



Histone H3 phosphorylation and cell division

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Histone H3 is specifically phosphorylated during both mitosis and meiosis in patterns that are specifically coordinated in both space and time. Histone H3 phosphorylation may initiate at different phases of the cell division in different organisms, but metaphase chromosomes are always found to be heavily phosphorylated. Upon exit of mitosis/meiosis a global dephosphorylation of H3 takes place. Potential candidates for H3 kinases are described and their hypothetical mechanism of action on highly condensed chromatin templates is discussed. In addition, a novel hypothesis for the role of histone H3 phosphorylation during cell division is proposed. This hypothesis, termed the 'ready production label' model, explains the results in the literature and suggests that phosphorylation of histone H3 is a part of a complex signaling mechanism. *Oncogene* (2001) 20, 3021–3027.

Keywords: histone H3; phosphorylation; mitosis; meiosis

Introduction

To ensure its correct proliferation, differentiation and apoptosis, eukaryotic cells react to a very large number of extracellular stimuli. These stimuli are registered at the cell surface and transduced via signaling pathways to the nucleus where they induce appropriate responses, reflected by the activation or repression of specific processes. Protein phosphorylation is the major post-translational modification involved in trans-cytoplasmic signaling pathways. An abundant literature describes these manifold signal transduction pathways and their modes of regulation (Hunter, 2000). However, the identification of physiological relevant substrates implicated in signaling pathways as well as the functional significance of multiple inter-pathway cross talks still remains a challenge. In this context, it has to be kept in mind that many of these signal transduction pathways converge towards a common, very complex and dynamic substrate, the chromatin fiber (Turner, 1999; Thomson *et al.*, 1999b; Cheung *et al.*, 2000a; Strahl and Allis, 2000).

In the nucleus, the highly compact nature of the chromatin fiber severely restricts the interactions of specific proteins with genomic DNA and affects important cellular processes such as transcription and chromosome assembly during mitosis and meiosis. The

chromatin fiber is composed of repetitive units, known as nucleosomes, which are comprised of an octamer of core histones (two of each H2A, H2B, H3 and H4), around which two ~80 bp superhelical turns of DNA are wrapped. Nucleosomal architecture is achieved mainly through histone–histone and histone–DNA interactions mediated by the conserved C-terminal 'histone fold' domains present in each of the four core histones (Arents *et al.*, 1991; Luger *et al.*, 1997). In addition, each histone contains a seemingly more flexible N-terminal histone tail domain, which are dispensable for the nucleosome formation (Luger *et al.*, 1997). These domains protrude from the surface of the nucleosome and are involved in the stabilization of the chromatin fiber (Tse and Hansen, 1997; Tse *et al.*, 1998; Garcia-Ramirez *et al.*, 1992; Makarov *et al.*, 1984). Interestingly, the tails are subjected to multiple post-translational modifications such as acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation. So far, the structural and functional consequences of histone acetylation have been the most studied since histone tail acetylation has been extensively correlated with transcriptional activation (Turner, 2000; Wolffe and Hayes, 1999; Khochbin and Wolffe, 1997; Wolffe and Pruss, 1996).

However, during the last few years numerous publications have concentrated on the role of histone H3 phosphorylation at serine 10. H3 phosphorylation is believed to be involved in two structurally opposed processes: transcriptional activation (Mahadevan *et al.*, 1991; Thomson *et al.*, 1999a,b; Sassone-Corsi *et al.*, 1999; Clayton *et al.*, 2000; Cheung *et al.*, 2000b; Crosio *et al.*, 2000; Nowak and Corces, 2000), requiring chromatin fiber decondensation, and chromosome compaction during cell division (Van Hooser *et al.*, 1998; Wei *et al.*, 1998, 1999; Hendzel *et al.*, 1997; Hsu *et al.*, 2000; de la Barre *et al.*, 2000; Kaszas and Cande, 2000). The role of histone H3 phosphorylation in transcription has been discussed in detail in several recent papers (reviewed in Thomson *et al.*, 1999b). This review focuses on histone H3 phosphorylation at mitosis and meiosis. We summarize the current data and present evidence that this histone H3 modification functions as a part of a complex signaling pathway.

Histone H3 phosphorylation at mitosis

Chromosome condensation at mitosis is accompanied not only with phosphorylation of histone H3 (Gurley

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et al., 1978; Allis and Gorovsky, 1981; Goto *et al.*, 1999), but also with hyperphosphorylation of linker histone H1 (Bradbury, 1992; Bradbury *et al.*, 1973), suggesting a functional role for these two post-translational modifications in chromosome formation. However, recent experiments have demonstrated that both chromosome and nucleus assembly are not affected by the absence of linker histones, thus arguing against a role of histone H1 phosphorylation at mitosis (Ohsumi *et al.*, 1993; Dasso *et al.*, 1994; Shen *et al.*, 1995). Recently, an extremely specific and high affinity polyclonal antibody was generated against a H3 peptide, phosphorylated at serine 10 (Hendzel *et al.*, 1997; Wei *et al.*, 1998). Thanks to this very valuable tool, it was demonstrated that in mitosis in organisms as divergent as *Tetrahymena thermophila*, *Aspergillus nidulans*, *Caenorhabditis elegans*, plants and vertebrates, chromosome condensation is accompanied by phosphorylation of histone H3 on serine 10 (Van Hooser *et al.*, 1998; Wei *et al.*, 1998, 1999; Hendzel *et al.*, 1997; Hsu *et al.*, 2000; de la Barre *et al.*, 2000; Kaszas and Cande, 2000; De Souza *et al.*, 2000). In addition, a mutant *Tetrahymena thermophila* strain, containing non-phosphorylatable histone H3, exhibited perturbed chromosome condensation and abnormal segregation, further confirming the important role of phosphorylation of histone H3 at serine 10 in cell division (Wei *et al.*, 1999). The above data suggest that this modification is required for proper chromosome compaction. It should be noted, however, that phosphorylation of histone H3 is not always essential for this process. For example, a yeast strain containing single mutation within histone H3 at serine 10 (S10A) exhibited generation times and cell cycle progression identical to the wild type strain (Hsu *et al.*, 2000). Thus, contrary to *Tetrahymena*, in *S. cerevisiae* a causal relationship between phosphorylation at serine 10 of histone H3 and chromosome dynamics is not observed. It has been proposed that *S. cerevisiae* could compensate this loss of serine 10 histone H3 phosphorylation by phosphorylation of other histones, namely H2B (Hsu *et al.*, 2000; Cheung *et al.*, 2000a).

During chromosome assembly global phosphorylation of histone H3 occurs in a step wise and ordered manner (Hendzel *et al.*, 1997; Sauve *et al.*, 1999; Van Hooser *et al.*, 1998). In mammalian cells in late G2 phase, phosphorylation is first detected in pericentromeric heterochromatin and, as mitosis proceeds, spreads throughout the whole chromosomes. It is completed in late prophase and maintained through metaphase. Dephosphorylation of histone H3 begins in anaphase and ends at early telophase. A strong correlation between the initial chromatin condensation and H3 phosphorylation was observed (Hendzel *et al.*, 1997; Van Hooser *et al.*, 1998). However, phosphorylation seems not to be essential for the maintenance of the compact chromosome state (Van Hooser *et al.*, 1998). Upon cell arrest in mitosis and subsequent brief incubation in hypotonic solution, strong dephosphorylation of H3 within chromosomes takes place. Interestingly, after release into tissue culture medium,

the cells again became heavily phosphorylated, indicating that histone H3 phosphorylation could be important for cell passage from metaphase to anaphase (Van Hooser *et al.*, 1998).

The mitotic pattern of H3 phosphorylation is different in plants (Kaszas and Cande, 2000). First, phosphorylation begins late in prophase where the chromosomes are already quite condensed and it is observed on discrete locations on some chromosomes only. Second, at the metaphase plate all chromosomes show histone H3 phosphorylation primarily in the pericentromeric chromatin, in contrast to mammalian metaphase chromosomes, which are phosphorylated along their entire lengths. Finally, pericentromeric H3 phosphorylation is maintained at anaphase and disappears at telophase. The initial phosphorylation of H3 in prophase observed on compacted chromosomes argues against an important role of this modification in chromosome condensation in plants.

Meiotic phosphorylation of histone H3

At meiosis, as in mitosis, a specific spacio-temporal phosphorylation of histone H3 at serine 10 was detected. In *Tetrahymena*, upon micronuclei entering in metaphase of meiosis I histone H3 is phosphorylated, and subsequently dephosphorylated at anaphase (Wei *et al.*, 1998). The temporal pattern of these events is similar at meiosis II: the condensed metaphase II chromosomes contain phosphorylated histone H3, while the exit of meiosis is associated with its dephosphorylation. In addition, an abnormal meiosis in the mutant S10A *Tetrahymena* strain having a non-phosphorylatable H3 was found: chromosome condensation was not completed and anaphase bridges were observed (Wei *et al.*, 1999). Thus, it seems that meiotic chromosome condensation in *Tetrahymena* is associated with H3 phosphorylation. However, again this conclusion cannot be generalized. For example, in *C. elegans* the absence of phosphorylation of histone H3, due to the depletion of BIR-1 protein and the aurora-like kinase AIR-2 by using RNA-mediated gene interference (RNAi), has no effect on chromosome condensation during meiotic prophase (Speliotes *et al.*, 2000).

Furthermore, during meiosis in maize histone H3 phosphorylation is demonstrated to correlate with changes in the maintenance of sister chromatid cohesion, rather than chromosome condensation (Kaszas and Cande, 2000). This organism is very suitable for chromatin compaction studies, since its chromosomes are very long (20–80 μm at the pachytene stage) and a large number of mutants in meiosis are available (Kaszas and Cande, 2000). It was reported that histone H3 phosphorylation occurs at the diakinesis/prometaphase I transition (where chromosomes are already in a highly compact state) and coincides with the breakdown of the nuclear envelope. The anti-phosphorylated histone H3 antibody stained the entire length of the condensed chromosomes and

the signals persisted into anaphase I. At telophase I phosphorylation is no longer detected and appeared again at metaphase II, but essentially in pericentromeric chromatin. No phosphorylation is detected at telophase II. In addition, experiments with a maize mutant *afd1*, where chromatid cohesion at metaphase II is absent, demonstrated an absence of phosphorylation of histone H3. Since the release of chromosome sister arm cohesion is taking place at anaphase I and centromere cohesion is released at anaphase II, the above data suggest that histone H3 phosphorylation is associated with chromosome cohesion (Kaszas and Cande, 2000).

Unexpected distribution of phosphorylated histone H3 during assembly of chromosomes in *Xenopus* egg extracts

Xenopus eggs are arrested in metaphase II of meiosis. They contain a very large number of different nuclear components and a reservoir of chromosome assembly factors and other factors needed for multiple rapid rounds of cell division. It is relatively easy to isolate cytoplasmic extracts from *Xenopus* eggs and chromosomes can be assembled *in vitro* by incubation of demembrated sperm in the extracts (Lohka and Masui, 1983; Hirano and Mitchison, 1993). These chromosomes exhibit structural properties which are very similar, if not identical, to the somatic ones (Houchmandzadeh and Dimitrov, 1999; Houchmandzadeh *et al.*, 1997). The *in vitro* chromosome assembly is a synchronous process; the demembrated sperm undergo a series of structurally and temporally well-defined rearrangement phases, beginning with a very rapid decondensation due to the uptake of protamine-like proteins (Dimitrov *et al.*, 1994) and culminating in the formation of condensed chromosomes (Hirano and Mitchison, 1993). Each phase of sperm nucleus rearrangement showed a characteristic H3 phosphorylation pattern (de la Barre *et al.*, 2000). Initially, after 5 min of incubation in the extract, histone H3 phosphorylation is observed only at the periphery of the decondensed nuclei. As assembly proceeds, in addition to the peripheral labeling, a punctuated labeling is detected 'inside' the nuclei. The fully compacted chromosomes exhibit a uniform pattern of histone H3 phosphorylation. Importantly, immunoblotting analysis showed that histone H3 phosphorylation is very quick and accompanied the initial rapid sperm nuclei decondensation. Saturation of the phosphorylation is essentially achieved within the first 10–15 min of sperm nuclei incubation in the extract when decondensation is completed (de la Barre *et al.*, 2000 and Scrittore L, Hans F, Angelov D, Charra M and Dimitrov S, unpublished data). Therefore, upon *in vitro* chromosome assembly an 'uncoupling' of chromosome condensation and histone H3 phosphorylation occurs, i.e. upon sperm nuclei decondensation, nearly maximal phosphorylation of histone H3 is realized. Subsequently, the decondensed nuclei containing

phosphorylated H3 is subjected to structural transitions leading to the formation of compact chromosomes.

In summary, histone H3 is phosphorylated during both mitosis and meiosis and upon assembly of chromosomes in *Xenopus* egg extracts. However, the spacio-temporal distribution of this histone H3 modification is different in the different systems studied. In addition, the mitotic phosphorylation pattern may differ from the meiotic one within the same organism.

Identification of histone H3 kinases functioning during cell division

The identification of the kinase/phosphatase system(s), responsible for the regulation of histone H3 phosphorylation at mitosis and meiosis is of primary importance for the comprehension of the role of this histone modification and of the chromosome assembly itself. During the last few years numerous efforts were undertaken in this regard. Recently, two different groups identified Ipl1/AIR-2 aurora kinase in *S. cerevisiae* and *C. elegans* (Hsu *et al.*, 2000) and NIMA kinase in *Aspergillus nidulans* (De Souza *et al.*, 2000) as histone H3 mitotic kinases. This was an interesting and at the same time surprising finding, since both enzymes belong to two structurally unrelated and evolutionary divergent kinase families (Bischoff and Plowman, 1999; Giet and Prigent, 1999; Giet *et al.*, 1999; Ye *et al.*, 1997).

In yeast, the aurora kinase Ipl1 (for increased in ploidy) is involved in cell cycle progression and more particularly in the segregation and disjunction of chromosomes (Chan and Botstein, 1993). Ipl1 expression is low at G1 phase and achieved maximal levels at S phase and mitosis (Biggins *et al.*, 1999). It phosphorylates serine 10 of histone H3 and to a lesser extent histone H2B in a mixture of free histones (Hsu *et al.*, 2000). In nucleosomes, both histones are phosphorylated to a similar level by the kinase. The *in vivo* involvement of Ipl1 in histone H3 phosphorylation was demonstrated by using a temperature-sensitive lethal Ipl1 strain. This strain, even when grown at permissive temperature, exhibited clearly reduced histone H3 phosphorylation (Hsu *et al.*, 2000).

Contrary to yeast, multiple Ipl1 homologues have been found in higher eukaryotes (reviewed in Giet and Prigent, 1999; Bischoff and Plowman, 1999). All these members of the aurora kinase family have been classified in three subgroups dependent upon their intracellular localization and role during the cell cycle. Among them, the *C. elegans* AIR2/human auroral is of particular interest because in the nematode AIR-2 associates with the chromosomes in metaphase of mitosis and meiosis (Schumacher *et al.*, 1998; Hsu *et al.*, 2000; Adams *et al.*, 2000; Speliotes *et al.*, 2000). In addition, nematode embryos, obtained after disruption of *air2* expression by RNA-mediated interference, are highly polyploid, disorganized and with decondensed chromosomes (Hsu *et al.*, 2000). Importantly, these embryos do not show detectable staining with anti-

phosphorylated histone H3 antibody, suggesting that AIR-2 is necessary for H3 phosphorylation (Hsu *et al.*, 2000).

In the filamentous fungus *Aspergillus nidulans*, NIMA activity is essential for entry in mitosis (Osmani *et al.*, 1991). NIMA level peaks at G2/M (Osmani *et al.*, 1987). Histone H3 phosphorylation is dependent on the activation of NIMA and it correlates with the transient localization of the kinase on chromatin early in mitosis (De Souza *et al.*, 2000). In addition, NIMA kinase phosphorylates *in vitro* histone H3 at serine 10 and its ectopic expression leads to the induction of chromatin condensation and H3 phosphorylation (De Souza *et al.*, 2000).

Taken together, the above data demonstrate that Ipl1 aurora and NIMA, although being evolutionary divergent proteins, share some common characteristics that could make them *bona fide* H3 mitotic kinases. If this is really the case, different kinases should function as mitotic H3 kinases in different organisms. In addition, it is conceivable that within any single organism many kinases can phosphorylate histone H3 during cell division. A typical example is maize, where as already described, histone H3 is phosphorylated only at the pericentromeric chromatin in both mitosis and meiotic metaphase II, but at metaphase I the chromosomes are stained with the anti-phosphorylated histone H3 antibody along their entire length. Thus, one could imagine that a specific kinase is responsible for the pericentromeric chromatin phosphorylation (a good candidate for this function is the aurora Ipl1/AIR2 kinase, which is localized mainly at metaphase on the centromeric chromosome region (Adams *et al.*, 2000). Another or several other kinases might be involved in histone H3 phosphorylation on chromosome arms. Such hypothetical H3 phosphorylation scenario is reminiscent of histone acetylation where a large number of different histone acetyltransferases are effecting 'targeted' (gene promoter or chromatin domain specific) or 'bulk' acetylation of histones (Perry and Chalkley, 1982; Wolffe and Hayes, 1999; Wolffe and Pruss, 1996).

How are histone H3 mitotic kinases recruited on chromosomes?

As we have already discussed, during mitosis and meiosis histone H3 phosphorylation is ordered and highly structurally and temporally coordinated. The question we will address in this section is how this may be achieved. Obviously, the H3 mitotic kinases should form a complex(es) with proteins that are able to target them to specific chromosomal regions. In this context the data of two recent publications are very informative. In the first it was shown that the 'chromosomal passenger' protein INCENP (INner CENtromere Protein) is stored as a complex with AIR-2 in *Xenopus* eggs (Adams *et al.*, 2000). Passenger proteins are believed to play an essential role in cell division and they exhibited specific localization (Earnshaw and

Bernat, 1991). At mitosis, INCENP is carried by chromosomes on the metaphase plate, where it is relocated on the centromeres as metaphase proceeds. At anaphase this protein migrates to the central spindle. In human cells a very precise colocalization of INCENP and AIR-2 was found. Furthermore, the localization of AIR-2 depends on that of INCENP and the perturbation of INCENP localization is accompanied by a similar one of AIR-2. Since during mitosis INCENP specifically associates with centromeres it may target AIR-2 to the centromeric chromatin. Interestingly, the authors identified Sli15p, one of the binding partners of Ipl1, as the yeast homologue of INCENP.

The second paper presented evidence that the survivin-like *C. elegans* BIR-1 protein functions in concert with AIR-2 during cell division (Speliotes *et al.*, 2000). At mitosis and meiosis BIR-1 was found localized to chromosomes and to the spindle midzone, suggesting that this protein may act in chromosomal and spindle zone processes. Indeed, upon blocking *bir-1* function by RNA interference (RNAi), *bir-1*(RNAi) embryos exhibited defects in chromosome alignment, segregation and cytokinesis. The same defects were observed in *air-2* (RNAi) embryos. In addition, the localization of AIR-2 on chromosomes required BIR-1. Importantly, phosphorylation of histone H3 is dependent on BIR-1 and AIR-2 presence on chromosomes. Thus, BIR-1 might form a complex with AIR2 and localize it to chromosomes.

How mitotic kinases phosphorylate histone H3 in condensed chromatin?

During cell divisions phosphorylation of histone H3 typically takes place within the condensed chromatin. This immediately raises the issue of how H3 kinases are able to access their substrate within this highly restrictive chromatin environment. No data are available in the literature. However, one could speculate by comparison to the mechanism of acetylation of histone H3 tail in chromatin by histone acetyltransferases (HAT's). Indeed, the recombinant HAT PCAF (rPCAF) is not able to acetylate histone H3 in chromatin (Herrera *et al.*, 2000). This inhibition effect is due to the presence of linker histones, since rPCAF acetylates efficiently histone H3 in linker histone-depleted oligonucleosomes. Nonetheless, the cellular PCAF, which exists as a multiprotein complex, seems to be able to perturb linker histone organization and to overcome the repression (Herrera *et al.*, 2000). One can envisage similar scenario for mitotic H3 kinases, since *in vivo* they are also part of a high molecular complex and the other proteins of this complex may induce chromatin rearrangement and help the enzyme in its function on chromatin templates. If necessary, the cell could use the assistance of ATP-dependent chromatin remodeling machines to open the compact chromatin structure and increase the accessibility of H3 tail to the kinase. Interestingly, it was recently shown that the

human SWI/SNF-B remodeling complex localizes to spindle poles and to kinetochores of mitotic chromosomes (Xue *et al.*, 2000). RSC, the yeast homologue of this complex, is required for cell cycle progression through mitosis (Cairns *et al.*, 1996). Given the localization of SWI/SNF-B, it seems likely that this complex may be involved in remodeling of the pericentromeric chromatin structure to allow access mitotic H3 kinases to phosphorylate histone H3. This mechanism could be operative in the cases where only pericentromeric histone H3 phosphorylation is observed. Additional remodeling machines, besides SWI/SNF-B, might be required for the phosphorylation of chromosomes along their entire length. Since histone H3 phosphorylation is highly coordinated, it is possible that some of the remodeling machines exist as a complex with the mitotic H3 kinases.

During mitosis histone H3 is also phosphorylated on serine 28

A recent paper reported the identification of a novel phosphorylation mitotic site of histone H3 (Goto *et al.*, 1999). The authors, by using immunochemical and biochemical approaches, demonstrated that serine 28 of histone H3 is phosphorylated at mitosis in a large number of mammalian cell lines. Histone H3 serine 28 phosphorylation coincided with chromosome condensation and showed a pattern very similar to that of H3 serine 10 phosphorylation: it initiates at prophase, at metaphase the chromosomes are heavily labeled and at late anaphase the phosphorylation disappears. H3 serine 28 phosphorylation level was found slightly lower than that of H3 serine 10. Interestingly, if histone extraction was carried out in the absence of okadaic acid (a serine/threonine phosphatase inhibitor) dephosphorylation of H3 serine 28, but not of serine 10, was observed. The above data suggest either the existence of at least two different kinase activities, each one using as a target only one of the two phosphorylatable histone H3 serines or two different phosphatases responsible for the dephosphorylation of each serine, or both. In yeast and nematodes, one of these kinase-phosphatase 'couples' operating on histone H3 serine 10 is likely to be the aurora Ipl1 kinase and Glc7/PP1 phosphatase, since they participate in the balance of mitotic histone H3 phosphorylation (Hsu *et al.*, 2000). Interestingly, the NIMA murine kinase homologue Nek2, which is observed associated with meiotic chromosomes, is found in a complex with PP1 (Helps *et al.*, 2000). Thus, different organisms could use distinct H3 mitotic kinases, but homologous phosphatases for acting on serine 10 of histone H3.

Potential candidates for a kinase/phosphatase couple, governing the balance of histone H3 phosphorylation at serine 28, are not suggested. Moreover, there are no reports in the literature addressing the casual relationship between H3 serine 28 phosphorylation,

chromosomal condensation and mitosis in general. It is not known if this histone H3 modification is realized during meiosis.

Why is histone H3 phosphorylated during cell division: the 'ready production label' hypothesis

It is difficult to give a definite answer to this question. The literature data suggest that in different organisms the cell may associate histone H3 phosphorylation either with condensation or with cohesion of chromosomes. Two models were proposed to explain the role of this H3 modification in chromosome condensation (Wei *et al.*, 1998, 1999; Cheung *et al.*, 2000a). According to the first, phosphorylation of histone H3 induces a local decondensation of the chromatin fiber, due to the reduction of the overall positive charge of its tail from +14 to +12. This local decondensation will help the interaction of chromosome assembly factors with chromatin and thus, assists the condensation of chromosomes. The second model implied the induction of direct interaction between chromosome condensation factors and histone H3 upon phosphorylation of its tail. However, by reducing the positive charge of histone H3 tail by two, it is very unlikely to decondense chromatin. Indeed, hard physicochemical data have shown that hyperacetylation of the tails of all four histones in native chromatin, which diminishes the histone positive tail charge by far by two, does not result in chromatin decondensation (McGhee *et al.*, 1983; Dimitrov *et al.*, 1986). Histone acetylation leads to extended chromatin conformation only in the absence of linker histones (Garcia-Ramirez *et al.*, 1995). An effect is also observed on the nucleosome level, which allowed an enhanced binding of transcription factors (Lee *et al.*, 1993; Vettese-Dadey *et al.*, 1996; Mutskov *et al.*, 1998). As for an increased affinity of chromosome assembly factors for phosphorylated histone H3 (the second model), the literature data do not really support this proposal: the two known factors involved in chromosome condensation, topoisomerase II and condensins, interact equally well with native and tailless nucleosomes (de la Barre *et al.*, 2000; Kimura and Hirano, 2000).

As it was discussed earlier, during cell division the spacio-temporal distribution of histone H3 phosphorylation is different in different organisms. Furthermore, the pattern of H3 phosphorylation in mitosis may differ from that of meiosis in the same organism. The phosphorylation may initiate at the very beginning of chromosome condensation (a typical example are mammalian cells) or on already heavily condensed structures (plant cells). It may affect the entire length of the chromosome or the pericentromeric chromosome only. However, histone H3 phosphorylation show a common feature in both cell divisions in the different experimental systems studied: chromosomes are always heavily phosphorylated at metaphase and dephosphorylated upon exit of mitosis or meiosis. This suggests that histone H3 phosphorylation may be used

by the cell to mark the metaphase chromosomes, once they are ready to go through anaphase and telophase. In other words, phosphorylation of histone H3 should present some type of 'ready production label' which has to be 'sticked' to chromosomes, once they have overcome the different checkpoints and have arrived at metaphase. This labeling may be realized at different phases of the cell divisions prior to metaphase and, in principle, it could be used by the cohesin complexes (Kaszas and Cande, 2000). Different kinases may be required to act in cells where the chromosomes have to be phosphorylated along their entire length. It is quite probable that chromatin remodeling complexes have to assist the kinases in their function. In some systems, as yeast, the H3 labeling may be redundant with the phosphorylation of other histones.

The 'ready production label' hypothesis postulates that once arriving at metaphase, chromosomes have to be phosphorylated and this may even not depend on their condensation state. We have two such examples in the literature. The partially decondensed and dephosphorylated mammalian metaphase chromosomes, obtained upon cell incubation in hypotonic solution, are again heavily phosphorylated, but not recondensed, when released into tissue culture medium (Van Hooser *et al.*, 1998). The second example is even more dramatic: upon assembly of chromosomes in *Xenopus* egg extracts (the extracts are arrested at metaphase II) phosphorylation of serine 10 of histone H3 correlates with the initial decompaction of sperm nuclei, but not with chromosome condensation (de la Barre *et al.*, 2000 and de la Barre AE, Scrittore L, Hans F, Angelov D, Charra M and Dimitrov S, unpublished data).

The 'ready production label' model implies indirectly that histone H3 phosphorylation may affect strongly processes taking place after metaphase. Indeed, the main defects observed in the *Tetrahymena* strain, containing non-phosphorylatable histone H3, are associated with abnormal segregation and extensive chromosome loss (Wei *et al.*, 1999). Additionally, mutants of the potential H3 mitotic kinase Ipl1, are defective in chromosome segregation (Hsu *et al.*, 2000). Elimination of the expression of *air-2*, the nematode homologue of Ipl1, besides of affecting chromosome

condensation, was also shown to perturb alignment, segregation, spindle midzone formation and cytokinesis (Speliotes *et al.*, 2000).

Concluding remarks

In this review we see that during cell division the serine 10 histone H3 phosphorylation pattern is coordinated and may be different in various organisms. Until now, this evolutionary conserved histone modification was mainly related to chromosome condensation. However, the analysis of the literature data allowed to conclude that histone H3 phosphorylation seems to be some type of labeling of metaphase chromosomes and to be essentially required for processes operating after metaphase. We have suggested, contrary to the views in the literature, that more than one kinase is phosphorylating histone H3 in the same organism. Since the phosphorylation is realized in most of the cases on already highly condensed chromatin templates, we have proposed that this enzymatic reaction should be helped by remodeling of the chromatin structure through the action of chromatin remodeling machines. Biochemical and functional experiments are required to test all these suggestions.

As already stressed, during mitosis H3 is phosphorylated at serine 10, but also at serine 28. Why is the cell using such double labeling? Is serine 28 phosphorylation important for mitosis? Is histone H3 phosphorylated at serine 28 in meiosis? If yes, is there a crosstalk between the two types of phosphorylation? These and many other questions are awaiting for an answer.

In conclusion, we would like to point out that some initial dissection of the role of histone H3 phosphorylation in cell division is being achieved and much remains to be done.

Acknowledgments

We thank Drs J Hayes, S Nonchev and S Khochbin for providing critical input. We appreciate the support of Dr Jean-Jacques Lawrence throughout the course of this work. This study was supported by INSERM and La Ligue Nationale Contre Le Cancer.

References

- Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmena M, Smythe C, Gerloff DL and Earnshaw WC. (2000). *Curr. Biol.*, **10**, 1075–1078.
- Allis CD and Gorovsky MA. (1981). *Biochemistry*, **20**, 3828–3833.
- Arents G, Burlingame RW, Wang BC, Love WE and Moudrianakis EN. (1991). *Proc. Natl. Acad. Sci. USA*, **88**, 10148–10152.
- Biggins S, Severin FF, Bhalla N, Sassoon I, Hyman AA and Murray AW. (1999). *Genes Dev.*, **13**, 532–544.
- Bischoff JR and Plowman GD. (1999). *Trends Cell Biol.*, **9**, 454–459.
- Bradbury EM. (1992). *Bioessays*, **14**, 9–16.
- Bradbury EM, Inglis RJ, Matthews HR and Sarner N. (1973). *Eur. J. Biochem.*, **33**, 131–139.
- Cairns BR, Lorch Y, Zhang M, Lacomis L, Erdjument-Bromage H, Tempst P, Du J, Laurent B and Kornberg RD. (1996). *Cell*, **87**, 1249–1260.
- Chan CS and Botstein D. (1993). *Genetics*, **135**, 677–691.
- Cheung P, Allis CD and Sassone-Corsi P. (2000a). *Cell*, **103**, 263–271.
- Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM and Allis CD. (2000b). *Mol. Cell*, **5**, 905–915.
- Clayton AL, Rose S, Barratt MJ and Mahadevan LC. (2000). *EMBO J.*, **19**, 3714–3726.

- Crosio C, Cermakian N, Allis CD and Sassone-Corsi P. (2000). *Nat. Neurosci.*, **3**, 1241–1247.
- Dasso M, Dimitrov S and Wolffe AP. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 12477–12481.
- de la Barre AE, Gerson V, Gout S, Creaven M, Allis CD and Dimitrov S. (2000). *EMBO J.*, **19**, 379–391.
- De Souza CP, Osmani AH, Wu LP, Spotts JL and Osmani SA. (2000). *Cell*, **102**, 293–302.
- Dimitrov S, Dasso MC and Wolffe AP. (1994). *J. Cell Biol.*, **126**, 591–601.
- Dimitrov S, Makarov V, Apostolova T and Pashev I. (1986). *FEBS Lett.*, **197**, 217–220.
- Earnshaw WC and Bernat RL. (1991). *Chromosoma*, **100**, 139–146.
- Garcia-Ramirez M, Dong F and Ausio J. (1992). *J. Biol. Chem.*, **267**, 19587–19595.
- Garcia-Ramirez M, Rocchini C and Ausio J. (1995). *J. Biol. Chem.*, **270**, 17923–17928.
- Giet R and Prigent C. (1999). *J. Cell Sci.*, **112**, 3591–3601.
- Giet R, Uzbekov R, Kireev I and Prigent C. (1999). *Biol. Cell.*, **91**, 461–470.
- Goto H, Tomono Y, Ajiro K, Kosako H, Fujita M, Sakurai M, Okawa K, Iwamatsu A, Okigaki T, Takahashi T and Inagaki M. (1999). *J. Biol. Chem.*, **274**, 25543–25549.
- Gurley LR, D'Anna JA, Barham SS, Deaven LL and Tobey RA. (1978). *Eur. J. Biochem.*, **84**, 1–15.
- Helps NR, Luo X, Barker HM and Cohen PT. (2000). *Biochem. J.*, **349**, 509–518.
- Hendzel MJ, Wei Y, Mancini MA, Van Hooser A, Ranalli T, Brinkley BR, Bazett-Jones DP and Allis CD. (1997). *Chromosoma*, **106**, 348–360.
- Herrera JE, West KL, Schiltz RL, Nakatani Y and Bustin M. (2000). *Mol. Cell Biol.*, **20**, 523–529.
- Hirano T and Mitchison TJ. (1993). *J. Cell Biol.*, **120**, 601–612.
- Houchmandzadeh B and Dimitrov S. (1999). *J. Cell Biol.*, **145**, 215–223.
- Houchmandzadeh B, Marko JF, Chatenay D and Libchaber A. (1997). *J. Cell Biol.*, **139**, 1–12.
- Hsu JY, Sun ZW, Li X, Reuben M, Tatchell K, Bishop DK, Grushcow JM, Brame CJ, Caldwell JA, Hunt DF, Lin R, Smith MM and Allis CD. (2000). *Cell*, **102**, 279–291.
- Hunter T. (2000). *Cell*, **100**, 113–127.
- Kaszas E and Cande WZ. (2000). *J. Cell Sci.*, **113**, 3217–3226.
- Khochbin S and Wolffe AP. (1997). *FEBS Lett.*, **419**, 157–160.
- Kimura K and Hirano T. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 11972–11977.
- Lee DY, Hayes JJ, Pruss D and Wolffe AP. (1993). *Cell*, **72**, 73–84.
- Lohka MJ and Masui Y. (1983). *Science*, **220**, 719–721.
- Luger K, Mader AW, Richmond RK, Sargent DF and Richmond TJ. (1997). *Nature*, **389**, 251–260.
- Mahadevan LC, Willis AC and Barratt MJ. (1991). *Cell*, **65**, 775–783.
- Makarov VL, Dimitrov SI, Tsaneva IR and Pashev IG. (1984). *Biochem. Biophys. Res. Commun.*, **122**, 1021–1027.
- McGhee JD, Nickol JM, Felsenfeld J and Rau DC. (1983). *Nucleic Acids Res.*, **11**, 4065–4075.
- Mutskov V, Gerber D, Angelov D, Ausio J, Workman J and Dimitrov S. (1998). *Mol. Cell Biol.*, **18**, 6293–6304.
- Nowak SJ and Corces V. (2000). *Genes Dev.*, **14**, 3003–3013.
- Ohsumi K, Katagiri C and Kishimoto T. (1993). *Science*, **262**, 2033–2035.
- Osmani AH, McGuire SL and Osmani SA. (1991). *Cell*, **67**, 283–291.
- Osmani SA, May GS and Morris NR. (1987). *J. Cell Biol.*, **104**, 1495–1504.
- Perry M and Chalkley R. (1982). *J. Biol. Chem.*, **257**, 7336–7347.
- Sassone-Corsi P, Mizzen CA, Cheung P, Crosio C, Monaco L, Jacquot S, Hanauer A and Allis CD. (1999). *Science*, **285**, 886–891.
- Sauve DM, Anderson HJ, Ray JM, James WM and Roberge M. (1999). *J. Cell Biol.*, **145**, 225–235.
- Schumacher JM, Golden A and Donovan PJ. (1998). *J. Cell Biol.*, **143**, 1635–1646.
- Shen X, Yu L, Weir JW and Gorovsky MA. (1995). *Cell*, **82**, 47–56.
- Spliotos EK, Uren A, Vaux D and Horvitz HR. (2000). *Mol. Cell*, **6**, 211–223.
- Strahl BD and Allis CD. (2000). *Nature*, **403**, 41–45.
- Thomson S, Clayton AL, Hazzalin CA, Rose S, Barratt MJ and Mahadevan LC. (1999a). *EMBO J.*, **18**, 4779–4793.
- Thomson S, Mahadevan LC and Clayton AL. (1999b). *Semin. Cell Dev. Biol.*, **10**, 205–214.
- Tse C and Hansen JC. (1997). *Biochemistry*, **36**, 11381–11388.
- Tse C, Sera T, Wolffe AP and Hansen JC. (1998). *Mol. Cell Biol.*, **18**, 4629–4638.
- Turner BM. (1999). *Semin. Cell Dev. Biol.*, **10**, 165–167.
- Turner BM. (2000). *Bioessays*, **22**, 836–845.
- Van Hooser A, Goodrich DW, Allis CD, Brinkley BR and Mancini MA. (1998). *J. Cell Sci.*, **111**, 3497–3506.
- Vettese-Dadey M, Grant PA, Hebbes TR, Crane-Robinson C, Allis CD and Workman JL. (1996). *EMBO J.*, **15**, 2508–2518.
- Wei Y, Mizzen CA, Cook RG, Gorovsky MA and Allis CD. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 7480–7484.
- Wei Y, Yu L, Bowen J, Gorovsky MA and Allis CD. (1999). *Cell*, **97**, 99–109.
- Wolffe AP and Hayes JJ. (1999). *Nucleic Acids Res.*, **27**, 711–720.
- Wolffe AP and Pruss D. (1996). *Cell*, **84**, 817–819.
- Xue Y, Canman JC, Lee CS, Nie Z, Yang D, Moreno GT, Young MK, Salmon ED and Wang W. (2000). *Proc. Natl. Acad. Sci. USA*, **97**, 13015–13020.
- Ye X, Xu G, Fincher RR and Osmani SA. (1997). *Methods Enzymol.*, **283**, 520–532.