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Chromosome condensation and decondensation during mitosis

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During eukaryotic cell division, nuclear chromatin undergoes marked changes with respect to shape and degree of compaction. Although already significantly compacted during interphase, upon entry into mitosis chromatin further condenses and individualizes to discrete chromosomes that are captured and moved independently by the mitotic spindle apparatus. Once segregated by the spindle, chromatin decondenses to re-establish its interphase structure competent for DNA replication and transcription. Although cytologically described a long time ago, the underlying molecular mechanisms of mitotic chromatin condensation and decondensation are still ill-defined. Here we summarize our current knowledge of mitotic chromatin restructuring and recent progress in the field.

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Introduction

The changes occurring during cell division, especially the fate of chromatin, have been fascinating biologists since the 19th century when chromatin could be visualized with novel cellular staining techniques. The iconic structure of X-shaped mitotic chromosomes not only decorates numerous covers of scientific journals but is nowadays also firmly anchored in common knowledge. Nevertheless, how this structure is formed remains highly controversial despite years of intensive efforts.

In animal cells, the nuclear envelope breaks down with the onset of mitosis so that a variety of cytoplasmic proteins can access chromatin. Although chromatin changes are already detectable before nuclear envelope breakdown, this leads to further chromatin condensation and allows assembly of the mitotic spindle, which will capture, move and align the individualized chromosomes at the metaphase plate and segregate the disengaged chromatids. At the end of mitosis a nuclear envelope reforms around the segregated and decondensing chromatin in each of the emerging daughter cells. Although in some yeast species the nuclear envelope is not dis-assembled and reassembled during mitosis, cytosolic factors similarly get access to the nucleoplasm with entry into mitosis allowing chromatin condensation and intra-nuclear spindle formation to occur. After chromatin segregation, the compacted chromatin is similarly decondensed to reestablish its interphase state.

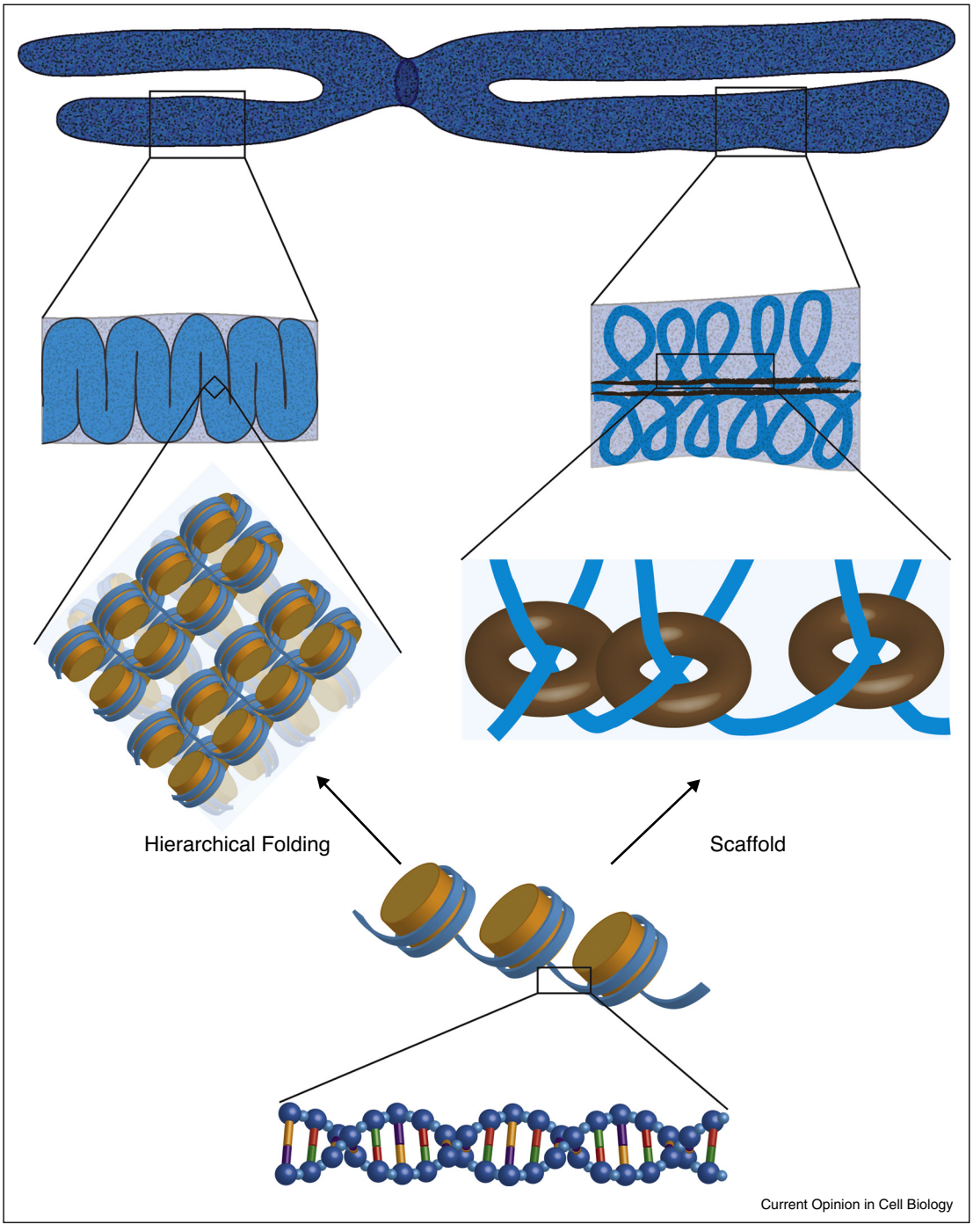
Although chromatin condensation in mitosis is, at least in animal and plant cells, already apparent by light microscopy, assessments of the degree of compaction of mitotic chromatin in relation to its interphase state differ considerably, from two to fiftyfold as estimated by volume occupancy of an EGFP-H2B signal or by distance measurements of chromosome loci, respectively [1,2]. Various models attempting to explain how mitotic chromatin is organized have been suggested. These models fall mainly into two broad categories (Figure 1): one class proposes that the DNA is hierarchically folded into increasingly higher order structures (e.g. [3,4]). The second class, which follows pioneering work of the Lämmli laboratory, suggests that mitotic chromatin forms series of loops which are attached to a central chromosome scaffold axis (e.g. [5]). Although at first sight the two models appear incompatible, it is possible that both organizational principles coexist in different domains of a condensed chromosome, for example, if radial loops consist of hierarchically folded fibers. In addition, both models could describe chromatin structure at different stages of mitotic compaction. For example, hierarchical folding might transiently contribute to initial condensation giving rise to chromatin structures which then locally melt but are arranged in radial loops.

Thus, the question of how mitotic chromosomes are structured is far from being resolved. We will nevertheless summarize our current knowledge about chromatin condensation and decondensation during mitosis integrating recent results both from yeast and animals.

Chromatin condensation

In early prophase of metazoan mitosis, the homogenously distributed chromatin of interphase begins to

Figure 1



Structure of mitotic chromosomes. Two classes of models have been proposed to describe the structure of mitotic chromosomes. Hierarchical folding models (left) suggest that chromatin fibers are folded into consecutive higher-order-structures starting from initial 11-nm-fibers ('beads-on-a-string'). The scaffold model (right) predicts the existence of a continuous, proteinaceous core at the center of chromosome arms to which loops are attached.

form visible thread-like structures. The driving force for this initial phase of chromatin compaction is highly debated. Condensin complexes play an important role during this stage of condensation since their depletion delays the process [6–8]. However, the details of their activation and their mechanistic contribution are far from clear.

The pentameric condensin complexes are composed of two proteins of the structural maintenance of

chromosomes (SMC) family and three non-SMC subunits, a kleisin and two HEAT repeat containing proteins. SMC proteins form long anti-parallel coiled coils flanked by an ATPase head domain and a hinge domain which is required for complex formation between SMC subunits [9]. The head domains interact with the kleisin subunit, so that both SMC and the kleisin subunit form a closed ring, while the HEAT-repeat proteins associate with the complexes largely through their interaction with their kleisin subunits [10,11]. This ring may entrap two strands of DNA from the same chromosome similar to the way cohesin (a structurally related complex involved in sister chromatid cohesion, DNA repair and transcriptional regulation [12]) encircles sister chromatids [13••,14••]. In addition to kleisin mediated ring formation, the ATPase domains of the SMC proteins interact with each other upon ATP binding and dissociate upon its hydrolysis [15]. The purpose of this ATPase cycle is largely unclear; however, interfering mutations abrogate condensin function *in vivo* [16]. The recent reconstitution of mitotic chromosome assembly using only six purified components may help to shed light on the mechanism of condensin function [17••].

Condensin mediated looping of linear chromatin segments with a size of 80–120 kb has been suggested to represent the initial event in a multi-step process [18] (Figure 2). This model is supported by polymer simulations and the analysis of mitotic chromosomes by chromosome conformation capture experiments [19••]. The size of these loops may be restricted by the distance between consecutive cohesin binding sites or be due to condensin-inherent properties [18]. Later in M phase, axial compression of chromosome arms requires further condensin activity in combination with sister chromatid resolution, which is mediated by topoisomerase IIα and the release of cohesin by the 'prophase pathway'. All metazoan species studied so far possess two different condensin complexes, condensin I and II, that differ in the non-SMC subunits. Whereas the condensin II complex is found on chromatin throughout mitosis, the condensin I complex will bind to chromosomes only after nuclear envelope breakdown [20]. Both types of condensins contribute to chromosome condensation with disparate effects on shape [21–23]. The latter may participate in condensation by stabilizing the condensed state and by compressing protruding fibers that have escaped condensin II action to promote lateral compaction.

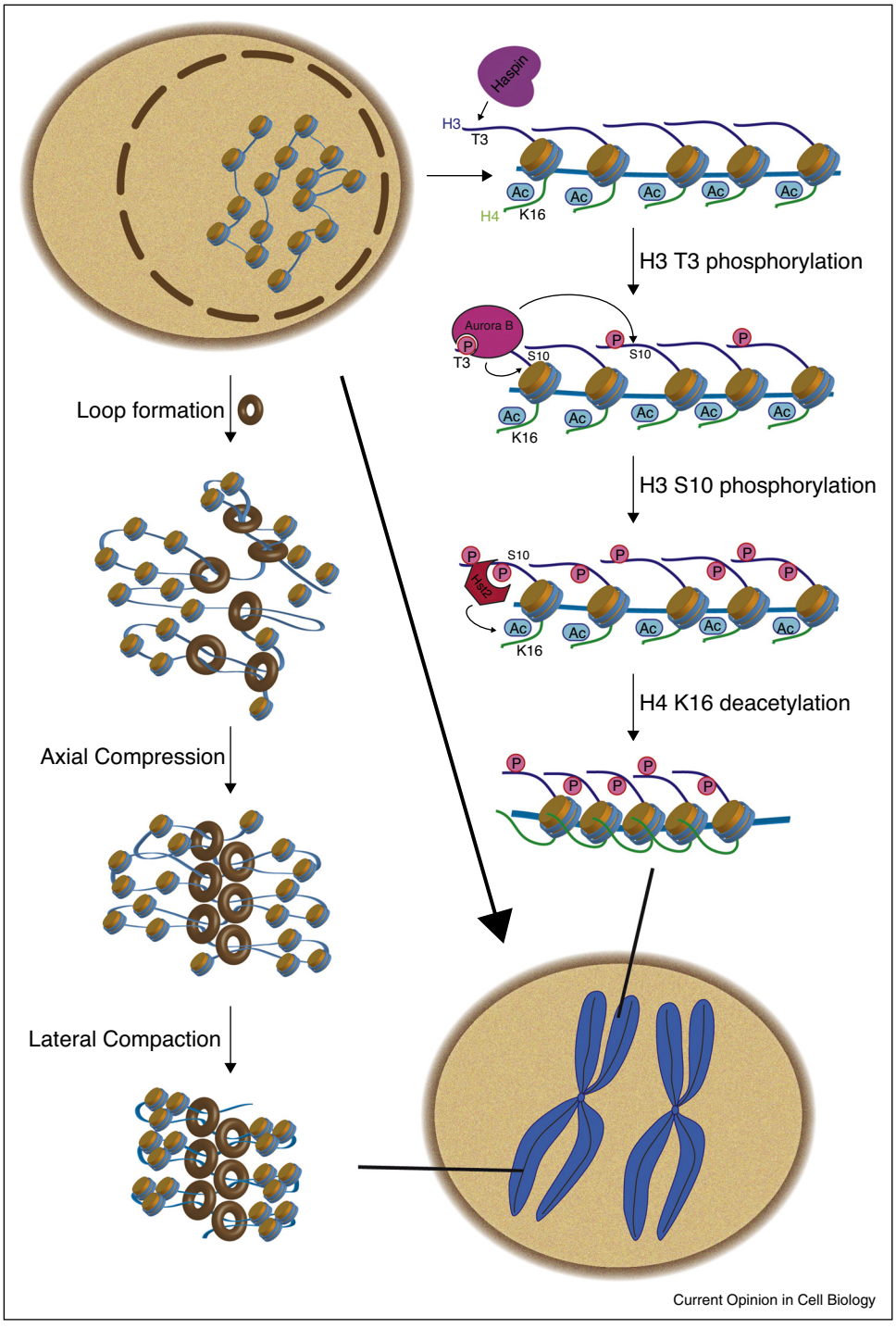
Controversy about the mechanism of mitotic chromosome condensation comes from the fact that condensed chromosomes still form in cells depleted of condensins, although with strongly reduced structural integrity [8,20,24]. Condensin inactivation interferes with the condensation of the rDNA locus in mitosis of budding yeast [25–29]. In fission yeast, temperature-sensitive mutants of the SMC proteins fail to condense and segregate chromosomes in mitosis but remain able to perform spindle elongation and cell division [30]. Analysis of mitotic condensation by fluorescence microscopy revealed an almost complete loss of condensation at restrictive temperature [31].

Condensation in animals is also affected, although not abolished, by depletion of condensin subunits. In vertebrates, initial condensation in prophase precedes condensin recruitment to the central axis, which may be interpreted as initially condensed states being stapled together by the association of condensin [32]. Shutting down expression of SMC2 in chicken DT40 cells [8,24] or knock-down by RNAi in *Caenorhabditis elegans* [6] severely impaired mitotic chromosome architecture but had only limited effects on condensation per se. This may be the result of incomplete condensin depletion in these systems, although protein levels were reduced beyond detectability. Recently, using floxed alleles of kleisin subunits, Nasmyth and colleagues showed that condensin II is essential for chromosome structure and rigidity in mouse meiosis [33••]. However, even in the almost complete absence of both condensin isoforms, interphase chromatin compacted into clumps.

The observation that condensation is almost normal after condensin depletion in chicken DT40 cells led to the postulation of a factor called 'regulator of chromosome architecture' (RCA) [24]. Indeed, several other proteins have been described as chromatin condensation factors, but their function is even less clear than that of condensins. Experiments in fission yeast have implicated a requirement for topoisomerase IIα in addition to its decatenating activity in chromatin condensation [34]. This agrees with work from the Lämmli lab, which identified the protein as a major non-histone component of the scaffold [35,36] involved in chromatin condensation [37,38]. Additionally, drugs targeting topoisomerase II partially inhibit chromatin condensation [39,40]. However, knockdown of topoisomerase IIα in fly and human cell lines impair chromosome segregation, but do not result in prominent condensation defects [41,42]. Chromokinesin KIF4 was shown to cooperate with condensin to drive axial shortening of chromosome arms [43]. However, the mechanism by which KIF4 acts is presently unclear.

Condensin-independent condensation is probably driven by a chromatin-inherent attractive force controlled by post-translational modifications on histone tails [44••] (Figure 2). This attraction between neighbouring nucleosomes is generated by binding of H4 tails to the acidic patch of another nucleosome, an interaction that is well-characterized *in vitro* [45,46]. Installation of UV-activatable cross-linker amino acids in yeast histones demonstrated that this interaction is predominantly present in mitosis [44••]. During interphase, the interaction is prevented by acetylation of H4 K16 [45,46]. Early in

Figure 2



Potential mechanism of mitotic chromosome formation as a multi-layered process. Condensin-driven condensation (left) in prophase leads to loop formation, which are subsequently compacted in axial and lateral direction. Histone-driven condensation (right) promotes local chromatin compaction mediated by interactions between neighbouring nucleosomes and controlled by post-translational modifications.

mitosis, phosphorylation of H3 T3 by Haspin and subsequently of H3 S10 by Aurora B kinase recruits the lysine deacetylase Hst2p to deacetylate H4 K16, triggering chromatin condensation.

This mechanism probably also acts in animals and plants since all the factors are evolutionarily and functionally conserved. It will be interesting to learn how this pathway cooperates with the canonical condensation machinery.

Inactivating condensin in budding yeast by employing a temperature-sensitive condensin allele does not prevent the association of the H4-tail with the acidic patch of another nucleosome, suggesting that chromosome architectural changes are dispensable for this interaction [47]. Vice versa, inactivation of Hst2 affects chromosome condensation by mechanisms beyond controlling the acetylation state of H4 K16 [47]. We postulate that condensin-mediated and histone-mediated condensation act in parallel at different levels of chromosome architecture. Although condensins drive large-scale rearrangements by loop formation, association between nucleosomes drives close-range chromosome compaction, for example within such loops. It seems likely that these mechanisms communicate to orchestrate the condensation process.

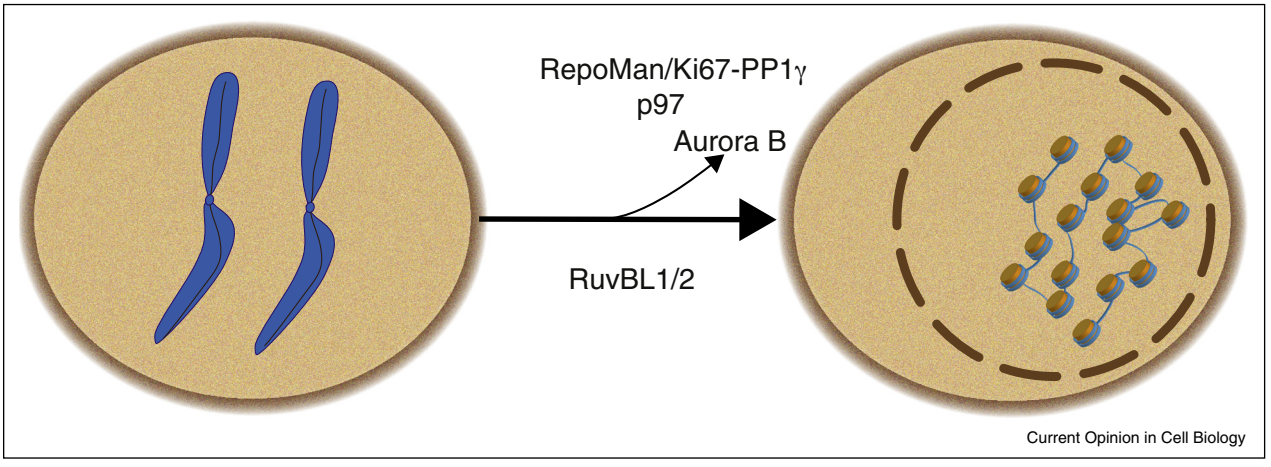
In animal cells, chromosomes reach their highest compaction level in anaphase during segregation [48]. This additional longitudinal condensation may prevent cleavage of lagging chromosome arms during cytokinesis. In budding yeast, the extent of this compaction increases with chromosome arm length, resulting in adaptation of the compaction level to the size of the spindle [49••]. This process depends on the kinase Aurora B and is driven by a direct attractive interaction between nucleosomes [44••].

Chromatin decondensation

In late anaphase and telophase the mitotic chromatin decondenses to re-establish its interphase structure (Figure 3). Decondensation is not a simple reversal of events leading to condensation. Whereas entry into mitosis is largely executed by mitotic kinases, mitotic exit requires both inactivation of the kinases and reversion of mitotic phosphorylation. In yeast, mitotic exit is driven by the phosphatase CDC14. In animal cells, PP1 and PP2A protein phosphatases are crucial players in this process. Depletion of PP2A as well as its regulatory subunit B55α and the scaffolding subunit R1a delay mitotic exit [50]. Whether this phosphatase has a direct role in chromatin decondensation is unclear. PP1γ dephosphorylates among others the mitotic histone marks Thr3, Ser10 and Ser28 on histone H3 [51]. It is tempting to speculate that these histone marks need to be reverted to trigger reacetylation of H4 K16 (and probably other downstream events) to allow chromatin decondensation. PP1γ is recruited to anaphase chromosomes by its targeting subunit Repo-Man [52]. Depletion of Repo-Man affects nuclear envelope formation but does not detectably impair chromatin decondensation [53]. However, PP1γ is also recruited to decondensing chromosomes by Ki-67 [54] and it is possible that this function can in part compensate for the loss of Repo-Man. PP1α is recruited to chromatin later, just before nuclear envelope enclosure, by its targeting subunit PNUTS. It has been suggested to regulate chromatin decondensation [55] but its precise targets and mechanisms remain elusive.

In addition to the action of phosphatases eliminating mitotic phosphorylations, removal of the mitotic kinase Aurora B from chromatin is required for chromatin decondensation [56••]. The AAA$^+$-ATPase p97, together with its cofactors UFD1 and NPL4, is required for this. As this machinery usually recognizes ubiquitinylated proteins, it is likely that Aurora B is ubiquitinylated at the end of mitosis. If so, the responsible ubiquitin ligases would be an interesting target to identify to understand the regulation of this process. As indicated above, the main mitotic target of Aurora B is Ser10 of Histone H3, but it is unlikely that preventing this phosphorylation is sufficient for chromatin decondensation. It is more likely that Aurora B has to be removed to prevent phosphorylation of other yet to be defined targets to allow for chromatin decondensation. For

Figure 3



Chromatin decondensation in animal cells: p97 removes Aurora B from mitotic chromatin, PP1γ is recruited to chromatin via Repo-Man and Ki-67 and dephosphorylates, among other potential targets, histone H3. The function of RuvBL1/RuvBL2 on decondensing chromatin still needs to be defined.

example, Aurora B phosphorylates and by that regulates condensin complexes [57] and it is tempting to speculate that removal of this kinase is required for condensins' activities to cease during mitotic exit.

Using a cell-free assay that recapitulates chromatin decondensation with Xenopus egg extracts, a second class of ATPases were identified as involved in the process, RuvBL1 and RuvBL2 [58••]. Both AAA-ATPases, also known as pontin and reptin, form a double hexameric complex that functions in a variety of cellular processes. Depletion of the complex renders the egg extracts incompetent for chromatin decondensation. Re-addition of the wildtype proteins, but not ATPase deficient mutants, rescues the depletion phenotype indicating that the ATPase-function of the complex is critically involved. The precise mechanism of how RuvBL1/2 function in chromatin decondensation remains to be resolved. Loss of condensin I complex, but also Histone H3 Ser10 phosphorylation, is unaffected by RuvBL1/2 depletion indicating that these ATPases are involved in another, probably later step. The fact that these ATPases are part of chromatin remodeling complexes could hint to a similar function in chromatin decondensation.

Summary and outlook

Although essential events in the eukaryotic life cycle, our understanding of the mechanisms of mitotic chromatin condensation and decondensation remains vague. Chromosome condensation appears to be a multi-layered process with different mechanisms operating at different levels. Condensins are key players but their precise contribution/function remains controversial. H4-tail mediated nucleosome interactions, regulated by the deacetylation of H4 K16, are important for chromatin condensation in budding yeast, and it is likely that similar mechanisms also contribute to mitotic chromatin compaction in metazoans.

Chromatin decondensation is embedded in the mitotic exit regulatory network. Phosphatases are presumably required to revert mitotic phosphorylations on key factors, but the nature of these targets remains to be identified. AAA-ATPases are involved in chromatin decondensation: p97 functions in removing Aurora B from chromatin; a complex of RuvB-like ATPases, RuvBL1 and RuvBL2, is crucial for chromatin decondensation probably due to its chromatin remodeling activity.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Vagnarelli P: **Mitotic chromosome condensation in vertebrates**. *Exp Cell Res* 2012, **318**:1435-1441.

2. Belmont AS: **Mitotic chromosome structure and condensation**. *Curr Opin Cell Biol* 2006, **18**:632-638.

3. Hansen JC: **Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions**. *Ann Rev Biophys Biomol Struct* 2002, **31**:361-392.

4. Maeshima K, Eltsov M: **Packaging the genome: the structure of mitotic chromosomes**. *J Biochem* 2008, **143**:145-153.

5. Paulson JR, Laemmli UK: **The structure of histone-depleted metaphase chromosomes**. *Cell* 1977, **12**:817-828.

6. Hagstrom KA, Holmes VF, Cozzarelli NR, Meyer BJ: ***C. elegans* condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis**. *Genes Dev* 2002, **16**:729-742.

7. Kaitna S, Pasierbek P, Jantsch M, Loidl J, Glotzer M: **The aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis**. *Curr Biol* 2002, **12**:798-812.

8. Hudson DF, Vagnarelli P, Gassmann R, Earnshaw WC: **Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes**. *Dev Cell* 2003, **5**:323-336.

9. Anderson DE, Losada A, Erickson HP, Hirano T: **Condensin and cohesin display different arm conformations with characteristic hinge angles**. *J Cell Biol* 2002, **156**:419-424.

10. Neuwald AF, Hirano T: **HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions**. *Genome Res* 2000, **10**:1445-1452.

11. Onn I, Aono N, Hirano M, Hirano T: **Reconstitution and subunit geometry of human condensin complexes**. *EMBO J* 2007, **26**:1024-1034.

12. Jeppsson K, Kanno T, Shirahige K, Sjogren C: **The maintenance of chromosome structure: positioning and functioning of SMC complexes**. *Nat Rev Mol Cell Biol* 2014, **15**:601-614.

13. •• Cuylen S, Metz J, Hruby A, Haering CH: **Entrapment of chromosomes by condensin rings prevents their breakage during cytokinesis**. *Dev Cell* 2013, **27**:469-478.
See note Ref. [14••].

14. •• Cuylen S, Metz J, Haering CH: **Condensin structures chromosomal DNA through topological links**. *Nat Struct Mol Biol* 2011, **18**:894-901.
Refs. 13 and 14 provide evidence for the ability of condensin to topologically enclose DNA in living cells.

15. Lammens A, Schele A, Hopfner KP: **Structural biochemistry of ATP-driven dimerization and DNA-stimulated activation of SMC ATPases**. *Curr Biol* 2004, **14**:1778-1782.

16. Hudson DF, Ohta S, Freisinger T, Macisaac F, Sennels L, Alves F, Lai F, Kerr A, Rappsilber J, Earnshaw WC: **Molecular and genetic analysis of condensin function in vertebrate cells**. *Mol Biol Cell* 2008, **19**:3070-3079.

17. •• Shintomi K, Takahashi TS, Hirano T: **Reconstitution of mitotic chromatids with a minimum set of purified factors**. *Nat Cell Biol* 2015, **17**:1014-1023.
Reconstitution of mitotic chromosome condensation using six purified factors (histones, three histone chaperones, topoisomerase II and condensin I).

18. Kschonsak M, Haering CH: **Shaping mitotic chromosomes: from classical concepts to molecular mechanisms**. *Bioessays* 2015, **37**:755-766.

19. •• Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA, Dekker J: **Organization of the mitotic chromosome**. *Science* 2013, **342**:948-953.
Chromosome conformation capture identifies a folding patter in mitotic chromosomes consistent with scaffolding models proposing consecutive chromatin loops.

20. Gerlich D, Hirota T, Koch B, Peters JM, Ellenberg J: **Condensin I stabilizes chromosomes mechanically through a dynamic interaction in live cells**. *Curr Biol* 2006, **16**:333-344.

21. Green LC, Kalitsis P, Chang TM, Cipetic M, Kim JH, Marshall O, Turnbull L, Whitchurch CB, Vagnarelli P, Samejima K *et al.*: **Contrasting roles of condensin I and condensin II in mitotic chromosome formation**. *J Cell Sci* 2012, **125**:1591-1604.

22. Shintomi K, Hirano T: **The relative ratio of condensin I to II determines chromosome shapes**. *Genes Dev* 2011, **25**:1464-1469.

23. Ono T, Losada A, Hirano M, Myers MP, Neuwald AF, Hirano T: **Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells**. *Cell* 2003, **115**:109-121.

24. Vagnarelli P, Hudson DF, Ribeiro SA, Trinkle-Mulcahy L, Spence JM, Lai F, Farr CJ, Lamond AI, Earnshaw WC: **Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis**. *Nat Cell Biol* 2006, **8**:1133-1142.

25. Bhalla N, Biggins S, Murray AW: **Mutation of YCS4, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior**. *Mol Biol Cell* 2002, **13**:632-645.

26. Biggins S, Bhalla N, Chang A, Smith DL, Murray AW: **Genes involved in sister chromatid separation and segregation in the budding yeast *Saccharomyces cerevisiae***. *Genetics* 2001, **159**:453-470.

27. Lavoie BD, Hogan E, Koshland D: **In vivo dissection of the chromosome condensation machinery: reversibility of condensation distinguishes contributions of condensin and cohesin**. *J Cell Biol* 2002, **156**:805-815.

28. Ouspenski II, Cabello OA, Brinkley BR: **Chromosome condensation factor Brn1p is required for chromatid separation in mitosis**. *Mol Biol Cell* 2000, **11**:1305-1313.

29. Strunnikov AV, Hogan E, Koshland D: **SMC2, a *Saccharomyces cerevisiae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family**. *Genes Dev* 1995, **9**:587-599.

30. Saka Y, Sutani T, Yamashita Y, Saitoh S, Takeuchi M, Nakaseko Y, Yanagida M: **Fission yeast Cut3 and Cut14, members of a ubiquitous protein family are required for chromosome condensation and segregation in mitosis**. *Embo J* 1994, **13**:4938-4952.

31. Petrova B, Dehler S, Kruitwagen T, Heriche JK, Miura K, Haering CH: **Quantitative analysis of chromosome condensation in fission yeast**. *Mol Cell Biol* 2013, **33**:984-998.

32. Kireeva N, Lakonishok M, Kireev I, Hirano T, Belmont AS: **Visualization of early chromosome condensation: a hierarchical folding, axial glue model of chromosome structure**. *J Cell Biol* 2004, **166**:775-785.

33. •• Houlard M, Godwin J, Metson J, Lee J, Hirano T, Nasmyth K: **Condensin confers the longitudinal rigidity of chromosomes**. *Nat Cell Biol* 2015, **17**:771-781.
So far the cleanest condensin inactivation in mammalian cells; shows that the condensin II complex is required for chromatin compaction.

34. Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K, Yanagida M: **DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe***. *Cell* 1987, **50**:917-925.

35. Earnshaw WC, Halligan B, Cooke CA, Heck MM, Liu LF: **Topoisomerase II is a structural component of mitotic chromosome scaffolds**. *J Cell Biol* 1985, **100**:1706-1715.

36. Lewis CD, Laemmli UK: **Higher order metaphase chromosome structure: evidence for metalloprotein interactions**. *Cell* 1982, **29**:171-181.

37. Adachi Y, Luke M, Laemmli UK: **Chromosome assembly in vitro: topoisomerase II is required for condensation**. *Cell* 1991, **64**:137-148.

38. Gasser SM, Laroche T, Falquet J, Boy de la Tour E, Laemmli UK: **Metaphase chromosome structure Involvement of topoisomerase II**. *J Mol Biol* 1986, **188**:613-629.

39. Andreassen PR, Lacroix FB, Margolis RL: **Chromosomes with two intact axial cores are induced by G2 checkpoint override: evidence that DNA decatenation is not required to template the chromosome structure**. *J Cell Biol* 1997, **136**:29-43.

40. Gorbsky GJ: **Cell cycle progression and chromosome segregation in mammalian cells cultured in the presence of the topoisomerase II inhibitors ICRF-187 [(+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane; ADR-529] and ICRF-159 (Razoxane)**. *Cancer Res* 1994, **54**:1042-1048.

41. Carpenter AJ, Porter AC: **Construction, characterization, and complementation of a conditional-lethal DNA topoisomerase IIalpha mutant human cell line**. *Mol Biol Cell* 2004, **15**:5700-5711.

42. Chang CJ, Goulding S, Earnshaw WC, Carmena M: **RNAi analysis reveals an unexpected role for topoisomerase II in chromosome arm congression to a metaphase plate**. *J Cell Sci* 2003, **116**:4715-4726.

43. Samejima K, Samejima I, Vagnarelli P, Ogawa H, Vargiu G, Kelly DA, de Lima Alves F, Kerr A, Green LC, Hudson DF *et al.*: **Mitotic chromosomes are compacted laterally by KIF4 and condensin and axially by topoisomerase IIalpha**. *J Cell Biol* 2012, **199**:755-770.

44. •• Wilkins BJ, Rall NA, Ostwal Y, Kruitwagen T, Hiragami-Hamada K, Winkler M, Barral Y, Fischle W, Neumann H: **A cascade of histone modifications induces chromatin condensation in mitosis**. *Science* 2014, **343**:77-80.
Reveals a chromatin-inherent driving force of condensation mediated by an association between nucleosomes and induced by a histone modification cascade in mitosis.

45. Robinson PJ, An W, Routh A, Martino F, Chapman L, Roeder RG, Rhodes D: **30 nm chromatin fibre decompaction requires both H4-K16 acetylation and linker histone eviction**. *J Mol Biol* 2008, **381**:816-825.

46. Shogren-Knaak M, Ishii H, Sun JM, Pazin MJ, Davie JR, Peterson CL: **Histone H4-K16 acetylation controls chromatin structure and protein interactions**. *Science* 2006, **311**:844-847.

47. Kruitwagen T, Denoth-Lippuner A, Wilkins BJ, Neumann H, Barral Y: **Axial contraction and short-range compaction of chromatin synergistically promote the condensation of mitotic chromosomes**. *eLIFE* 2015 http://dx.doi.org/10.7554/eLife.10396.

48. Mora-Bermudez F, Gerlich D, Ellenberg J: **Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase**. *Nat Cell Biol* 2007, **9**:822-831.

49. •• Neurohr G, Naegeli A, Titos I, Theler D, Greber B, Diez J, Gabaldon T, Mendoza M, Barral Y: **A midzone-based ruler adjusts chromosome compaction to anaphase spindle length**. *Science* 2011, **332**:465-468.
Identification of an Aurora B and H3 S10ph dependent pathway in yeast that adapts chromosome arm length to spindle size in anaphase.

50. Schmitz MH, Held M, Janssens V, Hutchins JR, Hudecz O, Ivanova E, Goris J, Trinkle-Mulcahy L, Lamond AI, Poser I *et al.*: **Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells**. *Nat Cell Biol* 2010, **12**:886-893.

51. Qian J, Lesage B, Beullens M, Van Eynde A, Bollen M: **PP1/Repo-man dephosphorylates mitotic histone H3 at T3 and regulates chromosomal aurora B targeting**. *Curr Biol CB* 2011, **21**:766-773.

52. Trinkle-Mulcahy L, Andersen J, Lam YW, Moorhead G, Mann M, Lamond AI: **Repo-Man recruits PP1 gamma to chromatin and is essential for cell viability**. *J Cell Biol* 2006, **172**:679-692.

53. Vagnarelli P, Ribeiro S, Sennels L, Sanchez-Pulido L, de Lima Alves F, Verheyen T, Kelly DA, Ponting CP, Rappsilber J, Earnshaw WC: **Repo-Man coordinates chromosomal reorganization with nuclear envelope reassembly during mitotic exit**. *Dev Cell* 2011, **21**:328-342.

54. Booth DG, Takagi M, Sanchez-Pulido L, Petfalski E, Vargiu G, Samejima K, Imamoto N, Ponting CP, Tollervey D, Earnshaw WC *et al.*: **Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery**. *Elife* 2014, **3**:e01641.

55. Landsverk HB, Kirkhus M, Bollen M, Kuntziger T, Collas P: **PNUTS enhances in vitro chromosome decondensation in a PP1-dependent manner**. *Biochem J* 2005, **390**:709-717.

56. •• Ramadan K, Bruderer R, Spiga FM, Popp O, Baur T, Gotta M, Meyer HH: **Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin**. *Nature* 2007, **450**:1258-1262.
Shows that p97 removes Aurora B from chromatin during chromatin decondensation.

57. Thadani R, Uhlmann F, Heeger S: **Condensin, chromatin crossbarring and chromosome condensation**. *Curr Biol CB* 2012, **22**:R1012-R1021.

58. •• Magalska A, Schellhaus AK, Moreno-Andres D, Zanini F, Schooley A, Sachdev R, Schwarz H, Madlung J, Antonin W: **RuvB-like ATPases function in chromatin decondensation at the end of mitosis**. *Dev Cell* 2014, **31**:305-318.
describes a cell free assay for chromatin decondensation and identifies a complex of the RuvB-like ATPases RuvBL1 and RuvBL2 as crucial chromatin decondensation factor.