Condensins, cohesins, and chromosome architecture: How to make and break a mitotic chromosome

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Condensins, Cohesins, and Chromosome Architecture: How to Make and Break a Mitotic Chromosome

Minireview

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The orderly packaging of DNA into chromosomes in preparation for mitosis is a structural problem common to all cells. The DNA in metaphase chromosomes of higher eukaryotic cells is compacted nearly 10,000-fold in length (a packaging ratio 20- to 100-fold greater than in interphase [Trask, et al., 1993]). Thus, a hypothetical metaphase chromosome 1 cm in length would have about 100 m of DNA within it, with the diameter of each sister chromatid being about 1 mm. This condensation is all the more amazing when one considers that the products of replication are intertwined, and that the consequences of improper condensation or resolution of decatenation are dire to the cell.

Putting the Usual Subjects on the Scaffold

Just how chromosomes are ordered has inspired numerous models, ranging from random spaghetti-like folding to successive orders of helical coiling of the DNA fibre (starting with DNA coiled around the nucleosome), to the involvement of a specific subset of nonhistone proteins anchoring loops of chromatin (reviewed in Earnshaw, 1991). Electron micrographs published two decades ago by Laemmli and colleagues of metaphase chromosomes from which the histones and the majority of the nonhistone proteins had been stripped inspired the "scaffold" model of mitotic chromosome architecture. The now familiar pictures showed loops of DNA emanating from a central proteinaceous core that resembled the native chromosome in shape (Paulson and Laemmli, 1977). Further biochemical analysis of scaffolds isolated from highly purified mitotic chromosomes illustrated a strikingly simple composition for this residual structure; two predominant bands at 170 and 135 kDa, named Sc1 and Sc2, were observed along with a number of more minor components.

Though scaffolds were often maligned as an artifact of precipitation, the scaffold model nonetheless played a crucial role in the understanding of chromosome architecture and dynamics through the cell cycle. The demonstration in 1985 that Sc1 was DNA topoisomerase II provided the first identification of a protein involved in mitotic chromosome function (reviewed in Earnshaw and Mackay, 1994). Indeed, in both Schizosaccharomyces pombe and Saccharomyces cerevisiae, genetic evidence revealed that mutations in topoisomerase II were lethal in mitosis, with sister chromatids failing to segregate properly. Further analysis in S. pombe then revealed that topoisomerase II was also required for the final stages of chromosome condensation. These genetic experiments were subsequently confirmed in biochemical studies using cell-free extracts prepared from Xenopus eggs. The extract studies demonstrated that interphase nuclei could be converted to mitotic chromosomes only if topoisomerase II was endogenously present or added back after depletion of the extract.

More recently, a similar convergence of data from divergent approaches has revealed the importance of the SMC (structural maintenance of chromosomes, formerly called stability of mini chromosomes) family of proteins in mitotic chromosome structure and function. Discovered originally in S. cerevisiae and now known to be present in species from mycoplasma to mammals, the SMCs comprise at least four distinct subfamilies (reviewed in Koshland and Strunnikov, 1996; also see Table 1). *smc1* mutants (S. cerevisiae) had a dramatic increase in the rate of chromosome loss as well as a defect in nuclear division, while *smc2* mutants exhibited a defect in chromosome segregation and partial chromosome decondensation in cells arrested in mitosis. This theme was reiterated in S. pombe, where it was shown that the Cut3 and Cut14 (for cell untimely torn) SMC proteins were essential for chromosome condensation and segregation; additionally, since *cut3* and topoisomerase II mutants were synthetically lethal, this suggested that these proteins may cooperate in chromosome condensation. The nematode DPY-27 SMC is involved in dosage compensation and specifically coats the X chromosome in somatic cells. In chicken, the abundant Sc2 component of the chromosome scaffold is an SMC protein. Finally, the Xenopus XCAP-C and XCAP-E (for Xenopus chromosome-associated protein) SMCs were found to be associated with mitotic chromatids assembled when demembranated sperm nuclei were incubated in egg mitotic extracts. Though these findings initially suggested that the SMCs might be primarily involved in chromosome packaging, recent evidence implies more wide-ranging functions, including a role in post-replication recombination repair. RC-1 (recombination complex) from calf thymus contains, in addition to two SMCs, DNA polymerase ϵ, DNA ligase III, and endonuclease (Jessberger et al., 1996). With an abundance of SMCs (even budding yeast has four family members), novel roles for the SMCs in chromatin mechanics are bound to emerge.

Not a Barren Field of Study

Recent advances in understanding the process of chromosome condensation have come through the identification of components interacting with both topoisomerase II and with SMC family members. The Drosophila *barren* gene (so named because the peripheral nervous system in mutant embryos had fewer cells than normal) encodes a molecule important for sister chromatid segregation; numerous chromosome bridges are observed in *barr* embryos (Bhat et al., 1996). The *barren* gene product has been shown to interact with topoisomerase II both in vivo and in vitro. The identification of SMCs and subsequent functional analysis in Drosophila, an organism amenable to cytological and genetic analysis, is in its infancy but should prove fruitful.

Table 1. SMC Subfamilies Discussed in This Minireview

Organism	SMC1	SMC2	SMC3	SMC4
Saccharomyces cerevisiae	Smc1	Smc2	Smc3	Smc4
Schizosaccharomyces pombe		Cut14		Cut3
Caenorhabditis elegans				DPY-27
Xenopus laevis		XCAP-E		XCAP-C
Gallus domesticus		Sc2		
Bovis domesticus	BSMC1	BSMC2		

A direct link between SMCs and Barren was demonstrated earlier this year when a Xenopus homolog of Barren was identified as one of the "XCAPs" (Hirano et al., 1997). The original profile of XCAPs consisted of the core histones, histone H1-like proteins, and a number of high molecular weight proteins. XCAP-B is identical to topoisomerase IIα; XCAP-C and XCAP-E are SMCs essential for chromosome condensation in vitro. Biochemical studies of these SMC proteins during in vitro chromosome assembly defined two complexes, termed condensins, sedimenting at 8S and 13S (Hirano et al., 1997). In addition to XCAP-C and XCAP-E (the sole components of 8S condensin), the 13S complex contained three additional subunits, XCAP-D2 (150 kDa), XCAP-G (130 kDa), and XCAP-H (100 kDa). Immunodepletion and rescue experiments revealed that 13S condensin was required for chromosome assembly in vitro. The function of 8S condensin is unknown. Microsequencing of the XCAP-H subunit revealed homology to the Barren protein. Notably, there are also two short stretches within the Barren/XCAP-H proteins that bear homology to C. elegans DPY-26, which is essential for X chromosome dosage compensation and meiotic chromosome segregation (Leib et al., 1996). The fact that DPY-26 requires DPY-27 (the nematode SMC) to associate with the X chromosome in somatic cells further emphasizes the interaction.

First Hints to Mechanism

Because of its ability to create double-stranded breaks in DNA and pass one strand through another, DNA topoisomerase II is well suited for the task of chromosome disentanglement. It has been postulated that one of the purposes of chromosome condensation is to facilitate the disentangling of replicated sister chromatids by displacing the topoisomerase II catenation/decationation equilibrium in the direction of decatenation (reviewed in Holm, 1994). Consistent with this idea are scanning electron micrographs of human lymphocytes in mitosis that showed that chromosomes first condense as a single, long cylinder that subsequently resolves into two parallel, thinner cylinders (Sumner, 1991). Condensation may be the prerequisite for sister chromatid separation.

But what about the SMCs? With Xenopus 13S condensin in hand, Kimura and Hirano have been able to define one activity of this complex (Kimura and Hirano, 1997). 13S condensin introduces positive supercoils into plasmid DNA in the presence of either E. coli or calf thymus topoisomerase I. The supercoiling reaction requires ATP hydrolysis, consistent with the finding that all SMC proteins share an NTP-binding motif in their amino-terminal domains. The condensin ATPase activity was stimulated ~5-fold in the presence of double-stranded, closed, circular DNA, but only ~2-fold with single-stranded DNA. Interestingly, cruciform DNA was preferred by 13S condensin as a binding substrate over duplex DNAs containing the same sequence. One implication of this result is that 13S condensin prefers structured DNA. Possibly, cruciforms mimic crossover points of two duplex DNAs (as would exist in supercoiled DNA), though the authors prefer the possibility that 13S condensin interacts with distorted (or bent) DNA. It is interesting to note that S/MARs (scaffold- or matrix-attachment regions), postulated to be *cis* sites for chromosome order in interphase and mitosis and involved in scaffold protein binding, are also not defined by specific sequence composition, but rather by the presence of highly AT-rich DNA sequences that produce a narrow, minor groove (reviewed in Laemmli et al., 1992). It is tempting to speculate that 13S condensin may have a preference for binding SARs, which also lie along the axes of mitotic chromatids (Saitoh and Laemmli, 1994).

Cut3 and Cut14 from S. pombe also form a DNA-binding oligomeric complex, which corresponds to Xenopus 8S condensin in terms of subunit composition (Sutani and Yanagida, 1997). This complex, but not the individual proteins, had potent DNA renaturation activity, possibly ~70-fold more efficient than RecA. Yet this activity was not dependent on ATP, nor was it inhibited by ATP analogs. In this respect, the DNA renaturation activity was similar to the activity of the SMC subunits within RC-1. Analysis of the temperature-sensitive mutants revealed a decrease in renaturation activity when the complex was heated. Interestingly, there appeared also to be an effect on overall chromatin structure in *cut14* cells grown at elevated temperature. Chromatin isolated from *cut14* cells was much more readily digested by S1 nuclease than chromatin from wild-type or *cut3* cells. It remains to be determined if the DNA renaturation activity of the Cut3/Cut14 complex is mechanistically related to the ATP-dependent supercoiling activity associated with 13S condensin.

The Awesome Power of Yeast Cytology

Until fairly recently, the awesome power of yeast genetics has been limited by the lack of adequate cytological techniques to examine chromosome dynamics. A number of techniques have now been developed, however, that overcome this hurdle. While chromosome condensation and segregation in budding yeast are invisible by conventional light or electron microscopy, FISH (fluorescence in situ hybridization) experiments with probes to two or more regions along a chromosome have very clearly demonstrated that yeast chromosomes do condense in mitosis (albeit to a much lower level than in higher eukaryotes) (Guacci et al., 1994). The use of GFP tagging has also been exploited to examine chromosome behavior in living yeast cells. In S. cerevisiae, the binding of GFP-Lac repressor fusion proteins to an integrated tandem array of Lac operator sites was utilized to show the timing of chromatid separation and that checkpoint-deficient mutants separate sister chromatids under circumstances in which they remain associated in wild-type cells. Using this methodology, the same group then elegantly demonstrated that even though budding yeast did not exhibit a conventional metaphase plate, cells did undergo anaphase A (movement of the chromosomes to the poles) (Straight et al.,

1997). In living S. pombe, GFP-tagged centromere protein Mis6 was also visualized during anaphase as separated sister chromatids moved to opposite spindle poles (Saitoh et al., 1997).

In this issue of *Cell*, the cast of characters integral to chromosomal dynamics is extended by the simultaneous identification by two groups of a new protein interacting genetically with Smc1p: named *MCD1* by Guacci et al. (1997), and *SCC1* by Michaelis et al. (1997). Both groups have utilized FISH and/or GFP tagging of chromosomes in S. cerevisiae to characterize the mutant phenotypes of two different alleles of the *MCD1*/*SCC1* gene.

MCD1 (mitotic chromosome determinant) was identified by Guacci et al. in two screens for genes encoding chromosomal structural proteins. Temperature-sensitive mutations were screened for enhanced lethality after mitotic arrest. Although mitotic inviability has also been observed for mitotic checkpoint defects, *MCD1* appears to have functional mitotic checkpoint control, as mutant cells fail to undergo new rounds of replication at the nonpermissive temperature. *MCD1* was also identified in a screen for genes interacting with *SMC1*, behaving as a high-copy suppressor of an *smc1* mutation. In addition to this genetic interaction, Mcd1p and Smc1p could be coimmunoprecipitated, further evidence for direct complex formation. At the nonpermissive temperature, *mcd1* cultures were enriched for cells with a stretched nuclear DNA mass as well as an increased frequency of large budded cells with short or partially elongated spindles (indicative of a chromosome segregation defect). To establish whether the segregation defect reflected problems with sister chromatid cohesion, populations synchronized in mid-M phase by nocodazole treatment were processed for FISH, using either centromere-proximal or -distal probes to assay cohesion at different sites along chromosomes I, IV, or XVI. Although the majority of wild-type cells had a single FISH signal in mid-M phase, most *mcd1* cells had two FISH signals per DNA mass, implying that sister chromatids had separated (this was true for both centromere-proximal and -distal probes). To determine whether *MCD1* was necessary for the maintenance of cohesion, cells were allowed to establish cohesion at permissive temperature, then shifted to nonpermissive temperature to inactivate Mcd1p. As double FISH signals were once again visualized, they concluded that Mcd1p was also required for the maintenance of cohesion.

Guacci et al. also used FISH to examine the status of chromosome condensation in *mcd1* mutants. While wild-type cells in mid-M phase had a single line-like signal for the rDNA locus in yeast (a 500 kb block of repetitive DNA), *mcd1* cells had an amorphous FISH signal, indicating a defect in rDNA condensation. The disruption in condensation was not limited to the rDNA, as "painting" chromosome VIII or XVI (with a number of probes for that chromosome) showed a defect as well, namely that the normally closely spaced signals seen in wild-type mitotic cells were dispersed in the *mcd1* cells. All is not entirely clear, however: despite the apparent role for *MCD1* in chromosome condensation and cohesion, the levels of the protein are highest in early S-phase cells, reduced by late S to a lower level that remains constant through telophase, and then nearly undetectable by G1.

Michaelis et al. discovered the same protein starting with a different premise: that the destruction of proteinaceous bridges holding sister chromatids together might be mediated by the anaphase-promoting complex, or APC. As APC mutants fail to separate sister chromatids, Michaelis et al. set out to isolate mutants that lose chromosomes at a high frequency at permissive temperature (using a colony sectoring assay) and are capable of separating sister chromatids in the absence of APC function (conditional inactivation at nonpermissive temperature). Eight mutations representing four different complementation groups were identified: *SMC1*, *SMC3*, *SCC1*, and *SCC2* (for sister chromatid cohesion). Michaelis et al. also found by FISH analysis using either centromere-proximal or -distal probes that two fluorescent signals were produced in *scc1* nocodazole-treated cells, confirming the role for Scc1p in chromatid cohesion. To extend their observations to living cells, they examined Tet repressor-GFP fusions bound to an integrated array of Tet operators. In *scc1* cells, sister chromatid separation occurred about 15 minutes earlier than in wild-type cells and could occur in the presence of the anti-microtubule drug nocodazole that normally blocks mitotic progression. Chromosome spreads were prepared for immunofluorescence to examine whether Scc1p was associated specifically with chromatin. As soon as the endogenous protein could be detected within early S-phase cells, Scc1p was chromosomal. Interestingly, in anaphase cells, Scc1p dissociated from chromatin (or was masked?), though the protein was not yet degraded. The association of Scc1p with chromatin was found to be dependent on Smc1p. This suggested an intriguing hypothesis: if the association of Scc1p with chromosomes was relevant to sister chromatid cohesion and Scc1p fails to associate in *smc1* cells, then *smc1* cells might also be defective in sister cohesion. Experiments using the Tet-GFP system confirmed this suspicion and showed that the other three genes discussed in this report (*SMC1*, *SMC3*, and *SCC2*) were also required to prevent separation of sister chromatids, hence the coining of the name "cohesins" for the group. Whether the two alleles examined in these reports cause different disruptions to the *MCD1*/*SCC1* protein remains to be determined.

Future Prospects

The working model for higher-order chromosome dynamics presented in Figure 1 takes into account the molecules mentioned in this minireview. It is by no means complete or without variation, depending on the experimental system. It may, however, be useful as a guide to formulate questions concerning chromosome architecture. Is the supercoiling activity of 13S condensin necessary for the full compaction of chromatin loops during mitosis (or possibly only required in eukaryotes with large amounts of DNA)? Will Xenopus *MCD1*/*SCC1* turn out to be one of the remaining components of 13S condensin? Are there distinct cohesin and condensin entities? It is striking that many of the molecules depicted have already been shown to have roles in both condensation and cohesion, thus blurring the distinction. Perhaps the activity differences will be determined

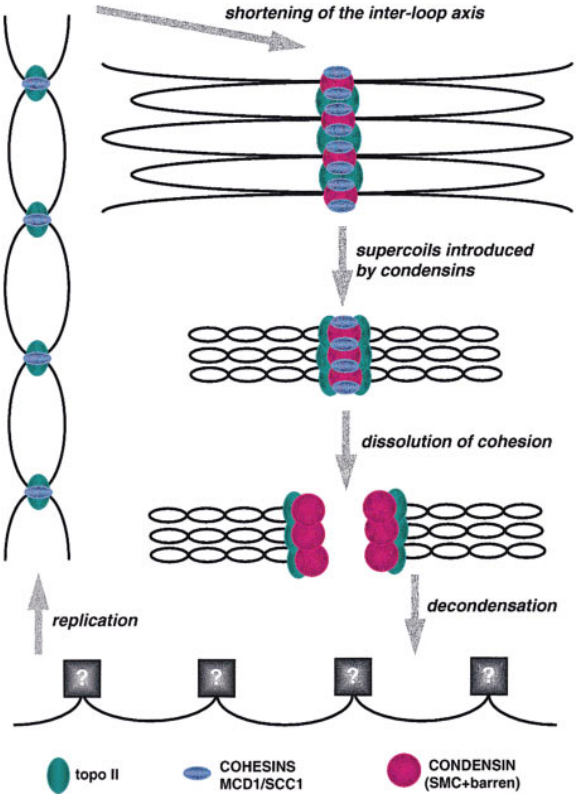

Figure 1. A Working Model for Higher-Order Chromosome Dynamics

The components discussed in this review are shown as green ovals (DNA topoisomerase II), blue ovals (cohesins), and red circles (condensins). The solid lines represent the chromatin fiber. The replicated, but not yet condensed, sister chromatids are shown at the left. Topo II and cohesins are present in S-phase cells, while condensin is involved in shortening of the interloop axis, and compaction of the chromatin loops by the introduction of supercoils. With the dissociation of cohesins, sister chromatids fall apart. Topo II is present in anaphase chromosomes but degraded at the end of mitosis. The black boxes in the ensuing G1 phase chromosomes represent the ill-defined postmitotic content of the "complexes" at the bases of chromatin loops. While the condensins and cohesins are shown as distinct entities for the sake of simplicity, further research will expose the full extent of overlap in composition and/or activity and how this may be regulated spatially and temporally. (Chromosomes are not drawn to scale.)

by the precise nature of subunit composition, phosphorylation state, quantity, and stoichiometry relative to the other components. The observations made so far can be explained by remodeling of complexes at the bases of chromatin loops during the cell cycle to accommodate new subunits, while others are proteolyzed in concert with disassembly, and modulation of activity by cell cycle–dependent phosphorylation.

The understanding of chromosome structural biology has come a long way from Flemming's drawings of mitosis published in 1882, where the longitudinal "splitting" of chromosomes was first described. The highly conserved nature of chromosome condensation has allowed the identification of proteins important to this process from a large number of model organisms. The analysis of the expression and localization of the components described in this minireview will undoubtedly shed light on the subtle nuances of chromosome behavior in different systems under distinct developmental constraints. The insights to be gleaned from analyzing the cast of players available will indeed continue to fascinate chromosome biologists for many years to come.

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