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REVIEW ARTICLES

# Chromosome Condensation Outside of Mitosis: Mechanisms and New Tools

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A basic principle of cell physiology is that chromosomes condense during mitosis. However, condensation can be uncoupled from mitotic events under certain circumstances. This phenomenon is known as ''premature chromosome condensation (PCC).'' PCC provides insights in the mechanisms of chromosome condensation, thus helping clarifying the key molecular events leading to the mitosis. Besides, PCC has proved to be an useful tool for analyzing chromosomes in interphase. For example, using PCC we can visualize genetic damage shortly after the exposure to clastogenic agents. More than 30 years ago, the first report of PCC in interphase cells fused to mitotic cells using Sendai virus was described (virus-mediated PCC). The method paved the way to a great number of fundamental discoveries in cytogenetics, radiation biology, and related fields, but it has been hampered by technical difficulties. The novel drug-induced PCC method was introduced about 10 years ago. While fusion-induced PCC exploits the action of external maturation/mitosis promoting factor (MPF), migrating from the inducer mitotic cell to the interphase recipient, drug-induced PCC exploits protein phosphatase inhibitors, which can activate endogenous intracellular MPF. This method is much simpler than fusion-induced PCC, and has already proven useful in different fields. J. Cell. Physiol. 209: 297–304, 2006. © 2006 Wiley-Liss, Inc.

## CHROMOSOME CONDENSATION IN INTERPHASE

In ordinary circumstances, chromosomes condense in mitotic phase only, under highly ordered molecular events. However, in certain circumstances, chromosome condensation may be uncoupled from mitotic events. This phenomenon is known as premature chromosome condensation (PCC). The first observation of PCC was reported in virus mediated multinucleated fused cells of interphase and mitotic cells (Kato and Sandberg, 1967). They observed a typical appearance of "shattered" or "pulverized" chromosomes following virus infection of cells, and they thought that the "pulverized" chromatin represented chromosomal fragments induced by the virus. The first correct interpretation of this phenomenon was given by Johnson and Rao following their carefully designed experiment (Johnson and Rao, 1970). They fused synchronized interphase cells (in G1, S, or G2 phase) to mitotic cells using Sendai virus (Fig. 1), and they found that the appearance of condensed chromatin was dependent on the cell phase of interphase cells at that time of fusion; that is, (1) G1-phase nuclei produced univalent chromosomes, (2) G2-phase nuclei showed bivalent chromosomes, similar to mitotic chromosomes, although less condensed, and (3) S-phase nuclei converted to a "pulverized" appearance that consisted of univalent and bivalent chromosomes. They concluded that the interphase chromatin was condensed "prematurely" following fusion to mitotic-like chromosomes, and they first named this phenomenon as "premature chromosome condensation (PCC)," and the condensed interphase chromatins "prematurely condensed chromosomes" (PCCs) (Fig. 2). PCC was soon recognized as a valuable tool for investigations in chromosome dynamics, such as in radiation cytogenetics and chromosome replication studies (Hittelman and Rao, 1974; Schor et al., 1975; Hittelman and Pollard, 1982; Mullinger and Johnson, 1983; Cornforth and Bedford, 1983a). Virus-induced fusion PCC method has, however, several drawbacks. First, fusion efficiency is highly dependent on the virus strain and viral activity. Second, the technique cannot be always applied, because the cell should express surface receptors to the fusogenic virus. Third, the chromosomes are somewhat confusing mixture of mitotic inducer and recipient cells, and differential replication staining (in mitotic cells) is needed to visualize the genetic material in the two cells (Cornforth and Bedford, 1983b). Finally, handling of viruses restricts the use of virus-mediated PCC method, and manipulation of the infectious virus is generally banned in non-specialized laboratories. Cell fusion can also be achieved by chemicals (Fig. 2), in particular polyethylene glycol (PEG), although the fusion yield is generally lower than that obtained by virus. Pantelias and Maillie (1983) first successfully applied PEG-fusion to PCC induction, and this method has been widely used in several laboratories, especially for radiation cytogenetics studies (Pantelias and Maillie, 1984; Iliakis et al., 1987; Suzuki et al., 1992; Watanabe et al., 1992; Okayasu and Iliakis, 1993; Durante et al., 1996b, 1998b).

## DRUG-INDUCED PCC

As described in the previous section, PEG- or virus-induced PCC (cell fusion-mediated PCC) is a useful method for chromosome analysis, but its use is problematic. Due to these restrictions, virus or PEG-mediated fusion-PCC is used in very limited number of laboratories only.

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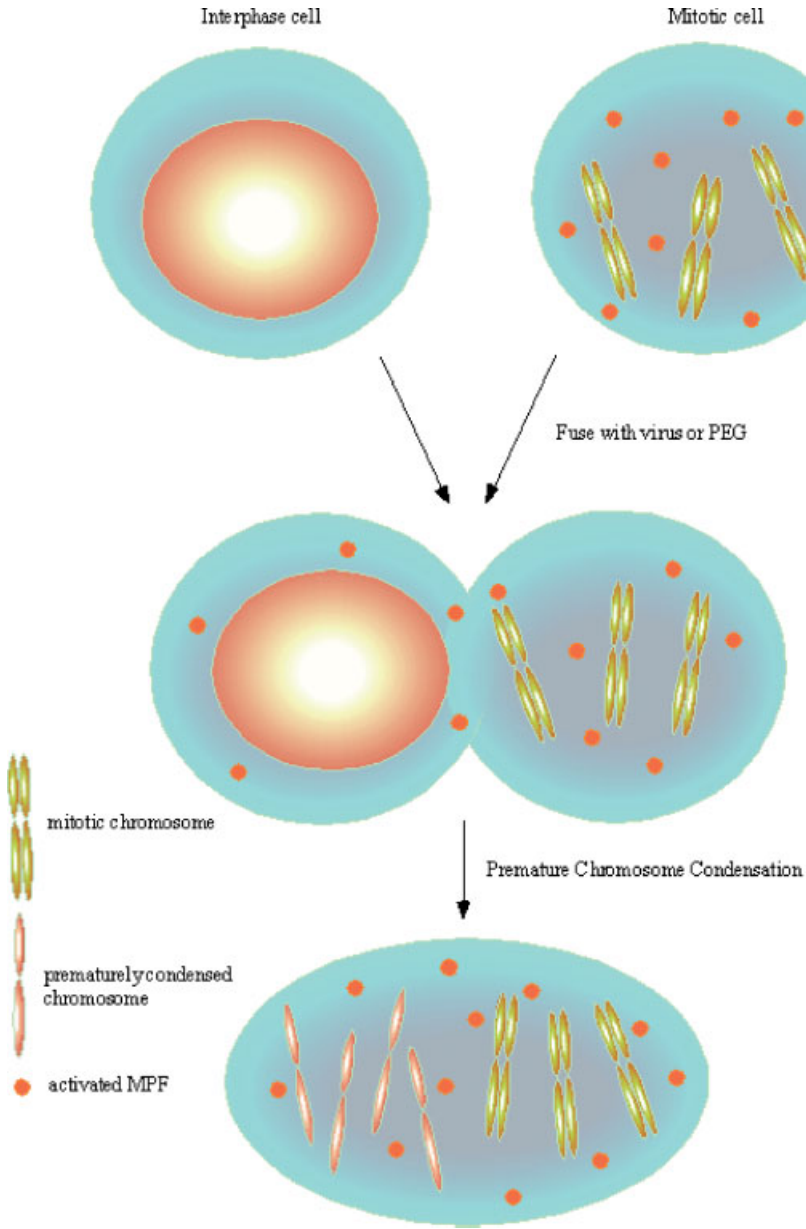


Fig. 1. Mechanism of fusion-induced PCC. Fusion is generally achieved either by virus or chemicals (PEG).

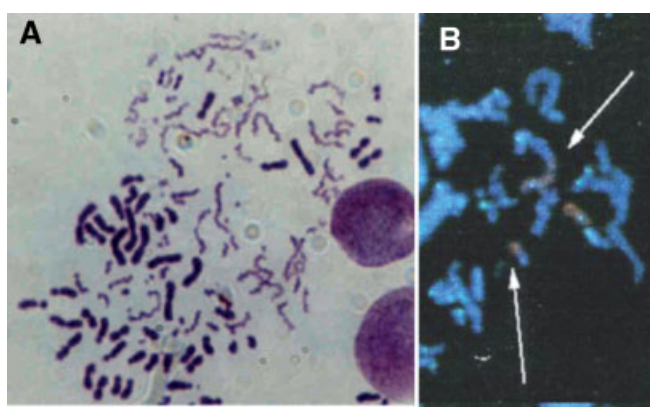


Fig. 2. Photomicrographs of fusion-induced PCCs. The images show prematurely condensed single-chromatid (i.e., univalent) human chromosomes from G0 peripheral blood lymphocytes fused by PEG, after exposure to 7 Gy γ-rays, to mitotic Chinese hamster cells. Note that the chromosomes are mixture of mitotic hamster cell and recipient human cell. **A**: Several fragments are visible by Giemsa-staining in the irradiated human chromosomes (pale purple). **B**: A dicentric (with associated acentric fragment, both pointed by arrows) in human PCC 4, visualized by FISH-painting with a human DNA whole chromosome probe (spectrum orange) combined to a pan-centromeric probe (spectrum green). Photographs from References Durante et al. (1996a,b), copyright Radiation Research Society, reproduced with permission.

Several approaches have been carried out to induce PCC using chemicals, which would make the procedure easier and reliable. First successes were obtained with caffeine and okadaic acid, but cells had to be synchronized in S-phase using DNA synthesis inhibitors such as hydroxyurea or thymidine (Schlegel and Pardee, 1986; Schlegel et al., 1990; Yamashita et al., 1990). The obtained chromosomes were, therefore, S-phase PCC only, which cannot be used for usual chromosome analysis, because they appear pulverized. The first report of PCC induction in somatic cells in any phase of cell cycle, without arresting the cells in S-phase, came from Gotoh et al. (1995). The authors used calyculin A or okadaic acid, specific inhibitors of type 1 and 2A protein phosphatases (Bialojan and Takai, 1988; Ishihara et al., 1989; Cohen et al., 1990) (Fig. 3). Calyculin A is, in particular, able to induce PCC in many kinds of cells, either in suspension or attached, and the PCC index is generally higher than with other protein phosphatase inhibitors. The method of drug-induced PCC is relatively easy whereby simply substitutes the use of colcemid to calyculin A in conventional chromosome preparation protocol (Gotoh et al., 1995; Durante et al., 1998a). Incubation time of only approximately 30 min, much shorter than colcemid block (2–4 h) can induce a substantial number of PCCs. In addition, PCC index (corresponds to mitotic index) is usually much higher (>20%) than mitotic index of colcemid protocol (~1–2%) (Gotoh et al., 1995; Asakawa and Gotoh, 1997; Durante et al., 1998a). Examples of PCCs in different phases of the cell cycle, visualized by Giemsa-stained or fluorescence in situ hybridization (FISH) with whole-chromosome human DNA probes, are given in Figure 4. Drug-induced PCC is becoming increasingly popular, and a lot of reports in the literature using this technique have been recently published, where this technique is applied to several mammalian cells, either attached or in suspension (Gotoh and Asakawa, 1996; Asakawa and Gotoh, 1997; Coco-Martin and Begg, 1997; Durante et al., 1998a, 1999; Gotoh et al., 1999, 2005; Prasanna et al., 2000; Ito et al., 2002; Bezrookove et al., 2003; Terzoudi et al., 2003; Malik et al., 2004; Blakely et al., 2005; El Achkar et al., 2005; Someya et al., 2006).

## MOLECULAR MECHANISMS OF CHROMOSOME CONDENSATION

The mechanism of chromosome condensation has been deeply investigated and several key molecules, such as condensin, cohesin, lamina, microtubules, histone H1, aurora kinase, and topoisomerase II (see for instance Kubiak, 1991; Rattner and Wang, 1992; Ishida et al., 1994; Van Hooser et al., 1998; Vass et al., 2003; Maton et al., 2005), have been identified and their mechanism described. However, the complete mechanism is still unclear. PCC is partly similar, yet not quite the same phenomenon as normal chromosome condensation (Ghosh et al., 1992; Rattner and Wang, 1992; Guo et al., 1995). It is expected that a deeper knowledge of PCC may lead to a better understanding of the normal mitotic chromosome condensation.

Incunabula, it was thought that PCC (chromosome pulverization) was caused by the nature of viruses used for cell fusion (Kato and Sandberg, 1967), because at that time pulverization of chromosome was thought to be a quintessence matter. However, as described above, this idea was turned over by the carefully designed experiment using synchronized cells (Johnson and Rao, 1970). They interpreted the mechanism of PCC as follows: (1) "certain molecules," which promote chromosome condensation, accumulate in mitotic nuclei, (2) these molecules work in the interphase cell nuclei after

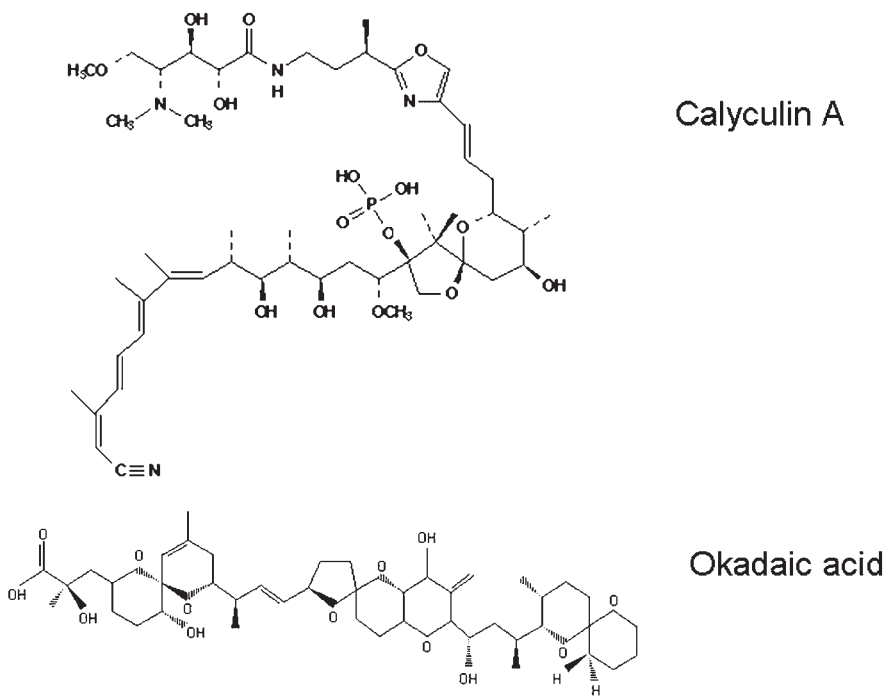


Fig. 3. Molecular structures of calyculin A and okadaic acid, two powerful protein phosphatase inhibitors and PCC inducers. Chemical characteristics are: Calyculin A: Molecular Formula, $C_{50}H_{81}N_4O_{15}P$; Molecular Weight, 1009.18; $IC_{50}$ for PP1, 0.5–2 nM; $IC_{50}$ for PP2A, 0.1–1 nM; Source, *Discodermia Calyx*. Okadaic Acid: Molecular Formula, $C_{44}H_{68}O_{13}$; Molecular Weight, 805.2; $IC_{50}$ for PPI, 10–60 nM; $IC_{50}$ for PP2A, 0.1–1 nM; Source, *Halichondria Okadai*.

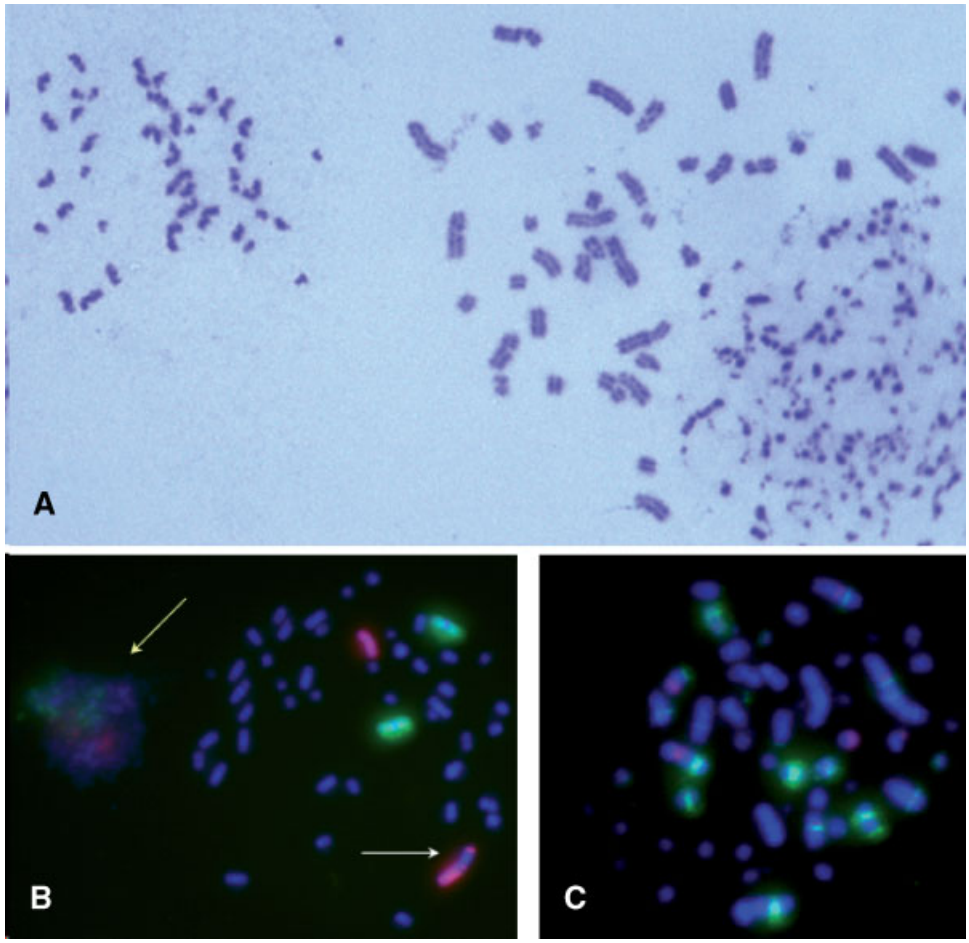


Fig. 4. Examples of PCC in human mononuclear lymphocytes induced by calyculin A. **A**: A Giemsa-stained image of three cells in the same area of the slide in three different phases of the cell cycle: left, univalent G1 chromosomes; center, bivalent G2 chromosomes; right, pulverized S-phase cell. **B**: An insertion in chromosome 2, FISH-painted in spectrum orange (pointed by a white arrow) in a cell from a patient during radiotherapy. The normal chromosome 1 is painted in green. An S-phase cell is also visible in the same image, pointed by the yellow arrow. Drug-induced PCC is preferable to conventional colcemid-block for biodosimetry after partial-body exposure, such as in radiotherapy. **C**: A multi-aberrant cell, showing several complex rearrangements in both chromosomes 1 and 2, followed exposure to 1 Gy of high-energy iron ions. Severe damage induced by densely ionizing radiation may be underestimated using metaphase analysis.

fusion to metaphase cells (3) the migrating molecules force the interphase chromosomes to condense prematurely (Johnson and Rao, 1970). The premature condensation process takes less than 1 h to complete: approximately 20 min are necessary for the condensation to proceed to a point where no DNA rejoining is possible (Cornforth and Bedford, 1993; Durante et al., 1998c).

The germinal vesicle breakdown (GVBD) is a similar phenomenon, where oocyte chromosomes condense prematurely at meiotic metaphase stage (egg maturation). The maturation-promoting factor (MPF) was first identified as the key molecule inducing GVBD induction (Masui, 1974, 1982, 2001). It was later revealed that MPF regulates chromosome condensation not only in meiotic but also in mitotic cells (Miake-Lye and Kirschner, 1985), therefore, MPF was re-named as maturation/mitosis promoting factor. MPF is actually a complex of p34cdc2/cyclinB (Dunphy et al., 1988; Gautier et al., 1988; Maller et al., 1989) and plays a central role in cell cycle regulation and cell growth control. The dephosphorylated form of p34cdc2/cyclinB complex (activated MPF) promotes chromosome condensation (Moreno et al., 1989). Therefore, after cell fusion by either viruses or PEG, the interphase nucleus in the fused cell is exposed to activated MPF which is supplied from the mitotic nucleus, thus resulting in prematurely chromosome condensation of interphase cell chromatin (Fig. 1).

Cdc25 is a cell cycle checkpoint protein which regulates the onset of mitosis along with p34cdc2/cyclinB (Ducommun et al., 1990; Moreno et al., 1990). Cdc25 is a tyrosine phosphatase that activates p34cdc2/cyclinB complex by dephosphorylation of tyrosine residue (Gould et al., 1990; Dunphy and Kumagai, 1991; Gautier et al., 1991; Kumagai and Dunphy, 1991; Strausfeld et al., 1991). The activity of cdc25 is regulated by auto-phosphorylation/dephosphorylation and is sensitive to PP1 and PP2A (type 1 and 2A protein phosphatase) (Izumi et al., 1992; Kumagai and Dunphy, 1992; Kinoshita et al., 1993). Okadaic acid and calyculin A, specific inhibitors of PP1 and PP2A, influence the activity of cdc25 and p34cdc2/cyclinB (Fig. 5), thus promoting the premature entry in mitotic stage (Kumagai and Dunphy, 1992; Kinoshita et al., 1993). Activated MPF (p34cdc2/cyclinB) may finally promote chromosome condensation in interphase. In this hypothesis, PCC is not directly induced by the chemicals, but should be caused by inhibition of protein phosphatase activity. Interestingly, other inhibitors of type 1 and type 2A protein phosphatase such as endothal and cantharidine are also able to induce PCC (Gotoh, 1996, 1998).

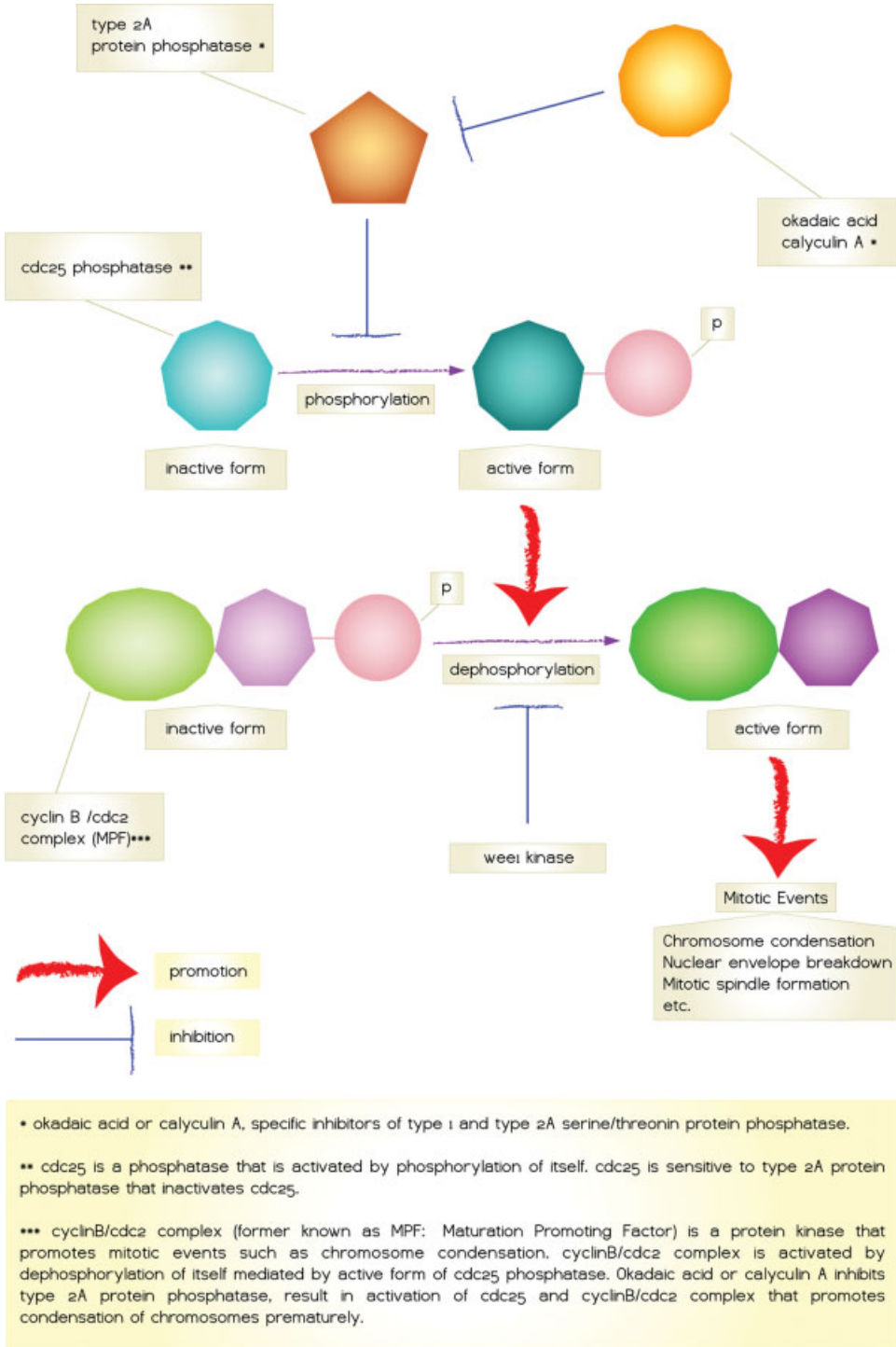


Fig. 5. Mechanism of chromosome condensation induced by protein phosphatase inhibitors.

Clearly, according to the mechanism described above, PCC will only be induced if MPF protein is available in the cell. It is well known that MPF activity is dependent on the cellular concentration of cyclins, in particular cyclin B (e.g., Doree and Galas, 1994). Cyclin B concentration oscillates through cell cycle: it is very low in G1, and increases gradually from S to G2, where it reaches its maximum, corresponding to the maximum in MPF activity (Fig. 6). Therefore, calyculin A or okadaic acid will induce PCC predominantly in G2-phase, followed by S-phase and only few G1-phase. A number of late telophase post-mitotic G1 are still visible by drug-induced PCC, but no PCC can be induced in G0 cells (Durante et al., 1998a). PCC induced by calyculin A in G2 takes only about 5 min (Gotoh et al., 1999), that is, it is probably faster than fusion-induced PCC (about 20 min).

The lack of G0-phase PCC after calyculin A or okadaic acid can represent a drawback as compared to fusion-induced PCC. In fact, if early events in G0 cells want to

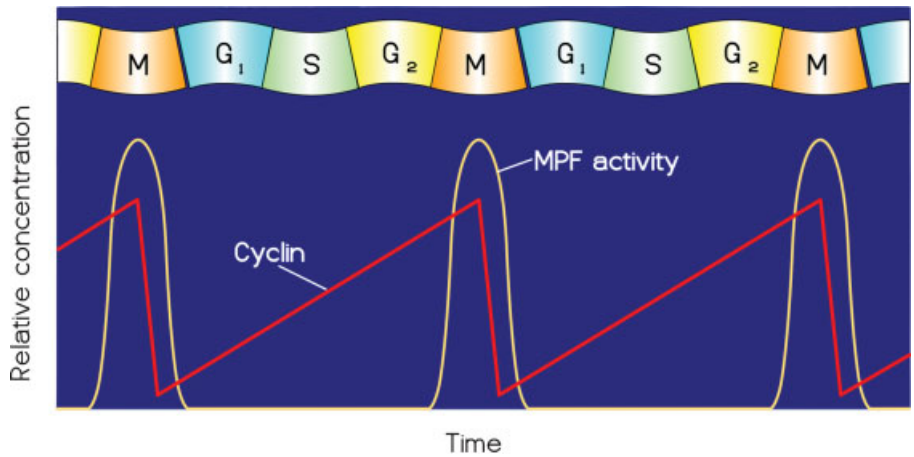


Fig. 6. Relationship between cyclin concentration and MPF activity during the cell cycle.

be studied, fusion-induced PCC is to be preferred to calyculin A. For instance, the kinetics of DNA repair in G0 cells exposed to ionizing radiation has been extensively studied using fusion-induced PCC (Cornforth and Bedford, 1983a, 1993; Durante et al., 1998b,c), while calyculin A was used to measure G2 kinetics (Gotoh et al., 1999; Kawata et al., 2000, 2004).

Efforts to induce PCC in G0, resting-state human cells have concentrated on other key molecules. Since cyclin B concentration is too low in G0, Prasanna et al. (2000) added adenosine triphosphate (ATP) and p34cdc2/cyclin B kinase to okadaic acid to induce PCC in G0 human lymphocytes. Although the authors observed condensation indexes as high as 40% at high concentrations of p34cdc2/cyclin B, the quality of the chromosome spread is hardly suitable for karyotyping or detection of structural chromosome aberrations. Nevertheless, the same authors have demonstrated that FISH-painting of these partly condensed spreads produce distinct color-domains for each painted chromosome pair, and radiation-induced breaks and rearrangements result in an increased number of hybridized spots (Prasanna and Blakely, 2005). This technique is then potentially very useful in biological dosimetry of ionizing radiation, especially when results are needed shortly after the exposure.

## PCC APPLICATIONS

The conventional colcemid-block protocol has been used popularly for analyzing chromosomes. Cytological material is, however, limited to mitotic chromosomes. In fact, colcemid inhibits the assembly of spindle body at mitotic phase, thus the cells arrest in mitosis with condensed chromosomes. In contrast, PCC forces the chromosomes to be condensed "prematurely" not only in mitosis but also in interphase nuclei, hence interphase nuclei are visualized as condensed chromosome form. Because of this unique property, PCC has been used for analyze various chromatin events occur during interphase, including (1) breakage and repair of chromosomes following exposure to either ionizing radiation (Cornforth and Bedford, 1983a; Pantelias, 1986; Iliakis et al., 1987; Bedford and Goodhead, 1989; Durante et al., 1996a) or chemical mutagens (Hittelman and Rao, 1974, 1975; Hittelman and Pollard, 1982; Sognier and Hittelman, 1986); (2) DNA replication (Hanks and Rao, 1980; Lau and Arrighi, 1980, 1981; Mullinger and Johnson, 1983), (3) conformational changes during cell cycle (Schor et al., 1975; Rao et al., 1977; Hittelman and Rao, 1978; Rao and Hanks, 1980; Hanks et al., 1983), or others. Drug-induced PCC has also been applied in several studies. PCC is extremely useful in biodosimetry of ionizing radiation, especially in a number of cases where metaphase analysis can fail. The application of PCC is proving the worth in overcoming some of the limitations of the conventional dicentric assay (IAEA, 2001). These include (Durante et al., 1997): partial body exposure, such as in radiotherapy, when unirradiated cells will be harvested sooner than exposed cells, delayed by radiation-induced cell-cycle blocks; exposure to densely ionizing radiation, which produces prolonged cell-cycle delays; very low mitotic indexes, such as in pathological situations, in the elderly, or in stress conditions; exposure to very high radiation dose, which invariably produce poor or no mitosis after stimulation.

A simple protocol for simultaneous analysis of interphase–metaphase chromosomes in human lymphocytes for biological dosimetry was described by Durante et al. (1998a), and since then has been widely applied to biodosimetry. In cancer patients undergoing radiotherapy, use of PCC induced by calyculin A resulted in accurate measurements of the yield of chromosomal aberrations in lymphocytes of patients during the treatment at different sites and with different radiation qualities (Durante et al., 2000). In all cases, the dose-response curve shows a saturation at high number of fractions, due to re-population from unirradiated bone marrow. In addition, it has been shown that the aberration yield strongly depends on the number of lymph nodes in the radiation field (d'Alesio et al., 2003), thus suggesting that most lymphocytes are not exposed while in the circulating blood, but in the lymph nodes.

For accidental overexposure to very high doses, dose estimates should be determined as quickly as possible to choose appropriate medical treatments for the victims. However, when human body is irradiated with large doses, the white cells remaining in the peripheral blood not only arrest at G2 or G1 phase and do not enter mitosis, but also undergo mitotic cell death or apoptosis. As a result, it is usually impossible to prepare chromosome samples for analysis. Before the 21st century, exposures over 10 Gy were invariably lethal (~2 times higher than LD50 of 6 and 4 Gy of humans with medical care or without medical care, respectively, Anno et al., 2003). Thus it was not practically required for the cytogenetic biodosimeter to be able to estimate whole

body doses over 10 Gy. However, with recent advances in medical technology such as stem cell transplantation and cytokine therapy, it became possible to save victims exposed to over 10 Gy whole body (Lloyd, 1997). Therefore, the need for developing a cytogenetic biodosimeter that covers the estimation doses over 10 Gy been emerged. This dose limitation of biodosimeter was first overcame using drug-induced PCC technique, where the maximum estimable dose is approximately 40 Gy $\gamma$-rays if combined to chromosome painting method (Gotoh and Asakawa, 1996). Following this report, other parameters have been proposed to analyze prematurely condensed chromosomes after very high doses: centric rings (Kanda et al., 1999), combined use of total chromosome number and G2-PCC index (Gotoh et al., 2005), and the length ratio of longest/shortest size of chromosome (Gotoh and Tanno, 2005). The drug-induced PCC has been indeed applied after the 1999 criticality accident at the uranium fuel conversion test facility of JCO in Tokai-mura, Japan. Three workers were exposed to very high doses (two of them died within a few months), and $^{24}$Na and $^{32}$P radioactivity measurements by whole-body counters suggested soon that two of them were exposed to lethal doses (Miyamoto et al., 2002). However, cytogenetic analysis by drug-induced PCC provided the best estimate of the absorbed dose (Kanda et al., 2002).

Further applications of drug-induced PCC include (1) mutagenic assays (Asakawa and Gotoh, 1997; Terzoudi et al., 2003; Malik et al., 2004) (2) karyotyping of chromosomes (Kowalska et al., 2003), (3) chromosome instability analysis (Bezrookove et al., 2003), (4) prenatal diagnosis (Srebniak et al., 2005), and (5) others (Johnson et al., 1999; Ito et al., 2002; El Achkar et al., 2005; Mochida et al., 2005; Someya et al., 2006).

## CONCLUSIONS

PCC has been long considered a useful tool for studies of chromosome dynamics. The new technique of drug-induced PCC by protein phosphatase inhibitors has several advantages over the previous one based on cell fusion, and has lead to a wide spectrum of research activities in several laboratories, from basic cell biology to practical applications in radiation medicine. Despite decades of careful molecular studies, the detailed mechanism of chromosome condensation in normal cells entering mitosis is still not completely understood. It is likely that studies on "forced," premature condensation will also decisively contribute to understanding this normal, fundamental cellular process.

## LITERATURE CITED


Anno GH, Young RW, Bloom RM, Mercier JR. 2003. Dose response relationships for acute ionizing-radiation lethality. Health Phys 84:565–575.

Asakawa Y, Gotoh E. 1997. A method for detecting sister chromatid exchanges using prematurely condensed chromosomes and immunogold-silver staining. Mutagenesis 12:175–177.

Bedford JS, Goodhead DT. 1989. Breakage of human interphase chromosomes by alpha particles and X-rays. Int J Radiat Biol 55:211–216.

Bezrookove V, Smits R, Moeslein G, Fodde R, Tanke HJ, Raap AK, Darroudi F. 2003. Premature chromosome condensation revisited: A novel chemical approach permits efficient cytogenetic analysis of cancers. Genes Chromosomes Cancer 38:177–186.

Bialojan C, Takai A. 1988. Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. Biochem J 256:283–290.

Blakely WF, Salter CA, Prasanna PG. 2005. Early-response biological dosimetry-recommended countermeasure enhancements for mass-casualty radiological incidents and terrorism. Health Phys 89:494–504.

Coco-Martin JM, Begg AC. 1997. Detection of radiation-induced chromosome aberrations using fluorescence in situ hybridization in drug-induced premature chromosome condensations of tumour cell lines with different radiosensitivities. Int J Radiat Biol 71:265–273.

Cohen P, Holmes CF, Tsukitani Y. 1990. Okadaic acid: A new probe for the study of cellular regulation. Trends Biochem Sci 15:98–102.

Cornforth MN, Bedford JS. 1983a. X-ray-induced breakage and rejoining of human interphase chromosomes. Science 222:1141–1143.

Cornforth MN, Bedford JS. 1983b. High-resolution measurement of breaks in prematurely condensed chromosomes by differential staining. Chromosoma 88:315–318.

Cornforth MN, Bedford JS. 1993. Ionizing radiation damage and its early development in chromosomes. Adv Radiat Biol 17:423–497.

d'Alesio V, Pacelli R, Durante M, Canale Cama G, Cella L, Gialanella G, Grossi G, Pugliese M, Punzo G, Sardi I, Scampoli P, Solla R, Salvatore M. 2003. Lymph nodes in the irradiated field influence the yield of radiation-induced chromosomal aberrations in lymphocytes from breast cancer patients. Int J Radiat Oncol Biol Phys 57:732–738.

Doree M, Galas S. 1994. The cyclin-dependent protein kinases and the control of cell division. FASEB J 8:1114–1121.

Ducommun B, Draetta G, Young P, Beach D. 1990. Fission yeast cdc25 is a cell-cycle regulated protein. Biochem Biophys Res Commun 167:301–309.

Dunphy WG, Kumagai A. 1991. The cdc25 protein contains an intrinsic phosphatase activity. Cell 67:189–196.

Dunphy WG, Brizuela L, Beach D, Newport J. 1988. The Xenopus cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. Cell 54:423–431.

Durante M, George K, Wu H, Yang TC. 1996a. Rejoining and misrejoining of radiation-induced chromatin breaks. I. experiments with human lymphocytes. Radiat Res 145:274–280.

Durante M, George K, Yang TC. 1996b. Biological dosimetry by interphase chromosome painting. Radiat Res 145:53–60.

Durante M, George K, Yang TC. 1997. Biodosimetry of ionizing radiation by selective painting of prematurely condensed chromosomes in human lymphocytes. Radiat Res 148:S45–S50.

Durante M, Furusawa Y, Gotoh E. 1998a. A simple method for simultaneous interphase-metaphase chromosome analysis in biodosimetry. Int J Radiat Biol 74:457–462.

Durante M, Furusawa Y, George K, Gialanella G, Greco O, Grossi G, Matsufuji N, Pugliese M, Yang TC. 1998b. Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particles. Radiat Res 149:446–454.

Durante M, George K, Wu HL, Yang TC. 1998c. Rejoining and misrejoining of radiation-induced chromatin breaks. III. Hypertonic treatment. Radiat Res 149:68–74.

Durante M, Furusawa Y, Majima H, Kawata T, Gotoh E. 1999. Association between G2-phase block and repair of radiation-induced chromosome fragments in human lymphocytes. Radiat Res 151:670–676.

Durante M, Yamada S, Ando K, Furusawa Y, Kawata T, Majima H, Nakano T, Tsujii H. 2000. X-rays vs. carbon-ion tumor therapy: Cytogenetic damage in lymphocytes. Int J Radiat Oncol Biol Phys 47:793–798.

El Achkar E, Gerbault-Seureau M, Muleris M, Dutrillaux B, Debatisse M. 2005. Premature condensation induces breaks at the interface of early and late replicating chromosome bands bearing common fragile sites. Proc Natl Acad Sci USA 102:18069–18074.

Gautier J, Norbury C, Lohka M, Nurse P, Maller J. 1988. Purified maturation-promoting factor contains the product of a Xenopus homolog of the fission yeast cell cycle control gene cdc2+. Cell 54:433–439.

Gautier J, Solomon MJ, Booher RN, Bazan JF, Kirschner MW. 1991. cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2. Cell 67:197–211.

Ghosh S, Paweletz N, Schroeter D. 1992. Failure of kinetochore development and mitotic spindle formation in okadaic acid-induced premature mitosis in HeLa cells. Exp Cell Res 201:535–540.

Gotoh E, Asakawa Y. 1996. Detection and evaluation of chromosomal aberrations induced by high doses of gamma-irradiation using immunogold-silver painting of prematurely condensed chromosomes. Int J Radiat Biol 70:517–520.

Gotoh E, Gotoh E, Assignee. 1996. Agents and a method of chromosome preparation using protein phosphatase inhibitors induced premature chromosome condensation (PCC) technique. England, France, Italy, Germany, Netherland, Sweden Patent EP0696730.

Gotoh E, Gotoh E, Assignee. 1998. Agents and a method of chromosome preparation using protein phosphatase inhibitors induced premature chromosome condensation (PCC) technique. The United States of America Patent 5,789,164.

Gotoh E, Tanno Y. 2005. Simple biodosimetry method for cases of high-dose radiation exposure using the ratio of the longest/shortest length of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). Int J Radiat Biol 81:379–385.

Gotoh E, Asakawa Y, Kosaka H. 1995. Inhibition of protein serine/threonine phophatases directly induces premature chromosome condensation in mammalian somatic cells. Biomed Res 16:63–68.

Gotoh E, Kawata T, Durante M. 1999. Chromatid break rejoining and exchange aberration formation following gamma-ray exposure: Analysis in G2 human fibroblasts by chemically induced premature chromosome condensation. Int J Radiat Biol 75:1129–1135.

Gotoh E, Tanno Y, Takakura K. 2005. Simple biodosimetry method for use in cases of high-dose radiation exposure that scores the chromosome number of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). Int J Radiat Biol 81:33–40.

Gould KL, Moreno S, Tonks NK, Nurse P. 1990. Complementation of the mitotic activator, p80cdc25, by a human protein-tyrosine phosphatase. Science 250:1573–1576.

Guo XW, Th'ng JP, Swank RA, Anderson HJ, Tudan C, Bradbury EM, Roberge M. 1995. Chromosome condensation induced by fostriecin does not require p34cdc2 kinase activity and histone H1 hyperphosphorylation, but is associated with enhanced histone H2A and H3 phosphorylation. Embo J 14:976–985.

Hanks SK, Rao PN. 1980. Initiation of DNA synthesis in the prematurely condensed chromosomes of G1 cells. J Cell Biol 87:285–291.

Hanks SK, Gollin SM, Rao PN, Wray W, Hittelman WN. 1983. Cell cycle-specific changes in the ultrastructural organization of prematurely condensed chromosomes. Chromosoma 88:333–342.

Hittelman WN, Pollard M. 1982. Induction and repair of DNA and chromosome damage by neocarzinostatin in quiescent normal human fibroblasts. Cancer Res 42:4584–4590.
Hittelman WN, Rao PN. 1974. Bleomycin-induced damage in prematurely condensed chromosomes and its relationship to cell cycle progression in CHO cells. Cancer Res 34:3433–3439.
Hittelman WN, Rao PN. 1975. The nature of adriamycin-induced cytotoxicity in Chinese hamster cells as revealed by premature chromosome condensation. Cancer Res 35:30–35.
Hittelman WN, Rao PN. 1978. Mapping G1 phase by the structural morphology of the prematurely condensed chromosomes. J Cell Physiol 95:333–341.
IAEA. 2001. Cytogenetic analysis for radiation dose assessment. A manual. Vienna: International Atomic Energy Agency.
Iliakis G, Pantelias GE, Okayasu R, Seaner R. 1987. 125IdUrd-induced chromosome fragments, assayed by premature chromosome condensation, and DNA double-strand breaks have similar repair kinetics in G1-phase CHO-cells. Int J Radiat Biol Relat Stud Phys Chem Med 52:705–722.
Ishida R, Sato M, Narita T, Utsumi KR, Nishimoto T, Morita T, Nagata H, Andoh T. 1994. Inhibition of DNA topoisomerase II by ICRF-193 induces polyploidization by uncoupling chromosome dynamics from other cell cycle events. J Cell Biol 126:1341–1351.
Ishihara H, Martin BL, Brautigan DL, Karaki H, Ozaki H, Kato Y, Fusetani N, Watabe S, Hashimoto K, Uemura D, et al. 1989. Calyculin A and okadaic acid: Inhibitors of protein phosphatase activity. Biochem Biophys Res Commun 159:871–877.
Ito S, Gotoh E, Ozawa S, Yanagi K. 2002. Epstein-Barr virus nuclear antigen-1 is highly colocalized with interphase chromatin and its newly replicated regions in particular. J Gen Virol 83:2377–2383.
Izumi T, Walker DH, Maller JL. 1992. Periodic changes in phosphorylation of the Xenopus cdc25 phosphatase regulate its activity. Mol Biol Cell 3:927–939.
Johnson RT, Rao PN. 1970. Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei. Nature 226:717–722.
Johnson RT, Gotoh E, Mullinger AM, Ryan AJ, Shiloh Y, Ziv Y, Squires S. 1999. Targeting double-strand breaks to replicating DNA identifies a subpathway of DSB repair that is defective in ataxia-telangiectasia cells. Biochem Biophys Res Commun 261:317–325.
Kanda R, Hayata I, Lloyd DC. 1999. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. Int J Radiat Biol 75:441–446.
Kanda R, Minamihisamatsu M, Hayata I. 2002. Dynamic analysis of chromosome aberrations in three victims of the Tokai-mura criticality accident. Int J Radiat Biol 78:857–862.
Kato H, Sandberg A. 1967. Chromosome pulverization in human binucleate cells following colcemid treatment. J Cell Biol 34:35–46.
Kawata T, Gotoh E, Durante M, Wu H, George K, Furusawa Y, Cucinotta FA. 2000. High-LET radiation-induced aberrations in prematurely condensed G2 chromosomes of human fibroblasts. Int J Radiat Biol 76:929–937.
Kawata T, Ito H, Uno T, Saito M, Yamamoto S, Furusawa Y, Durante M, George K, Wu H, Cucinotta FA. 2004. G2 chromatid damage and repair kinetics in normal human fibroblast cells exposed to low- or high-LET radiation. Cytogenet Genome Res 104:211–215.
Kinoshita N, Yamano H, Niwa H, Yoshida T, Yanagida M. 1993. Negative regulation of mitosis by the fission yeast protein phosphatase ppa2. Genes Dev 7:1059–1071.
Kowalska A, Srebniak M, Wawrzkiewicz A, Kaminski K. 2003. The influence of calyculin A on lymphocytes in vitro. J Appl Genet 44:413–418.
Kubiak JZ. 1991. Cell cycle-dependent behavior of microtubules in hybrids of mouse oocytes and blastomeres. Int J Dev Biol 35:421–427.
Kumagai A, Dunphy WG. 1991. The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. Cell 64:903–914.
Kumagai A, Dunphy WG. 1992. Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. Cell 70:139–151.
Lau YF, Arrighi FE. 1980. Studies of mammalian chromosome replication. I. BrdU-induced differential staining patterns in interphase and metaphase chromosomes. Cytogenet Cell Genet 27:176–183.
Lau YF, Arrighi FE. 1981. Studies of mammalian chromosome replication. II. Evidence for the existence of defined chromosome replicating units. Chromosoma 83:721–741.
Lloyd DC. 1997. Chromosomal analysis to assess radiation dose. Stem Cells 15 (Suppl 2):195–201.
Malik SI, Terzoudi GI, Pantelias GE. 2004. SCE analysis in G2 lymphocyte prematurely condensed chromosomes after exposure to atrazine: The non-dose-dependent increase in homologous recombinational events does not support its genotoxic mode of action. Cytogenet Genome Res 104:315–319.
Maller J, Gautier J, Langan TA, Lohka MJ, Shenoy S, Shalloway D, Nurse P. 1989. Maturation-promoting factor and the regulation of the cell cycle. J Cell Sci Suppl 12:53–63.
Masui Y. 1974. A cytostatic factor in amphibian oocytes: Its extraction and partial characterization. J Exp Zool 187:141–147.
Masui Y. 1982. Oscillatory activity of maturation promoting factor (MPF) in extracts of Rana pipiens eggs. J Exp Zool 224:389–399.
Masui Y. 2001. From oocyte maturation to the in vitro cell cycle: The history of discoveries of Maturation-Promoting Factor (MPF) and Cytostatic Factor (CSF). Differentiation 69:1–17.
Maton G, Lorca T, Girault JA, Ozon R, Jessus C. 2005. Differential regulation of Cdc2 and Aurora-A in Xenopus oocytes: A crucial role of phosphatase 2A. J Cell Sci 118:2485–2494.
Miake-Lye R, Kirschner MW. 1985. Induction of early mitotic events in a cell-free system. Cell 41:165–175.
Miyamoto K, Watanabe Y, Yukawa M, Takeda H, Nishimura Y, Ishigure N, Hirama T, Akashi M. 2002. Reconstruction of two victims' posturing based on the induced radioactivities in their bones in the criticality accident in Tokai-Mura, Japan. Health Phys 83:19–25.
Mochida A, Gotoh E, Senpuku H, Harada S, Kitamura R, Takahashi T, Yanagi K. 2005. Telomere size and telomerase activity in Epstein-Barr virus (EBV)-positive and EBV-negative Burkitt's lymphoma cell lines. Arch Virol 150:2139–2150.
Moreno S, Hayles J, Nurse P. 1989. Regulation of p34cdc2 protein kinase during mitosis. Cell 58:361–372.
Moreno S, Nurse P, Russell P. 1990. Regulation of mitosis by cyclic accumulation of p80cdc25 mitotic inducer in fission yeast. Nature 344:549–552.
Mullinger AM, Johnson RT. 1983. Units of chromosome replication and packing. J Cell Sci 64:179–193.
Okayasu R, Iliakis G. 1993. Ionizing radiation induces two forms of interphase chromosome breaks in Chinese hamster ovary cells that rejoin with different kinetics and show different sensitivity to treatment in hypertonic medium or beta-araA. Radiat Res 136:262–270.
Pantelias GE. 1986. Radiation-induced cytogenetic damage in relation to changes in interphase chromosome conformation. Radiat Res 105:341–350.
Pantelias GE, Maillie HD. 1983. A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol. Somatic Cell Genet 9:533–547.
Pantelias GE, Maillie HD. 1984. The use of peripheral blood mononuclear cell prematurely condensed chromosomes for biological dosimetry. Radiat Res 99:140–150.
Prasanna PG, Blakely WF. 2005. Premature chromosome condensation in human resting peripheral blood lymphocytes for chromosome aberration analysis using specific whole-chromosome DNA hybridization probes. Methods Mol Biol 291:49–57.
Prasanna PG, Escalada ND, Blakely WF. 2000. Induction of premature chromosome condensation by a phosphatase inhibitor and a protein kinase in unstimulated human peripheral blood lymphocytes: A simple and rapid technique to study chromosome aberrations using specific whole-chromosome DNA hybridization probes for biological dosimetry. Mutat Res 466:131–141.
Rao PN, Hanks SK. 1980. Chromatin structure during the prereplicative phases in the life cycle of mammalian cells. Cell Biophys 2:327–337.
Rao PN, Wilson B, Puck TT. 1977. Premature chromosome condensation and cell cycle analysis. J Cell Physiol 91:131–141.
Rattner JB, Wang T. 1992. Kinetochore formation and behaviour following premature chromosome condensation. J Cell Sci 103(Pt 4):1039–1045.
Schlegel R, Pardee AB. 1986. Caffeine-induced uncoupling of mitosis from the completion of DNA replication in mammalian cells. Science 232:1264–1266.
Schlegel R, Belinsky GS, Harris MO. 1990. Premature mitosis induced in mammalian cells by the protein kinase inhibitors 2-aminopurine and 6-dimethylaminopurine. Cell Growth Differ 1:171–178.
Schor SL, Johnson RT, Waldren CA. 1975. Changes in the organization of chromosomes during the cell cycle: Response to ultraviolet light. J Cell Sci 17:539–565.
Sognier MA, Hittelman WN. 1986. Mitomycin-induced chromatid breaks in HeLa cells: A consequence of incomplete DNA replication. Cancer Res 46:4032–4040.
Someya M, Sakata K, Matsumoto Y, Yamamoto H, Monobe M, Ikeda H, Ando K, Hosoi Y, Suzuki N, Hareyama M. 2006. The association of DNA-dependent protein kinase activity with chromosomal instability and risk of cancer. Carcinogenesis 27:117–122.
Srebniak MI, Trapp GG, Wawrzkiewicz AK, Kazmierczak W, Wiczkowski AK. 2005. The usefulness of calyculin a for cytogenetic prenatal diagnosis. J Histochem Cytochem 53:391–394.
Strausfeld U, Labbe JC, Fesquet D, Cavadore JC, Picard A, Sadhu K, Russell P, Doree M. 1991. Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. Nature 351:242–245.
Suzuki M, Watanabe M, Suzuki K, Nakano K, Matsui K. 1992. Heavy ion-induced chromosome breakage studied by premature chromosome condensation (PCC) in Syrian hamster embryo cells. Int J Radiat Biol 62:581–586.
Terzoudi GI, Malik SI, Pantelias GE, Margaritis K, Manola K, Makropoulos W. 2003. A new cytogenetic approach for the evaluation of mutagenic potential of chemicals that induce cell cycle arrest in the G2 phase. Mutagenesis 18:539–543.
Van Hooser A, Goodrich DW, Allis CD, Brinkley BR, Mancini MA. 1998. Histone H3 phosphorylation is required for the initiation, but not maintenance, of mammalian chromosome condensation. J Cell Sci 111(Pt 23): 3497–3506.
Vass S, Cotterill S, Valdeolmillos AM, Barbero JL, Lin E, Warren WD, Heck MM. 2003. Depletion of Drad21/Scc1 in Drosophila cells leads to instability of the cohesin complex and disruption of mitotic progression. Curr Biol 13:208–218.
Watanabe M, Suzuki M, Suzuki K, Nakano K, Yatagai F. 1992. Radiation-induced chromosome damage in G1 phase cells as breaks in premature chromosome condensation (PCC) and its biological meaning. J Radiat Res (Tokyo) 33 (Suppl):87–94.
Yamashita K, Yasuda H, Pines J, Yasumoto K, Nishitani H, Ohtsubo M, Hunter T, Sugimura T, Nishimoto T. 1990. Okadaic acid, a potent inhibitor of type 1 and type 2A protein phosphatases, activates cdc2/H1 kinase and transiently induces a premature mitosis-like state in BHK21 cells. EMBO J 9:4331–4338.