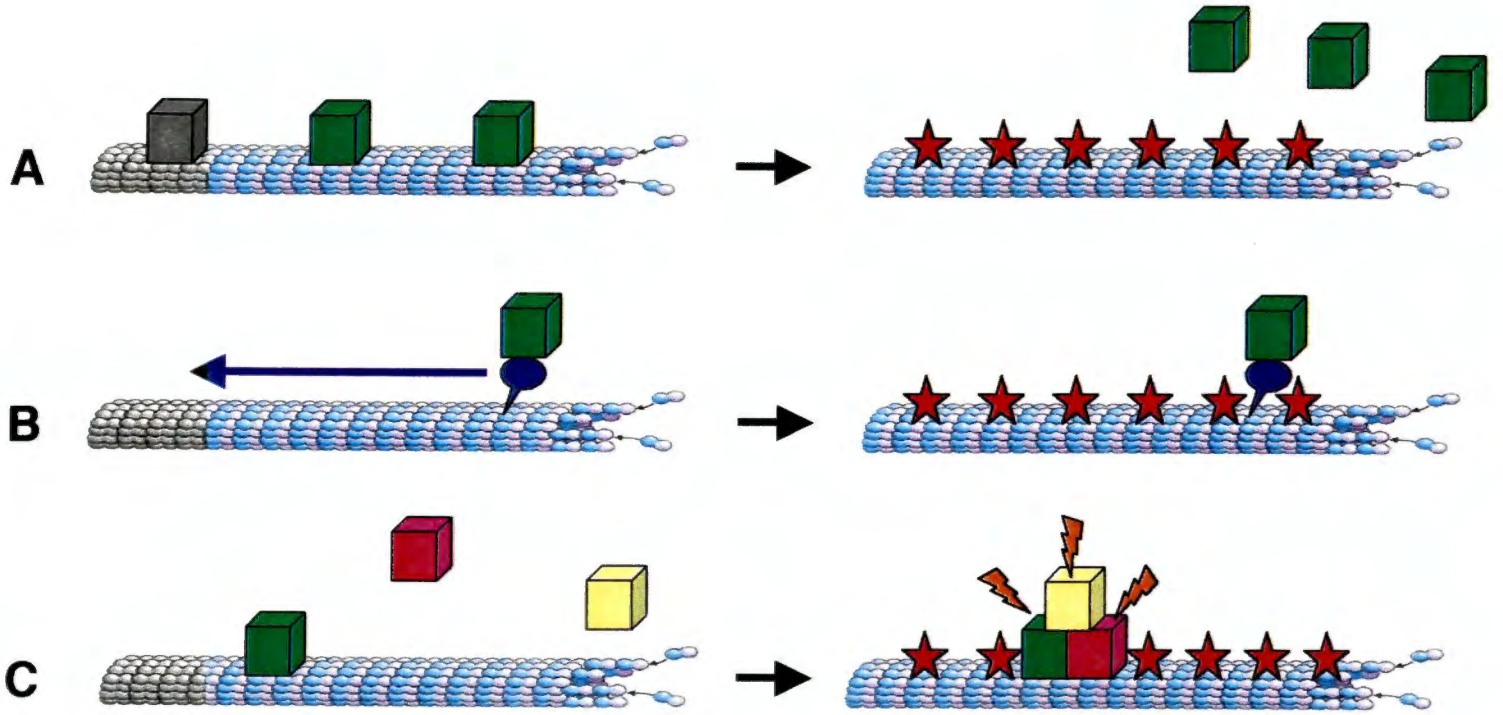
Three mechanisms have been proposed for the MT to directly activate its downstream signal transduction pathways (26). (i) Due to its wide distribution over the entire cytoplasm, the MT has a hugh protein surface to sequester cellular proteins, including those in various signal transduction pathways. (ii) Signaling proteins can be delivered to specific cellular compartments by MT-dependent motor proteins, such as kinesin and dynein. (iii) MTs may function as a scaffolding structure to facilitate interactions among signaling proteins in a cascade (**Figure 3**). For all of the above mechanisms, interactions between MTs and signaling proteins can be regulated by the MT status, which is altered by PTX binding.

Intriguingly, tubulin itself is a G-protein. GTP binds to β-tubulin reversibly and tubulin, when assembled into the MT lattice, acts as a GTPase activating protein (GAP) (18). Although other small G-proteins such as the Ras, Rac, and Rho family are important signaling proteins (31), it is unclear whether tubulin has a similar role in the JNK signal transduction pathway. Nevertheless, tubulins and MTs have been shown to interact with Rac (6), a Dbl family member Lfc (25)(24), and a MAPK kinase kinase MLK2 (mixed linkage kinase) (71).

Rac can directly bind to tubulin *in vitro* and it has been demonstrated to be co-localized with



(Figure 2) Activated JNK is required for the early phase of paclitaxel-induced apoptosis.



(Figure 3) Potential mechanisms of microtubule-regulated signal transduction. ***A,*** sequestering and release. ***B,*** motor protein-dependent delivery of signaling proteins. ***C,*** microtubules as a scaffolding protein. Signaling proteins (green, purple, and yellow cubes), paclitaxel (orange stars), motor protein (blue ballon), movement of motor protein (blue arrow).

MTs *in vivo* (6). Furthermore, the PH (Pleckstrin homology) domain of Lfc has been proposed to mediate its localization to MT, recruiting Rac to MTs to activate JNK (24). Lfc acts as GEF (guanine nucleotide exchanging factor) that specifically stimulates GDP dissociation from Rho, but not those from Rac, Cdc42, or Ras. Since Lfc binds to Rac equally well when Rac is nucleotide-depleted, or in the GDP- or GTP-bound state (25), Lfc may collaborate with MT as scaffolding proteins. On the other hand, Lfc can activate JNK and this activation is blocked by dn Rac, suggesting that Lfc still functions through Rac (24). It is possible that PTX treatment may modulate interactions between MTs, Lfc, Rac, and JNK. Further elucidation of effects of PTX on the Rac-MT interaction may provide explanation to the observation that dn Rac protects BR cells from PTX-induced apoptosis (**Part IV, Table I and Figure 2**).

A kinesin-like KIF3 family member, KIF3A, and its putative target KAP3A have been shown to be colocalized with MLK2 as punctate structures on MTs, suggesting a formation of complex (71). When expressed in fibroblasts, MLK2 colocalizes with active, dually phosphorylated, endogenous JNK to punctate structures along MTs (71). The kinesin-like identity of KIF3A raises the possibility that MTs may regulate the JNK pathway when the function of motor proteins is disrupted by PTX binding (**Figure 3B**). The complex formation between MTs and signaling proteins of the JNK pathway also suggests that MTs may function as a scaffolding structure (**Figure 3C**).

HIGH CONCENTRATIONS OF PACLITAXEL MAY BE REQUIRED FOR ITS FUNCTIONS OTHER THAN MITOTIC ARREST

The fate of cells exposed to PTX is determined by: (i) the cell type (for example, transformed *vs* nontransformed) (93), (ii) concentrations of PTX (40)(53), and (iii) duration of exposure (53). For example, short times (1 h) of exposure of leukemia HL-60 cells to low concentrations (20 nM) of PTX induce a reversible mitotic block without apoptosis, whereas either 60 nM PTX for 1 h or 10 nM for 12 h induces apoptosis (53).

It has been well documented that PTX concentrations required for blocking mitotic progression are low (IC_{50} = 8nM in HeLa cells), whereas high concentrations of PTX (IC_{50} = 330 nM in HeLa cells) are required for stabilizing polymerization (measured by MT mass) and massive MT damage (5)(19)(40)(74). Furthermore, in the studies where PTX has been demonstrated to exert functions other than mitotic arrest, the concentrations of PTX used were often high, ranging from 200 nM to 30 μM

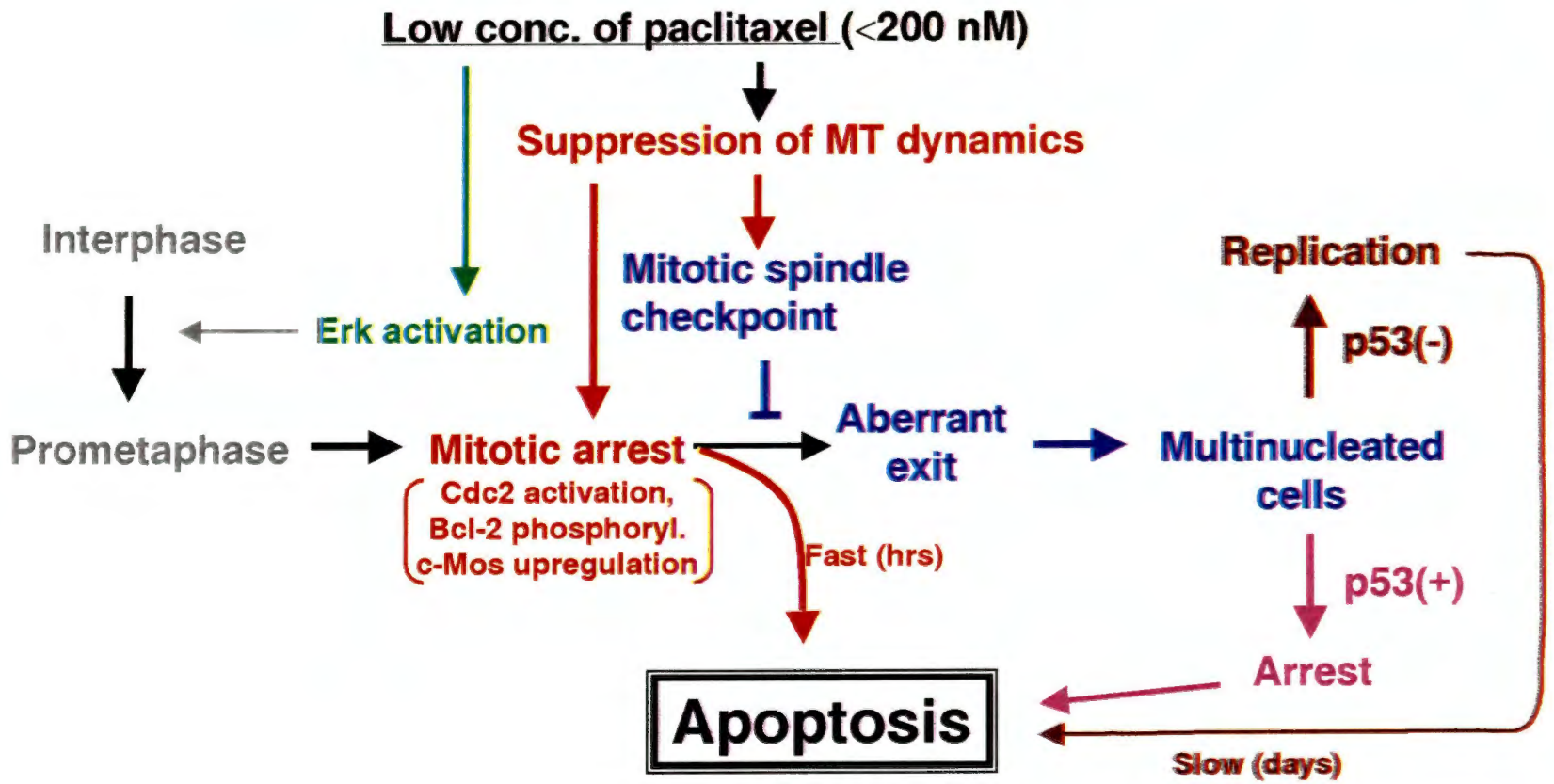
(9)(8)(12)(36)(46)(49)(47)(48)(58)(69). In agreement with the notion that higher concentrations of PTX may be required to exert functions in addition to mitotic arrest, my dose-effect data also exhibit a positive correlation between fold activation of JNK and PTX concentrations from 10 nm to 10 μM (**Part III, Table I**). Therefore, the activation of JNK pathway may be more associated with MT damage (induced by high concentrations of PTX) than with mitotic arrest (by low concentrations of PTX).

CELLS TREATED WITH LOW CONCENTRATIONS OF PACLITAXEL

Sensitivity of MTs to PTX may differ depending on the cell type, however, it is believed that mitotic spindles are the most sensitive to PTX (41)(53). Possible cellular events in cells treated with low concentrations of PTX are schematically summarized in **Figure 4**. When cells are treated with low concentrations of PTX (such as between 10 and 100 nM), suppression of MT dynamics inhibits formation of mitotic spindles, resulting in mitotic arrest. Dysfunctional mitotic MTs may trigger the mitotic spindle checkpoint. The mitotic spindle checkpoint can be considered as a signal transduction pathway that links a kinetochore-associated sensor, which monitors the appropriate connections between mitotic spindles and chromosomes, to an output that arrests cell cycle (87). It is noteworthy that PTX at low concentrations did not affect the overall architecture of the MT cytoskeleton (40). Interestingly, low (20 nM) concentrations of PTX have been shown to be able to accelerate the progression of the cell cycle to the mitotic phase (54).

Cells arrested at the G_2/M phase are characterized with Cdc2 activation (19)(36)(38)(78) and Bcl-2 phosphorylation (8)(30)(29)(76), both contributing to apoptosis. The duration of normal mitosis varies from 45 min to about 3 h (75) and , after a prolonged period (several hours), cells usually undergo apoptosis through unclear mechanisms (40). In some situations, the mitotic spindle checkpoint fails to sustain the mitotic arrest and cells undergoes aberrant exit from the abnormal mitosis, becoming multinucleated (60). The wt p53-containing multinucleated cells arrest and directly undergo apoptosis because chromosomal DNA is damaged during the formation of multinuclei (103). Those multinucleated cells with mutant p53 may continue to cycle and eventually die through apoptosis (87).

The mechanism by which PTX-treated cells exit mitosis (**Figure 4**) remains unclear. A process of adaptation has been proposed that the cell may progressively become less sensitive to the checkpoint signal as the mitotic delay lengthens (68)(92)(99). According to data from yeast, adaptation through cdc55 and cdc28 (68)(92)(99) occurs, in which cells override the checkpoint despite the persistence of MT



(Figure 4) Possible events leading to apoptosis in cells treated with low concentrations of paclitaxel.

damage. For instance, cdc55 mutant *S. cerevisiae* are able to inactivate mitosis-promoting factor and exit mitosis, through inhibitory phosphorylation of cdc28. However, the human homologs of these yeast genes are yet to be identified (87). It is also unknown whether PTX has effects on modulation of these gene products.

CELLS TREATED WITH HIGH CONCENTRATIONS OF PACLITAXEL

In cultured cells, high concentrations of PTX have been shown to cause massive MT damage (40)(41)(101), regulate gene expression (39)(46)(49)(47)(56)(55)(57)(69)(88), activate JNK (2)(48)(98), Cdc2, and other kinases (19)(36)(38)(73)(77)(78) (**Figure 5**). So far, the MT is the only known cellular target that physically interacts with PTX; no cell membrane-associated or cytoplasmic receptor has been identified (89)(101). It remains unknown, however, whether MT damage is responsible for all known effects of PTX or whether PTX also directly activates some other cellular proteins.

PTX-induced activations of JNK (97) and Cdc2 (19)(36)(38)(77) have been closely associated with PTX-induced apoptosis. Among genes that have been shown to be upregulated by PTX, Bak (57)(39), Bax (88), and TNF-α (46) are known to promote apoptosis; whereas cyclin B1 (56)(55) may activate Cdc2 activation, leading to apoptosis. PTX-induced activation of Raf-1(9)(8) has been proposed to be involved in Bcl-2 phosphorylation, which may also promote apoptosis. It is noteworthy that apoptosis induced by these pathway does not requires mitotic arrest, suggesting that it may occur in cells at any phase of the cell cycle (**Figure 5**).

OTHER CONSIDERATIONS FOR HIGH CONCENTRATIONS OF PACLITAXEL

An intriguing observation is that treatment with 10 nM PTX for 20 h resulted in high intracellular drug accumulation (8.3 μM) (41). Does this mean that, no matter what concentrations of PTX are used, the cell eventually responds in the same way as it were treated with high concentrations of PTX? Not necessarily, because both (i) the differential PTX sensitivity of tubulin at the ends versus in the shaft of MTs and (ii) the temporal factor are also crucial.

Due to a high affinity of PTX for MTs, after a long time of treatment (20 h) even at low concentration (10 nM), most PTX gradually accumulates in the MT-bound compartment, explaining the

high intracellular drug accumulation. The effects of PTX on MTs, however, may be different in the cases where (i) a few molecules of PTX bind to MTs in cells treated with low concentrations of PTX and (ii) high concentrations of PTX bombards MTs. The speculation is based on reports where PTX in the nM concentration range suppresses assembly dynamics of MT without overstabilization of them, suggesting that ends of MTs, where the assembly dynamics are the most active, are more sensitive to PTX than the shaft (17)(40). Recent evidence also indicates the sensitivities to PTX even differ at the plus and minus ends of MTs (16)(100). Therefore, the effects by gradually added MT-bound PTX at 8.3 μM concentration on MT or other targets could be very different from those exerted by a sudden increase of cytosolic PTX to the same μM concentration range.

Furthermore, while low concentrations of PTX may only suppress assembly dynamics of MTs, its high concentrations have been shown to exhibit many more functions (discussed in p. 199) and some cellular responses, such as JNK activation (**Part III, Figure 2**), only occur during a limited time window. When treated with nM concentrations of PTX, by the time cells accumulate PTX to μM concentrations, the time window for transient activation processes, such as JNK, may have passed. Alternatively, the cell populations responding well to high concentrations of PTX may be limited to non-mitotic cells. After treatment with 10 nM PTX for 20 h, however, the majority of cycling cells would be blocked at the mitotic phase. These are speculations yet to be tested.

The use of high concentrations (>200 nM) of PTX, the concentrations within the clinically achievable plasma concentrations (ranging from 0.1 to 10 μM) (86), may have various impacts on cancer therapy. Since high concentrations of PTX may induce apoptosis independently of mitotic arrest, a potential advantage is that high concentrations of PTX may be able to kill dormant cancer cells. Cancer dormancy is a state where the population of tumor cells is not progressively increasing in number. It can result from a situation where the majority of tumor cells are in cell-cycle arrest or that there is a balance between proliferation and death (94). On the other hand, the potential disadvantage is that PTX may kill normal, differentiated cells as well. For instance, it is important to study whether the use of higher concentrations of PTX aggravates side effects such as neurotoxicity and cardiotoxicity.

USEFUL METHODOLOGY

Insufficient transfection efficiency perhaps is the most common problem encountered by

researchers when using transient transfection procedures. Several methods have been developed to circumvent this problem. For instance, in assays of CAT reporter, luciferase reporter, HA-JNK (59), and HA-Akt activities (70), a "reporter" construct is coexpressed with the gene(s) to be studied. Based on a commonly accepted assumption that co-transfected genes express within the same cell, activities of reporters thereby reflect the effects of coexpressed gene of interest. Another way to overcome the low transfection problem is to isolate only cell populations that successfully express a cotransfected marker on the cell membrane, such as CD20, by using a fluorochrome-conjugated anti-CD20 antibody and flow cytometric sorting (96).

In my study, the use of pEGFP proved to be straightforward and useful in analyses for apoptosis and in flow cytometric sorting (97). Two experimental procedures used have successfully circumvented the limited transfection efficiencies (20 % in BR cells and 15% in MCF-7 cells at best), allowing me to be able to examine the effects of various dn signaling proteins of the JNK pathway on the PTX-initiated apoptosis.

(i) Flow cytometric sorting: I have cotransfected cells with pEGFP and expression vectors for dn signaling proteins and isolated green cells with flow cytometric sorting for further Western blot analyses, cell cycle analysis, DNA fragmentation assays, or caspase-3 activity assay (**Part IV, Figures 3-8**). Coexpression of pEGFP and dn expression vectors was verified by Western blots showing the presence of dn signaling protein in green cells but not in non-green cells (**Part IV, Figure 3**). Separation by this method appears to be specific, however, the worst drawback is that this method is very time-consuming. The highest acceptable sorting speed in the FACStar^{PLUS} that I have used is 2,000 cells/second, at that speed the "aborted" rate is about 10 %. When two or more cells reside together in a single drop of normal saline passing through the nozzle of flow cytometer, the computer detects them and aborts the collection of this drop. Further increasing flow speed to more than 2,000 cells/second in that machine usually greatly elevates aborted rates, without a much higher collecting efficiency. Newer flow cytometric sorters designed to sort at speed higher than 10,000 cells/second should alleviate this time-consuming disadvantage.

(ii) Three-color analysis for early apoptosis in cells expressing transfected genes: I have cotransfected cells with pEGFP and genes of interest, then done flow cytometric analyses for apoptosis using PE-conjugated AV and 7-AAD. Three fluorochromes, green protein as a result of pEGFP expression, PE-conjugated AV, and 7-AAD, are excited by a laser tuned to 488 nm and emissions are

detected at 507, 575, and 650 nm, respectively (**Part IV, Figure 1**). Intensities of these three parameters of every cell can be acquired and analyzed by flow cytometry. The combined use of AV and 7-AAD can differentiate the early apoptotic cells (AV positive/7-AAD negative) from dead cells (7-AAD positive). This procedure is quick because no sorting is required. I would predict that it shall become a popular screening method to test the apoptotic effects of any gene.

DIRECTIONS FOR FURTHER RESEARCH

Results from my study have suggested a linkage between MT dysfunction and activation of the JNK/SAPK pathway (98). A pro-apoptotic role of JNK/SAPK in PTX-induced apoptosis has also been demonstrated (97). However, these data have raised more questions than they have answered. Three obvious questions extending from what I have observed are: (i) how does MT dysfunction signal Ras, Rac, and ASK1? (ii) how does the PTX-activated JNK promote apoptosis? and (iii) what are the mechanisms of the later phase of PTX-induced apoptosis? The ultimate direction for clinically related research on PTX mechanisms, however, is to develop strategies of using taxanes more efficiently.

MECHANISTIC CONNECTIONS AMONG MICROTUBULES, RAS, RAC, AND ASK1

Exploration of these connections should be focused on proteins that may directly interact with MTs. Known MT-interacting proteins include Rac (6), MLK2, KIFs, JNK (71), Lfc (24), and several MAPs.

The first step is to examine how PTX treatment changes the interactions among these proteins and MTs. This can be achieved by co-staining of these proteins followed by fluorescent microscopy. Furthermore, comparisons of these patterns with or without PTX treatment among the PTX-resistant cell lines, PTX10 and PTX22, and the PTX-sensitive parental 1A9 cells should be able to verify the role of PTX binding to MTs in regulation of these changes. Alternatively, since the amino acid residue mutations in β-tubulin that abolish PTX binding to MTs are known, the genes for wt or mutant β-tubulin can be constructed to be tagged with EGFP and expressed in the cells. If EGFP does not interfere with tubulin functions, we can follow the dynamics of MT assembly before and after PTX treatment. Expression of EGFP also facilitates our observation on MT-interacting proteins using fluorescent microscopy.

Among proteins that have not been identified to be associated with MTs, one candidate is ASK1. ASK1 has been to demonstrated to be activated by PTX treatment (98). Similar to MLK2, ASK1 is also a MAPK kinase kinase. It would be of great interest to examine whether ASK1 directly interacts with MTs and how PTX treatment modulates the interactions. Investigation on this hypothesis can be achieved by colocalization under fluorescent microscopy, coimmunoprecipitation, and the GST-fusion protein pull down assay. To further discover novel tubulin-interacting proteins, the yeast two-hybrid assay with the tubulin gene as the bait can be used to screen cDNA library for other candidates.

MAPs are believed to be important in regulating assembly and stability of MT. Although I am not aware of any study demonstrating that PTX-bound MTs have effects on MAPs, it would not be surprising if dysfunctional MTs are proved to be able to signal through MAPs.

All of the approaches described above apply for studies in cells at any phase of the cell cycle. Some proteins interacting with MTs, however, may only function in cells arrested in the mitotic state. For example, is the spindle assembly checkpoint involved in the PTX-induced JNK activation? Several mammalian spindle assembly checkpoint genes, such as MAD2 (51)(52), BUB1 (91), BUB3 (90) (above are kinetochore-localized), PP2A, and MAPK have been identified (87), and overexpression of dn Bub1 in HeLa cells reduces the nocodazole-triggered apoptosis (91)(87). It would be interesting to examine whether PTX (and, if so, at what concentrations) has any effect on these checkpoint gene products and whether these gene products interact with signaling proteins of the JNK pathway.

HOW DOES ACTIVATED JNK CAUSE APOPTOSIS IN THE EARLY PHASE OF PACLITAXEL-INDUCED APOPTOSIS?

JNK has been shown to regulate activating protein-1 (AP-1) transcriptional activity *in vivo* (104)(105). The transactivation activity of AP-1, however, may be regulated by: (i) different dimerization of AP-1 factors from members of the Jun family (c-Jun, JunB, JunD) (15), Fos family (c-Fos, FosB, Fra1, Fra2) (62)(107), and ATF family (ATF2, ATF3/LRF1, B-ATF) (20)(35), thereby eliciting differential binding to the TRE or CRE (28)(44)(84), (ii) phosphoregulation of subunits of AP-1 by specific protein kinases and phosphatases (1)(10)(27)(34)(95), and (iii) further interactions between AP-1 and transcriptional coactivators (3)(4)(14)(43)(44)(45).

It is not surprising, therefore, that the role of AP-1 in apoptosis may be completely opposite,

depending on the cell type and stimuli (44), despite the frequent associations between JNK and apoptosis (**Part I, Table 17**). An obvious question is "Is AP-1 transcriptional activity required for PTX-induced apoptosis?"

Results from my study (98) and others (2)(48) demonstrate that activation of functional AP-1 result from PTX-induced activation of the JNK pathway (**Part III, Figure 4**). It remains unknown, however, whether: (i) transcriptional activation of AP-1 activity is required for PTX-induced apoptosis, (ii) it has no role in this apoptosis, or (iii) it may even protect the cell from apoptosis.

The involvement of transcriptional activation in PTX-induced apoptosis can be tested by using available inhibitors for RNA and protein synthesis. A caveat is that these inhibitors by themselves may induce apoptosis, confounding apoptotic analysis. For example, a commonly used transcriptional inhibitor, actinomycin-D, efficiently induces apoptosis (**Part IV, Figure 7**). It is also worth searching database, such as EMBL/GenBank, for apoptosis-related genes and examine whether there are TRE and/or CRE in the promoter region. The bioinformatic search should narrow down the scope of candidate genes.

The expression vector for dn c-Jun (pCMV-TAM67) is very effective in inhibiting AP-1 activity (**Part III, Figure 4B**) and can be used to examine whether inhibition of c-Jun can protect cells from, at least the first phase of, PTX-induced apoptosis. However, careful titrations to determine an optimal plasmid concentration for cotransfection experiments appear to be critical. In cotransfection experiments using pEGFP and TAM67 at ratio 1:3, I have observed high cytotoxicity of TAM67 overexpression. To determine the appropriate concentration of TAM67 that is high enough to express enough dn c-Jun to block JNK-activated AP-1 activity but low enough to avoid undesired cytotoxicity, two experimental parameters should be carefully monitored during the titration procedures. (i) The lowest concentration of TAM67 that still can sufficiently suppress AP-1 activity can be determined by using a TRE reporter and PTX treatment. (ii) The highest possible concentration of TAM67 should be limited by monitoring percentage of surviving green cells that are cotransfected with pEGFP and increasing amount of pCMV-TAM67.

A systematic way to examine gene regulation by treatment with PTX is screening different pools of mRNA isolated from either PTX-treated cells or control cells with biochips which are coated with thousand of oligonucleotides representing the human cDNA library (33)(72)(83)(85). Briefly in this method, mRNAs are isolated from treated cells, subjected to reverse transcription to generate cDNAs in

the presence of fluorochrome-conjugated dNTP, and the labeled cDNAs are hybridized with biochips. The intensities of fluorescence among thousands of oligonucleotides can be recorded by special readers and analyzed by computer. Upregulation and downregulation of genes by PTX treatment can be easily detected by this method. Biochips provide a powerful way to study: (i) what genes are regulated by PTX treatment, (ii) what concentration ranges of PTX are able to regulate gene expression, (iii) the cell type specificity, such as proliferating tumor cells versus normal differentiated cells, and even (iv) the cell cycle phase specificity, if it exists, for PTX-induced gene regulation. For the last aim, mRNA can be isolated from either G_1 phase or G_2/M phase PTX-treated cells separated by centrifugal elutriation (see Part IV, Appendix).

On the other hand, it remains possible that transactivation through AP-1 may not be required for the PTX-initiated apoptotic pathway. Instead, activated JNK may activate caspases either directly or through interactions with other proteins. For instance, JNK may interact with p21 (81), which in turn may interact with Cdc2 (5)(13)(106) or other CDKs (79). Recent studies suggest an increasingly important role of CDKs as an effector of apoptosis (22)(32)(36)(50)(64)(80)(82). In addition to c-Jun and ATF2, JNK has been shown to be associated with a variety of proteins, including p53 (66), MAPK-APK-3 (MAPK-activated protein kinase-3 (61) (these two are its substrates) and non-substrate such as Jun B (21). Likewise, it remains possible that JNK may turn out to be able to interact with some regulators of the apoptotic pathway, including AIF, Apaf-1, cytochrome *c*, caspases-8, -9,-10, or some yet-to-be-identified apoptogenic regulators and effectors. The use of GST-JNK to affinity precipitate its interacting proteins can be used for this screening.

WHAT ARE THE MECHANISMS OF THE LATER PHASE OF PACLITAXEL-INDUCED APOPTOSIS?

The observation on involvement of the JNK pathway in the early phase of PTX-induced apoptosis only provides a partial answer for the fundamental question: "how does PTX kill cancer cells?". Apoptotic mechanisms for the later phase still remain unknown. Prolonged mitotic arrest remains the most likely culprit, although the mechanism remains unknown. Nevertheless, other factors may also be involved.

Since endogenous JNK activity can be sufficiently suppressed by expression of dn signaling proteins of the JNK pathway up to 48 h (**Part IV, Figure 3**), the use of expression vector for dn JNK can

dissect out the JNK effects, allowing us to study the JNK-independent, PTX-induced apoptosis. For instance, to examine the role of G_2/M arrest in the later phase of apoptosis, we can test whether PTX can still induce apoptosis in the dn JNK-expressing cells that are arrested in the G_1 phase by the use of adenoviral vectors for p16 and/or those for p27 (**Part III, Appendix**). If the G_2/M arrest is the only cause for the JNK-independent late phase of PTX-induced apoptosis, cell death induced by PTX treatment should be markedly inhibited in the dn JNK-expressing G1-arrested cells. If so, the following phase of research should focus on identification of apoptogenic factors that are responsible for apoptosis following G_2/M arrest. Apoptogenic factors, which are activated in cells arrested in the G_2/M phase, might be identified from cells expressing dn JNK that are arrested in the G_2/M phase by PTX treatment for 36 to 48 h. Apoptogenecity of these factors can be verified by overexpression in the G_1-arrested cells, followed by measurement for apoptosis. Centrifugal elutriation (**Part IV, Appendix**) can be used to isolate G_1 and G_2/M phase cells.

Two methods are useful for screening for potential apoptogenic factors. The first method is the yeast two-hybrid assays using known apoptotic regulators as baits, such as the Bcl-2 family members, Apaf-1, the caspase family members, and various CDKs. The second is to use biochips (described in p. 205) to analyze genes that are upregulated or downregulated in this particular population of cells.

Alternative approach to examine apoptogenecity of the JNK-independent G_2/M arrest is the use of low concentrations of PTX, such as 10 nM, which did not significantly activate JNK (**Part III, Table 1**). In the present study, I have not examined the effects of PTX on JNK activation in cells treated for more than 24 h. However, I would predict that, at 36 to 48 h treatment, the majority of BR cells should be arrested in the G_2/M phase and still undergo apoptosis without the apoptogenic influence by activated JNK. From those apoptotic cells, we may be able to characterize the apoptotic mechanism after a prolonged mitotic arrest or even identify some novel factors responsible for the mitotic arrest-induced apoptosis.

Since PTX has been known to stimulate expression of *mdr* gene (7)(37), one potential pitfall of this approach is that treatment with low concentrations of PTX for several days favors for PTX-resistant cells with upregulated *mdr*. This may explain the observations that one out of four cell lines treated with 12 nM of PTX for four days survived and continued growing (11). Cotreatments with verapamil (5 μg/ml) has been reported to be able to inhibit the P-glycoprotein functions (23) and should be added into culture media in these experiments.

On the other hand, if cells can not be protected from PTX-initiated apoptosis by simultaneous G_1 phase arrest of the cell cycle and suppression of JNK activity, apoptogenic mechanisms independent of G_2/M arrest are indicated and novel apoptogenic factors could be subsequently identified from these cells.

DIRECTION TO THE ULTIMATE GOAL

Our improved understanding of mechanisms of PTX action should allow development of more efficient strategies of using taxane-based chemotherapy. Demonstration of a role for JNK activation in PTX-initiated apoptosis in this study only contributes a tiny share to our understanding on action mechanisms of PTX and, by inference, the role of MTs as regulators of apoptosis. To achieve the ultimate objective of using taxanes more efficiently to treat patients with cancers, we should continue working on: (i) how to increase the specificity of taxanes against cancer cells, (ii) how to enhance its effects by adjuvant agents, (iii) how to decrease the resistance of cancer cells to taxanes, and (iv) how to lessen its adverse side effects.

LITERATURE CITED IN PART V

1. **Adler, V., A. Polotskaya, F. Wagner, and A. S. Kraft.** 1992. Affinity-purified c-Jun amino-terminal protein kinase requires serine/threonine phosphorylation for activity. J. Biol. Chem. **267:**17001-5.
2. **Amato, S. F., J. M. Swart, M. Berg, H. J. Wanebo, S. R. Mehta, and T. C. Chiles.** 1998. Transient stimulation of the c-Jun-NH2-terminal kinase/activator protein 1 pathway and inhibition of extracellular signal-regulated kinase are early effects in paclitaxel-mediated apoptosis in human B lymphoblasts. Cancer Res. **58:**241-7.
3. **Arias, J., A. S. Alberts, P. Brindle, F. X. Claret, T. Smeal, M. Karin, J. Feramisco, and M. Montminy.** 1994. Activation of cAMP and mitogen responsive genes relies on a common nuclear factor. Nature **370:**226-229.
4. **Bannister, A. J., T. Oehler, D. Wilhelm, P. Angel, and T. Kouzarides.** 1995. Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. Oncogene **11:**2509-14.
5. **Barboule, N., P. Chadebech, V. Baldin, S. Vidal, and A. Valette.** 1997. Involvement of p21 in mitotic exit after paclitaxel treatment in MCF-7 breast adenocarcinoma cell line. Oncogene **15:**2867-75.
6. **Best, A., S. Ahmed, R. Kozma, and L. Lim.** 1996. The Ras-related GTPase Rac1 binds tubulin. J. Biol. Chem. **271:**3756-62.
7. **Bhalla, K., Y. Huang, C. Tang, S. Self, S. Ray, M. E. Mahoney, V. Ponnathpur, E. Tourkina, A. M. Ibrado, G. Bullock, and et al.** 1994. Characterization of a human myeloid leukemia cell line highly resistant to taxol. Leukemia **8:**465-75.
8. **Blagosklonny, M. V., P. Giannakakou, W. S. el-Deiry, D. G. Kingston, P. I. Higgs, L. Neckers, and T. Fojo.** 1997. Raf-1/bcl-2 phosphorylation: a step from microtubule damage to cell death. Cancer Res. **57:**130-5.
9. **Blagosklonny, M. V., T. Schulte, P. Nguyen, J. Trepel, and L. M. Neckers.** 1996. Taxol-induced apoptosis and phosphorylation of Bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. Cancer Res. **56:**1851-4.
10. **Bocco, J. L., A. Bahr, J. Goetz, C. Hauss, T. Kallunki, C. Kedinger, and B. Chatton.** 1996. In vivo association of ATFa with JNK/SAP kinase activities. Oncogene **12:**1971-80.
11. **Cameron, M. R., M. R. Caudle, W. R. Sullivan, Jr., J. J. Peluso, and J. Wimalasena.** 1995. The steroidogenic and morphological effects of paclitaxel on cultured ovarian cancer cells. Oncol. Res. **7:**145-56.
12. **Carles, G., D. Braguer, G. Sabeur, and C. Briand.** 1998. The effect of combining antitubulin agents on differentiated and undifferentiated human colon cancer cells. Anticancer Drugs **9:**209-21.
13. **Chen, J., T. Willingham, M. Shuford, D. Bruce, E. Rushing, Y. Smith, and P. D. Nisen.** 1996. Effects of ectopic overexpression of p21(WAF1/CIP1) on aneuploidy and the malignant phenotype of human brain tumor cells. Oncogene **13:**1395-403.

14. **Claret, F. X., M. Hibi, S. Dhut, T. Toda, and M. Karin.** 1996. A new group of conserved coactivators that increase the specificity of AP-1 transcription factors. Nature **383:**453-7.
15. **Deng, T., and M. Karin.** 1993. JunB differs from c-Jun in its DNA-binding and dimerization domains, and represses c-Jun by formation of inactive heterodimers. Genes Dev. **7:**479-90.
16. **Derry, W. B., L. Wilson, and M. A. Jordan.** 1998. Low potency of taxol at microtubule minus ends: implications for its antimitotic and therapeutic mechanism. Cancer Res. **58:**1177-84.
17. **Derry, W. B., L. Wilson, and M. A. Jordan.** 1995. Substoichiometric binding of taxol suppresses microtubule dynamics. Biochemistry **34:**2203-11.
18. **Desai, A., and T. J. Mitchison.** 1997. Microtubule polymerization dynamics. Annu. Rev.Cell. Dev. Biol. **13:**83-117.
19. **Donaldson, K. L., G. L. Goolsby, P. A. Kiener, and A. F. Wahl.** 1994. Activation of p34cdc2 coincident with taxol-induced apoptosis. Cell Growth Differ. **5:**1041-50.
20. **Dorsey, M. J., H. J. Tae, K. G. Sollenberger, N. T. Mascarenhas, L. M. Johansen, and E. J. Taparowsky.** 1995. B-ATF: a novel human bZIP protein that associates with members of the AP-1 transcription factor family. Oncogene **11:**2255-65.
21. **Fuchs, S. Y., B. Xie, V. Adler, V. A. Fried, R. J. Davis, and Z. Ronai.** 1997. c-Jun NH2-terminal kinases target the ubiquitination of their associated transcription factors. J. Biol. Chem. **272:**32163-8.
22. **Furukawa, Y., S. Iwase, Y. Terui, J. Kikuchi, T. Sakai, M. Nakamura, S. Kitagawa, and M. Kitagawa.** 1996. Transcriptional activation of the cdc2 gene is associated with Fas- induced apoptosis of human hematopoietic cells. J. Biol. Chem. **271:**28469-77.
23. **Giannakakou, P., D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, T. Fojo, and M. S. Poruchynsky.** 1997. Paclitaxel-resistant human ovarian cancer cells have mutant β-tubulins that exhibit impaired paclitaxel-driven polymerization. J. Biol. Chem. **272:**17118-17125.
24. **Glaven, J. A., I. Whitehead, S. Bagrodia, R. Kay, and R. A. Cerione.** 1999. The Dbl-related protein, Lfc, localizes to microtubules and mediates the activation of Rac signaling pathways in cells. J. Biol. Chem. **274:**2279-85.
25. **Glaven, J. A., I. P. Whitehead, T. Nomanbhoy, R. Kay, and R. A. Cerione.** 1996. Lfc and Lsc oncoproteins represent two new guanine nucleotide exchange factors for the Rho GTP-binding protein. J. Biol. Chem. **271:**27374-81.
26. **Gundersen, G. G., and T. Cook a.** 1999. Microtubules and signal transduction. Curr. Opin. Cell Biol. **11:**81-94.
27. **Gupta, S., D. Campbell, B. Derijard, and R. J. Davis.** 1995. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science **267:**389-93.
28. **Hai, T., and T. Curran.** 1991. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. U S A **88:**3720-4.
29. **Haldar, S., A. Basu, and C. M. Croce.** 1998. Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. Cancer Res. **58:**1609-15.
30. **Haldar, S., N. Jena, and C. M. Croce.** 1995. Inactivation of Bcl-2 by phosphorylation. Proc. Natl. Acad. Sci. USA **92:**4507-11.
31. **Hall, A.** 1998. Rho GTPases and the actin cytoskeleton. Science **279:**509-14.
32. **Harvey, K. J., J. F. Blomquist, and D. S. Ucker.** 1998. Commitment and effector phases of the physiological cell death pathway elucidated with respect to Bcl-2 caspase, and cyclin-dependent kinase activities. Mol. Cell. Biol. **18:**2912-22.
33. **He, B., and F. Regnier.** 1998. Microfabricated liquid chromatography columns based on collocated monolith support structures. J. Pharm. Biomed. Anal. **17:**925-32.
34. **Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin.** 1993. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes Dev. **7:**2135-48.
35. **Hsu, J. C., T. Laz, K. L. Mohn, and R. Taub.** 1991. Identification of LRF-1, a leucine-zipper protein that is rapidly and highly induced in regenerating liver. Proc. Natl. Acad. Sci. U S A **88:**3511-5.
36. **Huaug, T. S., C. H. Shu, W. K. Yang, and J. Whang-Peng.** 1997. Activation of CDC 25 phosphatase and CDC 2 kinase involved in GL331- induced apoptosis. Cancer Res. **57:**2974-8.
37. **Huang, Y., A. M. Ibrado, J. C. Reed, G. Bullock, S. Ray, C. Tang, and K. Bhalla.** 1997. Co-expression of several molecular mechanisms of multidrug resistance and their significance for paclitaxel cytotoxicity in human AML HL-60 cells. Leukemia **11:**253-7.
38. **Ibrado, A. M., C. N. Kim, and K. Bhalla.** 1998. Temporal relationship of CDK1 activation and mitotic arrest to cytosolic accumulation of cytochrome C and caspase-3 activity during Taxol-induced apoptosis of human AML HL-60 cells. Leukemia **12:**1930-6.
39. **Jones, N. A., J. Turner, A. J. McIlwrath, R. Brown, and C. Dive.** 1998. Cisplatin- and paclitaxel-induced apoptosis of ovarian carcinoma cells and the relationship between bax and bak up-regulation and the functional status of p53. Mol Pharmacol **53:**819-26.
40. **Jordan, M. A., R. J. Toso, D. Thrower, and L. Wilson.** 1993. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc. Natl. Acad. Sci. U S A **90:**9552-6.
41. **Jordan, M. A., K. Wendell, S. Gardiner, W. B. Derry, H. Copp, and L. Wilson.** 1996. Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res. **56:**816-25.
42. **Jordan, M. A., and L. Wilson.** 1998. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr. Opin. Cell Biol. **10:**123-30.
43. **Kamei, Y., L. Xu, T. Heinzel, J. Torchia, R. Kurokawa, B. Gloss, S.-C. Lin, R. A. Heyman, D. W. Rose, C. K. Glass, and M. G. Rosenfeld.** 1996. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell **85:**403-414.
44. **Karin, M., Z. Liu, and E. Zandi.** 1997. AP-1 function and regulation. Curr. Opin. Cell Biol. **9:**240-6.
45. **Kwok, R. P., J. R. Lundblad, J. C. Chrivia, J. P. Richards, H. P. Bachinger, R. G. Brennan, S. G. Roberts, M. R. Green, and R. H. Goodman.** 1994. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature **370:**223-

6.
46. **Lanni, J. S., S. W. Lowe, E. J. Licitra, J. O. Liu, and T. Jacks.** 1997. p53-independent apoptosis induced by paclitaxel through an indirect mechanism. Proc. Natl. Acad. Sci. U S A **94:**9679-83.
47. **Lee, L. F., J. S. Haskill, N. Mukaida, K. Matsushima, and J. P. Ting.** 1997. Identification of tumor-specific paclitaxel (Taxol)-responsive regulatory elements in the interleukin-8 promoter. Mol. Cell. Biol. **17:**5097-105.
48. **Lee, L. F., G. Li, D. J. Templeton, and J. P. Ting.** 1998. Paclitaxel (Taxol)-induced gene expression and cell death are both mediated by the activation of c-Jun NH2-terminal kinase (JNK/SAPK). J. Biol. Chem. **273:**28253-60.
49. **Lee, L. F., C. C. Schuerer-Maly, A. K. Lofquist, C. van Haaften-Day, J. P. Ting, C. M. White, B. K. Martin, and J. S. Haskill.** 1996. Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. Cancer Res. **56:**1303-8.
50. **Levkau, B., H. Koyama, E. W. Raines, B. E. Clurman, B. Herren, K. Orth, J. M. Roberts, and R. Ross.** 1998. Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. Mol. Cell **1:**553-63.
51. **Li, Y., and R. Benezra.** 1996. Identification of a human mitotic checkpoint gene: hsMAD2. Science **274:**246-8.
52. **Li, Y., C. Gorbea, D. Mahaffey, M. Rechsteiner, and R. Benezra.** 1997. MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. Proc. Natl. Acad. Sci. U S A **94:**12431-6.
53. **Lieu, C. H., Y. N. Chang, and Y. K. Lai.** 1997. Dual cytotoxic mechanisms of submicromolar taxol on human leukemia HL-60 cells. Biochem. Pharmacol. **53:**1587-96.
54. **Lieu, C. H., C. C. Liu, T. H. Yu, K. D. Chen, Y. N. Chang, and Y. K. Lai.** 1998. Role of mitogen-activated protein kinase in taxol-induced apoptosis in human leukemic U937 cells. Cell Growth Differ. **9:**767-76.
55. **Ling, Y. H., U. Consoli, C. Tornos, M. Andreeff, and R. Perez-Soler.** 1998. Accumulation of cyclin B1, activation of cyclin B1-dependent kinase and induction of programmed cell death in human epidermoid carcinoma KB cells treated with taxol. Int. J. Cancer **75:**925-32.
56. **Ling, Y. H., Y. Yang, C. Tornos, B. Singh, and R. Perez-Soler.** 1998. Paclitaxel-induced apoptosis is associated with expression and activation of c-Mos gene product in human ovarian carcinoma SKOV3 cells. Cancer Res **58:**3633-40.
57. **Liu, Q. Y., and C. A. Stein.** 1997. Taxol and estramustine-induced modulation of human prostate cancer cell apoptosis via alteration in bcl-xL and bak expression. Clin. Cancer Res. **3:**2039-2046.
58. **Liu, Y., K. Bhalla, C. Hill, and D. G. Priest.** 1994. Evidence for involvement of tyrosine phosphorylation in taxol-induced apoptosis in a human ovarian tumor cell line. Biochem. Pharmacol. **48:**1265-72.
59. **Liu, Z. G., H. Hsu, D. V. Goeddel, and M. Karin.** 1996. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. Cell **87:**565-76.
60. **Long, B. H., and C. R. Fairchild.** 1994. Paclitaxel inhibits progression of mitotic cells to G1 phase by interference with spindle formation without affecting other microtubule functions during anaphase and telephase. Cancer Res. **54:**4355-61.
61. **Ludwig, S., K. Engel, A. Hoffmeyer, G. Sithanandam, B. Neufeld, D. Palm, M. Gaestel, and U. R. Rapp.** 1996. 3pK, a novel mitogen-activated protein (MAP) kinase-activated protein kinase, is targeted by three MAP kinase pathways. Mol. Cell. Biol. **16:**6687-97.
62. **Matsui, M., M. Tokuhara, Y. Konuma, N. Nomura, and R. Ishizaki.** 1990. Isolation of human fos-related genes and their expression during monocyte-macrophage differentiation. Oncogene **5:**249-55.
63. **Maundrell, K., B. Antonsson, E. Magnenat, M. Camps, M. Muda, C. Chabert, C. Gillieron, U. Boschert, E. Vial-Knecht, J.-C. Martinou, and S. Arkinstall.** 1997. Bcl-2 undergoes phosphorylation by c-Jun N-terminal kinase/stress-activated protein kinases in the presence of the constitutively active GTP-binding protein Rac1. J. Biol. Chem. **272:**25238-25242.
64. **Meikrantz, W., and R. Schlegel.** 1996. Suppression of apoptosis by dominant negative mutants of cyclin- dependent protein kinases. J. Biol. Chem. **271:**10205-9.
65. **Milas, L., N. R. Hunter, B. Kurdoglu, K. A. Mason, R. E. Meyn, L. C. Stephens, and L. J. Peters.** 1995. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. Cancer Chemother. Pharmacol. **35:**297-303.
66. **Milne, D. M., L. E. Campbell, D. G. Campbell, and D. W. Meek.** 1995. p53 is phosphorylated in vitro and in vivo by an ultraviolet radiation- induced protein kinase characteristic of the c-Jun kinase, JNK1. J. Biol. Chem. **270:**5511-8.
67. **Milross, C. G., K. A. Mason, N. R. Hunter, W. K. Chung, L. J. Peters, and L. Milas.** 1996. Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. J. Natl. Cancer Inst. **88:**1308-14.
68. **Minshull, J., A. Straight, A. D. Rudner, A. F. Dernburg, A. Belmont, and A. W. Murray.** 1996. Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast. Curr. Biol. **6:**1609-20.
69. **Moos, P. J., and F. A. Fitzpatrick.** 1998. Taxane-mediated gene induction is independent of microtubule stabilization: induction of transcription regulators and enzymes that modulate inflammation and apoptosis. Proc. Natl. Acad. Sci. U S A **95:**3896-901.
70. **Murga, C., L. Laguinge, R. Wetzker, A. Cuadrado, and J. S. Gutkind.** 1998. Activation of Akt/protein kinase B by G protein-coupled receptors. A role for alpha and beta gamma subunits of heterotrimeric G proteins acting through phosphatidylinositol-3-OH kinasegamma. J. Biol. Chem. **273:**19080-5.
71. **Nagata, K., A. Puls, C. Futter, P. Aspenstrom, E. Schaefer, T. Nakata, N. Hirokawa, and A. Hall.** 1998. The MAP kinase kinase kinase MLK2 co-localizes with activated JNK along microtubules and associates with kinesin superfamily motor KIF3. EMBO. J. **17:**149-58.
72. **Persidis, A.** 1998. Biochips. Nat. Biotechnol. **16:**981-3.
73. **Ponnathpur, V., A. M. Ibrado, J. C. Reed, S. Ray, Y. Huang, S. Self, G. Bullock, A. Nawabi, and K. Bhalla.** 1995. Effects of modulators of protein kinases on Taxol-induced apoptosis of human leukemic cells possessing disparate levels of p26BCL-2 protein. Clin. Cancer Res. **1:**1399-1406.

74. **Pulkkinen, J. O., L. Elomaa, H. Joensuu, P. Martikainen, K. Servomaa, and R. Grenman.** 1996. Paclitaxel-induced apoptotic changes followed by time-lapse video microscopy in cell lines established from head and neck cancer. J. Cancer Res. Clin. Oncol. **122:**214-8.

75. **Rieder, C. L., A. Schultz, R. Cole, and G. Sluder.** 1994. Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. J.Cell Biol. **127:**1301-10.

76. **Roth, W., B. Wagenknecht, C. Grimmel, J. Dichgans, and M. Weller.** 1998. Taxol-mediated augmentation of CD95 ligand-induced apoptosis of human malignant glioma cells: association with bcl-2 phosphorylation but neither activation of p53 nor G2/M cell cycle arrest. Br. J. Cancer **77:**404-11.

77. **Scatena, C. D., Z. A. Stewart, D. Mays, L. J. Tang, C. J. Keefer, S. D. Leach, and J. A. Pietenpol.** 1998. Mitotic phosphorylation of Bcl-2 during normal cell cycle progression and Taxol-induced growth arrest. J. Biol. Chem. **273:**30777-30784.

78. **Shen, S. C., T. S. Huang, S. H. Jee, and M. L. Kuo.** 1998. Taxol-induced p34cdc2 kinase activation and apoptosis inhibited by 12-O- tetradecanoylphorbol-13-acetate in human breast MCF-7 carcinoma cells. Cell Growth Differ. **9:**23-9.

79. **Sherr, C. J., and J. M. Roberts.** 1995. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev. **9:**1149-63.

80. **Shi, L., W. K. Nishioka, J. Th'ng, E. M. Bradbury, D. W. Litchfield, and A. H. Greenberg.** 1994. Premature p34cdc2 activation required for apoptosis. Science **263:**1143-5.

81. **Shim, J., H. Lee, J. Park, H. Kim, and E. J. Choi.** 1996. A non-enzymatic p21 protein inhibitor of stress-activated protein kinases. Nature **381:**804-6.

82. **Shimizu, T., O. C. PM, K. W. Kohn, and Y. Pommier.** 1995. Unscheduled activation of cyclin B1/Cdc2 kinase in human promyelocytic leukemia cell line HL60 cells undergoing apoptosis induced by DNA damage. Cancer Res. **55:**228-31.

83. **Skolnick, A. A.** 1996. Russian and US researchers develop 'biochips' for faster, inexpensive biomedical tests. JAMA **275:**581-2.

84. **Smeal, T., P. Angel, J. Meek, and M. Karin.** 1989. Different requirements for formation of Jun: Jun and Jun: Fos complexes. Genes Dev. **3:**2091-100.

85. **Solinas-Toldo, S., S. Lampel, S. Stilgenbauer, J. Nickolenko, A. Benner, H. Dohner, T. Cremer, and P. Lichter.** 1997. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer **20:**399-407.

86. **Sonnichsen, D. S., and M. V. Relling.** 1998. Chapter 17. Paclitaxel and docetaxel, p. 375-394. *In* L. B. Grochow, and M. M. Ames (ed.), A clinician's guide to chemotherapy pharmacokinetics and pharmacodynamics. Williams & Wilkins, Baltimore.

87. **Sorger, P. K., M. Dobles, R. Tournebize, and A. A. Hyman.** 1997. Coupling cell division and cell death to microtubule dynamics. Curr. Opin. Cell Biol. **9:**807-14.

88. **Srivastava, R. K., A. R. Srivastava, S. J. Korsmeyer, M. Nesterova, Y. S. Cho-Chung, and D. L. Longo.** 1998. Involvement of microtubules in the regulation of Bcl2 phosphorylation and apoptosis through cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **18:**3509-17.

89. **Suffness, M.** 1994. Is taxol a surrogate for a universal regulator of mitosis? in vivo **8:**867-878.

90. **Taylor, S. S., E. Ha, and F. McKeon.** 1998. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. J. Cell Biol. **142:**1-11.

91. **Taylor, S. S., and F. McKeon.** 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. Cell **89:**727-35.

92. **Toczyski, D. P., D. J. Galgoczy, and L. H. Hartwell.** 1997. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. Cell **90:**1097-106.

93. **Trielli, M. O., P. R. Andreassen, F. B. Lacroix, and R. L. Margolis.** 1996. Differential Taxol-dependent arrest of transformed and nontransformed cells in the G1 phase of the cell cycle, and specific-related mortality of transformed cells. J. Cell Biol. **135:**689-700.

94. **Uhr, J. W., R. H. Scheuermann, N. E. Street, and E. S. Vitetta.** 1997. Cancer dormancy: opportunities for new therapeutic approaches. Nat. Med. **3:**505-9.

95. **van Dam, H., D. Wilhelm, I. Herr, A. Steffen, P. Herrlich, and P. Angel.** 1995. ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents. EMBO. J. **14:**1798-811.

96. **van den Heuvel, S., and E. Harlow.** 1993. Distinct roles for cyclin-dependent kinases in cell cycle control. Science **262:**2050-2054.

97. **Wang, T. H., D. M. Popp, H. S. Wang, M. Saitoh, J. G. Mural, D. C. Henley, H. Ichijo, and J. Wimalasena.** 1999. Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells. J. Biol. Chem. :in press.

98. **Wang, T. H., H. S. Wang, H. Ichijo, P. Giannakakou, J. S. Foster, T. Fojo, and J. Wimalasena.** 1998. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress- activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J. Biol. Chem. **273:**4928-36.

99. **Wang, Y., and D. J. Burke.** 1997. Cdc55p, the B-type regulatory subunit of protein phosphatase 2A, has multiple functions in mitosis and is required for the kinetochore/spindle checkpoint in Saccharomyces cerevisiae. Mol. Cell. Biol. **17:**620-6.

100. **Waters, J. C., T. J. Mitchison, C. L. Rieder, and E. D. Salmon.** 1996. The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. Mol. Biol. Cell **7:**1547-58.

101. **Wilson, L., and M. A. Jordan.** 1994. Pharmacological probes of microtubule function., p. 59-83. *In* J. S. Hyams, and C. W. Lloyd (ed.), Microtubules, vol. 13. John Wiley and Sons, Inc.,, New York.

102. **Wolfson, M., C. P. Yang, and S. B. Horwitz.** 1997. Taxol induces tyrosine phosphorylation of Shc and its association with Grb2 in murine RAW 264.7 cells. Int. J. Cancer. **70:**248-52.

103. **Woods, C. M., J. Zhu, P. A. McQueney, D. Bollag, and E. Lazarides.** 1995. Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. Mol. Med. **1:**506-26.

104. **Yang, D., C. Tournier, M. Wysk, H. T. Lu, J. Xu, R. J. Davis, and R. A. Flavell.** 1997. Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. Proc. Natl. Acad. Sci. U S A **94**:3004-9.

105. **Yang, D. D., C. Y. Kuan, A. J. Whitmarsh, M. Rincon, T. S. Zheng, R. J. Davis, P. Rakic, and R. A. Flavell.** 1997. Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the JNK3 gene. Nature **389**:865-70.

106. **Yu, D., T. Jing, B. Liu, J. Yao, M. Tan, T. J. McDonnell, and M. C. Hung.** 1998. Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of p21Cip1, which inhibits p34Cdc2 kinase. Mol. Cell **2**:581-91.

107. **Zerial, M., L. Toschi, R. P. Ryseck, M. Schuermann, R. Muller, and R. Bravo.** 1989. The product of a novel growth factor activated gene, fos B, interacts with JUN proteins enhancing their DNA binding activity. EMBO. J. **8**:805-13.

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Publications

1. **Lee JN, Wang TH, Yang MY.** Litocin- a novel neurohypophyseal hormone in human placenta. *Placental and Endometrial Proteins.* (1988) pp. 43-50.
2. **Wang TH, Yang WK, Hoyt PR, Ch'ang LY, Savin TJ.** Effect of maternal age on transgene heritability in transgenic founder mice derived from zygotes microinjected with retroviral long terminal repeat-containing recombinant DNA. *Biology of Reproduction* (1993), **48:** 1104-1112.
3. **Hoyt PR, Wang TH, Henley DC, Yang DM, Ch'ang L-Y, Yang WK.** Carbon tetrachloride induces rapid changes in liver nuclear protein factors capable of sequence-specific binding to regulatory elements in the long terminal repeat of polytropic-class endogenous murine leukemia virus-related provirus. *Molecular Carcinogenesis* (1993), **8:** 245-54.
4. **Wang TH, Wang HS.** Chlamydial infection of the female genital tract: its impact on human reproduction and comparison of diagnostic methods. *Journal of Reproduction and Infertility* (1994), **3:** 1-11.
5. **Wang TH, Wang HS.** Gestational trophoblastic diseases: current trends and perspectives. *Journal of Formosan Medical Association* (1995), **94:** 449-457.
6. **Wang TH, Wang HS.** P53, apoptosis and human cancers. *Journal of Formosan Medical Association* (1996), **95:** 509-522.
7. **Saavedra HI, Wang TH, Hoyt PR, Popp D, Yang WK, Stambrook PJ.** Interleukin-3 increases the incidence of 5-azacytidine-induced thymic lymphomas in pBOR-Il-3 mice. *Cellular Immunology* (1996), **173:** 116-123.
8. **Shore VH, Wang TH, Wang CL, Torry RJ, Caudle MR, Torry DS.** of vascular endothelial growth factor, placental growth factor and their receptors in isolated human trophoblast. *Placenta* (1997) **18:** 657-665.
9. **Wang TH, Wang HS, Ichijo H, Giannakakou P, Foster JS, Fojo T, Wimalasena J.** Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. *Journal of Biological Chemistry* (1998) **273:** 4928-4936.
10. **Torry DS, Wang HS, Wang TH, Caudle MR, Torry RJ.** Preeclampsia is associated with reduced serum levels of placenta growth factor. *American Journal of Obstetrics and Gynecology* (1998): **179:** 1539-1544.
11. **Wang TH, Popp DM, Wang HS, Saitoh M, Mural JG, Henley DC, Ichijo H, Wimalasena J.** Microtubule dysfunction induced by paclitaxel initiates apoptosis through both c-Jun N-terminal kinase (JNK)-dependent and -independent pathways in ovarian cancer cells. *Journal of Biological Chemistry* (1999): in press.