

Perspective

Dynamic Regulation of Effector Protein Binding to Histone Modifications

The Biology of HP1 Switching

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ABSTRACT

Post-translational modifications of histone proteins, the basic building blocks around which eukaryotic DNA is organized, are crucially involved in the regulation of genome activity as they control chromatin structure and dynamics. The recruitment of specific binding proteins that recognize and interact with particular histone modifications is thought to constitute a fundamental mechanism by which histone marks mediate biological function. For instance, tri-methylation of histone H3 lysine 9 (H3K9me3) is important for recruiting heterochromatin protein 1 (HP1) to discrete regions of the genome, thereby regulating gene expression, chromatin packaging, and heterochromatin formation. Until now, little was known about the regulation of effector-histone mark interactions, and in particular, of the binding of HP1 to H3K9me3. Recently, we and others presented evidence that a "binary methylation-phosphorylation switch" mechanism controls the dynamic release of HP1 from H3K9me3 during the cell cycle: phosphorylation of histone H3 serine 10 (H3S10ph) occurs at the onset of mitosis, interferes with HP1-H3K9me3 interaction, and therefore, ejects HP1 from its binding site. Here, we discuss the biological function of HP1 release from chromatin during mitosis, consider implications why the cell controls HP1 binding by such a methylation-phosphorylation switching mechanism, and reflect on other cellular pathways where binary switching of HP1 might occur.

CHROMATIN, HISTONE MODIFICATIONS, EFFECTOR PROTEINS AND HETEROCHROMATIN PROTEIN 1

DNA in the nucleus of eukaryotic cells is stored in close association with a set of abundant proteins in a complex called chromatin. In the nucleosome, the fundamental packaging unit of chromatin, DNA is wrapped around an octamer of core histone proteins (two copies each of H2A, H2B, H3 and H4), leading to a significant compaction of the DNA fiber.¹ Histone proteins undergo a large variety of post-translational modifications, such as acetylation, phosphorylation, methylation, or ubiquitination.^{2,3} In recent years, it has been uncovered that different covalent histone modifications (histone marks) play a key role in the regulation of chromatin structure and function, impacting on important cellular processes such as transcription, replication, mitosis, DNA repair, and apoptosis.⁴ On a molecular level, certain histone marks function by directly regulating internucleosomal contacts and controlling higher order chromatin folding.^{5,6} Other histone modifications appear to exert their effects more indirectly by recruiting specific binding proteins (effectors) to distinct areas of the genome.⁷⁻⁹

Particularly well studied among the effectors of histone modifications is heterochromatin protein 1 (HP1), a small, conserved chromosomal protein with important functions in chromatin organization and chromosomal dynamics.^{10,11} Three variants of HP1 are found in higher eukaryotes, HP1 α , HP1 β , and HP1 γ . All these isoforms localize to regions of constitutive heterochromatin and repetitive DNA elements,¹² as well as some euchromatic areas.^{13,14} In general, HP1 binding correlates with gene repression, although cases of HP1 localization to transcriptionally active zones of the genome have been reported^{15,16} (reviewed in ref. 17).

HP1 proteins consist of two highly conserved domains, an amino-terminal chromo domain¹⁸ and a carboxyl-terminal chromoshadow domain,¹⁹ connected by a more variable hinge region. The chromo domain of all HP1 isoforms binds to tri-methylated lysine 9 on the amino-terminal tail of histone H3 (H3K9me3).^{20,21} This interaction is important for the recruitment of HP1 proteins to heterochromatic areas of the genome,²²⁻²⁵ and loss of the methyltransferase mediating methylation of H3K9 results in delocal-

ization of HP1.²⁰ In vitro, the binding of the chromo domain to H3K9me3 is rather weak. In agreement, a large fraction of the cellular HP1 molecules are not stably associated with chromatin, but display rapid on-off kinetics from their subnuclear target areas.²⁶⁻²⁸ In the few cases, where more steady association of HP1 with chromatin is observed, additional interactions are thought to contribute to local stabilization. In this regard, the chromoshadow domain has been implicated in a wide variety of protein-protein contacts, including dimerization^{29,30} as well as interactions with other components of chromatin, transcriptional regulators, chromatin modifiers, and factors implicated in replication, the cell cycle, and nuclear architecture (see refs. 12 or 31 for an overview). These manifold interactions might also account for putative H3K9me3-independent chromatin association of HP1.^{25,31-33}

Early genetic experiments had uniformly implicated HP1 in gene silencing and heterochromatin formation.¹¹ Indeed, artificial targeting of HP1 to euchromatic sites is sufficient to induce gene repression and local condensation of chromatin in several experimental systems.³⁴⁻³⁷ On a molecular level, HP1 might induce heterochromatin formation via cross-linking of nucleosomes in combination with the recruitment of other chromatin-modifying proteins.^{38,39} The few instances where artificial HP1 targeting did not induce silencing⁴⁰ or the startling situations where HP1 has been implicated in gene activation^{16,41,42} might be due to unique behavior of HP1 isoforms, distinct local chromatin environments, and the differential and regulated interaction with additional factors.¹⁷

MITOTIC RELEASE OF HP1 FROM CHROMATIN

Chromatin association of HP1 proteins as it is observed in interphase (Fig. 1) changes dramatically during mitosis (Fig. 2). Different studies using different cell lines and experimental approaches have shown partial to complete loss of HP1 from mitotic chromatin.⁴³⁻⁴⁸ The consensus seems that at this stage of the cell cycle, all of HP1 β and HP1 γ , as well as the majority of HP1 α , dissociate from chromatin. Only a fraction of HP1 α remains localized at the centromeres. There it appears to be involved in the cohesion of sister centromeres (Fig. 2).^{47,49,50} While dissociation of almost all of HP1 from chromatin is observed, most surprisingly its binding site, H3K9me3, persists during M-phase.⁵¹⁻⁵³ Results presented in several recently published studies⁵³⁻⁵⁵ have shed light on this paradoxical interruption of the signaling axis HP1-H3K9me3.

First, the timing of the mitotic release of HP1 proteins from chromatin was found closely correlated to the chromosome-wide appearance of H3 serine 10 phosphorylation (H3S10ph).⁵⁵ Second,

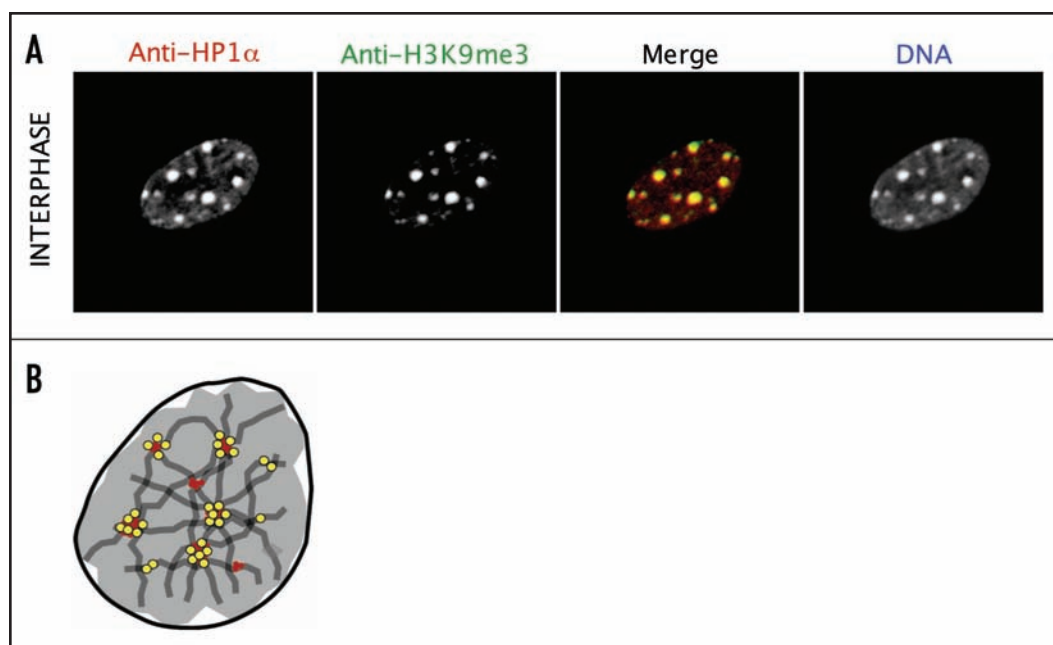


Figure 1. Distribution of HP1 α and H3K9me3 in an interphase cell. (A) Anti-HP1 α and anti-H3K9me3 immunofluorescence staining of an interphase 10T1/2 mouse fibroblast cell nucleus show an overlapping punctate pattern. The dots correspond to regions of condensed pericentric heterochromatin as visualized by the DNA staining (DAPI). (B) Schematic illustration of the localization of H3K9me3 (red) and HP1 (yellow) in the nucleus of an interphase cell. H3K9me3 is mainly localized to regions of condensed, pericentric heterochromatin. Some interspersed H3K9me3 containing euchromatic areas are also shown. The distribution of HP1 largely overlaps with its recruiting H3K9me3 mark.

and in contrast to earlier in vitro findings,⁵⁶ mass spectrometric and immunological approaches unambiguously identified the K9me and S10ph marks present on the same histone H3 tail at the beginning of M-phase (Fig. 2B).^{53,54} Third, immunofluorescence studies verified that the dual mark combination of H3K9me3S10ph and HP1 proteins exclude each other from chromatin.^{53,54} Indeed, in in vitro binding studies, HP1 interaction with a dually, K9me3S10ph-modified H3 peptide is much weaker compared to a singly, K9me3-modified H3 tail.⁵³⁻⁵⁵ The kinase responsible for mitotic phosphorylation of H3S10 is Aurora B, a component of the chromosomal passenger complex (CPC).^{57,58} In vitro phosphorylation by this kinase complex is sufficient to interrupt the interaction of the different HP1 chromo domains with a H3K9me3 peptide.⁵³ In agreement with a crucial role of H3S10 phosphorylation in the mitotic release of HP1 proteins from chromatin, depletion of Aurora B by RNA interference or antibodies,⁵³⁻⁵⁵ inhibition of enzymatic Aurora B activity with the small molecule inhibitor hesperadin,^{53,54,59} or overexpression of a H3 mutant that cannot be phosphorylated at serine 10 (H3S10A)⁵⁵ all result in retention of the effectors on chromosome arms.

Overall, the findings support an interpretation where release of HP1 proteins from mitotic chromatin is achieved by a “binary methylation-phosphorylation switch” mechanism, as depicted in (Fig. 3).⁶⁰ Phosphorylation of H3S10 by the chromosomal passenger complex disrupts the interaction of the chromo domain of HP1 proteins with the H3K9me3-target site. As a consequence, the dynamic equilibrium between bound and unbound HP1 is shifted towards the unbound state and HP1 is released from chromatin. At the end of mitosis, dephosphorylation of H3S10 reestablishes the interaction of HP1 proteins with the recruiting H3K9me3 mark and therefore allows the reassociation of the effectors with chromatin.

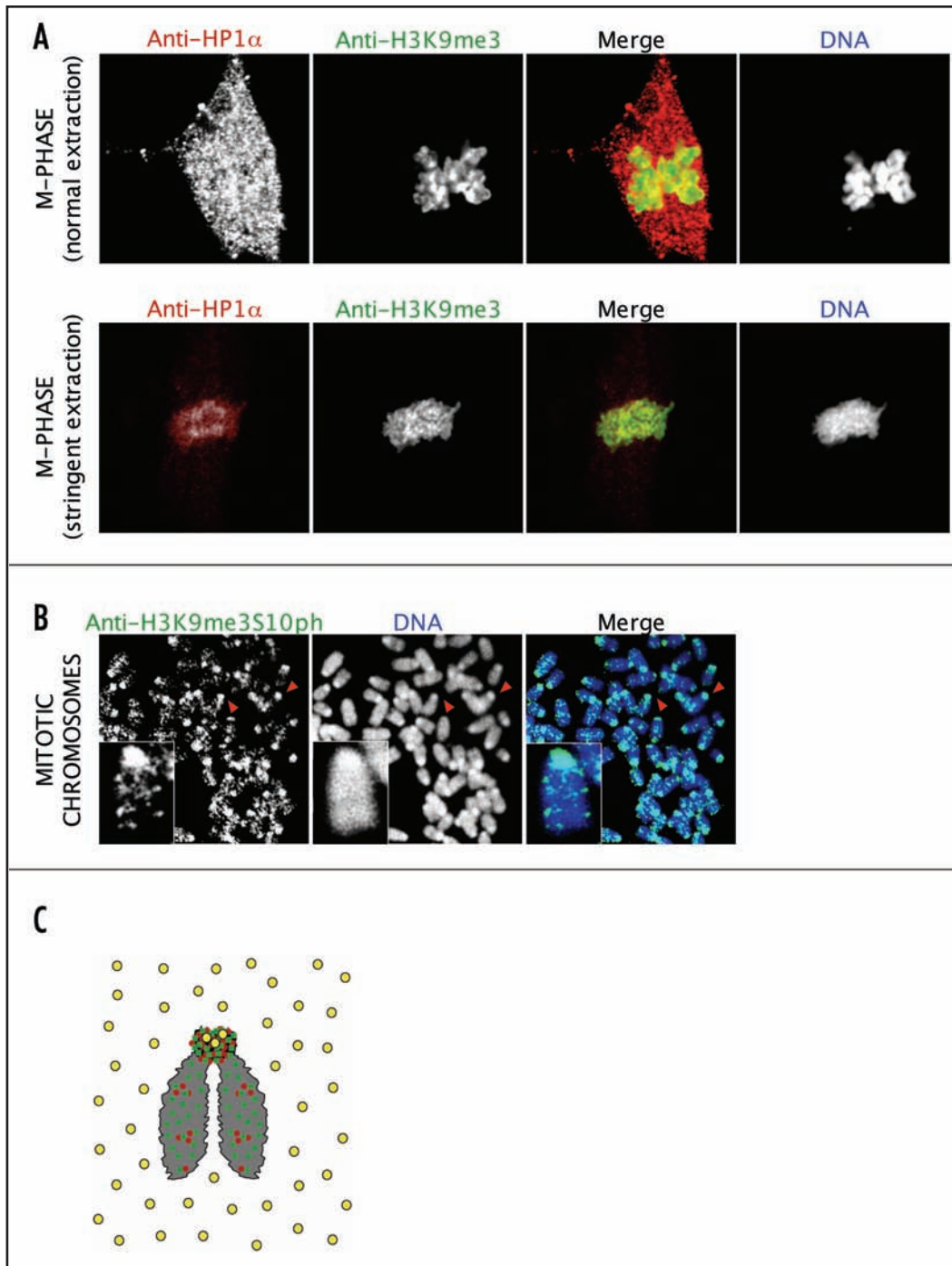


Figure 2. Distribution of HP1 α and H3 histone marks during mitosis. (A) Anti-HP1 α and anti-H3K9me3 immunofluorescence staining of 10T1/2 mouse fibroblasts in M-phase. top: Immunostaining under the same conditions as in Figure 1A (extraction with 0.1% NP-40 after fixation). HP1 α is distributed throughout the cell. Some overlap with the H3K9me3 mark on chromatin (visualized by DAPI staining, DNA) is observed. bottom: Immunostaining where the cells were extracted with 0.2% NP-40 before fixation reveals the persisting association of a fraction of HP1 α with the centromeres. (B) Mitotic chromosome spreads stained with an antiserum specific for the dual mark combination of H3K9me3S10ph. Red arrow-heads indicate the region around the centromere, which shows the strongest anti-H3K9me3S10ph staining. In addition, weaker dual mark staining is detected at a few sites on the chromosome arms. Inset: enlarged individual mitotic chromosome. Note that mouse chromosomes are acrocentric. (C) Schematic illustration of the localization of H3K9me3 (red), H3S10ph (green) and HP1 (yellow) on a mitotic chromosome. H3 is globally phosphorylated at serine 10, which interferes with HP1 binding to H3K9me3 and causes its release from mitotic chromatin. Via unknown mechanisms, a small fraction of HP1 α remains stably associated with the centromeres throughout mitosis.

While the new reports agree that H3S10 phosphorylation plays a seminal role in the dissociation of HP1 proteins from chromatin, it is not clear at this point whether this event is actually sufficient. Two studies found decreased binding of all HP1 isoforms

and in particular their chromo domains to synthetic H3 peptides containing the dual mark combination of H3K9me3S10ph⁵³ or to H3K9me3 peptides phosphorylated by recombinant Aurora B in *in vitro* binding experiments.⁵⁴ However, Terada using a purified

CPC complex detected almost no loss of binding of the full-length HP1 α protein from an H3K9me3 peptide upon H3S10 phosphorylation.⁵⁵ This behavior is in stark contrast to the induced dissociation of the chromo domains of all HP1 isoforms and of full-length HP1 β observed in the same enzymatic experimental set-up.^{53,55} More confusingly, another study detected an increased interaction of full-length HP1 α with peptides carrying the dual H3K9me3S10ph mark. Only the presence of an additional acetylation mark on H3K14 (H3K9me2S10phK14ac) abolished this interaction.⁵²

The discrepancies in the different studies might not only have originated from varying experimental systems, but might also reflect isoform-specific behavior of the HP1 proteins. Especially, the retention of a fraction of HP1 α at the centromere during mitosis has to be considered (see Fig. 2A). Mutational analysis indicated that the mitotic release of all three HP1 isoforms is depending on the chromo domain whereas the retention of HP1 α at the centromere requires sequences carboxyl-terminal to this region.⁴⁶ If phosphorylation of H3S10 is indeed sufficient to abolish interaction of the chromo domain of all HP1 isoforms with H3K9me3, globally releasing them from mitotic chromatin, then separate mechanisms must exist to anchor HP1 α at the centromere. Interestingly, HP1 α itself is subject to extensive post-translational modifications,⁴⁴ which appear to regulate its association with other chromatin components.⁶¹ In vitro, the CPC complex phosphorylates residues in the HP1 α hinge region (our unpublished observations), which might mediate the retention of this isoform at the centromere, possibly via interaction with the CPC and/or phosphorylated histone H3. In this scenario, the enzymatic activity of the CPC could be responsible for both, interruption of the HP1 chromodomain-H3K9me3 interaction with concomitant release of all HP1 isoforms from chromatin as well as retention of HP1 α at the centromere via chromodomain independent mechanisms and direct CPC-phosphoHP1 α interactions. Post-translational modifica-

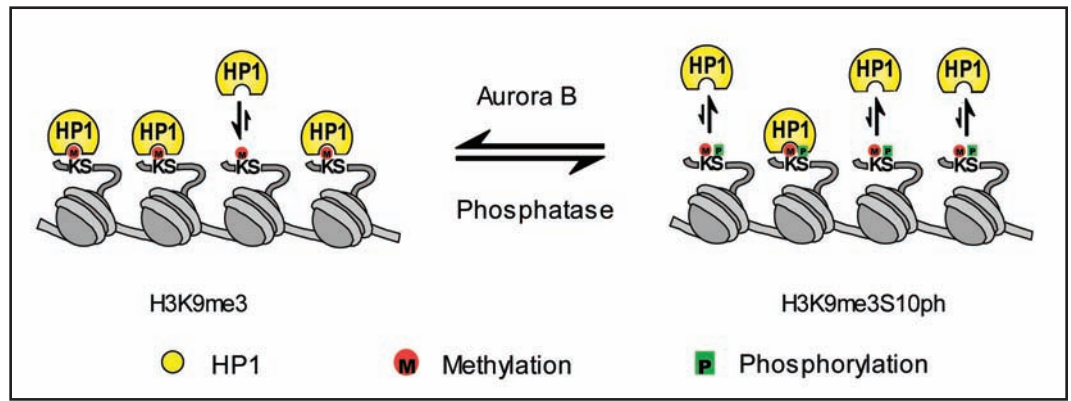


Figure 3. Principle of the binary methylation-phosphorylation switch. At the onset of mitosis, Aurora B mediates the phosphorylation of H3S10 right next to the HP1 binding site, H3K9me3. The additional modification causes a significant reduction in the affinity of HP1 to the H3 tail, therefore shifting the binding equilibrium between bound and unbound HP1 to the unbound state. At telophase, removal of the phosphate reestablishes HP1 binding. Thus, phosphorylation of H3S10 dynamically regulates HP1 binding to the stable H3K9me mark during the cell cycle.

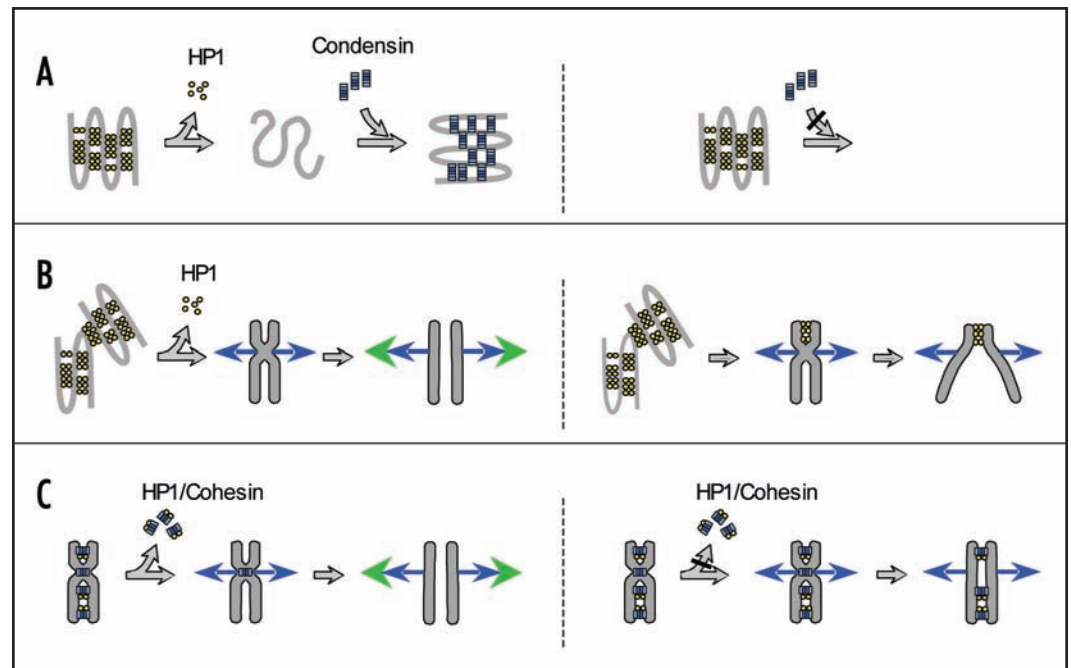


Figure 4. Steps during mitosis that may require release of HP1. (A) HP1 dissociation might be required for full mitotic chromatin condensation. left: In a cell that progresses through M-phase, HP1 is released at the onset of mitosis. This process allows condensin to access chromatin, therefore promoting mitotic chromatin condensation. right: If HP1 does not dissociate, condensin cannot get full access to chromatin and mitotic chromatin condensation is inhibited. (B) HP1 release may be necessary for proper resolution/segregation of chromatids. left: If HP1 is released at the onset of mitosis, the pulling forces exerted by the spindle microtubuli segregate the chromatids into the two daughter cells (as indicated by the green arrow heads). right: If HP1 is not removed, different chromatids (in this case sister chromatids) may remain attached to each other via HP1 cross-linking. This entanglement inhibits proper resolution of chromatids and may cause faulty chromosome segregation. (C) Dissociation of HP1 may be required for the release of condensin from chromosome arms. left: HP1 release at the onset of mitosis also removes cohesin, which is associated with HP1, from chromosome arms. This allows proper segregation of chromatids into the two daughter cells to occur. right: If HP1 is not released, cohesin remains associated with the chromosome arms. As a consequence, the chromatids cannot separate resulting in missegregation of chromosomes.

tion of HP1 might further regulate the functionality of the effector, switching from interphase chromatin compaction to specific functions in sister chromatin cohesion and centromere biology.

Evidently, more work is necessary to determine the degrees of overlapping and distinct biology of the three HP1 isoforms. Additional experiments are required to find out whether other

mechanisms besides methylation-phosphorylation switching impact on the mitotic release of HP1 proteins. Here, especially the exact role of additional histone modifications in the localization of the CPC and the release of the effectors need to be investigated.^{62,63} Further, the role of H3K9 demethylases in the regulation of HP1 chromatin association needs to be defined.

SIGNIFICANCE OF MITOTIC RELEASE OF HP1

Whereas the new studies are shedding light on the thus far enigmatic role of mitotic H3S10 phosphorylation,⁶⁴ the biological purpose of the dissociation of the H3K9me3 effectors during M-phase remains unclear. In this section, we will discuss three steps during mitosis that might require the dissociation of HP1: (1) condensin-mediated chromatin condensation, (2) proper resolution/segregation of chromatids and (3) removal of cohesin from chromosome arms.

The mechanism of mitotic chromatin compaction seems to be distinct from HP1-mediated heterochromatin formation. To permit mitotic chromosome condensation, HP1 proteins may need to be released from chromatin (Fig. 4A). One protein complex important for metaphase chromosome condensation is condensin, which is composed of two SMC subunits (Structural Maintenance of Chromosomes, members of a large family of ATPases with important roles in higher-order chromosome organization and dynamics (reviewed in ref. 65)), and three non-SMC subunits.⁶⁶ Although the exact mechanism of condensin action is still unclear, it binds to DNA and induces positive supercoiling.⁶⁶⁻⁷⁰ This process is thought to contribute to chromosome compaction. HP1 effector proteins might block the access of condensin to DNA, inhibit its binding, and thus interfere with condensation. Consistent with this model, in fission yeast *cut17* mutant cells defective in CPC function, condensin is not localized to mitotic chromosomes and chromosomes fail to properly condense.⁷¹ Centromeric localization of condensins in HeLa cells also requires Aurora B.⁷² Further, it seems that the timing of HP1 release is clearly before the start of condensin loading. The CPC-dependent release of HP1 may help condensin to function properly in mitotic chromosome condensation.

Possibly related to the function of condensins, HP1 proteins may have to dissociate from mitotic chromosomes to facilitate individualization of chromatids (Fig. 4B). During DNA replication, sister chromatids become topologically intertwined. Since sister chromatids attach to opposing poles of the spindle for their proper segregation during anaphase, any unresolved topological linkages will hold the chromatids together, leading to lagging chromosomes, chromosome bridges, and aneuploidy (reviewed in ref. 73). HP1 effectors might cross-link nucleosomes on sister chromatids during metaphase via dimerization or interaction with other chromatin components. If HP1 was not removed before anaphase, the consequence could be segregation defects similar to catenation. According to this model, mitotic release of HP1 proteins would be important for the resolution and separation of chromatids.

The release of HP1 effectors could also be required for the removal of cohesin from chromosome arms (Fig. 4C). The cohesin complex, similar to condensin, is comprised of two SMC subunits, which are accessorized by two non-SMC subunits.⁷⁴ It is required for establishing and maintaining the cohesion of sister chromatids until metaphase to anaphase transition.⁷⁵ In fission yeast, cohesin is recruited to heterochromatic domains, such as the pericentromeric region and the mating type locus, by the HP1 homologue Swi6.^{76,77}

While Swi6 is necessary for the cohesion at these heterochromatic domains, it is not required for the cohesion at chromosome arms, which persists in yeast until anaphase. In vertebrates, by contrast, a majority of the cohesin bound to the chromosome arms dissociates already in prophase, while only a small population of cohesin, including that at the centromere, stays associated with chromatin until anaphase.^{74,78,79} This prophase dissociation of cohesin is required for the efficient resolution of sister chromatids and is mediated in part by Plk1 and Aurora B.^{80,81} The mechanism by which Aurora B regulates the dissociation of cohesin is unknown, but HP1 proteins might be part of this pathway. The release of HP1 effectors from chromatin occurs at a similar stage of the cell cycle as cohesin dissociation.^{53,55} Since cohesin and HP1 proteins interact, cohesin removal could be accomplished by Aurora B via the ejection of HP1 effectors by H3S10 phosphorylation. The physiological function of the prophase-removal of cohesin from chromosome arms is, however, unknown at this point. While important for the resolution of sister chromatids, the dissociation of cohesin seems not to be essential for anaphase segregation of chromatids.⁸¹ Nevertheless, the function of HP1 release might be to mediate the removal of cohesin and thereby aid in the resolution of sister chromatids.

IMPLICATIONS OF MITOTIC HP1 SWITCHING

In recent years, a number of effector proteins for histone modifications have been identified.^{9,82} The search for additional effectors recruited by histone modifications and the regulation of their chromatin association are active fields of research. Besides binary switching,⁶⁰ several other mechanisms have to be considered to explain the binding and release of effector proteins to and from histone modification marks. These include enzymatic removal of the histone mark, replacement of modified histones, and proteolytic clipping of histone tails (see refs. 83 or 84). While these mechanisms might be used for certain effector/mark combinations and during specific cellular situations, it is intriguing that the mitotic release of HP1 proteins is accomplished by a methylation-phosphorylation switching mechanism. A closer look at this pathway reveals that it has certain characteristics that set it apart from the other mechanisms and that may be of particular importance in the specific cellular context of mitosis.

In methylation-phosphorylation switching, the recruitment of an effector protein is controlled solely by a kinase-phosphatase reaction. In many cases, kinases appear to control rapid changes in protein-protein interactions. Examples include the recruitment of adaptor proteins in signal transduction pathways,⁸⁵ the control of enzymatic activity via conformational changes⁸⁶ or the disassembly of the lamin network underlying the nuclear envelope during mitotic prophase.⁸⁷ In the context of the rapid chromatin changes required for the faithful progression through mitosis, a kinase reaction might be the most efficient way to achieve a highly regulated and fast global release of the HP1 effectors.

The removal of an effector protein by methylation-phosphorylation switching is exquisitely specific. The phosphate group interferes only with the association of proteins that bind in the direct vicinity of the phosphorylation site, while effector proteins binding at other regions of the histone are not affected. Histone exchange or tail clipping, on the other hand, may unspecifically remove many different histone marks and all the interaction partners associated with them.

At the same time, methylation-phosphorylation switching is readily reversible, as only the removal of a single phosphate group

by a phosphatase is required to reestablish binding. Consequently, rapid reassociation of HP1 can be accomplished at a minimal energy cost for the cell. In comparison, histone tail clipping or histone exchange would require large-scale resynthesis and specific methylation of H3 before HP1 proteins could reassociate with chromatin.⁸³

Lastly, methylation-phosphorylation switching fully preserves the epigenetic information encoded in the methyl-marks. This is in contrast to release of effector proteins by enzymatic removal of modifications through demethylation, which would completely erase the “epigenetic memory” of the H3K9 methyl-mark (reviewed in ref. 88).

HP1 SWITCHING IN OTHER CONTEXTS?

Besides their involvement in constitutive heterochromatin, HP1 proteins also localize to specific regions in euchromatin, where they can repress gene expression, as has been shown in flies and mammals.^{13,14,89-92} On a molecular level, several transcriptional repressors interact with HP1 effectors, such as TIF1 α (transcription intermediary factor 1 α),⁹³ TIF1 β /KAP1 (KRAB-associated protein 1),^{94,95} E2F,¹³ Rb (Retinoblastoma),¹⁴ and CtBP (C-terminal binding protein),⁹⁶ supporting the idea that HP1 has a role in local gene silencing. Interestingly, a recent study found that induced transcriptional activation of a silenced locus involves the dissociation of previously bound HP1 proteins.⁹⁷ Obviously, HP1-mediated gene silencing is reversible and mechanisms must have evolved to overcome this form of transcriptional repression.

In this context it is worth noting that phosphorylation of H3S10 has also been observed in gene regulation. Treatment of quiescent mammalian cells with mitogens triggers complex intracellular signals that result in rapid induction of gene expression and cell growth, a process called immediate early (IE) gene response. IE gene activation of different genes is associated with rapid phosphorylation of histone H3 (the nucleosomal response).⁹⁸ As opposed to the global, genome-wide phosphorylation of H3S10 during mitosis, this

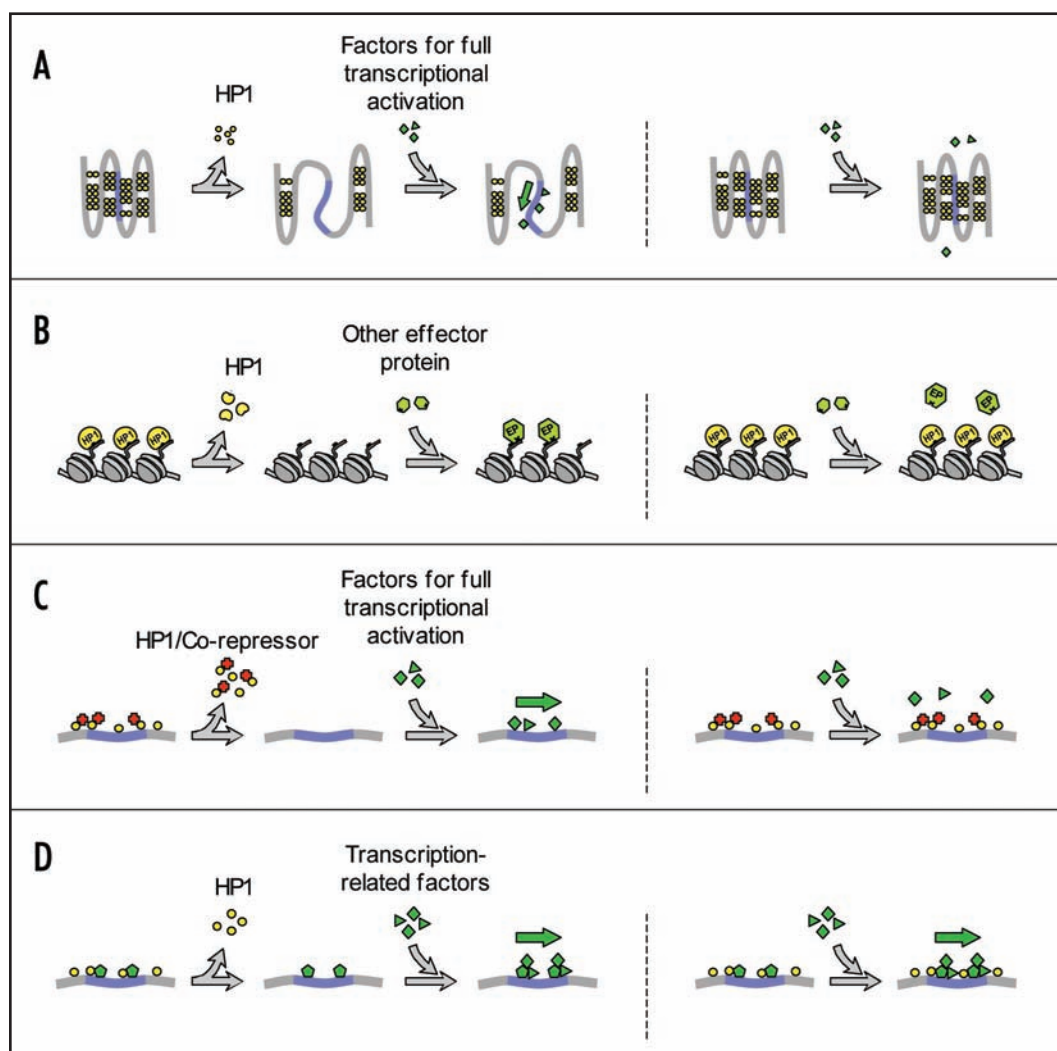


Figure 5. Mechanisms by which release of HP1 might facilitate transcription. (A) Dissociation of HP1 may be required for decondensation of a gene locus. left: HP1 release results in decondensation of a gene locus (depicted in blue). The open chromatin conformation of the decondensed locus allows access of factors required for full transcriptional activation (green arrow). right: If HP1 is not removed, the gene locus remains compacted. This significantly reduces the accessibility of the locus and thus impedes efficient transcription. (B) Dissociation of HP1 may be necessary to give full access to the histone tail. left: Upon release of HP1, the H3 tails become fully accessible to histone acetyltransferases, histone-modifying enzymes and other effector proteins (EP) that may be required for transcription. right: If HP1 remains associated with the histone tail, steric hindrance by HP1 obstructs full access to the H3 tail. (C) left: Dissociation of HP1 also results in the removal of associated transcriptional corepressors. Full gene activation (green arrow) is observed. right: Failure of HP1 to dissociate also leads to retention of associated corepressors. Transcriptional activation of the locus is inhibited. (D) HP1 dissociation might be required to counteract the inhibitory effects of HP1 on transcription-promoting factors. left: Certain factors essential for transcription are present at the locus, but are inhibited by their interaction with HP1. Upon dissociation of HP1, the factors are released and can contribute to rapid transcriptional activation of the locus. bottom: If HP1 remains associated with the locus, the transcription-promoting factors continue to be inhibited by their interaction with HP1. This antagonizes full transcriptional activation.

transcription-dependent phosphorylation is limited to a subset of genes and correlates with transcriptional activation.⁹⁹⁻¹⁰⁶ Several IE kinases targeting H3S10 have been identified. These include Msk1 (mitogen- and stress-activated kinase 1),¹⁰⁷ Rsk2 (ribosomal S6 kinase 2)¹⁰⁸ and IKK- α (I κ B kinase α).^{101,102}

At first sight, the correlation of H3S10ph with both, mitosis and gene activation, seems puzzling: the same histone modification correlating with two apparently opposing changes in chromatin structure. At the onset of mitosis, H3S10 phosphorylation correlates with chromatin condensation, while in gene activation it is likely linked

to the formation of a more open, accessible chromatin structure.¹⁰⁹ However, the suggested methylation-phosphorylation switching mechanism of HP1 might provide a possible explanation for this puzzle. While the changes of chromatin structure during mitosis and transcriptional activation are quite different, both situations might require the release of HP1 proteins or other repressors. Removal of HP1 effectors from repressed genes could at least in part be mediated via phosphorylation of H3S10.

Local loss of HP1 proteins might support transcriptional activation in several ways: (1) through changes in chromatin conformation, (2) by giving activating proteins full access to the N-terminal tail of histone H3, (3) by removing repressive factors recruited by HP1 proteins, and (4) by eliminating local inhibitory effects of HP1.

HP1 recruitment is believed to mediate gene silencing by reducing local chromatin accessibility and stabilizing a compact nucleosomal arrangement that confers transcriptional repression.³⁹ In reverse, release of HP1 proteins might lead to a more open chromatin structure. During transcriptional activation of a silenced transgene, the release of HP1 effectors indeed correlates with chromatin decondensation.⁹⁷ In a more open chromatin structure, general DNA accessibility will be enhanced, thus facilitating the recruitment of factors required for full transcriptional activation (such as histone acetyltransferases (HATs) and others, see below) as well as the transcriptional machinery itself. Therefore, the release of HP1 proteins might be an important step for rapid gene induction and efficient initiation of transcription.

Alternatively, dissociation of HP1 effectors from chromatin may be necessary to permit full access to the N-terminal tail of histone H3, which is otherwise partially blocked by bound HP1 (Fig. 5B). Several steps during transcriptional activation depend on direct access to the histone N-terminal tails. For example, most genes require acetylation of lysine residues in the N-terminal tails of histones H3 and H4 for full transcriptional activation.^{110,111} Histone acetylation is observed during immediate early gene activation,¹¹²⁻¹¹⁶ and localization of several proteins with histone acetylation activity to specific IE gene promoters has been reported, including CBP (CREB binding protein), p300, or PCAF (p300/CBP-associated factor).¹¹⁷ Whereas it had been initially suggested that H3S10ph could directly enhance subsequent histone acetylation by increasing the binding affinity of the histone tail for HAT complexes,^{115,118,119} recent results question such a simple interpretation.¹²⁰ Might it be possible that H3S10 phosphorylation facilitates transcription by removing repressors such as HP1 proteins, leading to improved access to the H3 tail, which in turn may support H3 acetylation? Besides HATs, there are other histone-modifying enzymes or effector proteins of H3 marks that might get better access upon release of HP1 proteins. These include effectors of H3K4me, a mark generally associated with active transcription.¹²¹⁻¹²⁴

Dissociation of HP1 proteins from repressed genes upon H3 phosphorylation could also promote transcription by removing corepressors, which are associated with HP1 (Fig. 5C). Several transcriptional repressors and corepressors have been shown to bind to HP1 (TIF1 α ,⁹³ TIF1 β /KAP1,^{94,125,126} Rb,¹⁴ Su(z)12,¹²⁷ HDACs¹²⁸). Such transcriptional repressors might dissociate together with HP1 proteins, thus contributing to efficient transcriptional activation.

Removal of HP1 proteins could alternatively promote gene expression by releasing inhibition of factors involved in transcriptional activation (Fig. 5D). For example, HP1 proteins have been shown to interact with TAF_{II}130, a component of the general

transcription factor TFIID.¹²⁹ It has been speculated that this interaction could keep TFIID in a repressed status, thus prohibiting activation while keeping TFIID poised for rapid transcription.¹²

Could H3S10 phosphorylation during gene activation, as it is observed in the IE response, indeed be part of a binary switch regulating HP1 association? Preliminary in vitro data support such a conclusion: Phosphorylation of premethylated H3 tail peptides by the IE kinase MSK1 proceeds with efficiencies comparable to those of unmodified peptides. Furthermore, in in vitro binding assays this phosphorylation is able to release recombinant HP1 effectors from methylated histone tail peptides (our unpublished results). Methylation-phosphorylation switching could allow the cell to rapidly regulate HP1 association with genes. Since IE genes must be shut down quickly again after the stimulus has faded, any regulatory mechanism has to be readily reversible. As outlined before, among the different pathways that can accomplish such a task, methylation-phosphorylation switching seems ideal for this purpose.

Besides mitosis and gene expression, switching might be important in other cellular processes. For example, HP1 proteins might have to be released for accurate progression of meiosis where phosphorylation of H3S10 has also been reported.¹³⁰⁻¹³³ Besides, HP1 methylation-phosphorylation switching might play a role in the definition of heterochromatic domains: In *Drosophila*, reduced levels of the JIL-1 H3S10 kinase result in spreading of HP1 effectors and H3K9me2 to ectopic locations on chromosome arms.¹³⁴ One possible explanation for this observation is that H3S10 phosphorylation by JIL-1 leads to HP1 switching, therefore preventing HP1 association with target sites and blocking the spreading of heterochromatin. Other instances where binding of effector proteins may be regulated by methylation-phosphorylation switching include the recently reported interaction of HP1 proteins with methylated linker histone H1.4,¹³⁵ the binding of Polycomb to methylated H3K27, or the interaction of CHD1 with methylated H3K4.¹³⁶ All of these methyl-marks are in close proximity of potential phospho-acceptors (H1.4 serine 27 (H1.4S27), H3 threonine 3 (H3T3), and serine 28 (H3S28)). Finally, the existence of "phospho/methyl switching" in nonhistone proteins remains a formal possibility (for a recent conceptual review of this general topic, see ref. 85).

CONCLUSION

Proteins specifically interacting with post-translational modifications play a crucial role in mediating the functionality of covalent histone marks.^{7,8,85} The biological outcome of a given post-translational mark is obviously not only depending on the presence of the modification, but especially on the recruitment and presence of a specific effector. As illustrated here on the example of the HP1 methylation-phosphorylation switch, cross-talk between different marks could be of central importance in regulating the binding of an effector protein to a particular histone modification.¹³⁷ With the high density of post-translational marks on the core histones,⁶⁰ it is likely that chromatin association of other proteins besides HP1 might be regulated in a similar way. Alongside multiple collaborating or counteracting histone marks, additional modulating mechanisms, such as post-translational modification and stability of effector proteins might further determine the level of read-out of a given mark.⁶¹ Whereas much has already been learned about the important regulatory functions of chromatin and its molecular mechanisms within the cell, it seems that there are many more layers of complexity of this regulatory system to be discovered.

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