Helsinki University Biomedical Dissertations No. 62

FUNCTIONS OF THE COREGULATOR PROTEIN SNURF (RNF4) IN TRANSCRIPTION AND CELL GROWTH

MARIKA HÄKLI

INSTITUTE OF BIOMEDICINE / PHYSIOLOGY
UNIVERSITY OF HELSINKI
FINLAND

ACADEMIC DISSERTATION

To be publicly discussed with the permission of
the Medical Faculty of the University of Helsinki, in the lecture hall 2,
Biomedicum Helsinki, Haartmaninkatu 8, on March 19th, 2005,
at 12 o'clock

Helsinki 2005

Supervised by

Professor Olli A. Jänne

University of Helsinki

&

Professor Jorma J. Palvimo

University of Kuopio

Reviewed by

Docent Asta Pirskanen

University of Oulu

&

Docent Ilkka Julkunen

National Public Health Institute,

Helsinki

ISBN 952-10-2368-6 (paperback)

ISBN 952-10-2369-4 (PDF)

ISNN 1457-8433

http://ethesis.helsinki.fi

Yliopistopaino

Helsinki 2005

Äidille ja Isälle

CONTENTS

ABSTRACT

Gene expression is a strictly regulated process, which involves RNA polymerase II, sequence-specific transcription factors, general transcription factors and coregulatory proteins that cooperate with each other to achieve transcription activation or repression from a given gene promoter at a given time (development, growth, and homeostasis) and place (tissue/cell specificity). Androgen receptor (AR) is a sequence-specific transcription factor that delivers the messages of male steroids (androgens) to transcription machinery, and thus is responsible for normal sexual development and maintenance of spermatogenesis. The AR-mediated gene transcription is initiated when androgen binds to the ligand-binding domain (LBD) of AR, which then leads to nuclear translocation, homodimerization and binding to the androgen response element (ARE) via the DNA-binding domain (DBD). Increasing number of coregulatory proteins that bind androgen receptor have been identified. They enhance (coactivators) or repress (corepressors) AR-mediated transcription via modifying chromatin structures by histone acetylation or deacetylation and by ATPase-mediated chromatin remodelling. They can also connect the enhancer binding proteins to basal transcription machinery or can recruit additional regulatory proteins into the transcription machinery. The activities of transcription factors and cofactors are regulated by covalent modifications such as phosphorylation, methylation, acetylation, ubiquitination and sumoylation, which contribute to protein stability and structure, subcellular localization, or protein-protein and protein-DNA interactions.

In the present study, we have characterized the functions of a novel RING finger protein, SNURF (small nuclear RING finger), that was discovered as an AR-interacting protein. SNURF enhances the transcriptional activity of both nuclear receptors and hormone-independent transcription factors, such as promoter specificity protein 1 (Sp1), and thus acts as a transcriptional coactivator. In addition to steroid-receptors, SNURF interacts with many different proteins of transcriptional control such as steroidogenic factor 1 (SF-1) and TATA-binding protein (TBP). Moreover, it exhibits binding activity towards nucleic acids and nucleosomes. SNURF binds various types of DNA molecules without sequence-specificity and the DNA-binding activity of SNURF correlates with its coactivation function in Sp1-regulated transcription. SNURF specifically regulates the expression of luteinizing hormone (LH), which is involved in the synthesis of sex steroids in ovaries and testis, from the LHβ-promoter by connecting two promoter elements, the distal and the proximal regulatory elements, through interactions with SF-1 and Sp1. SNURF possesses RING finger-dependent ubiquitin E3 ligase activity and cooperates with various ubiquitin-conjugating E2 enzymes in ubiquitin-mediated protein degradation pathway. The human counterpart of SNURF, the RNF4, has been shown to inhibit cell growth also in a RING finger-dependent fashion. SNURF is covalently modified by self-ubiquitination, sumoylation and phosphorylation, but the role of these modifications in SNURF function remains elusive. SNURF associates with promyelocytic leukemia protein 3 (PML-3) through a non-covalent interaction with small ubiquitin-like modifier 1 (SUMO-1). PML-3 is able to abolish the coactivation function of SNURF in Sp1-regulated transcription, which parallels the ability of PML-3 to recruit nucleoplasmic SNURF to PML nuclear bodies. Taken together, these results indicate that SNURF is a multifunctional transcriptional coregulator and suggest an important role for SNURF in cell growth control.

ORIGINAL PUBLICATIONS

The thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Moilanen A-M, Poukka H, Karvonen U, Häkli M, Jänne OA, and Palvimo JJ (1998) Identification of a novel RING finger proteins as a coregulator in steroid receptor-mediated gene transcription. Mol Cell Biol 18: 5128-5139

II Häkli M, Karvonen U, Jänne OA, and Palvimo JJ (2001) The RING finger protein SNURF is a bifunctional protein possessing DNA binding activity. J Biol Chem 276: 23653-23660

III Curtin D, Ferris H, Häkli M, Gibson M, Jänne OA, Palvimo JJ, and Margaret A. Shupnik (2004) Small nuclear RING finger protein (SNURF) stimulates the rat luteinizing hormone-β promoter by interacting with Sp1 and steroidogenic factor-1 and protects from androgen suppression. Mol Endocrinol 18: 1263-1276

IV Häkli M, Lorick K, Weissman AM, Jänne OA, and Palvimo JJ (2004) Transcriptional coregulator SNURF (RNF4) possesses ubiquitin E3 ligase activity. FEBS Lett 560: 56-62

V Häkli M, Karvonen U, Jänne OA, and Palvimo JJ (2005) SUMO-1 promotes association of SNURF (RNF4) with PML nuclear bodies. Exp Cell Res 304: 224-233

In addition, some unpublished data are presented.

Original publication I was also included in the thesis “Novel coregulators of androgen receptor action” by Anu-Maarit Moilanen, and “SNURF and Ubc9 as coregulators of androgen receptor function” by Hetti Poukka.

ABBREVIATIONS

AF	activation function
AR	androgen receptor
ARE	androgen response element
ATP	adenosine triphosphate
bp	base pair
CBP	CREB-binding protein
cDNA	complementary deoxyribonucleic acid
COS-1	simian virus 40-transformed monkey kidney cell line
CTD	C-terminal domain of the largest subunit of RNA polymerase II
CV-1	monkey kidney cell line
DBD	DNA-binding domain
DHT	5α-dihydrotestosterone
DRIP	vitamin D receptor-interacting protein
EMSA	electrophoretic mobility shift assay
ER	estrogen receptor
F9	murine embryonal carcinoma cell line
GFP	green fluorescent protein
GnRH	gonadotropin hormone-releasing hormone
GR	glucocorticoid receptor
GRIP1	glucocorticoid receptor-interacting protein 1
GST	glutathione S-transferase
GTF	general transcription factor
HAT	histone acetyltransferase
HDAC	histone deacetylase
HeLa	human cervix carcinoma cell line
HMG	high mobility group protein
Hsp	heat shock protein
kDa	kilodalton
LβT2	secretory gonadotrope cell line
LBD	ligand-binding domain
LH	luteinizing hormone
LUC	luciferase
MR	mineralocorticoid receptor
mRNA	messenger ribonucleic acid
NLS	nuclear localization signal
NR	nuclear receptor
PCAF	p300/CBP-associated factor
PIC	preinitiation complex
PML	promyelocytic leukemia gene product
PML NB	PML nuclear body
Pol II	RNA polymerase II
PPAR	peroxisome proliferator-activated receptor
PR	progesterone receptor
RAR	retinoid acid receptor
RNF4	RING finger 4 protein
RXR	retiboid X receptor
SF-1	steroidogenic factor 1
Sf9	Spodoptera frugiperda insect cell line

SNURF	small nuclear RING finger protein
Sp1	promoter specificity protein 1
SRC	steroid receptor coactivator
SUMO	small ubiquitin-like modifier
SWI/SNF	Switch/sucrose non-fermenting
TAF	TBP-associated factor
TBP	TATA-binding protein
TR	thyroid hormone receptor
TRAP	TR-associated protein
Ub	ubiquitin
UBA	ubiquitin-associated domain
Ubc	ubiquitin-conjugating enzyme
UIM	ubiquitin-interacting motif
VDR	vitamin D_3 receptor

REVIEW OF THE LITERATURE

1. TRANSCRIPTIONAL REGULATION

1.1 Regulatory elements of the gene

Eukaryotes contain three RNA polymerases (RNA polymerases I, II, and III) of which RNA polymerase II is responsible for the transcription of protein-coding genes (mRNA genes). Gene expression is regulated by promoter regions, which are typically located upstream of transcription start site (+1). The core promoter region, which is generally situated within ~ -35 to + 35 of the start site, is recognized by the basal RNA polymerase II (Pol II) transcriptional machinery. The core promote is the minimal DNA region required for the transcription initiation complex assembly and initiation of RNA transcription *in vitro*. The core promoter may contain DNA elements such as TATA-box, TFIIB recognition element (BRE), the initiator element (Inr), and the downstream core promoter (DPE). Each of these DNA elements is found in only a subset of core promoters; certain promoters may contain all, none or only some of these elements. The TATA-box is found approximately in one third of human core promoters (Suzuki et al. 2001), located around −25 to −30 of the start site and is recognized by TATA-binding protein (TBP). The Inr element contains transcription start site(s), associates with TBP-associated proteins (TAFIIs) of the TFIID complex and RNA polymerase II, and can function without TATA-box element. BRE element, which is recognized by the TFIIB, is located upstream of the TATA-box (Lagrange et al. 1998, Evans et al. 2001). The DPE element mostly exist in TATA-less promoters and is located downstream of transcription start site (at + 28 to +32). In the DPE-dependent basal transcription, the Inr element is required for TFIID interaction (Burke and Kadonaga 1997, Kutach and Kadonaga 2000). These different combinations of core promoter elements contribute to the differential regulation of gene expression. Proximal promoters are 6-20-nt long sequences (such as CCAAT box, Sp1 box) located in the near vicinity of core promoter region (at -100 to -200), and typically contribute to the efficiency of transcription initiation. The distal transcription regulatory regions (enhancers and silencers) are located several kilobases (up- or downstream) from the transcription start site.

The general transcription factors (GTFs), such as Pol II, TBP, and TFIIB, bind to the core promoter region. Sequence-specific transcription factors usually recognise proximal and distal regulatory regions and stimulate or repress the recruitment of the GTFs and Pol II to the promoter.

The third major class of transcription regulators is the group of coregulators, which function is to connect sequence-specific transcription factors to the GTFs or to modify chromatin structure.

1.2 General transcription machinery

The basal transcription by RNA polymerase II is a multi-step process, where preinitiation-stage is followed by initiation, elongation, termination, and mRNA processing. The preinitiation complex (PIC) is composed of Pol II and GTFs (TFIID, -B, -E, -F, -H, and -A), and it is assembled on the core promoter (reviewed by Orphanides et al. 1996). The PICs can be composed of different set of factors at distinct promoters (reviewed Müller and Tora 2003). TBP-type factors (TLFs) may play complementing roles in transcriptional regulation from the TATA-less promoters (reviewed by Dantonel et al. 1999 and Ohbayashi et al. 2003). Also TAFIIs, subunits of the TFIID complex, can bind the Inr and BRE element. Also TBP-free TAFII-complexes, such as yeast SAGA, may functionally replace the TFIID from certain promoters.

At the TATA-box containing promoters, TBP binds the core promoter and forms a binding surface for other components of the transcription machinery by bending DNA, while some of TAFIIs interact with Inr and DPE-element (Oelgeschläger et al. 1996, Burke et al. 1997, Chalkley et al. 1999). The BRE element facilitates TFIIB binding to the TBP-DNA complex, and TFIIB and TFIIA stabilize the TBP-DNA complex on the core promoter. Then TFIIB recruits TFIIF together with Pol II to the promoter and transcription is initiated when TFIIE and TFIIH incorporate into the PIC. TFIIH possesses helicase activity and catalyzes ATP-dependent melting of the promoter at transcription start site (open complex formation) and is required for the promoter clearance during transcription. The carboxy-terminal domain of the largest subunit of Pol II (CTD) is phosphorylated by TFIIH-associated kinases during transcription initiation (Liu Y et al. 2004). The Pol II, TFIIB TFIIH and TFIIF dissociate from the promoter and leave the remaining PIC complex (TFIID-TFIIA) at the promoter, or alternatively, the mediator coregulatory complex may remain associated with the core promoter together with TFIIA, -D, -E, and -H for waiting for the assembly of the second transcription complex (reinitiation) (Yudkovsky et al. 2000). The entry of RNA polymerase II into progressive elongation stage is followed by dephosphorylation on Ser5, and phosphorylation of Ser2 within the CTD (Komarnitsky et al. 2000). mRNA synthesis by Pol II is boosted by TFIIF and elongation factors such as the Elongings and ELL (Eissenberg et al. 2002, Garret et al. 1994). The mRNA processing events (5´-end capping, intron splicing and 3´-end maturation) occur while the nascent mRNA is being synthesized by Pol II. mRNA processing factors associate with the CTD of elongating Pol II and perform their action at a certain time point. The transcription termination, where the transcription

complex dissociates, generally occurs 4 kbp beyond of the poly(A) signal. Gene transcription is repetitive and thus is able to synthesize multiple copies of identical mRNA molecule from the same template by contacting the remaining PIC complex at transcription start site (transcription reinitiation). Reinitiation may be down-regulated by CTD dephosphorylation by specific phosphatases, or Pol II transcription components can be ubiquitinated and degraded by the proteasome (Reviewed by Lin et al. 2002 and Tansey 2001, Mitsui and Sharp 1999). Recent study suggests that long yeast genes are able to form loops, where the promoter and termination regions are brought together in the early stages of transcriptional activation, and thus explain the use of the same factors such as TFIIB and TFIID in transcription and termination processes (O´Sullivan et al. 2004).

1.3 Sequence-specific transcription factors

In eukaryotes, there are thousands of protein-coding genes, whose transcription by Pol II is predominantly mediated by a network of numerous sequence-specific DNA-binding transcription factors. These factors bind to the proximal promoter and distal transcriptional regulatory regions, enhancers or silencers, located upstream of the promoter and induce or repress gene expression via association with Pol II, GTFs, and cofactors (Reviewed by Tjian and Maniatis 1994). A typical sequence-specific transcription factor has a DNA-binding domain (DBD) that mediates the binding of the protein to specific DNA sequences. There are many different DBDs, such as basic domains helix-loop-helix (bHLH) and leucine zipper (bZip) found in myogenic transcription factor D (MyoD) and activator protein-1 (AP-1), respectively (Ma et al. 1994, Glover and Harrison 1995). The zinc finger (Cys_2His_2) is found in promoter specificity protein-1 (Sp1) and nuclear receptors (Kadonaga et al. 1998, Schwabe et al. 1991). The helix-loop-helix domain (homeo- and ETS-domains) is found in Hox8, and a domain with the β-scaffold is found in the nuclear factor kappa B (NF-κB) and p53 (reviewed by Grimm and Baeuerle 1993, Cho et al. 1994). Also, the regions outside the DBD and protein-protein interactions may act to increase the specificity of DNA-binding (reviewed by Marmorstein and Fitzgerald 2003, Janknecht et al. 1994). The bHLH-, the bZIP- and zinc finger domains allow their dimerization when binding to DNA. The DNA-binding module can be joined to other functional modules, such as activation domain (AD) or repression domain (ID), di- and multimerization domain, regulatory domain and nuclear localization signal (NLS). Transcription factors are translocated to nucleus via NLS or nuclear translocation can be delivered along with other proteins. The transactivation and repression domains are highly variable regions and they act as a platform for other transcriptional regulators, such as coactivators and corepressors. For instance, Sp1 contains two glutamine-rich transactivation domains, which bind other transcription proteins such as

TAFII130 (Courey and Tjian 1988, Gill et al. 1994). Some transcription factors are controlled via the regulatory region, such as nuclear receptors via ligand-binding domain (LBD).

1.3.1 General characteristics of the nuclear receptor family

Nuclear receptors (NRs) comprise the largest family of transcription factors and are involved in the regulation of a wide variety of cellular processes from development to homeostasis. They are a family of ligand-inducible transcription factors that regulate gene expression in response to small lipophilic molecules such as steroid hormones, T3/T4 thyroid hormone, vitamin D, retinoids and eicosanoids. The human genome contains 48 genes encoding for NRs, and the total amount of different NRs is even higher due to alternative splicing and/or alternative promoter usage (Robinson-Rechavi et al. 2001, Kastner et al. 1990). All members of the NR family share a modular structure consisting of distinct domains (Fig. 1).

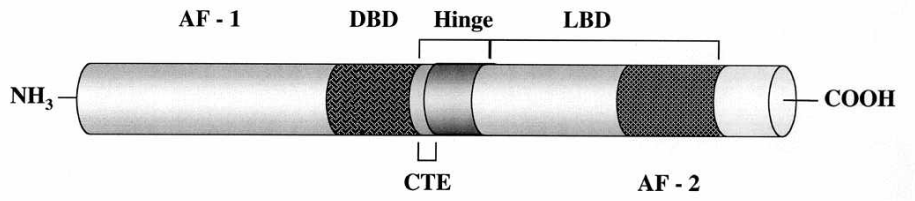


Fig. 1. The structural and functional domains of a nuclear receptor. AF-1, the activation function 1; DBD, the DNA binding domain; CTE, the carboxyl-terminal extension; The hinge region; LBD, the ligand-binding domain; AF-2, the activation function 2.

1.3.1.1 DNA-binding domain

The DNA-binding domain assembles with the NRs in a defined configuration into the response-element (RE) of the ligand-responsive gene (reviewed by Glass 1994). The DBD consisting of 66-68 amino acids is the most conserved region among NRs. Three dimensional structures of many DBDs have been determined in a complex with the DNA response element and have revealed that DBDs are structurally conserved as well. The DBD contains two zinc-binding modules, where four conserved cysteine residues (Cys_4) coordinate one zinc ion in each binding module (Freedman et al. 1988, reviewed by Khorasanizadeh and Rastinejad 2001). The DBD involves two α-helices; the α-helix1 resides after the first zinc finger, and it is responsible for specific binding of the factor to the major groove of the half-site (six to eight nucleotides) within the RE; the α-helix2 is located after the second

zinc finger, and it is not in contact with DNA, but is important for the overall folding of the DBD (Schwabe et al. 1993, Alroy and Freedman 1992, Luisi et al. 1991, reviewed by Freedman 1992). Many NRs bind DNA as homo- or heterodimers, where the N-terminal residues of the second zinc finger in both receptors mediate dimerization (Forman et al. 1992, Kurokawa et al. 1993, Dahlman-Wright et al. 1991, reviewed by Glass 1994). Receptor dimerization is dependent on the DNA, and the response element must contain two half-sites in a specific orientation (inverted or direct repeat, non-repeat) and spacing (1-5 nucleotides) (Khorasaizadeh et al. 2001). Some NRs such as thyroid hormone receptor (T3R, Quack et al. 2001), constitutive androstane receptor (CAR, Frank et al. 2003) and SF-1 (Wilson et al. 1993), can bind RE as a monomer, where the carboxyl-terminal extension (CTE) of the DBD associates with the 5´ flanking sequence of the DNA half-site (Harding and Lazar 1995, Meinke and Sigler 1999). The CTE is a part of the hinge region and a variable region among NRs. The CTD of TR and VDR forms a third α-helix, which is needed for higher affinity DNA binding and correct spacing with RXR heterodimer by making extensive contacts along the phosphate backbone of DNA (Rastinejad et al. 1995, Shaffer and Gewirth 2002). In addition to DNA-binding and dimerization, DBD binds various regulatory proteins such as non-histone proteins HMGB1- and -2, which facilitate NR binding to DNA and the transcription activity of NR (Melvin et al. 2002). The border region between the DBD and hinge region in steroid receptors typically contains a NLS for nuclear transport (Poukka et al. 2000).

1.3.1.2 Ligand-binding domain

Numerous three-dimensional structures of the ligand-binding domains (LBDs) bound to their appropriate ligands have been solved (reviewed by Renaud and Moras 2000). The C-terminal LBD is positioned as three-layer sandwich to form a hydrophobic pocket for the ligand (Wurtz et al. 1996, Bourguet et al. 2000_a). Helix 12 sticks out from the LBD core in the absence of cognate ligand, but it is rearranged in response to ligand binding by folding against the core of the LBD and creates a lid over the ligand-binding pocket (Bourguet et al. 1995, Renaud et al. 1995, Kallenberger et al. 2003). Helix 12 contains the activation function 2, the AF-2 domain, which is important for the binding of coregulators (Danielian et al. 1992). In the agonist-induced conformation, the LBD displaces co-repressors and reveals a binding surface for coactivators with a leucine-rich consensus sequence, the LXXLL-motif (L=leucine, X= any amino acid, also called NR box) (Chen H et al. 1997, Ogryzko et al. 1996). In the antagonist-bound ER, helix 12 is displaced from the position required for coactivator binding and blocks the binding regions of coactivators in helix 3 and 4 (Brzozowski et al. 1997, Shiau et al. 1998). Some antagonists, so-called inverse-agonists, are able to facilitate corepressor binding via a conformational change in LBD (Xu et al. 2002). In the absence of a ligand, corepressors bind to

LBD and partially stabilize the ligand-free conformation (Pissios et al. 2000, Pratt and Toft 1997). Some NRs, such as liver receptor homolog 1 (LHR-1) and estrogen-related receptor 3 (ERR3), are in an active conformation with an empty ligand pocket (Sablin et al. 2003, Greschik et al. 2002) or the ligand-binding pocket can be occupied with the side chains of the NR (Kallen et al. 2004). Interestingly, nur-related factor 1 (Nurr1) lacks the ligand-binding pocket and cofactor binding sites, but it is continuously active because of its fold that mimics the agonist-bound LBD (Wang Z et al. 2003). NRs, whose physiological ligands are not yet known, are called orphan receptors, such as steroidogenic factor 1 (SF-1) (Wilson et al. 1993), Nurr1 and testis receptors (TR2 and TR4). In addition to binding to ligands and coregulators, LBD is able to bind heat shock proteins and form dimers in the absence of DNA (Lee et al. 1996, Glass 1994, Bourguet et al. 2000_b, Depoix et al. 2001, reviewed by Pratt and Toft 1997).

1.3.1.3 Activation function 1

The activation function 1 (AF-1) region is located in the N-terminal region of NR, which is the most variable region among NRs. Activities of different AF-1 domains vary considerably. The activity of AF-1 is not dependent on the ligand and has been defined to be continuously active (Tora et al. 1989). AF-1 is important in mediating cell type- and promoter-specific responses (Meyer et al. 1992, McInerney and Katzenellenbogen 1996, Ikonen et al. 1997). AF-1 can function independently, but it communicates with AF-2 to gain the full response in transcription (Kraus et al. 1995, Ikonen et al. 1997, Tetel et al. 1999, Ali et al. 1993). *In vitro* binding studies with purified AF-1 and AF-2 fragments of progesterone receptor (PR) showed that these two domains bind each other directly (Tetel et al. 1999). Under physiological conditions, ligand binding to PR induces a conformation change and thus facilitates the interaction between AF-1 and AF-2, and coactivators have been shown to stabilize this structure (Tetel et al. 1999). In addition to coactivator binding, AF-1 is able to associate with GTFs (Hentschke and Borgmeyer 2003, Deblois and Giguere 2003, reviewed by Beato and Sanchez-Pacheco 1996).

NRs have been divided into six subfamilies on the basis of the evolution of the conserved DNA- and ligand-binding domains, and differences in the mechanism of action (DNA binding and dimerization) (Laudet 1997, NRNC, nuclear receptor nomenclature committee 1999, and an update of the nomenclature is available in a web site, http://www.ens-lyon.fr/LBMC/laudet/nomenc.html). The subfamily I is the largest subfamily containing thyroid receptor (TR), peroxisome proliferator activated receptors (PPAR), vitamin D receptor (VDR), and *all-trans* retinoic acid receptors (RAR). The subfamily II contains *9-cis* retinoid acid receptor (RXR), and testis receptors (TR2 and TR4).

Steroid receptors, including androgen receptor, estrogen receptors (ERα and -β), progesterone receptor (PR), mineralocorticoid receptor (MR), glucocorticoid receptor (GR), and estrogen receptor-regulated receptors (ERRs), which bind DNA as a homodimer upon ligand binding belong to the group of the subfamily III. The subfamily IV includes the nur-related factor 1 (Nurr1), and the subfamily V contains steroidogenic factor 1 (SF-1) that binds DNA as a monomer. The subfamily VI contains RTR that forms homodimers. Finally, there is also an additional subfamily called subfamily 0 that contains receptors that lack one of the conserved domains (DBD or LBD), such as DAX1. Members of subfamilies I and IV are able to form heterodimers with RXR while binding to DNA.

1.3.2 Androgen receptor (AR)

Androgen receptor (AR) plays a key role in the proper development and function of male reproductive organs in response to the androgens (testosterone and 5α-dihydrotestossterone, DHT). AR is expressed in a variety of genital tissues (testis, prostate, and ovaries) and also in non-genital tissues, such as the brain (reviewed by Quigley et al. 1995). The human AR gene is located on the long arm on X chromosome (q11-12) and encodes a protein with 919 amino acid residues containing N-terminal AF-1 domain (142-485 aa), DBD (529-618 aa), LBD (662-919 aa) and the hinge region with a bipartite nuclear localization signal (608-625 aa). The AR gene is autoregulated by androgens, and up- and down-regulation have been reported in different cell lines. Transcription factors that upregulate AR are promoter specificity protein 1 (Sp1), cAMP-response element binding protein (CREB) and c-myc, while NF-κB and NF-1 have been shown to down-regulate the AR gene (Chen S et al. 1997, Mizokami et al. 1994, Grad et al. 1999, Supakar et al. 1995, Song et al. 1999). Examples of androgen-induced genes are prostate-specific antigen (PSA), probasin, and antiapoptotic factor p21, while androgen-repressed genes are the tumor suppressing genes serpin and maspin (Nelson et al. 2002, Eder et al. 2003, Jiang and Wang 2003, Umar 2003a and 2003b).

The N-terminal region of AR contains a number of amino acid repeat sequences, including poly-glutamine (Q), poly-glycine (G) and poly-proline (P) repeats. The N-terminus is structurally flexible and adopts a more stable helical conformation upon specific protein-protein interactions with e.g. TFIIF or p160, and consequently it is more potent for additional protein binding (reviewed Reid et al. 2002_a, 2002_b, and 2003). The N-terminal region of AR is highly active compared to other NRs. The primary transcription activation domain of AR is located in the N-terminal region, whereas the LBD harbors a comparatively weak activation function (MacLean et al. 1997, Simental et al. 1991). Interaction between the N- and C-terminal LBD regions, where helix 12 of LBD and the FXXLF sequence of N-terminus forms the intramolecular interaction, is important for AR-dependent gene

activation (Steketee et al. 2002, Ikonen et al. 1997, reviewed by He and Wilson 2002). AR binds DNA as a homodimer, recognizes the sequence of DNA often as a direct repeat sequence and adopts the "head-to-head" binding configuration.

Since AR is located on X chromosome and therefore present only as a single copy in males, mutations within this gene will result in a direct phenotypic manifestation. The shorter length of the N-terminal poly-Q stretch has an increased risk of prostate cancer and patterned baldness, while shorter poly-Q repeats correlates with infertility (Correa-Cerro et al. 1999, Edwards et al. 1999, Ellis et al. 2001, reviewed by McEwan 2001b). Expansion of the poly-Q repeat to more than 40 residues results into misfolding and aggregation of AR and causes a spial bulbar muscular atrophy (Kennedy´s disease), a neurodegenerative condition associated with selective neuronal cell death in brainstem and spinal cord (reviewed by McEwan 2001_b). Natural AR mutations that cause partial or complete androgen insensitivity syndrome (AIS) have proven the significance of AR in normal male sex differentiation. In complete AIS, males are phenotypically females with female external genitalia. In partial AIS, the male phenotype varies from near-normal male to near-normal female (reviewed by Quigley et al. 1995 and Avila et al. 2001).

1.3.3 Androgen action

Testis is responsible for the production of sperm and the synthesis of testosterone in the adult male, but also the adrenal glands produce other less potent androgens, such as androstenedione (Konety et al. 2001). Both testicular functions are regulated by the central nervous system (CNS) via follicle-stimulating hormone (FSH) and luteinizing hormone (LH). LH regulates testosterone (T) synthesis in Leydig cells, and FSH controls the spermatogenesis in Sertoli cells. Mutations in LH leads to the absence of Leydig cells, azoospermic and lack of spontaneous puberty (Weiss et al. 1992). Secretion of LH and FSH from the anterior pituitary is regulated by gonadotropin hormone-releasing hormone (GnRH), which is synthesized in hypothalamus and secreted as pulses. GnRH recognizes and binds to its receptors on pituitary gonatropes, which then leads to the release of LH and FSH (Clarke and Cummins 1982). Testicular hormones (testosterone, estradiol (E2) and inhibin) decrease FSH and LH secretion by decreasing the sensitivity of the pituitary to GnRH stimulation and GnRH production (Matsumoto and Bremner 1984, Sheckter et al. 1989). In testis, LH stimulates directly the synthesis of a steroidogenic acute regulatory (StAR) protein, which has an essential role in the transfer of cholesterol from the outer to the inner mitochondrial membrane where cholesterol is converted to pregnenolone. Thereafter, steroid hormone biosynthesis takes place in the smooth endoplasmic reticulum. Testosterone, the most abundant androgen, is released from Leydic cells into circulation and diffuses into various cells. As a lipophilic ligand, testosterone enters the cytoplasm by diffusing

through the plasma membrane (Fig. 2). In certain types of cells, testosterone is converted by the 5α-reductase to 5α-dihydrotestosterone (DHT), which is the more active androgen. Testosterone regulates the feedback of gonadotrophin synthesis and secretion, spermatogenesis and sexual differentation of wolffian ducts, whereas DHT regulates the differentation and development of the prostate, the external genetalia and several secondary male characteristics during puberty.

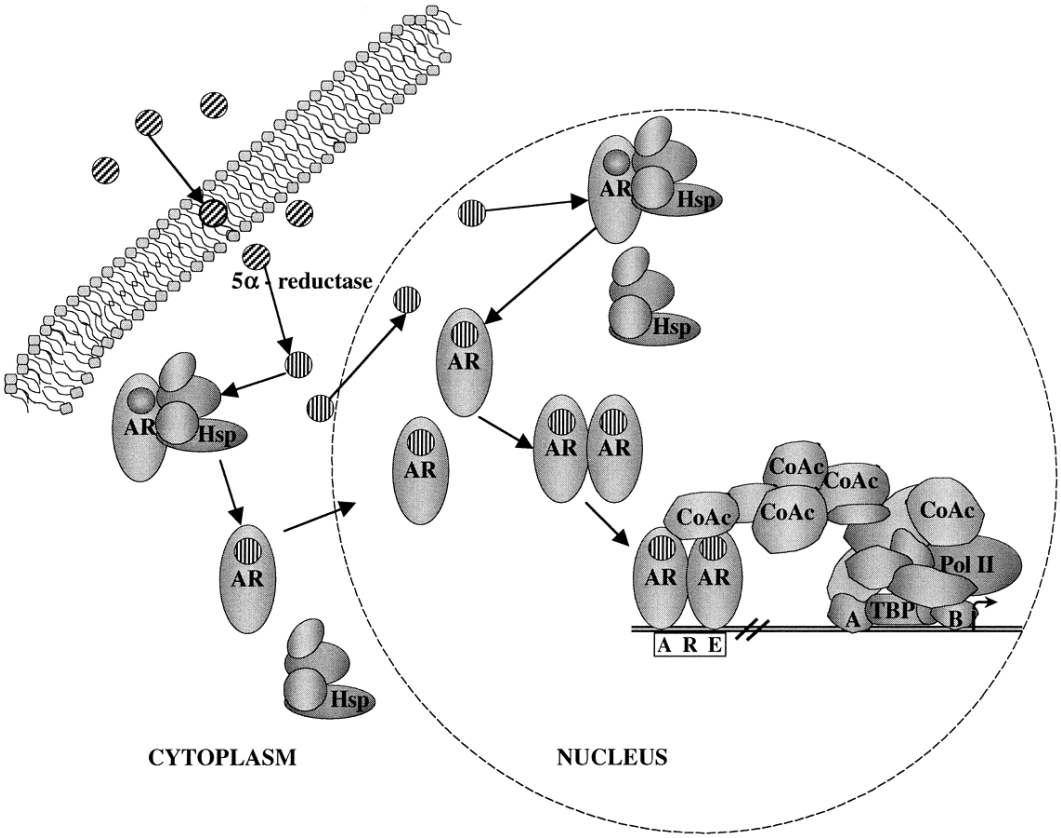


Fig. 2. Simplified model of the androgen action in cell. The lipid hormone, testosterone, enters to cytoplasm through plasma membrane by diffusion. Testosterone can be converted to the more active 5α-dihydrotestosterone (DHT) by the 5α-reductase. When androgen binds to AR, AR-bound inhibitory proteins (hsp, heat scock protein) dissoate and AR is transported into the nucleus. Alternatively, AR may bind hormone within the nucleus. Further, AR dimerizes, and binds to appropriate response elements (ARE) and regulates gene transcription. Coregulator proteins (CoAc) contact the AR dimer and PIC complex (Pol II, TBP, TFIIA and B components are indicated) and modulate hormone-dependent transcription.

The newly synthesized AR is bound by a number of molecular chaperones such as the heat shock protein (Hsp) -90, -70, -54, -56, p23 and by certain immunophilins (Davies et al. 2002, Pratt and Toft 1997) and stays in an inactive form (Fig. 2). These molecular chaperones appear to maintain the receptor in a conformation capable of binding its ligand (reviewed by Pratt and Toft 1997 and McEwan et al. 2001_a). Upon testosterone or DHT binding to LBD, AR undergoes a series of conformational changes, which leads to the release of Hsps and results in the translocation of activated

AR into the nucleus. It has also shown that these molecular chaperons may accompany the receptor into the nucleus (Kaul et al. 2002). In the nucleus, AR homodimerizes and DBD recognizes the androgen response elements (AREs) of the androgen-responsive genes.

The PSA gene is the most well-studied AR target gene. In addition to Sp1 and AP-1 binding sites, the regulatory regions of PSA contains at least three AREs, two of which locate in the proximal promoter region and the third one in the enhancer region (Cleutjens et al. 1997). Chromatin immunoprecipitation (ChIP) with antibodies against acetylated histones showed that upon androgen stimulation, the regions around the three AREs contain acetylated histones, indicating the rearrangement of chromatin structure by ligand-bound AR (Shang et al. 2002). In the same study, coactivators such as CBP and GRIP1 were also shown to be recruited to the AREs upon ligand-binding (Shang et al. 2002), but in the case of antagonist (bicalutamide) binding to AR, the proximal promoter AREs were associated by NCoR and SMRT, which indicates that antagonist-bound AR was recruiting histone deacetylase activity (Shang et al. 2002). AR may communicate with the general transcription machinery by direct protein-protein interactions or indirectly through coregulators (reviewed by Heinlein and Chang 2002). AR may also regulate transcription by enhancing the assembly of the PIC complex, clearance of the promoter region and elongation. (McEwan and Gustafsson 1997, Lee et al. 2000, Lee et al. 2001, Kang et al. 2002).

1.4 Transcriptional coregulators

Coregulators are proteins that can interact with sequence-specific transcription factors and play an important role in mediating or facilitating the effects of these factors to the basal transcription machinery either via direct interactions with components of the basal transcription machinery or through modification of chromatin structure (Fig. 3). These factors can either enhance (coactivators) or repress (corepressors) gene transcription (reviewed by McKenna and O´Malley 2002, reviewed by Näär et al. 2001, Robyr et al. 2000). Coregulators can be divided into four groups. The first group consists of histone covalent modifiers, histone acetyltransferases (HATs), histone methyltransferases (HMTs), and histone deacetylases (HDACs), that can add acetyl- and methyl-groups or remove acetyl-groups from chromatin, respectively, and thus regulate the chromatin access for other transcription regulators (reviewed by Schreiber and Bernstein 2002 and Cheung 2000 and Jenuwein and Allis 2001). The second group contains ATP-dependent chromatin-modelling complexes, such as the switch/sucrose non-fermentable (SWI/SNF) family of proteins, which disrupt the condensed structure of chromatin and increase the accessibility of transcription regulators in a non-covalent

manner. The third group includes mediators, such as TRAP/DRIP, which act as bridging factors between general transcription machinery and transcription factors through protein-protein interactions.

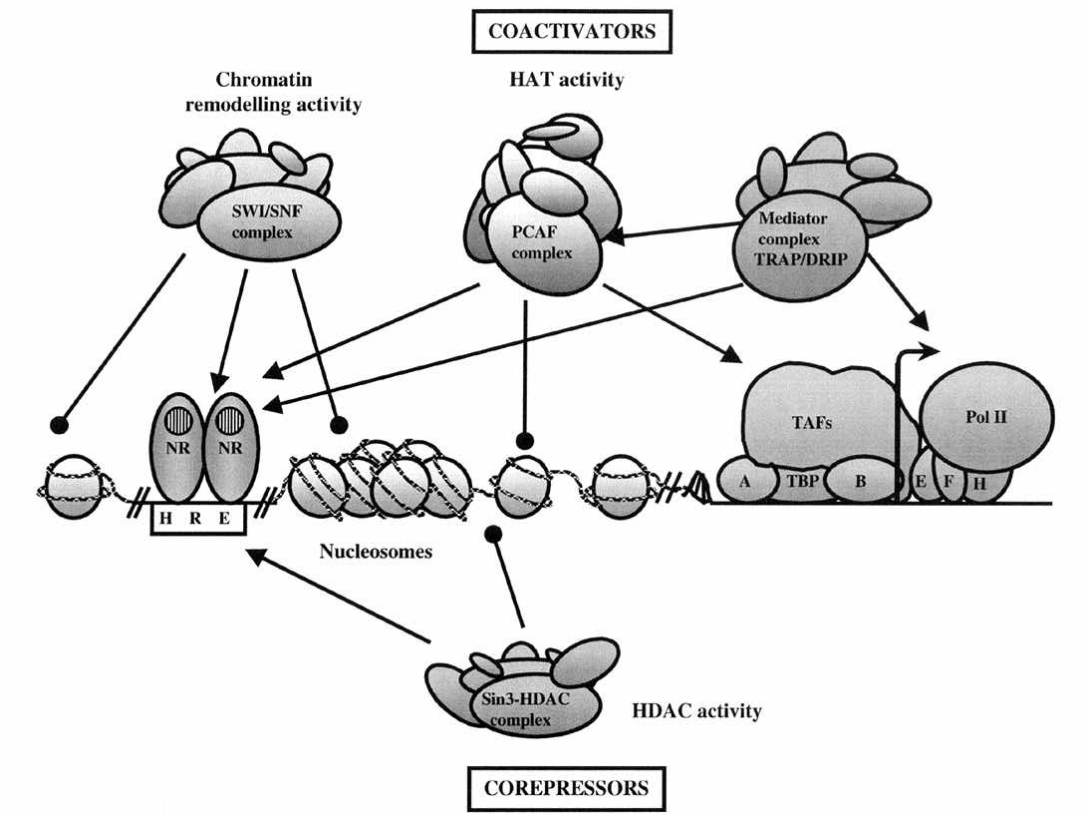


Fig. 3. The action of transcriptional coregulators. Coactivators; the chromatin remodelling complex (e.g. SWI/SNF complex) modifies the chromatin structure; the histone acetylase complex (HATs, e.g. PCAF complex) modifies the chromatin structure via covalent histone acetylation; the mediator complex (e.g. TRAP and TRIP complexes) connects sequence-specific transcription factors to basal transcription machinery via protein-protein-interactions. Corepressors; the histone deacetylase complex (HDACs, e.g. Sin3-HDAC) modifies chromatin structure by removing acetyl-groups of histone tails. NR; nuclear receptor, TBP; TATA-binding protein, TAFs; TBP-associating proteins, A-, B-, E-, F-, H-; TFIIA, TFIIB, TFIIE, TFIIF, TFIIH, respectively, Pol II; RNA polymerase II. ⟶ ; protein-protein-interactions; ⟶● ; chromatin modifying activity.

And the fourth group is characterised by coregulators of diverse or unknown function (reviewed by Näär et al. 2001, Roeder 1998, McKenna et al. 1999). The breast cancer susceptibility gene 1 (BRCA1) is one member of the fourth group. BRCA1 possesses ubiquitin ligase activity in combination with BARD (BRCA1-associated RING domain protein 1), it interacts with various transcription proteins, and acts as a coactivator for p53 and AR, and corepressor for unliganded NRs (Brzovic et al. 2001, Hashizume et al. 2001, Scully et al. 1997, Bochar et al. 2000, Yarden and Brody 1999, Zhang et al. 1998, Park et al. 2000). The ubiquitin-conjugating enzyme UbcH7 is a coactivator for steroid receptors (Verma et al. 2004). PIAS (protein inhibitor of activated signal transducer and

activator of transcription) proteins are SUMO ligases that have been shown to modulate the activities of NRs (Kotaja et al. 2002, Tan et al. 2002). The non-histone proteins HMG-1 and -2 are known to enhance the transcription of steroid receptors and p53 via increasing DNA-binding activity of receptors (Boonyaratanakornkit et al. 1998, Melvin and Edwards 1999). Nuclear receptor corepressors N-CoR (NR corepressor) and SMRT (silencing mediator for RAR and TR) bind unliganded NRs and recruit histone deacetylases for silencing gene expression (Chen and Evans 1995, Hörlein et al. 1995, Li et al. 2000, Wen et al. 2000).

It has now become clear that the transcriptional coactivation or corepression involves a dynamic interplay of multiple distinct coregulator complexes, and rapid promoter association and dissociation of various coregulators occurs temporally and in a cyclic manner (Burakov et al. 2002, Shang et al. 2000, Acevedo et al. 2003, Liu XF et al. 2004). In addition, it seems that there is no specific order for the function of coregulators; rather, each promoter possesses its own characteristic order in recruiting and displacing transcription factors and coregulators.

1.4.1 Covalent modifiers

Histone covalent modifiers alter the chromatin structure by modifying the N-terminal tails of core histones. Acetylation of lysine residues by histone acetyltransferases is thought to neutralize the basic charge of histone tails and thus decrease their affinity towards negatively charged DNA and loosen the chromatin structure. Furthermore, histone acetylation attracts other transcription coregulators such as the chromatin modelling complexes (Lee et al. 1993, Anderson et al. 2001, Sewack et al. 2001). Histone deacetylases catalyze removal of acetyl groups from histones resulting in gene silencing. Methylation of histones by histone methyltransferases is associated with gene silencing and activation depending on the specific lysine residue and the level of the modification (mono, di or tri).

1.4.1.1 Histone acetyltransferases, HATs

Four families of histone acetyltransferases have been identified in the nucleus; PCAF/GCN5, MYST, p300/CBP and the p160 protein family. They all contain an acetyl-CoA binding site and they have been found as a part of large complexes, such as SAGA, ADA and NuA4. Each of these complexes contains a specific composition of subunits, and thus has distinct histone substrates and target complexes to distinct gene promoters via interaction with different transcription factors (reviewed by

Roth 2001). CREB-binding protein (CBP) and p300 share several conserved functional domains and are rather general and diversified transcriptional coactivators (Arany et al. 1994, and reviewed by Janknecht and Hunter 1996). Ito T et al. (2000) showed that acetylation of histones by p300/CBP favors H2A/H2B dimers to escape from nucleosomes and enhance transcription. Many nuclear receptors and other transcription factors, such as p53 and general transcription factors TBP, TFIIB and TFIIF, interact with p300/CBP, indicating that p300/CBP also acts as a connector between sequence-specific and general transcription factors (reviewed by Chan 2001). p300/CBP has also been shown to form multicomponent coactivator complexes with other HATs, like pCAF, SRC-1 and SCR-3 (Ogryzko et al. 1996, Yao et al. 1996, Chen H et al. 1997). Steroid receptor coactivator 1, SRC-1 (NcoA-1), a member of the p160 family, is a common coactivator for nuclear receptors and harbors the histone acetyltransferase activity in its C-terminal region. The central region of SRC-1 contains a nuclear receptor-interacting region within three LXXLL-motifs (NR-box) (Ornate et al. 1995). SRC-1 knock out mice revealed that SRC-1 is needed for efficient steroid hormone action especially for estrogen and progesterone action in the uterus and mammary gland and for androgen action in the prostate and testis (Xu et al. 1998). Later on, it was reported that SRC-1 and AR expression levels were elevated in recurrent prostate cancer (Gregory et al. 2001). SRC-2 (TIF2/GRIP1/NcoA-2) and SRC-3 are coactivators for NRs, but they are able to coactivate other transcription factors. Finally, several HATs like p300/CBP, TIP60 and PCAF can acetylate non-histone proteins such as p53, HMG-1, and AR (Sakaguchi et al. 1998, Barlev et al. 2001, Munshi et al. 1998, Fu et al. 2000, Gaughan et al. 2002). Acetylation of HMG-1 results in the disassembly of enhancesomes and silencing of transcription (Munshi et al. 1998). Acetylation of AR controls co-regulation recruitment and has been shown to promote growth of prostate cancer (Fu et al. 2000, Fu et al. 2003).

1.4.1.2 Histone deacetylases, HDACs

Gene silencing is often associated with deacetylation of histones. Histone deacetylase complexes, HDACs, catalyze removal of acetyl groups from lysine residues not only from histones, but also from non-histone proteins. Histone acetylases HDAC1 and HDAC2 are subunits of switch-independent 3 protein (Sin3) complex and ATPase-dependent chromatin remodelling NuRD complex (reviewed by Khochbin 2001). In lymphocytes, the DNA-binding protein Ikaros can recruit NuRD complex to the regions of heterochromatin, and it has been suggested to maintain the inactive state of chromatin or remodel active chromatin structure to inaccessible structure (Kim J et al. 1999). Also a transcription repressor, KRAB-zinc finger protein (KAP-1), recruits NuRD to specific promoters to repress transcription (Schultz et al. 2001). Corepressors of nuclear receptors, SMRT and NcoR, interact directly with HDAC3 and stimulate its deacetylase activity (Fischle et al. 2002, Li et al. 2000, Zhang et al. 2002, Wen et al. 2000). Interestingly, antiestrogen- and promoter-bound ER is able to

sequentially recruit N-CoR-HDAC3-complex and NuRD-HDAC1 to the promoter region, which results in H3 and H4 deacetylation and release of Poll II from the promoter. The latter event might occur through remodelling chromatin structure by NuRD (Liu XF et al. 2004).

1.4.1.3 Histone methyltransferases, HMTs

Histones H3 and H4 can be methylated on lysine or arginine residues by lysine methyltransferases, such as Suv39HI and arginine methyltransferases like CARM1/PRMT4 and PRMT1 (Rea et al. 2000, Strahl et al. 2001, Ma et al. 2001). CARM 1 is a coactivator for nuclear receptors, but this activity takes place only in the presence of p300/CBP and p160 (Chen et al. 1999, Koh et al. 2001). Interestingly, CARM1 was found to be integrated with SWI/SNF remodelling components to form nuclear methylation activator complex, NUMAC (Xu et al. 2004). NUMAC coactivated ER-mediated transcription, and CARM1 stimulated the chromatin remodelling activity of NUMAC. HMT activity can be recruited to chromatin by the methyl-CpG-binding protein MeCP2, which has shown to enhance methylation of lysine 9 in H3 and lead to gene silencing (Fuks et al. 2003). On the contrary, the H3-Lys9 methylation is required for DNA methylation (Tamaru and Selker 2001). PRMT1 and CARM1 can also catalyze methylation of non-histone proteins like STAT1 and CBP, respectively, and in both systems methylation inhibits recruitment of coregulators (Mowen et al. 2001, Xu et al. 2001).

1.4.2 ATP-dependent chromatin remodelling

ATP-dependent chromatin remodelling increases the accessibility for regulatory proteins to recognize and interact with their specific target elements on DNA through various mechanisms. They can, for instance, remove histones from the promoter region, change nucleosome positions or replace histones with histone variants (Reinke and Horz 2003, Fazzio et al. 2003, Krogan et al. 2003). Chromatin remodelling complexes all have an ATP-hydrolyzing core that exhibits homology to the helicase family proteins, which, in turn, catalyze the progressive separation of duplex DNA into single-stranded DNA (reviewed by Flaus and Owen-Hughes 2001). Other subunits of these complexes are thought to modulate the remodelling activity of the ATPase subunit, or they participate in targeting the remodelling complexes to specific promoters (Ito et al. 1999, reviewed by Längst and Becker 2001, Xu et al. 2004). There are four major chromatin remodelling family: ISWI, SWI2, CHD and Ino80. ISWI-family members, such as human chromatin accessibility complex hCHRAC and human nucleosome remodelling factor, hNURF, contain C-terminal SAINT-like domain, which binds both DNA and proteins, within ATPase subunit (Grüne et al. 2003). The complex remodels nucleosomes without disruption or displacement of the histone octamer, but catalyze nucleosome sliding and this

activity is dependent of histone H4 tails (Hamiche et al. 1999, Längst et al. 1999, Clapier et al. 2001, Schwanbeck et al. 2004). CHD-family members like human NuRD contains the chromodomain that mediates specific interaction with proteins (Kelley et al. 1999). The NuRD complex contains also histone deacetylation subunits and has been suggested to play a repressive role in nuclear receptor-mediated transcription (Feng and Zhang 2003, Underhill et al. 2000, Fujita et al. 2003, Fujita et al. 2004, Liu XF et al. 2004). The SWI/SNF-family members, such as human NUMAC and BAF, and yeast SWI/SNF and RSC, are characterized by ATPases with a bromodomain that is involved in binding with acetylated peptide (Martens et al. 2003). The SWI/SNF complexes that contain BRG-1 as an ATPase subunit, have been proposed to be essential in steroid receptor-dependent chromatin remodelling and gene regulation (Direnzo et al. 2000, Fryer and Archer 1998, Huang et al. 2003, Xu et al. 2004). In the Ino80 family, the ATPase domain is split into two segments by an insert, and so far, only yeast members, Ino80.com and SWR.com, of this family have been identified. The remodelling activity of CWR complex can exchange canonical H2A for H2A variants (Krogan et al. 2003, Mizuguchi et al. 2004).

1.4.3 Mediators

The Mediator complex was initially identified in yeast cells, while searching proteins that interact with the unphosphorylated C-terminal domain (CTD) of the largest subunit of Pol II (Flanagan et al. 1991, Thompson et al. 1993, Hengartner et al. 1995, Kim et al. 1994). The yeast and metazoan Mediators are multisubunit complexes containing from 7 to 25 different proteins. The human mediator complexes isolated by different laboratories include the TRAP, DRIP, ARC, SMCC, NAT, and PC2/CRSP complexes (Fondell et al. 1996, Fondell et al. 1999, Boyer et al. 1999, Ito et al. 2002, Näär et al. 1999, Sun et al. 1998, Rachez et al. 1998, Rachez et al. 1999, reviewed by Malik and Roeder 2000, Ryu et al. 1999). These complexes share similar subunit composition with each other, and part of their subunits are related to yeast Mediator components. Thyroid-hormone-receptor-associated protein (TRAP) and vitamin D-interacting proteins (DRIP) were copurified with ligand-bound TR and VDR, respectively, and were found to act as coactivators (Fondell et al. 1996, Rachez et al. 1999). The 220-kDa subunit of these complexes, referred to as TRAP220 and DRIP230, contains the LXXXL-motif for direct NR binding (Yan et al. 2000). TRAP220 knock-out mice fail to develop beyond 10.5 days postconception, and fibroblast derived from TRAP220 -/- embryos do not maintain efficient TR-mediated activation (Ito M et al. 2000). The negative regulator of activated transcription (NAT) and SRB/mediator coactivation complex (SMCC) are also able to repress both induced and basal transcription (Akoulitchev et al. 2000, Gu et al. 1999, Sun et al. 1998). Mediators can interact with Pol II and general transcription factors TBP, TFIIB, TFIIE and TFIIF (reviewed by Malik and

Roeder 2000, Park et al. 2001). Structural studies of yeast Mediator, CRSP (cofactor required for Sp1 activation) and TRAP complexes by electron microscopy have revealed that mediators can bind Pol II through multiple domains (Asturias et al. 1999, Taatjes et al. 2002, Dotson et al. 2000, Näär 2002) and that the conformation of mediator is significantly altered upon binding to Pol II or activator protein. Interestingly, Mediator and p300/CBP-SRC function synergistically during ER-mediated transcription, and ER-bound mediator promotes the formation of the PIC complex for subsequent rounds of transcription reinitiation (Acevedo et al. 2003).

2. RING FINGER PROTEINS

The RING finger motif was originally named after a protein that was encoded by the Really Interesting New Gene (Freemont 2000, Lovering et al.1993). To date, hundreds of members of the RING finger protein family have been identified. RING finger motifs are found in many regulatory proteins throughout the plant, animal, fungal, viral and protozoan kingdom. RING finger proteins are localized both in the cell nucleus and cytoplasm. These proteins function in a many cellular processes, including oncogenesis, apoptosis, development, viral replication and protein degradation. Several RING proteins are implicated in human diseases. For example, PML is responsible for acute promyelocytic leukemia when it forms a fusion protein with retinoid acid receptor α (RARα) (Jensen et al. 2001) and RING finger motif of BRCA1 (Breast Cancer gene 1) is a site for numerous mutations found in families genetically predisposed to breast and ovarian cancer (Ruffner et al. 2001). Parkin protein is disrupted in autosomal recessive familial juveline parkinsonism (AP-JP). Many viral RING finger proteins are also critical for virus replication (Saurin et al. 1996).

2.1 RING finger domain structure

Zinc-binding domains are common, relatively small protein motifs that fold around one or more zinc ions. These domains have been divided into a number of classes, based primarily on the number and the arrangement of the zinc-chelating histidine and cysteine residues (Schwabe and Klug 1994). For instance, the classical zinc finger motif (ZnF), exemplified by the ZnFs of the transcription factor TFIIIA (Miller et al. 1985), is characterised by two conserved cysteines and histidines (C_2H_2), which bind tetrahedrally to a zinc atom. Another class of zinc finger proteins has been described in the nuclear receptor family of proteins, where the motif binds two zinc atoms to form a single folded domain with four cysteine ligands for each zinc (reviewed by Schwabe and Rhodes 1991). The zinc finger found in GAL4 DNA binding domain (Marmorstein et al. 1992, Kraulis et al. 1992, Baleja et al. 1992), binds two zinc atoms through six cysteines with the metals sharing two of the ligands.

GATA-type ZnF contains a zinc atom coordinated by the conserved four cysteines (de Pater et al. 1996). RING finger (C_3HC_4, $C_3H_2C_3$), FYVE finger (C_5HC_2 or C_8), PHD finger (C_4HC_3), DnaJ cysteine-rich domain (C_8), LIM ($C_2HC_4C/H/D$) and GCM domain (C_6H_2) each posses two binding pockets formed by eight zinc-coordinating residues (Barlow et al. 1994, Misra and Hurley 1999, Pascual et al. 2000, Martinez-Yamout et al. 2000, Cohen et al.2003, Freyd et al. 1990, Crawford et al. 1994). RING, PHD and Fyve motifs use a cross-brace zinc ligation system, but the

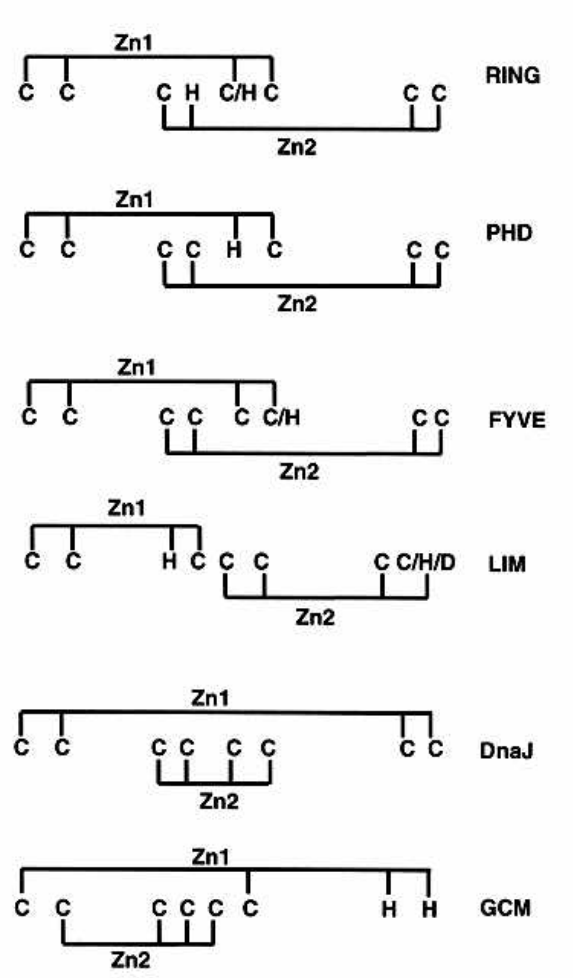


Fig. 4. The cysteine (C)/histidine (H) arrangement in the RING finger and RING-finger-like (PHD, Fyve, LIM, DnaJ, and GCM) motifs. Quartettes of zinc-coordinating residues are connected by lines.

LIM domain displays a striking structural similarity to domains of the GR and GATA-1 transcription factors (Fig. 4).

Only relatively scarce structural data of RING finger domains are presently available, partly because these domains have a tendency to aggregate and precipitate when expressed and concentrated. RING finger is a motif of 40 to 60 residues, where the conserved eight metal binding residues

(cysteines and histidines) bind two divalent zinc ions. There are two different variants, the C_3HC_4 (RING-CH) and a $C_3H_2C_3$ -type (RING-H2), which are clearly related despite the presence of cysteine (C) or histidine (H) in the fifth position. The spacing of cysteines and histidine in the RING-finger motif is C-x_2-C-$x_{(9-39)}$-C-$x_{(1-3)}$-H-$x_{(2-3)}$-C/H-x_2-C-$x_{(4-48)}$-C-x_2-C, where x is any amino acid. Four pairs of metal-binding residues sequester two zinc atoms at distinct tetrahedral sites (Zheng et al. 2000). The first and third pairs (C_4-pair) ligate the zinc ion in position 1, while the zinc in position 2 is ligated by the second and the fourth pair (C_3H) of the RING finger motif. The structure of the zinc ligation is unique and is referred to as the "cross-brace" motif (Fig. 5), which is also found in PHD and Fyve domains. Within the RING domain the sulphydryl group of cysteine and the imidazolyl nitrogen of histidine are ligating the metal ions (Barlow et al. 1994, Everett et al. 1993). Conserved metal-binding residues can be substituted for other metal-binding amino acids (Asp and Thr). Exceptionally in the RAG-1 (recombination-activating gene 1) and Rbx-1 (Ring box protein-1, also known as ROC1 and HRT1) the first zinc-binding site of RING finger structure is a part of an unique binuclear cluster with the cysteine bridging two zinc atoms, leading to co-ordination of three zinc ions.

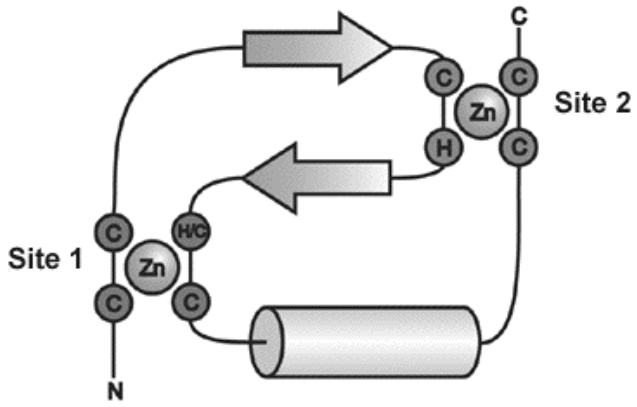


Fig. 5. Simple model of the “cross-brace” structure of the RING finger domain showing conserved cysteines (C) and histidines (H) and secondary structure elements (Dodd et al. 2004). Zn; a zinc ion.

Metal binding has been shown to stabilize the RING structure of PML (promyelocytic leukemia protein) and MAT-1 (Menage a trois) (Borden 1995). In addition to zinc(II) binding, RING finger can ligate cobolt(II) and cadmium(II) but with a lower affinity (Lovering et al. 1993, von Arnim and Deng 1993, Upton et al.1994). Interestingly, the C_4-site (site one) in the RING finger domain of BRCA1 or HDM2 (human double minute 2) possesses a higher affinity towards zinc atom, whereas the C_3H -site (site 2) possesses a lower zinc-binding activity (Roehm and Berg 1997, Lai et al. 1998).

The metal binding is anticooperative, since the zinc-binding affinity of the C_3H -site decreases ~ 20-fold, when a metal ion binds to the C_4-site.

The atomic resolution structures have been solved for RING domains of RAG-1, PML, MAT-1, IEEVH and EL-5. Also the structures of BRCA1-BARD1 (BRCA1-associated ring domain protein 1) heterodimeric RING-RING complex, c-Cbl-Ubc7 complex and Rbx-1 containing SCF (Skp1-Cullin-F-box protein) ubiquitin ligase complex have been solved. The RING C_3HC_4 secondary structure adopts a ββαβ fold in Herpes simplex virus type 1 immediate-early protein Vmw110 (IEEVH), RAG-1, BRCA1 and MAT-1 (Fig. 6), but PML has a βββ-loop-α-β-fold (Everett et al. 1993). Conserved residues within the α-helix, β-sheets and loops contribute to a compact hydrophobic

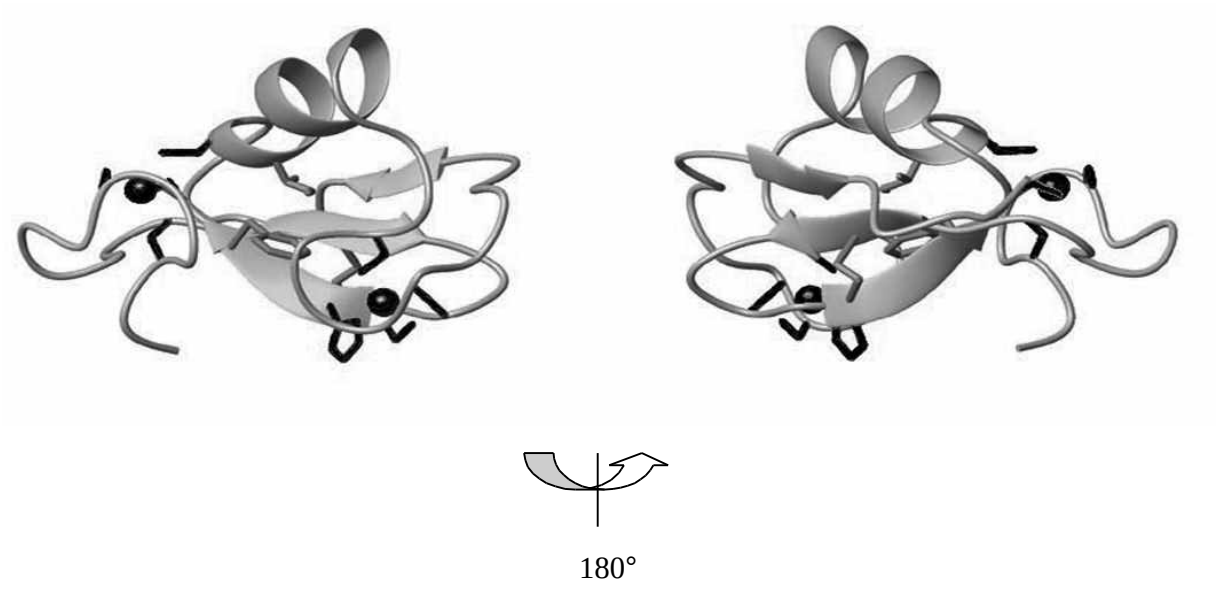


Fig. 6. The three-dimensional structure (NMR) of the RING finger domain of IEEHV presented as a ribbon diagram. The right site shows the molecule after a 180°C rotation around the vertical axis compared the left side (Gervais et al. 2001). Black sphere presents zinc atom. Zinc ligating residues are shown in black.

core in IEEHV (immediate early protein from Equine herpes virus type 1) and MAT1 (Barlow et al. 1994, Gervais et al. 2001). The variable spacing in the consensus sequence leads to differences in three-dimensional fold around the zinc-binding site 2 in PML, IEEHV and RAG1. The distance between the two zinc-binding sites is likely to be the same in all known RING fingers, given the absolute conservation of the spacing of the two residues between histidine ligand of the C_3H-site and the first cysteine ligand of the C_4-site. For instance, the inter-zinc distance in PML, RAG1 and IEEHV

RING finger structure is the same (14Å) (Barlow et al. 1994, Borden et al. 1995, Bellon et al. 1997). Also the charge distribution at the surface of RING finger domains could be an essential factor that modulates RING finger activity, since, for example, the mutations of charged amino acids within the RING domain affect the function of PML and IEEVH (Boddy et al. 1997). Generally, there is very little sequence homology between RING proteins outside the consensus RING sequence, but these regions regulate the specificity of protein-protein interactions and the structural diversity within this family of proteins (Zheng et al. 2000, Brzovic et al. 2001). It appears that the RING finger motif adopts significantly varying three-dimensional structures, while maintaining some structural conservation including the overall topology of the central β-strands, the cross-braced Zn^{2+} binding system and the packing of conserved residues which form the hydrophobic core of the molecule. This suggests that the RING finger motif forms a convenient scaffold, which can be structurally varied to reflect the diversity in its molecular function. The RING finger domain cannot be substituted even between closely related RINGs without a change in function. For instance, even though the RING fingers of BRCA1 and Rpt1 have more than 90% sequence identity, a BRCA1 form containing the $RING_{Rpt1}$-substitution does not maintain the BAP1 interaction anymore (Jensen et al. 1998).

RING fingers are often associated with distinct domains. For instance, the RING finger domains of TRAF 2-5 are followed by five zinc fingers, a coiled-coil and a TRAF domain (Schwabe and Klug 1994). The DNA repair proteins RAD5 and RAD16 have a RING finger that is interleaved with ATPase domains. The inhibitor of apoptosis (IAP3) contains three BIR (baculovirus IAP repeat) domains in front of the RING finger (Laren et al. 2003). Many RING finger proteins, such as Parkin and the human homologue of *Drosophila* Ariadne (HHARI), that are involved in protein ubiquitination and degradation are characterized by the presence of two RING finger domains separated by the cysteine-rich IBR (the in between RING fingers) domain or DRIL (double RING finger linked) domain (Moynihan et al. 1999, Zhang et al. 2000). This tripartite domain is called as a TRIAD (two RING fingers and a DRIL). Mutations in the RING-IBR-RING of Parkin cause the AR-JP, and RING2 mutations are found in a rare form of parkinsonism (Morett and Bork 1999, Shimura et al. 2001). Interestingly, a NMR structural study of the RING2 in TRIAD of HHARI revealed that RING2 has a totally different three-dimensional fold when compared to the classical RING finger, and it binds only one zinc atom. In addition, the RING2 of HHARI possesses ubiquitin E3 ligase activity (Capili et al. 2004). Most frequently, RING domains are associated with cysteine-rich zinc-binding domains, B-boxes, and α-helical coiled-coil domain and referred to as RBCC-domains. This domain is found in PML, estrogen-responsive RING finger (Efp), TIFα1, neuregulin receptor degradation pathway protein 1 (Nrdp1) and KAP-1 (Fagioli et al. 1998, Inoue et al. 1991, Qiu and Goldberg 2002,

reviewed by Saurin et al. 1996). The RBCC domain appears to be an integral structural unit requiring every one of its subdomains for proper function of the protein in which it is found.

2.2 Function of the RING domain

A small zinc-ligating domain can facilitate multiple intermolecular interactions between nucleic acids and proteins. GATA-type ZnF can bind both DNA and proteins, and the proteins with multiple GATA-type motifs can play a complex role in regulating transcription through an interplay of these different binding selectivities and affinities. Other ZnFs have more specific functions, such as DNA-binding ZnFs in the nuclear hormone receptor proteins and small-molecule-binding ZnFs in protein kinase C. When the RING finger was initially identified, it was thought that it acted as a specific DNA-binding domain (Freemont et al. 1991, van Lohuizen et al. 1991, Haupt et al. 1991), but RING finger together with PHD, Fyve and LIM domains appear to act exclusively in protein-protein interactions. Several intriguing characteristics evident from the studies of RING domains have made it difficult to establish a single biochemical function for RINGs.

RING finger proteins are known to be involved in the assembly of large protein complexes. RING finger is able to interact with other RING finger domains and non-RING containing sequences. Further, RING finger can use both zinc-binding regions independently for binding different proteins (Roehm and Berg 1997, reviewed by Kentsis and Borden 2000). The arenaviral protein Z is the smallest known RING finger protein (90 aa) constituted almost entirely by its RING domain (reviewed by Riviere et al. 1987). The Z protein self-assembles into ordered spherical structures via its RING finger domain *in vitro* and these structures resemble the nuclear domains formed by Z protein during virus infection (Kentsis et al. 2002_a). Isolated RING domains of BRCA1, KAP-1, mel-18 and PML are able to self-assembly *in vitro* (Kentsis et al. 2002_b). Mutating the first zinc-binding site of RING finger abolishes the capacity to self-assemble *in vitro* and also *in vivo*, but in contrast, the second zinc-binding site mutation does not destroy the RING finger domain structure or eliminate the self-assembly activity (Kentsis et al. $2002_{a,b}$, Peng et al. 2000, Borden et al. 1995, Campbell et al. 2000). Interestingly, the BRCA1 cancer predisposing RING mutant (C64G) protein fails to self-assemble *in vitro*, form nuclear domains or suppress tumor growth *in vivo* (Kentsis et al. 2002_b, Jin et al. 1997). In addition to homodimerization, BRCA1 can form heterodimers with another RING finger protein BARD1 through $RING_{BRCA1}$-$RING_{BARD1}$ interaction. Also BARD1 homodimerizes (Brzovic et al. 1998, Meza et al. 1999). The RING finger domain of the PML is important for the formation of PML multiprotein complexes that are referred to as PML nuclear bodies (PML NBs). Disruption of RING domain destroys the PML NBs and further correlates with a loss of growth suppression and

apoptotic activities (Borden et al. 1997, Mu et al. 1994). Also the polycomb group protein Bmi1 exists in a large 2-5 MDa protein complexes, and mutations in the conserved residues within RING finger of Bmi1 disperses the Polycomp complexes and leads to anterior-posterior transformation of the axial skeleton (Alkema et al. 1997). The sequence determinants for RING finger binding of RING-less proteins are not well characterized. However, one potential proline-rich consensus sequence has been defined as PxBxPJxP, where B is Leu/Val, J is Ala/ser and X is any amino acid, and is called as FRODO (Funky RING oligomerization domain) (Kentsis and Borden 2000). The first zinc-binding site of the RING finger has been shown to maintain the interaction with the FRODO.

There is a lot of evidence that the RING domain mediates ubiquitin E3 ligase activity (reviewed by Jackson et al. 2000 and Joazeiro et al. 2000, Lorick et al. 1999). Generally, the RING finger of E3 binds the ubiquitin-conjugation enzyme (E2) (Lorick et al. 1999, Zheng et al. 2000). The crystal structure study of ubiquitin E3 ligase, c-Cbl, complexed with E2 enzyme, Ubc7, revealed that the hydrophobic groove formed by the helix and the two zinc-chelating loops of the c-Cbl RING finger forms the major contact region with loops 1 and 2 of Ubc7, but also the region preceding the RING finger participates in E2 binding (Zheng et al. 2000). The multisubunit E3s, such as APC and SCF, always contain a RING finger subunit such as Apc11 and Rbx1, respectively. The organization and the function of these complex E3s are critically dependent on the RING finger (Seol et al 1999). Mutations in the first zinc-binding site reduces ubiquitin ligase activity of SCF, but the second zinc-binding site is irreparable for ubiquitin ligation (Ohta et al. 1999), indicating that the protein-interactions by the zinc-binding site 2 are critical for normal function of the SCF complex. The RING finger of Rbx1 is the primary binding region for E2 and serves as a secondary binding region for Cul1 in SCF complex (Zheng et al. 2002). The BRCA1-BARD1 complex functions as a RING ubiquitin E3 ligase (Hashizume et al. 2001, Kentsis et al. 2002_b). *In vitro* ubiquitination reactions examined by electron microscopy, showed that $RING_{BRCA1:BARD1}$ bodies efficiently scaffold multiple UbcH5Cs on their surface with several chains of polyubiquitins (Kentsis et al. 2002_b). In addition, a RING-like domain (SP-RING;Siz/PIAS-RING) of PIAS proteins, which has been suggested to have a similar three-dimensional structure as RING finger, binds directly to SUMO-conjugating enzyme (E2) Ubc9 and is required for PIAS SUMO E3 ligase activity (Kahyo et al. 2001, Kotaja et al. 2002).

RING finger proteins involve in many cellular processes. Many of the biological functions of the PML, such as growth and transformation suppressive action, are mediated through PML NBs and require an intact RING finger domain (Melnick and Licht 1999, Borden at al. 1997, Mu et al. 1994). RING-mediated oligomerization of KAP-1 is required for its association with the DNA-dependent transcriptional repression domain (KRAB) of KOX-1, thereby it mediates transcriptional repression

(Peng et al. 2000). Also the RING finger LIM domain-binding protein, RLIM, is a corepressor that recruits histone deacetylases HDAC2 and Sin3 (Bach et al. 1999). Interestingly, also the RING-mediated oligomerization stimulates catalytic activity of MAPKKK/MEKK1 and its autophosphorylation, which is needed for the JNK activation (Baud et al. 1999). RING ubiquitin E3 ligases MDM2, IPC0, BRCA1-complex, pirh2, COP1 and Topors are involved in ubiquitination and degradation of the tumor suppressor protein p53 in addition to other protein targets (Kubbutat et al. 1997, Haupt et al. 1997, Boutell and Everett 2003, Leng et al. 2003, Dong et al. 2003, Dornan et al. 2004, Rajendra et al. 2004). c-Cbl attenuates signaling by the growth factor receptors EGFR and PGDFR via inducing their ubiquitination and degradation. Interestingly, oncogenic variants of c-Cbl have been shown to contain mutated forms of the RING (Levkowitz et al. 1998, Miyake et al. 1998, Joazeiro et al. 1999, Blake et al. 1991, Langdon et al. 1989).

3. THE UBIQUITINATION SYSTEM

Protein proteolysis is increasingly understood to be an important general mechanism by which cells regulate protein levels and consequently their function at specific times. Constant protein turnover serves many critical regulatory roles, including quality control by confirming degradation of misfolded proteins due to mutations or damage in the protein. This function is especially important in non-dividing cells, such as neurons, where the accumulation of malfunctioning proteins would be highly deleterious (reviewed by Kopito 2000 and Sherman and Goldberg 2001 and Petrucelli and Dawson 2004). In eukaryotic cells, the main mechanism for such control involves specific covalent modification by ubiquitin/polyubiquitin, which labels target proteins for proteolysis and subsequent degradation by the 26S proteasome or, as in the case of cell surface receptors, the ubiquitin conjugation causes their down-regulation by the endosomal-lysosomal pathway (reviewed by Schwartz and Ciechanover 1999). Ubiquitination can also directly alter the function and intracellular localization of proteins. The ubiquitination pathway is an ATP-dependent and a multistep process, involving at least three types of enzymes, known as E1, E2 and E3. Numerous studies have demonstrated that ubiquitination plays an important regulatory role in many cellular events, such as cell cycle, apoptosis, inflammation, DNA repair, and stress responses.

3.1 The ubiquitination pathway and proteasome

Ubiquitin is transcribed as an inactive precursor molecule with a C-terminal extension of several amino acids, which is processed by a specific protease, ubiquitin carboxy-terminal hydrolase, to make the carboxy-terminal double glycine (Gly76-Gly77) motif available for conjugation. Ubiquitination is

initiated by the activation of ubiquitin at the C-terminal Gly76 by ubiquitin-activating enzyme (E1) in an ATP-dependent manner (Fig. 7). Synthesized C-terminal adenylate intermediate serves as the

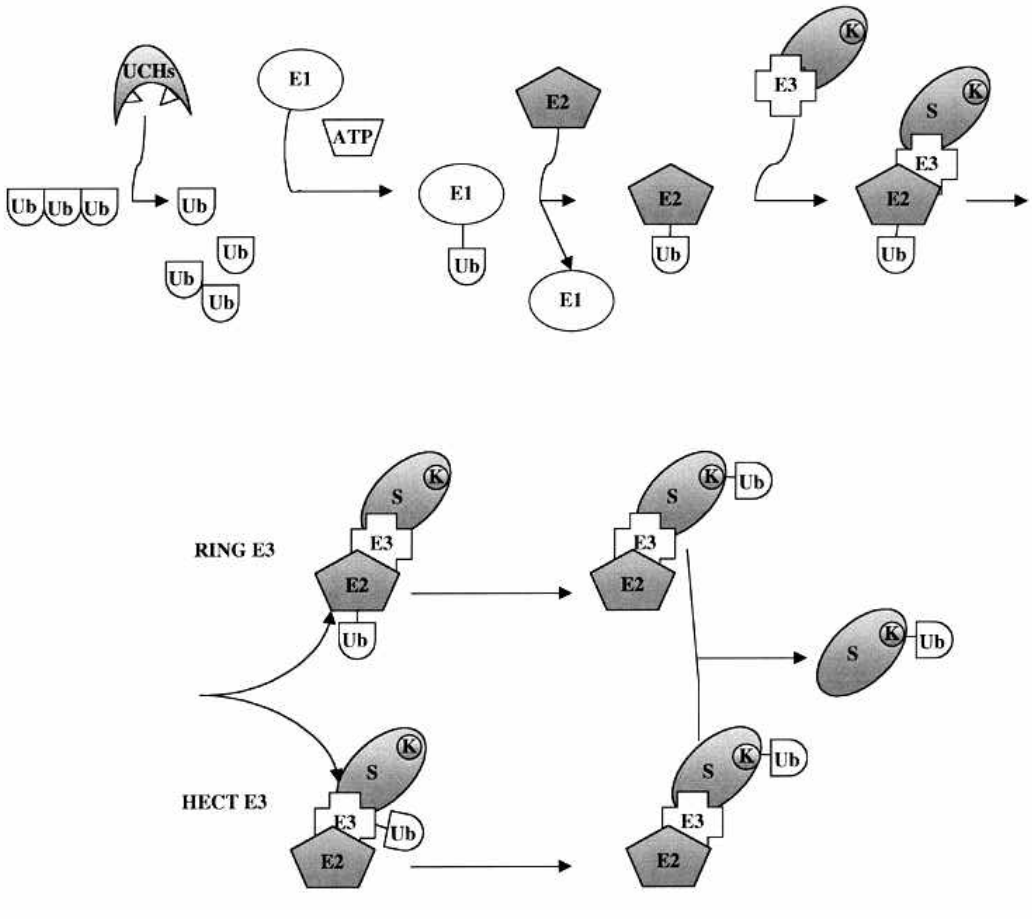


Fig. 7. The ubiquitination pathway. Free ubiquitin (Ub) is cleaved from the ubiquitin precursor by ubiquitin carboxy-terminal hydrolase (UCH). Ubiquitin is activated in an ATP-dependent manner with the formation of a thiol-ester linkage between E1 and the carboxy terminus of ubiquitin. Ubiquitin is transferred to E2. E2 associates with E3, which may have the substrate already bound. For HECT domain E3s, ubiquitin is transferred to the active site cysteine of the HECT domain followed by ubiquitin transfer to the substrate (S). For RING E3, current evidence indicates that ubiquitin is transferred directly from E2 to the substrate (reviewed by Weissman 2001).

donor of ubiquitin to a cysteine (Cys) in the E1 active site and the thiol ester bond is formed between ubiquitin and E1. Then the ubiquitin-conjugating enzyme (E2) accepts the activated ubiquitin from E1 with its own active Cys residue and, again, the thiol ester bond is formed. Finally, E2 ubiquitinates the substrate in cooperation with ubiquitin E3 ligase. The HECT-type E3 accepts ubiquitin from E2, and forms thiol linkage with ubiquitin and then shifts it to protein substrate. The RING-type ubiquitin E3 ligases do not form a thiol linkage with ubiquitin, rather they facilitate direct ubiquitin transfer from E2 to the substrate. E3s dictate the specificity of ubiquitination by recognizing the target protein. In

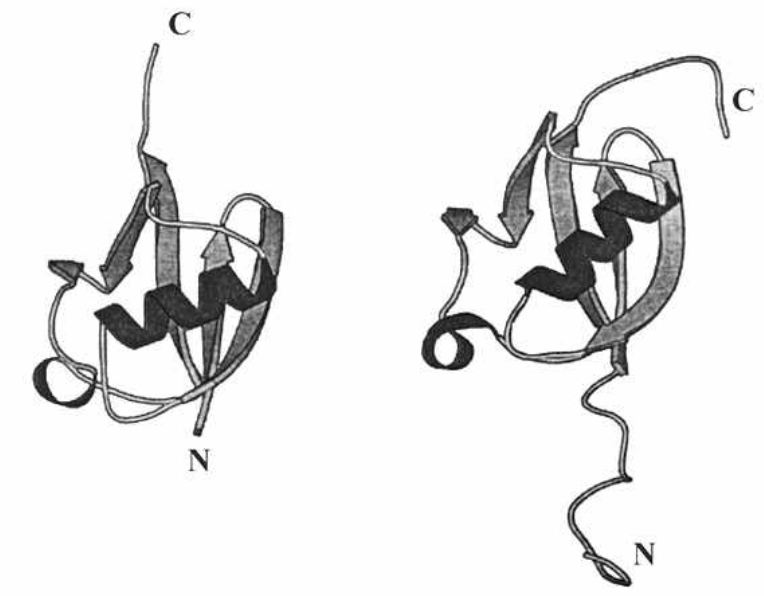
most cases, the ubiquitination of proteins is mediated by lysine dependent-ubiquitination pathway, where ubiquitin is conjugated into ε-NH_2 group of an internal Lys residue. For instance, Lys21 and Lys22 of IκBα have been shown to be the targets of ubiquitin conjugation, but in cyclin B any single lysine residue can serve as an ubiquitin acceptor (King et al. 1996).

Proteasomes are present both in the cytoplasm and the nucleus. In the cytoplasm, proteasomes associate with the cytoskeletal networks (reviewed by Wojcik and DeMartino 2003) and the outer surface of the endoplasmic reticulum (ER) (reviewed by Brodsky et al. 1999). In the nucleus, proteasomes are spread throughout the nucleoplasm, but are absent in the nucleoli, and sometimes associate with discrete subnuclear domains called the PML nuclear bodies (PML NBs). Proteasomes exist in multiple forms such as a free 20S proteasome, a 26S proteasome, a 20S proteasome associated with PA28 and a hybrid proteasome (reviewed by DeMartino and Slaughter 1999 and Glickman and Ciechanover 2002). Polyubiquitinated proteins are recognized and degraded by the 26S proteasome (Chu-Ping et al. 1994). The S26 proteasome is a 2.4 MDa complex composed of two subcomplexes; a 20S core particle, which carries the protease complex, and one or two 19S regulatory particles, which regulate the function of the former and select the substrates. The polyubiquitinated proteins are recognized and bound by the proteasome (Thrower et al. 2000) and the ubiquitin chain is cleaved from the protein substrate by deubiquitination enzymes (DUBs). It has been suggested that specific ubiquitin chain-recognition adaptors serve to distinguish between these ubiquitin chains and they are likely play a role in the substrate delivery to the proteasome. For instance, HHR23 (human homologue of yeast RAD23) translocates ubiquitinated target proteins to the proteasome via its UDP (ubiquitin-domain protein) and UBA (ubiquitin-associated) domains, which bind catalytically active proteasome and ubiquitin chains of the target protein, respectively (Chen and Madura 2002, Elsasser et al. 2004) The protein is then unfolded and translocated into the 20S core proteasome in an ATP-dependent manner and subsequently the protein is hydrolyzed into small peptides. Ubiquitin is spared from degradation through its release from the substrate by ubiquitin hydrolases, and it is recycled back into the ubiquitin pathway (Swaminathan et al. 1999, reviewed by Hershko and Ciechanover 1998). Interestingly, not all ubiquitinated proteins are fully degraded, since some proteins, such as the transcription factor p105 (a precursor of p50), and Spt23, are processed into a truncated form by proteasome and thus the proteasome can regulate the activity of the protein (Palombella et al. 1994, Hoppe et al. 2000). Proteasomes have also a capacity to degrade certain non-ubiquitinated substrates, which can either be directly recognized by the proteasome or be presented to the proteasome by cofactors, like antizyme in the case of ornithine decarboxylase (Murakami et al. 1992). Ubiquitination is a reversible process due to deubiquitination enzymes (DUBs). DUBs are cysteine proteases that specifically hydrolyse the amide bond immediately adjacent to the carboxy-terminal residue and these

proteases are divided into two categories; UCHs (ubiquitin-COOH hydrolases) and UBPs (ubiquitin-specific proteases). UCHs remove short and flexible peptide chains from the COOH terminus of ubiquitin, whereas UBPs cleave the isopeptide bond between ubiquitins (Ub-Ub) or between the Ub and the protein. DUBs are involved in the generation of free ubiquitin, recycling of ubiquitin, editing polyubiquitin chains (Kovalenko et al. 2003, Trompouki et al. 2003), and aiding proteasome-dependent degradation (Reviewed by D'Andrea and Pellman 1998). Inhibitors of the ubiquitin-proteasome pathway directly target and inhibit the 20S proteasome, rather than the upstream ubiquitination. Lactacystin and its derivative *clasto*-lactacystin β-lactone, metabolites of *Streptomyces lactacystinaeus*, irreversibly inhibit the 20S proteasome, as it forms a covalent bond with the catalytic N-terminal residue of the proteasomal β-subunit (Fenteany et al. 1995, Ostrowska et al. 1997, Dick et al. 1996). Another natural inhibitor, epoxomicin (EXM) is a peptide (originally isolated from a species of Actinomycetes) that has a relatively high specificity for the 20S proteasome, and it covalently binds to the catalytic subunits of the proteasome (Meng et al. 1999). Synthetic peptide aldehydes, MG-132 (benzyloxycarbonyl-leucinyl-leucinyl-leucinal), MG-115 (carbobenzoxy-L-leucyl-L-leucyl-L-nor-valinal) and PSI (carbo-benzoxy-L-isoleucyl-gamma-t-butyl-L-glutamyl-L-alanyl-L-leucinal) arrest reversibly the proteasome activity by inhibiting the chymotrypsin-like activity (Rock et al. 1994, Wilk and Figueiredo-Pereira 1993). Since the ubiquitin-proteasome pathway is critical for the proliferation and survival of cells, including cancer cells, proteasome inhibitors are of great interest as potential therapeutics for cancer. In general, proteasome inhibition tends to induce apoptosis in proliferating cells (Drexler 1997). The first proteasome inhibitor has been approved for use in patients with relapsed and refractory multiple myeloma. Bortezomib (PS-341) is a modified dipeptidyl boronic acid that binds reversibly to the chymotryptic site in the proteasome and induces apoptosis in different cancer cell lines (Adams et al. 1999).

Ubiquitin

Ubiquitin (Ub) was first discovered as a lymphocyte differentiation-promoting factor in 1975 (Goldstein 1975). Ubiquitin is a small 8.5-kDa polypeptide (76 amino acid residues) that is ubiquitously expressed in all eukaryotic cells, and it is found throughout the cell (*ubique*, latin for everywhere). Ubiquitin is highly conserved during evolution, with only three amino acid changes from yeast to human. Ubiquitin consisting of both alpha-helices and beta-sheets (ββαββαβ-fold), that form a highly stable, globular structure containing a hydrophobic core with C-terminal glycine residues (Gly76-Gly77) protruding from the main body of the protein (Fig. 8) (Vijay-Kumar 1987). Figure 8 also shows the 3D structure of SUMO-1 (small ubiquitin-like modifier 1), which is conjugated to proteins in a similar fashion as ubiquitin, but with different enzyme components and different effect on the function of the target protein (see section 4. SUMO-1 conjugation).



Ubiquitin **SUMO-1**

Fig. 8. The three-dimensional structure of the ubiquitin and the small ubiquitin-like modifier 1 (SUMO-1). The crystal structure of human ubiquitin and the NMR structure of human SUMO-1 are shown (Vijay-Kumar et al. 1987, Bayer et al. 1998, reviewed by Melchior 2000).

Ubiquitin is not expressed as a free ubiquitin, but instead it exists in an unfolded form attached to itself or to certain ribosomal protein subunits. These ubiquitin precursors are processed by DUBs into a free form and therefore, it is found both as a free monomer as well as covalently linked to itself (polyubiquitin chain) and other proteins (ubiquitination) with its lysine-linked chain (reviewed by Hershko and Ciechanover 1998). In protein ubiquitination, ubiquitin is covalently conjugated to protein through isopeptide bond formed between carboxyl-terminal Gly76 and the ε-amino group of a lysine residue in the target protein. Recent findings show that ubiquitin can be linked to a target protein also via bonding between α-NH_2 group of the N-terminal residue of target protein and the Gly76 residue of ubiquitin (Ciechanover et al. 2004). In polyubiquitin chain formation, ubiquitins are linked similarly to each other via the isopeptide bond between carboxyl-terminal Gly76 and lysine residues of the previously conjugated ubiquitin (reviewed by Hershko and Ciechanover 1998).

3.2 Enzymes of the ubiquitination pathway

Ubiquitination is a dynamic and reversible process involving enzymes E1, E2, E3 and deubiquitination enzymes, DUBs. There is only one E1 enzyme, multiple E2s, which can serve

several E3 enzymes, and an even a greater number of E3s. This multi-step reaction involving many different enzyme components enables protein ubiquitination to achieve a high degree of specificity and diversity. Another large group of proteins involved in this process is the deubiquitinating enzymes, a family of ubiquitin-specific proteases that cleave ubiquitin from ubiquitin-conjugated proteins and are thought to act at different steps in the ubiquitin pathway.

3.2.1 Ubiquitin-activating E1 enzyme

A single E1 enzyme (UBA) in human and yeast is required to activate ubiquitin for all conjugating reactions and to transfer it to all known E2s. E1 is essential for cell viability, since the deletion of UBA1 in yeast is lethal (McGrath 1991). E1 is found in the nucleus and cytosol, and the localization of E1 is cell cycle-regulated with a predominant nuclear localization in the G2 phase (Grenfell et al.1994, Trausch, et al. 1993). E1 is also phosphorylated in mammalian cells by cdc2 kinase (Nagai et al. 1995). Mutation of Cys residue of the active site abolishes the E1 activity (Hatfield and Vierstra 1992).

3.2.2 Ubiquitin-conjugating E2 enzymes

There are 13 isoforms of E2s in yeast and more than 20 in mammals (reviewed by Jentsch 1992 and Hochstrasser 1996). E2s all share an active site ubiquitin binding Cys residue within the core structure, Ubc domain, required for binding E3s. E2s are subdivided into four classes. Examples of class I are Ubc4, Ubc5, Ubc7 and Ubc9-13, which contain UBC domain and require E3s for substrate recognition and the ubiquitin transfer to target proteins. Several E2 enzymes are more complex than the class I members and have unique N-terminal (class II) or C-terminal (class III) extensions of various lengths or both of them (class IV). Class II contains Ubc1, Ubc2 (RAD6), Ubc3(Cdc34), Ubc6 and Ubc8. The C-terminal extension of Ubc2 appears to mediate interactions with histones and is also needed for histone ubiquitination (Sung et al. 1988). Also the C-terminal extension is needed for anchoring Ubc6 to the cytosolic side of endoplasmic reticulum (ER) (Sommer and Jentsch 1993). Ubc2/RAD6 acts along with Ubr1/E3α to target N-end rule substrates (Xie and Varshavsky 1999). Ubc4 and ubc5 E2s participate in the turnover of short-lived and misfolded proteins. Ubc13 is involved in error-free postreplicative repair pathway in yeast (Brusky et al. 2000). It also forms a complex with Mms2p, an ubiquitin-conjugating enzyme variant (UEV) without a catalytic cysteine within UBC domain and causes the assembly of polyubiquitin chains linked through Lys-63 (Hofmann and Pickart 2001). Ubc6 associates with Ubc7 and they have been implicated in

endoplasmic ER-associated degradation (ERAD) pathway (Chen et al.1993, Kim BW et al. 2003). Two UBC domain-containing proteins, Ubc9 and Ubc12, do not conjugate ubiquitin, but conjugate ubiquitin-like molecules, SUMO (small ubiquitin-like modifier) and RUB-1/Nedd8 (related to ubiquitin 1/Neural precursor cell-Expressed Developmentally down-regulated), respectively (Johnson and Blodel 1997, Gong and Yeh 1999). The E2 terminology is not standardized across different species, since similar names given to yeast and mammalian enzymes do not reflect functional or structural homology. For instance, human UBCH1 is not the human homolog of yeast Ubc1, but rather the homolog of yeast Ubc2/RAD6. Human UbcH5 is one of the most active E2s and it is involved in ubiquitination of p53 and IκBα (Scheffner et al.1994, Gonen, et al.1999). Human UBCH6 and UBCh7 are involved in targeting of soluble proteins in the cytosol (Nuber et al.1996).

3.2.3 Ubiquitin E3 ligases

Ubiquitin E3 ligases play a critical roles in the ubiquitination cascade by recruiting E2s with ubiquitin and recognizing the target protein and thereby facilitating ubiquitin transfer from E2 to the target protein. The E3s can be divided into two groups: the homologous to E6-AP carboxy terminus (HECT) domain-containing E3s and the RING domain-containing E3s. RING-type E3s are further divided into single subunit and multisubunit E3s. Also RING-like ubiquitin ligase domains, plant homeodomain (PHD) domain, the U-box and HUL-1, have been identified (van Sant et al. 2001, Hagglund and Roizman 2003).

3.2.3.1 HECT-type ubiquitin E3 ligases

The first enzyme described in this family was E6-AP (E6-associated protein), which targets p53 for degradation in the presence of human papillomavirus (HPV) oncoprotein E6 (Scheffner et al. 1993). E6-AP was found to promote ubiquitination of p53 and SRC family kinase Blk in the absence of E6 (Scheffner et al. 1993, Oda et al. 1999), suggesting its role as an ubiquitin E3 ligase. Mutations in E6-AP have been implicated in the pathogenesis of Angelman syndrome, a severe form of inherited mental and motor retardation (Kishino et al. 1997). Later on, the family of proteins harboring HECT, which shares a region of ~350 amino acid residues similar to the C-terminal domain of the E6-AP, was revealed. This domain contains conserved Cys-residue that forms a covalent thiol-ester intermediate with ubiquitin. In addition to the chemistry of ubiquitination, the C-terminal HECT domain mediates the binding of distinct subclass of E2s, such as human UbcH5, UbcH7 and UbcH8 (Scheffner et al. 1994, Nuber et al. 1996, Kumar et al. 1997). HECT domain proteins contain an N-terminal protein kinase C-related C2 domain and WW (two conserved tryptophan residues) domains

responsible for cellular localization and substrate recognition. The target proteins of HECT E3 ligases predominately possess proline-rich motif, PpxY, for WW domain recognition. Yeast has only one HECT E3 ligase, Rsp5p, which regulates a number of important cellular processes including mitochondrial inheritance, internalization of cell surface receptors, and transcription. The disruption of the rsp5 gene is also lethal (Fisk and Yaffe 1999, Huibregtse et al. 1997, Hein et al. 1995, reviewed by Rotin 2000). Higher eukaryotes have several related Nedd4 proteins, such as Nedd4-1 and -2, Smurf1 and -2, Bul1 and Itch. Nedd4 ubiquitinates subunits of the epithelial sodium channel leading to down-regulation of the number of active channels (Kamynina et al. 2001). The Smad ubiquitin regulatory factor 1 and 2 (Smurf 1 and 2) participate in the control of cell growth, differentiation and apoptosis via playing distinct functions in the regulation of signaling pathways triggered by the TGF-β superfamily (Arora and Warrior 2001).

3.2.3.2 RING-type ubiquitin E3 ligases

In contrast to HECT-type E3s, RING-type E3s do not form a thioester bond with ubiquitin, but rather bring the ubiquitin-E2 complex and substrate into close proximity and promote ubiquitin transfer directly from E2 to the substrate (reviewed by Hershko and Ciechanover 1998). The RING finger protein E3 family is composed of two distinct groups, single and multisubunit proteins. In single protein RING-type E3s, such as MDM2, Cbl and Parkin, the RING finger domain and the substrate recognition site are in the same molecule. For instance, Cbl E3 ligase is a 120-kDa protein, harboring RING finger domain for recruiting E2-ubiquitin complex and TKB domain for binding the target protein, tyrosine phosphorylated receptor tyrosine kinases. The crystal structure of Cbl-RING and Ubc7 shows that the interaction surface for Ubc7 in RING domain is similar to that of HECT domain, and also Ubc7 uses the same structural elements for the interaction with both domains, although there is no sequence similarity between RING finger and HECT domain. MDM2 has E3 ligase activity towards itself (autoubiquitination) and as well as to tumor suppressor protein p53. Autoubiquitination of MDM2 does not only render proteins for degradation by the proteasome, but it also reduces its ubiquitination activity towards p53. Autoubiquitination of IAP E3 ligase induces its degradation and promotes apoptosis.

Many RING finger proteins are members of multisubunit ubiquitin E3 ligase complex, such as APC (anaphase promoting complex), SCF (Skp1-Cullin1-F-box) and VCB-CUL2 (von-Hippel-Lindau-ElongingC/B-Cul2). The small Rbx1/Hrt1/Roc1 RING finger protein is involved in E2-ubiquitin recruitment together with Cullin in SCF or VCB-CUL2 E3s and assembly of other components of the complex. The F-protein such as Skip2 in SCF complex recognizes the substrate. The most complex multisubunit E3 is APC, which contains at least 13 subunits (reviewed by Hershko

and Ciechanover 1998, Vodermaier 2004). The APC and SCF complexes are the key players in the control of cell cycle, where APC/C seems to inactivate many cell cycle-advancing protein activities from the previous cell cycle stage, whereas SCF removes blocking agents of the cell cycle progression (reviewed by Page and Hieter 1999). Interestingly, degradation of the SCF component Skp2 is mediated by APC in the phase G1 of the cell-cycle, and therefore APC influences also the SCF-dependent event (Wei et al. 2004).

3.3 Substrate specificity and regulation of ubiquitination

Targeting of a protein via the ubiquitin system must require specific recognition signals for appropriate E3 ligases, but thus far our knowledge of these signals is limited. Only a few motifs or structures that identify proteins as proteolytic substrates have been defined precisely. Certain amino acid sequences called destruction signals or degrees appear to be the signals for degradation. Many short-lived proteins contain a PEST sequence, a short stretch of approximately eight amino acids enriched with proline (P), glutamic acid (E), serine (S) and threonine (T) (Rogers et al. 1986, reviewed by Rechsteiner and Rogers 1996). PEST sequences often contain minimum consensus phosphorylation sites (S/TP) for certain protein kinases (Yaglom et al. 1995). Phosphorylation within the PEST region seems to be required for ubiquitination and degradation of NF-κB1 p105, the inhibitor of the NF-κB, and transcription activator Gcn4 (Kornitzer et al. 1994, Lang et al. 2003). The N-end rule pathway targets proteins carrying destabilizing N-terminal residues called N-degron for degradation. The N-degron is recognized by ubiquitin E3 ligase, which then ubiquitinates the lysine residue of the target protein. The relationship between the nature of the N-terminal amino acid residues of the protein and its half-life is called the N-end rule (Bachmair et al. 1986, Varshavsky 1997). The E3α/Ubr1 has N-degron recognition site for substrates with basic NH_2-termini and hydrophobic termini, but also for non-N-end rule substrates (Kwon et al. 1998, Reiss, et al. 1988). For example, DIAP1 (Drosophila inhibitor-of-apoptosis protein 1) degradation by the N-end rule pathway is needed for its regulation activity in apoptosis (Ditzel et al. 2004). New degrons continue to be identified at a regular rate. The Cdc20-APC/cyclosome (APC/C) ubiquitin E3 ligase complex recognizes and binds the destruction box (D-box; Arg-xx-Leu-xxxx-Asn) of cyclin B and securin and directs them for proteolysis during metaphase/anaphase (Yamano et al. 1998, Zur and Brandeis 2001). Also APC/C-Cdh1 binds KEN-box (Lys-Glu-Asn) of hTK1 (human thymidine kinase 1) (Ke and Chang 2004) and directs its to the proteasome. Signals may also be buried in the hydrophobic core. Misfolded proteins probably expose similar hydrophobic pockets, which are buried in the correctly folded structure, and these signals may be seen by the Ub-machinery yielding the protein to become ubiquitinated and proteolytically processed (Sadis et al. 1995, Gilon et al. 1998.

Interaction of E3 and degron can be modulated by posttranslational modifications occurring at different stages of cellular events. Phosphorylation triggers degradation of CDK regulators at appropriate points in the cell cycle (reviewed by Peters 2002 and Deshaies and Ferrell 2001) or stabilizes the protein such as Pds1, an inhibitor of anaphase initiation, in response to DNA damage (Agarwal et al. 2003). Hydroxylation on proline residue and acetylation of HIF-1α (hypoxia inducible factor-1α) are critical for recognition by the pVHL ubiquitination complex (Ivan et al. 2001, Jaakkola et al. 2001). Also RNA polymerase II needs phosphorylation and proline hydroxylation for recognition by the pVHL complex and thus its ubiquitination (Kuznetsova et al. 2003). p53 protein is acetylated and ubiquitinated on the same Lys residues, and it seems that acetylation blocks also ubiquitination of unacetylated Lys residues and thus stabilizes p53 (Li M et al. 2002). In addition to acetylation, also methylation and sumoylation can be modifying same lysine residue as ubiquitin, and it is possible that these modifications regulate each other by competing for the same lysine residues. Sumoylation of lysine residue in IκBα and Smad4 has been demonstrated to block ubiquitination at an identical site, protecting these proteins from degradation (Desterro et al. 1998, Lin et al. 2003). Also protein association and dissociation of substrate can regulate its ubiquitination. Heterodimerization of the yeast mating factors MATa1 and MAT2, or homodimerization of nuclear factor NF-IL6 may mask an element that otherwise serves as the target for ubiquitination and proteasome degradation (Johnson et al. 1998, Hattori et al. 2003). It is also possible that dimerization activates subsequent proteasome processing such as in the case of dimerization of transcription factors ATF2 (activating transcription factor 2) and c-Jun (Fuchs and Ronai 1999).

3.4 Alternative ubiquitin signals and their function

A single protein can be ubiquitinated on one or several lysines with a single ubiquitin (monoubiquitination) moiety, with lysine-linked chains of ubiquitin (polyubiquitination) or combination of these two. Polyubiquitin chains can be built to the lysine residue of the target protein through repetitive conjugation of ubiquitin via distinct Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) of preceding ubiquitin (Peng et al. 2003). The fate of a target protein depends on the number of ubiquitin molecules conjugated as well as the lysine linkage in the ubiquitin chain (Fig. 9). In some cases, polyubiquitination of protein requires additional ubiquitin chain elongating factor, named E4 (Koegl et al. 1999). In yeast, E4 binds to the ubiquitin moieties of short conjugates and catalyzes ubiquitin elongation in conjunction with E1, E2, and E3. Interestingly, transcription coactivator p300 appears to possess the E4 activity, and polyubiquitinates p53, but p53 must be first monoubiquitinated by MDM2 (Grossman et al. 2003). Proteins modified with ubiquitin

polymer, where a Gly78-Lys48 linkage links four or more ubiquitins to one another, are generally directed to the 26S proteasome for degradation. Also ubiquitin chains assembled via Lys29 are involved in degradation of substrate (Johnson et al. 1995). The ubiquitin-proteasome system regulates transcription through removing transcription factors from the promoter region. For instance, the Pol II is polyubiquitinated upon DNA damage, and this is thought to lead to Pol II degradation during transcription-coupled repair (Lee et al. 2002, Woudstra et al. 2002). Lys6-linked ubiquitin chain on

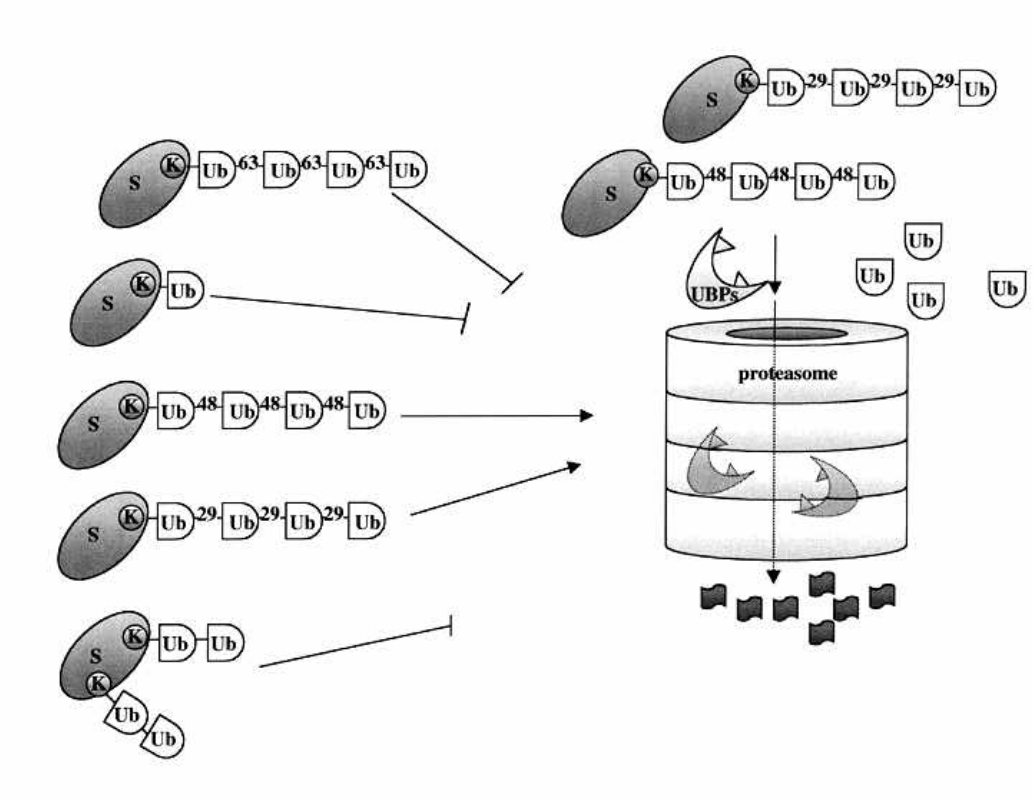


Fig. 9. Ubiquitin signals for degradation by the ubiquitin-specific proteases (UBPs) in the proteasome. Proteins that are modified by polyubiquitin chains containing Lys48- or Lys29-link between ubiquitins are directed to the proteasome for proteolysis. Ubiqutin is spared from degradation through its cleavage from target proteins, and it is recycled into the ubiquitination pathway. The target protein is hydrolyzed into small peptides within the proteasome. Other types of ubiquitin signals can regulate function of the target protein.

BRCA1 is generated by autoubiquitination activity of BRCA1-BARD1 dimer during DNA damage, and this autoubiquitination does not appear to serve a signal for proteasome, rather it enhances the E3 activity of BRCA1/BARD1 (Nisikawa et al. 2004, Mallery et al. 2002, Morris and Salomon 2004, Wu-Baer et al. 2003). Neither does a polyubiquitin chain linked through Lys63 serve a signal for the proteasome; it mediates kinase activation and DNA repair (Deng et al. 2000). The solution structure of Lys63-linked di-ubiquitin chain determined by NMR differs from that of Lys48-linked chain and probably the conformation of the latter chain is more compatible with the proteasomal recognition

signal (Varadan et al. 2004). In addition, Lys11, -27 and -33 ubiquitin chains has been observed, but the functions of these chains are unknown (Peng, et al. 2003). Lys-11 polyubiquitins on BAG-1 protein does not lead to degradation, but stimulation of the degradation-independent association of BAG-1 with the proteasome (Alberti, et al. 2002). Monoubiquitination does not normally target a protein for degradation, but appears to act as a signal for trafficking, DNA repair, gene silencing and vesicle sorting or as a substrate for polyubiquitin conjugation (reviewed by Johnson 2002 and Pickart 2000). Sumoylated lysine of PCNA (proliferating cell nuclear antigen) becomes monoubiquitinated during DNA damage by UV light (Stelter and Ulrich 2003). The same lysine can be further Lys63-polyubiquitinated, which is important in error-free repair of the damaged DNA (Hoege et al. 2002). Monoubiquitination is the principal signal responsible for the movement of receptor tyrosine kinases (RTKs) from the plasma membrane to the lysosome (Haglund et al. 2003, Jeong et al. 2002). Moreover, the monoubiquitination of p53 by MDM2 E3 ligase is critical for its nuclear export (Li M et al. 2003). Monoubiquitination of transcription regulators has been shown to be involved in transcription activation. Monoubiquitination of coactivator protein CIITA, which regulates the expression of major histocompatibility complex (MHC) II class gene, is suggested to enhance the assembly of CTIIA into a transcriptional complex at the promoter and thus increase gene expression (Greer et al. 2003).

Since ubiquitination plays an essential role in a broad array of cellular functions, the malfunction of this pathway will cause numerous diseases. In neurodegenerative diseases, such as the Angelman sydrome and Parkinson the ubiquitin E3 ligase, E6-AP or Parkin, respectively, or the ubiquitin carboxy-terminal hydrolase-1 (UCH-L1) have been shown to be mutated (Kishino et al. 1997, Shimura et al. 2000, Saigoh et al. 1999). The ubiquitin protease UCH BAP1 has been shown to enhance the growth inhibitory activity of BRCA1, and mutations in *BAP1* gene have been observed in some lung carcinoma cell lines (Jensen et al. 1998). Also mutations that interfere with ubiquitin E3 ligase activity of BRCA1 are found in breast cancer (Ruffner et al. 2001). MDM2 is overexpressed in a significant number of human tumors, which is thought to suppress normal p53 levels and suffocate the p53 response to cell stress (Reifenberger et al. 1993, reviewed by Michael and Oren 2003). Additionally, alterations of genes of the APC subunits have been found in colon cancer cell lines (Wang Q et al. 2003).

3.5 Ubiquitin-binding domains

The information of ubiquitin signals can be transmitted within the cell by proteins that bind noncovalently to ubiquitin and ubiquitinated proteins. Ubiquitin and poly-ubiquitin chains can be

recognized by different classes of ubiquitin-binding domains; the UIM (ubiquitin-interacting motif), the UBA (ubiquitin-associated), the CUE (coupling of ubiquitin conjugation to ER degradation), the UEV (ubiquitin E2 variant/UBC-like), the NZF (Npl4 zinc finger), the GAT (GGA/Tom1) and the PAZ (polyubiquitin associated zinc finger). The UBA domain consists of three helices and exposes hydrophobic interface, which contacts ubiquitin (Mueller and Feigon 2002). The UBA domain of RAD23 and that of Dsk2p have shown binding preference to Lys48-linked ubiquitin chains (Funakoshi et al. 2002, Raasi and Pickart 2003) and to be essential for their function in proteolysis (Rao and Sastry 2002). CUE and UBA are structurally similar and bind both mono-, polyubiquitin and ubiquitinated proteins (Bertolaet et al. 2001). The UIM motif forms an amphipathic α-helix and two or three UIMs can be present in a protein (Swanson et al. 2003). UIM and CUE domains promote monoubiquitination of proteins containing these sequences (Shih et al. 2003, Polo et al. 2002). The UIM domains of both Vps27 and Hse1 are needed for sorting of ubiquitinated proteins for degradation at the endosome (Bilodeau et al. 2002). The UEV domain is related to the catalytic domain of E2 enzymes, but lacks the catalytic cysteine that is essential for ubiquitin conjugation. In the Ubc13-Mms2 heterodimer the Mms2 protein binds ubiquitin through its UEV domain, which is required for linking ubiquitin molecules to one to another via Lys-63 isopeptide bond (Mckenna et al. 2001, Mckenna et al. 2003_a, Mckenna et al. 2003_b). Tsg101/Vsp23 UEV domain binds ubiquitin with a different interface than the UEV domain of Msm2. Ubiquitin binding of Tsg101/Vsp23 does not involve linking ubiquitin chains, but is required for sorting ubiquitinated proteins into the internal vesicles of multivesicular bodies (Katzmann et al. 2001, Bilodeau et al. 2003, Teo et al. 2004). The ubiquitin binding activity of the GAT domain in GGA protein is needed for ubiquitination within its GAT domain and ubiquitin-dependent transport to endosomes (Shiba et al. 2004, Scott et al. 2004). The PAZ domain of HDAC6 (histone deacetylase 6) interacts only with polyubiquitin and similar zinc finger domains are present in several DUBs and in BRCA1-associated proteins 1 and 2 (Hook et al. 2002). Ubiquitin binding of the Vsp36 NZF domain is required for efficient vacuolar sorting of ubiquitinated proteins in yeast (Alam et al. 2004). Although the NZF domain is structurally distinct from UIM, GAT, UBA and CUE, all of these domains bind the same hydrophobic surface (the "Ile-44" hydrophobic surface) of ubiquitin (Alam et al. 2004, Mueller and Feigon 2002, Kang et al. 2003, Swanson et al. 2003, Scott et al. 2004).

4. SUMO-1 CONJUGATION

Over the past 15 years, a number of proteins related to ubiquitin, UBLs (ubiquitin-like proteins), have been discovered. Most of these proteins resemble ubiquitin in their primary and higher order structure, but they have unique properties. These UBLs can be divided into two subclasses: Type-1 UBLs

SUMO, RUB/Nedd8 (Parry and Estelle 2004), FAT10 (Raasi et al. 2001), ISG15 (reviewed Ritchie and Zhang 2004), Ufm1 (Komatsu et al. 2004), Hub1/UBL5 (Luders et al. 2003), Atg12 and Atg8 (reviewed by Ohsumi and Mizushima 2004) are conjugated to target proteins in a manner similar, but not identical, to the ubiquitination pathway, whereas type-2 UBLs (also called UDP, ubiquitin-domain proteins), such as Parkin, RAD23, and DSK2 contain an ubiquitin-like domain that is not covalently conjugated to other proteins, but has been found to direct proteins to the proteasome (Chen and Madura 2002, Leggett et al. 2002). SUMO (small ubiquitin-like modifier) is probably the most investigated of the UBLs over the past few years.

4.1 The SUMO pathway

SUMOs (small ubiquitin-related modifiers) belong to a highly conserved protein family found in all eukaryotes. SUMO is structurally related to ubiquitin containing the ββαββαβ ubiquitin-fold, although SUMO-1 and ubiquitin share only ~18 % primary sequence identity (Bayer et al. 1998, Jin et al. 2001) (Fig. 8). However, the surface charge distribution of SUMO is very different from that of ubiquitin. SUMOs have also unstructured N-terminal extension that is not present in ubiquitin and that may provide an additional surface for protein interactions (Bayer et al. 1998). *Saccharomyces cerevisiae* contain a single SUMO protein encoded by the *SMT3* gene, while mammals contain three different SUMOs; SUMO-1 (also called PIC1, GMP1, Ubl1 and Smt3c), SUMO-2 (sentrin-3, Smt3a), and SUMO-3 (sentrin-2, Smt3b) (reviewed by Melchior 2000, Müller et al. 2001, Seeler and Dejean, 2003). SUMO-1 shares 48 % identity with SUMO-2, and SUMO-2 and SUMO-3 are 95% identical to each other. The crystal structure of the SUMO-2 is similar to that of SUMO-1 (Huang et al. 2004). Localization studies of SUMO variants showed that SUMO-1, -2 and -3 share a similar distribution all over the nucleoplasm, including PML NBs. However, SUMO-1 is also localized to nucleoli, the nuclear envelope and cytoplasmic foci. The distribution of different SUMOs change rapidly during cell cycle. During mitosis, SUMO-1 is more avidly localized to the mitotic spindle, and during late anaphase, it is recruited to re-formed nuclear envelopes and further accumulates in the nucleus. SUMO-2 and SUMO-3 share similar cell cycle-dependent localization, which is distinct from that of SUMO-1. For instance, they are not concentrated in spindle microtubules and they are found in chromatin regions earlier than SUMO-1 (Ayaydin and Dasso 2004). Recently, a human SUMO-4 gene was discovered, and its protein product has 87% homology with SUMO-2. Interestingly, a polymorphism in the SUMO-4 gene appears to be associated with type 1 diabetes (Bohren et al. 2004, Guo et al. 2004). Plants (*Aradopsis*), in turn, might contain up to eight SUMO genes (Lois et al. 2003, Kurepa et al. 2003).

Like ubiquitin, the precursor of SUMO requires processing to remove four amino acids from its C terminus by cysteine proteases (SUMO proteases) to reveal diglycine motif (Gly-Gly) for conjugation. Covalent attachment of SUMO on target protein is catalyzed by three enzymatic steps, which are analogous to the ubiquitination pathway, consisting of the SUMO-activating (E1) enzyme (heterodimer of SAE1/Aos1/Sua1 and SAE2/Uba2), the SUMO-conjugating (E2) enzyme (Ubc9), and several SUMO E3 ligases (PIAS/Siz, RanBP2, and Pc2). First, SUMO is activated by E1 in the presence of ATP and then it is transmitted to the active cysteine of E2 from where it is directed to the ε-amino group of lysine in the target protein. In contrast to ubiquitination pathway where ubiquitin ligases are responsible for target recognition, Ubc9 efficiently transfers SUMO to selected targets *in vitro*. However, the specific SUMO E3 ligase appears to be required for efficient modification *in vivo* (Fig. 10).

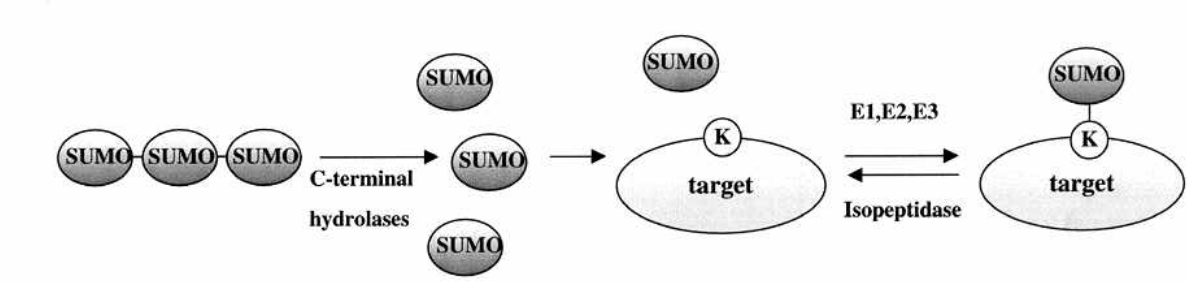


Fig. 10. The SUMO conjugation pathway. After SUMO is proteolytically processed by C-terminal hydrolases, it serves as the substrate in the ATP-dependent formation of an isopeptide bond between SUMO and the lysine in the target protein. This reaction is mediated by SAE1/SAE2 (E1), Ubc9 (E2) and SUMO ligases (E3). Sumoylation is a reversible reaction, since the isopeptide bond can be cleaved by isopeptidases (Adapted from Melchior 2000).

In most cases, the lysine residue is embedded in a consensus sequence, ψKxE/D, where ψ is a large hydrophobic amino acid (Ile, Leu, or Val), K is lysine, X is any amino acid residue, and E and D are glutamic and aspartic acid, respectively. Both Ubc9 and E3s contribute to substrate specificity. Sumoylation of the target protein can be regulated by other posttranslational modification. Phosphorylation of c-Jun, p53, IκBα and PML reduces SUMO-1 modification, whereas the heat shock factor 1 (HSF1) must be phosphorylated in order to be sumoylated (Müller et al. 1998, Hietakangas et al. 2003, Desterro et al. 1998, Everett et al. 1999). Also other lysine-dependent modifications, acetylation, methylation and ubiquitination may regulate sumoylation by modifying the same lysine residue (Sapetschnig et al. 2002, Braun et al. 2001, Desterro et al. 1998, Ross et al. 2003).

Very little is know about the functions of SUMO-2 and SUMO-3, but at least they have been implicated to play a role in the response to environmental stress (Saitoh and Hinchey 2000). As

SUMO-2 and SUMO-3 contain the ψKXE/D consensus motif in their N-terminal region, they can form SUMO chains (Tatham et al. 2001). Although SUMO-1 lacks this sequence, it may be able to form SUMO chains via different lysine acceptors (Pichler et al. 2002). SUMO chains may have important functions *in vivo*, since, for instance, they may affect amyloid protein processing and thus could be involved in the pathogenesis of Alzheimer´s disease (Li Y et al. 2003). Like ubiquitination, sumoylation is a reversible and dynamic process. Removal of SUMO from modified proteins is executed by SUMO-specific isopeptidases of the SUSP/SENP family.

4.2 Enzymes of the sumoylation pathway

4.2.1 SUMO-activating E1 enzyme

Most organisms have only a single SUMO-activating enzyme, E1, which is required for conjugation of all SUMO variants to their target proteins. The SUMO E1 is a heterodimer (SAE1/SAE2), which is structurally and functionally related to ubiquitin-activating E1 enzyme, UBA1. The SAE1 (SUMO activating enzyme 1, also called Aos1 and Sua1) subunit resembles the N-terminal half of UBA1, whereas the SAE2 (also called Uba2) subunit corresponds to the C-terminal part of UBA1 and contains the active site cysteine (Desterro et al. 1999, Gong et al. 1999, Okuma et al. 1999). However, Aradopsis has two SAE1 genes, whose products probably form a heterodimer with SAE2 (Kurepa et al. 2003). In the SUMO activation step, SAE1/SAE2 consumes ATP to adenylate the C-terminal glycine of SUMO-1 before it forms the high-energy thiolester bond between the C-terminal glycine of SUMO and the active site cysteine in SAE2.

4.2.2 SUMO-conjugating E2 enzymes

The SUMO moiety is transferred from SAE1/SAE2 to the active site cysteine (Cys 93) of the SUMO-conjugating enzyme (E2), forming a SUMO-E2 thioester complex. SUMO pathway involves only a single SUMO E2, Ubc9, which shares substantial sequence and structural similarity with ubiquitin E2s (Tong et al. 1997). The positively charged N-terminal region of Ubc9 binds non-covalently to the negatively charged surface of all SUMOs, but not with the positively charged surface of ubiquitin (Tatham et al. 2003, Liu et al. 1999). This may have a role in modifier discrimination since Ubc9 is specific to SUMO and does not function with ubiquitin (Desterro et al. 1997, Gong et al. 1997). The SUMO binding region of Ubc9 overlaps with that of E1, and E1 binding of Ubc9 is needed for the thiolester bond formation (Bencsath et al. 2002). Non-covalent SUMO binding of Ubc9 is important for the transfer of SUMO from E1 to E2, although it is distant from the C-terminal active site cysteine of Ubc9. This C-terminal region of Ubc9 also binds the ψKXE/D consensus motif in the substrate

(Tatham et al. 2003, Tong et al. 1997, Bernier-Villamor et al. 2002). Most of the SUMO targeted proteins are able to bind Ubc9.

4.2.3 SUMO E3 ligases

Even though Ubc9 is capable of recognizing and binding the SUMO modification site in a substrate, a specific SUMO E3 ligase may be required for efficient and properly targeted modification *in vivo*. SUMO E3 ligases have the capacity to associate with Ubc9 and increase the rate of target protein modification. To this date, only three kinds of SUMO E3 ligases have been discovered. The Siz/PIAS (Sap and Miz/protein inhibitors of activated STAT) protein family shares some similarity with RING-finger ubiquitin E3 ligases, particularly an essential RING-like motif (Johnson and Gupta 2001, Kahyo et al. 2001, Sachdev et al. 2001). In *S. cerevisiae*, Siz1p is required for sumoylation of septins *in vivo*, and it enhances *in vitro* sumoylation (Johnson and Gupta 2001, Takahashi et al. 2001). Mammalian PIAS proteins (PIAS1, -3, -x and -y, and splicing derivatives) bind Ubc9 with their RING finger-like domain and are able to bind the target protein (Kotaja et al. 2002, Liang et al. 2004) and SUMO. SUMO ligation activity is clearly dependent on the RING finger-like motif. PIAS proteins act as SUMO E3s for LEF1, p53, AR and c-Jun (Kahyo et al. 2001, Sachdev et al. 2001, Schmidt and Müller 2002, Kotaja et al. 2002). Recently, RanBP2 (Ran-binding protein 2)/Nup358 and Pc2 (Polycomb protein) were shown to have SUMO E3 ligase activity (Pichler et al. 2002, Kagey et al. 2003). RanBP2 has SUMO E3 activity toward MDM2, Sp100 and HDAC4, heterogeneous nuclear ribonucleoproteins, hnRNP C and M proteins (Kirsh et al. 2002, Pichler et al. 2002, Miyauchi et al. 2002, Vassileva and Matunis 2004). Pc2 is able to sumoylate the corepressor protein CtBP (carboxyl-terminal binding protein) (Kagey et al. 2003). Also SUMO E3 ligases are themselves sumoylated (Kotaja et al. 2002). Interestingly, all three ligase types localize to specific subcellular complexes suggesting that these structures may serve as modification sites. PIAS proteins are found in the close proximity of PML or other nuclear bodies, Pc2 in Polycomb group (PcG) bodies and RanBP2 in nuclear pore complex (NPC). The role of E3 ligases may be to stabilize the interaction between Ubc9 and the target protein.

4.2.4 SUMO proteases

SUMO proteases can act both in the maturation of the pre-SUMO (carboxy-terminal hydrolases) and in the cleavage of SUMO from modified proteins (isopeptidases). These cysteine proteases are distinct from ubiquitin-specific proteases, and they all contain a C-terminal ULP-domain (200 aa), which harbors the catalytically active region (Mossessova and Lima 2000). SUMO proteases have variable

N-terminal domains, which localize the enzymes to different regions in the cells and thus appear to be responsible for desumoylation of different proteins. *Saccharomyces cerevisiae* contains two SUMO proteases, Upl1 and Ulp2/Smt4 (Li and Hochstrasser 1999 and 2000). Ulp2p is predominantly located in the nucleus, whereas Ulp1p colocalizes with nuclear pore complex proteins. Ulp1 is capable of processing both SUMO maturation and SUMO substrate cleavage, and it is required for the G2/M transition of the cell cycle (Li and Hochstrasser 1999). Ulp2 is localized in the nucleus, but it is not essential for cell viability (Li and Hochstrasser 2000). In mammals, SENP1 is primarily a nuclear protease that is found in PML nuclear bodies. It has been shown to cleave SUMO from PML and histone deacetylase 1 (HDAC1) (Gong et al. 2000, Bailey and O´Hare 2004, Cheng et al. 2004). SENP2 in turn is a nuclear envelope-associated protease (Hang and Dasso 2002). SuPr-1, a splice variant of mouse SENP2, is able to alter the distribution of Daxx, CBP and Sp3 in PML NBs (Best et al. 2002, Ross et al. 2002). SENP3/SMT3IP1 is localized in the nucleolus (Nishida et al. 2000) whrereas SENP6/SUSP1 is found in the cytosol (Kim et al. 2000).

4.3 Function of SUMO-1

A number of transcription factors, and chromatin-associated and DNA repair proteins have shown to be posttranslationally modified by SUMO-1. SUMO-1 modification regulates protein-protein interactions, protein stability, activity and conformation, and subcellular localization. For instance, sumoylated form of the RanGAP, a GTPase-activating protein that plays a role in nuclear import, binds more actively to the nuclear pore complex (NCP)-anchored RanBP2. This may enable RanGAP to be recruited from cytoplasm to the NCP (Matunis et al. 1996, Mahajan et al. 1997). In addition to SUMO-1 conjugation, other regions of RanGAP are needed for proper interaction with RanBP2, suggesting that the binding is mediated through regions of SUMO-1 and RanBP2 or the sumoylation induces structural changes, which then reveals the RanBP2-binding element of RanGAP (Matunis et al. 1998 499). Moreover, the SUMO-1-modified RanGAP is found in the spindle microtubules during mitosis (Joseph et al. 2002). Translocation of the MAP kinase MEK1 and the NF-κB essential regulator protein (NEMO) between the nucleus and the cytosol is regulated by sumoylation (Galy et al. 2002, Huang et al. 2003). Subnuclear localization of PML is regulated by SUMO-1 conjugation, while SUMO-1-modified PML exists in nuclear PML NBs (Kamitani et al. 1998, Duprez et al. 1999). SUMO-1-modified PML recruits many transcription factors and chromatin modifiers, such as Sp100, CBP, Daxx, to PML NBs (Kamitani et al. 1998, Müller et al. 1998, Duprez et al. 1999, Ishov et al. 1999). Many of these proteins are also SUMO-1 modified (reviewed by Zhong et al. 2000), but it

seems that sumoylation of PML rather than sumoylation of other proteins is the controlling factor in PML NB formation (Hofmann et al. 2000, Fogal et al. 2000), e.g. sumoylation deficient Sp100 is recruited PML NBs (Sternsdorf et al. 1997). However, SUMO-1 conjugation of Sp100 enhances its interaction with heterochromatin protein 1 (HP1) *in vitro* (Seeler et al. 2001), suggesting that this PML-associated protein may recruit additional proteins via its SUMO-1 modification. Interestingly, different SUMO variants may direct certain SUMO target proteins to different subcellullar compartments (Saitoh and Hinchey 2000). Transcription factor SATB2 localizes to nuclear dots when conjugated to SUMO-1, but to the nuclear periphery when conjugated to SUMO-3 (Dobreva et al. 2003).

Many transcription factors and coregulators are sumoylated, suggesting that sumoylation plays a role in the regulation of gene expression. In most cases, sumoylation appears to repress transcription. For example, when SUMO-1 is fused to the DNA-binding domain of Gal4, this fusion protein represses transcription. This result may indicate that SUMO-1 is able to recruit transcriptional repression proteins, such as HDACs, to the promoter (Ross et al. 2002, Yang et al. 2003). Interestingly, the coactivator protein p300 interacts with the HDAC6 only when the repressor domain of p300 is sumoylated (Girdwood et al. 2003). Transcription is also repressed when sumoylated transcription factor Elk-1 binds to HDAC2 (Yang and Sharrocks 2004). In addition, sumoylation of histone H4 represses transcription, which is likely to occur through interaction with corepressor proteins HDAC1 and HP1 (Shiio and Eisenman 2003). An interesting example of sumoylation is that of glucocorticoid receptor (GR). GR contains so called synergy control motifs, which are found in several transcription factors, like in AR, and attenuate the activity of promoters having multible GR-binding elements (GREs), but not that of promoters with single GRE-sites (Iniguez-Lluhi and Pearce 2000). These synergy control motifs harbor the classical SUMO-1 attachment sites, and they are indeed sumoylated, which is suggested to prevent the synergistic action of multible GRs bound to the same promoter (Iniguez-Lluhi and Pearce 2000, Tian et al. 2002, Subramanian et al. 2003, Poukka et al. 2000_b). Although sumoylation of transcription factors is mainly correlated with transcriptional repression, SUMO-1 has also been shown to have positive effects on transcription. For example, sumoylation of the coactivator GRIP1 (SRC-2) facilitates AR-interaction and thus enhances AR-mediated transcription (Kotaja et al. 2002b). In addition, it has been suggested that sumoylation of the transcription factor Tcf-4 is needed for its activation function (Yamamoto et al. 2003).

SUMO-1 modification has been shown to protect some proteins from proteasome-mediated degradation. Sumoylation stabilizes IκBα and Smad4 likely by competing with the same lysine residue involved in ubiquitination (Desterro et al. 1998, Lin et al. 2003). Interestingly, degradation of

CREB by ubiquitin-proteasome system leads to induction of proinflamatory genes right after hypoxic stress, but it is stabilized via sumoylation one or two days after the stress (Comerford et al. 2003). Yeast proliferating cell nuclear antigen (PCNA) can be modified on the same lysine by SUMO-1 and ubiquitin. However, the SUMO-1 modification regulates the function rather than the stability of PCNA (Stelter and Ulrich 2003, Haracska et al. 2004). Sumoylation of yeast PCNA may promote replication by increasing the activity of translesion DNA polymerase and reducing the recombinant-dependent bypass mechanism in the S phase, while ubiquitination of PCNA may promote DNA repair processes (Stelter and Ulrich 2003, Haracska et al. 2004, Hoege et al. 2002).

SUMO-1 is able to alter the activity of thymine DNA glycosylase (TDG) that is involved in base excision repair during DNA damage (Hardeland et al. 2002). The unmodified TDG binds and excises a mutant base, becomes sumoylated and then dissociates from the DNA product. The affinity of TDG towards its DNA substrate is reduced possibly due to a conformational change of TDG, where an intramolecular interaction occurs between the SUMO-1-binding motif of TDG and the conjugated SUMO-1. Further, SUMO proteases might restore the enzymatic activity of TDG. In the case of heat shock factors 1 and 2 (HSF1 and HSF2), sumoylation enhances their binding activity to DNA *in vitro* (Hong et al. 2001, Goodson et al. 2001). *In vivo*, heat shock treatment induces sumoylation of HSF1, which is linked to the activation of stress-induced gene expression.

Several proteins have been shown to bind non-covalently to SUMO-1 and some SUMO-1-binding motifs have been identified (Minty et al. 2000, Engelhardt et al. 2003, Rosendorff et al. 2004 , Song et al. 2004). According to Minty et al. (2000), protein motifs containing Ser/Thr- and Glu/Asp-rich sequences preceded by hydrophobic residues are able to mediate the interaction with free SUMO-1. Rosendorff et al. (2004) found that acidic amino acid-containing regions followed by hydrophobic residues [consensus: $(D/E)_3V/TIEV$] provide binding platform for SUMO-1. A recent study by Song et al. (2004), in turn, showed that hydrophobic regions possessing V/IxV/IV/I motif (SMB) mediates SUMO-1 interaction rather than the consensus sequence proposed by Minty et al. (2000). Indeed, SUMO-1-binding proteins like PML, PIASx and SAE-2 contain the latter SMB-motif. In addition, SMB is also capable of recognizing all three SUMO variants (SUMO-1, -2, and -3) (Song et al. 2004). Many proteins that bind noncovalently to SUMO-1, such as Daxx, IE2 and Dnmt3b, PIAS and PML, are also SUMO-1 modified (Ryu et al. 2000, Kotaja et al. 2002, Kang et al. 2001, Ahn et al. 2001). It has been suggested that both covalent and non-covalent SUMO-1 binding enhances the complex formation between various SUMO-1 modified proteins. For instance, PML NB-associated proteins, such as Sp100, Daxx, CBP and HIPK2, contain the latter SMB-motif. Also HDAC6 and HDAC2 that

are recruited by sumoylated p300 and Elk-1, respectively, contain the SMB (Bernier-Villamout et al. 2002, Yang and Sharrocks 2004).

Sumoylation has been reported to play a role in some diseases, such as in neurodegenerative disorders and infections. In Huntington´s disease (HD), SUMO modification of Huntingtin protein appears to contribute to HD pathology (Steffan et el. 2004). Several viral and bacterial pathogens may benefit from the host cell sumoylation system. For instance, *Yersinia pestis* contains a SUMO protease homology protein YopJ, whose activity contributes to downregulation of host immune response (reviewed by Wilson and Rangasamy 2001). An interesting study on Gam1 protein, which is essential for the replication of the avian adenovirus CELO, demonstrates that Gam1 is able to interfere with the SUMO-1 pathway and thus may alter gene expression of the host cell. Gam1 binds E1 heterodimer complex, which results in the inhibition of E1 activity and whole sumoylation pathway *in vitro* and *in vivo*. In addition, the protein levels of SAE-1 and -2, and Ubc9 are down-regulated upon CELO infection or Gam1 overexpression (Boggio et al. 2004). Moreover, many tumor suppressors and oncogenes are modified and regulated by SUMO and, therefore, they may contribute to the development of cancer. Interestingly, the acute promyelocytic leukaemia (APL) is successfully treated with arsenic trioxide, which restores sumoylation of the PML-RARα-fusion protein (Müller et al. 1998).

5. NUCLEAR STRUCTURES

The nucleus is the place for many vital and primary biochemical events. Recent advances in imaging technologies have confirmed that the nucleus contains compartments and domains with specialized functions in a fashion similar to the cytoplasm. Some nuclear domains have a dynamic structure and rapid exchange occurs between many domains and the nucleoplasm (reviewed by Misteli 2001). The nucleus is surrounded by a nuclear envelope, which is a double membrane structure that harbors nuclear pores through which the material between the nucleus and cytoplasm is transported (Stofler et al. 1999). Nuclear DNA is organized into distinct chromosomal territories, where different chromosomes display different gene densities, transcriptionally silent heterochromatin and transcriptionally active euchromatin (Boyle et al. 2001, Tanabe et al. 2002). Also various protein-rich regulatory compartments are represented with distinct domains, such as the PML nuclear bodies, Cajal bodies and nucleolus. Nucleoli are sites of ribosomal RNA synthesis and processing and assembly of ribosomal subunits (Spector 1993). Cajal bodies (or coiled bodies) contain pre-mRNA splicing factors and they are implicated in snRNP assembly, snRNA metabolism and posttranscriptional modification of newly assembled of splicesomal snRNAs (Matera 1999, Sleeman et al. 1999, Stanik et al. 2003).

PML bodies have been suggested to play a role in transcriptional regulation and to be targets of viral infections. The nuclear matrix is maintaining the spatial arrangement of the genome and other nuclear components. Below, the chromatin structures, PML NBs, transcriptionally active regions and nuclear matrix is presented in more detail in light of transcription control.

5.1 Chromatin

Mammalian DNA has to be firmly packaged in order to fit inside the nucleus. Chromatin is composed of nucleosomes in which 146-bp of DNA is wrapped around a histone octamer that consists of two molecules each of core histones H2A, H2B, H3 and H4. Each nucleosome is separated from the next one by a region of linker DNA to which the linker histone H1 and high mobility group proteins (HMGs) bind (reviewed by Hill 2001). This string of nucleosomes can be further condensed to a 30-nm fibre and further wound into higher order structures, larger solenoid structure and finally into chromosomes (Horn and Peterson 2002). The majority of DNA is not expressed and thus forms condensed heterochromatin structures. Current study of chromatin fiber structure of the human genome by Gilbert et al. (2004) showed that heterochromatin is surprisingly heterogeneous structure and there is not a simple structural division between heterochromatin and transcriptionally active euchromatin. Gilbert and coworkers also presented that open chromatin fibers (active region) correlate with regions of the highest gene density, but not necessarily with gene expression in view of the fact that inactive genes can be found in domains of open chromatin, and conversely active genes within regions of low gene density can be found in condensed chromatin fibers. Thus, the ability of a gene to be activated does not simply depend on the condensation state of chromatin density. It was thought that heterochromatin contains a network of stable protein-protein interactions that block access of transcription factors to the silent genes.

Recent studies, which demonstrate the mobility of the heterochromatic proteins in living cells by FRAP-technique (fluorescence recovery after photobleaching), have revealed that heterochromatin-interacting proteins are not stably-bound but are rather in a constant flux (Cheutin et al. 2003, Festenstein et al. 2003). Non-histone HP1 is recruited by methylation of histone H3 tail (N terminus) and is believed to promote the formation of these dense chromatin structures and to act as a corepressor of genes within transcriptionally active chromatin (Grewal and Elgin 2002, reviewed by Li Y et al. 2002). The mobility of HP1 increases during cell activation, and the phosphoacetylation of H3 tails release the HP1 from chromatin (Mateescu et al. 2004).

Modulation of chromatin structure plays a central role in the regulation of cellular processes, such as DNA replication, repair, recombination and transcription (reviewed Kornberg and Lorch 1999). Two most important enzymatic activities that regulate the chromatin access during transcription are chromatin remodelling and covalent chromatin modification activities. The histone acetyltransferase complexes and ATP-dependent chromatin remodelling complexes cooperate with sequence-specific transcription factors to help the transcriptional machinery gain access to the promoter of certain genes (Näär et al. 2001).

Covalent modifications of chromatin that include acetylation, methylation, phosphorylation, ADP-ribosylation, sumoylation and ubiquitination usually occur on the N-terminal tails of core histone that protrude from the nucleosomes, with the exception of ubiquitination that occurs at the C-terminal tails of H2A and H2B (reviewed by Strahl and Allis 2000 and Berger 2001). Acetylation on lysine residues of histone tails by histone acetyltransferases correlates with the gene activity. In transcription activation, the HAT component acetylates nucleosomes in the vicinity of the promoter and recruits chromatin remodelling components, such as SWI/SNF (Vignali et al. 2000, Syntichaki et al. 2000). Then these remodelers mobilize nucleosomes by remodelling nucleosomes (the bulging mechanism) or removing them from the promoter (Boeger et al. 2003, Reinke and Hortz 2003). Acetylation of the promoter region can also stabilize the interactions of transcription machinery with the active chromatin region. The changes in chromatin followed from histone acetylation are reversed by deacetylation by histone deacetylases (reviewed by Kurdistani and Grunstein 2003, Robyr et al. 2002).

Also DNA methylation on CpG nucleotides silences gene expression, possibly through recruitment of HDACs and histone methyltransferases by methyl CpG-binding proteins or induction of the formation of chromatin structures that decrease the efficiency of Pol II elongation (reviewed by Li 1999 and Bird and Wolffe 1999, El-Osta et al. 2000, Lorincz et al. 2004, Fuks et al. 2003). Phosphorylation of all five histones has been reported (van Holde 1989). Phosphorylation of linker histone H1 stabilizes higher order chromatin structure and hinders the access of transcriptional coactivators to the promoter DNA (Horn et al. 2002, Lee and Archer 1998, Cheung et al. 2002, Hill and Imbalzano 2000). Phosphorylation of H3 on Ser10 is crucial for chromosome condensation and cell cycle progression during mitosis and meiosis (Gurley et al. 1978), but it is also important for gene expression, probably via acting as a binding site for transcription machinery (Barratt et al. 1994).

Methylation occurs both on arginine and lysine residues of H3 and H4 by histone methyltransferases (Bannister et al. 2002, reviewed by Fischle et al. 2003 and Kouzarides 2002).

Histone methylation have multiple effects on chromatin structure, since dimethylation of Lys9 and trimethylation of Lys27 in H3 are associated with gene silencing, while methylation of Lys4, Lys36 and Lys79 is associated with gene activation (reviewed by Kouzarides 2002). Also ubiquitination of H2B correlates with gene expression (Nickel et al. 1989). Yeast H2B ubiquitination has been suggested to have an overlapping function with acetylation of H3, through the parallel recruitment of chromatin remodeling factors (Johnson 2002). However, ubiquitin-conjugation to H2B can also lead to H3 methylation and gene silencing in yeast (Sun and Allis 2002, Dover et al. 2002). Interestingly, sumoylation of H4 leads to association with HDAC and HP1, and at least within an artificial reporter model; this correlates with gene silencing (Shiio and Eisenman 2003).

5.2 Nuclear matrix

The nuclear matrix or nucleoskeleton is a structural framework within nucleus that has been implicated in a number of cellular events and arrangement of chromatin (reviewed by Nickerson 2001). The nuclear matrix consists of the nuclear lamina that lies at the interface between chromatin and inner nuclear membrane, and the internal fibrogranular ribonucleoprotein framework, which contains over 200 nuclear matrix proteins, such as lamins, heterogeneous ribonucleoprotein (hnRNP) and nuclear mitotic apparatus protein (NuMA) (Nickerson et al. 1997, Monneron and Bernhardt 1969, Fey et al. 1986, Mattern et al. 1996, reviewed by Mancini et al. 1996, Goldman et al. 2002). The protein composition of nuclear matrix varies in a cell type- and developmental stage-dependent manner (Fey and Penman 1988, Dworetzky et al. 1990). Various nuclear proteins interact with the nuclear matrix, which supports their assembly into functional multisubunit complexes involved in transcription, RNA splicing and DNA replication (Nickerson 2001). Nuclear matrix proteins, such as SAF-A (scaffold attachment factor A) and ARBP (attachment region binding protein), that specifically bind AT-rich DNA sequences called S/MARS (scaffold/matrix-associated regions) generate an active and silenced chromatin state by recruiting HATs and HDACs, respectively (Martens et al. 2002). The SABT1 (special AT-rich sequence binding 1) targets chromatin remodelling factors to specific chromatin domains and it interacts with RNA (Yasui et al. 2002, Durrin and Krontiris 2002). Nuclear matrix proteins can also bind chromatin via association with HP1 (Ye and Worman 1996) and transcription factors, such as retinoblastoma protein (Rb), GR, AR and active phosphorylated Pol II (Mancini et al. 1994, Markiewicz et al. 2002, van Steensel et al. 1995, Tang and DeFranco 1996, Patturajan et al. 1998, Nayler et al. 1998). Interestingly, disruption of normal nuclear lamin assembly significantly inhibits Poll II activity, but not that of Pol I or Pol III (Spann et al. 2002, Kumaran et al. 2002). Taken together, these results suggest that nuclear matrix plays an important role in gene expression.

5.3 Sites of transcription

To identify the sites of gene expression in the nucleus it is important to elucidate the relationship of nuclear architecture and genomic function. The sites of RNA synthesis have been examined by labelling permeabilized cells with bromouridine triphosphate or bromouridine and light and electron microscopy. The majority of labelled transcripts is concentrated at 500-1000 transcription foci (40-80 nm) in proliferating mammalian cell (Jackson et al. 1998, Pombo et al. 1999, Elbi et al. 2002, Grande et al. 1997), but Pol II is also localized to numerous domains outside these “transcription factories” (Jackson et al. 1998). A recent study by Osborne et al. (2004) shows that many transcriptionally active genes colocalize into the same transcription factory at a high frequency and inactive genes are localized apart from these sites. Thus, these results suggest that activated genes are recruited to preassembled Pol II compartments rather than that each gene assembles its own transcription complex.

5.4 PML nuclear bodies

The promyelocytic leukaemia (PML) bodies (also known as PML nuclear body, PML NB; PML oncogenic domain, POD; nuclear domain 10, ND10; Kremer body, Kr-body) are nuclear domains that are disrupted in human acute promyelocytic leukaemia (APL) cells (Dyck et al. 1994, Koken et al. 1994). A typical cell nucleus contains about 10-30 PML NBs with the appearance of doughnut-shape and size variation from 0.2 to 1.0 μm in diameter. The number of PML NBs varies between cell types and the stage of cell cycle (Zhong et al. 2000b, Everett et al. 1999). Several models have been proposed for the function of the PML NBs. They have been thought to serve as storage pools for proteins waiting for degradation or to modulate nuclear protein concentrations by preserving them at PML bodies until needed (reviewed by Maul et al. 2000). In addition, PML NBs may serve as a location of post-translational modification of its protein components (Hofmann et al. 2002, D´Orazi et al. 2002, Bernassola et al. 2004). The major structural component of PML NBs is the PML protein. PML knock-out mice are viable, but sensitive to tumor-promoting agents and display chromosome instability, suggesting that PML is a tumor suppressor protein (Wang et al. 1998a and 1998b). Chromosomal translocation of the PML gene on chromosome 15 into the proximity of the RARα gene on chromosome 17 in APL cells results in PML-RARα-fusion protein that mislocalizes PML and thus destroys PML NBs into smaller domains (Melnick and Licht 1999, Koken et al. 1994, Dyck et al. 1994, Daniel 1993). Treatment of leukemic cells with retinoic acid or arsenic trioxide (As_2O_3) results in reformation of PML NBs (Huang et al. 1988, Zhu et al. 1997 and 2002). More than forty proteins,

including SUMO-1, nuclear antigen Sp100, p53 and CBP are listed to localize either transiently or constitutively with these domains (Zhong et al. 2000a, Hodges et al. 1998, Dellaire et al. 2003, Zhong et al. 2000b).

PML NBs are thought to represent sites of specific nuclear processes, such as transcription, DNA replication or repair, and appear to be the targets of viral infections (reviewed by Maul 1998 and Everett 2001). PML NBs are regularly found in juxtaposition to distinct nuclear structures, such as splicing speckle domains and Cajal bodies, and are tightly associated with nuclear matrix (Dyck et al. 1994). Recent studies have proven that PML NBs are mobile (Muratani et al. 2002, Görisch et al. 2004) and that the dynamics and accessibility of chromatin may target nuclear bodies to specific nuclear subcompartments where they carry out their biological function (Görisch et al. 2004). Interestingly, PML NBs are stably surrounded by chromatin, but during inhibition of transcription and chromatin condensation or cleavage, the PML NBs breakdown into microstructures, “PML microbodies”, suggesting that chromatin integrity and condensation stage can influence also PML NB structure and stability (Eskiw et al. 2004). Similar to transcription inhibition, PML NBs are disrupted by heat-shock or heavy metal stress, but the released subunits of PML NBs are distinct from “PML microbodies”, since they lack Sp100 and SUMO-1 (Eskiw et al. 2003). PML NBs may also regulate chromatin structure, since HP1 and chromatin-modifying proteins HATs and HDACs accumulate in PML NBs (Seeler et al. 1998, Wu et al. 2001, Doucas et al. 1999, Boisvert et al. 2001). Many transcription regulators, such as HIPK2, p53, BRCA1 and MDM2, concentrate into PML NBs, and the transcriptional activity of these nuclear bodies is further supported by demonstration of nascent RNA on the surface of the PML NBs and association specific gene loci with PML NBs (Kim YH et al. 1999, Louria-Hayon et al. 2003, Xu et al. 2003, Boisvert et al. 2000, Shiels et al. 2001, Sun et al. 2003). PML may regulate p53- and the Sp1-mediated transcription by recruiting these transcription factors into PML NBs (Vallian et al. 1998, Fogal et al. 2000). On the other hand, RNA polymerase II, TFIIF or DNA has not been found in these nuclear domains (Boisvert et al. 2001), arguing against PML NBs to be the sites of active transcription.

AIMS OF THE STUDY

We were interested in identifying androgen receptor (AR)-interacting proteins that interact with the DNA-binding domain (DBD) and the hinge region of AR. The aim of this study was to characterize the biological function of one of these newly discovered AR-binding proteins, termed small nuclear RING finger protein (SNURF), in more detail. The specific aims of this study were:

- To examine the function of SNURF in the AR-dependent and -independent transcriptional regulation
- To investigate the functional regions of SNURF by searching for interaction partners
- To study the potential role of SNURF as a RING ubiquitin E3 ligase
- To study SNURF as a target of covalent modifications
- To examine subcellular localization of SNURF

MATERIALS AND METHODS

Materials and methods used in this study are presented in the original publications (I-V) as indicated in Table 1, or under the title of *Methods of unpublished results.*

Table 1. The methods used in original publications.

Method	**Original publication**
yeast two-hybrid screening and interaction assays	I
cDNA cloning and sequencing	I
northern blotting	I
cell culture and transfections	I, II, III, IV, V
mammalian two-hybrid interaction assays	I
immunohistochemistry	I
immunocytochemistry	I, II
confocal microscopy	V
immunoprecipitation	I, IV, V
chromatin immunoprecipitation (ChIP)	V
SDS-PAGE and immunoblotting	I, II, III, IV, V
plasmid construction and recombinant DNA techniques	
- in bacteria	I, II, III, IV, V
- the baculovirus/insect cell system	I
- in mammalian cells	I, II, III, IV, V
GST pull-down assay	I, II, III, V
electrophoretic mobility shift assay (EMSA)	II
gel filtration chromatography	IV
in vitro ubiquitination	IV
in vitro sumoylation	V

Methods of unpublished results

RNA- EMSA

A central region of SNURF gene (71-280 bp) was inserted between *Pst*I and *Hinc*II of pGem3Z vector (Promega*). In vitro* transcribed RNA (232 ribonucleotides) was produced as described in *in vitro* RNA synthesis protocol of Promega. Shortly, pGem3Z-SNURF(71-280 bp) was linearized with *Xba*I and the template DNA was purified by phenol/chloroform extraction and ethanol precipitation. The DNA template was incubated with Rnase inhibitor, ATP, UTP, GTP, SP6 RNA polymerase and [γ^{32}P]CTP and incubated for 20 minutes at 37 °C. Samples were treated with DnaseRQ1 for 15 minutes at 37 °C and the transcribed RNA was purified with gel filtration. GST-SNURF (60 ng) was incubated with ^{32}P-RNA-probe and 4 units of Rnase inhibitor in a buffer containing 20 mM Hepes (pH 7.9), 50 mM KCl, 1 mM $MgCl_2$, 10% (v/v), 2 mM DTT, 0.35 mM EDTA, 0.025% (v/v) Nonidet P-40, 0.25 mM PMSF, 2 µg/ml pepstatin A, 2 µg/ml leupeptin, and 2 µg/ml aprotinin in a reaction vol of 20 µl for 30 minutes at room temperature. Competitors, yeast tRNA (1 µg) (Sigma) and pGem3z-vector (200 ng) were included before addition of the ^{32}P-RNA probe. Protein-RNA complexes were resolved on 4% polyacrylamide gel in 0.25 x Tris-borate-EDTA at °4C. Complexes were detected by autoradiography.

In vitro phosphorylation

GST-SNURF protein was produced in bacteria and purified as described (Häkli et al. 2000). Casein kinase II (CKII) was obtained from Promega. GST-SNURF (1 µg) was incubated with 10 ng of CKII, 100 µM ATP, 1 µCi [γ^{32}P]ATP in a buffer containing 25 mM Tris-HCl (pH 7.4), 200 mM NaCl, 1 mM DTT, 10 mM $MgCl_2$, in a volume of 40 µl. Samples were incubated at 37 °C for 1 h and reactions were terminated by adding concentrated SDS-PAGE buffer and heat denaturation. Samples were fractioned by 15% SDS-PAGE and detected by autoradiography. Alternatively, GST-SNURFs (1 µg) resolved on SDS-PAGE gels were stained with Coomassie staining.

RESULTS AND DISCUSSION

1. SNURF POSSESSES PROTEIN- AND DNA-BINDING ACTIVITY (I, II, III, IV and V)

Characteristics of SNURF

In addition to mediating dimerization and binding to response element in DNA, the DNA-binding domain of nuclear receptor can act as a binding platform for regulatory proteins. We were interested to find out proteins that interact with the DBD and the hinge region of androgen receptor. Yeast two-hybrid screening was used to screen a mouse embryonic day 10.5 cDNA domain library, in which the amino acid residues 554–644 comprising the DBD and N-terminal part of the hinge region of human AR fused to LexA DNA-binding domain was used as a bait. A novel protein termed small nuclear RING finger (SNURF) was one of the discovered proteins. SNURF mRNAs of 3-kb and 1.6-kb in size were identified when SNURF cDNA was used as a probe in Northern blotting of various rat and human tissues. The 3-kb SNURF mRNA was found in all tissues, but the smaller, 1.6-kb mRNA was expressed only in the rat testis. The human and mouse SNURF homolog, which is called RNF4, was identified by Chiariotti and coworkers in the same year as SNURF (Chiariotti et al. 1998). RNF4 shows expression pattern similar to that of SNURF, and its gene was mapped to chromosome 4p16.3, which is associated with different neoplastic diseases (Chiariotti et al. 1998, Galili et al. 2000, Shivapurkar 1999, Caron et al. 1996).

Full-length SNURF cDNA was isolated from rat testis cDNA library by conventional hybridization methods, and an open reading frame (ORF) with 194 amino acid residues was revealed. The mouse and human RNF4 exhibit 97% and 91% sequence identity with SNURF, respectively (Fig. 11A), but no other homologs are found. Based on the primary SNURF sequence, a bipartite NLS in the N-terminal part, a SV-40-like NLS in the central region of SNURF, and a RING finger (C_3HC_4) structure in the C terminus (amino acids 136-180) (Fig. 11B), which is fully conserved in RNF4 (Fig. 11A), were identified in rat SNURF.

A

```
                          20
SNURF 1   MSTRNPQRKRRGGAVNSRQTQKRTRETTSTPEISLEAEPIELVETVGDEIVDLTCESLEPVVVDL 65
mRNF4 1   MSTRNPQRKRRGGTVNSRQTQKRTRETTSTPEVSLETEPIELVETVGDEIVDLTCESLEPVVVDL 65
hRNF4 1   MST----RKRRGGAINSRQAQKRTREATSTPEISLEAEPIELVETAGDEIVDLTCESLEPVVVDL 61
                 +  + + + +          -  -  -  -  -  -  -  -

                                                                      121
SNURF 66  THNDSVVIVEERRRPRRNGRRLRQDHADSCVVSSDDEELSKDKDVYVTTHTPRSTKDEGTTGLRP 130
mRNF4 66  THNDSVVIVEERRRPRRNGRRLRQDHADSCVVSSDDEELSRDKDVYVTTHTPRSTKDDGATGPRP 130
hRNF4 62  THNDSVVIVDERRRPRRNARRLPQDHADSCVVSSDDEELSRDRDVYVTTHTPRNARDEGATGLRP 126
                  + + + +          -  -  -

              136                                                 180
SNURF 131 SGTVSCPICMDGYSEIVQNGRLIVSTECGHVFCSQCLRDSLKNANTCPTCRKKINHKRYHPIYI 194
mRNF4 131 SGTVSCPICMDGYSEIVQNGRLIVSTECGHVFCSQCLRDSLKNANTCPTCRKKINHKRYHPIYI 194
hRNF4 127 SGTVSCPICMDGYSEIVQNGRLIVSTECGHVFCSQCLRDSLKNANTCPTCRKKINHKRYHPIYI 190
                                                          +  +  +
```

B

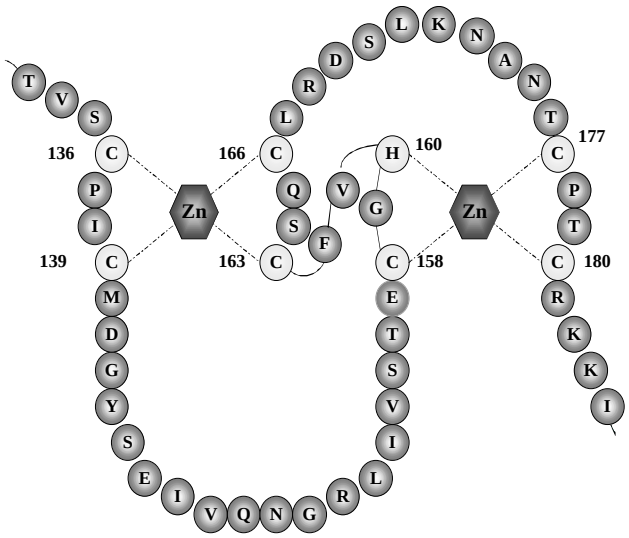


Fig. 11. A, Sequence alignment of SNURF and its mouse (mRNF4) and human (hRNF4) counterparts. RING finger (C_3HC_4) motif is indicated by dark grey shading and the cysteine and histidine residues mediating putative zinc binding within the RING finger motif are bolded. Bolded italics present amino acid residues that differ between the three homologous sequences, and dash lines indicate absent amino acid residues. Underlined sequences present NLSs, and the grey shading region between amino acids 20-121 is AR-interacting region. Basic and acidic aa regions are indicated + and –, respectively. Protein database sequence identification numbers: SNURF (AAC35248), mRNF4 (AAF00620) and hRNF4 (AAC52022). **B**, Schematic "cross-braced" structure of SNURF/RNF4 RING finger motif. Zinc (Zn)-coordinating amino acid residues are numbered and circled with light grey spheres.

SNURF is a hydrophilic protein, and its charge distribution is asymmetrical with alternating basic and acidic amino acid clusters. Such charged regions have been involved in DNA binding and transcriptional activation (van Hoy et al.1993, Niessing et al. 2003). This electrical asymmetry of

SNURF may be responsible for the slower migration rate (~35 kDa) on SDS-PAGE than expected from the molecular mass calculations (~22 kDa).

SNURF and protein-protein interactions

RING fingers have been shown to mediate protein-protein interactions and the formation multi-protein complexes (Saurin et al. 1996). The interaction partners of SNURF/RNF4 and their corresponding interaction regions in SNURF/RNF4 are presented in Figure 12. The binding partners of SNURF found in this study are presented below the schematic structure of SNURF.

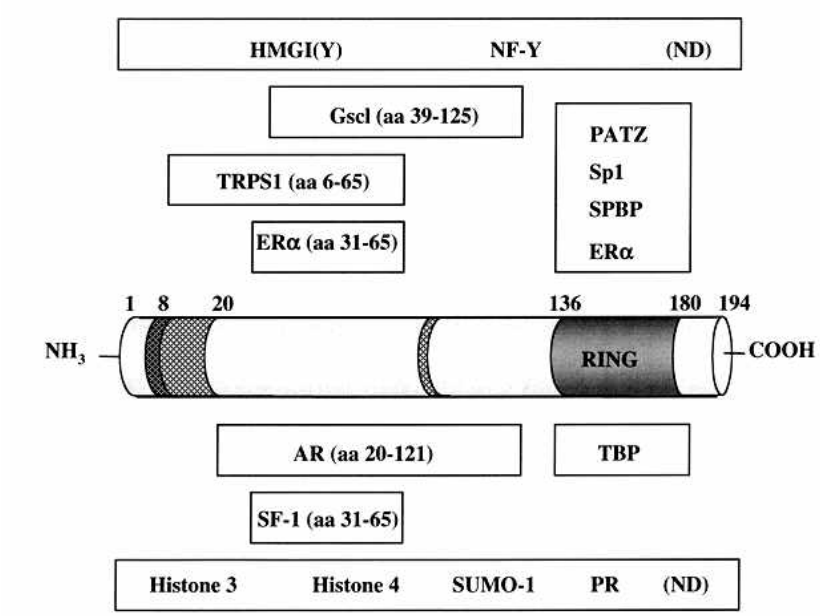


Fig. 12. Interaction partners of SNURF. Schematic presentation of the interaction sites of SNURF binding partners, and SNURF amino acid (aa) region important for the protein binding is indicated. ND; SNURF binding region not detected, RING; RING finger; ▨ , NLS; ▩ , DNA-binding region. Interaction partners of SNURF that are identified by this study (boxes under the cartoon of SNURF) and by others (boxes above SNURF). References; HMGI(Y) and PATZ by Fedele et al. 2000; Gscl by Galili et al. 2000; Sp1 by Poukka et al. 2000a; SPBP by Lyngsø et al. 2000; ERα by Saville et al. 2002; TRPS1 by Kaiser et al. 2003; NF-Y by Wu et al. 2004.

The interaction between AR and SNURF revealed by yeast two-hybrid screening was confirmed by *in vitro*- and *in vivo*-binding studies. In the GST-pull down assay, *in vitro* translated SNURF bound the GST-fusion of AR DBD, and in yeast cells (a yeast two-hybrid assay), full-length AR interacted with SNURF in an androgen-dependent manner. In addition to AR, full-length SNURF was able to bind DBD of other steroid receptors such as progesterone (PR) and estrogen (ER) receptor in hormone-dependent fashion in yeast cells. Ectopically expressed FLAG-SNURF and AR were also

observed to form a complex in mammalian cells (COS-1), when co-immunoprecipitated with anti-FLAG antibody and furthermore, the N-terminal part of SNURF RING finger was shown to be required for this interaction. SNURF interaction region in AR localizes to the region flanking the AR DBD and hinge region as revealed by yeast two-hybrid screening. Later on, Poukka et al. 2000a performed more detailed examination of this binding region, and found that the bipartite NLS in the vicinity of the DBD/hinge-borderline was critical for the interaction. In addition, AR DBD point mutations that are observed in patients with partial androgen insensitivity syndrome and male breast cancer weakened the interaction between AR-DBD and SNURF (Poukka et al. 2000a). In the interactions with ERα, both the RING and the central acidic region (aa 31-65) of SNURF were needed for the interaction (Saville et al. 2002). The same acidic region of SNURF was also needed for the interaction with the orphan nuclear receptor steroidogenic factor 1 (SF-1 or NR5A1). In addition to sequence-specific transcription factors, SNURF was able to bind to the basal transcription machinery protein TBP with its RING finger region that was also needed for binding to other transcription factors, such as POZ AT-hook zinc finger protein (PATZ), promoter specificity protein 1 (Sp1), and the activator of stromelysin 1 gene transcription (SPBP) (Lyngsø et al. 2000, Fedele et al. 2000, Poukka et al. 2000_a). SNURF interacts also with the repressor of GATA-mediated transcription, the TRPS1 (trichorhino-phalangeal syndrome) protein with its amino-terminal region (aa 6-65) (Kaiser et al. 2003).

Interestingly, when SNURF region of amino acids 21-186 was used as a bait in the yeast two-hybrid screening SUMO-1 and Ubc9 were identified as SNURF-interacting proteins (Poukka, unpublished results), which suggests that SNURF might be involved in SUMO modifications. We studied these interactions by using the GST-pull down assay and found that SNURF bound efficiently to GST-SUMO-1, but not to GST-Ubc9, suggesting that additional protein(s) mediate the SNURF-Ubc9 interaction in yeast cells. Several proteins have been shown to bind noncovalently to SUMO, but little is known about the effects of this function. However, noncovalent SUMO binding has been shown to influence protein localization and activity (Minty et al. 2000, Kotaja et al. 2002, Engelhardt et al. 2003). A recent study by Song et al. (2004) revealed that hydrophobic consensus sequence V/IxV/IV/I is required for binding to all three SUMO variants (SUMO-1, -2,and -3). SNURF contains one perfect SUMO-1 binding motif, ^{71}VVIV^{74}, in the central region of SNURF. However, the SNURF mutant (Δ66-98) lacking this region was still able to bind SUMO-1 in GST-pull down assays, albeit less efficiency than the wild-type protein, indicating that there is an additional SUMO-1 binding site in SNURF (unpublished results). This secondary SUMO-1-binding region in SNURF could be the ^{61}VVVD^{64} sequence (Song et al. 2004). We examined the possible interaction between SNURF and another RING finger- and well-known SUMO-binding- protein PML3 (promyelocytic leukemia

protein 3) by using immunoprecitation techniques. Immunoprecipitation with anti-SNURF antibody revealed that PML3 was complexed with SNURF, but only when SUMO-1 was coexpressed. Some of the SNURF-interacting protein partners, such as SF-1 an AR, have been found to be sumoylated (Poukka et al. 2000b, Chen et al. 2004), and thus SUMO-1 may be involved in mediating these interactions. Finally, SNURF was found to reside in large (≥500-kDa) multiprotein complexes, as assessed by size fractionation of protein lysates of F9 cells and mouse testis by gel filtration chromatography. In addition, *E. coli*-produced purified SNURF was eluted in fractions of ≥100-kDa, suggesting that SNURF is able to self-assemble. Taken together, SNURF is able to interact with many diverse transcription regulators via multiple regions and *in vivo* it appears to be a component in a multiprotein complex.

SNURF and DNA/nucleosome binding

It has been speculated that RING finger structure possesses DNA-binding activity similar to other zinc-binding molecules such as Gal4, but up until now none of the DNA-binding RING finger proteins have been shown to contact DNA via their RING fingers. Nucleotide-binding ability of SNURF was examined by electrophoretic mobility shift assay (EMSA) *in vitro*. Recombinant SNURF was found to bind DNA in a non-sequence specific manner with different types of DNA (single- and double-stranded DNA, supercoiled and linear plasmid DNA, and four-way junction DNA), but there was no clear binding preference between linear or branched DNA. The capacity to bind normal and abnormal DNA without sequence specificity is a common property of architectural proteins, such as HP1 (reviewed by Zlatanova and van Holde 1998). GST-SNURF interacted with DNA in a cooperative fashion that was dependent on the length of the DNA fragment. Also human RNF4 showed a similar DNA-binding behavior (Wu et al. 2004). A comparable DNA-binding capacity has been characterized for HP1 and BRCA1 (Zhao et al. 2000, Paull et al. 2001). BRCA1 interacts with DNA as a multimer and is able to generate DNA loops (Paull et al. 2001). Since SNURF is also able to self-associate (Lyngsø et al. 2000), it is possible that SNURF can bind DNA as a multimer. The RING finger structure of SNURF was not needed for its DNA-binding activity, but the deletion of the positively charged N-terminal region (aa 1-20) abolished the DNA-binding activity of SNURF. Further examination revealed that basic amino acids ^{8}RKRR^{11} play a critical role in DNA binding. Many DNA-binding proteins, such as Bicoid, mel-8 and HMGY(I), associate with DNA through their basic amino acid regions (Niessing et al. 2000). Since Arg-rich clusters are also involved in RNA recognition (Muchardt et al. 2002, Burd et al. 1994), we examined whether SNURF is able to bind RNA as well. GST-SNURF bound the RNA-probe (232 nucleotides) efficiently also in the presence of a cold competitor transfer RNA (tRNA) (90-fold excess) (Fig. 13, lanes 2 and 3). However, a lower

(0.3-fold) molar ratio of linearized plasmid DNA was able to compete with RNA, since smaller SNURF-RNA complexes started to appear (Fig. 13, lane 4).

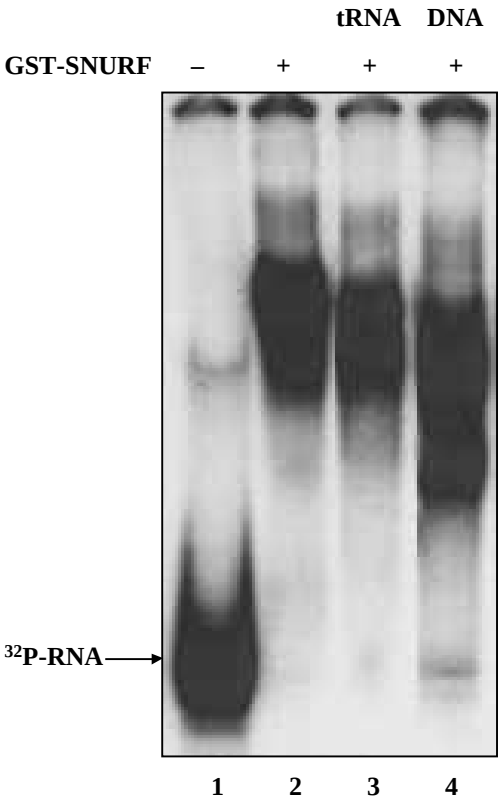


Fig. 13. SNURF is a RNA-binding protein. In EMSA, GST-SNURF was incubated with *in vitro*-transcribed ^{32}P-RNA (lanes 2-4). In lanes 3 and 4, RNA-binding of SNURF is competed with tRNA (90-fold excess) and linearized plasmid DNA (0.3-fold excess), respectively.

Since SNURF was able to interact with proteins and various DNA molecules, we were interested in studying whether SNURF is able to bind nucleosomes. We reconstituted DNA on mononucleosomes *in vitro* and performed EMSA assays with different amounts of GST-SNURF. SNURF was able to bind efficiently to nucleosomes, albeit with a lower activity compared to naked DNA. The DNA binding-deficient SNURF mutant (Δ1-20) was also practically unable to bind nucleosomes. GST-SNURF pull-down experiments showed that SNURF is able to recruit histones H3 and H4, suggesting that both DNA- and protein recognitions are involved in nucleosome binding. Interestingly, HP1α that is also a small (191 amino acids) protein with DNA- and RNA-binding activities binds to nucleosomes and histones H3 and H4 (Polioudaki et al. 2001, Zhao et al. 2000, Meehan et al. 2003, Murchardt et al. 2002). This suggests that SNURF and HP1 share similar functional properties that are involved in the regulation of chromatin function. In addition, SNURF binds non-histone chromatin modeling protein HMGI(Y), which is involved in transcription and cell

growth regulation (Fedele et al. 2000). Since SNURF is able to bind a variety of transcription regulators, DNA and nucleosomes, it may promote assembly of nucleoprotein structures involved in transcription control.

2. SNURF AS A TRANSCRIPTIONAL COREGULATOR (I, II, III, and V)

Since SNURF was able to bind AR, we studied whether SNURF was influencing AR-dependent transcription. We performed reporter gene assays, in which SNURF was coexpressed with AR in cultured CV-1 and COS-1 cells. These assays showed that SNURF was able to enhance transcriptional activity of AR from different AR-regulated promoters, such as minimal ARE_2-TATA-LUC and natural and more complex rat probasin-LUC, in an androgen-dependent fashion. This activity of SNURF was dependent on the AR-binding region of SNURF. Interestingly, SNURF was also able to enhance basal transcription from probasin-promoter and minimal TATA-LUC reporters, which was dependent on the RING finger of SNURF. Later on, Poukka and coworkers showed that the activity of SNURF in AR-mediated transcription depends on the cell type. Namely, in COS-1 cells, the coactivator function of the SNURF RING finger mutant (SNURF-C177/180S), in which the second zinc-binding site was mutated, was practically inactive, but this mutant was active in CV-1 cells (Poukka et al. 2000a). Interestingly, the PATZ protein is capable of switching SNURF coactivation function to corepression function in AR-mediated transcription by interacting with SNURF (Pero et al. 2002).

SNURF is not specific for AR, since it was able to enhance the activity of other steroid receptors, such as the progesterone receptor (PR) and glucocorticoid receptor (GR) in the presence of appropriate hormone. In addition, SNURF enhanced transcription from promoters containing binding sites for Sp1 and activator protein 1 (AP-1). The latter coactivator function of SNURF was dependent on the intact RING finger, which supports the Sp1 interaction. However, also the N-terminal region was required for both Sp1- and AR-regulated transcription (Poukka et al. 2000a). Since SNURF uses its N-terminal region for DNA binding, we examined whether DNA binding is required for SNURF action in transcription regulation. We cotransfected COS-1 cells with $Sp1_2$-TATA-LUC or ARE_2-TATA-LUC together with SNURF or its DNA-binding mutants. We found that the DNA-binding activity of SNURF clearly correlates with its coactivation function in both cases (AR; unpublished results). Therefore, the DNA-binding activity of SNURF may also be needed in ERα-regulated transcription, since the N-terminal deletion of SNURF abolishes the coactivation of ERα, although SNURF was still maintaining ER-interaction and nuclear localization (Saville et al. 2000). We also examined whether SNURF enhanced Sp1 binding to a GC-rich promoter sequence in EMSA, but

there was no clear cooperativity between Sp1 and SNURF. Neither was AR binding to its corresponding promoter element influenced by SNURF (AR; unpublished result). Interestingly, RNF4 has been shown to promote both DNA binding and transcriptional activity of SPBP (Lyngø et al. 2000).

Since SNURF mRNA is also expressed in mouse brain, we were interested in studying whether SNURF is able to influence the regulation of luteinizing hormone β -gene expression in pituitary tissues, where GnRH plays a stimulative role, and androgens and AR play a repressive role (negative feedback). We were able to detect endogenous SNURF protein in mouse pituitary tissues and in pituitary gonadotrope LβT2 cell line by anti-SNURF antibody. Furthermore, endogenous SNURF physically associated with the native LHβ promoter in LβT2 cells as, revealed by anti-SNURF antibodies and chromatin immunoprecipitation (ChIP) assay. In transient transfection assays, ectopically expressed SNURF enhanced the basal and GnRH-regulated transcription from the LHβ-promoter. Enhancer regions of the LHβ gene, where the distal enhancer harbors binding sites for Sp1 and the proximal enhancer contains binding sites for SF-1 and Egr-1, were both needed for full SNURF activity. The stimulatory effect of SNURF on the LHβ-promoter was abolished, when the Sp1- or the SF-1-binding region of SNURF was deleted. Interestingly, SNURF was able to prevent the androgen-mediated suppression of GnRH-stimulation of LHβ. AR has previously been shown to interfere with the transcription factors that stimulate the LHβ promoter (Curtin et al. 2001, Jorgensen et al. 2001); AR may form a complex with SF-1, which leads to repression of LHβ transcription (Jorgensen et al. 2001). Our results suggest that SNURF is also able to block the entry of AR to the LHβ promoter via binding to SF-1 and Sp1. cAMP signaling system has been shown to regulate the LHβ promoter via Sp1, Egr-1 and SF-1 in LβT2 cells, but the precise mechanism of its action on the LHβ promoter is not known. SF-1 is not mandatory for the cAMP-induced activation of the LHβ promoter, but it amplifies the cAMP-response via an unknown mechanism (Horton and Halvorson 2004). Interestingly, SNURF expression is enhanced by forskolin and PMA (phorbol myristate, another LH agonist) in cultured granulosa cells of follicles (Hirvonen-Santti et al. 2003). Therefore, it would be interesting to study whether SNURF is participating in the cAMP-induced activation of the LHβ promoter in pituitary tissue. In view of this, it is interesting that the transcription factor NF-Y has been shown to regulate the bovine LHβ promoter and contribute to cAMP signalling (Keri et al. 2000, Zhong et al. 2000). Moreover, a recent study showed that RNF4 is a coactivator for NF-Y and it can enhance transcription of GTP cyclohydrolase I (GCH), which is an essential gene in the synthesis of neurotransmitters dopamine and serotonin (Wu et al. 2004).

SNURF fulfills only some of the characteristics of a “classical” coregulator. It interacts with many transcription factors, whose transcription activity SNURF either enhances (e.g. AR and Sp1) or represses (TRPS1) (Kaiser et al. 2003). Since SNURF can bind to DNA, a large number of enhancer-binding proteins and to proteins of general transcription machinery, SNURF may bridge the sequence-specific transcription factors to the general transcription machinery, and thus enhance or repress transcription. In agreement with the latter observation, SNURF has been shown to cooperate with TBP in coactivation of ERα-mediated transactivation (Saville et al. 2002).

3. SNURF HAS UBIQUITIN E3 LIGASE ACTIVITY (IV)

Since many RING finger-containing proteins mediate ubiquitin E3 ligase activity, we have studied the ability of SNURF to cooperate with E2 enzyme by using *in vitro* ubiquitination assays, in which immobilized GST-SNURF was incubated with recombinant labelled ubiquitin, ubiquitin-activating enzyme (E1) and various E2s. Our result showed that SNURF is able to mediate the ubiquitin E3 ligase activity via cooperation with different E2s, such as Ubch5A/B, HHR6B (RAD6), E2-25K, MmUbc7 and UbcH13. This multiple usage of E2 is a rare quality among the known ubiquitin E3s. UbcH5 has previously been shown to cooperate with many E3s, such as MDM2, BRCA1-BRAD1, SCF and APC, and to ubiquitinate p53 (Ostendorff et al. 2002, Brzovic et al. 2003). HHR6 (RAD6) has been implicated in the regulation of chromatin structure via histone ubiquitination (Robzyk et al. 2000, Haas et al. 1990). As in the case of many other E3s, such as BRCA1 and MDM2, also SNURF was self-ubiquitinated in a RING-finger-dependent manner (Fang et al. 2000, Chen et al. 2002). We used several SNURF-interacting partners, such as AR, Sp1, SF-1, and PML-3, to screen for substrate(s) for the SNURF ubiquitin E3 ligase activity. SNURF was, however, not able to enhance the ubiquitination level of these proteins, thus the specific substrate(s) other than SNURF itself was not discovered. Since the endogenous SNURF was found in large multiprotein complexes in F9 cells, SNURF may act as an essential RING finger subunit in an E3 multiprotein complex. Further work should be performed to identify potential endogenous interaction partners of SNURF. It should be possible to purify SNURF-multiprotein complex by using SNURF-affinity chromatography and identify SNURF-bound proteins with mass spectrometric analyses. Unfortunately, our current antibodies were not suitable for such an approach. Mammalian two-hybrid screening with full-length SNURF as a bait could provide another tool to study the components of SNURF-containing complexes. Results from these experiments may also lead us to identify specific ubiquitination targets of SNURF. Interestingly, MDM2 ubiquitin E3 ligase has recently been shown to repress transcription activity of p53 via Nedd8 (ubiquitin-like molecule) conjugation (Xirodimas et al. 2004). Therefore, it

would be interesting to study whether SNURF is able to mediate conjugation of ubiquitin-like molecules, such a Nedd8, ISG15 and FAT10.

SNURF has been suggested to play a role in spermatid maturation (Yan et al. 2002). SNURF is down-regulated in germ cell tumors and RAS-transformed cells (Pero et al. 2001, Zuber et al. 2000, Hirvonen-Santti et al. 2003), suggesting a role for SNURF in the pathogenesis of testicular germ cell cancer. Interestingly, overexpression of SNURF inhibits cell proliferation (Pero et al. 2001). The RING finger SNURF mutants that are inactivate as ubiquitin E3 ligases show attenuated coregulatory function as well as inhibitory function in cell growth. Similarly, RING finger mutated BRCA1 fails to inhibit the cell growth and also the ubiquitin E3 ligase activity of the BRCA1/BARD heterodimer is lost as a consequence of the mutation within the RING of BRCA1. Therefore, it has been suggested that the E3 ubiquitin ligase activity of BRCA1-BARD1 would promote tumor suppression (Chen et al. 2002, Jin et al. 1997, reviewed by Baer and Ludwig 2002). Accordingly, it would be important to study the ubiquitin E3 ligase activity of SNURF in the context of cell growth regulation in more detail.

4. COVALENT MODIFICATIONS OF SNURF (IV, V)

SNURF is mainly mono- and di-ubiquitinated *in vitro* and *in vivo*, but the addition of MG-132, a proteasome inhibitor, to cultured cells increases total SNURF ubiquitination level and especially, polyubiquitinated SNURF forms become more evident. SNURF contains nine lysine residues. Although we have used a versatile deletion series of SNURF (Poukka et al. 2000a), we were not able to pinpoint the target lysines, suggesting that SNURF can simultaneously be ubiquitinated on several lysine residues or a deletion of one ubiquitination site may lead to ubiquitination of another lysine. Some proteins, such as c-Jun, exhibit seemingly random ubiquitination patterns, while others, such as IκBα, are ubiquitinated with high specificity (Weissman 2001). Mutations in the RING finger motif did not stabilize the SNURF protein, suggesting that SNURF autoubiquitination is not simply targeting SNURF for degradation, but rather modifies SNURF function, possibly its subcellular localization, DNA binding or protein-protein interactions. MDM2, for instance, regulates its degradation by auto-ubiquitination, since the destruction of its RING finger structure stabilizes MDM2 in cells (Fang et al. 2000, Geyer et al. 2000), whereas in the case of BRCA1, autoubiquitination does not lead to degradation, rather to a modulation of its function (Nishikawa et al. 2004).

Since SNURF was able to bind non-covalently to SUMO-1 *in vitro,* we studied whether SNURF is also covalently conjugated to SUMO-1. SNURF does not contain precise sumoylation consensus sequence, ψKXE/D. However, many proteins appear to be sumoylated through lysine residues, which surrounding sequences do not fulfill the SUMO consensus sequence (Hoege et al. 2002, Lee et al. 2003). We performed *in vitro* sumoylation assays and observed that SNURF can be conjugated to two SUMO-1 molecules in the presence of SUMO-activating enzyme (E1), Ubc9 and SUMO-1. Studies in COS-1 cells suggested that at least three SUMO-1 molecules can be conjugated to SNURF. SNURF has one potential site Lys121 within the TKDE sequence, but the deletion of this SNURF region (deletion Δ121-143) did not alter the sumoylation pattern of SNURF. Interestingly, the RING finger SNURF mutant was more avidly sumoylated than the wild-type SNURF in COS-1 cells, suggesting that sumoylation and autoubiquitination compete for the same target lysine(s) in SNURF or that the destruction of RING finger can reveal hidden sumoylation sites. Interestingly, SUMO-1 conjugation of MDM2 shifts its E3 activity towards p53 ubiquitination and diminishes its autoubiquitination and thus its degradation (Buschmann et al. 2001). SUMO conjugation of SNURF may regulate the stability, protein-protein interactions, DNA-binding or subnuclear localization of SNURF. However, SUMO-1-mediated SNURF-PML3 complex formation was independent on the covalent SUMO-1 conjugation.

SNURF sequence contains many consensus target sites for various kinases, such as protein kinase A (PKA), protein kinase C (PKC) and casein kinase II (CKII). There are seven potential phosphorylation sites for CKII, which lie within the AR-binding region of SNURF. We performed *in vitro* phosphorylation reactions, where various bacterially produced and purified GST–SNURF proteins were incubated with CKII and [γ^{32}P]ATP. A shown in Fig. 14, wild-type SNURF was efficiently phosphorylated by CKII (lanes 2 and 9), but the SNURF mutants 1-94 and Δ99-118 were not phosphorylated (lanes 3 and 6). Next, we mutated two serine residues (Ser98 and Ser99) simultaneously (lane 8 and 10) or separately (lanes 11 and 12) within this region, and found that both serines were targets of CKII phosphorylation. Lack of CKII phosphorylation resulted in a slight increase in SNURF coactivation function in both Sp1- and AR-regulated transcription (data not shown), suggesting that CKII-mediated phosphorylation of SNURF does not play a major role in transcription. Phosphorylation may, in turn, regulate sumoylation and/or ubiquitination of SNURF, since, in the case of IκBα, phosphorylation inhibits SUMO modification and enhances ubiquitination (Desterro et al. 1998).

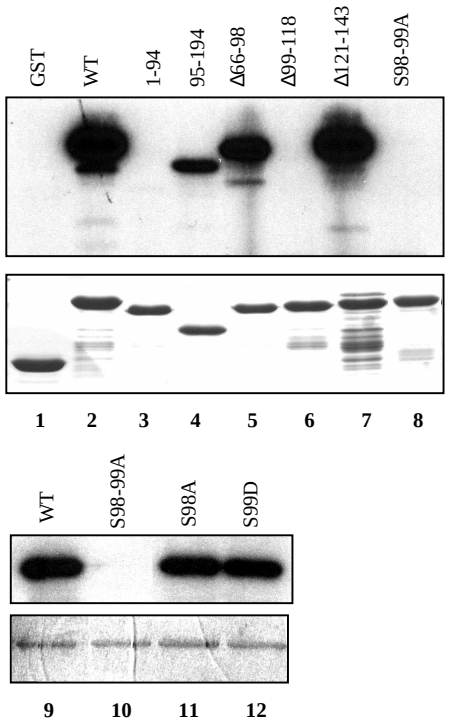


Fig. 14. SNURF is phosphorylated by casein kinase II *in vitro*. GST (lane 1), GST-SNURF wild-type (lanes 2 and 9) and its mutants (lanes 3-8 and 10-12) were incubated with CKII and [γ^{32}P]ATP. Samples were fractionated by 15% SDS-PAGE gel and detected by autoradiography.

5. SUBCELLULAR LOCALIZATION OF SNURF (I and V)

SNURF mRNA was found to be expressed in various mouse and human tissues as revealed by Northern blotting. Immunohistochemistry of rat prostate epithelial cells by anti-SNURF antibodies revealed that SNURF is localized into the nucleus. Furthermore, immunocytochemical staining of cultured CV-1 cells revealed that the endogenous SNURF was localized into small nuclear speckles in CV-1 cells. Kaiser et al. (2003) have obtained similar results with primary human fibroblast. Interestingly, in the cells of the nervous system of developing mouse embryo and adult mouse, the localization of RNF4 was primarily cytoplasmic (Galili et al. 2000), suggesting that the localization of SNURF is regulated in a tissue-specific manner and that SNURF may participate in shuttling information between the cytoplasm and the nucleus. Poukka et al. (2000c) showed that SNURF is capable of modulating nuclear targeting of AR by facilitating nuclear import during ligand induction and retarding export of AR after hormone withdrawal in CV-1 cells.

Since SUMO-1 is an essential factor in the formation of the PML nuclear bodies (PML NBs) and many RING finger proteins, such as PML, BRCA1 and MDM2, are found to colocalize to these structures, we investigated potential colocalization of SNURF, SUMO-1 and PML in HeLa cells by using immunostaining and confocal microscopy. Interestingly, a significant portion of the endogenous SNURF colocalized with PML and SUMO-1 into the same nuclear structures. In addition, ectopic expression of SNURF and PML isoforms, PML3 (PML IV) and PML-L (PML III), showed that SNURF is specifically recruited to PML NBs by PML3. Likewise, the EGFP-SUMO-1 was localized with ectopically expressed SNURF in the nucleus and the localization was independent on the RING finger structure of SNURF. Furthermore, triple staining of ectopically expressed SUMO-1, PML3 and SNURF revealed perfect localization into PML NBs. Interestingly, overexpression of PML3, but not PML-L, was able to repress SNURF coactivition function in Sp1-regulated transcription, suggesting that PML3 was able to regulate SNURF via recruiting SNURF into PML NBs and store SNURF in a transcriptionally inactive form. Also Daxx and p53 are specifically recruited to PML NBs and their transcription activity is repressed by PML3 (Fogal et al. 2000, Kim et al. 2003). PML can be considered as a tumor suppressor protein (Wang et al. 1998a and 1998b). Its expression is down-regulated in many cancers, such as in germ cell tumors, and absent in progressive tumors of prostate and central nervous system (Gurrieri et al. 2004). SNURF is down-regulated in germ cell tumors and overexpression of SNURF inhibits cell proliferation (Yan et al. 2002, Galili et al. 2000), therefore, we suggest that SNURF and PML may communicate in the regulation of transcription and cell growth.

CONCLUSIONS

In the present work, we have examined the functional characteristics of rat small nuclear RING finger protein SNURF. Highly conserved SNURF homologs in human and mouse are called RNF4, but no other close homologs or related proteins exist. SNURF is a hydrophilic protein that contains zinc-binding RING-finger motif, which is known to maintain protein-protein interactions and ubiquitin E3 ligase activity. The results of this study are summarized below:

- SNURF is a transcriptional coregulator protein. SNURF interacts with steroid receptors as well as other transcription regulators, such as, SF-1 and TBP, and enhances transactivation function of AR and Sp1. SNURF is able to stimulate LHβ gene expression via interaction with Sp1 and SF-1 that are bound to distal and proximal regulatory elements within the LHβ promoter. SNURF may act as a bridging factor between sequence-specific transcription factors and the basal transcription machinery.

- SNURF is able to bind DNA and RNA. SNURF binds to various types of DNA without apparent sequence specificity. Furthermore, SNURF is able to bind to nucleosomes. The DNA-binding activity of SNURF correlates with its transcriptional coactivation function. SNURF may thus promote the assembly of nucleoprotein structures involved in transcriptional control.

- SNURF is a ubiquitin E3 ligase capable of cooperating with various ubiquitin-conjugating E2 enzymes. The E3 function is dependent on SNURF RING finger structure. SNURF may serve as an essential RING component in a multiprotein E3 complex.

- SNURF is able to ubiquitinate itself (autoubiquitination), and it is covalently modified by the SUMO-1 conjugation and phosphorylation.

- SNURF is a nuclear protein that is recruited to PML nuclear bodies via interaction with PML3 and SUMO-1. Localization of SNURF to PML NBs represses its activity in transcription regulation.

ACKNOWLEDGEMENTS

Tämä väitöskirjatyö laadittiin professori Olli Jänteen ja professori Jorma Palvimon tutkimusryhmässä Helsingin yliopiston Biolääketieteen laitoksen fysiologian osastolla. Kiitän Olli Jännettä ohjauksesta ja loistokkaasti varustetusta työympäristöstä Biomedicum Helsinki-tutkimuskeskuksessa. Olen syvästi kiitollinen Jorma Palvimolle asiantuntevasta ja innostavasta ohjauksesta sekä itsenäisyydestä.

Kiitokset Asta Pirskaselle ja Ilkka Julkuselle rakentavista huomautuksista ja ehdotuksista tarkastaessanne väitöskirjaani.

Kiitokset Helsinki Biomedical graduate school -tutkijakoululle saamastani rahoituksesta ja koulutuksesta. Lämmin kiitos Emil Aaltosen Säätiölle, Otto A. Malmin lahjoitusrahastolle, Jalmari ja Rauha Ahokkaan Säätiölle ja Farmoksen Tutkimus- ja Tiedesäätiölle saamastani tuesta ja kannustuksesta

Marikki Laiholle ja Ilkka Julkuselle lämmin kiitos toimiessanne tutkimusprojektini seurantaryhmässä avustavana ja kannustavana jäsenenä.

I am grateful to Denis Curtin, Hearther Ferris, Matthew Gibson, Ulla Karvonen, Kevin Lorick, Anu-Maarit Moilanen, Hetti Poukka, Margaret Shupnik and Allan Weissmann for productive collaboration.

Leenalle, Seijalle, Pirjolle, Saijalle, Johannalle, Katjalle, Riitalle, Katille, Heikille ja Birgitille erityislämminkiitos saamastani avusta ja mukavasta työskentelyseurasta. Annelle, Pirjolle, Mallalle ja Niinalle kiitokset käytännönasioiden hoidosta. Kiitokset Marc Baumannille avusta proteiinikemian osa-alueella.

Lämmin kiitos kaikille OAJ- ja ”Orphan group”-tutkimusryhmämme jäsenille mukavasta työympäristöstä, sekä yhteistyöstä että avunannosta. Leenalle, Seijalle ja Marikalle sydämellinen kiitos kannustuksesta ja välittämisestä. Sirpalle, Karoliinalle ja Lauralle kiitos virkistävästä yhteiselosta Penkereen alakerran tutkijakammiossa ja Sirpalle vielä kiitos SNURF-hetkistä. Försti Ullalle erityiskiitos värikkäistä huumorinkukkasista. Ne olivat tarpeen! Kiitokset Shalle, Nooralle, Andriille, Tanyalle, Henrikille, Fu-Pingille, Zhigangille ja Jamekselle hauskoista hetkistä. Minnaa ja Annaa kiitän suuresti avuliaasta ja piristävästä yhteistyöstä.

Annelle, Jaanalle, Marjalle, Nathalielle, Päiville, Sirpalle, Tarjalle ja Terhille kiitos ystävyydestä.

Olen kiitollinen pikkuauringoista, Rikistä ja muista ”karvanapeista”, jotka ovat vaeltaneet rinnallani tämän pitkän työrupeaman aikana.

Äidille, Isälle, Tiitukselle, Marjaanalle, Mikalle ja Stefanille kiitokset saamastani tuesta ja paljosta muusta.

Helsinki, maaliskuu 2005

Marika Hähli

REFERENCES

Acevedo ML, Kraus WL (2003) Mediator and p300/CBP-steroid receptor coactivator complexes have distinct roles, but function synergistically, during estrogen receptor alpha-dependent transcription with chromatin templates. Mol Cell Biol 23:335-348

Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott PJ (1999) Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 59:2615-2622

Agarwal R, Tang Z, Yu H, Cohen-Fix O (2003) Two distinct pathways for inhibiting pds1 ubiquitination in response to DNA damage. J Biol Chem 278:45027-45033

Ahn JH, Xu Y, Jang WJ, Matunis MJ, Hayward GS (2001) Evaluation of interactions of human cytomegalovirus immediate-early IE2 regulatory protein with small ubiquitin-like modifiers and their conjugation enzyme Ubc9. J Virol 75:3859-3872

Akoulitchev S, Chuikov S, Reinberg D (2000) TFIIH is negatively regulated by cdk8-containing mediator complexes. Nature 407:102-106

Alam SL, Sun J, Payne M, Welch BD, Blake BK, Davis DR, Meyer HH, Emr SD, Sundquist WI (2004) Ubiquitin interactions of NZF zinc fingers. EMBO J 23:1411-1421

Alberti S, Demand J, Esser C, Emmerich N, Schild H, Hohfeld J (2002) Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J Biol Chem 277:45920-45927

Ali S, Metzger D, Bornert JM, Chambon P (1993) Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region. EMBO 12:1153-1160

Alkema MJ, Bronk M, Verhoeven E, Otte A, van 't Veer LJ, Berns A, van Lohuizen M (1997) Identification of Bmi1-interacting proteins as constituents of a multimeric mammalian polycomb complex. Genes Dev 11:226-240

Alroy I, Freedman LP (1992) DNA binding analysis of glucocorticoid receptor specificity mutants. Nucleic Acids Res 20:1045-1052

Anderson JD, Lowary PT, Widom J (2001) Effects of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites. J Mol Biol 307:977-985

Arany Z, Sellers WR, Livingston DM, Eckner R (1994) E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. Cell 77:799-800

Arora K, Warrior R (2001) A new Smurf in the village. Dev Cell 1:441-442

Asturias FJ, Jiang YW, Myers LC, Gustafsson CM, Kornberg RD (1999) Conserved structures of mediator and RNA polymerase II holoenzyme. Science 283:985-987

Avila DM, Zoppi S, McPhaul MJ (2001) The androgen receptor (AR) in syndromes of androgen insensitivity and in prostate cancer. J Steroid Biochem Mol Biol 76:135-142

Ayaydin F, Dasso M (2004) Distinct In Vivo Dynamics of Vertebrate SUMO Paralogues. Mol Biol Cell 15:5208-5218

Bach I, Rodriguez-Esteban C, Carriere C, Bhushan A, Krones A, Rose DW, Glass CK, Andersen B, Izpisúa Belmonte JC, Rosenfeld MG (1999) RLIM inhibits functional activity of LIM homeodomain transcription factors via recruitment of the histone deacetylase complex. Nat Genet 22:394-399

Bachmair A, Finley D, Varshavsky A (1986) In vivo half-life of a protein is a function of its amino-terminal residue. Science 234:179-186

Baer R, Ludwig T (2002) The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. Curr Opin Genet Dev 12:86-91

Bailey D, O'Hare P (2002) Herpes simplex virus 1 ICP0 co-localizes with a SUMO-specific protease. J Gen Virol 83:2951-2964

Bailey D, O'Hare P (2004) Characterization of the localization and proteolytic activity of the SUMO-specific protease, SENP1. J Biol Chem 279:692-703

Baleja JD, Marmorstein R, Harrison SC, Wagner G (1992) Solution structure of the DNA-binding domain of Cd2-GAL4 from S. cerevisiae. Nature 356:450-453

Bannister AJ, Schneider R, Kouzarides T (2002) Histone methylation: dynamic or static? Cell 109:801-806

Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, Berger SL (2001) Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Mol Cell 8:1243-1254

Barlow PN, Luisi B, Milner A, Elliott M, Everett R (1994) Structure of the C3HC4 domain by 1H-nuclear magnetic resonance spectroscopy. A new structural class of zinc-finger. J Mol Biol 237:201-211

Barratt MJ, Hazzalin CA, Cano E, Mahadevan LC (1994) Mitogen-stimulated phosphorylation of histone H3 is targeted to a small hyperacetylation-sensitive fraction. Proc Natl Acad Sci U S A 91:4781-4785

Batonnet S, Leibovitch MP, Tintignac L, Leibovitch SA (2004) Critical role for lysine 133 in the nuclear ubiquitin-mediated degradation of MyoD. J Biol Chem 279:5413-5420

Baud V, Liu ZG, Bennett B, Suzuki N, Xia Y, Karin M (1999) Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev 13:1297-1308

Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J (1998) Structure determination of the small ubiquitin-related modifier SUMO-1. J Mol Biol 280:275-286

Beato M, Sanchez-Pacheco A (1996) Interaction of steroid hormone receptors with the transcription initiation complex. Endocr Rev 17:587-609

Bellon SF, Rodgers KK, Schatz DG, Coleman JE, Steitz TA (1997) Crystal structure of the RAG1 dimerization domain reveals multiple zinc-binding motifs including a novel zinc binuclear cluster. Nat Struct Biol 4:586-591

Bencsath KP, Podgorski MS, Pagala VR, Slaughter CA, Schulman BA (2002) Identification of a multifunctional binding site on Ubc9p required for Smt3p conjugation. J Biol Chem 277:47938-47945

Berger SL (2001) An embarrassment of niches: the many covalent modifications of histones in transcriptional regulation. Oncogene 20:3007-3013

Bernassola F, Salomoni P, Oberst A, Di Como CJ, Pagano M, Melino G, Pandolfi PP (2004) Ubiquitin-dependent degradation of p73 is inhibited by PML. J Exp Med 199:1545-1557

Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD (2002) Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell 108:345-356

Bertolaet BL, Clarke DJ, Wolff M, Watson MH, Henze M, Divita G, Reed SI (2001) UBA domains of DNA damage-inducible proteins interact with ubiquitin. Nat Struct Biol 8:417-422

Best JL, Ganiatsas S, Agarwal S, Changou A, Salomoni P, Shirihai O, Meluh PB, Pandolfi PP, Zon LI (2002) SUMO-1 protease-1 regulates gene transcription through PML. Mol Cell 10:843-855

Bilodeau PS, Urbanowski JL, Winistorfer SC, Piper RC (2002) The Vps27p Hse1p complex binds ubiquitin and mediates endosomal protein sorting. Nat Cell Biol 4:534-539

Bird AP, Wolffe AP (1999) Methylation-induced repression--belts, braces, and chromatin. Cell 99:451-454

Blake TJ, Shapiro M, Morse HC 3rd, Langdon WY (1991) The sequences of the human and mouse c-cbl proto-oncogenes show v-cbl was generated by a large truncation encompassing a proline-rich domain and a leucine zipper-like motif. Oncogene 6:653-657

Bloom J, Amador V, Bartolini F, DeMartino G, Pagano M (2003) Proteasome-mediated degradation of p21 via N-terminal ubiquitinylation. Cell 115:71-82

Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F, Shiekhattar R (2000) BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. Cell 102:257-265

Boddy MN, Duprez E, Borden KL, Freemont PS (1997) Surface residue mutations of the PML RING finger domain alter the formation of nuclear matrix-associated PML bodies. J Cell Sci 110:2197-2205

Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2003) Nucleosomes unfold completely at a transcriptionally active promoter. Mol Cell 11:1587-1598

Boggio R, Colombo R, Hay RT, Draetta GF, Chiocca S (2004) A Mechanism for Inhibiting the SUMO Pathway. Mol Cell 16:549-561

Bohren KM, Nadkarni V, Song JH, Gabbay KH, Owerbach D (2004) A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. J Biol Chem 279:27233-27238

Boisvert FM, Hendzel MJ, Bazett-Jones DP (2000) Promyelocytic leukemia (PML) nuclear bodies are protein structures that do not accumulate RNA. J Cell Biol 148:283-292

Boisvert FM, Kruhlak MJ, Box AK, Hendzel MJ, Bazett-Jones DP (2001) The transcription coactivator CBP is a dynamic component of the promyelocytic leukemia nuclear body. J Cell Biol 152:1099-1106

Boonyaratanakornkit V, Melvin V, Prendergast P, Altmann M, Ronfani L, Bianchi ME, Taraseviciene L, Nordeen SK, Allegretto EA, Edwards DP (1998) High-mobility group chromatin proteins 1 and 2 functionally interact with steroid hormone receptors to enhance their DNA binding in vitro and transcriptional activity in mammalian cells. Mol Cell Biol 18:4471-4487

Borden KL (2000) RING domains: master builders of molecular scaffolds? J Mol Biol 295:1103-1112

Borden KL, Boddy MN, Lally J, O'Reilly NJ, Martin S, Howe K, Solomon E, Freemont PS (1995) The solution structure of the RING finger domain from the acute promyelocytic leukaemia proto-oncoprotein PML. EMBO J 14:1532-1541

Borden KL, CampbellDwyer EJ, Salvato MS (1997) The promyelocytic leukemia protein PML has a pro-apoptotic activity mediated through its RING domain. FEBS Lett 418:30-34

Bourguet W, Germain P, Gronemeyer H (2000_a) Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci 21:381-388

Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. Nature 375:377-382

Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, Moras D (2000_b) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol Cell 5:289-298

Boutell C, Everett RD (2003) The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with and Ubiquitinates p53. J Biol Chem 278:36596-36602

Boyer TG, Martin ME, Lees E, Ricciardi RP, Berk AJ (1999) Mammalian Srb/Mediator complex is targeted by adenovirus E1A protein. Nature 399:276-279

Boyle S, Gilchrist S, Bridger JM, Mahy NL, Ellis JA, Bickmore WA (2001) The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. Hum Mol Genet 10:211-219

Braun H, Koop R, Ertmer A, Nacht S, Suske G (2001) Transcription factor Sp3 is regulated by acetylation. Nucleic Acids Res 29:4994-5000

Breitschopf K, Bengal E, Ziv T, Admon A, Ciechanover A (1998) A novel site for ubiquitination: the N-terminal residue, and not internal lysines of MyoD, is essential for conjugation and degradation of the protein. EMBO J 17:5964-5973

Brodsky JL, McCracken AA (1999) ER protein quality control and proteasome-mediated protein degradation. Semin Cell Dev Biol 10:507-513

Brusky J, Zhu Y, Xiao W (2000) UBC13, a DNA-damage-inducible gene, is a member of the error-free postreplication repair pathway in Saccharomyces cerevisiae. Curr Genet 37:168-174

Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox D 3rd, Fukuda M, Ohta T, Klevit R (2003) Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci U S A 100:5646-5651

Brzovic PS, Meza J, King MC, Klevit RE (1998) The cancer-predisposing mutation C61G disrupts homodimer formation in the NH2-terminal BRCA1 RING finger domain. J Biol Chem 273:7795-7799

Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE (2001) Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol 8:833-837

Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753-758

Burakov D, Crofts LA, Chang CP, Freedman LP (2002) Reciprocal recruitment of DRIP/mediator and p160 coactivator complexes in vivo by estrogen receptor. J Biol Chem 277:14359-14362

Burd CG, Dreyfuss G (1994) Conserved structures and diversity of functions of RNA-binding proteins. Science 265:615-621

Burke TW, Kadonaga JT (1997) The downstream core promoter element, DPE, is conserved from Drosophila to humans and is recognized by TAFII60 Drosophila. Genes Dev 11:3020-31

Buschmann T, Lerner D, Lee CG, Ronai Z (2001) The Mdm-2 amino terminus is required for Mdm2 binding and SUMO-1 conjugation by the E2 SUMO-1 conjugating enzyme Ubc9. J Biol Chem 276:40389-40395

Campbell Dwyer EJ, Lai H, MacDonald RC, Salvato MS, Borden KL (2000) The lymphocytic choriomeningitis virus RING protein Z associates with eukaryotic initiation factor 4E and selectively represses translation in a RING-dependent manner. J Virol 74:3293-3300

Capili AD, Edghill EL, Wu K, Borden KL (2004) Structure of the C-terminal RING finger from a RING-IBR-RING/TRIAD motif reveals a novel zinc-binding domain distinct from a RING. J Mol Biol 340:1117-1129

Caron H, van Sluis P, Buschman R, Pereira do Tanque R, Maes P, Beks L, de Kraker J, Voute PA, Vergnaud G, Westerveld A, Slater R, Versteeg R (1996) Allelic loss of the short arm of chromosome 4 in neuroblastoma suggests a novel tumour suppressor gene locus. Hum Genet 97:834-837

Chalkley GE, Verrijzer CP (1999) DNA binding site selection by RNA polymerase II TAFs: a TAF(II)250-TAF(II)150 complex recognizes the initiator. EMBO J 18:4835-4845

Chan HM, La Thangue NB (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J Cell Sci 114:2363-2373

Chen A, Kleiman FE, Manley JL, Ouchi T, Pan ZQ (2002) Autoubiquitination of the BRCA1*BARD1 RING ubiquitin ligase. J Biol Chem 277:22085-22092

Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR (1999) Regulation of transcription by a protein methyltransferase. Science 284:2174-2177

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569-580

Chen JD, Evans RM (1995) A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454-457

Chen L, Madura K (2002) Rad23 promotes the targeting of proteolytic substrates to the proteasome. Mol Cell Biol 22:4902-4913

Chen P, Johnson P, Sommer T, Jentsch S, Hochstrasser M (1993) Multiple ubiquitin-conjugating enzymes participate in the in vivo degradation of the yeast MAT alpha 2 repressor. Cell 74:357-369

Chen S, Supakar PC, Vellanoweth RL, Song CS, Chatterjee B, Roy AK (1997) Functional role of a conformationally flexible homopurine/homopyrimidine domain of the androgen receptor gene promoter interacting with Sp1 and a pyrimidine single strand DNA-binding protein. Mol Endocrinol 11:3-15

Chen WY, Lee WC, Hsu NC, Huang F, Chung BC (2004) SUMO modification of repression domains modulates function of nuclear receptor 5A1 (steroidogenic factor-1). J Biol Chem 279:38730-3875

Chen Z, Pickart CM (1990) A 25-kilodalton ubiquitin carrier protein (E2) catalyzes multi-ubiquitin chain synthesis via lysine 48 of ubiquitin. J Biol Chem 265:21835-21842

Cheng J, Wang D, Wang Z, Yeh ET (2004) SENP1 enhances androgen receptor-dependent transcription through desumoylation of histone deacetylase 1. Mol Cell Biol 24:6021-6028

Cheung E, Zarifyan AS, Kraus WL (2002) Histone H1 represses estrogen receptor alpha transcriptional activity by selectively inhibiting receptor-mediated transcription initiation. Mol Cell Biol 22:2463-2471

Cheung P, Allis CD, Sassone-Corsi P (2000) Signaling to chromatin through histone modifications. Cell 103:263-271

Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T (2003) Maintenance of stable heterochromatin domains by dynamic HP1 binding. Science 299:721-725

Chiariotti L, Benvenuto G, Fedele M, Santoro M, Simeone A, Fusco A, Bruni CB (1998) Identification and characterization of a novel RING-finger gene (RNF4) mapping at 4p16.3. Genomics 47:258-265

Cho Y, Gorina S, Jeffrey PD, Pavletich NP (1994) Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science 265:346-355

Chu-Ping M, Vu JH, Proske RJ, Slaughter CA, DeMartino GN (1994) Identification, purification, and characterization of a high molecular weight, ATP-dependent activator (PA700) of the 20 S proteasome. J Biol Chem 269:3539-3547

Ciechanover A, Ben-Saadon R (2004) N-terminal ubiquitination: more protein substrates join in. Trends Cell Biol 14:103-106

Clapier CR, Langst G, Corona DF, Becker PB, Nightingale KP (2001) Critical role for the histone H4 N terminus in nucleosome remodeling by ISWI. Mol Cell Biol 21:875-883

Clarke IJ, Cummins JT (1982) The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 111:1737-1739

Cleutjens KB, van der Korput HA, van Eekelen CC, van Rooij HC, Faber PW, Trapman J (1997) An androgen response element in a far upstream enhancer region is essential for high, androgen-regulated activity of the prostate-specific antigen promoter. Mol Endocrinol 11:148-161

Cohen SX, Moulin M, Hashemolhosseini S, Kilian K, Wegner M, Muller CW (2003) Structure of the GCM domain-DNA complex: a DNA-binding domain with a novel fold and mode of target site recognition. EMBO J 22:1835-1845

Comerford KM, Leonard MO, Karhausen J, Carey R, Colgan SP, Taylor CT (2003) Small ubiquitin-related modifier-1 modification mediates resolution of CREB-dependent responses to hypoxia. Proc Natl Acad Sci U S A 100:986-991

Correa-Cerro L, Wohr G, Haussler J, Berthon P, Drelon E, Mangin P, Fournier G, Cussenot O, Kraus P, Just W, Paiss T, Cantu JM, Vogel W (1999) (CAG)nCAA and GGN repeats in the human androgen receptor gene are not associated with prostate cancer in a French-German population. Eur J Hum Genet 7:357-362

Courey AJ, Tjian R (1988) Analysis of Sp1 in vivo reveals multiple transcriptional domains, including a novel glutamine-rich activation motif. Cell 55:887-898

Crawford AW, Pino JD, Beckerle MC (1994) Biochemical and molecular characterization of the chicken cysteine-rich protein, a developmentally regulated LIM-domain protein that is associated with the actin cytoskeleton. J Cell Biol 124:117-127

Curtin D, Jenkins S, Farmer N, Anderson AC, Haisenleder DJ, Rissman E, Wilson EM, Shupnik MA (2001) Androgen suppression of GnRH-stimulated rat LHbeta gene transcription occurs through Sp1 sites in the distal GnRH-responsive promoter region. Mol Endocrinol 15:1906-1917

Dahlman-Wright K, Wright A, Gustafsson JA, Carlstedt-Duke J (1991) Interaction of the glucocorticoid receptor DNA-binding domain with DNA as a dimer is mediated by a short segment of five amino acids. J Biol Chem 266:3107-3112

D'Andrea A, Pellman D (1998) Deubiquitinating enzymes: a new class of biological regulators. Crit Rev Biochem Mol Biol 33:337-352

Daniel MT, Koken M, Romagne O, Barbey S, Bazarbachi A, Stadler M, Guillemin MC, Degos L, Chomienne C, de The H (1993) PML protein expression in hematopoietic and acute promyelocytic leukemia cells. Blood 82:1858-1867

Danielian PS, White R, Lees JA, Parker MG (1992) Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors EMBO J 11:1025-1033

Dantonel JC, Wurtz JM, Poch O, Moras D, Tora L (1999) The TBP-like factor: an alternative transcription factor in metazoa? Trends Biochem Sci 24:335-339

Davies TH, Ning YM, Sanchez ER (2002) A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem 277:4597-4600

de Pater S, Greco V, Pham K, Memelink J, Kijne J (1996) Characterization of a zinc-dependent transcriptional activator from Arabidopsis. Nucleic Acids Res 24:4624-4631

Deblois G, Giguere V (2003) Ligand-independent coactivation of ERalpha AF-1 by steroid receptor RNA activator (SRA) via MAPK activation. J Steroid Biochem Mol Biol 85:123-131

Dellaire G, Farrall R, Bickmore WA (2003) The Nuclear Protein Database (NPD): sub-nuclear localisation and functional annotation of the nuclear proteome. Nucleic Acids Res 31:328-330

DeMartino GN, Slaughter CA (1999) The proteasome, a novel protease regulated by multiple mechanisms. J Biol Chem 274:22123-22126

Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, Chen ZJ (2000) Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell 103:351-361

Depoix C, Delmotte MH, Formstecher P, Lefebvre P (2001) Control of retinoic acid receptor heterodimerization by ligand-induced structural transitions. A novel mechanism of action for retinoid antagonists. J Biol Chem 276:9452-9459

Deshaies RJ, Ferrell JE Jr (2001) Multisite phosphorylation and the countdown to S phase. Cell 107:819-822

Desterro JM, Rodriguez MS, Hay RT (1998) SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. Mol Cell 2:233-239

Desterro JM, Rodriguez MS, Kemp GD, Hay RT (1999) Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. J Biol Chem 274:10618-10624

Desterro JM, Thomson J, Hay RT (1997) Ubch9 conjugates SUMO but not ubiquitin. FEBS Lett 417:297-300

Dick LR, Cruikshank AA, Grenier L, Melandri FD, Nunes SL, Stein RL (1996) Mechanistic studies on the inactivation of the proteasome by lactacystin: a central role for clasto-lactacystin beta-lactone. J Biol Chem 271:7273-7276

DiRenzo J, Shang Y, Phelan M, Sif S, Myers M, Kingston R, Brown M (2000) BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. Mol Cell Biol 20:7541-7549

Ditzel M, Wilson R, Tenev T, Zachariou A, Paul A, Deas E, Meier P (2003) Degradation of DIAP1 by the N-end rule pathway is essential for regulating apoptosis. Nat Cell Biol 5:467-473

Dobreva G, Dambacher J, Grosschedl R (2003) SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. Genes Dev 17:3048-3061

Dodd RB, Allen MD, Brown SE, Sanderson CM, Duncan LM, Lehner PJ, Bycroft M, Read RJ (2004) Solution structure of the kaposi's sarcoma-associated herpesvirus K3 N-terminal domain reveals a novel E2-binding C4HC3-type RING domain. J Biol Chem 279:53840-53847

Dong Y, Hakimi MA, Chen X, Kumaraswamy E, Cooch NS, Godwin AK, Shiekhattar R (2003) Regulation of BRCC, a holoenzyme complex containing BRCA1 and BRCA2, by a signalosome-like subunit and its role in DNA repair. Mol Cell 12:1087-1099

D'Orazi G, Cecchinelli B, Bruno T, Manni I, Higashimoto Y, Saito S, Gostissa M, Coen S, Marchetti A, Del Sal G, Piaggio G, Fanciulli M, Appella E, Soddu S (2002) Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. Nat Cell Biol 4:11-19

Dornan D, Wertz I, Shimizu H, Arnott D, Frantz GD, Dowd P, O'Rourke K, Koeppen H, Dixit VM (2004) The ubiquitin ligase COP1 is a critical negative regulator of p53. Nature 429:86-92

Dotson MR, Yuan CX, Roeder RG, Myers LC, Gustafsson CM, Jiang YW, Li Y, Kornberg RD, Asturias FJ (2000) Structural organization of yeast and mammalian mediator complexes. Proc Natl Acad Sci U S A 97:14307-14310

Doucas V, Tini M, Egan DA, Evans RM (1999) Modulation of CREB binding protein function by the promyelocytic (PML) oncoprotein suggests a role for nuclear bodies in hormone signaling. Proc Natl Acad Sci U S A 96:2627-2632

Dover J, Schneider J, Tawiah-Boateng MA, Wood A, Dean K, Johnston M, Shilatifard A (2002) Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. J Biol Chem 277:28368-28371

Drexler HC (1997) Activation of the cell death program by inhibition of proteasome function. Proc Natl Acad Sci U S A 94:855-860

Duprez E, Saurin AJ, Desterro JM, Lallemand-Breitenbach V, Howe K, Boddy MN, Solomon E, de The H, Hay RT, Freemont PS (1999) SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. J Cell Sci 112:381-393

Durrin LK, Krontiris TG (2002) The thymocyte-specific MAR binding protein, SATB1, interacts in vitro with a novel variant of DNA-directed RNA polymerase II, subunit 11. Genomics 79:809-817

Dworetzky SI, Fey EG, Penman S, Lian JB, Stein JL, Stein GS (1990) Progressive changes in the protein composition of the nuclear matrix during rat osteoblast differentiation. Proc Natl Acad Sci U S A 87:4605-4609

Dyck JA, Maul GG, Miller WH Jr, Chen JD, Kakizuka A, Evans RM (1994) A novel macromolecular structure is a target of the promyelocyte-retinoic acid receptor oncoprotein. Cell 76:333-343

Eder IE, Haag P, Basik M, Mousses S, Bektic J, Bartsch G, Klocker H (2003) Gene expression changes following androgen receptor elimination in LNCaP prostate cancer cells. Mol Carcinog 37:181-191

Edwards SM, Badzioch MD, Minter R, Hamoudi R, Collins N, Ardern-Jones A, Dowe A, Osborne S, Kelly J, Shearer R, Easton DF, Saunders GF, Dearnaley DP, Eeles RA (1999) Androgen receptor polymorphisms: association with prostate cancer risk, relapse and overall survival. Int J Cancer 84:458-465

Eissenberg JC, Ma J, Gerber MA, Christensen A, Kennison JA, Shilatifard A (2002) dELL is an essential RNA polymerase II elongation factor with a general role in development Proc Natl Acad Sci U S A 99: 9894-9899

Elbi C, Misteli T, Hager GL (2002) Recruitment of dioxin receptor to active transcription sites. Mol Biol Cell 13:2001-2015

Ellis JA, Stebbing M, Harrap SB (2001) Polymorphism of the androgen receptor gene is associated with male pattern baldness. J Invest Dermatol 116:452-255

El-Osta A, Wolffe AP (2000) DNA methylation and histone deacetylation in the control of gene expression: basic biochemistry to human development. Gene Expr 9:63-75

Elsasser S, Chandler-Militello D, Muller B, Hanna J, Finley D (2004) Rad23 and Rpn10 serve as alternative ubiquitin receptors for the proteasome. J Biol Chem 279:26817-26822

Engelhardt OG, Boutell C, Orr A, Ullrich E, Haller O, Everett RD (2003) The homeodomain-interacting kinase PKM (HIPK-2) modifies ND10 through both its kinase domain and a SUMO-1 interaction motif and alters the posttranslational modification of PML. Exp Cell Res 283:36-50

Eskiw CH, Dellaire G, Bazett-Jones DP (2004) Chromatin contributes to structural integrity of promyelocytic leukemia bodies through a SUMO-1-independent mechanism. J Biol Chem 279:9577-9585

Eskiw CH, Dellaire G, Mymryk JS, Bazett-Jones DP (2003) Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. J Cell Sci 116:4455-4466

Everett RD (2001) DNA viruses and viral proteins that interact with PML nuclear bodies. Oncogene 20:7266-7273

Everett RD, Barlow P, Milner A, Luisi B, Orr A, Hope G, Lyon D (1993) A novel arrangement of zinc-binding residues and secondary structure in the C3HC4 motif of an alpha herpes virus protein family. J Mol Biol 234:1038-1047

Everett RD, Lomonte P, Sternsdorf T, van Driel R, Orr A (1999) Cell cycle regulation of PML modification and ND10 composition. J Cell Sci 112:4581-4588

Fagioli M, Alcalay M, Tomassoni L, Ferrucci PF, Mencarelli A, Riganelli D, Grignani F, Pozzan T, Nicoletti I, Grignani F, Pelicci PG (1998) Cooperation between the RING + B1-B2 and coiled-coil domains of PML is necessary for its effects on cell survival. Oncogene 16:2905-2913

Fang S, Jensen JP, Ludwig RL, Vousden KH, Weissman AM (2000) Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. J Biol Chem 275:8945-8951

Fazzio TG, Tsukiyama T (2003) Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. Mol Cell 12:1333-1340

Fedele M, Benvenuto G, Pero R, Majello B, Battista S, Lembo F, Vollono E, Day PM, Santoro M, Lania L, Bruni CB, Fusco A, Chiariotti L (2000) A novel member of the BTB/POZ family, PATZ, associates with the RNF4 RING finger protein and acts as a transcriptional repressor. J Biol Chem 275:7894-7901

Feng Q, Zhang Y (2003) The NuRD complex: linking histone modification to nucleosome remodeling. Curr Top Microbiol Immunol 274:269-290

Fenteany G, Standaert RF, Lane WS, Choi S, Corey EJ, Schreiber SL (1995) Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacystin. Science 268:726-731

Festenstein R, Pagakis SN, Hiragami K, Lyon D, Verreault A, Sekkali B, Kioussis D (2003) Modulation of heterochromatin protein 1 dynamics in primary Mammalian cells. Science 299:719-721

Fey EG, Krochmalnic G, Penman S (1986) The nonchromatin substructures of the nucleus: the ribonucleoprotein (RNP)-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. J Cell Biol 102:1654-1665

Fey EG, Penman S (1988) Nuclear matrix proteins reflect cell type of origin in cultured human cells. Proc Natl Acad Sci U S A 85:121-125

Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W, Verdin E (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 9:45-57

Fischle W, Wang Y, Allis CD (2003) Histone and chromatin cross-talk. Curr Opin Cell Biol 15:172-183

Fisk HA, Yaffe MP (1999) A role for ubiquitination in mitochondrial inheritance in Saccharomyces cerevisiae. J Cell Biol 145:1199-1208

Flanagan PM, Kelleher RJ 3rd, Sayre MH, Tschochner H, Kornberg RD (1991) A mediator required for activation of RNA polymerase II transcription in vitro. Nature 350:436-438

Flaus A, Owen-Hughes T (2001) Mechanisms for ATP-dependent chromatin remodelling. Curr Opin Genet Dev 11:148-154

Fogal V, Gostissa M, Sandy P, Zacchi P, Sternsdorf T, Jensen K, Pandolfi PP, Will H, Schneider C, Del Sal G (2000) Regulation of p53 activity in nuclear bodies by a specific PML isoform. EMBO J 19:6185-6195

Fondell JD, Ge H, Roeder RG (1996) Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc Natl Acad Sci U S A 93:8329-8333

Fondell JD, Guermah M, Malik S, Roeder RG (1999) Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID. Proc Natl Acad Sci U S A 96:1959-1964

Forman BM, Casanova J, Raaka BM, Ghysdael J, Samuels HH (1992) Half-site spacing and orientation determines whether thyroid hormone and retinoic acid receptors and related factors bind to DNA response elements as monomers, homodimers, or heterodimers. Mol Endocrinol 6:429-442

Frank C, Gonzalez MM, Oinonen C, Dunlop TW, Carlberg C (2003) Characterization of DNA complexes formed by the nuclear receptor constitutive androstane receptor. J Biol Chem 278:43299-43310

Freedman LP (1992) Anatomy of the steroid receptor zinc finger region. Endocr Rev 13:129-145

Freedman LP, Luisi BF, Korszun ZR, Basavappa R, Sigler PB, Yamamoto KR (1988) The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. Nature 334:543-546

Freemont PS (2000) RING for destruction? Curr Biol 10:R84-R87

Freemont PS, Hanson IM, Trowsdale J (1991) A novel cysteine-rich sequence motif. Cell 64:483-484

Freyd G, Kim SK, Horvitz HR (1990) Novel cysteine-rich motif and homeodomain in the product of the Caenorhabditis elegans cell lineage gene lin-11. Nature 344:876-879

Fryer CJ, Archer TK (1998) Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. Nature 393:88-91

Fu M, Rao M, Wang C, Sakamaki T, Wang J, Di Vizio D, Zhang X, Albanese C, Balk S, Chang C, Fan S, Rosen E, Palvimo JJ, Jänne OA, Muratoglu S, Avantaggiati ML, Pestell RG (2003) Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. Mol Cell Biol 23:8563-8575

Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, Pestell RG (2000) p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 275:20853-20860

Fuchs SY, Ronai Z (1999) Ubiquitination and degradation of ATF2 are dimerization dependent. Mol Cell Biol 19:3289-3298

Fujita N, Jaye DL, Kajita M, Geigerman C, Moreno CS, Wade PA (2003) MTA3, a Mi-2/NuRD complex subunit, regulates an invasive growth pathway in breast cancer. Cell 113:207-219

Fujita N, Kajita M, Taysavang P, Wade PA (2004) Hormonal regulation of metastasis-associated protein 3 transcription in breast cancer cells. Mol Endocrinol 18:2937-2949

Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem 278:4035-4040

Funakoshi M, Sasaki T, Nishimoto T, Kobayashi H (2002) Budding yeast Dsk2p is a polyubiquitin-binding protein that can interact with the proteasome. Proc Natl Acad Sci U S A 99:745-750

Galili N, Nayak S, Epstein JA, Buck CA (2000) Rnf4, a RING protein expressed in the developing nervous and reproductive systems, interacts with Gscl, a gene within the DiGeorge critical Dev Dyn 218:102-111

Galy A, Neron B, Planque N, Saule S, Eychene A (2002) Activated MAPK/ERK kinase (MEK-1) induces transdifferentiation of pigmented epithelium into neural retina. Dev Biol 248:251-264

Gaughan L, Logan IR, Cook S, Neal DE, Robson CN (2002) Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor. J Biol Chem 277:25904-25913

Gervais V, Busso D, Wasielewski E, Poterszman A, Egly JM, Thierry JC, Kieffer B (2001) Solution structure of the N-terminal domain of the human TFIIH MAT1 subunit: new insights into the RING finger. J Biol Chem 276:7457-7464

Geyer RK, Yu ZK, Maki CG (2000) The MDM2 RING-finger domain is required to promote p53 nuclear export. Nat Cell Biol 2:569-573

Gilbert N, Boyle S, Fiegler H, Woodfine K, Carter NP, Bickmore WA (2004) Chromatin architecture of the human genome: gene-rich domains are enriched in open chromatin fibers. Cell 118:555-566

Gill G, Pascal E, Tseng ZH, Tjian R (1994) A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the component of the Drosophila TFIID complex and mediates transcriptional activation. Proc Natl Acad Sci U S A 91:192-196

Gilon T, Chomsky O, Kulka RG (1998) Degradation signals for ubiquitin system proteolysis in Saccharomyces cerevisiae. EMBO J 17:2759-2766

Girdwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, Garcia-Wilson E, Perkins ND, Hay RT (2003) P300 transcriptional repression is mediated by SUMO modification. Mol Cell 11:1043-1054

Glass CK (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev 15:391-407

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373-428

Glover JN, Harrison SC (1995) Crystal structure of the heterodimeric bZIP transcription factor c-Fos-c-Jun bound to DNA. Nature 373:257-261

Goldman RD, Gruenbaum Y, Moir RD, Shumaker DK, Spann TP (2002) Nuclear lamins: building blocks of nuclear architecture. Genes Dev 16:533-547

Goldstein G, Scheid M, Hammerling U, Schlesinger DH, Niall HD, Boyse EA (1975) Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. Proc Natl Acad Sci U S A 72:11-15

Gonen H, Bercovich B, Orian A, Carrano A, Takizawa C, Yamanaka K, Pagano M, Iwai K, Ciechanover A (1999) Identification of the ubiquitin carrier proteins, E2s, involved in signal-induced conjugation and subsequent degradation of IkappaBalpha. J Biol Chem 274:14823-14830

Gong L, Kamitani T, Fujise K, Caskey LS, Yeh ET (1997) Preferential interaction of sentrin with a ubiquitin-conjugating enzyme, Ubc9. J Biol Chem 272:28198-28201

Gong L, Li B, Millas S, Yeh ET (1999) Molecular cloning and characterization of human AOS1 and UBA2, components of the sentrin-activating enzyme complex. FEBS Lett 448:185-189

Gong L, Millas S, Maul GG, Yeh ET (2000) Differential regulation of sentrinized proteins by a novel sentrin-specific protease. J Biol Chem 275:3355-3359

Gong L, Yeh ET (1999) Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J Biol Chem 274:12036-12042

Goodson ML, Hong Y, Rogers R, Matunis MJ, Park-Sarge OK, Sarge KD (2001) Sumo-1 modification regulates the DNA binding activity of heat shock transcription factor 2, a promyelocytic leukemia nuclear body associated transcription factor. J Biol Chem 276:18513-18518

Görisch SM, Wachsmuth M, Ittrich C, Bacher CP, Rippe K, Lichter P (2004) Nuclear body movement is determined by chromatin accessibility and dynamics. Proc Natl Acad Sci U S A 101:13221-13226

Grad JM, Dai JL, Wu S, Burnstein KL (1999) Multiple androgen response elements and a Myc consensus site in the androgen receptor (AR) coding region are involved in androgen-mediated up-regulation of AR messenger RNA. Mol Endocrinol 13:1896-1911

Grande MA, van der Kraan I, de Jong L, van Driel R (1997) Nuclear distribution of transcription factors in relation to sites of transcription and RNA polymerase II. J Cell Sci 110:1781-1791

Greer SF, Zika E, Conti B, Zhu XS, Ting JP (2003) Enhancement of CIITA transcriptional function by ubiquitin. Nat Immunol 4:1074-1082

Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM (2001) A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 61:4315-4319

Grenfell SJ, Trausch-Azar JS, Handley-Gearhart PM, Ciechanover A, Schwartz AL (1994) Nuclear localization of the ubiquitin-activating enzyme, E1, is cell-cycle-dependent. Biochem J 300:701-708

Greschik H, Wurtz JM, Sanglier S, Bourguet W, van Dorsselaer A, Moras D, Renaud JP (2002) Structural and functional evidence for ligand-independent transcriptional activation by the estrogen-related receptor 3. Mol Cell 9:303-313

Grewal SI, Elgin SC (2002) Heterochromatin: new possibilities for the inheritance of structure. Curr Opin Genet Dev 12:178-187

Grimm S, Baeuerle PA (1993) The inducible transcription factor NF-kappa B: structure-function relationship of its protein subunits. Biochem J 290:297-308

Grossman SR, Deato ME, Brignone C, Chan HM, Kung AL, Tagami H, Nakatani Y, Livingston DM (2003) Polyubiquitination of p53 by a ubiquitin ligase activity of p300. Science 300:342-344

Grüne T, Brzeski J, Eberharter A, Clapier CR, Corona DF, Becker PB, Müller CW (2003) Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. Mol Cell 12:449-460

Gu W, Malik S, Ito M, Yuan CX, Fondell JD, Zhang X, Martinez E, Qin J, Roeder RG (1999) A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol Cell 3:97-108

Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, Wang J, Dong Z, Brusko T, Atkinson M, Pozzilli P, Zeidler A, Raffel LJ, Jacob CO, Park Y, Serrano-Rios M, Larrad MT, Zhang Z, Garchon HJ, Bach JF, Rotter JI, She JX, Wang CY (2004) A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. Nat Genet 36:837-841

Gurley LR, D'Anna JA, Barham SS, Deaven LL, Tobey RA (1978) Histone phosphorylation and chromatin structure during mitosis in Chinese hamster cells. Eur J Biochem 84:1-15

Gurrieri C, Capodieci P, Bernardi R, Scaglioni PP, Nafa K, Rush LJ, Verbel DA, Cordon-Cardo C, Pandolfi PP (2004) Loss of the tumor suppressor PML in human cancers of multiple histologic origins. J Natl Cancer Inst 96:269-279

Haas A, Reback PM, Pratt G, Rechsteiner M (1990) Ubiquitin-mediated degradation of histone H3 does not require the substrate-binding ubiquitin protein ligase, E3, or attachment of polyubiquitin chains. J Biol Chem 265:21664-21669

Hagglund R, Roizman B (2003) Herpes simplex virus 1 mutant in which the ICP0 HUL-1 E3 ubiquitin ligase site is disrupted stabilizes cdc34 but degrades D-type cyclins and exhibits diminished neurotoxicity. J Virol 77:13194-13202

Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I (2003) Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. Nat Cell Biol 5:461-466

Halvorson LM, Ito M, Jameson JL, Chin WW (1998) Steroidogenic factor-1 and early growth response protein 1 act through two composite DNA binding sites to regulate luteinizing hormone beta-subunit gene expression. J Biol Chem 273:14712-14720

Hamiche A, Sandaltzopoulos R, Gdula DA, Wu C (1999) ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell 97:833-842

Hang J, Dasso M (2002) Association of the human SUMO-1 protease SENP2 with the nuclear pore. J Biol Chem 277:19961-19966

Haracska L, Torres-Ramos CA, Johnson RE, Prakash S, Prakash L (2004) Opposing effects of ubiquitin conjugation and SUMO modification of PCNA on replicational bypass of DNA lesions in Saccharomyces cerevisiae. Mol Cell Biol 24:4267-4274

Hardeland U, Steinacher R, Jiricny J, Schar P (2002) Modification of the human thymine-DNA glycosylase by ubiquitin-like proteins facilitates enzymatic turnover. EMBO J 21:1456-1464

Harding HP, Lazar MA (1995) The monomer-binding orphan receptor Rev-Erb represses transcription as a dimer on a novel direct repeat. Mol Cell Biol 15:4791-4802

Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T (2001) The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem 276:14537-14540

Hatfield PM, Vierstra RD (1992) Multiple forms of ubiquitin-activating enzyme E1 from wheat. Identification of an essential cysteine by in vitro mutagenesis. J Biol Chem267:14799-14803

Hattori T, Ohoka N, Inoue Y, Hayashi H, Onozaki K (2003) C/EBP family transcription factors are degraded by the proteasome but stabilized by forming dimer. Oncogene 22:1273-1280

Haupt Y, Alexander WS, Barri G, Klinken SP, Adams JM (1991) Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphomagenesis in E mu-myc transgenic mice. Cell 65:753-763

Haupt Y, Maya R, Kazaz A, Oren M (1997) Mdm2 promotes the rapid degradation of p53. Nature 387:296-299

Hauser HP, Bardroff M, Pyrowolakis G, Jentsch S (1998) A giant ubiquitin-conjugating enzyme related to IAP apoptosis inhibitors. J Cell Biol 141:1415-1422

He B, Wilson EM (2002) The NH(2)-terminal and carboxyl-terminal interaction in the human androgen receptor. Mol Genet Metab 75:293-298

Hein C, Springael JY, Volland C, Haguenauer-Tsapis R, Andre B (1995) NPl1, an essential yeast gene involved in induced degradation of Gap1 and Fur4 permeases, encodes the Rsp5 ubiquitin-protein ligase. Mol Microbiol 18:77-87

Heinlein CA, Chang C (2002) Androgen receptor (AR) coregulators: an overview. Endocr Rev 23:175-200

Hengartner CJ, Thompson CM, Zhang J, Chao DM, Liao SM, Koleske AJ, Okamura S, Young RA (1995) Association of an activator with an RNA polymerase II holoenzyme. Genes Dev 9:897-910

Hentschke M, Borgmeyer U (2003) Identification of PNRC2 and TLE1 as activation function-1 cofactors of the orphan nuclear receptor ERRgamma. Biochem Biophys Res Commun 312:975-982

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425-479

Hietakangas V, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI, Mikhailov A, Palvimo JJ, Pirkkala L, Sistonen L (2003) Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. Mol Cell Biol 23:2953-2968

Hill DA (2001) Influence of linker histone H1 on chromatin remodeling. Biochem Cell Biol 79:317-324

Hill DA, Imbalzano AN (2000) Human SWI/SNF nucleosome remodeling activity is partially inhibited by linker histone H1. Biochemistry 39:11649-11656

Hirvonen-Santti SJ, Rannikko A, Santti H, Savolainen S, Nyberg M, Jänne OA, Palvimo JJ (2003) Down-regulation of estrogen receptor beta and transcriptional coregulator SNURF/RNF4 in testicular germ cell cancer. Eur Urol 44:742-747

Hirvonen-Santti SJ, Sriraman V, Anttonen M, Savolainen S, Palvimo JJ, Heikinheimo M, Richards JS, Jänne OA (2004) Small nuclear RING finger protein expression during gonad development: regulation by gonadotropins and estrogen in the postnatal ovary. Endocrinology 145:2433-2444

Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Annu Rev Genet 30:405-439

Hodges M, Tissot C, Howe K, Grimwade D, Freemont PS (1998) Structure, organization, and dynamics of promyelocytic leukemia protein nuclear bodies. Am J Hum Genet 63:297-304

Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419:135-141

Hofmann H, Floss S, Stamminger T (2000) Covalent modification of the transactivator protein IE2-p86 of human cytomegalovirus by conjugation to the ubiquitin-homologous proteins SUMO-1 and hSMT3b. J Virol 74:2510-2524

Hofmann RM, Pickart CM (2001) In vitro assembly and recognition of Lys-63 polyubiquitin chains. J Biol Chem 276:27936-27943

Hofmann TG, Moller A, Sirma H, Zentgraf H, Taya Y, Droge W, Will H, Schmitz ML (2002) Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. Nat Cell Biol 4:1-10

Hong Y, Rogers R, Matunis MJ, Mayhew CN, Goodson ML, Park-Sarge OK, Sarge KD, Goodson M (2001) Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. J Biol Chem 276:40263-40267

Hook SS, Orian A, Cowley SM, Eisenman RN (2002) Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes. Proc Natl Acad Sci U S A 99:13425-13430

Hoppe T, Matuschewski K, Rape M, Schlenker S, Ulrich HD, Jentsch S (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102:577-586

Hörlein AJ, Näär AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK (1995) Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397-404

Horn PJ, Carruthers LM, Logie C, Hill DA, Solomon MJ, Wade PA, Imbalzano AN, Hansen JC, Peterson CL (2002) Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. Nat Struct Biol 9:263-267

Horn PJ, Peterson CL (2002) Molecular biology. Chromatin higher order folding--wrapping up transcription. Science 297:1824-1827

Horton CD, Halvorson LM (2004) The cAMP signaling system regulates LHbeta gene expression: roles of early growth response protein-1, SP1 and steroidogenic factor-1. J Mol Endocrinol 32:291-306

Huang ME, Ye YC, Chen SR, Chai JR, Lu JX, Zhoa L, Gu LJ, Wang ZY (1988) Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567-572

Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S (2003) Sequential modification of NEMO/ IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. Cell 115:565-576

Huang ZQ, Li J, Sachs LM, Cole PA, Wong J (2003) A role for cofactor-cofactor and cofactor-histone interactions in targeting p300, SWI/SNF and Mediator for transcription. EMBO J 22:2146-2155

Huibregtse JM, Yang JC, Beaudenon SL (1997) The large subunit of RNA polymerase II is a substrate of the Rsp5 ubiquitin-protein ligase. Proc Natl Acad Sci U S A 94:3656-3661

Ikonen T, Palvimo JJ, Jänne OA (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. J Biol Chem 272:29821-29828

Iniguez-Lluhi JA, Pearce D (2000) A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. Mol Cell Biol 20:6040-6050

Inoue S, Kondo S, Hashimoto M, Kondo T, Muramatsu M (1991) Isolation of estrogen receptor-binding sites in human genomic DNA. Nucleic Acids Res 19:4091-4096

Ishov AM, Sotnikov AG, Negorev D, Vladimirova OV, Neff N, Kamitani T, Yeh ET, Strauss JF 3rd, Maul GG (1999) PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. J Cell Biol 147:221-234

Ito M, Okano HJ, Darnell RB, Roeder RG (2002) The TRAP100 component of the TRAP/Mediator complex is essential in broad transcriptional events and development. EMBO J 21:3464-3475

Ito M, Yuan CX, Okano HJ, Darnell RB, Roeder RG (2000) Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. Mol Cell 5:683-693

Ito T, Ikehara T, Nakagawa T, Kraus WL, Muramatsu M (2000) p300-mediated acetylation facilitates the transfer of histone H2A-H2B dimers from nucleosomes to a histone chaperone. Genes Dev 14:1899-1907

Ito T, Levenstein ME, Fyodorov DV, Kutach AK, Kobayashi R, Kadonaga JT (1999) ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev 13:1529-1539

Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr (2001) HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292:464-468

Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim Av, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ (2001) Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science 292:468-472

Jackson DA, Iborra FJ, Manders EM, Cook PR (1998) Numbers and organization of RNA polymerases, nascent transcripts, and transcription units in HeLa nuclei. Mol Biol Cell 9:1523-1536

Jackson PK, Eldridge AG, Freed E, Furstenthal L, Hsu JY, Kaiser BK, Reimann JD (2000) The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases. Trends Cell Biol 10:429-439

Janknecht R, Hunter T (1996) Transcription. A growing coactivator network. Nature 383:22-23

Janknecht R, Zinck R, Ernst WH, Nordheim A (1994) Functional dissection of the transcription factor Elk-1. Oncogene 9:1273-1278

Jensen DE, Proctor M, Marquis ST, Gardner HP, Ha SI, Chodosh LA, Ishov AM, Tommerup N, Vissing H, Sekido Y, Minna J, Borodovsky A, Schultz DC, Wilkinson KD, Maul GG, Barlev N, Berger SL, Prendergast GC, Rauscher FJ 3rd (1998) BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. Oncogene 16:1097-1112

Jensen K, Shiels C, Freemont PS (2001) PML protein isoforms and the RBCC/TRIM motif. Oncogene 20:7223-7233

Jentsch S (1992) The ubiquitin-conjugation system. Annu Rev Genet 26:179-207

Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074-1080

Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW (2002) Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell 111:709-720

Jiang F, Wang Z (2003) Identification of androgen-responsive genes in the rat ventral prostate by complementary deoxyribonucleic acid subtraction and microarray. Endocrinology 144:1257-1265

Jin C, Shiyanova T, Shen Z, Liao X (2001) Heteronuclear nuclear magnetic resonance assignments, structure and dynamics of SUMO-1, a human ubiquitin-like protein. Int J Biol Macromol 28:227-234

Jin Y, Xu XL, Yang MC, Wei F, Ayi TC, Bowcock AM, Baer R (1997) Cell cycle-dependent colocalization of BARD1 and BRCA1 proteins in discrete nuclear domains. Proc Natl Acad Sci U S A 94:12075-12080

Joazeiro CA, Weissman AM (2000) RING finger proteins: mediators of ubiquitin ligase activity. Cell 102:549-552

Joazeiro CA, Wing SS, Huang H, Leverson JD, Hunter T, Liu YC (1999) The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. Science 286:309-312

Johnson ES (2002) Ubiquitin branches out. Nat Cell Biol 4:E295-E298

Johnson ES, Blobel G (1997) Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. J Biol Chem 272:26799-26802

Johnson ES, Gupta AA (2001) An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 106:735-744

Johnson ES, Ma PC, Ota IM, Varshavsky A (1995) A proteolytic pathway that recognizes ubiquitin as a degradation signal. J Biol Chem 270:17442-17456

Johnson PR, Swanson R, Rakhilina L, Hochstrasser M (1998) Degradation signal masking by heterodimerization of MATalpha2 and MATa1 blocks their mutual destruction by the ubiquitin-proteasome pathway. Cell 94:217-227

Jorgensen JS, Nilson JH (2001) AR suppresses transcription of the LHbeta subunit by interacting with steroidogenic factor-1. Mol Endocrinol 15:1505-1516

Kadonaga JT, Carner KR, Masiarz FR, Tjian R (1987) Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. Cell 51:1079-1090

Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. Cell 113:127-137

Kahyo T, Nishida T, Yasuda H (2001) Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol Cell 8:713-718

Kaiser FJ, Möröy T, Chang GT, Horsthemke B, Ludecke HJ (2003) The RING finger protein RNF4, a co-regulator of transcription, interacts with the TRPS1 transcription factor. J Biol Chem 278:38780-38785

Kaiser UB, Halvorson LM, Chen MT (2000) Sp1, steroidogenic factor 1 (SF-1), and early growth response protein 1 (egr-1) binding sites form a tripartite gonadotropin-releasing hormone response element in the rat luteinizing hormone-beta gene promoter: an integral role for SF-1. Mol Endocrinol 14:1235-1245

Kallen J, Schlaeppi JM, Bitsch F, Filipuzzi I, Schilb A, Riou V, Graham A, Strauss A, Geiser M, Fournier B (2004) Evidence for ligand-Independent transcriptional activation of the human estrogen-related receptor alpha: Crystal structure of ERRalpha LBD in complex with PGC-1alpha. J Biol Chem 279:49330-49337

Kallenberger BC, Love JD, Chatterjee VK, Schwabe JW (2003) A dynamic mechanism of nuclear receptor activation and its perturbation in a human disease. Nat Struct Biol 10:136-140

Kamitani T, Kito K, Nguyen HP, Wada H, Fukuda-Kamitani T, Yeh ET (1998) Identification of three major sentrinization sites in PML. J Biol Chem 273:26675-26682

Kamynina E, Debonneville C, Bens M, Vandewalle A, Staub O (2001) A novel mouse Nedd4 protein suppresses the activity of the epithelial Na+ channel. FASEB J 15:204-214

Kang ES, Park CW, Chung JH (2001) Dnmt3b, de novo DNA methyltransferase, interacts with SUMO-1 and Ubc9 through its N-terminal region and is subject to modification by SUMO-1. Biochem Biophys Res Commun 289:862-868

Kang RS, Daniels CM, Francis SA, Shih SC, Salerno WJ, Hicke L, Radhakrishnan I (2003) Solution structure of a CUE-ubiquitin complex reveals a conserved mode of ubiquitin binding. Cell 113:621-630

Kang Z, Pirskanen A, Jänne OA, Palvimo JJ (2002) Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277:48366-48371

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, Chambon P (1990) Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603-1614

Katzmann DJ, Babst M, Emr SD (2001) Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. Cell 106:145-155

Kaul S, Murphy PJ, Chen J, Brown L, Pratt WB, Simons SS Jr (2002) Mutations at positions 547-553 of rat glucocorticoid receptors reveal that hsp90 binding requires the presence, but not defined composition, of a seven-amino acid sequence at the amino terminus of the ligand binding domain. J Biol Chem 277:36223-36232

Ke PY, Chang ZF (2004) Mitotic degradation of human thymidine kinase 1 is dependent on the anaphase-promoting complex/cyclosome-CDH1-mediated pathway. Mol Cell Biol 24:514-526

Kentsis A, Borden KL (2000) Construction of macromolecular assemblages in eukaryotic processes and their role in human disease: linking RINGs together. Curr Protein Pept Sci 1:49-73

Kentsis A, Gordon RE, Borden KL (2002_a) Self-assembly properties of a model RING domain. Proc Natl Acad Sci U S A 99:667-672

Kentsis A, Gordon RE, Borden KL (2002_b) Control of biochemical reactions through supramolecular RING domain self-assembly. Proc Natl Acad Sci U S A 99:15404-15409

Keri RA, Bachmann DJ, Behrooz A, Herr BD, Ameduri RK, Quirk CC, Nilson JH (2000) An NF-Y binding site is important for basal, but not gonadotropin-releasing hormone-stimulated, expression of the luteinizing hormone beta subunit gene. J Biol Chem 275:13082-13088

Khochbin S, Verdel A, Lemercier C, Seigneurin-Berny D (2001) Functional significance of histone deacetylase diversity. Curr Opin Genet Dev 11:162-166

Khorasanizadeh S, Rastinejad F (2001) Nuclear-receptor interactions on DNA-response elements. Trends Biochem Sci 26:384-390

Kim BW, Zavacki AM, Curcio-Morelli C, Dentice M, Harney JW, Larsen PR, Bianco AC (2003) Endoplasmic reticulum-associated degradation of the human type 2 iodothyronine deiodinase (D2) is mediated via an association between mammalian UBC7 and the carboxyl region of D2. Mol Endocrinol 17:2603-2612

Kim EJ, Park JS, Um SJ (2003) Identification of Daxx interacting with p73, one of the p53 family, and its regulation of p53 activity by competitive interaction with PML. Nucleic Acids Res 31:5356-5367

Kim J, Sif S, Jones B, Jackson A, Koipally J, Heller E, Winandy S, Viel A, Sawyer A, Ikeda T, Kingston R, Georgopoulos K (1999) Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. Immunity 10:345-355

Kim KI, Baek SH, Jeon YJ, Nishimori S, Suzuki T, Uchida S, Shimbara N, Saitoh H, Tanaka K, Chung CH (2000) A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. J Biol Chem 275:14102-14106

Kim YH, Choi CY, Kim Y (1999) Covalent modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. Proc Natl Acad Sci U S A 96:12350-12355

Kim YJ, Bjorklund S, Li Y, Sayre MH, Kornberg RD (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell 77:599-608

King RW, Glotzer M, Kirschner MW (1996) Mutagenic analysis of the destruction signal of mitotic cyclins and structural characterization of ubiquitinated intermediates. Mol Biol Cell 7:1343-1357

Kirsh O, Seeler JS, Pichler A, Gast A, Muller S, Miska E, Mathieu M, Harel-Bellan A, Kouzarides T, Melchior F, Dejean A (2002) The SUMO E3 ligase RanBP2 promotes modification of the HDAC4 deacetylase. EMBO J 21:2682-2691

Kishino T, Lalande M, Wagstaff J (1997) UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15:70-73

Koegl M, Hoppe T, Schlenker S, Ulrich HD, Mayer TU, Jentsch S (1999) A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. Cell 96:635-644

Koh SS, Chen D, Lee YH, Stallcup MR (2001) Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. J Biol Chem 276:1089-1098

Koken MH, Puvion-Dutilleul F, Guillemin MC, Viron A, Linares-Cruz G, Stuurman N, de Jong L, Szostecki C, Calvo F, Chomienne C (1994) The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J 13:1073-1083

Komarnitsky P, Cho EJ, Buratowski S (2000) Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev 14:2452-2460

Komatsu M, Chiba T, Tatsumi K, Iemura S, Tanida I, Okazaki N, Ueno T, Kominami E, Natsume T, Tanaka K (2004) A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. EMBO J 23:1977-1986

Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10:524-530

Kornberg RD, Lorch Y (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98:285-294

Kornitzer D, Raboy B, Kulka RG, Fink GR (1994) Regulated degradation of the transcription factor Gcn4. EMBO J 13:6021-6030

Kotaja N, Karvonen U, Jänne OA, Palvimo JJ (2002_a) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 22:5222-5234

Kotaja N, Karvonen U, Jänne OA, Palvimo JJ (2002_b) The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. J Biol Chem 277:30283-30288

Kouzarides T (2002) Histone methylation in transcriptional control. Curr Opin Genet Dev 12:198-209

Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G (2003) The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 424:801-805

Kraulis PJ, Raine AR, Gadhavi PL, Laue ED (1992) Structure of the DNA-binding domain of zinc GAL4. Nature 356:448-450

Kraus WL, McInerney EM, Katzenellenbogen BS (1995) Ligand-dependent, transcriptionally productive association of the amino- and carboxyl-terminal regions of a steroid hormone nuclear receptor. Proc Natl Acad Sci U S A 92:12314-12318

Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V, Richards DP, Wu X, Emili A, Hughes TR, Buratowski S, Greenblatt JF (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell 12:1565-1576

Kubbutat MH, Jones SN, Vousden KH (1997) Regulation of p53 stability by Mdm2. Nature 387:299-303

Kumar S, Kao WH, Howley PM (1997) Physical interaction between specific E2 and Hect E3 enzymes determines functional cooperativity. J Biol Chem 272:13548-13554

Kumaran RI, Muralikrishna B, Parnaik VK (2002) Lamin A/C speckles mediate spatial organization of splicing factor compartments and RNA polymerase II transcription. J Cell Biol 159:783-793

Kurdistani SK, Grunstein M (2003) Histone acetylation and deacetylation in yeast. Nat Rev Mol Cell Biol 4:276-284

Kurepa J, Walker JM, Smalle J, Gosink MM, Davis SJ, Durham TL, Sung DY, Vierstra RD (2003) The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis. Accumulation of SUMO1 and –2 conjugates is increased by stress. J Biol Chem 278:6862-6872

Kurokawa R, Yu VC, Näär A, Kyakumoto S, Han Z, Silverman S, Rosenfeld MG, Glass CK (1993) Differential orientations of the DNA-binding domain and carboxy-terminal dimerization interface regulate binding site selection by nuclear receptor heterodimers. Genes Dev 7:1423-1435

Kutach AK, Kadonaga JT (2000) The downstream promoter element DPE appears to be as widely used as the TATA box in Drosophila core promoters. Mol Cell Biol 20:4754-4764

Kuznetsova AV, Meller J, Schnell PO, Nash JA, Ignacak ML, Sanchez Y, Conaway JW, Conaway RC, Czyzyk-Krzeska MF (2003) von Hippel-Lindau protein binds hyperphosphorylated large subunit of RNA polymerase II through a proline hydroxylation motif and targets it for ubiquitination. Proc Natl Acad Sci U S A 100:2706-2711

Kwon YT, Reiss Y, Fried VA, Hershko A, Yoon JK, Gonda DK, Sangan P, Copeland NG, Jenkins NA, Varshavsky A (1998) The mouse and human genes encoding the recognition component of the N-end rule pathway. Proc Natl Acad Sci U S A 95:7898-7903

Lagrange T, Kapanidis AN, Tang H, Reinberg D, Ebright RH (1998) New core promoter element in RNA polymerase II-dependent transcription: sequence-specific DNA binding bytranscription factor IIB. Genes Dev 12:34-44

Lai Z, Freedman DA, Levine AJ, McLendon GL (1998) Metal and RNA binding properties of the hdm2 RING finger domain. Biochemistry 37:17005-17015

Lang V, Janzen J, Fischer GZ, Soneji Y, Beinke S, Salmeron A, Allen H, Hay RT, Ben-Neriah Y, Ley SC (2003) betaTrCP-mediated proteolysis of NF-kappaB1 p105 requires phosphorylation of p105 serines 927 and 932. Mol Cell Biol 23:402-413

Langdon WY, Hartley JW, Klinken SP, Ruscetti SK, Morse HC 3rd (1989) v-cbl, an oncogene from a dual-recombinant murine retrovirus that induces early B-lineage lymphomas. Proc Natl Acad Sci U S A 86:1168-1172

Längst G, Becker PB (2001) Nucleosome mobilization and positioning by ISWI-containing chromatin-remodeling factors. J Cell Sci 114:2561-2568

Längst G, Bonte EJ, Corona DF, Becker PB (1999) Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. Cell 97:843-852

Lareu RR, Lacher MD, Bradley CK, Sridaran R, Friis RR, Dharmarajan AM (2003) Regulated expression of inhibitor of apoptosis protein 3 in the rat corpus luteum. Biol Reprod 68:2232-2240

Laudet V (1997) Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. J Mol Endocrinol 19:207-226

Lee DK, Duan HO, Chang C (2000) From androgen receptor to the general transcription factor TFIIH. Identification of cdk activating kinase (CAK) as an androgen receptor NH(2)-terminal associated coactivator. J Biol Chem 275:9308-9313

Lee DK, Duan HO, Chang C (2001) Androgen receptor interacts with the positive elongation factor P-TEFb and enhances the efficiency of transcriptional elongation. J Biol Chem 276:9978-9984

Lee DY, Hayes JJ, Pruss D, Wolffe AP (1993) A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell 72:73-84

Lee HL, Archer TK (1998) Prolonged glucocorticoid exposure dephosphorylates histone H1 and inactivates the MMTV promoter. EMBO J 17:1454-1466

Lee HS, Aumais J, White JH (1996) Hormone-dependent transactivation by estrogen receptor chimeras that do not interact with hsp90. Evidence transcriptional repressors. J Biol Chem 271:25727-25730

Lee KB, Wang D, Lippard SJ, Sharp PA (2002) Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II in vitro. Proc Natl Acad Sci U S A 99:4239-4244

Lee PS, Chang C, Liu D, Derynck R (2003) Sumoylation of Smad4, the common Smad mediator of transforming growth factor-beta family signaling. J Biol Chem 278:27853-27863

Leggett DS, Hanna J, Borodovsky A, Crosas B, Schmidt M, Baker RT, Walz T, Ploegh H, Finley D (2002) Multiple associated proteins regulate proteasome structure and function. Mol Cell 10:495-507

Leng RP, Lin Y, Ma W, Wu H, Lemmers B, Chung S, Parant JM, Lozano G, Hakem R, Benchimol S (2003) Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. Cell 112:779-791

Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, Yarden Y (1998) c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. Genes Dev 12:3663-36674

Li E (1999) The mojo of methylation. Nat Genet 23:5-6

Li J, Wang J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J (2000) Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. EMBO J 19:4342-4350

Li M, Brooks CL, Wu-Baer F, Chen D, Baer R, Gu W (2003) Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. Science 302:1972-1975

Li M, Luo J, Brooks CL, Gu W (2002) Acetylation of p53 inhibits its ubiquitination by Mdm2. J Biol Chem 277:50607-50611

Li SJ, Hochstrasser M (1999) A new protease required for cell-cycle progression in yeast. Nature 398:246-251

Li SJ, Hochstrasser M (2000) The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. Mol Cell Biol 20:2367-2377

Li Y, Kirschmann DA, Wallrath LL (2002) Does heterochromatin protein 1 always follow code? Proc Natl Acad Sci U S A 99:16462-16469

Li Y, Wang H, Wang S, Quon D, Liu YW, Cordell B (2003) Positive and negative regulation of APP amyloidogenesis by sumoylation. Proc Natl Acad Sci U S A 100:259-264

Liang M, Melchior F, Feng XH, Lin X (2004) Regulation of Smad4 sumoylation and transforming growth factor-beta signaling by protein inhibitor of activated STAT1. J Biol Chem 279:22857-22865

Lin PS, Marshall NF, Dahmus ME (2002) CTD phosphatase: role in RNA polymerase II cycling and the regulation of transcript elongation. Prog Nucleic Acid Res Mol Biol 72:333-365

Lin X, Liang M, Liang YY, Brunicardi FC, Feng XH (2003) SUMO-1/Ubc9 promotes nuclear accumulation and metabolic stability of tumor suppressor Smad4. J Biol Chem 278:31043-31048

Littlepage LE, Ruderman JV (2002) Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. Genes Dev 16:2274-2285

Liu Q, Jin C, Liao X, Shen Z, Chen DJ, Chen Y (1999) The binding interface between an E2 (UBC9) and a ubiquitin homologue (UBL1). J Biol Chem 274:16979-16987

Liu XF, Bagchi MK (2004) Recruitment of distinct chromatin-modifying complexes by tamoxifen-complexed estrogen receptor at natural target gene promoters in vivo. J Biol Chem 279:15050-15058

Liu Y, Kung C, Fishburn J, Ansari AZ, Shokat KM, Hahn S (2004) Two cyclin-dependent kinases promote RNA polymerase II transcription and formation of the scaffold Mol Cell Biol 24:1721-1735

Lois LM, Lima CD, Chua NH (2003) Small ubiquitin-like modifier modulates abscisic acid signaling in Arabidopsis. Plant Cell 15:1347-1359

Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc Natl Acad Sci U S A 96:11364-11369

Lorincz MC, Dickerson DR, Schmitt M, Groudine M (2004) Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nat Struct Mol Biol 11:1068-1075

Louria-Hayon I, Grossman T, Sionov RV, Alsheich O, Pandolfi PP, Haupt Y (2003) The promyelocytic leukemia protein protects p53 from Mdm2-mediated inhibition and degradation. J Biol Chem 278:33134-33141

Lovering R, Hanson IM, Borden KL, Martin S, O'Reilly NJ, Evan GI, Rahman D, Pappin DJ, Trowsdale J, Freemont PS (1993) dentification and preliminary characterization of a protein motif related to the zinc finger. Proc Natl Acad Sci U S A 90:2112-2116

Luders J, Pyrowolakis G, Jentsch S (2003) The ubiquitin-like protein HUB1 forms SDS-resistant complexes with cellular proteins in the absence of ATP. EMBO Rep 4:1169-1174

Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Sigler PB (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature 352:497-505

Lyngsø C, Bouteiller G, Damgaard CK, Ryom D, Sanchez-Munoz S, Norby PL, Bonven BJ, Jorgensen P (2000) Interaction between the transcription factor SPBP and the positive cofactor RNF4. An interplay between protein binding zinc fingers. J Biol Chem 275:26144-26149

Ma H, Baumann CT, Li H, Strahl BD, Rice R, Jelinek MA, Aswad DW, Allis CD, Hager GL, Stallcup MR (2001) Hormone-dependent, CARM1-directed, arginine-specific methylation of histone H3 on a steroid-regulated promoter. Curr Biol 11:1981-1985

Ma PC, Rould MA, Weintraub H, Pabo CO (1994) Crystal structure of MyoD bHLH domain-DNA complex: perspectives on DNA recognition and implications transcriptional activation. Cell 77:451-459

Malik S, Roeder RG (2000) Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. Trends Biochem Sci 25:277-283

Mallery DL, Vandenberg CJ, Hiom K (2002) Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. EMBO J 21:6755-6762

Mancini MA, He D, Ouspenski II, Brinkley BR (1996) Dynamic continuity of nuclear and mitotic matrix proteins in the cell cycle. J Cell Biochem 62:158-164

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH (1994) The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci U S A 91:418-422

Markiewicz E, Dechat T, Foisner R, Quinlan RA, Hutchison CJ (2002) Lamin A/C binding protein LAP2alpha is required for nuclear anchorage of retinoblastoma protein. Mol Biol Cell 13:4401-4413

Marmorstein R, Carey M, Ptashne M, Harrison SC (1992) DNA recognition by GAL4: structure of a protein-DNA complex. Nature 356:408-414

Marmorstein R, Fitzgerald MX (2003) Modulation of DNA-binding domains for sequence-specific DNA recognition. Gene 304:1-12

Martens JH, Verlaan M, Kalkhoven E, Dorsman JC, Zantema A (2002) Scaffold/matrix attachment region elements interact with a p300-scaffold attachment factor A complex and are bound by acetylated nucleosomes. Mol Cell Biol 22:2598-2606

Martinez-Yamout M, Legge GB, Zhang O, Wright PE, Dyson HJ (2000) Solution structure of the cysteine-rich domain of the Escherichia coli chaperone protein DnaJ. J Mol Biol 300:805-818

Mateescu B, England P, Halgand F, Yaniv M, Muchardt C (2004) Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3. EMBO Rep 5:490-496

Matera AG (1999) Nuclear bodies: multifaceted subdomains of the interchromatin space. Trends Cell Biol 9:302-309

Matsumoto AM, Bremner WJ (1984) Modulation of pulsatile gonadotropin secretion by testosterone in man. J Clin Endocrinol Metab 58:609-614

Mattern KA, Humbel BM, Muijsers AO, de Jong L, van Driel R (1996) hnRNP proteins and B23 are the major proteins of the internal nuclear matrix of HeLa S3 cells. J Cell Biochem 62:275-289

Maul GG (1998) Nuclear domain 10, the site of DNA virus transcription and replication. Bioessays 20:660-667

Maul GG, Negorev D, Bell P, Ishov AM (2000) Properties and assembly mechanisms of ND10, PML bodies, or PODs. J Struct Biol 129:278-287

McEwan IJ (2001_a) Bakers yeast rises to the challenge: reconstitution of mammalian steroid receptor signalling in S. cerevisiae. Trends Genet 17:239-243

McEwan IJ (2001_b) Structural and functional alterations in the androgen receptor in spinal bulbar muscular atrophy. Biochem Soc Trans 29:222-227

McEwan IJ, Gustafsson J (1997) Interaction of the human androgen receptor transactivation function with the general transcription factor TFIIF. Proc Natl Acad Sci U S A 94:8485-8490

McGrath JP, Jentsch S, Varshavsky A (1991) UBA 1: an essential yeast gene encoding ubiquitin-activating enzyme. EMBO J 10:227-236

McInerney EM, Katzenellenbogen BS (1996) Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. J Biol Chem 271:24172-24178

McKenna NJ, Lanz RB, O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321-344

McKenna NJ, O'Malley BW (2002) Minireview: nuclear receptor coactivators--an update. Endocrinology 143:2461-2465

McKenna S, Hu J, Moraes T, Xiao W, Ellison MJ, Spyracopoulos L (2003_a) Energetics and specificity of interactions within Ub.Uev.Ubc13 human ubiquitin conjugation complexes. Biochemistry 42:7922-7930

McKenna S, Moraes T, Pastushok L, Ptak C, Xiao W, Spyracopoulos L, Ellison MJ (2003_b) An NMR-based model of the ubiquitin-bound human ubiquitin conjugation complex Mms2.Ubc13. The structural basis for lysine 63 chain catalysis. J Biol Chem 278:13151-13158

McKenna S, Spyracopoulos L, Moraes T, Pastushok L, Ptak C, Xiao W, Ellison MJ (2001) Noncovalent interaction between ubiquitin and the human DNA repair protein Mms2 is required for Ubc13-mediated polyubiquitination. J Biol Chem 276:40120-40126

Meehan RR, Kao CF, Pennings S (2003) HP1 binding to native chromatin in vitro is determined by the hinge region and not by the chromodomain. EMBO J 22:3164-3174

Meinke G, Sigler PB (1999) DNA-binding mechanism of the monomeric orphan nuclear receptor NGFI-B. Nat Struct Biol 6:471-477

Melchior F (2000) SUMO--nonclassical ubiquitin. Annu Rev Cell Dev Biol 16:591-626

Melnick A, Licht JD (1999) Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood 93:3167-3215

Melvin VS, Edwards DP (1999) Coregulatory proteins in steroid hormone receptor action: the role of chromatin high mobility group proteins HMG-1 and –2. Steroids 64:576-586

Melvin VS, Roemer SC, Churchill ME, Edwards DP (2002) The C-terminal extension (CTE) of the nuclear hormone receptor DNA binding domain determines interactions functional response to the HMGB-1/-2 co-regulatory proteins. J Biol Chem 277:25115-25124

Meng L, Mohan R, Kwok BH, Elofsson M, Sin N, Crews CM (1999) Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci U S A 96:10403-10408

Meyer ME, Quirin-Stricker C, Lerouge T, Bocquel MT, Gronemeyer H (1992) A limiting factor mediates the differential activation of promoters by the human progesterone receptor isoforms. J Biol Chem 267:10882-10887

Meza JE, Brzovic PS, King MC, Klevit RE (1999) Mapping the functional domains of BRCA1. Interaction of the ring finger domains of BRCA1 and BARD1. J Biol Chem 274:5659-5665

Michael D, Oren M (2003) The p53-Mdm2 module and the ubiquitin system. Semin Cancer Biol 13:49-58

Miller J, McLachlan AD, Klug A (1985) Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO J 4:1609-1614

Minty A, Dumont X, Kaghad M, Caput D (2000) Covalent modification of p73alpha by SUMO-1. Two-hybrid screening with p73 identifies novel SUMO-1-interacting proteins and a SUMO-1 interaction motif. J Biol Chem 275:36316-36323

Misra S, Hurley JH (1999) Crystal structure of a phosphatidylinositol 3-phosphate-specific membrane-targeting motif, the FYVE domain of Vps27p. Cell 97:657-666

Misteli T (2001) Protein dynamics: implications for nuclear architecture and gene expression. Science 291:843-847

Mitsui A, Sharp PA (1999) Ubiquitination of RNA polymerase II large subunit signaled by phosphorylation of carboxyl-terminal Proc Natl Acad Sci U S A 96:6054-6059

Miyake S, Lupher ML Jr, Druker B, Band H (1998) The tyrosine kinase regulator Cbl enhances the ubiquitination and degradation of the platelet-derived growth factor receptor alpha. Proc Natl Acad Sci U S A 95:7927-7932

Miyauchi Y, Yogosawa S, Honda R, Nishida T, Yasuda H (2002) Sumoylation of Mdm2 by protein inhibitor of activated STAT (PIAS) and RanBP2 enzymes. J Biol Chem 277:50131-50136

Mizokami A, Yeh SY, Chang C (1994) Identification of 3',5'-cyclic adenosine monophosphate response element and other cis-acting elements in the human androgen receptor gene promoter. Mol Endocrinol 8:77-88

Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303:343-348

Mizushima T, Hirao T, Yoshida Y, Lee SJ, Chiba T, Iwai K, Yamaguchi Y, Kato K, Tsukihara T, Tanaka K (2004) Structural basis of sugar-recognizing ubiquitin ligase. Nat Struct Mol Biol 11:365-370

Monneron A, Bernhard W (1969) Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruct Res 27:266-288

Morett E, Bork P (1999) A novel transactivation domain in parkin. Trends Biochem Sci 24:229-231

Morris JR, Solomon E (2004) BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. Hum Mol Genet 13:807-817

Mossessova E, Lima CD (2000) Ulp1-SUMO crystal structure and genetic analysis reveal conserved interactions and a regulatory element essential for cell growth in yeast. Mol Cell 5:865-876

Mowen KA, Tang J, Zhu W, Schurter BT, Shuai K, Herschman HR, David (2001) Arginine methylation of STAT1 modulates IFNalpha/beta-induced transcription. Cell 104:731-741

Moynihan TP, Ardley HC, Nuber U, Rose SA, Jones PF, Markham AF, Scheffner M, Robinson PA (1999) The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. J Biol Chem 274:30963-30968

Mu ZM, Chin KV, Liu JH, Lozano G, Chang KS (1994) PML, a growth suppressor disrupted in acute promyelocytic leukemia. Mol Cell Biol 14:6858-6867

Muchardt C, Guilleme M, Seeler JS, Trouche D, Dejean A, Yaniv M (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. EMBO Rep 3:975-981

Mueller TD, Feigon J (2002) Solution structures of UBA domains reveal a conserved hydrophobic surface for protein-protein. J Mol Biol 2002 319:1243-1255

Müller F, Tora L (2003) The multicoloured world of promoter recognition complexes. EMBO J 23:2-8

Müller S, Hoege C, Pyrowolakis G, Jentsch S (2001) SUMO, ubiquitin's mysterious cousin. Nat Rev Mol Cell Biol 2:202-210

Muller S, Matunis MJ, Dejean A (1998) Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. EMBO J 17:61-70

Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D (1998) Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. Mol Cell 2:457-467

Murakami Y, Matsufuji S, Kameji T, Hayashi S, Igarashi K, Tamura T, Tanaka K, Ichihara A (1992) Ornithine decarboxylase is degraded by the 26S proteasome without ubiquitination. Nature 360:597-599

Muratani M, Gerlich D, Janicki SM, Gebhard M, Eils R, Spector DL (2002) Metabolic-energy-dependent movement of PML bodies within the mammalian cell nucleus. Nat Cell Biol 4:106-110

Näär AM, Beaurang PA, Zhou S, Abraham S, Solomon W, Tjian R (1999) Composite co-activator ARC mediates chromatin-directed transcriptional activation. Nature 398:828-832

Näär AM, Lemon BD, Tjian R (2001) Transcriptional coactivator complexes. Annu Rev Biochem 70:475-501

Näär AM, Taatjes DJ, Zhai W, Nogales E, Tjian R (2002) Human CRSP interacts with RNA polymerase II CTD and adopts a specific CTD-bound conformation. Genes Dev 16:1339-1344

Nayler O, Stratling W, Bourquin JP, Stagljar I, Lindemann L, Jasper H, Hartmann AM, Fackelmayer FO, Ullrich A, Stamm S (1998) SAF-B protein couples transcription and pre-mRNA splicing to SAR/MAR elements. Nucleic Acids Res 26:3542-3549

Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, Hood L, Lin B (2002) The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci U S A 99:11890-11895

Nickel BE, Allis CD, Davie JR (1989) Ubiquitinated histone H2B is preferentially located in transcriptionally active chromatin. Biochemistry 28:958-963

Nickerson J (2001) Experimental observations of a nuclear matrix. J Cell Sci 114:463-474

Nickerson JA, Krockmalnic G, Wan KM, Penman S (1997) The nuclear matrix revealed by eluting chromatin from a cross-linked nucleus. Proc Natl Acad Sci U S A 94:4446-4450

Niessing D, Driever W, Sprenger F, Taubert H, Jackle H, Rivera-Pomar R (2000) Homeodomain position 54 specifies transcriptional versus translational control by Bicoid. Mol Cell 5:395-401

Nishida T, Tanaka H, Yasuda H (2000) A novel mammalian Smt3-specific isopeptidase 1 (SMT3IP1) localized in the nucleolus at interphase. Eur J Biochem 267:6423-6427

Nishikawa H, Ooka S, Sato K, Arima K, Okamoto J, Klevit RE, Fukuda M, Ohta T (2004) Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. J Biol Chem 279:3916-3924

Nuber U, Schwarz S, Kaiser P, Schneider R, Scheffner M (1996) Cloning of human ubiquitin-conjugating enzymes UbcH6 and UbcH7 (E2-F1) and characterization of their interaction with E6-AP and RSP5. J Biol Chem 271:2795-2800

Oda H, Kumar S, Howley PM (1999) Regulation of the Src family tyrosine kinase Blk through E6AP-mediated ubiquitination. Proc Natl Acad Sci U S A 96:9557-9562

Oelgeschläger T, Chiang CM, Roeder RG (1996) Topology and reorganization of a human TFIID-promoter complex. Nature 382:735-738

Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953-959

Ohbayashi T, Shimada M, Nakadai T, Wada T, Handa H, Tamura T (2003) Vertebrate TBP-like protein (TLP/TRF2/TLF) stimulates TATA-less terminal deoxynucleotidyl transferase promoters in a transient reporter assay, and TFIIA-binding capacity of TLP is required for this function. Nucleic Acids Res 31:2127-2133

Ohsumi Y, Mizushima N (2004) Two ubiquitin-like conjugation systems essential for autophagy. Semin Cell Dev Biol 15:231-236

Ohta T, Michel JJ, Schottelius AJ, Xiong Y (1999) ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. Mol Cell 3:535-541

Okuma T, Honda R, Ichikawa G, Tsumagari N, Yasuda H (1999) In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. Biochem Biophys Res Commun 254:693-698

Onate SA, Tsai SY, Tsai MJ, O'Malley BW (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354-1357

Orphanides G, Lagrange T, Reinberg D (1996) The general transcription factors of RNA polymerase II. Genes Dev 10:2657-2683

Osborne CS, Chakalova L, Brown KE, Carter D, Horton A, Debrand E, Goyenechea B, Mitchell JA, Lopes S, Reik W, Fraser P (2004) Active genes dynamically colocalize to shared sites of ongoing transcription. Nat Genet 36:1065-1071

Ostendorff HP, Peirano RI, Peters MA, Schluter A, Bossenz M, Scheffner M, Bach I (2002) Ubiquitination-dependent cofactor exchange on LIM homeodomain transcription factors. Nature 416:99-103

Ostrowska H, Wojcik C, Omura S, Worowski K (1997) Lactacystin, a specific inhibitor of the proteasome, inhibits human platelet lysosomal cathepsin A-like enzyme. Biochem Biophys Res Commun 234:729-732

O'Sullivan JM, Tan-Wong SM, Morillon A, Lee B, Coles J, Mellor J, Proudfoot NJ (2004) Gene loops juxtapose promoters and terminators in yeast. Nat Genet 36:1014-1018

Page AM, Hieter P (1999) The anaphase-promoting complex: new subunits and regulators. Annu Rev Biochem 68:583-609

Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 78:773-785

Park JJ, Irvine RA, Buchanan G, Koh SS, Park JM, Tilley WD, Stallcup MR, Press MF, Coetzee GA (2000) Breast cancer susceptibility gene 1 (BRCAI) is a coactivator of the androgen receptor. Cancer Res 60:5946-5949

Park JM, Gim BS, Kim JM, Yoon JH, Kim HS, Kang JG, Kim YJ (2001) Drosophila Mediator complex is broadly utilized by diverse gene-specific transcription factors at different types of core promoters. Mol Cell Biol 21:2312-2323

Parry G, Estelle M (2004) Regulation of cullin-based ubiquitin ligases by the Nedd8/RUB ubiquitin-like proteins. Semin Cell Dev Biol 15:221-229

Pascual J, Martinez-Yamout M, Dyson HJ, Wright PE (2000) Structure of the PHD zinc finger from human Williams-Beuren syndrome transcription factor. J Mol Biol 304:723-729

Patturajan M, Wei X, Berezney R, Corden JL (1998) A nuclear matrix protein interacts with the phosphorylated C-terminal domain of RNA polymerase II. Mol Cell Biol 18:2406-2415

Paull TT, Cortez D, Bowers B, Elledge SJ, Gellert M (2001) Direct DNA binding by Brca1. Proc Natl Acad Sci U S A 98:6086-6091

Peng H, Begg GE, Schultz DC, Friedman JR, Jensen DE, Speicher DW, Rauscher FJ 3rd (2000) Reconstitution of the KRAB-KAP-1 repressor complex: a model system for defining the molecular anatomy of RING-B box-coiled-coil domain-mediated protein-protein interactions. J Mol Biol 295:1139-1162

Pero R, Lembo F, Di Vizio D, Boccia A, Chieffi P, Fedele M, Pierantoni GM, Rossi P, Iuliano R, Santoro M, Viglietto G, Bruni CB, Fusco A, Chiariotti L (2001) RNF4 is a growth inhibitor expressed in germ cells but not in human testicular tumors. Am J Pathol 159:1225-1230

Pero R, Lembo F, Palmieri EA, Vitiello C, Fedele M, Fusco A, Bruni CB, Chiariotti L (2002) PATZ attenuates the RNF4-mediated enhancement of androgen receptor-dependent transcription. J Biol Chem 277:3280-3285

Peters JM (2002) The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol Cell 9:931-943

Petrucelli L, Dawson TM (2004) Mechanism of neurodegenerative disease: role of the ubiquitin proteasome system. Ann Med 36:315-320

Pichler A, Gast A, Seeler JS, Dejean A, Melchior F (2002) The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 108:109-120

Pickart CM (2000) Ubiquitin in chains. Trends Biochem Sci 25:544-548

Pissios P, Tzameli I, Kushner P, Moore DD (2000) Dynamic stabilization of nuclear receptor ligand binding domains by hormone or corepressor binding. Mol Cell 6:245-253

Polioudaki H, Kourmouli N, Drosou V, Bakou A, Theodoropoulos PA, Singh PB, Giannakouros T, Georgatos SD (2001) Histones H3/H4 form a tight complex with the inner nuclear membrane protein LBR and heterochromatin protein 1. EMBO Rep 2:920-925

Polo S, Sigismund S, Faretta M, Guidi M, Capua MR, Bossi G, Chen H, De Camilli P, Di Fiore PP (2002) A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. Nature 416:451-455

Pombo A, Jackson DA, Hollinshead M, Wang Z, Roeder RG, Cook PR (1999) Regional specialization in human nuclei: visualization of discrete sites of transcription by RNA polymerase III. EMBO J 18:2241-53

Poukka H, Aarnisalo P, Santti H, Jänne OA, Palvimo JJ (2000_a) Coregulator small nuclear RING finger protein (SNURF) enhances Sp1- and steroid receptor-mediated transcription by different mechanisms. J Biol Chem 275:571-579

Poukka H, Karvonen U, Jänne OA, Palvimo JJ (2000_b) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci U S A 97:14145-14150

Poukka H, Karvonen U, Yoshikawa N, Tanaka H, Palvimo JJ, Jänne OA (2000_c) The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. J Cell Sci 113:2991-3001

Pratt WB, Toft DO (1997) Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev 18:306-360

Qiu XB, Goldberg AL (2002) Nrdp1/FLRF is a ubiquitin ligase promoting ubiquitination and degradation of the epidermal growth factor receptor family member, ErbB3. Proc Natl Acad Sci U S A 99:14843-14848

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271-321

Raasi S, Pickart CM (2003) Rad23 ubiquitin-associated domains (UBA) inhibit 26 S proteasome-catalyzed proteolysis by sequestering lysine 48-linked polyubiquitin chains. J Biol Chem 278:8951-8959

Raasi S, Schmidtke G, Groettrup M (2001) The ubiquitin-like protein FAT10 forms covalent conjugates and induces apoptosis. J Biol Chem 276:35334-35343

Rachez C, Lemon BD, Suldan Z, Bromleigh V, Gamble M, Näär AM, Erdjument-Bromage H, Tempst P, Freedman LP (1999) Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. Nature 398:824-828

Rachez C, Suldan Z, Ward J, Chang CP, Burakov D, Erdjument-Bromage H, Tempst P, Freedman LP (1998) A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transactivation in a cell-free system. Genes Dev 12:1787-1800

Rajendra R, Malegaonkar D, Pungaliya P, Marshall H, Rasheed Z, Brownell J, Liu LF, Lutzker S, Saleem A, Rubin EH (2004) Topors functions as an E3 ubiquitin ligase with specific E2 enzymes and ubiquitinates p53. J Biol Chem 279:36440-36444

Rao H, Sastry A (2002) Recognition of specific ubiquitin conjugates is important for the proteolytic functions of the ubiquitin-associated proteins Dsk2 and Rad23. J Biol Chem 277:11691-11695

Rastinejad F, Perlmann T, Evans RM, Sigler PB (1995) Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature 375:203-211

Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, Opravil S, Mechtler K, Ponting CP, Allis CD, Jenuwein T (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406:593-599

Rechsteiner M, Rogers SW (1996) PEST sequences and regulation by proteolysis. Trends Biochem Sci 21:267-271

Reid J, Betney R, Watt K, McEwan IJ (2003) The androgen receptor transactivation domain: the interplay between protein conformation and protein-protein interactions. Biochem Soc Trans 31:1042-1046

Reid J, Kelly SM, Watt K, Price NC, McEwan IJ (2002_a) Conformational analysis of the androgen receptor amino-terminal domain involved in transactivation. Influence of structure-stabilizing solutes and protein-protein interactions. J Biol Chem 277:20079-20086

Reid J, Murray I, Watt K, Betney R, McEwan IJ (2002_b) The androgen receptor interacts with multiple regions of the large subunit of general transcription factor TFIIF. J Biol Chem 277:41247-41253

Reifenberger G, Liu L, Ichimura K, Schmidt EE, Collins VP (1993) Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. Cancer Res 53:2736-2739

Reinke H, Horz W (2003) Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol Cell 11:1599-1607

Reiss Y, Kaim D, Hershko A (1988) Specificity of binding of NH2-terminal residue of proteins to ubiquitin-protein ligase. Use of amino acid derivatives to characterize specific binding sites. J Biol Chem 263:2693-2698

Renaud JP, Moras D (2000) Structural studies on nuclear receptors. Cell Mol Life Sci 57:1748-1769

Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic. Nature 378:681-689

Ritchie KJ, Zhang DE (2004) ISG15: the immunological kin of ubiquitin. Semin Cell Dev Biol 15:237-246

Riviere Y (1987) Mapping arenavirus genes causing virulence. Curr Top Microbiol Immunol 133:59-65

Robinson-Rechavi M, Carpentier AS, Duffraisse M, Laudet V (2001) How many nuclear hormone receptors are there in the human genome? Trends Genet 17:554-556

Robyr D, Suka Y, Xenarios I, Kurdistani SK, Wang A, Suka N, Grunstein M (2002) Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. Cell 109:437-446

Robyr D, Wolffe AP, Wahli W (2000) Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 14:329-347

Robzyk K, Recht J, Osley MA (2000) Rad6-dependent ubiquitination of histone H2B in yeast. Science 287:501-504

Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL (1994) Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 78:761-771

Roeder RG (1998) Role of general and gene-specific cofactors in the regulation of eukaryotic transcription. Cold Spring Harb Symp Quant Biol 63:201-218

Roehm PC, Berg JM (1997) Sequential metal binding by the RING finger domain of BRCA1. Biochemistry 36:10240-10245

Rogers S, Wells R, Rechsteiner M (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234:364-368

Rosendorff A, Illanes D, David G, Lin J, Kieff E, Johannsen E (2004) EBNA3C coactivation with EBNA2 requires a SUMO homology domain. J Virol 78:367-377

Ross S, Best JL, Zon LI, Gill G (2002) SUMO-1 modification represses Sp3 transcriptional activation and modulates its subnuclear localization. Mol Cell 10:831-842

Roth SY, Denu JM, Allis CD (2001) Histone acetyltransferases. Annu Rev Biochem 70:81-120

Rotin D, Staub O, Haguenauer-Tsapis R (2000) Ubiquitination and endocytosis of plasma membrane proteins: role of Nedd4/Rsp5p family of ubiquitin-protein ligases. J Membr Biol 176:1-17

Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM (2001) Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proc Natl Acad Sci U S A 98:5134-5139

Ryu S, Zhou S, Ladurner AG, Tjian R (1999) The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. Nature 397:446-450

Ryu SW, Chae SK, Kim E (2000) Interaction of Daxx, a Fas binding protein, with sentrin and Ubc9. Biochem Biophys Res Commun 279:6-10

Sablin EP, Krylova IN, Fletterick RJ, Ingraham HA (2003) Structural basis for ligand-independent activation of the orphan nuclear receptor LRH-1. Mol Cell 11:1575-1585

Sachdev S, Bruhn L, Sieber H, Pichler A, Melchior F, Grosschedl R (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev 15:3088-3103

Sadis S, Atienza C Jr, Finley D (1998) Synthetic signals for ubiquitin-dependent proteolysis. Mol Cell Biol 15:4086-4094

Saitoh H, Hinchey J (2000) Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J Biol Chem 275:6252-6258

Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E (1998) DNA damage activates p53 through a phosphorylation-acetylation cascade. Genes Dev 12:2831-2841

Sapetschnig A, Rischitor G, Braun H, Doll A, Schergaut M, Melchior F, Suske G (2002) Transcription factor Sp3 is silenced through SUMO modification by PIAS1. EMBO J 21:5206-5215

Saurin AJ, Borden KL, Boddy MN, Freemont PS (1996) Does this have a familiar RING? Trends Biochem Sci 21:208-214

Saville B, Poukka H, Wormke M, Jänne OA, Palvimo JJ, Stoner M, Samudio I, Safe S (2002) Cooperative coactivation of estrogen receptor alpha in ZR-75 human breast cancer cells by SNURF and TATA-binding protein. J Biol Chem 277:2485-2497

Scheffner M, Huibregtse JM, Howley PM (1994) Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. Proc Natl Acad Sci U S A 91:8797-8801

Scheffner M, Huibregtse JM, Vierstra RD, Howley PM (1993) The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell 75:495-505

Schmidt D, Müller S (2002) Members of the PIAS family act as SUMO ligases for c-Jun and p53 and repress p53 activity. Proc Natl Acad Sci U S A 99:2872-2877

Schreiber SL, Bernstein BE (2002) Signaling network model of chromatin. Cell 111:771-778

Schultz DC, Friedman JR, Rauscher FJ 3rd (2001) Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes Dev 15:428-443

Schwabe JW, and Klug A (1994) Zinc mining for protein domains. Nat Struct Biol 1:345-349

Schwabe JW, Fairall L, Chapman L, Finch JT, Dutnall RN, Rhodes D (1993) The cocrystal structures of two zinc-stabilized DNA-binding domains illustrate different ways of sequence-specific DNA recognition. Cold Spring Harb Symp Quant Biol 58:141-147

Schwabe JW, Klug A (1994) Zinc mining for protein domains. Nat Struct Biol 1:345-349

Schwabe JW, Rhodes D (1991) Beyond zinc fingers: steroid hormone receptors have a novel structural motif for DNA recognition. Trends Biochem Sci 16:291-296

Schwanbeck R, Xiao H, Wu C (2004) Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. J Biol Chem 279:39933-39941

Schwartz AL, Ciechanover A (1999) The ubiquitin-proteasome pathway and pathogenesis of human diseases. Annu Rev Med 50:57-74

Scott PM, Bilodeau PS, Zhdankina O, Winistorfer SC, Hauglund MJ, Allaman MM, Kearney WR, Robertson AD, Boman AL, Piper RC (2004) GGA proteins bind ubiquitin to facilitate sorting at the trans-Golgi network. Nat Cell Biol 6:252-259

Scully R, Anderson SF, Chao DM, Wei W, Ye L, Young RA, Livingston DM, Parvin JD (1997) BRCA1 is a component of the RNA polymerase II holoenzyme. Proc Natl Acad Sci U S A 94:5605-5610

Seeler JS, Dejean A (2003) Nuclear and unclear functions of SUMO. Nat Rev Mol Cell Biol 4:690-699

Seeler JS, Marchio A, Losson R, Desterro JM, Hay RT, Chambon P, Dejean A (2001) Common properties of nuclear body protein SP100 and TIF1alpha chromatin factor: role of SUMO modification. Mol Cell Biol 21:3314-3324

Seeler JS, Marchio A, Sitterlin D, Transy C, Dejean A (1998) Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. Proc Natl Acad Sci U S A 95:7316-7321

Seol JH, Feldman RM, Zachariae W, Shevchenko A, Correll CC, Lyapina S, Chi Y, Galova M, Claypool J, Sandmeyer S, Nasmyth K, Deshaies RJ, Shevchenko A, Deshaies RJ (1999) Cdc53/cullin and the essential Hrt1 RING-H2 subunit of SCF define a ubiquitin ligase module that activates the E2 enzyme Cdc34. Genes Dev 13:1614-1626

Sewack GF, Ellis TW, Hansen U (2001) Binding of TATA binding protein to a naturally positioned nucleosome is facilitated by histone acetylation. Mol Cell Biol 21:1404-1415

Shaffer PL, Gewirth DT (2002) Structural basis of VDR-DNA interactions on direct repeat response elements. EMBO J 21:2242-2252

Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843-852

Shang Y, Myers M, Brown M (2002) Formation of the androgen receptor transcription complex. Mol Cell 9:601-610

Sheckter CB, Matsumoto AM, Bremner WJ (1989) Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary. J Clin Endocrinol Metab 68:397-401

Sherman MY, Goldberg AL (2001) Cellular defenses against unfolded proteins: a cell biologist thinks about neurodegenerative diseases. Neuron 29:15-32

Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL (1998) The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927-937

Shiba Y, Katoh Y, Shiba T, Yoshino K, Takatsu H, Kobayashi H, Shin HW, Wakatsuki S, Nakayama K (2004) GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination. J Biol Chem 279:7105-7111

Shiels C, Islam SA, Vatcheva R, Sasieni P, Sternberg MJ, Freemont PS, Sheer D (2001) PML bodies associate specifically with the MHC gene cluster in interphase nuclei. J Cell Sci 114:3705-3716

Shih SC, Prag G, Francis SA, Sutanto MA, Hurley JH, Hicke L (2003) A ubiquitin-binding motif required for intramolecular monoubiquitylation, the CUE domain. EMBO J 22:1273-1281

Shiio Y, Eisenman RN (2003) Histone sumoylation is associated with transcriptional repression. Proc Natl Acad Sci U S A 100:13225-13230

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. Science 293:263-269

Shivapurkar N, Sood S, Wistuba II, Virmani AK, Maitra A, Milchgrub S, Minna JD, Gazdar AF (1999) Multiple regions of chromosome 4 demonstrating allelic losses in breast carcinomas. Cancer Res 59:3576-3580

Simental JA, Sar M, Lane MV, French FS, Wilson EM (1991) Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 266:510-518

Sleeman JE, Lamond AI (1999) Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. Curr Biol 9:1065-1074

Sommer T, Jentsch S (1993) A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. Nature 365:176-179

Song CS, Jung MH, Supakar PC, Chatterjee B, Roy AK (1999) Negative regulation of the androgen receptor gene promoter by NFI and an adjacently located multiprotein-binding site. Mol Endocrinol 13:1487-1496

Song J, Durrin LK, Wilkinson TA, Krontiris TG, Chen Y (2004) Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. Proc Natl Acad Sci U S A 101:14373-14378

Song S, Kim SY, Hong YM, Jo DG, Lee JY, Shim SM, Chung CW, Seo SJ, Yoo YJ, Koh JY, Lee MC, Yates AJ, Ichijo H, Jung YK (2003) Essential role of E2-25K/Hip-2 in mediating amyloid-beta neurotoxicity. Mol Cell 12:553-563

Spann TP, Goldman AE, Wang C, Huang S, Goldman RD (2002) Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription. J Cell Biol 156:603-608

Spector DL (1993) Macromolecular domains within the cell nucleus. Annu Rev Cell Biol 9:265-315

Steffan JS, Agrawal N, Pallos J, Rockabrand E, Trotman LC, Slepko N, Illes K, Lukacsovich T, Zhu YZ, Cattaneo E, Pandolfi PP, Thompson LM, Marsh JL (2004) SUMO modification of Huntingtin and Huntington's disease pathology. Science 304:100-104

Steketee K, Berrevoets CA, Dubbink HJ, Doesburg P, Hersmus R, Brinkmann AO, Trapman J (2002) Amino acids 3-13 and amino acids in and flanking the 23FxxLF27 motif modulate the interaction between the N-terminal and ligand-binding domain of the androgen receptor. Eur J Biochem 269:5780-5791

Stelter P, Ulrich HD (2003) Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature 425:188-191

Sternsdorf T, Jensen K, Will (1997) Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. J Cell Biol 139:1621-1634

Strahl BD, Allis CD (2000) The language of covalent histone modifications. Nature 403:41-45

Strahl BD, Briggs SD, Brame CJ, Caldwell JA, Koh SS, Ma H, Cook RG, Shabanowitz J, Hunt DF, Stallcup MR, Allis CD (2001) Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. Curr Biol 11:996-1000

Subramanian L, Benson MD, Iniguez-Lluhi JA (2003) A synergy control motif within the attenuator domain of CCAAT/enhancer-binding protein alpha inhibits transcriptional synergy through its PIASy-enhanced modification by SUMO-1 or SUMO-3. J Biol Chem 278:9134-9141

Sun X, Zhang Y, Cho H, Rickert P, Lees E, Lane W, Reinberg D (1998) NAT, a human complex containing Srb polypeptides that functions as a negative regulator of activated transcription. Mol Cell 2:213-222

Sun Y, Durrin LK, Krontiris TG (2003) Specific interaction of PML bodies with the TP53 locus in Jurkat interphase nuclei. Genomics 2003 82:250-252

Sun ZW, Allis CD (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418:104-108

Sung P, Prakash S, Prakash L (1988) The RAD6 protein of Saccharomyces cerevisiae polyubiquitinates histones, and its acidic domain mediates this activity. Genes Dev 2:1476-1485

Supakar PC, Jung MH, Song CS, Chatterjee B, Roy AK (1995) Nuclear factor kappa B functions as a negative regulator for the rat androgen receptor gene and NF-kappa B activity increases during the age-dependent desensitization of the liver. J Biol Chem 270:837-842

Swaminathan S, Amerik AY, Hochstrasser M (1999) The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast. Mol Biol Cell 10:2583-2594

Swanson KA, Kang RS, Stamenova SD, Hicke L, Radhakrishnan I (2003) Solution structure of Vps27 UIM-ubiquitin complex important for endosomal sorting and receptor downregulation. EMBO J 22:4597-4606

Syntichaki P, Topalidou I, Thireos G (2000) The Gcn5 bromodomain co-ordinates nucleosome remodelling. Nature 404:414-417

Taatjes DJ, Naar AM, Andel F 3rd, Nogales E, Tjian R (2002) Structure, function, and activator-induced conformations of the CRSP coactivator. Science 295:1058-1062

Takahashi Y, Toh-e A, Kikuchi Y (2001) A novel factor required for the SUMO1/Smt3 conjugation of yeast septins. Gene 275:223-231

Tamaru H, Selker EU (2001) A histone H3 methyltransferase controls DNA methylation in Neurospora crassa. Nature 414:277-283

Tan JA, Hall SH, Hamil KG, Grossman G, Petrusz P, French FS (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J Biol Chem 277:16993-7001

Tanabe H, Muller S, Neusser M, von Hase J, Calcagno E, Cremer M, Solovei I, Cremer C, Cremer T (2002) Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates. Proc Natl Acad Sci U S A 99:4424-4429

Tang Y, DeFranco DB (1996) ATP-dependent release of glucocorticoid receptors from the nuclear matrix. Mol Cell Biol 16:1989-2001

Tansey WP (2001) Transcriptional activation: risky business. Genes Dev 2001 15:1045-1050

Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, Naismith JH, Hay RT (2001) Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. J Biol Chem 276:35368-35374

Tatham MH, Kim S, Yu B, Jaffray E, Song J, Zheng J, Rodriguez MS, Hay RT, Chen Y (2003) Role of an N-terminal site of Ubc9 in SUMO-1, -2, and -3 binding and conjugation. Biochemistry 42:9959-9969

Teo H, Veprintsev DB, Williams RL (2004) Structural insights into endosomal sorting complex required for transport (ESCRT-I) recognition of ubiquitinated proteins. J Biol Chem 279:28689-28696

Tetel MJ, Giangrande PH, Leonhardt SA, McDonnell DP, Edwards DP (1999) Hormone-dependent interaction between the amino- and carboxyl-terminal domains of progesterone receptor in vitro and in vivo. Mol Endocrinol 13:910-924

Thompson CM, Koleske AJ, Chao DM, Young RA (1993) A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell 73:1361-1375

Thrower JS, Hoffman L, Rechsteiner M, Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. EMBO J 19:94-102

Tian S, Poukka H, Palvimo JJ, Jänne OA (2002) Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J 367:907-911

Tjian R, Maniatis T (1994) Transcriptional activation: a complex puzzle with few easy Cell 77:5-8

Tong H, Hateboer G, Perrakis A, Bernards R, Sixma TK (1997) Crystal structure of murine/human Ubc9 provides insight into the variability of the ubiquitin-conjugating system. J Biol Chem 272:21381-21387

Tora L, White J, Brou C, Tasset D, Webster N, Scheer E, Chambon P (1989) The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell 59:477-487

Trausch JS, Grenfell SJ, Handley-Gearhart PM, Ciechanover A, Schwartz AL (1993) Immunofluorescent localization of the ubiquitin-activating enzyme, E1, to the nucleus and cytoskeleton. Am J Physiol 264:C93-C102

Trompouki E, Hatzivassiliou E, Tsichritzis T, Farmer H, Ashworth A, Mosialos G (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature 424:793-796

Umar A, Luider TM, Berrevoets CA, Grootegoed JA, Brinkmann AO (2003) Proteomic analysis of androgen-regulated protein expression in a mouse fetal vas deferens cell line. Endocrinology 144:1147-1154

Umar A, Ooms MP, Luider TM, Grootegoed JA, Brinkmann AO (2003) Proteomic profiling of epididymis and vas deferens: identification of proteins regulated during rat genital tract development. Endocrinology 144:4637-4647

Underhill C, Qutob MS, Yee SP, Torchia J (2000) A novel nuclear receptor corepressor complex, N-CoR, contains components of the mammalian SWI/SNF complex and the corepressor KAP-1. J Biol Chem 275:40463-40470

Upton C, Schiff L, Rice SA, Dowdeswell T, Yang X, McFadden G (1994) A poxvirus protein with a RING finger motif binds zinc and localizes in virus factories. J Virol 68:4186-4195

Vallian S, Chin KV, Chang KS (1998) The promyelocytic leukemia protein interacts with Sp1 and inhibits its transactivation of the epidermal growth factor receptor promoter. Mol Cell Biol 18:7147-7156

van Holde KE (1989) Chromatin, Springer-Verlag: New York

van Hoy M, Leuther KK, Kodadek T, Johnston SA (1993) The acidic activation domains of the GCN4 and GAL4 proteins are not alpha helical but form beta sheets. Cell 72:587-594

van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H, Berns A (1991) Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. Cell 65:737-752

van Sant C, Hagglund R, Lopez P, Roizman B (2001) The infected cell protein 0 of herpes simplex virus 1 dynamically interacts with proteasomes, binds and activates the cdc34 E2 ubiquitin-conjugating enzyme, and possesses in vitro E3 ubiquitin ligase activity. Proc Natl Acad Sci U S A 98:8815-8820

van Steensel B, Jenster G, Damm K, Brinkmann AO, van Driel R (1995) Domains of the human androgen receptor and glucocorticoid receptor involved in binding to the nuclear matrix. J Cell Biochem 57:465-478

Varadan R, Assfalg M, Haririnia A, Raasi S, Pickart C, Fushman D (2004) Solution conformation of Lys63-linked di-ubiquitin chain provides clues to functional diversity of polyubiquitin signaling. J Biol Chem 279:7055-7063

Varshavsky A (1997) The N-end rule pathway of protein degradation. Genes Cells 2:13-28

Vassileva MT, Matunis MJ (2004) SUMO modification of heterogeneous nuclear ribonucleoproteins. Mol Cell Biol 24:3623-3632

Verma S, Ismail A, Gao X, Fu G, Li X, O'Malley BW, Nawaz Z (2004) The ubiquitin-conjugating enzyme UBCH7 acts as a coactivator for steroid hormone receptors. Mol Cell Biol 24:8716-8726

Vignali M, Steger DJ, Neely KE, Workman JL (2000) Distribution of acetylated histones resulting from Gal4-VP16 recruitment of SAGA and NuA4 complexes. EMBO J 19:2629-2640

Vijay-Kumar S, Bugg CE, Cook WJ (1987) Structure of ubiquitin refined at 1.8 Å resolution. J Mol Biol 194:531-544

Vodermaier HC (2004) APC/C and SCF: controlling each other and the cell cycle. Curr Biol 14:R787-R796

von Arnim AG, Deng XW (1993) Ring finger motif of Arabidopsis thaliana COP1 defines a new class of zinc-binding domain. J Biol Chem 268:19626-19631

Wang Q, Moyret-Lalle C, Couzon F, Surbiguet-Clippe C, Saurin JC, Lorca T, Navarro C, Puisieux A (2003) Alterations of anaphase-promoting complex genes in human colon cancer cells. Oncogene 22:1486-1490

Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP, Perlmann T (2003) Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. Nature 423:555-560

Wang ZG, Rivi R, Delva L, Konig A, Scheinberg DA, Gambacorti-Passerini C, Gabrilove JL, Warrell RP Jr, Pandolfi PP (1998_a) Arsenic trioxide and melarsoprol induce programmed cell death in myeloid leukemia cell lines and function in a PML and PML-RARalpha independent manner. Blood 92:1497-1504

Wang ZG, Ruggero D, Ronchetti S, Zhong S, Gaboli M, Rivi R, Pandolfi PP (1998_b) PML is essential for multiple apoptotic pathways. Nat Genet 20:266-272

Wei W, Ayad NG, Wan Y, Zhang GJ, Kirschner MW, Kaelin WG Jr (2004) Degradation of the SCF component Skp2 in cell-cycle phase G1 by the anaphase-promoting 428:194-198

Weiss J, Axelrod L, Whitcomb RW, Harris PE, Crowley WF, Jameson JL (1992) Hypogonadism caused by a single amino acid substitution in the beta subunit of luteinizing hormone. N Engl J Med 326:179-183

Weissman AM (2001) Themes and variations on ubiquitylation. Nat Rev Mol Cell Biol 2:169-178

Wen YD, Perissi V, Staszewski LM, Yang WM, Krones A, Glass CK, Rosenfeld MG, Seto E (2000) The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci U S A 97:7202-7207

Wilk S, Figueiredo-Pereira ME (1993) Synthetic inhibitors of the multicatalytic proteinase complex (proteasome). Enzyme Protein 47:306-313

Wilson TE, Fahrner TJ, Milbrandt J (1993) The orphan receptors NGFI-B and steroidogenic factor 1 establish monomer binding as a third paradigm of receptor-DNA interaction. Mol Cell Biol 13:5794-5804

Wilson VG, Rangasamy D (2001) Viral interaction with the host cell sumoylation system. Virus Res 81:17-27

Wojcik C, DeMartino GN (2003) Intracellular localization of proteasomes. Int J Biochem Cell Biol 35:579-589

Woudstra EC, Gilbert C, Fellows J, Jansen L, Brouwer J, Erdjument-Bromage H, Tempst P, Svejstrup JQ (2002) A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. Nature 415:929-933

Wu SM, Kuo WC, Hwu WL, Hwa KY, Mantovani R, Lee YM (2004) RNF4 is a coactivator for nuclear factor Y on GTP cyclohydrolase I proximal promoter. Mol Pharmacol 66:1317-1324

Wu WS, Vallian S, Seto E, Yang WM, Edmondson D, Roth S, Chang KS (2001) The growth suppressor PML represses transcription by functionally and physically interacting with histone deacetylases. Mol Cell Biol 21:2259-2268

Wu-Baer F, Lagrazon K, Yuan W, Baer R (2003) The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. J Biol Chem 278:34743-34746

Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, Gronemeyer H (1996) A canonical structure for the ligand-binding domain of nuclear receptors. Nat Struct Biol 3:87-94

Xie Y, Varshavsky A(1999) The E2-E3 interaction in the N-end rule pathway: the RING-H2 finger of E3 is required for the synthesis of multiubiquitin chain. EMBO J 18:6832-6844

Xirodimas DP, Saville MK, Bourdon JC, Hay RT, Lane DP (2004) dm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell 118:83-97

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT, Parks DJ, Moore JT, Kliewer SA, Willson TM, Stimmel JB (2002) Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. Nature 415:813-817

Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ, O'Malley BW (1998) Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 279:1922-1925

Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M, Evans RM (2001) A transcriptional switch mediated by cofactor methylation. Science 294:2507-2511

Xu W, Cho H, Kadam S, Banayo EM, Anderson S, Yates JR 3rd, Emerson BM, Evans RM (2004) A methylation-mediator complex in hormone signaling. Genes Dev 18:144-156

Xu ZX, Timanova-Atanasova A, Zhao RX, Chang KS (2003) PML colocalizes with and stabilizes the DNA damage response protein TopBP1. Mol Cell Biol 23:4247-4256

Yaglom J, Linskens MH, Sadis S, Rubin DM, Futcher B, Finley D (1995) p34Cdc28-mediated control of Cln3 cyclin degradation. Mol Cell Biol 15:731-741

Yamamoto H, Ihara M, Matsuura Y, Kikuchi A (2003) Sumoylation is involved in beta-catenin-dependent activation of Tcf-4. EMBO J 22:2047-2059

Yamanaka K, Ishikawa H, Megumi Y, Tokunaga F, Kanie M, Rouault TA, Morishima I, Minato N, Ishimori K, Iwai K (2003) Identification of the ubiquitin-protein ligase that recognizes oxidized IRP2. Nat Cell Biol 5:336-340

Yamano H, Tsurumi C, Gannon J, Hunt T (1998) The role of the destruction box and its neighbouring lysine residues in cyclin B for anaphase ubiquitin-dependent proteolysis in fission yeast: defining the D-box receptor. EMBO J 17:5670-5678

Yan W, Hirvonen-Santti SJ, Palvimo JJ, Toppari J, Jänne OA (2002) Expression of the nuclear RING finger protein SNURF/RNF4 during rat testis development suggests a role in spermatid maturation. Mech Dev 118:247-253

Yao TP, Ku G, Zhou N, Scully R, Livingston DM (1996) The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. Proc Natl Acad Sci U S A 93:10626-10631

Yarden RI, Brody LC (1999) BRCA1 interacts with components of the histone deacetylase complex. Proc Natl Acad Sci U S A 96:4983-4988

Yasui D, Miyano M, Cai S, Varga-Weisz P, Kohwi-Shigematsu T (2002) ATB1 targets chromatin remodelling to regulate genes over long distances. Nature 419:641-645

Ye Q, Worman HJ (1996) Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1. J Biol Chem 271:14653-14656

Yoshida Y, Chiba T, Tokunaga F, Kawasaki H, Iwai K, Suzuki T, Ito Y, Matsuoka K, Yoshida M, Tanaka K, Tai T (2002) E3 ubiquitin ligase that recognizes sugar chains. Nature 418:438-442

Yoshida Y, Tokunaga F, Chiba T, Iwai K, Tanaka K, Tai T (2003) Fbs2 is a new member of the E3 ubiquitin ligase family that recognizes sugar chains. J Biol Chem 278:43877-43884

Yudkovsky N, Ranish JA, Hahn S (2000) A transcription reinitiation intermediate that is stabilized by activator. Nature 408 225-229

Zhang H, Somasundaram K, Peng Y, Tian H, Zhang H, Bi D, Weber BL, El-Deiry WS (1998) BRCA1 physically associates with p53 and stimulates its transcriptional activity. Oncogene 16:1713-1721

Zhang J, Kalkum M, Chait BT, Roeder RG (2002) The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. Mol Cell 9:611-623

Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM (2000) Parkin functions as an E2-dependent ubiquitin- protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. Proc Natl Acad Sci U S A 97:13354-13359

Zhao T, Heyduk T, Allis CD, Eissenberg JC (2000) Heterochromatin protein 1 binds to nucleosomes and DNA in vitro. J Biol Chem 275:28332-28338

Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepp DM, Elledge SJ, Pagano M, Conaway RC, Conaway JW, Harper JW, Pavletich NP (2002) Structure of the Cul1-Rbx1-Skp1-F boxSkp2 SCF ubiquitin ligase complex. Nature 416:703-709

Zheng N, Wang P, Jeffrey PD, Pavletich NP (2000) Structure of a c-Cbl-UbcH7 complex: RING domain function in ubiquitin-protein ligases. Cell 102:533-539

Zhong S, Muller S, Ronchetti S, Freemont PS, Dejean A, Pandolfi PP (2000_a) Role of SUMO-1-modified PML in nuclear body formation. Blood 95:2748-2752

Zhong S, Salomoni P, Pandolfi PP (2000_b) The transcriptional role of PML and the nuclear body. Nat Cell Biol 2:E85-E90

Zhong ZD, Hammani K, Bae WS, DeClerck YA (2000) NF-Y and Sp1 cooperate for the transcriptional activation and cAMP response of human tissue inhibitor of metalloproteinases-2. J Biol Chem 275:18602-18610

Zhu J, Chen Z, Lallemand-Breitenbach V, de The H (2002) How acute promyelocytic leukaemia revived arsenic. Nat Rev Cancer 2:705-713

Zhu J, Koken MH, Quignon F, Chelbi-Alix MK, Degos L, Wang ZY, Chen Z, de The H (1997) Arsenic-induced PML targeting onto nuclear bodies: implications for the treatment of acute promyelocytic leukemia. Proc Natl Acad Sci U S A 94:3978-3983

Zlatanova J, van Holde K (1998) Binding to four-way junction DNA: a common property of architectural proteins? FASEB J 12:421-431

Zuber J, Tchernitsa OI, Hinzmann B, Schmitz AC, Grips M, Hellriegel M, Sers C, Rosenthal A, Schafer R (2000) A genome-wide survey of RAS transformation targets. Nat Genet 24:144-152

Zur A, Brandeis M (2001) Securin degradation is mediated by fzy and fzr, and is required for complete chromatid separation but not for cytokinesis. EMBO J 20:792-801