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Chromatin remodeling during mitosis: a structure-based code?

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1. ABSTRACT

Histone modifications have been associated with particular states of transcriptional activity and are thought to serve as an "information code". However, this principle does not apply to histone phosphorylation, which can be detected in two, seemingly contrasting situations, i.e., in a transcriptionally hyperactive state following growth factor stimulation and in transcriptionally paused mitotic chromosomes. There are several indications that mitotic phosphorylation of histone H3 at serine-10 by the Aurora B kinase and trimethylation at lysine-9 by the methyl transferase Suvar3,9 operate as a "binary switch", which determines recruitment or eviction of heterochromatin-specific proteins from pericentromeric repeats. Moreover, threonine-3 phosphorylation of histone H3 by the newly identified haspin kinase seems to promote chromatid cohesion during mitosis. We discuss here emerging information and new ideas suggesting that these modifications, in combination to upstream and downstream marks, constitute a system of intrinsic folding determinants that facilitate chromatin condensation and confer topological specificity to mitotic chromosomes.

2. INTRODUCTION

Chromatin is a protean assembly: its molecular composition varies and its folding state differs, depending on nuclear locale. Loosely packed euchromatin, compacted heterochromatin and highly condensed mitotic chromosomes are only a few of the different chromatin "states" that are observed *in vivo*, yielding a colorful kaleidoscope of structures and interfaces that are difficult to describe in conventional cytological terms.

Chromatin is also a very dynamic entity. Its building blocks, the nucleosome core particles, exhibit "breathing", i.e., a perpetual, millisecond-scale wrapping and unwrapping of DNA around the histone octamer. They undergo reversible disassembly during the S phase and occasionally break in two semi-somes during transcription. Chromatin fibers entangle and disentangle, organize in loops of variable length and form catenated or extended structures, depending on cell cycle phase and differentiation state (1,2).

Is there a principle, by which we could understand and rationalize the polymorphic and dynamic

features of "living" chromatin? Perhaps there is: like protein folding, chromatin folding occurs in a hierarchical manner and is in part spontaneous and in part assisted. The basic plan for chromatin folding is determined by the (invariable) physico-chemical properties of histones and DNA. Thus, 147bp of DNA wrap around the core histone octamer ($(H3_2 \ . \ H4_2)$- $(H2A \ . \ H2B)_2$), forming 1.7 left-handed superhelical turns. A fifth histone (H1/H5) that sits at the points of entry-exit of the nucleosome assists the folding of another 20bp of DNA, so that the second superhelical turn is completed and exactly 167bp of DNA are wrapped around the histone octamer. Depending on nucleosome repeat length, the initial beads-on-a-string structure can form compacted fibers that have various diameters and contain from 6-11 nucleosomes /11nm (3,4).

Intrinsic parameters that likely play a role in the differential packing of chromatin are: (i) the local recruitment (or removal) of linker histones; (ii) the introduction of multiple post-translational modifications in the core histones; (iii) the replacement of the "standard" histones with specialized variants; and (iv) the methylation of DNA. On the other hand, extrinsic factors that possibly regulate chromatin organization include: (i) various chromatin remodeling machines; (ii) chromatin "modifies", such as Polycomb and HP1; (iii) "scaffolding" proteins, such as condensins and topoisomerase II; and (iv) specific "adaptors" that tether chromatin to the nuclear envelope or the kinetochore microtubules.

Of all the factors that play a direct or indirect role in chromatin folding, histone modifications deserve a special mention, because their combinatorial repertoire amounts to millions (5). And, as conventional wisdom has it, even if some of these modifications occur in clusters or obligatory combinations, we are still left with an astounding number of chromatin "colors" that have no apparent reason to exist, unless they serve some coding function.

The idea of a "histone code" has been proposed several years ago (6,7) and dominated the literature ever since. However, it is still not clear whether post-translational modifications represent signals that are "read" and "deciphered" by specific effectors, or whether these chemical alterations affect directly the physical chemistry of the chromatin components (8). In other words, we do not know whether the "histone code" is an information-based, or a structure-based, code.

One approach to distinguish between these two alternatives, that are not necessarily mutually exclusive, is to ask this question: would the mere modification of the core histones cause a change of chromatin state (e.g., the degree of condensation), or is it always necessary to have "readers" and "translators", which mediate this process? Or else, is chromatin itself the "reader" of its own modification, or is it just the repository from which the regulatory factors extract "instructions" and "information"? We tackle this problem here, discussing critically some new information on chromosome condensation during mitosis and chromatin folding *in vitro*.

3. CHROMOSOME CONDENSATION: THE ROLE OF EXTRINSIC AND INTRINSIC FACTORS

Chromosome condensation is a complex process that occurs with high precision and fidelity each time the cells enter mitosis. Pioneering work published in the ′90s has suggested that this process is not spontaneous. Instead, the ~500-fold compaction of intephase chromatin and the packaging into mitotic chromosomes requires the action of specific proteins, termed condensins (9). Condensins belong to the SMC family are now known to form two distinct complexes, I and II. Each complex contains two ATPases, Smc2-CAP-E and Smc4-CAP-C and three non-SMC subunits (reviewed by 10,11).

The condensin-based hypothesis dominated the literature for several years. Yet, new evidence indicates that key aspects of the original model should now be revised (12). Recent observations indicate that formation of uniformly condensed chromosomes at prophase precedes the recruitment of condensin subunits in the chromosomal axis, where they supposedly act (13). Furthermore, RNAi knockdown experiments show that normal levels of condensin I and II complexes are not required for condensation during prometaphase (14,15). In fact, after condensing depletion, chromosome condensation is delayed (16,17), but a metaphase alignment is eventually achieved and cells enter anaphase (14,18)

This does not mean that condensins are obsolete. In some capacity, condensin II is indeed required for normal chromosome condensation in early prophase and condensing I is necessary for normal timing of progression through prometaphase and metaphase and for complete dissociation of cohesins from chromosomal arms at a later stage (14). Recent studies demonstrate that whereas condensin I is constantly exchanged on and off chromatin, condensin II is stably bound to mitotic chromosomes (19). After depletion of condensin I, centromeres exhibit increased stretching and re-compaction in metaphase, suggesting impaired resistance against the pulling forces of the mitotic spindle. However, consistent with previous findings, even when both condensin complexes are depleted, stretched centromeres re-compact immediately after removal of the spindle forces, suggesting that these proteins maintain rather than establish the compaction state.

If condensins are not the main effectors of chromosome condensation, how is this process triggered at the beginning of mitosis? Mozziconacci et al. (20) have proposed an interesting new concept: that chromosome condensation could be initiated by an internal structural change of the nucleosome core particle, termed "gaping". Gaping involves the detachment of H2A/H2B dimers from each other, by loosening H2A-H2A, H2A-H3 and H2B-H4 contacts, leading to an opening of the particle in the manner of a gaping oyster (21). If this opening is followed by a twist of about 2 bp per linker, the external faces of neighboring nucleosomes come into close contact (stacking). And stacking of adjacent nucleosomes is exactly what would be required for transforming a "slender" chromatin fiber with approximately 6 nucleosomes per

11nm into a compact fiber, similar to that found in mitotic chromosomes, with 11-15 nucleosomes per 11nm.

Another idea that relates chromatin compaction to intrinsic structural parameters comes from *in vitro* reconstitution experiments. Robinson and co-workers have recently examined the effect of varying the length of the linker DNA (nucleosome repeat length) in the assembly of chromatin fibers. They have found that, depending on linker length, nucleosome arrays fold in two distinct ways, yielding either fibers with a diameter of 33nm and a density of 11 nucleosomes per 11nm, or fibers with a diameter of 44nm and a density of 15 nucleosomes per 11nm (4). Although this change in linear density does not account directly for miotic chromatin condensation, it does represent a classic example of how intrinsic structural elements can affect more global aspects of chromatin architecture.

4. INVOLVEMENT OF THE HISTONE TAILS IN HIGH-ORDER CHROMATIN FOLDING

Despite initial conclusions based on nucleosome reconstitution experiments (22), genetic experiments in yeast have shown that simultaneous deletion of histone H2A and H2B or H3 and H4 tails is lethal, while deletion of one from either group is not (23).

Early studies have suggested that removal of the core histone tails does not change significantly the structure or salt-dependent stability of nucleosome core particles below 0.7 M NaCl (24). However, dramatic alterations in the melting profiles of the nucleosome are observed upon selective removal of the tails by trypsinization, suggesting a role in the maintenance of thermal stability. Besides that, we now know that the tail domains are also required for proper folding of nucleosomal arrays into fully condensed chromatin fibers and for fiber–fiber self-association under conditions of physiological ionic strength (25,26,27).

Inter-nucleosomal interactions are believed to involve extensive histone tail bridging (28,29). Molecular Dynamics simulations indicate that under certain conditions histone tails can extend, bridge with one another and form condensed systems (30,31). H3 and H4 tails appear to be more involved than other histone tails in building attractive interactions and compacting chromatin, since the removal of both H3 and H4 tails prevents chromatin from complete folding (27,32). However, more recent analyses indicate an elaborate "division of labour": the H4 tails are more important for inter-nucleosomal interactions, especially in highly compact chromatin with linker histones, whereas the H3 tails are crucial in screening electrostatic repulsion between the entering-exiting DNA linkers. On the other hand, the H2A and H2B tails, situated as they are in the periphery of chromatin fibers, could be crucial in fiber-fiber interactions (33). Consistent with these predictions, H4 tails emanating from one nucleosome are seen to contact acidic patches of adjacent nucleosomes in crystals (34), while H3 tails have been shown to participate in intra-nucleosomal interactions when nucleosome arrays are decondensed and in inter-nucleosomal interactions upon salt-dependent folding (35). Finally, the prediction that the H2A-H2B tails mediate fiber-fiber interactions is in line with experiments demonstrating that these tails are crucial for oligomerization of nucleosomal arrays at high salt concentrations, where the H3 and H4 tails are dispensable (27,36).

It is widely believed that the end domains of the core histones are "floppy" and rather unstructured. Early work has shown that free histone tails exhibit random coil conformations in solution (37,38), while the amino- and carboxy-terminal regions of the histones cannot be traced in the X-ray structure of the nucleosome core (34). Despite that, structure predictions suggest that certain parts of H2A, H2B, H3 and H4 have a propensity to form α-helices (8), a hypothesis also supported by circular dichroism (39,40) and folding/unfolding (41) studies.

These views are not mutually exclusive. Employing a light scattering assay to study the structure of isolated mono-nucleosomes, Bertin et al. (42) have demonstrated that histone H3 and H4 tails are folded at low salt, but assume an extended configuration under conditions of higher ionic strength. Moreover, core particles deprived of both H3/H4 tails yield scattering curves that are indicative of a more opened conformation at the entry-exit DNA sides than the one seen in "wild type" nucleosomes. These observations lend support to an idea initially proposed by Hansen, *i.e.*, that the histone tails are able to adopt various different configurations, depending on the conditions and the binding interfaces that are available in their immediate neighbourhood (43). This property is known as "*intrinsic protein disorder*".

Intrinsically disordered proteins (IDPs) do not possess a defined 3-D structure on their own, but could adopt distinct conformations by associating with other molecules. In other words, this class of proteins exhibits a concerted folding-binding behaviour (reviewed in 44). This differs in a fundamental way from "induced fit", in which binding affects the equilibrium between two well defined protein conformations, and imparts distinct properties to IDPs: they can rapidly access the molecular environment and associate with a variety of targets through a "fly-casting" mechanism (45).

The molecular imprints of IDPs are two-fold. First, all these proteins show a bias in favour of specific hydrophilic amino acids (R, N, E, R, P and S) in their amino acid composition. Hydrophobic residues, especially C, I, L, F, W, Y, and V, are largely absent, permitting prediction of "intrinsic disorder" from the primary structure (PONDR algorithm; reviewed in 46). Second, IDPs are distinguished by their readiness to post-translational modifications, susceptibility to proteolysis and their spectral characteristics (sharp peaks in NMR, far-UV absorption typical of unfolded proteins) and their large radius of gyration, which resembles that of proteins that are fully denatured by urea or guanidinium salts (47).

Interestingly, the amino acid composition of H2A and H4 differs from that of H2B and H3 (43). Although the

significance of this dichotomy is not clear, at least H3 exhibits a typical IDP behaviour in solution: an extended, largely disordered peptide corresponding to the amino-terminal tail of this histone can acquire a defined conformation and fill in a β-sheet in several occasions (binding to the chromodomain of HP1 or Polycomb; 48, 49, 50). Specific associations of the amino-terminal tails of H3 and H4 to (adjacent) nucleosomes, Sir3p and p300 have also been demonstrated, but it is not entirely clear how this occurs and whether these associations involve a folding-binding process (51,52,53).

5. THE ROLE OF POST-TRANSLATIONAL MODIFICATIONS

Post-translational modifications are expected to affect profoundly the molecular features of IDPs, and, therefore, the properties of the histone tails. For instance, lysine acetylation, that eliminates positive charge and caps the residue by a hydrophobic methyl group, should decrease the "disorderly" character of the polypeptide chain, while addition of phosphate groups to serine and threonine residues is expected to promote intrinsic disorder. Methylation at lysine and arginine residues could affect protein structure in more subtle ways. In these cases, we have to deal with a spectrum of similar, yet different, modifications (mono-, di- and tri-methylation of lysine ε-amino groups and mono-methylation and symmetric or asymmetric di-methylation of the arginine guanido-group). Methylation does not erase positive charge, but replaces 1-3 amino-groups capable of hydrogen bonding with equivalent number of less polar methyl groups. All these alterations would affect dramatically the conformation of the histone tails in the "basic" (unbound) state and may promote the formation of defined structures by binding to chromatin proteins that happen to possess "strategically" located residues of like or opposite charge on their binding surfaces.

In the last few years, firm correlations between the histone modification status and the physical or transcriptional state of chromatin have been established. For example, histone H3 tri-methylated at lysine-9 and mono-methylated at lysine-27, as well as histone H4 tri-methylated at lysine-20, are enriched in constitutive heterochromatin, whereas H3 tri-methylated at lysine-4 and H4 acetylated in multiple sites are found primarily in the neighborhood of transcribed genes (54).

The simple rules that describe the relationship between histone methylation or acetylation with transcriptional activity do not apply to histone phosphorylation. This modification is observed in two seemingly contrasting situations: transcriptional activation after growth factor stimulation and transcriptional pause during mitotic condensation (for comments see 55).

The most extensively phosphorylated core histone is H3 (57,58,59). Serines-10, 28 and 31 (in the variant H3.3) are modified by various protein kinases, but mitotic phosphorylation is catalyzed by the Aurora B kinase (60,61,62,63,64,65,66). Aurora B is a chromosomal passenger protein localized in the inner centromere from late G2 through metaphase, at the spindle mid-zone of anaphase cells and at the post-mitotic bridge of telophase cells. It is activated by autophosphorylation upon binding to the inner centromere proteins and its depletion results in segregation defects and failure of cytokinesis (reviewed in 67,68).

Another site of mitotic phosphorylation of histone H3 is threonine-3 (69). This site is modified by the newly identified kinase haspin (70). Haspin represents a divergent member of the ePK superfamily that cannot be classified in any of the previously described protein kinase subgroups. It contains a divergent amino-terminal domain and a distinctive carboxy-terminal domain that exhibits serine/threonine kinase activity (reviewed in 71). At least one haspin form is nuclear in interphase and its overexpression inhibits cell proliferation (72). In transfected cells, EGFP-haspin or myc-haspin associates with chromosomes and is more concentrated at their centric regions during mitosis (70).

Finally, histone H3 is mitotically phosphorylated at threonine-11 by the Dlk/ZIP kinase (73). This is a kinase of unknown function that phosphorylates both nuclear (core histones) and cytoplasmic (myosin light chain) substrates (74). Normally, Dlk/ZIP is localized in the nucleus and interacts with transcription and splicing factors (75). However, under certain conditions the enzyme is retained in the cytoplasm and apparently participates in apoptotic pathways (74,76).

To this date, the functional significance of H3 phosphorylation remains unclear. Co-existence of lysine-9 tri-methylation and serine-10 phosphorylation is believed to provide a "binary switch", which determines recruitment or eviction of HP1 proteins from pericentromeric heterochromatin (77). This would justify the dissociation of most HP1 proteins from mitotic chromatin, but could not explain the persistent association of HP1α (one of the three HP1 variants) with the highly phosphorylated pericentromeric regions of metaphase chromosomes (78,79). Furthermore, there is now new evidence suggesting that lysine-9 tri-methylation in combination with serine-10 phosphorylation occur naturally in post-mitotic cells, in which HP1 remains bound to heterochromatin (80).

Van Hooser and co-workers have thoroughly examined the role of H3 phosphorylation in chromosome condensation. The results of this archetypical study suggested that serine-10 phosphorylation is required for the initiation of chromosome condensation at prophase (81). Consistent with this idea is the fact that Aurora B (the enzyme responsible for this modification) is also required for loading of condensin I onto chromosomes in prometaphase and for the maintenance of the complex on chromosomes in later stages of mitosis (82). However, when the enzyme is depleted, the cells exhibit mainly chromosome segregation defects and the effects on chromosome compaction are rather variable (83,63,84,16).

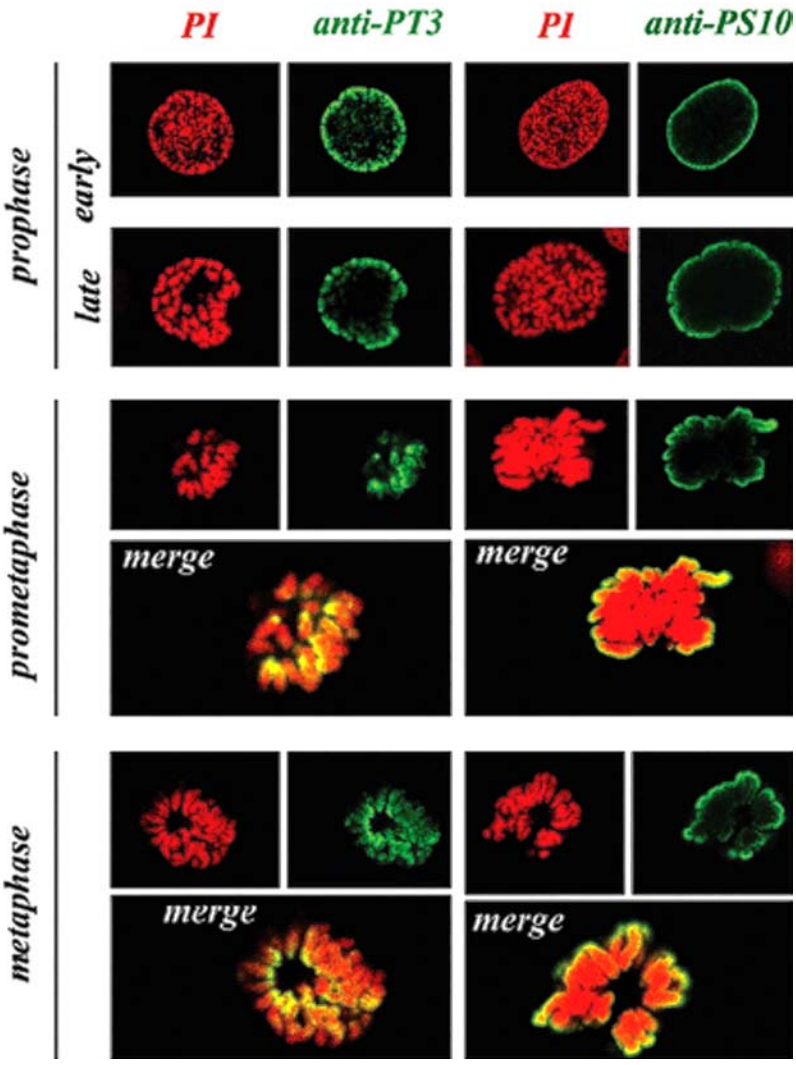

Figure 1. Comparison of threonine-3 and serine-10 phosphorylation of histone H3 during mitosis. Immunostaining of C127 cells with anti-phosphothreonine 3-specific antibodies (anti-PT3) or anti-phosphoserine 10-specificantibodies (anti-PS10). The phases of mitosis are indicated on the left. The specimens were counterstained with propidium iodide (PI). For clarity, merged images have been slightly magnified in relation to individual sections. Reproduced from (69).

Likewise, in *Xenopus* egg extracts, chromatin compaction is not particularly affected by Aurora B depletion (85).

Although at an early stage, the functional analysis of H3 threonine-3 phosphorylation (by manipulating the corresponding kinase, haspin) has already led to interesting results. When the levels of this enzyme are lowered by RNAi knockdown, there is a visible effect on sister chromatid cohesion (86). This effect can be explained in a number of ways. Haspin may phosphorylate a subunit of cohesin, rendering it resistant to proteolysis. Alternatively, haspin may collaborate with Sgot1 and Aurora B kinase to regulate sister chromatid cohesion during mitosis. Finally, the loss of cohesion may be a consequence of impaired centromeric/pericentromeric structure, which develops as a result of reduced threonine-3 phosphorylation. The latter idea is very tempting in view of the spatio-temporal pattern of threonine-3 phosphorylation during cell division.

The distribution of threonine-3 phosphorylated histone H3 is very similar to that reported for threonine-11 phosphorylated histone H3. However, examination of mitotic cells by confocal microscopy reveals a significant difference between serine-10 and threonine-3 specific phosphorylation. Although both modifications appear to commence at areas of prophase chromosomes neighboring with the nuclear envelope, upon progression of mitosis, the two signals become distinct: threonine 3-phosphorylated H3 is more concentrated in the central region of the metaphase plate than to chromosome arms, while serine-10 phosphorylated H3 is more prominent in the periphery of the metaphase plate (Figure 1; for more details see 69).

Thus, it is likely that threonine-3 phosphorylation, but not necessarily serine-10 phosphorylation, may contribute to chromatid cohesion by providing "sticking" surfaces or selective interaction sites around the centromeres (for more details on the potential mechanisms see below).

6. EPIGENETIC MODIFICATIONS AS DETERMINANTS OF CHROMATIN FOLDING

Could post-translational modifications, in particular H3 phosphorylation, affect chromosome compaction? From what we have discussed so far, it would be reasonable to deduce that phosphorylation could alter the status of histone tail bridging and, therefore, the regime of inter-nucleosomal interactions and the state of chromatin. However, the functional significance of each phosphorylation site might be different. For example, while *in vitro* experiments addressing the role of serine-10 phosphorylation in the compaction of nucleosomal arrays have been unsuccessful (50), molecular dynamics analysis addressing the role of threonine-3 phosphorylation that we have conducted in our laboratory has yielded very promising results: in contrast to unmodified H3 tails, amino-terminal peptides that bear phosphorylated threonine-3 and other modifications at neighboring residues seem to exhibit a strong tendency to form multiple hydrogen bonds and condense into oligomeric structures (Y. Markaki, A. Christogianni, G. Papamokos, A. S. Politou, and S. D. Georgatos, *in preparation*). A similar conclusion, on other phosphorylated H3 sites, has been reached in a recently published study (87).

A glance at the histone H3 sequence reveals that threonine-3 is a "strategically located" phosphorylation site, flanked by two residues (arginine-2 and lysine-4) amenable to methylation and forming a potential "methylation-phosphorylation-methylation" motif. Interestingly, this "cassette", is repeated in a permutated form a few residues downstream, where two methylation sites (arginine-8 and lysine-9) are followed by two phosphorylation sites (serine-10 and threonine-11) and then three sites (lysines 14, 18 and 23) that could exist either in a non-modified (positively charged), or in an acetylated (no charge) form. The quasi-regularly distributed, alternating charge in the amino-terminal end of the H3 molecule could be significant in two ways. First, when the tails are extended, patches of negative and positive charge may neutralize one another (intra- or, best, inter-molecularly), reducing the overall charge and promoting hydrophobicity. In this way, binding of two or more IDP-like H3 tails may result in the establishment of a distinct "fold" that stabilizes or enhances histone tail bridging. On the other hand, since H3 tails are thought to "screen" the electrostatic repulsion between the entering/exiting DNA linkers (33), phosphorylation might affect predominantly the bending of these regions and, therefore, the degree of inter-digitation in a zig-zagged chromatin fiber context (for a relevant model see 88).

That histone H3 phosphorylation affects inter-nucleosomal interactions or chromatin fiber packing in a direct way is a testable scenario that needs to be seriously considered in future studies. Nonetheless, another idea that deserves an equal amount of attention is whether H3 phosphorylation affects chromatin condensation indirectly, by promoting a gaping-like state, or stabilizing nucleosome gaping itself. Stabilization of the gaping state is necessary, because this is a metastable condition and has a significant energy cost (21).

How could that work? The model shown in Figure 2 postulates that phosphorylation of histone H3 tails will affect the entry-exit angle of the linker DNA, because, as we discussed previously, the charged H3 tails have a unique tendency to attach to the stretch that enters and exits the nucleosome core. This change of linker angle might then allow the optimum stacking of adjacent nucleosomes, without extreme bending of the linker regions, as required in the original nucleosome gaping model proposed by Mozziconacci-Victor (cf. Figure 2 in 21).

By means of the same charge-neutralizing effect, phosphorylation of H3 tails may loosen the wrapping of DNA around the core particle. This, in turn, might de-stabilize the two H2A-H2B dimers (whose binding to the H3-H4 tetramer is DNA-dependent), decrease the overall "cohesion" of the particle, and promote a gaping-like state.

The intelligent feature of the latter model, *i.e*., the phosphorylation-dependent nucleosome gaping, or a gaping-like state, with a subsequent compaction of the chromatin fiber, lies in its simplicity: phosphorylation does not need to "sweep" the entire H3 molecule or the entire surface of the chromosomes, as required in the previous hypothetical model (where "structure-forming" interactions among IDP-like histone tails would require "saturating" levels of histone H3 phosphorylation). Instead, even the partial phosphorylation at "strategic" sites could easily trigger the process and initiate chromosome condensation at the beginning of mitosis. In this manner, the reversal of condensation, *i.e.*, post-mitotic chromatin de-condensation, would also be facile: "editing" signals that are based on site-specific phosphorylation is "standard procedure" at late phases of cell division, when most of the mitotic kinases lose their activity and powerful protein phosphatases gradually take over.

7. SUMMARY AND PERSPECTIVE

From what we have discussed here, it looks that mitotic histone modifications will be a major focus of interest in future studies. Central in this endeavor will be, in our opinion, to examine the role of epigenetic marks in nucleosome gaping. This is clearly an interesting mechanism for compacting chromatin and may have a bearing to mitotic chromosome condensation.

However, as explained in previous sections, chromosome condensation is a very complex process and not a simple compaction of the chromatin fiber. There is kinetics to it, there is dynamics and there is also a spectrum of unresolved issues that concerned the sub-structure of mitotic chromosomes. Most of these questions have not been adequately answered yet. For instance, it is not clear whether or not condensed chromosomes carry the

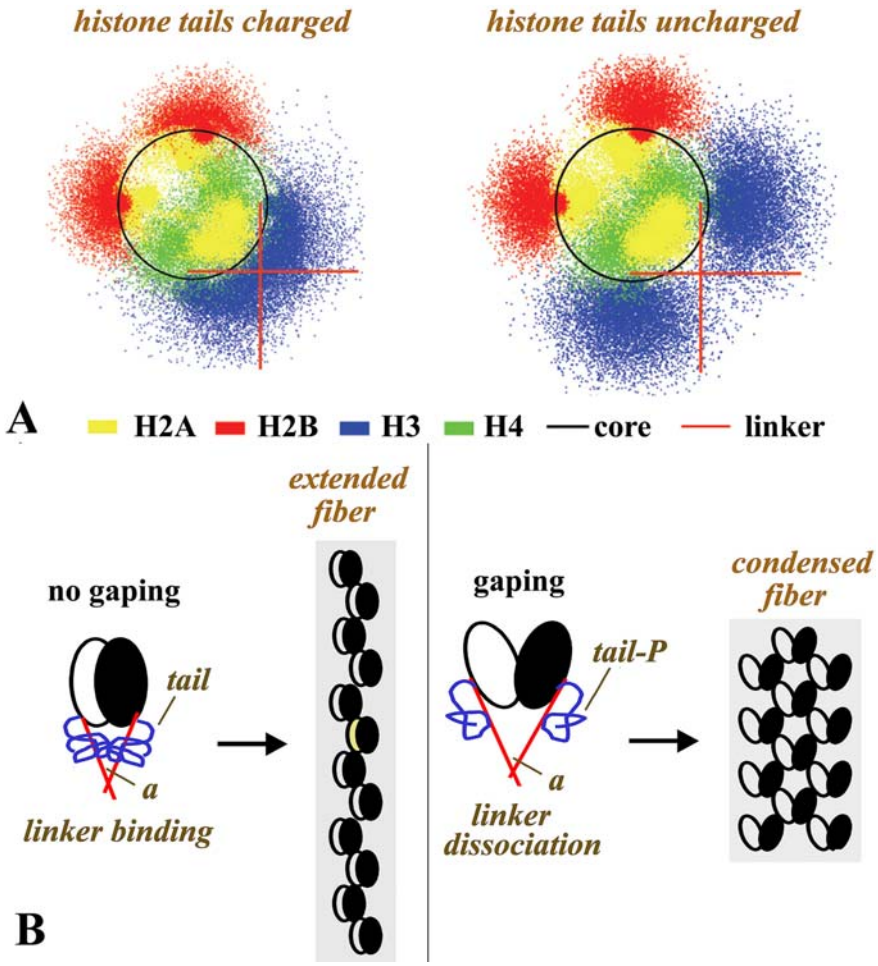

Figure 2. Hypothetical model showing how mitotic phsophorylation of the histone H3 tail could promote nucleosome gaping. (A) Charge neutralization alters the disposition of the core histone tails along the surface of the nucleosome (original data, as described in (33). (B) We postulate that mitotic phosphorylation of histone H3 eliminates the quasi-regularly repeated, alternating charge of its amino-terminal tail (blue line), leading to dissociation from the linker region (red line). In this manner, the entry-exit angle (α) is increased, a process that might facilitate or stabilize the partial opening of the core particle (white and black ovals) and promote chromatin fiber compaction as proposed in (20,21). For more details see text.

"memory" of cell-type specific epigenetic modifications. Are mitotic chromosomes from circulating lymphocytes and, say, fibroblasts *exactly* the same? Are the "hills" and "valleys" on the surface of a specific chromosome (*e.g.* human chromosome 1) superimposable, irrespective of cell type? And how histone modifications contribute to chromosome fine structure and diversity?

To tackle these issues, we need both, better *in vivo* assays and more precise structural data on multimeric chromatin assemblies (*e.g.*, nucleosome arrays, chromatin fibers, *etc*). Manipulating the genes that control mitotic modifications of the histone proteins maybe an avenue towards the first objective. As for the second one, it is clear that we cannot do without sophisticated biophysical methods (*e.g.*, use of optical tweezers and atomic force microscopy). Assessing the role of intrinsic and extrinsic factors in chromatin condensation and understanding whether histone modifications operate as a structure-based code is a difficult task and the tools required to confront this problem will not be conventional.

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