11

Eidetic Analysis of the Premature Chromosome Condensation Process

Dorota Rybaczek
Department of Cytophysiology, University of Łódź
Poland

'Why does this written doe bound through these written woods?
(…) Perched on four slim legs borrowed from the truth,
She pricks up her ears beneath my fingertips (…)'
(Szymborska, 1993)

1. Introduction

An exact transfer of genetic information depends on the accuracy of mechanisms duplicating DNA molecules in the S-phase and the precise division sister chromosomes during mitosis. The regulation systems of these processes (checkpoints) not only control the activation course of the factors imposing different metabolic specificity on each of the cell cycle phases, but first of all – supervising the proper chronology of events – they condition the behavior of the structural and functional genome integrity. Checkpoints receive signals of all abnormalities or structural damages to DNA and in response evoke reactions inhibiting successive transitions through the cell cycle to enable the expression of specific genes and activation of DNA repair factors. One of the easily perceptible effects of disorders in this signaling system is the induction of premature chromosome condensation (PCC).
The present chapter is a review of the ways and mode of the induction of PCC. The term 'PCC' is inseparably associated with Johnson & Rao (1970) and their experiments on the premature mitosis induced by fusion of interphase and mitotic HeLa cells (G1/M, S/M and G2/M) which were originally carried out using Sendai virus. PCC process can be also induced by chemical signals. Drug-induced PCC provides the new knowledge that DNA replication is tightly coupled with the premature chromosome condensation and that the genome stability results first of all from the alternation of the S-phase and mitosis. The main objective of this review is to show that the PCC induction is possible from various subperiods of cell cycle. Moreover, it has been shown that there are cause-and-effect relationships between the chromosome structure defining 'PCC phenotype' and subperiods, e.g. of the S-phase, initiating the biosynthesis of 'early' or 'late' replicons. Attempts have been made to find answers to questions such as: How to force cells to break out of the rules being developed by Nature for billions of years? How – despite the interrupted, still unterminated process of genome replication – to force a cell to initiate its division? What mechanisms annihilate the subordination principle verified in the course of evolution: first create (DNA-duplicating S-phase) and then divide (mitosis – a stage of DNA condensation and formation of sister

descendant nuclei)? Interference in the regulatory systems of cell cycle is not a simple matter. The gene pool, whose products participate in the creation of these systems, is constantly changing with time to continually form new systems and new interactions. Huge difficulties in the development of effective and selective methods that would arrest the proliferation of cancer cells result from their multiplicity and complication degree, as well as from the possibilities of starting the mechanisms of substitutive and biochemical emergency systems. Studies on the mechanisms inhibiting cell divisions seem to be the shortest way to reach the desired end. This chapter shows the usefulness of attempts to force divisions in cells, simultaneously taking into account the strategy of anticancer therapy.

Therefore the PCC phenomenon constitutes in reality not only a significant fundamental problem in the biology of cell cycle, but it is also an issue of paramount importance in view of practical applications. The radio- and chemotherapy methods used in the treatment of malignant diseases lead to extensive damages to DNA, arresting the replication process of genetic material. Despite this fact, the inhibition of cancer cell proliferation most often is of temporary character or it comprises only part of their population. Drug-induced PCC gives a novel tool to characterize the role of the chromosome instability in cancer development. In this chapter, an attempt is also made to explain the molecular base of PCC induction, for which the starting-point is the biochemical organization of the S-phase checkpoints that block mitosis initiation and the mechanisms which make it possible to suppress their restrictive interactions.

2. Discovery of the premature chromosome condensation (PCC)

The process of separate mitotic chromosome formation from chromatin of an interphase cell nucleus is associated with the construction of giant complexes or macromolecules this consequence being a specific expression of molecular morphogenesis. Simultaneously, it results from action of a complicated regulatory system, causing long, replicated DNA molecules to assume a form adapted to the biomechanical processes of mitosis. Control over these processes is provided on many planes of molecular chromatin organization, e.g. by the association of their components with the nuclear matrix, by specific phosphorylations and dephosphorylations conditioned by changes in the activity of protein kinases and phosphatases or by translocations of some molecules along the length of the fibrils of condensing chromatin or along the arms of existing chromosomes. The degree of packing achieved by chromatin throughout its domain organization before the G2→M transition falls short of the culminant metaphase condensation, the concomitant structural changes always resulting from biochemical modifications that proceed in the protein scaffold of the chromosome under formation.

Attentiveness to the integrity of genome determines the fundamental principles governing the regulation of cell cycle: the replication of each DNA molecule during S-phase can take place only once. The second condition involves the initiation of mitosis: this cannot begin before the complete termination of DNA replication. Control over the course of successive phases of cell cycle is extraordinarily precise and rigorous since initiation of the S-phase is restricted exclusively to unreplicated post-mitotic chromatin. Meanwhile, it is known that competence to initiate mitosis is not always conditioned by the replicated state of chromatin (this does not mean however that control over this process is not precise; simply, it results from the closely specified timing involved in setting-up factors liberating the activity of MPF [i.e. Cdk1 kinase and cyclin B complex or maturation/mitosis promoting factor] and its co-operation with aspects of the activators' and inhibitors' character).

Carefully designed experiments by Johnson & Rao (1970) have resulted in the first correct interpretation of the phenomenon of premature chromosome condensation (PCC). The term PCC appeared in the description of phenomena observed during the fusion of interphase and mitotic malignant HeLa cells. It has been shown that the PCC phenomenon is accompanied by disappearance of the nuclear envelope, chromatin condensation and the formation of mitotic spindle. Subsequent investigations have shown that the induction of PCC is inseparably connected with the activity of MPF complex. The experiments, involving fusion of interphase and mitotic cells have clearly shown that the cells in all the subperiods of interphase are characterized by capability to induce PCC. Thus: (i) if the fusion took place between a mitotic cell and an interphase cell in G1 phase, then PCC resulted in the formation of chromosomes consisting of a single chromatid only (univalent chromosomes); (ii) if the fusion took place between a mitotic cell and an interphase cell in S-phase, strongly fragmented chromosomes were formed in the PCC process, and their morphology constituted a specific reminder of the nucleoplasm organization in the period of the activity of the cell replication apparatus (a 'pulverized' appearance that consisted of univalent and bivalent chromosomes); (iii) if a mitotic cell and interphase cell in G2-phase participated in the combination, PCC resulted in the formation of chromosomes consisting of two chromatids apparently non-differentiated morphologically as compared to normal chromosomes, although probably less condensed (bivalent chromosomes). Despite the fact that the first observation of PCC was reported by Kato & Sandberg (1967) in virus mediated multinucleated fused cells of interphase and mitotic cells, it was Johnson & Rao who in 1970 properly defined the observed phenomenon as 'premature chromosome condensation' (PCC), and the condensed interphase chromatin as 'prematurely condensed chromosomes' (PCCs) (Gotoh & Durante, 2006). Generally, the PCC method facilitates the visualization of interphase chromatin as a condensed form of chromosome structure (Gotoh, 2007). Nowadays, the efficiency and scope of PCC induction have been proved by combining techniques.

Premature chromosome condensation became a method used to: (1) distinguish a cell cycle stage (Cadwell et al., 2011) and the Rabl-orientation of interphase chromosomes, e.g. G1-PCCs and G2-PCCs (Cremer et al., 1982); (2) investigations in chromosome dynamics, also that in interphase chromatin, chromosome replication studies and DNA repair analysis (Gotoh & Durante, 2006, as cited in Cornforth & Bedford, 1983; Hittelman & Pollard, 1982; Hittelman & Rao, 1974; Mullinger & Johnson, 1983; Schor et al., 1975); (3) perform mutagenic assay (Gotoh, 2009, as cited in Cornforth & Bedford, 1983; Durante et al., 1996); (4) chromosome instability analysis (Bezrookove et al., 2003); (5) prenatal diagnosis (Gotoh & Durante, 2006, as cited in Srebniak et al., 2005); (6) karyotyping of chromosomes (Kowalska et al., 2003); and (6) cytogenetic analysis of cancers (Darroudi et al., 2010).

2.1 Induction of PCC in historical and methodological terms

Virus-mediated PCC was first reported more than 40 years ago. In 1983, cell fusion could be achieved by means of polyethylene glycol (PEG-induced or chemically-mediated; Pantelias & Maillie, 1983). This allowed the external MPF to migrate from the inducing mitotic cell to the interphase recipient. A few years later it was possible to obtain chemically-induced PCC (drug-induced PCC): initially from synchronized cells and later still from each phase of the cell cycle (Gotoh & Durante, 2006, as cited in Schlegel & Pardee, 1986, Schlegel et al., 1990; Yamashita et al., 1990). Detailed data concerning PCC induction reported so far in the world literature are presented in Table 1.

The way of induction of PCC	References
A. Fusion-induced PCC Exploits the action of external MPF	
A1. Virus-induced fusion	
(a) UV inactivated Sendai virus-induced PCC	Kato & Sandberg, 1967 *The first observation of PCC* Johnson & Rao, 1970 *The first correct interpretation of PCC*
(b) PCC-type remodeling of the donor nucleus after somatic cell nuclear transfer (SCNT)	Le Bourhis et al., 2010
A2. Chemically-induced fusion	
(a) (PEG)-induced fusion	Pantelias & Maillie, 1983 *The first successfully applied polyethylene glycol (PEG)-induced fusion just before PCC induction*
B. Drug-induced PCC Exploits the activation of endogenous MPF	
B1. With synchronization Cells had to be synchronized in G1- or S- or G2-phase before PCC induction	
B1.1. Induced by protein phosphatase inhibitors	
(a) Okadaic acid	Schlegel & Pardee, 1986 *The first one successfully applied in chemically-induced PCC in S phase cells* Ghosh et al., 1998; Schlegel et al., 1990; Yamashita et al., 1990
(b) Sodium metavanadate	Ghosh et al., 1998; Rybaczek & Kowalewicz-Kulbat, 2011;
B1.2. Induced by protein kinase inhibitors	
(a) Caffeine	Schlegel & Pardee, 1986 *The first one successfully applied in chemically-induced PCC in S phase cells* Schlegel et al., 1990; Yamashita et al., 1990; Nghiem et al., 2001
(b) Caffeine 2-aminopurine Staurosporine 6-dimethylaminopurine	Sen & Ghosh, 1998; Rybaczek et al., 2008; Rybaczek & Kowalewicz-Kulbat, 2011; Steinmann et al., 1991
B2. Without synchronization PCC induction occurs without synchronization (in any phase of cell cycle)	
B2.1. Induced by protein phosphatase inhibitors	
(a) Okadaic acid Calyculin A	Bialojan & Takai, 1988; Cohen et al., 1990; Gotoh et al., 1995; Gotoh & Tanno, 2007; Ishihara et al., 1989; Prasanna et al., 2000
B2.2. Adriamycin (Doxorubicin)	Hittelman & Rao, 1975
C. Spontaneous PCC (SPCC)	
(a) In the ontogenesis of generative cells and during the development of endosperm	cited by Tam & Schlegel, 1995
(b) During heat exposure	Mackey et al., 1988; Swanson et al., 1995;
(c) In normal and transformed mammal cells	Kovaleva et al., 2007
(d) After X-irradiation of HeLa cells	Ianzini & Mackey, 1997

Table 1. Data concerning PCC induction reported in the word literature

A specific change occurred when the preliminary cell-free cytoplasmic extracts (obtained by the centrifugation of *Xenopus* egg cells) were used *in vitro* to study the assembly of 'synthetic nuclei' involving the conversion of chromatin sperms into mitotic chromosomes. The resulting expansion of knowledge concerning the action of inhibitors and/or activators used for PCC induction has created new opportunities and perspectives for researchers (Prokhorova et al., 2003). In cells with disturbed DNA structure or those blocked during DNA biosynthesis, PCC-type processes can be induced by various chemical compounds such as: (i) inhibitors of protein kinases, e.g. 2-aminopurine (Herbig et al., 2004), caffeine (Wang et al., 1999, Gabrielli et al., 2007), staurosporine, 7-hydroxystaurosporine (UCN-01), CEP-3891 (Kohn et al., 2002; Syljuåsen et al., 2005), wortmannin (WORT) (Liu et al., 2007) and Gö6976 (Jia et al., 2008) as well as (ii) inhibitors of protein phosphatases, e.g. calyculin A (CalA), okadaic acid (OA) and sodium metavanadate (Van) (Hosseini & Mozdarani, 2004; Rybaczek & Kowalewicz-Kulbat, 2011). In order to induce the PCC phenomenon, an incubation with a strong inhibitor, e.g. of a given kinase or phosphatase or alternating incubation in two different inhibitors, of which the former slows down the course of S phase (e.g. hydroxyurea or aphidicolin) and the latter specifically influences the activity of selected kinases or phosphatases is a frequently used approach. Such substances restore the activity of protein kinases and control the phosphorylation of subordinate proteins in the regulatory pathways of cell cycle simultaneously creating conditions necessary for the initiation of prophase chromosome condensation, thereby fulfilling the role of the inductors of Cdk1-cyclin B complexes and realising mitotic phosphorylations (Sturgeon et al., 2008).

Disturbance of the efficiency of cell cycle checkpoints can result from the action of many factors followed by overriding or breakage of control over genome integrity and the course of various interphase subperiods and finally PCC induction. The premature mitosis induced in meristems of *V. faba* roots by prolonged incubation with a mixture of hydroxyurea and caffeine is characterized by strong differentiation between the morphological forms of chromosomes, which allows one to separate several different cell classes. The degree of chromosome fragmentation (number of sections lost in anaphase) probably determines the level of genetic material disintegration in cells blocked by hydroxyurea in S-phase. Such observations suggest that there is a relationship between the chromosome structure determining 'PCC phenotype' and S-phase subperiod (initiating the biosynthesis of 'early' or 'late' replicons), in which replication block has taken place. This assumes that the period of time elapsing between the beginning of PCC induction and the appearance of first cells with symptoms of premature mitosis depends on the number of replication units in which the biosynthesis processes of complementary DNA strands have been initiated but not terminated. The same mechanism may also explain the considerable differentiation between cells showing morphological features of premature mitosis (Figure 1A).

Modern models assume that phosphorylation of the N-terminal fragments of H3 histones constitutes a preliminary molecular signal which makes initiation of chromosome condensation possible by creating conditions for the assembly of other proteins directly engaged in the structural metamorphoses of chromatin (e.g. condesin complexes). Therefore, the post-translation modifications of H3 molecules are only one of the symptoms reflecting the action of complicated regulatory system which leads to the increase in chromatin packing during prophase chromosome condensation. This view is consistent with observations pointing to considerable asynchrony of the period of intensified H3 histone phosphorylation and the initial stages of mitotic condensation among various organisms, as well as to the phosphorylation of H3 molecules in plant cells taking place in condensed

chromosomes. Meanwhile, S10 phosphorylation of H3 histone has turned out to be an excellent marker in phenotyping PCC from various subperiods of e.g. S-phase (Figure 1B).

The G2→M transition is a period of an increased sensitivity of cells also to the action of factors that directly do not lead DNA damage. For example, when cells entering the early stages of prophase are subjected to hypothermia, anoxia, osmotic shock or other stresses, their chromosomes are decondensed followed by a return to late G2 phase. This phenomenon usually is reversible following a period of stress adaptation or regression of mitosis (Mikhailov & Rieder, 2002). So, the question - what is the beginning of mitosis? – is not trivial, especially in the context of its irreversibility. It is known that almost always the very fact of initiation of S phase determines future mitosis. On the other hand, the fact of DNA replication involving a successful transition through the cell cycle checkpoints gives the process of genetic material segregation to two daughter cells the status of authority or even necessity. It helps when seeking the answer to the question 'what is the beginning of mitosis?' to recall the term 'antephase', historically associated with the paper by Furlough & Johnson published in 1951 (Pines & Rieder, 2001, as cited in Furlough & Johnson, 1951).

The term 'antephase' is used to describe the final stage of G2 phase directly preceding the first perceptible symptoms of prophase chromosome condensation. Cells in the middle stages of prophase (cf. neuroblasts of grasshopper, cells of newt or PtK_1 cell line) subjected to ionizing radiation gradually decondense the chromosomes under formation, their proteins are dephosphorylated and the course of cell cycle is arrested. The same cells irradiated during late stages of prophase, despite strong chromosome fragmentation, initiate further mitosis stages. A similar process – gradual chromosome decondensation – is also observed when the disassembly of microtubules by nocodazole takes place before cell transition through the middle stage of prophase. The action of nocodazole in later periods of prophase induces colcemid-mitoses – the division of chromosomes deprived of communication with the microtubular spindle apparatus leading to the formation of polyploid nuclei. Thus there is a specified point, i.e. the final period of 'antephase', after which a cell is unable to return to the interphase condition. In the cells of many animal species (vertebrates), 'the point of the last chance to return to G2 phase' occurs during the final stages of prophase, the border here between interphase and mitosis being set considerably later than usual (Pines & Rieder, 2001).

Thus, there are cells in which advanced stages of chromosome condensation are an easily recognizable indicator of G2 phase termination ('antephase'), whence they become convenient subjects for studying the transient G2→M period at a molecular level. The activity of kinase Cdk1-cyclin A complexes increases during the G2 phase and reaches its maximum at the moment of nuclear envelope decomposition. The microinjection of these complexes into G2 cells induces a violent chromosome condensation but inhibitors of Cdk1 kinase (such as $p21^{Waf1/Cip1}$) block the transition to mitosis and then the early-prophase cells return to the interphase state. This is a period during which inactive Cdk1-cyclin B complexes remain in the area of cytoplasm (Furuno et al., 1999). Thus, it seems that preparation for the entry into mitosis taking place in vertebrate cells during 'antephase' is mainly controlled by Cdk1-cyclin A complex and not by the association of Cdk1 with cyclin B. An important role is also played by Plk1 kinase, which controls organization of the cell centrosomal apparatus and, while phosphorylating Cdc25 phosphatase, indirectly activates Cdk1-cyclin B complexes (Kumagai & Dunphy, 1996). Another protein kinase, Aurora B (animals) or its homologue in yeast cells (Ipl1) induces chromosome condensation via phosphorylation of S10 in H3 histone (Hsu et al., 2000). In many types of cells, changes occur at the level of chromosome condensation causing centrosomes to interact with a

growing number of γ-tubulin molecules. Meanwhile, normal cells (and not the transformed ones) are subjected to the control mechanisms of G2 phase which, by the intervention of ATM/ATR kinases, monitor the condition of DNA structure and make it possible to arrest the cell cycle just before the initiation of mitosis.

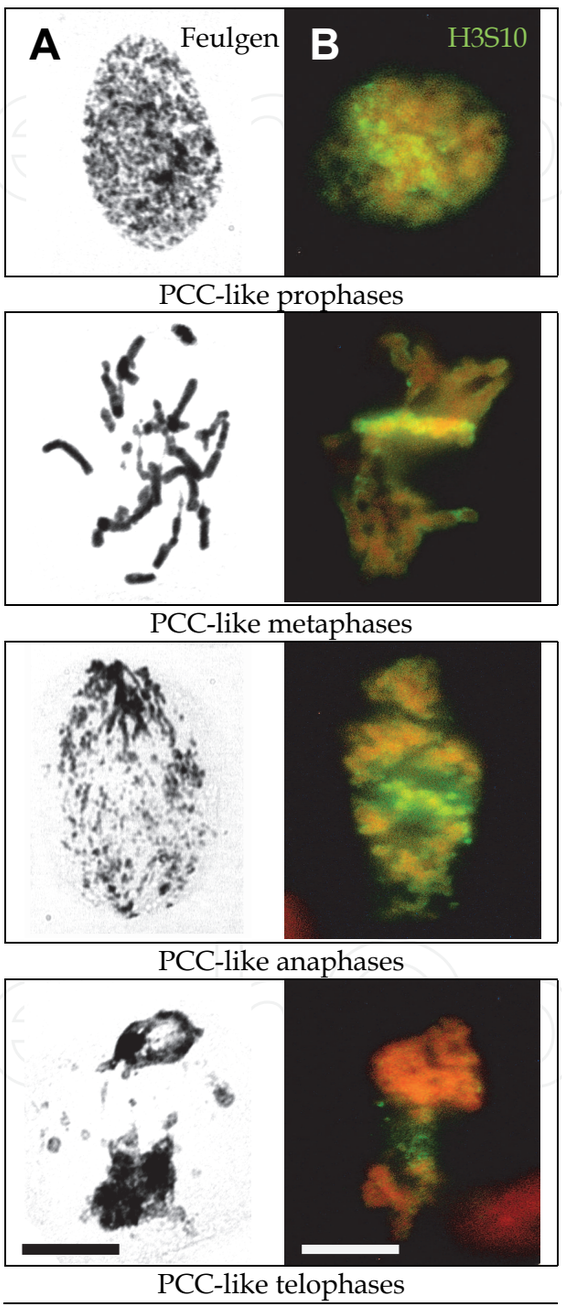


Fig. 1. A. Caffeine-induced PCC in Feulgen-stained root meristem cells of *Vicia faba*. The full array of aberrations included: chromosomal breaks and gaps, lost and lagging chromatids and chromosomes, acentric fragments and micronuclei. B. Immunofluorescence of phospho-H3 (S10; Cell Signaling) after caffeine-induced PCC in root meristem cells of *Vicia faba*. *Bar* 20 μm

3. Molecular origin of PCC induction

Hypotheses describing the mechanism of PCC induction are based on the results of genetic and biochemical analyses of yeast cells and human and animal cells *in vitro*. Among plants, the induction of premature chromosome condensation sometimes spontaneously occurs in the ontogenesis of generative cells and during the development of endosperm (cited by Tam & Schlegel, 1995). The special nature of these phenomena, the role played in them by phytohormonal factors and conditioning connected with the specificity of plant cell mitotic divisions have not yet been recognized. Neither have the organization of checkpoints blocking the initiation of mitosis, nor the mechanism that could overcome their restrictive interactions, been explained.

During the cell cycle, there are mechanisms governing transition 'to' and 'from' proliferation and coordinating of the complexes responsible for the successive stages of cell cycle transition. The first regulatory system is connected with the transition from G0 phase to cell cycle, which results in the transcription of appropriate genes, this being realization of the program responsible for leaving the resting condition. This leads to the commencement of DNA synthesis and consequently the initiation of mitotic division. The action of the second regulatory system involves arresting the course of G1 phase – before the start of DNA replication – or G2 phase – before the initiation of mitosis.

In cell cycles of the G1-S-G2-M type, the function of two main checkpoints is directly associated with the coordination of transient stages determining the maintenance of genetic identity. The G1 phase checkpoint monitors the metabolic conditions of cells in the G1 phase, the integrity of the nuclear DNA structure prior to the beginning of the synthesis of the complementary chromosome set and the level of necessary substrates, enzymes and replication factors. In plant cytology, it is known as the 'Principal Control Point 1' (PCP1), being the counterpart of the START point in yeast and of the Restriction Point in animal cells. The second checkpoint, PCP2, of a more conservative character, plays a similar role in G2 phase. Its functions are connected with the assessment of internal and external conditions of the cell environment during mitosis and cytokinesis. PCP2 controls if the replication of DNA is properly terminated and also monitors the integrity of chromatin structure before its mitotic condensation (Del Campo et al., 2003). The checkpoint pathways functioning in G1 and G2 phases of cell cycle constitute however only part of the complex system formed by checkpoint mechanisms of the whole cell cycle (Bucher & Britten, 2008). For example, the mechanisms determining S-phase initiation and development are functionally connected with the system of three S-phase checkpoints: (i) the intra-S-phase checkpoint or DSB-induced replication-independent intra-S-phase checkpoint which block mitosis initiation in the case of structural DNA damage; (ii) the replication checkpoint or replication-dependent intra-S-phase checkpoint which block mitosis initiation in the case of inhibition of DNA biosynthesis; and (iii) the replication-dependent S-M checkpoint which ensures that the G2 phase and mitosis can begin after complete genome replication (Bartek et al., 2004).

In many respects, the control mechanisms of G2 phase are similar to the regulatory systems that protect a cell against too rapid initiation of DNA replication. Still more analogies can be found by comparing both of them with the complex molecular systems of the S phase checkpoints. Admitting some simplification, it can be assumed that this continual repetition is not unusual: if monitoring of the DNA structure integrity is the basic aim of complicated biochemical mechanisms, the means serving that end do not have to be adapted for the realization of many targets, but just one – the most important. On the other hand, their

molecular construction must take into account the specificity of the successive phases of the cell cycle.

The mentioned analogies have probably contributed to the fact that the best known function of checkpoints in G2 phase consists in blocking the initiation of mitosis in case of DNA damage. Detection of structural anomalies liberates the action of two complementary and partly convergent molecular pathways centred on ATM/ATR kinases and a common purpose – maintenance the Cdk1 kinase complexes with cyclin B inactive (Mikhailov & Rieder, 2002; Paulsen & Cimprich, 2007). In one pathway, ATM directly (or indirectly) activates part of the regulatory system, with a key factor p53. This protein induces the synthesis of inhibitors of cyclin-dependent kinases, e.g. $p21^{Waf1/Cip1}$. In the other pathway, independent of p53, ATM activates effector kinases Chk1 and Chk2 that, in turn, prevent the activation of Cdc25C phosphatase (sometimes by Plk1 kinase). Undoubtedly, we have here an excellent example of two coincident regulatory systems that mutually intensify the effectiveness of their individual interactions, while at the same time providing an example of 'economical' use of the same metabolic networks in three different cell cycle phases (Brown & Baltimore, 2007; Matthews et al., 2007; Peddibhotla et al., 2009).

Mitosis is the most dramatic and potentially most 'dangerous' cell cycle phase – involving the condensation of replicated chromosomes, their association with the kinetochores and finally the segregation of sister chromatids to opposite poles. Not only chromosomes replicated in S phase are divided (karyokinesis) but also all organelles (cytokinesis). Thus the checkpoints of mitosis monitor all the transient stages of this complicated process but primarily they control the condition of mitotic spindle by detecting abnormalities in its structural and functional organization (Cortez & Elledge, 2000). Chromatin condensation forming mitotic chromosomes under physiological conditions takes place only during mitosis. Condensins are the key elements in this process. Condensin I and II occur among vertebrates. Condensin I obtains access to chromosomes always after the nuclear envelope breakdown (NEBD) and then, in cooperation with condensin II responsible for the initial stages of condensation in prophase nuclei, promotes the assembly of metaphase chromosomes. Condensins are regulated by phosphorylation dependent on Cdk1, which was demonstrated in the studies utilizing *Xenopus* egg extract. Active MPF initiates the nuclear envelope breakdown to allow condensin I to acquire chromosomes. Hypothetically, it is assumed that Cdk1-cyclin A complex phosphorylates and activates condensin II to initiate the early stage of chromosome condensation inside the prophase nucleus. Next, directly after the nuclear envelope breakdown, Cdk1-cyclin B complex phosphorylates and activates condensin I (Hirano, 2005).

Cell cycle checkpoints act via the principle of establishing a cause-effect relationship between separate biochemical processes (Hartwell & Weinert, 1989) involving feedback loops (Elledge, 1996). The term 'checkpoint' refers to a definite subset of internal and external regulatory mechanisms that link further processes to the realization of earlier ones. At the same time, one has to remember to always take into account the existence of hysteresis in the molecular interaction of the control network in the cell cycle system. Hysteresis means that it takes more to push a system from point A to point B than it does to keep the system at stage B (Sha et al., 2003; Solomon, 2003). Generally, there are two classes of regulatory systems in the cell cycle: (i) intrinsic systems of a constitutive character sorting out the events directly connected with the cell cycle, and (ii) extrinsic systems that are revealed under the influence of inducing factors and are engaged only when DNA damage is detected (Elledge, 1996).

Each of the cell cycle checkpoints comprises three essential parts: (i) capability of sensing that a cell cycle event is aberrant or incomplete, (ii) means by which this signal is transmitted, and finally, (iii) effectors that delay or block the cell cycle transitions until the problem is resolved. The position of arrest within the cell cycle varies depending on the phase during which the damage is sensed. Since the main role of all these checkpoints is to make a decision whether or not the cell division cycle can be continued, their particular elements deserve special attention as promising targets for pharmacological treatment of cancer (Deckert et al., 2009). The action of checkpoints of cell cycle crucially depends on the effectiveness of the system transmitting signals released by the cell sensory apparatus. The activation of mitotic protein kinases (M-Cdk) is then blocked which makes it possible to effect repairs or to terminate DNA replication or apoptosis induction (Khanna & Jackson, 2001; Zhou & Elledge, 2000).

The PCC phenomenon results from the overriding of S-M checkpoint. It blocks the ability of cells to make mitotic divisions after extensive DNA damage or under conditions of unfinished replication. DNA damage activates in the first place sensor kinases from the PIKK family, ATM and/or ATR which subsequently inhibit the formation of active MPFs by the phosphorylation of Chk2 and Chk1 kinases, which blocks the onset of mitosis. The initiated cascade of signals simultaneously activates repair factors including DNA-PK kinase which is essential for the repair of DNA suffering NHEJ-type damage. Blocking of the function of sensor ATM and/or ATR kinases can bring about avoidance of the restrictive interactions of S phase checkpoints causing premature mitosis (Block et al., 2004).

Knowledge of PCC mechanisms and S-M checkpoint action goes hand in hand with studies of malignant diseases and eagerness to maximize the beneficial effects of radio- and chemotherapy (Erenpreisa & Cragg, 2007). Despite evidence that overexpression of Cdk1-cyclin B complexes can promote PCC, it is not clear if these complexes initiate PCC in human cells. Studies of the import of cyclin B1 in human beings and in asteroid oocytes have shown that it is imported within the period of last several minutes of prophase, just after the initiation of chromosome condensation. These observations suggest that normal chromosome condensation is not initiated by Cdk1-cyclin B1 complexes. On the other hand, there is evidence indicating that the chromosome condensation and other phenomena occurring in the early prophase are initiated by Cdk2-cyclin A complexes present in this period within the cell nucleus. The initiation of cytoplasmic phenomena of mitotic character such as reorganization of Golgi apparatus and microtubular changes probably requires no import of Cdk1-cyclin B1 complexes into the nucleus (Takizawa & Morgan, 2000). On the other hand, the initiation of mitotic division occurs once Cdc25 phosphatase has dephosphorylated phosphate groups (both Y15 and T14) from the area of exposed pocket binding ATP within kinase $p34^{cdc2}$. Thus Cdc25 phosphatase is the activator of mitosis.

Dysfunctions of S phase checkpoints also occur in mutated cells (Krause et al., 2001). Deletions of *wee1* and *mik1* genes cause disappearance of proteins transmitting signals about DNA structure damage or blocked replication. Such cells initiate mitosis, but underreplications result in broken chromosomes being lost in the central spindle zone. Similar results follow from changes in the activity balance of protein kinases and phosphatases caused by overexpression of *cdc25* genes. Disappearance of intra-S-phase checkpoint function, caused e.g. by the lack (or mutation) of *atr*, also brings about the initiation of mitosis by cells that contain partly replicated genetic material (as opposed to normal cells in which replication forks activate ATR if they meet a defect impeding the biosynthesis of DNA which leads to the activation of S phase checkpoints and cell cycle

inhibition). In the embryonic evolution of *Drosophila melanogaster*, grapes (*grp*) – whose product is a homolog of Chk1 kinase – is one of the genes of checkpoints functioning in this period of morphogenesis. Grapes mutants show a shortened course of interphase, a defective chromosome condensation and delayed metaphase initiation (Yu et al., 2000). In this case, premature mitosis is caused firstly by overriding of function of S phase checkpoints (then chromosome condensation is not dependent on the termination of S phase) and secondly, by the moment of initiation of chromosome condensation (ICC) occurring with no delay. Thus, only the period between ICC and metaphase is shortened, which seems to be the direct cause of incomplete chromosome condensation. To sum up, in *D. melanogaster* the delay in *grp* embryo entry into metaphase is caused by chromatin condensation defects rather than by partial DNA replication (Royou et al., 2005).
The facts presented above that barely outline extensive problems connected with the cellular control mechanisms show a huge complexity of both stimulating and inhibiting biochemical systems. The associated regulatory network comprises processes that activate the expression of genes at the moment desired for the cell and block the course of chemical reactions when their products could accumulate in excessive amount or prematurely encourage cell cycle transitions. It seems that all eukaryotic cells are equipped with sensory factors, signal transmitting systems and effector factors. The significance of these consists in the fact that they make DNA replication and chromosome division possible without endangering the whole information contained in them which is indispensable for the organism development and maintenance of species continuity.

4. PCC and DNA damages

The successive phases of prematurely initiated mitosis follow an aberrated course. The loss of relatively large chromosome sections suggests that disturbances of post-replication repair processes in the G2 (G2-PCC) are responsible for this. On the other hand, much higher disintegration of genetic material in some chromosomes leads to the conclusion that this is symptomatic of mitosis initiated from cell subpopulations that have not yet finished the DNA replication process (S-PCC; Rybaczek et al., 2008). Some influence on the degree of DNA fragmentation can be exerted by chemical compounds used in studies of PCC induction, e.g. caffeine which additionally intensifies chromosome destruction during their individualization. It is certain that the losses or breaks in the chromosome continuity: (i) either illustrate the unreplicated areas of genome; (ii) or result from physical stresses created during the mitotic condensation and segregation of chromosomes; (iii) or originate from relative fragility of single-stranded DNA sections generated during retardation of replication forks under the conditions of nucleotide triphosphate deficiency (i.e. after the use of replication inhibitors, e.g. hydroxyurea) (Cimprich, 2003; El Achkar et al., 2005).
The appearance of double strand breaks (DSBs) in DNA molecules is connected with the formation of immunofluorescence foci associated with the phosphorylated form of H2AX histones at S139 (DSB marker). The family of lysine-like H2A histones includes three subfamilies of proteins (H2A1-H2A2, H2AZ and H2AX). In mammalian cells, H2AX amounts to about 2 to 25% (probably almost 100% in *S. cerevisiae*) (Rogakou et al., 1998). The C-terminal motif of AQ(D/E)(I/L/Y) is the sequence distinguishing H2AX among H2A histones, while S139, ahead of it, is the site of γ-phosphorylation. Formation of γ-H2AX molecules resulting from the exposure of mammalian cells (or living mice) to the action of ionizing radiation (in sublethal or lethal doses) is an extremely violent process (Kurose et al.,

2006). Half of the γ-H2AX histones appears after a 1-minute period of irradiation, while the maximal level is reached within 3 to 10 minutes of exposure (per 1 Gy of radiation about 1% of H2AX histone molecules undergoes γ-phosphorylation, which corresponds to about $2x10^6$ bp DNA in the area of the double-strand break of its structure) (Paull et al., 2000; Rogakou et al., 1998). It is assumed that each group of these molecules indicates a single DSB region, therefore the formation of H2AX foci phosphorylated at S139 constitutes a sensitive test revealing the presence of structural genome damage (Friesner et al., 2005). Literature reports in recent years show the occurrence of fluorescence foci of H2AX molecules within the area of S139 during PCC induction (Huang et al., 2006; Rybaczek et al., 2007; Rybaczek & Kowalewicz-Kulbat, 2011). On the other hand, Stevens and his co-workers (2010) clearly indicate that PCC is γ-H2AX negative, and that γ-H2AX phosphorylation is only a hallmark of a chromosome fragmentation phenomenon. However, simultaneously they define PCC as occurring in interphase cells exposed to active MPF vs that occurring during mitosis chromosome fragmentation. According to other investigations, however, the actual definition of drug-induced PCC involves both the described phenomena, i.e. PCC and chromosome fragmentation (Riesterer et al., 2009; Rybaczek et al., 2008; Terzoudi et al., 2010).

To protect against disturbances during DNA biosynthesis, cells have developed a network of biochemical reactions known as DNA-replication-stress-response. The basic strategy of this response is retardation of processes, whose continuation would result, among other things, in the transfer of affected DNA molecules to a new cell generation. Therefore under the conditions of replication stress, the DNA biosynthesis rate is slowed down and onset of mitosis is - most frequently - completely blocked. This continues until the expression of specific genes and activation of repair factors (Deckert et al., 2009; Mosesso et al., 2010). Each structural disturbance (e.g. DSB) causes the rate of production of replication forks to slow down. Additionally, any limitation of the replication apparatus effectiveness (resulting, e.g. from the deficiency of nucleotide triphosphate or polymerase dysfunction) can facilitate DNA damage. In such a situation, the action of checkpoint sensory factors releases a cascade of signals supplied to various effector proteins through intermediary elements.

The detection of double-strand breaks in DNA molecules activates the biochemical pathway in which ATM kinase is the superior element. The processes proceeding with its contribution are triggered in all the phases of cell cycle, while the factors participating here may also assist the second pathway going in parallel. ATR is also activated by damage resulting in disruption of continuity in both DNA strands, but here the induction process occurs more slowly (Scott & Pandita, 2006). The pathway subordinated to ATR kinase is specialized first of all in reacting to disturbance of the replication fork function. These disturbances can results from endogenous interactions, chemotherapy or experimental procedures leading to the inhibition or disturbance of replication processes caused by hydroxyurea (HU) or ultraviolet radiation (UV). In both biochemical pathways, Cdc25 phosphatase is the target substrate.

Mec1 and Tel1 kinases in *S. cerevisiae* and Rad3 and Tel1 kinases in humans are homologues of both conservative signaling ATM and ATR kinases (Garber et al., 2005). Their substrates, kinases of the CHK family, are subordinate factors activated by ATM/ATR/Mec1/Rad3. Such groups jointly form the central pathway module of response to replication stress, which both records incoming information on the DNA condition and sends signals to replication forks. In human and *Xenopus* cells, ATR kinase is indispensable in the phosphorylation of Chk1 kinase and it occurs in stable combination with ATR-interacting

protein (ATRIP), there complexes being concentrated in those areas of cell nucleus showing DNA damage. The activator of ATR-ATRIP complexes in vertebrate cells consists of TopBP1 protein. Investigations utilizing cytoplasmic extracts of *Xenopus* egg cells have also shown that the association of ATR with chromatin takes place during the period of DNA replication, whereas it disappears after the termination of replication (Freire et al., 2006; Harper & Elledge, 2007).

It is uncertain whether disturbances of DNA replication are detected by means of only one, universal, sensory mechanism. Maybe, regardless of the type of the factor blocking S phase, the DNA structure generated by replication forks is identical at the damage site. However, one cannot exclude the possibility that each type of the DNA structure disturbance exerts different, specific influence on replication forks and that the factors associated with particular types of damage are also different.

5. Consequences of PCC induction

Literature shows that proper functioning of all multicellular organisms, including *Homo sapiens,* depends not only on their ability to produce new cells but also on the ability of each cell to annihilate itself when it becomes unwanted or damaged. This takes place also when the control mechanisms of the cell cycle have been overridden during a simultaneous strong and/or long-lasting action of stress stimulus. Thus, among cells induced to enter premature, unauthorized division, there are also those that choose the apoptosis pathway (Sahu et al., 2009). Therefore premature mitoses/PCC are described as a mitotic catastrophe, abortive or suicidal. During the induction of apoptosis – following PCC induction – chromatin undergoes drastic changes: previously usually dispersed, it suddenly begins to condense into one or more aggregates in the vicinity of a nuclear membrane (Figure 2A). Changes connected with the initial phase of apoptosis also involve formation of intranuclear membranous structures (sometimes strongly developed and multi-layered) adhering to the nuclear envelope (Figure 2B-D).

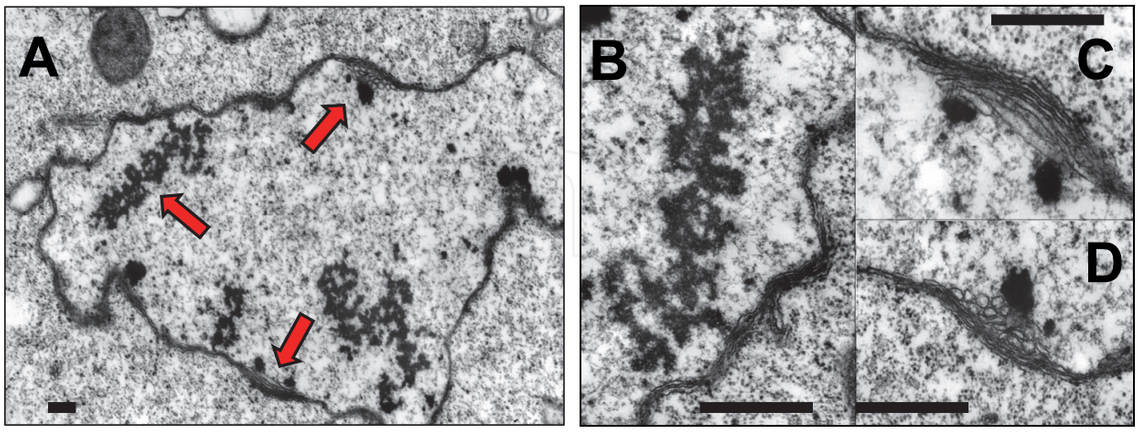


Fig. 2. Ultrastructure of an apoptotic cell of *V. faba* root meristem (after PCC induction caused by 5 mM caffeine). Selected fragments of the above micro-photography (marked with arrows) at magnification. *Bar* 1µm

Among unicellular organisms, irregularities in the organization of cell cycle control systems result in a decreased reproduction potential, while among multicellular mechanisms they

cause uncontrolled proliferation, cancer development and genetic disease transfer (Hartwell & Weinert, 1989; Russell, 1998).

6. Conclusion

What are the practical implications and prospects of PCC induction? Could PCC induction serve as a novel anti-cancer approach? Undoubtedly, the phenomenon of premature mitosis is an essential characteristic of cell biology, therefore an important issue in respect of potential medical applications. It is so because, according to many researchers, the chemotherapy commonly used for the treatment of malignancy leads to extensive DNA damage, whereas PCC induction (resulting from the stimulation of biochemical mechanisms overriding the action of S-M checkpoint) can intensify therapeutic effects. Recently, drug-induced PCC was optimized to assist analysis of the behavior of cancer cells with minimal side effects. However, PCC will also contribute to the understanding of normal cellular processes.

7. Acknowledgement

The author expresses her cordial thanks to Professor Joanna Deckert for her support and inspiration, to Anna Pastucha for her assistance with immunocytochemical assays employing anti-H3S10, and to Małgorzata Fronczak M.Sc. for her help in preparing this manuscript in English. This work was funded by the Ministry of Science and Higher Education, grant N N303 355935 (contract no. 3559/B/P01/2008/25).

8. References

Bartek, J., Lukas, C. & Lukas, J. (2004) Checking on DNA damage in S phase. *Nat Rev Mol Cell Biol* 5, 792-804.

Bezrookove, V., Smits, R., Moeslein, G., Fodde, R., Tanke, H.J., Raap, A.K. & 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.

Block, W.D., Merkle, D., Meek, K. & Lees-Miller, S.P. (2004) Selective inhibition of the DNA-dependent protein kinase (DNA-PK) by the radiosensitizing agent caffeine. *Nucleic Acids Res* 32, 1967-1972.

Brown, E.J. & Baltimore, D. (2007) Essential and dispensable roles of ATR in cell cycle arrest and genome maintenance. *Genes Dev* 17, 615-628.

Bucher, N. & Britten, C.D. (2008) G2 treatment abrogation and checkpoint kinase-I targeting in the treatment of cancer. *Br J Cancer* 98, 532-528.

Cadwell, K.K., Curwen, G.B., Tawn, E.J., Winther, J.F., Boice, J.D. Jr. (2011) G2 checkpoint control and G2 chromosomal radiosensitivity in cancer survivors and their families. *Mutagenesis* 26, 291-294.

Cimprich, K.A. (2003) Fragile sites: Breaking up over a slowdown. *Curr Biol* 13, R231–R233.

Cohen, P., Holmes, C.F. & Tsukitani, Y. (1990) Okadaic acid: A new probe for the study of cellular regulation. *Trends Biochem Sci* 15, 98-102.

Cortez, D. & Elledge, S.J. (2000) Conducting the mitotic symphony. *Nature* 406, 354-356.

Cremer, T., Cremer, C., Baumann, H., Luedtke, E-K., Sperling, K., Teuber, V. & Zorn, C. (1982) Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. *Hum Genet* 60, 46-56.

Darroudi, F., Bergs, J.W.J., Bezrookove, V., Buist, M.R., Stalpers, L.J. & Franken, N.A.P. (2010) PCC and COBRA-FISH a new tool to characterize primary cervical carcinomas: To asses hall-marks and stage specificity. *Cancer Lett* 287, 67-74.

Deckert, J., Pawlak, S. & Rybaczek, D. (2009) The nucleus as a 'headquarters' and target in plant cell stress reactions, In: *Compartmentation of Responses to Stresses in Higher Plants, True or False*, Waldemar Maksymiec, pp. 61-90, Transworld Research Network, ISBN: 978-81-7895-422-6, Kerala, India.

Del Campo, A., Samaniego, R., Giménez-Abián, J.F., Giménez-Martín, G., López-Sáez, J.F., Moreno Díaz de la Espina, S. & De la Torre C. (2003) G2 checkpoint targets late replicating DNA. *Biology of the Cell* 95, 521-526.

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.

Elledge, S.J. (1996) Cell cycle checkpoint: preventing an identity crisis. *Science* 274, 1664-1672.

Erenpreisa, J. & Cragg, M.S. (2007) Cancer: A matter of life cycle? *Cell Biol Int* 31, 1507-1510.

Freire, R., van Vugt, M.A.T.M., Mamely, I. & Medema R.H. (2006) Claspin. Timing the cell cycle arrest when the genome is damaged. *Cell Cycle* 5, 2831-2834.

Friesner, J.D., Liu, B., Culligan, K. & Britt, A.B. (2005) Ionizing radiation-dependent γ-H2AX focus formation requires ataxia telangiectasia mutated and ataxia telangiectasia mutated and Rad3-related. *Mol Biol Cell* 16, 2566-2576.

Furuno, N., den Elzen, N. & Pines, J. (1999) Human cyclin A is required for mitosis until mid-prophase. *J Cell Biol* 147, 295-306.

Gabrielli, B., Chau, Y.Q., Giles, N., Harding, A., Stevens, F. & Beamish, H. (2007) Caffeine promotes apoptosis in mitotic spindle checkpoint-arrested cells. *J Biol Chem* 10, 6954-6964.

Garber, P.M., Vidanes, G.M. & Toczyski, D.P. (2005) Damage in transition, *Trends Biochem Sci* 30, 63-66.

Ghosh, S., Paweletz, N. & Schroeter, D. (1998) Cdc2-independent induction of premature mitosis by okadaic acid in HeLa cells. *Exp Cell Res* 242, 1-9.

Gotoh, E. & Durante, M. (2006) Chromosome condensation outside of mitosis: mechanisms and new tools. *J Cell Physiol* 209, 297-304.

Gotoh, E. & Tanno, Y. (2007) 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. (2007) Visualizing the dynamics of chromosome structure formation coupled with DNA replication. *Chromosoma* 116, 453-462.

Gotoh, E. (2009) Drug-induced premature chromosome condensation (PCC) protocols : cytogenetic approaches in mitotic chromosome and interphase chromatin, In : *Chromatin Protocols: Second Edition, series: Methods in Molecular Biology*, Srikumar P. Chellappan, 523, 83-92, Humana Press, 523, 83-92.

Gotoh, E., Asakawa, Y. & Kosaka, H. (1995) Inhibition of protein serine/threonine phosphatases directly induces premature chromosome condensation in mammalian somatic cells. *Biomed Res* 16, 63-68.

Harper, J.W. & Elledge, S.J. (2007) The DNA damage response: ten years after. *Mol Cell* 28, 739-745

Hartwell, L.H. & Weinert, T.A. (1989) Checkpoints: controls that ensure the order of cell cycle events. *Science* 246, 629-634.

Herbig, U., Jobling, W.A., Chen, B.P.C., Chen, D.J. & Sedivy, J.M. (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and $p21^{CIP1}$, but not $p16^{INK4a}$. *Mol Cell* 4, 501-513.

Hirano, T. (2005) Condensins: organizing and segregating the genome. *Curr Biol* 15, 265-275.

Hittelman, W.N. & Rao, P.N. (1975) The nature of adriamycin-induced cytotoxicity in Chinese hamster cells as revealed by premature chromosome condensation. *Cancer Res* 35, 27-35.

Hosseini, S. & Mozdarani, H. (2004) Induction of premature chromosome condensation (PCC) by calyculin A for biodosimetry. *Iran J Radiat Res* 1, 1-6.

Hsu, J-Y., Sun, Z-W., Li, X., Reuben, M., Tatchell, K., Bishop, D.K., Grushcow, J.M., Brame, C.J., Caldwell, J.A. & Hunt, D.F. (2000) Mitotic phosphorylation of histone H3 is governed by Ipl1/aurora kinase and glc7/PP1 phosphatase in budding yeast and nematodes. *Cell* 102, 279-291.

Huang, X., Kurose, A., Tanaka, T., Traganos, F., Dai, W. & Darzynkiewicz, Z. (2006) Sequential phosphorylation of *Ser*-10 on histone H3 and *Ser*-139 on histone H2AX and ATM activation during premature chromosome condensation: relationship to cell-cycle phase and apoptosis. *Cytometry Part A* 69A, 222-229.

Ianzini, F. & Mackey, M.A. (1997) Spontaneous premature chromosome condensation and mitotic catastrophe following irradiation of HeLa S3 cells. *Int J Radiat Biol* 72, 409-421.

Ishihara, H., Martin, B.L., Brautigan, D.L., 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.

Jia, X.Z., Yang, S.Y., Zhou, J., Li, S.L., Ni, J.H., An, G.S. & Jia, H.T. (2008) Inhibition of CHK1 kinase by Gö6976 converts 8-chloro-adenosine-induced G2/M arrest into S arrest in human myelocytic leukemia K562 cells. *Biochem Pharmacol* 5, 770-780.

Johnson, R.T. & Rao, P.N. (1970) Mammalian cell fusion: Induction of premature chromosome condensation in interphase nuclei. *Nature* 226, 717-722.

Kato, H. & Sandberg, A. (1967) Chromosome pulverization in human binucleate cells following colcemid treatment. *J Cell Biol* 34, 35-46.

Khanna, K.K. & Jackson, S.P. (2001) DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 27, 247-254.

Kohn, E.A., Ruth, N.D., Brown, M.K., Livingstone, M. & Eastman, A. (2002) Abrogation of the S phase damage checkpoint results in S phase progression or premature mitosis depending on the concentration of 7-hydroxystaurosporine and the kinetics of Cdc25C activation. *J Biol Chem* 277, 26553-26564.

Kovaleva, O.A., Glazko, T.T., Kochubey, T.P., Lukash, L.L. & Kudryavets, Y.I. (2007) Spontaneous premature condensation of chromosomes in normal and transformed mammal cells. *Exp Oncol* 29, 18-22.

Kowalska, A., Srebniak, M., Wawrzkiewicz, A. & Kaminski, K. (2003) The influence of calyculin A on lymphocytes in vitro. *J Appl Genet* 44, 413-418.

Krause, S.A., Loupert, M.-L., Vass. S., Schoefelder, S., Harrison, S. & Heck, M.M.S. (2001) Loss of cell cycle checkpoint control in *Drosophila* RFC4 mutants. *Mol Cell Biol* 21, 5156-5168.

Kumagai, A. & Dunphy, W.G. (1996) Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from *Xenopus* egg extracts. *Science* 273, 1377-1380.

Kurose, A., Tanaka, T., Huang, X., Traganos, F. & Darzynkiewicz, Z. (2006) Synchronization in the cell cycle by inhibitors of DNA replication induces histone H2AX phosphorylation : an induction of DNA damage. *Cell Prolif* 39, 231-240.

Le Bourhis, D., Beaujean, N., Ruffini, S., Vignon, X. & Gall, L. (2010) Nuclear remodeling in bovine somatic cell nuclear transfer embryos using MG132-treated recipient oocytes. *Cell Reprogram* 12, 729-738.

Liu, Y., Jiang, N., Wu, J., Dai, W. & Rosenblum, J.S. (2007) Polo-like kinases inhibited by wortmannin: labeling site and downstream effects. *J Biol Chem* 4, 2505-2511.

Mackey, M.A., Morgan, W.F. & Dewey, W.C. (1988) Nuclear fragmentation and premature chromosome condensation induced by heat shock in S-phase Chinese hamster ovary cells. *Cancer Res* 48, 6478-6483.

Matthews, D.J., Yakes, F.M., Chen, J., Tadano, M., Bornheim, L., Clary, D.O., Tai, A., Wagner, J.M., Miller, N., Kim, Y.D., Robertson, S., Murray, L. & Karnitz, L.M. (2007) Pharmacological abrogation of S-phase checkpoint enhances the anti-tumor activity of gemcitabine in vivo. *Cell Cycle* 6, 104-110.

Mikhailov, A. & Rieder, C.L. (2002) Cell cycle: stressed out of mitosis. *Curr Biol* 12, R331-R333.

Mosesso, P., Palitti, F., Pepe, G., Pinero, J., Bellacima, R., Ahnstrom, G. & Natarajan, A.T. (2010) Relationship between chromatin structure, DNA damage and repair following X-irradiation of human leukocytes. *Mutat Res* 701, 86-91.

Nghiem, P., Park, P.K., Kim, Y-S, Vaziri, C. & Schreiber, S.L. (2001) ATR inhibition selectively sensitizes G_1 checkpoint-deficient cells to lethal premature chromatin condensation. *Proc Natl Acad Sci USA* 98, 9092-9097.

Pantelias, G.E. & Maillie, H.D. (1983) A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol. *Somatic Cell Genet* 9, 533-547.

Paull, T.T., Rogakou, E.P., Yamazaki, V., Kirchgessner, C.U., Gellert, M. & Bonner, W.M. (2000) A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* 10, 886-895.

Paulsen, R.D. & Cimprich, K.A. (2007) The ATR pathway: Fine-tuning the fork. *DNA Repair* 6, 953-966.

Peddibhotla, S., Lam, M.H., Gonzalez-Rimbau , M. & Rosen J.M. (2009) The DNA-damage effector checkpoint kinase 1 is essential for chromosome segregation and cytokinesis. *Proc Natl Acad Sci USA* 106, 5159-5164.

Pines, J. & Rieder, C.L. (2001) Re-staging mitosis: a contemporary view of mitotic progression. *Nat Cell Biol* 3, E3-E6.

Prasanna, P.G.S., Escalada, N.D. & Blakely, W.F. (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. *Mutation Res* 466, 131-141.

Prokhorova, T.A., Mowrer, K., Gilbert, C.H. & Walter, J.C. (2003) DNA replication of mitotic chromatin in Xenopus egg extracts. *Proc Natl Acad Sci USA* 100, 13241-13246.

Riesterer, O., Matsumoto, F., Wang, L., Pickett, J., Molkentine, D., Giri, U., Milas, L. & Raju, U. (2009) A novel Chk inhibitor, XL-844, increases human cancer cell rediosensitivity through promotion of mitotic catastrophe. *Invest New Drugs* 29, 514-522.

Rogakou, E.P., Pilch, D.R., Orr, A.H., Ivanova, V.S. & Bonner, W.M. (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 273, 5858–5868.

Royou, A., Macias, H. & Sullivan, W. (2005) The Drosophila Grp/Chk1 DNA damage checkpoint controls entry into anaphase. *Curr Biol* 15, 334-339.

Russell, P. (1998) Checkpoint on the road to mitosis. *Trends Biol Sci* 23, 399-402.

Rybaczek, D. & Kowalewicz-Kulbat, M. (2011) Premature chromosome condensation induced by caffeine, 2-aminopurine, staurosporine and sodium metavanadate in S-phase arrested HeLa cells is associated with a decrease in Chk1 phosphorylation, formation of phospho-H2AX and minor cytoskeletal rearrangements. *Histochem Cell Biol 135*, 263-280.

Rybaczek, D., Bodys, A., Maszewski, J. (2007) H2AX foci in late S/G2- and M-phase cells after hydroxyurea- and aphidicolin-induced DNA replication stress in *Vicia*. *Histochem Cell Biol* 128, 227-241.

Rybaczek, D., Żabka, A., Pastucha, A. & Maszewski, J. (2008) Various chemical agents can induce premature chromosome condensation in *Vicia faba*. *Acta Physiol Plant* 30, 663-672.

Sahu, R.P., Batra, S. & Srivastava, S.K. (2009) Activation of ATM/Chk1 by cucrumin causes cell cycle arrest and apoptosis in human pancreatic cancer cells. *Br J Cancer* 100, 1425-1433.

Schlegel, R. & Pardee A.B. (1986) Caffeine-induced uncoupling of mitosis from the completion of DNA replication in mammalian cells. *Science* 232, 1264-1266.

Schlegel, R., Belinsky, G.S., Harris, MO. (1990) Premature mitosis induced by mammalian cells by the protein kinase inhibitors 2-aminopurine and 6-dimentylaminopurine. *Cell Growth Differ* 1, 171-178.

Scott, S.P. & Pandita, T.K. (2006) The cellular control of DNA double-strand breaks. *J Biol Chem* 99, 1463-1475.

Sen, R. & Ghosh, S. (1998) Induction of premature mitosis in S-blocked onion cells. *Cell Biol Int* 22, 867-874.

Sha,W., Moore, J., Chen, K., Lassaletta, A.D., Yi, C-S. & Tyson, J.J., (2003) Hysteresis drives cell-cycle transitions in *Xenopus laevis* egg extracts. *Proc Natl Acad Sci USA* 100, 975-980.

Solomon, M.J. (2003) Hysteresis meets the cell cycle. *Proc Natl Acad Sci USA* 100, 771-772.

Steinmann, K.E., Belinsky, G.S., Lee, D. & Schlegel, R. (1991) Chemically induced premature mitosis: Differential response in rodent and human cells and the relationship to cyclin B synthesis and $p34^{cdc2}$/cyclin B complex formation. *Cell Biology* 88, 6843-6847.

Stevens, J.B., Abdallah, b.Y., Regan, S.M., Liu, G., Bremer, S.W., Ye, C.J. & Heng, H.H. (2010) Comparison of mitotic cell death by chromosome fragmentation to premature chromosome condensation. *Molecular Cytogenetics* 3, 20-30.

Sturgeon, C.M., Cinel, B., Díaz-Marrero, A.R., McHardy, L.M., Ngo, M., Andersen, R.J. & Roberge, M. (2008) Abrogation and inhibition of nuclear export by *Cryptocarya* pyrones. *Cancer Chemother Pharmacol* 3, 407-413.

Swanson, P.E., Carroll, S.B., Zhang, X.F. & Mackey, M.A. (1995) Spontaneous premature chromosome condensation, micronucleus formation, and non-apoptotic cell death in heated HeLa S3 cells. *Am J Pathol* 146, 963-971.

Syljuåsen, R.G., SØrennsen, C.S., Hansen, L.T., Fugger, K., Lundin, C., Johansson, F., Helleday, T., Sehested, M., Lukas, J. & Bartek, J. (2005) Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol Cell Biol* 9, 3553-3562.

Szymborska, W. (1993). *Poems New and Collected*. English translation by Barańczak S. & Cavanagh C. (1998), Harcourt Inc., ISBN 0-15-601146-8, Orlando, Florida, USA

Takizawa, C.G. & Morgan D.O. (2000) Control of mitosis by changes in the subcellular location of cyclin-B1-Cdk1 and Cdc25. *Curr Op Cell Biol* 12, 658-665.

Tam, S.W. & Schlegel, R. (1995) Quantification of premature and normal mitosis, In: *Cell Cycle - Materials and Methods*, Michele Pagano, pp. 93-99, Springer-Verlag, ISBN 3-540-58066-2, Berlin-Heidelberg-New York.

Terzoudi, G.I., Hatzi, V.I., Donta-Bakoyianni D. & Pantelias G.E. (2010) Chromatin dynamics during cell cycle mediate conversion of DNA damage into chromatin breaks and affect formation of chromosomal aberrations: Biological and clinical significance. *Mutation Res* 711, 174-186.

Waldren, C.A. & Johnson, R.T. (1974) Analysis of interphase chromosome damage by means of premature chromosome condensation after X- and ultraviolet-irradiation. *Proc Nat Acad Sci USA* 71, 1137-1141.

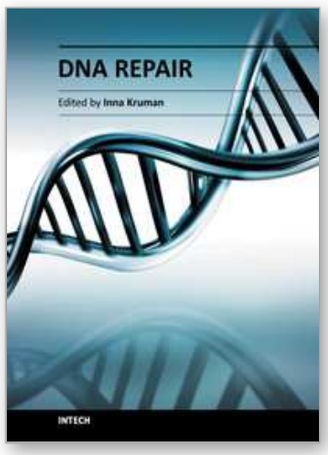
Wang, S.-W., Norbury, C., Harris, A.L. & Toda, T. (1999) Caffeine can override the S-M checkpoint in fission yeast. *J Cell Sci* 112, 927-937.

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 transciently induces a premature mitosis-like state in BHK21 cells. *EMBO J* 9, 4331-4338.

Yu, K.R., Saint, R.B. & Sullivan, W. (2000) The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation. *Nat Cell Biol* 2, 609-615.

Zhou B.-B.S. & Elledge S.J. (2000) The DNA damage response: putting checkpoints in perspective. *Nature* 408, 433-439.

IntechOpen



DNA Repair

Edited by Dr. Inna Kruman

ISBN 978-953-307-697-3
Hard cover, 636 pages
Publisher InTech
Published online 07, November, 2011
Published in print edition November, 2011

The book consists of 31 chapters, divided into six parts. Each chapter is written by one or several experts in the corresponding area. The scope of the book varies from the DNA damage response and DNA repair mechanisms to evolutionary aspects of DNA repair, providing a snapshot of current understanding of the DNA repair processes. A collection of articles presented by active and laboratory-based investigators provides a clear understanding of the recent advances in the field of DNA repair.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Dorota Rybaczek (2011). Eidetic Analysis of the Premature Chromosome Condensation Process, DNA Repair, Dr. Inna Kruman (Ed.), ISBN: 978-953-307-697-3, InTech, Available from: http://www.intechopen.com/books/dna-repair/eidetic-analysis-of-the-premature-chromosome-condensation-process

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen