Phosphorylation of serine 10 in histone H3, what for?

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Summary

Eukaryotic cells must possess mechanisms for condensing and decondensing chromatin. Chromatin condensation is particularly evident during mitosis and cell death induced by apoptosis, whereas chromatin decondensation is necessary for replication, repair, recombination and transcription. Histones are among the numerous DNA-binding proteins that control the level of DNA condensation, and post-translational modification of histone tails plays a critical role in the dynamic condensation/decondensation that occurs during the cell cycle. Phosphorylation of Ser10 in the tails of histone H3 has been extensively studied in many organisms. Interestingly, this modification is involved in both transcription and cell division, two events requiring opposite alterations in the degree of chromatin compaction. How does one and the same modification of histone H3 fulfil such roles? For instance, in interphase, phosphorylation of H3 correlates with chromatin relaxation and gene expression, whereas in mitosis it correlates with chromosome condensation. What is the kinase and under what circumstances does Ser10 becomes phosphorylated? Most importantly, what are the consequences of phosphorylation of this residue?

Key words: Chromatin, Histone H3, Serine 10, Phosphorylation, Mitosis

Introduction

Cells protect their DNA by organising it as a higher-order nucleoprotein complex termed chromatin in which the basic unit is the nucleosome. Each nucleosome is composed of an octamer of core histones (two each of H2A, H2B, H3 and H4), around which two super-helical turns (80 base pairs) of DNA are wrapped (van Holde, 1988). Crystallographic data reveal that the histone octamer exhibits a tripartite structure formed by a centrally located $(H3\text{-}H4)_2$ tetramer flanked by two H2A-H2B dimers (Arents et al., 1991). The core histones within the nucleosome share a common structural motif, the histone fold, and non-structured N-termini (Luger et al., 1997).

The organization of DNA into nucleosomes reduces its length about sixfold. Each nucleosome is linked to its neighbours by a segment of linker DNA, with which a distinct 'linker histone' interacts. This results in the packaging of chromatin into 30 nm fibers (van Holde, 1988). Further folding of these fibers generates higher-order chromatin structures (Woodcock and Dimitrov, 2001; Ridgway and Almouzni, 2001). DNA is most compact in mitotic chromosomes, and this requires additional proteins, such as the SMC (structural maintenance of chromosomes) proteins.

Such compaction gives a highly protected DNA molecule and makes it difficult for regulatory proteins to bind to DNA. Regulatory proteins that locally regulate DNA compaction by interacting with histones therefore control the accessibility of DNA sequences. It has long been thought that post-translational modification of histone tails controls the different levels of DNA organisation. Indeed, they can be acetylated, methylated, ADP-ribosylated, ubiquitylated and phosphorylated on several residues. Additionally, post-translational modification of one residue influences modification of the neighbouring residue.

The four tails provided by the conventional core histones are supplemented by new tails brought in by histone-variants such as CENP-A, which replaces H3 in the centromeric nucleosomes (Choo, 2001). Given the number of chromosomes per cell, the number of nucleosomes per chromosome, the number of different histone tails, and the number of different modifications, the potential complexity of regulation is immense. Strahl and Allis have therefore proposed the histone code hypothesis (Strahl and Allis, 2000), according to which each combination of post-translational modifications on a histone tail has a specific function (Jenuwein and Allis, 2001).

Histone phosphorylation was first observed in the sixties (Gutierrez and Hnilica, 1967), and the kinase responsible was shown to be an AMP-dependent kinase (Langan, 1968). Shoemaker and Chalkley subsequently reported that histone H3 is phosphorylated in vivo during metaphase at a single tryptic peptide by a cAMP-independent protein kinase (Shoemaker and Chalkley, 1978). Taylor then identified the first kinase able to phosphorylate H3 in vitro at Ser10 as cAMP-dependent kinase (Taylor, 1982). Since that time, an increasing number of protein kinases have been reported as true in vivo histone H3 Ser10 kinases, including PKA (DeManno et al., 1999; Schmitt et al., 2002). These kinases can be divided into kinases that function in signal transduction and mitotic kinases, which indicates that Ser10 phosphorylation might have different functions (Cheung et al., 2000a; Descamps and Prigent, 2001).

What are these functions? Here, we discuss two different approaches used to address this question: the elimination of a

putative kinase (De Souza et al., 2000; Giet and Glover, 2001; Hsu et al., 2000; MacCallum et al., 2002; Murnion et al., 2001; Petersen et al., 2001; Scrittori et al., 2001) and replacement of histone H3 Ser10 in vivo by non-phosphorylable residue (Hsu et al., 2000; Wei et al., 1999). We pay particular attention to the kinases involved and go on to discuss the different hypotheses that have been proposed to explain Ser10 phosphorylation.

Phosphorylation of H3 Ser10 in interphase

Phosphorylation of H3 Ser10 in interphase seems to be highly dependent on post-translational modification of neighbouring residues amino acids (Fig. 1). For instance, on native chromatin Ser10 phosphorylation depends on a methyltransferase activity that selectively methylates the Lys9 (Rea et al., 2000): an increase in phosphorylation of Ser10 is observed in methyltransferase-deficient cells, and Ser10 phosphorylation is reduced when Lys9 is dimethylated. Acetylation of Lys9 or Lys14 also influences Ser10 phosphorylation and vice versa. The H3 tail is a better substrate for Ser10 kinase when either residue is previously acetylated (Rea et al., 2000), and peptides that mimic the H3 tail and carry a phospho-Ser10 are better substrates for the histone acetyltransferase (HAT) that targets Lys14 (Lo et al., 2000; Cheung et al., 2000b). The situation in vivo, however, is more complex since Ser10 phosphorylation and Lys14 acetylation seem promoter and kinase specific (Lo et al., 2001; Merienne et al., 2001). Note that most of the in vitro experiments used non-physiological substrates (free histone H3 in solution or H3 tail peptides). In order to validate the above conclusions nucleosomes or nucleosomal arrays reconstituted with recombinant histones must be used as substrates.

At interphase, in contrast to at mitosis and meiosis (see below), the phosphorylation of histone H3 does not affect the whole genome but only a subset of genes. Ser10 phosphorylation correlates with transcriptional activation of these genes. For instance, cellular differentiation induced by follicular stimulating hormone (FSH) is accompanied by an increase in H3 phosphorylation on Ser10 and activation of PKA, which has been proposed to be the basis for PKA-dependent gene transcription in granulosa cells (DeManno et al., 1999). Similarly, Ser10 phosphorylation within the *Fos* gene occurs during activation of transcription by mitogens. Both the Rsk2 (ribosomal S6 kinase 2) (Sassone-Corsi et al., 1999) and Msk1 (mitogen- and stress-activated kinase 1) (Thomson et al., 1999) kinases are involved. Histone H3 is also phosphorylated in vivo at NF-κB-regulated promoters (such as IκBα promoter) during inflammatory responses triggered by cytokines (Saccani et al., 2002). The kinase involved has recently been identified as IκB kinase α (IKKα), a new Ser10 H3 kinase (Yamamoto et al., 2003).

The use of kinases inhibitors such as H89 for Msk1 (Thomson et al., 1999), PD98059 for MEK1 (MAP kinase kinase) (Zhong et al., 2000) or Sb202190 for p38 MAP kinase (Zhong et al., 2000) indicated that kinases from MAP kinase pathways are responsible for phosphorylation of H3 in interphase – the kinase used depending on the stimulus or stress.

Another immediate early gene, *Jun*, is also induced concomitantly with local Ser10 phosphorylation. Local phosphorylation of H3 at MAP-kinase-activated genes is associated with acetylation of the same tail (Clayton et al., 2000), indicating strong cooperation between both post-translational modifications in this particular situation. But this is not always so obvious; in the case of the retinoic acid receptor β2 (RARβ2) promoter, for instance, both histone H3 and H4 are constitutively acetylated. In the presence of ligand, rapid Ser10 phosphorylation occurs at the RARβ2 promoter but not at promoters of other genes, which indicates that it is the induction of phosphorylation that triggers gene activation (Lefebvre et al., 2002).

Under heat shock, acetylation of *Drosophila* polytene chromosomes remains unchanged whereas global phosphorylation of H3 decreases. This might seem contradictory but examination of heat shock loci reveals a local increase in H3 phosphorylation that depends on heat shock transcription factors (Nowak and Corces, 2000). Evidence from a variety of systems thus seems to support the idea that Ser10 phosphorylation at interphase is associated with activation of transcription.

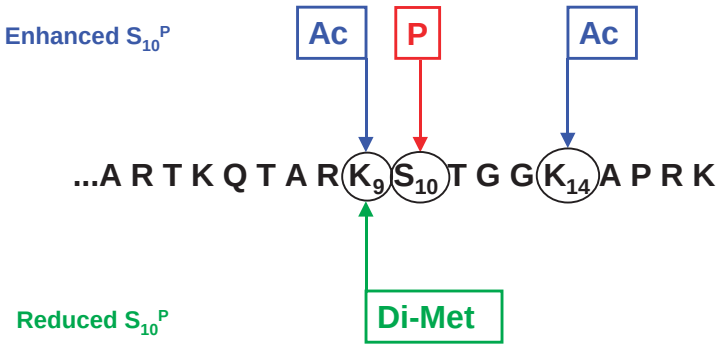


Fig. 1. The histone H3 tail. Amino acid sequence around serine 10 of H3 showing both lysine residues that can be acetylated (Ac) and/or methylated (Met) as indicated.

Phosphorylation of histone H3 at Ser10 during mitosis

During mitosis, histone H3 is phosphorylated at Ser10 in all eukaryotes, but since this observation the function of this post-translational modification has continually been debated (de la Barre et al., 2000; De Souza et al., 2000; Hendzel et al., 1997; Hsu et al., 2000; Kaszas and Cande, 2000; Van Hooser et al., 1998; Wei et al., 1998; Wei et al., 1999). Drugs that induce either phosphorylation or dephosphorylation of Ser10 have been used extensively, and induction of phosphorylation in interphase has been shown to correlate with chromosome condensation prior to mitosis, whereas chromosome decondensation is observed when dephosphorylation is prematurely induced in mitosis (Ajiro et al., 1996a; Ajiro et al., 1996b). The development of an antibody against the histone H3 phospho-Ser10 has allowed in vivo studies of this event (Hendzel et al., 1997), and there is a strong correlation between Ser10 phosphorylation and chromosome condensation during cell division (Hendzel et al., 1997; Van Hooser et al., 1998). In late G2 phase, phosphorylation

of histone H3 occurs on pericentromeric chromatin only. As mitosis proceeds, this spreads along the chromosomes and is complete at prophase. At the end of mitosis, histone H3 is dephosphorylated (Hendzel et al., 1997; Sauve et al., 1999; Van Hooser et al., 1998) (Fig. 2).

Van Hooser et al. have also examined whether cells can enter mitosis without phosphorylated Ser10 (Van Hooser et al., 1998) by microinjecting Ser10-sequence-carrying peptides into S-phase cells to saturate the kinase that phosphorylates Ser10 during the G2/M transition: the cells arrested in late G2 phase (Van Hooser et al., 1998). This does not directly demonstrate that phosphorylation of Ser10 is necessary for the G2/M transition but strongly suggests that the kinase is. Analogous experiments using similar competitive peptides during in vitro condensation of mitotic chromosomes in *Xenopus* egg extracts produced data in agreement with those of Van Hooser et al. (de la Barre et al., 2000; Van Hooser et al., 1998).

Some genetic experiments in *Tetrahymena thermophila* have produced data that are in agreement with those described above (Wei et al., 1999). A *tetrahymena* mutant strain in which Ser10 of histone H3 is replaced by an alanine residue exhibits altered chromosome condensation. In addition, abnormal chromosome segregation was observed in this strain, which the authors hypothesize is associated with the perturbation of chromosome condensation.

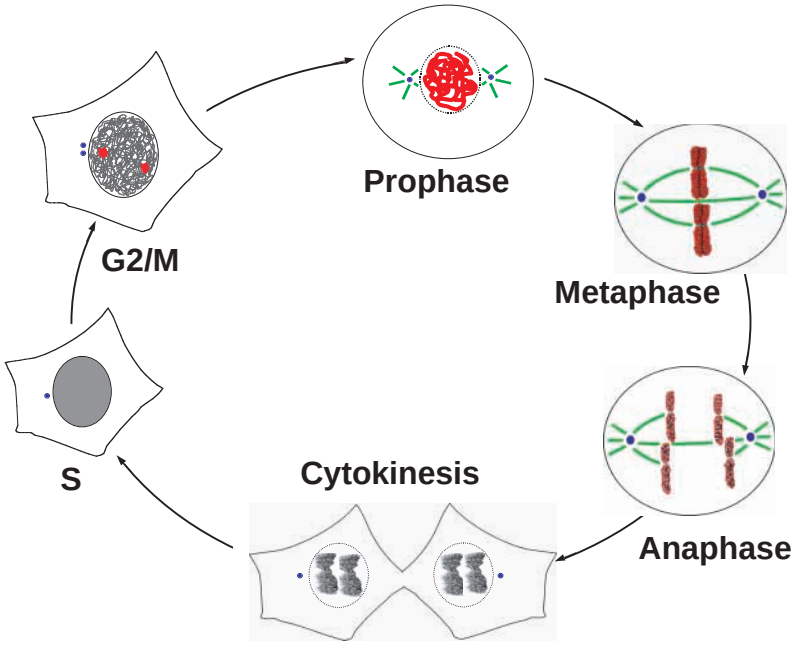


Fig. 2. Ser10 phosphorylation during cell cycle progression. Chromatin containing histone H3 phosphorylated at Ser10 is indicated in red, chromatin containing unphosphorylated Ser10 is grey, centrosomes are blue and microtubules green. Ser10 phosphorylation begins in G2 at pericentromeric chromatin, is complete in prophase and decreases upon exit from mitosis.

Other genetic data, however, suggest that histone H3 phosphorylation is not required for chromosome condensation. Yeast mutants lacking Ser10 (Ser10Ala) show generation times and cell cycle progression identical to those of the wild-type strain (Hsu et al., 2000). Thus, in *S. cerevisae*, a relationship between phosphorylation at Ser10 of histone H3 and chromosome dynamics has not been observed. Condensation of mitotic chromosomes induced by incubation of demembranated sperm nuclei in *Xenopus* egg extract is, as in cell culture, accompanied by a phosphorylation of histone H3. During this process a very fast decondensation (~10 minutes) is followed by a relatively long (2-3 hours) condensation step. The phosphorylation of histone H3 is complete, however, during decondensation, and thus chromosome condensation and histone H3 phosphorylation are uncoupled in this system.

Competition experiments using reconstituted chimeric nucleosomes have shown that the N-termini of the core histones are essential for mitotic chromosome compaction (de la Barre et al., 2001). Crucially, these experiments have demonstrated that the N-terminus of histone H2B, but not that of histone H3 or its phosphorylation, is required for chromosome condensation in *Xenopus* egg extracts (de la Barre et al., 2001). In addition, chromosomes condense properly but do not exhibit phosphorylated H3 in *Xenopus* extracts depleted of the kinase aurora B (MacCallum et al., 2002). These data argue against a role for histone H3 phosphorylation in chromosome condensation and are further supported by in vivo experiments in *Drosophila*, which reveal a very weak correlation between the level of histone H3 phosphorylation and the degree of chromosome compaction (Adams et al., 2001b). The role of this modification at mitosis is thus more controversial than that in interphase.

Phosphorylation of histone H3 at Ser10 during meiosis

Ser10 of histone H3 is also phosphorylated during meiosis in all organisms studied (Kaszas and Cande, 2000; Speliotes et al., 2000; Wei et al., 1998). The H3 phosphorylation in *Tetrahymena* in both meiosis I and II is similar to that in mitosis: upon entry into meiosis, the histone is phosphorylated and subsequently dephosphorylated at anaphase when cells exit meiosis. Again, the mutant H3 Ser10Ala strain exhibits abnormal condensation and segregation defects, and the H3 phosphorylation correlates with chromosome condensation (Wei et al., 1998). There is no such correlation in maize, however, where histone H3 phosphorylation is clearly associated with the maintenance of sister chromatid cohesion and not chromosome condensation (Kaszas and Cande, 2000). In addition, in *C. elegans*, chromosome condensation can occur in the absence of phosphorylation of histone H3 (Speliotes et al., 2000).

The mitotic H3 Ser10 kinase

The phenotype of the H3 Ser10Ala mutant in *Tetrahymena thermophila* resembles that of the *Saccharomyces cerevisiae* mutant lacking the only yeast aurora kinase Ipl1p (Chan and Botstein, 1993; Wei et al., 1999). In addition, the temperature-sensitive mutant strain *Ipl1-2* shows a reduction in the level of Ser10 phosphorylation in mitosis, and Ipl1p can phosphorylate

Ser10 of H3 in vitro (Hsu et al., 2000). Likewise in *Schizosaccharomyces pombe*, the single aurora kinase (Ark1) is associated with Ser10 phosphorylation in mitosis (Petersen et al., 2001). Multicellular organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster* possess two aurora kinases – aurora A and aurora B (Schumacher et al., 1998a; Schumacher et al., 1998b; Glover et al., 1995; Reich et al., 1999), whereas mammals possess an additional spermatogenesis-dedicated kinase, aurora C (Giet and Prigent, 1999; Adams et al., 2001a). Examination of Ser10 phosphorylation with antiphosphoSer10 antibodies in nematode and fruit fly cells in which expression of aurora A or aurora B has been eliminated by RNA interference has revealed that aurora B but not aurora A is required for Ser10 phosphorylation (Hsu et al., 2000; Giet and Glover, 2001; Adams et al., 2001b). In vertebrates there is also evidence for a role of aurora B in the phosphorylation of Ser10. For instance, both aurora A and aurora B phosphorylates Ser10 in vitro, and expression of both kinases correlates with the appearance of the phosphoSer10 in vivo (Scrittori et al., 2001; Crosio et al., 2002; Sugiyama et al., 2002; Murnion et al., 2001). However, treatment of mammalian cells in culture with hesparadin, a novel aurora B kinase inhibitor, leads to a strong decrease of histone H3 phosphorylation (Hauf et al., 2003). In agreement with this, RNA interference ablation of aurora B is also associated with a reduction in the phosphorylation of histone H3 at Ser10 (Hauf et al., 2003). Interestingly, aurora A overexpression can lead to cell transformation (Bischoff et al., 1998; Zhou et al., 1998). Whether this transformation correlates with hyperphosphorylation of Ser10 remains to be determined.

Because of the subcellular localisations of the kinases, aurora B makes a better Ser10 kinase than does aurora A. Aurora A is mainly a centrosomal protein (Gopalan et al., 1997) whereas aurora B is a passenger protein – it first localises at chromosome kinetochores from G2 phase to metaphase and then at the midbody from anaphase to telophase (Terada et al., 1998). Experiments depleting aurora B from *Xenopus laevis* egg extracts support the importance of aurora B. It is tightly associated with INCENP (INner CENtromeric Protein) and survivin, and depletion of the two produces a reduction in Ser10 phosphorylation (Adams et al., 2000; Bolton et al., 2002). Interestingly, chromosome condensation and condensin recruitment remain normal in the aurora-B- and INCENP-depletion experiments (Adams et al., 2001b). Only the association of XCAP-F (*Xenopus* chromosome associated protein F) with condensed chromosomes was affected (MacCallum et al., 2002). Chromosome condensation occurs normally in XCAP-F-depleted *Xenopus* egg extract, whereas nucleosome spacing is affected. These data suggest that phosphorylation of H3 is not directly involved in chromosome condensation but rather indirectly in complex and subtle mechanisms of chromosome remodelling.

After aurora B depletion, MacCallum et al. (MacCallum et al., 2002) observed a residual Ser10 phosphorylation that they attributed to an additional protein kinase. Indeed, De Souza et al. have reported that another protein kinase is responsible for Ser10 phosphorylation in *Aspergillus nidulans*, the kinase NIMA (never in mitosis) (De Souza et al., 2000). NIMA, like many other kinases, can phosphorylate Ser10 in vitro (De Souza et al., 2000). Because phosphorylation of Ser10 was thought to be involved in chromosome condensation, NIMA became a good candidate for several reasons. It triggers chromosome condensation in cells arrested in S phase (Ye et al., 1995; Osmani et al., 1988), which is accompanied by phosphorylation of Ser10 (De Souza et al., 2000). There is also a strong correlation between the localisation of NIMA and the appearance of the phosphoSer10 epitope (De Souza et al., 2000). Cells cannot enter mitosis without NIMA, which implies that mitotic phosphorylation of Ser10 cannot occur. Mammalian cells possess several NIMA-related kinases (Neks) but apparently only one functional ortholog of NIMA, Nercc1 kinase (Roig et al., 2002). Nercc1 phosphorylates H3 exclusively on serine and threonine residues but whether it is indeed a Ser10 kinase remains to be shown.

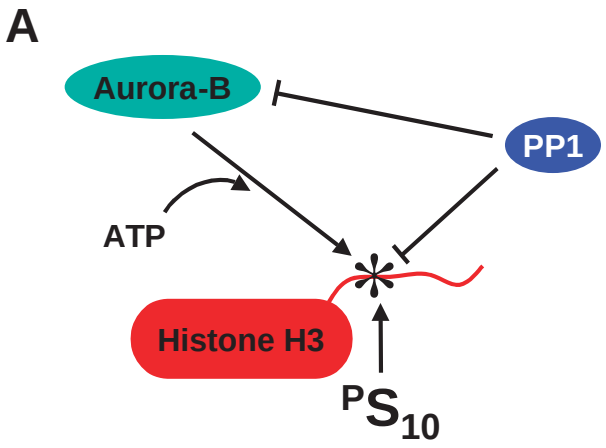
The mitotic H3 Ser10 phosphatase

Unlike the identity of the mitotic Ser10 kinase, that of mitotic Ser10 phosphatase is more clear: it is likely to be PP1. Given the identification of Ipl1p as the budding yeast mitotic Ser10 kinase (Hsu et al., 2000; Scrittori et al., 2001), it was fairly easy to define the Ser10 phosphatase since Glc7p had been shown to be the phosphatase that counteracts Ipl1p (Francisco et al., 1994). This was later confirmed when Ndc10p was identified as an Ipl1p kinase substrate and a Glc7p type 1 phosphatase substrate (Sassoon et al., 1999; Biggins et al., 1999). In *C. elegans*, the residue is also dephosphorylated in vivo by a type 1 phosphatase, namely CeGlc7p, although the precise role of its α and β isoforms remains to be clarified (Hsu et al., 2000).

In vertebrates, both aurora B and PP1 are associated with mitotic chromosomes (Murnion et al., 2001). Not only does PP1 dephosphorylate Ser10 as suggested in *C. elegans* and *S. cerevisiae*, but it also dephosphorylates and inactivates aurora B itself (Murnion et al., 2001). This is also true for aurora A (Tsai et al., 2003; Eyers et al., 2003). This adds a level of complexity, because elimination of PP1 might thus have two cumulative effects: constitutive phosphorylation of Ser10 (which cannot be dephosphorylated) and generation of constitutively active aurora B (which keeps phosphorylating Ser10) (Fig. 3A) (Murnion et al., 2001). The same is true of another aurora B substrate in *C. elegans*, REC-8. RNAi knockdown of Glc7α and Glc7β alters the localisation of both aurora B and REC-8 (Rogers et al., 2002). Aurora B is also activated by okadaic acid, a PP1/PP2A inhibitor, which indicates that the kinase should interact with the phosphatase, although direct interactions have not been reported yet (Sugiyama et al., 2002). Aurora A directly binds to PP1 through two domains, NB1 (K162) and NB2 (K343) (Fig. 3B) (Katayama et al., 2001), but aurora B and aurora C each have only one potential PP1-binding sequence. An alignment of aurora B sequences shows that this sequence is conserved and is always located after subdomain 1 of the kinase catalytic domain (Fig. 3C). This suggests that aurora B, like aurora A, binds directly to PP1, although this remains to be proven.

Phosphorylation of histone H3 at Ser28

Observed later and less studied than Ser10 phosphorylation, phosphorylation of Ser28 in the tail of H3 has kinetics very



B

Consensus		R/K	-	V/I	-	X	-	F
Hs Aurora-A	162	K	-	V	-	L	-	F
	343	R	-	V	-	E	-	F
Hs Aurora-B	106	K	-	V	-	L	-	F
Hs Aurora-C	72	K	-	V	-	L	-	F

C

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CeAurB    .gkfgsvylartktghfhvaikvlfksqli.
DmAurB    .gkfgrvylarerhshylvamkvmfkeelr.
XlAurB    .gkfgnvylarekqnkfimalkvlfksqle.
MmAurB    .gkfgnvylarekksrfivalkilfksqie.
RnAurB    .gkfgnvylarekksrfivalkilfksqie.
HsAurB    .gkfgnvylarekkshfivalkvlfksqie.
          Kinase          PP1
          subdomain I     binding?
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Fig. 3. PP1 and aurora B. (A) The function of aurora B and PP1 in H3 Ser10 phosphorylation. Ser10 is phosphorylated by aurora B in mitosis and dephosphorylated by PP1 that can also dephosphorylate and inactivate aurora B. (B) Potential consensus sequences for the type 1 phosphatase-binding site in human aurora kinases. (C) Alignment of a segment of aurora B from *C. elegans*, *D. melanogaster*, *X. laevis*, *M. musculus*, *R. norvegicus* and *H. sapiens* containing the end of the kinase subdomain 1 and the conserved potential PP1-binding site.

similar to those of Ser10. Both serines lie in the same consensus sequence -ARKS- (Fig. 4), and both phosphorylations occur early in mitosis when chromosomes begin to condense and during premature chromosome condensation induced in S-phase cells (Goto et al., 1999). Moreover, PKA phosphorylates both Ser10 and Ser28 in vitro (Shibata et al., 1990). Aurora B, the major mitotic Ser10 kinase in vivo, was thus a good candidate for the mitotic Ser28 kinase. Although aurora B can phosphorylate Ser28 in vitro (Sugiyama et al., 2002), no direct proof has been reported in vivo, but there is some circumstantial evidence. The localisation of aurora B on chromosomes during early mitosis, for instance, coincides with the areas where Ser10 and Ser28 phosphorylation is observed (Goto et al., 2002). Furthermore, like Ser10 phosphorylation, Ser28 is sensitive to treatment with PP1 (Goto et al., 2002), the phosphatase that counteracts aurora. The function of this phosphorylation, however, is not yet known. In contrast to Ser10 phosphorylation, phosphorylation of Ser28 has never been observed in interphase. Instead it seems to be a mitotic event strictly related to mitotic chromosome condensation (Goto et al., 1999).

Phosphorylation of CENP-A

Histone variants are found in all eukaryotes. They exhibit numerous sequence changes compared with the conventional histones (van Holde, 1988), and their function is not known (Tsanev et al., 1993). Histone H3 is replaced by its variant CENP-A in centromeric chromatin (Choo, 2001). Ablation of CENP-A results in non-functional centromeres and mislocalization of important kinetochore components (Blower et al., 2002; Buchwitz et al., 1999; Oegema et al., 2001). Therefore, the presence of CENP-A in centromeric nucleosomes is required for kinetochore organization and function. This role of CENP-A is played by its N-terminal domain, which differs from that of the conventional histone H3. Alignment of the CENP-A N-terminal domain with that of H3 indicates that it possesses a serine residue at position 7 that is the equivalent of Ser10 of histone H3. Ser7 is likely to be phosphorylated by aurora B in mitosis and is associated with completion of cytokinesis (Zeitlin et al., 2001). Cells carrying a Ser7Ala mutation in CENP-A show a mislocalisation of aurora B, PP1γ1 and INCENP: three proteins required for cytokinesis. Sequence alignment also reveals that the aurora B-phosphorylation consensus sequence is repeated twice in the CENP-A tail: at Ser7 and Ser17 (Fig. 5). Ser17 of CENP-A may thus be the equivalent of Ser28 of histone H3. This idea is supported by the fact that phosphorylation of the CENP-A Ser7Ala mutant is reduced by only 50% compared with phosphorylation of wild-type CENP-A (Zeitlin et al., 2001). Phosphorylation of Ser17 has never been studied, however.

Phosphorylation of H3 at Ser10 during apoptosis

During cell death induced by apoptosis one of the first changes observed in the cell is chromosome condensation (Wyllie, 1980). Because, in mitosis, phosphorylation of Ser10 of histone H3 accompanies chromosome condensation, a similar mechanism might act during apoptosis. In some situations, depending on the apoptosis inducer used, there is indeed a correlation between Ser10 phosphorylation and apoptotic chromosome condensation, for example, when apoptosis is induced by gliotoxin (Waring et al., 1997). This drug also triggers an increase in the cellular c-AMP level, however, and a rise in PKA activity. This could be a coincidence and both events be completely independent, since although an increase in PKA activity is rapidly followed by a massive Ser10 phosphorylation, this does not trigger chromosome condensation (Schmitt et al., 2002). During mitosis numerous proteins are phosphorylated on specific residues by kinases such as $p34^{cdc2}$ and polo-like kinase, and these residues can be revealed by the monoclonal antibody MPM-2. During apoptosis, in most cases, chromosome condensation occurs in the absence of $p34^{cdc2}$ activity, no MPM-2 epitope is observed

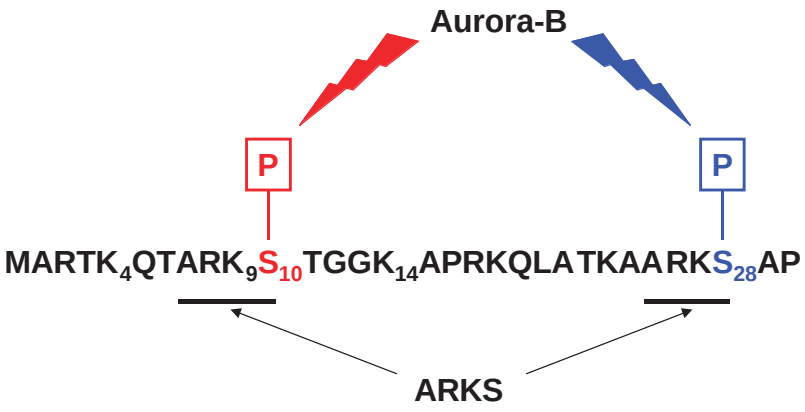

Fig. 4. Comparison between histone H3 Ser10 and Ser28. The sequence around H3 Ser10 and H3 Ser28. Both sequences correspond to the same consensus sequence: ARKS – easy to remember as 'aurora-related kinase serine'.

and condensed chromosomes have unphosphorylated histones H1 and H3 (Yoshida et al., 1997). These data indicate that phosphorylation of H3 and apoptotic chromosome condensation are unrelated events; they also suggest that chromosome condensation can occur without phosphorylation of Ser10.

The function of histone H3 phosphorylation in mitosis

Different models have been proposed to explain the role of histone H3 in mitosis. One of the first models is built on the hypothesis that H3 tail phosphorylation significantly affects histone H3-DNA interactions (Sauve et al., 1999). UV crosslinking experiments by the proposers of this model indicate that, in interphase, the histone H3 tail is bound to DNA (only a small pool of H3 is phosphorylated) but binding is reduced in mitosis (when all H3 molecules are phosphorylated) (Sauve et al., 1999). According to the model, the tail of histone H3 unbound to DNA is unstructured but adopts an α-helical conformation upon binding (Banéres et al., 1997). Phosphorylation of Ser10 was therefore proposed to neutralise positive charges in the H3 tail and thereby help to increase its α-helical potential. In this model, during interphase the unphosphorylated tail of H3 is bound to DNA and participates in chromatin compaction (together with other histone tails). Phosphorylation at Ser10 during mitosis weakens histone-tail–DNA interactions and favours DNA-polyamine binding. Polyamines then neutralise the negative charge of DNA more extensively, thus minimising repulsion between nucleosomes and allowing formation of the highly compacted mitotic chromosome. The model also offers an explanation for the dephosphorylation of H3 in anaphase and telophase: this would restore and increase the affinity of the H3 tail for DNA, which would favour displacement of polyamines and promote chromatin decondensation.

This model, however, has several drawbacks. First, upon phosphorylation of histone H3 the positive charge of its tail will decrease from 14 to 12, and this is very unlikely to result in disruption of the interaction with linker DNA (Hans and Dimitrov, 2001). Indeed, numerous reports have shown that hyperacetylation of histone tails, which reduces the positive charges of the tails from by far more than two, does not prevent the tails from interacting with nucleosomal DNA (Mutskov et al., 1998) and does not result in chromatin decondensation (McGhee et al., 1983; Dimitrov et al., 1986). Some small decondensation effects associated with histone hyperacetylation are only detectable in H1-depleted chromatin (Garcia-Ramirez et al., 1995).

Second, the model implies that H3 adopts an α-helical conformation upon binding to DNA, which is stabilized upon phosphorylation of Ser10. No firm data, however, have demonstrated that this is really the case. Third, the decreased efficiency of UV crosslinking of histone H3 to DNA could reflect a slightly modified mode of binding of the H3 tail to linker DNA. It should be noted that the UV laser crosslinking depends on both the close contact of the amino acid residues with the bases and their proper spatial orientation relative to each other (for a review, see Pashev et al., 1991). Hence, a slightly modified binding of the tail H3 to the linker DNA could not allow a proper orientation of the H3 tail residues relative to the nucleobases, which would result in smaller crosslinking efficiency. Finally, no unambiguous data have shown that higher amounts of endogenous polyamines bind to mitotic chromosomes compared with those bound to interphase chromatin.

A second model is based on the idea that the condensation factors are recruited to the chromosomes through direct interactions with phosphorylated histone H3 tail or, in general, with chromatin containing phosphorylated histone H3 (Wei et al., 1999; Cheung et al., 2000a). Thus, phosphorylation of histone H3 would be viewed as a trigger for chromosome condensation. The model is not, however, in agreement with the available data, since the two factors known to be involved in chromosome condensation, topoisomerase II and the SMC proteins, bind with identical, very low affinity to both native

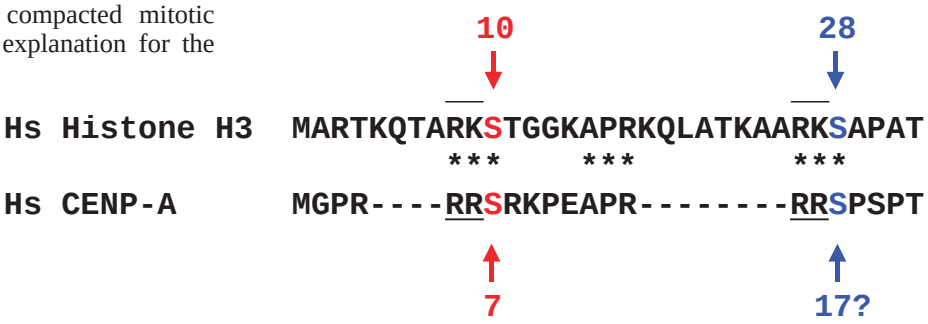

Fig. 5. Comparison between the histone H3 and CENP-A tails. Sequence alignment of the histone H3 and CENP-A tails. The aurora-B-phosphorylation sites are indicated.

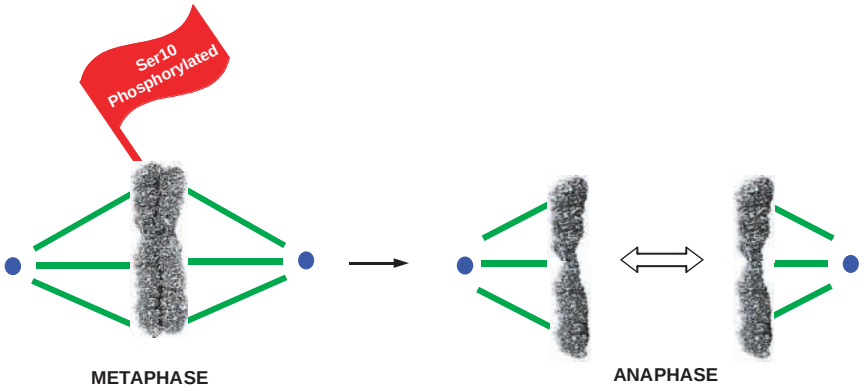

Fig. 6. The 'ready production label' model. Ser10 phosphorylation reaches its maximum in metaphase. Upon metaphase/anaphase transition the phosphorylation must be removed. The presence of phosphorylated Ser10 signals to the cell that it has reached the metaphase stage, but this is independent of the condensation state of the chromosome.

and tailless nucleosomes (de la Barre et al., 2000; Kimura and Hirano, 2000). Another condensation factor, different from topoisomerase II and the SMC proteins, might bind preferentially to H3 phosphorylated chromatin. This does not seem to be the case, since chromosomes can be efficiently assembled without histone H3 phosphorylation (MacCallum et al., 2002).

The two main models proposed in the literature are not in good agreement with the experimental data. Thus, what is the function of histone H3 Ser10 phosphorylation during mitosis? Presently, it is difficult to answer this question. However, during cell division in mitosis there are two facts that have been confirmed: the first fact is that histone H3 is always heavily phosphorylated on Ser10 on metaphase chromosomes; the second fact is that the same residue becomes dephosphorylated as soon as cells exit mitosis. Phosphorylation of Ser10 might thus be used to mark chromosomes in such a way that the cell knows that it is progressing from metaphase to telophase. Hence, as initially suggested by Hans and Dimitrov (Hans and Dimitrov, 2001), histone H3 phosphorylation might be viewed as some type of 'ready production label'. This label must stick to the chromosomes once they have successfully passed through the different checkpoints and arrived at metaphase. The presence of such a label is an indication to the cell that the chromosomes are ready to continue through anaphase. Once the cells have passed through anaphase, the labelling is not necessary anymore and it is removed.

The 'ready production label' hypothesis (Fig. 6) does not imply a relationship between chromosome condensation and histone H3 phosphorylation. It simply implies that once the cell has arrived at metaphase its chromosomes should be phosphorylated, and this is independent of their state of condensation. This explains two 'paradoxical' phenomena that cannot be explained by the two other models discussed above: the correlation of chromosome assembly in *Xenopus* egg extract with sperm decondensation and the heavy phosphorylation of decondensed human chromosomes (obtained upon cell incubation in hypotonic solution) upon their release into the culture medium.

The 'ready production label' model predicts that processes taking place after metaphase are associated with histone H3 phosphorylation. This prediction is in agreement with the available data for the *Tetrahymena* strain containing non-phosphorylatable histone H3 (Wei et al., 1999). Indeed, these strains exhibit abnormal segregation and chromosome loss (Wei et al., 1999). It should be noted, however, that histone H3 phosphorylation may not play an essential role in some organisms. For example, no causal relationship between phosphorylation of Ser10 of histone H3 and chromosome dynamics was observed in *S. cerevisiae* (Hsu et al., 2000). Neither the mitotic mechanism that identifies phosphorylated Ser10 nor the nature of the information given to the cell by phosphorylated Ser10 is known.

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