Building and Breaking Bridges between Sister Chromatids

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ABBREVIATIONS

ABC	ATP Binding Cassette
ATP	Adenosine 5' triphosphate
APC/C	Anaphase Promoting Complex/Cyclosome
CAR	Cohesin Associated Region
ChIP	Chromatin Immunoprecipitation
DNA	Deoxyribonucleic Acid
NTP	Nucleoside 5' triphosphate
PCNA	Proliferating Cell Nuclear Antigen
PLK	Polo-like Kinase
RFC	Replication Factor C
RNAi	Ribonucleic Acid interference
SMC	Structural Maintenance of Chromosomes

SUMMARY

Eukaryotic chromosomes undergo dramatic changes and movements during mitosis. These include the individualization and compaction of the two copies of replicated chromosomes (the sister chromatids) and their subsequent segregation to the daughter cells. Two multi-subunit protein complexes termed 'cohesin' and 'condensin', both composed of SMC (Structural Maintenance of Chromosomes) and kleisin subunits, have emerged as crucial players in these processes. Cohesin is required for holding sister chromatids together whereas condensin, together with topoisomerase II, has an important role in organizing individual axes of sister chromatids prior to their segregation during anaphase. SMC and kleisin complexes also regulate the compaction and segregation of bacterial nucleoids. New research suggests that these ancient regulators of chromosome structure might function as topological devices that trap chromosomal DNA between 50 nm long coiled coils.

INTRODUCTION

The transmission of genetic information from generation to generation forms the basis for the continuity of life. During cell proliferation, DNA replication produces two identical copies of every chromosome named sister chromatids. These are segregated to opposite poles of the cell prior to its division in a process called mitosis. During gametogenesis, two successive rounds of chromosome segregation following only a single round of DNA replication produce cells with only a single set of chromosomes (haploids) from cells initially bearing two copies (diploids). These specialized divisions are called meiosis. Chromosome segregation is not only a central aspect of all life but it is also of great medical importance. Missegregation produces cells bearing too few or too many chromosomes, with often fatal consequences. Mistakes in chromosome segregation during meiosis can lead to the inheritance of two instead of one particular maternal chromosome (trisomy). Trisomies are the major cause of spontaneous fetal abortions and hence infertility. Trisomy of chromosome 21 causes Down's syndrome [(1)]. Meanwhile, mistakes during mitotic chromosome segregation are frequently associated with malignant tumor cells and are thought to facilitate their evolution [(2)].

Sister chromatids are moved to opposite poles of the cell by forces generated by microtubules, which attach to specialized structures at centromeric regions of the chromatids called kinetochores. Kinetochores regulate the action of motor proteins and microtubule depolymerization to induce polewards movement of chromatids. This process can only deliver a complete set of the cell's chromosomes to each daughter cell if the kinetochore of each chromatid is attached to microtubules with opposite orientations to those attached to its sister, which is known as amphitelic attachment or bi-orientation (Fig. 1A). This co-ordination is made possible by connections between sister chromatids that hold them together until bi-orientation has been achieved. It is thought that microtubule attachments are made at least partly at random but that cells selectively eliminate those resulting in sister kinetochores being attached to the same pole (known as syntelic attachment, Fig. 1A). Amphitelic but not syntelic attachment results in a tug of war between microtubules attempting to pull sister chromatids apart, and cohesion between sisters resists this splitting force. The resulting tension is thought to stabilize kinetochore-microtubule attachments, which are otherwise highly labile. Very little is known about the mechanism by which microtubules attach and detach from kinetochores. It is nevertheless clear that sister chromatid cohesion must have a key role in generating the tension needed to stabilize

attachments. It is sister chromatid cohesion that makes it possible for cells to segregate their chromosomes long after their duplication. The gap between S- and M-phases, known as G2, can last up to five decades in the case of human oocytes. Only when all chromosomes are bi-oriented on the metaphase plate, do cells set about destroying the connections between sisters, which triggers their segregation to opposite poles during anaphase.

What kind of connection could hold sister chromatids together tightly enough to withstand the pulling forces of the mitotic spindle? The finding that short artificial linear chromosomes are more frequently missegregated than long ones led to the proposal that it might be the inter-twining (catenation) of sister DNA molecules [3]. This inter-catenation is a legacy of DNA replication. It undoubtedly exits and topoisomerase II (topo II) is required to decatenate sisters. However, there is currently no evidence either that inter-twining can 'resist' spindle forces or that topo II is suddenly activated at the onset of anaphase. It is moreover clear that small circular sister DNA molecules remain tightly tethered together in yeast long after decatenation has been completed [4].

THE COHESIN COMPLEX

The discovery that the ubiquitin protein ligase called the Anaphase Promoting Complex or Cyclosome (APC/C) responsible for destroying mitotic cyclins at the metaphase to anaphase transition is also required for sister chromatid separation provided an impetus to the search for cohesion proteins using genetic studies, initially in yeast. Over a dozen proteins necessary for cohesion have now been identified in various species. Four such proteins, namely Smc1, Smc3, Scc1 (Mcd1, Rad21) and Scc3, form a soluble 'cohesin' complex in yeast [5-7], flies [8], worms [9] and vertebrates [10, 11]. A substantial amount of evidence is consistent with the notion that cohesin might be the actual glue between sister chromatids. In yeast, cohesin associates with chromosomes in late G1-phase, remains bound during S- and G2-phases and dissociates again at the metaphase to anaphase transition; that is precisely when cohesion is dissolved (Fig. 1B). Mutations in any of its four subunits lead to the failure to hold sister chromatids together after they have been generated by DNA replication. Crucially, the proteolytic cleavage of cohesin's Scc1 subunit by the separase protease upon activation of the APC/C triggers the disjunction of sister chromatids at the onset of anaphase[12, 13]. In vertebrates, most cohesin bound to chromosome arms during interphase is released during the early stages of mitosis; that is, soon after break down of the nuclear membrane and during chromosome condensation [10] (Fig. 1B). How does this fit with the idea that cohesin holds sisters together during metaphase? This paradox was resolved by the finding that a small fraction of cohesin remains bound to centromeric regions until metaphase [14]. Even smaller amounts may also persist along the inter-chromatid axes of chromosome arms. There is still no final proof that the cohesin persisting at centromeres keeps sisters connected until the onset of anaphase. Nevertheless, the discovery that expression of a version of Scc1 that cannot be cleaved by separase prevents proper chromosome segregation in human cells [15] suggests not only that cohesin holds sister chromatids together after prophase but also that cohesin's cleavage is necessary to trigger

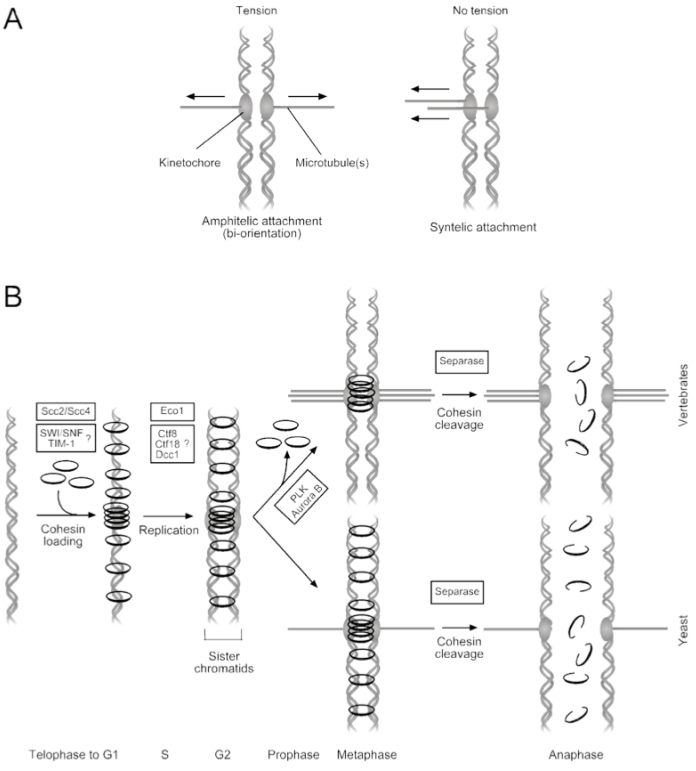
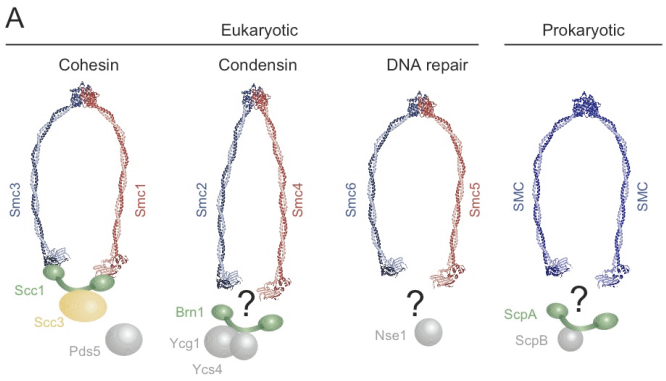


Figure 1 Chromosome segregation during mitosis. (A) To successfully segregate complete sets of chromosomes, the kinetochores of sister chromatid pairs have to be connected to microtubules emanating from opposite cell poles, known as amphitelic or bi-polar attachment. Amphitelic attachment generates tension, which is counteracted by cohesion between the sisters. No tension is generated if both sister kinetochores have been attached to microtubules from the same pole, known as syntelic attachment. **(B)** The cohesin cycle. Cohesin complexes are loaded onto chromosomes before the onset of DNA replication, varying in timing from telophase of the previous cell cycle (vertebrates) to late G1 phase (yeast). An Scc2/Scc4 protein complex is required for cohesin's association with chromosomes in yeast. A SWI/SNF chromatin remodeling complex and the TIM-1 protein have been reported to be involved in cohesin's loading onto chromosomes in human and *C. elegans*, respectively. Cohesion between sister chromatids is established during DNA replication in S-phase. The Eco1 protein and an alternative RFC containing Ctf8, Ctf18 and Dcc1 subunits might be involved in converting cohesin into cohesion. In vertebrates, the bulk of cohesin dissociates from chromosome arms during prophase, while centromeric cohesin stays bound and presumably keeps sister chromatids connected. Cohesin's dissociation during prophase is regulated by PLK and Aurora B kinases. In yeast, cohesin stays bound to chromosome arms during prophase. At metaphase, once bi-polar attachment of all sister kinetochores has been achieved, proteolytic cleavage of cohesin's Scc1 subunit by separase releases cohesin from chromosomes and resolves cohesion, triggering the movement of sister chromatids to opposite poles of the cell during anaphase. Chromosomes are symbolized by DNA double helices.

progress into anaphase.

Why do cells use a proteinaceous structure instead of chromatid intertwining to hold sister chromatids together? If catenation held sisters together, it would have to be resolved by a sudden increase in topo II activity once all chromosomes had bi-oriented. What would happen if a single catenation were not resolved? The pulling forces exerted by the mitotic spindle would lead to the breakage of one of the two sister chromatid fibers. The use instead of a multisubunit protein complex whose integrity depends on noncovalent interactions between its subunits means that failures to resolve individual connections can instead be resolved by disengagement of noncovalent protein subunit interactions, which are presumably far weaker than a chromatid fiber.

How does cohesin physically hold sister chromatids together and how does separase so abruptly break this tight linkage? Studies of cohesin's individual subunits and how they assemble into a complex have shed some insight into these questions. Cohesin's Smc1 and Smc3 subunits belong to the SMC (Structural Maintenance of Chromosomes) protein family, which has members present in all kingdoms of life. All SMC proteins appear to regulate chromosome morphology in some way or another. They have been implicated in chromosome condensation, recombination, repair, and gene dosage compensation as well as sister chromatid cohesion. The hallmark of these large (1000-1500 amino acids) proteins is a unique structural design. The globular amino- and carboxy-terminal domains of SMC proteins are separated by two long stretches of amphipathic alpha-helical regions, which are separated in the molecule's center by a third globular domain. This central domain orients the helical stretches in a way that they form an intramolecular [16, 17] anti-parallel [18] coiled coil about 50 nm in length, which brings the molecule's amino- and carboxy-terminal domains together to form the so-called 'head domain'. The amino-terminal domain contains an NTP binding motif called the P-loop or Walker A motif. The carboxy-terminal domain contains a so-called DA-box, which was initially thought to be a helix-loop-helix DNA binding motif [19]. Due to the identification of a short conserved LSGG motif characteristic for ATPases of the ATP Binding Cassette (ABC) transporter family preceding the DA-box, the DA-box is now known to correspond to the Walker B site of two-part ATP binding folds [20, 21]. An SMC head domain therefore contains all sequence motifs necessary to form a functional ATPase. The crystal structure of a bacterial SMC head domain indeed shows an ABC ATPase fold [22].



B

		S. cerevisiae	*S. pombe*	*X. laevis*	*H. sapiens*	*C. elegans*	*D. melanogaster*
Cohesin	SMCs	Smc1	Psm1	XSMC1	hSMC1α hSMC1β[a]	SMC-1	DmSMC1
		Smc3	Psm3	XSMC3	hSMC3	SMC-3	DmSMC3
	Kleisin α	Scc1 Rec8[a]	Rad21 Rec8[a]	XRAD21	hSCC1 hRec8[a]	COH-2 REC-8[a]	DmRAD21
		Scc3	Psc3 Rec11[a]	XSA1, XSA2	hSA1, hSA2 hSTAG3[a]	SCC-3	DmSA
		Pds5	Pds5	XPDS5	hPDS5	PDS-5	DmPDS5
Condensin	SMCs	Smc4	Cut3	XCAP-C	hCAP-C	SMC-4	Gluon
		Smc2	Cut14	XCAP-E	hCAP-E	MIX-1	DmSMC2
	Kleisin γ	Brn1	Cnd2	XCAP-H	hCAP-H	KLE-2[b]	Barren
		Ycg1	Cnd3	XCAP-G	hCAP-G		
		Ycs4	Cnd1	XCAP-D2	hCAP-D2		
	SMCs	Smc5	Spr18		hSMC5	F54D5.14	DmSMC5
		Rhc18	Rad18		hSMC6	C23H4.6	CG5524
		Nse1					AF161451

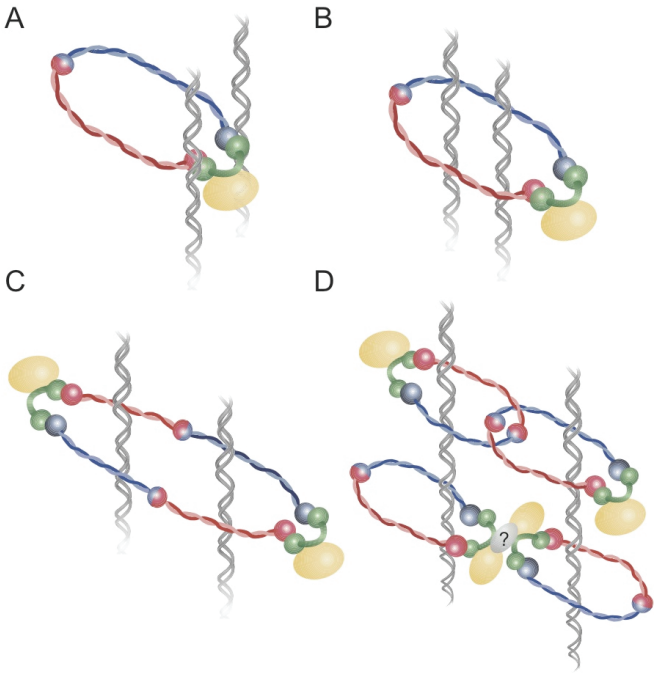
SMC proteins dimerize via their central domains to form V-shaped molecules [16, 18, 23] whose arms are composed of coiled coils and whose apices are composed of the ATPase containing head domains (Fig. 2). Both electron micrographic images and biophysical measurements suggest that the two SMC arms emerge from the central dimerization domain with flexible angles [18, 23, 24], which is the reason why the central dimerization domain was originally termed a 'hinge'. While two identical SMC proteins homodimerize in prokaryotes, two different SMC proteins heterodimerize in specific pairs in eukaryotes. In the case of cohesin, Smc1 dimerizes with Smc3.

Smc1 and Smc3 associate with the non-SMC subunits Scc1 and Scc3 to form the cohesin complex. A fifth protein, Pds5, appears to bind less tightly to cohesin complexes [25-27]. In electron micrographs of cohesin purified from human or frog cells, the non-SMC subunits appear to be situated in the vicinity of the SMC head domains [23]. Biochemical analysis of recombinant yeast cohesin subcomplexes showed that the amino-terminal domain of the Scc1 subunit binds to

Figure 2 Composition and proposed architecture of SMC protein complexes. (A) In cohesin, the head domains of an Smc1/Smc3 heterodimer are connected by different termini of the Scc1 (kleisin α) subunit. The Scc3 subunit binds to the central region of Scc1. A fifth subunit called Pds5 is less tightly associated with cohesin. In condensin, an Smc2/Smc4 heterodimer associates with three non-SMC subunits called Brn1, Ycg1 and Ycs4 in yeast. Sequence homology of Brn1 (kleisin γ) to the SMC binding domains of Scc1 suggests that Brn1 might connect the head domains of Smc2 and Smc4. An Smc5/Smc6 heterodimer has been found to function in DNA damage repair and associates with the Nse1 protein. Prokaryotic SMC proteins form homodimers. In *B. subtilis*, SMCs bind ScpA and ScpB proteins. Question marks indicate where exact complex architectures have not yet been determines. **(B)** Summary of eukaryotic SMC complexes from different species. [a]Additional cohesin subunits exclusively expressed during meiosis [b]Functional replacement by a kleisin β protein subfamily member.

Smc3's head while Scc1's carboxy-terminal domain binds to Smc1's head [16]. Sequences within the center of Scc1 bind Scc3 and thereby link this fourth subunit to the Smc1/Smc3 dimer (Fig. 2). The amino- and carboxy-terminal domains of Rec8, a homolog of Scc1 found in meiotic cohesin complexes [28-31], bind to Smc3 and Smc1 heads in a similar manner to Scc1 [32]. The finding that two differently tagged versions of any single cohesin subunit cannot be co-immunoprecipitated after cohesin has been released from chromatin [16] suggests that the two ends of a single Scc1 subunit connect the head domains of the same Smc1/Smc3 heterodimer, thereby forming a large ring (Fig. 2). Such a structure is consistent with electron micrographs of monomeric soluble cohesin and condensin complexes [23].

Does cohesin form such rings *in vivo* when it is bound to chromatin and when it mediates sister chromatid cohesion? Separase cleaves Scc1 into amino- and a carboxy-terminal fragments, both of which subsequently dissociate from chromatin. If cohesin had formed a ring on chromatin, then the two Scc1 cleavage fragments released from chromatin by separase should still be linked via their association with the two head domains of an Smc1/Smc3 dimer. This appears indeed to be the case. The two Scc1 fragments released from chromatin by separase at the onset of anaphase co-immunoprecipitate [32] in a manner that depends on the integrity of the Smc1/Smc3 dimer's arms, as severance of Smc3's coiled coil due its cleavage by a recombinant protease at artificially introduced sites destroys the link between the Scc1 fragments. Chromosomal cohesin must therefore form a ring structure.



COHESIN'S BROTHERS: CONDENSIN ET AL.

Chromosome segregation depends on another SMC protein complex called condensin, which is composed of a heterodimer of Smc2 and Smc4 associated with three additional proteins called Brn1 (Barren, CAP-H), Ycg1 (Cnd3, CAP-G) and Ycs4 (Cnd1, CAP-D2), reviewed by Losada and Hirano [33]. Might condensin also possess a ring-like architecture? The finding that the amino- and carboxy-terminal aminoacid sequences of condensin's Brn1 subunit have homology to the conserved SMC binding domains at the termini of Scc1 and Rec8 proteins [34] suggests that Brn1 homologs might associate with the head domains of Smc2/Smc4 heterodimers in a manner that resembles Scc1's association with the Smc1 and Smc3 (Fig. 2). In which case, it is conceivable that Brn1 connects the heads of Smc2 and Smc4. Scc1, Rec8 and Brn1 homologs belong to a new protein superfamily called 'kleisins' (from the Greek word for 'closure'). Besides Scc1/Rec8 (kleisin α) and Brn1 (kleisin γ), most animal and plant genomes (but not fungal ones) encode a third type of kleisin protein (kleisin β). In *C. elegans,* which lacks kleisin γ, RNAi-mediated knock down of kleisin β (KLE-2) produces a chromosomal phenotype similar if not indistinguishable to that of Smc2 or Smc4. The function of condensin in *C. elegans* is presumably mediated by a complex containing Smc2, Smc4, and kleisin β. Vertebrate genomes encode both kleisin β and γ proteins and most animal cells may therefore possess two different types of condensin complex [34]. A third SMC protein complex composed of Smc5 (Spr18) and Smc6 (Rad18) proteins is involved in DNA damage repair [35, 36]. Besides the finding that it associates with a novel protein of unknown function called Nse1 in budding yeast [37], the Smc5/Smc6 complex awaits further characterization.

Figure 3 Models for cohesin's association with chromosomes. (A) One model proposes that each of the two head domains of the Smc1/Smc3 heterodimer (red and blue) directly binds one sister chromatid (illustrated by DNA double helices), possibly in conjunction with the non-SMC subunits Scc1 (green) and Scc3 (yellow). **(B)** The cohesin ring model predicts that two sister chromatids are held together by their passage through the same cohesin ring. With a theoretical diameter of ~35 nm, the ring would be large enough to embrace two 10 nm chromatin fibers. **(C)** In a variation of the ring model, Scc1 subunits might connect the head domains of different Smc1/Smc3 heterodimers to form dimeric rings potentially large enough to encircle two 30 nm chromatin fibers. **(D)** Alternatively, two cohesin rings, each encircling a single sister chromatid, might be connected to hold two sisters together. The connection between two such rings on chromatin could either be made by their intercatenation (top), or by a yet unknown factor (bottom). There is however no evidence available that more than one of each cohesin subunit is found in one complex, as models (C) and (D) would predict. Even though cohesin's chromosomal association is topological in models (B) to (D), neither model rules out additional direct interactions between the cohesin ring(s) and the chromatids.

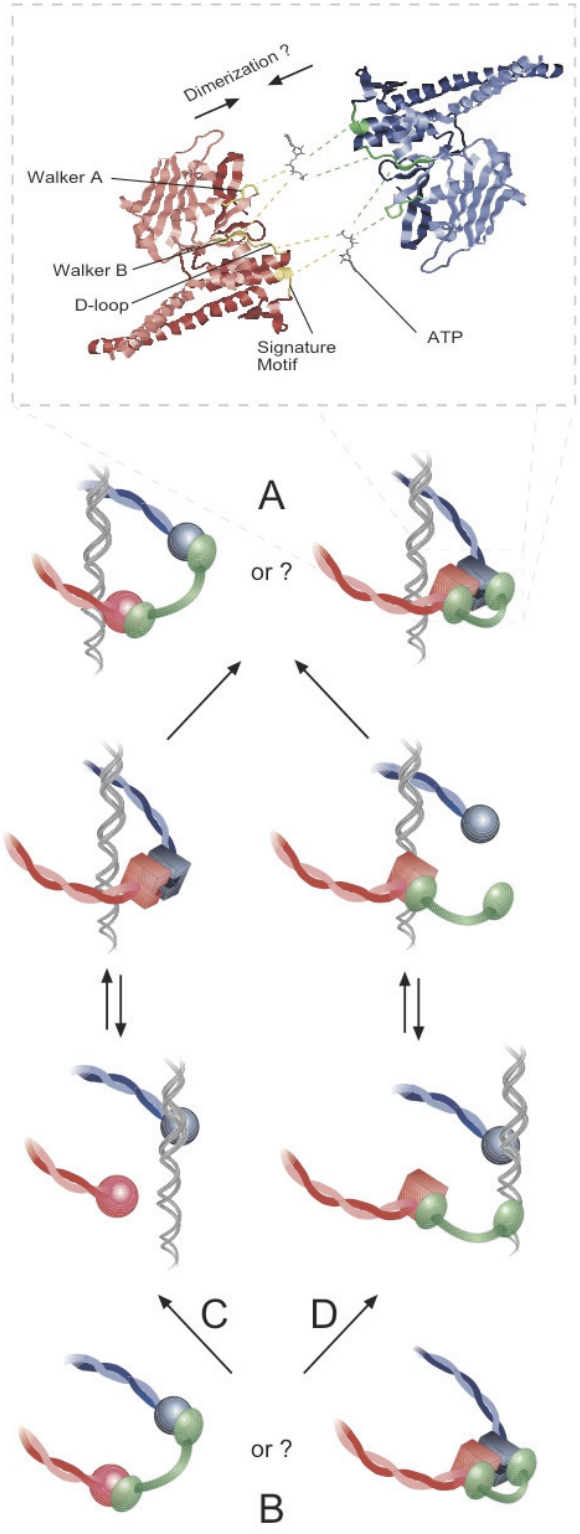
Prokaryotic genomes encode only a single SMC protein or an SMC-related protein of the MukB family. Mutations in SMC or MukB cause chromosome partitioning defects and a failure to compact nucleoids in *B. subtilis* and *E.coli*, respectively [38-40], which leads to the formation of anucleate cells. Bacterial SMC complexes presumably act in a similar manner to their eukaryotic counterparts in organizing chromosomes, reviewed by Graumann [41]. Unlike eukaryotic SMCs, prokaryotic ones form homodimers. In *B. subtilis*, the binding of ScpA and ScpB proteins to the SMC dimer appears to augment or promote SMC function [42]. The MukE and MukF proteins do so for the SMC-like MukB protein in *E. coli* [43]. Interestingly, ScpA and its homologs also belong to the kleisin protein superfamily [34], suggesting that ScpA might bind to the head domains of its associated SMC homodimer (Fig. 2A). SMC-kleisin complexes appear therefore to be extremely ancient chromosomal constituents.

COHESIN'S ASSOCIATION WITH CHROMATIN

Does our knowledge about cohesin's architecture explain how cohesin binds to chromosomes and mediates cohesion? Ideally, one would like to have a model to explain both of these phenomena. One model proposes that each ATPase containing head domain of the Smc1/Smc3 heterodimer binds a sister chromatid, possibly in conjunction with Scc1 and Scc3 subunits [23] (Fig. 3A). This model does not provide a particularly satisfactory explanation for why cohesin forms a ring. One can only suppose that Scc1 would reinforce the link between the head domains of Smc1/Smc3 in addition to their connection through the coiled coils and central dimerization domains. And why does cleavage of Scc1 by separase causes a catastrophic loss of cohesion at the metaphase to anaphase transition and releases cohesin from chromatin? According to the model, cleavage might weaken somewhat but not destroy cohesion. A third and potentially even more serious problem is that cohesin does not appear to be capable of binding DNA very tightly *in vitro* [44].

A more attractive albeit more radical alternative is that cohesin holds sister chromatids together by trapping them both inside its ring [16] (Fig. 3B). According to this "ring" model, the association of cohesin with chromatin has a largely but possibly not exclusively (see further below) topological nature. Such a mode of binding is consistent with cohesin's release from chromatin and the destruction of cohesion by cleavage of either Scc1 or Smc3's coiled coil [32]. It furthermore explains why treatment of a chromatin-containing pellet with salt concentrations high enough to solubilize chromatin-bound nucleosomes does not release cohesin [45]. With a diameter of ~35 nm, cohesin's ring would be wide enough to encircle with ease two 10 nm chromatin fibers; that is, nucleosomal DNA. However, it would not be wide enough to encompass more than one 30 nm chromatin fiber, unless two of these fibers were closely inter-locked. Larger rings could in principle be created if kleisin subunits connected not the heads of a single Smc1/Smc3 heterodimer but instead the heads of different heterodimers (Fig. 3C). Another variation of the ring model supposes that each chromatid is trapped inside different cohesin rings that are somehow connected (Fig. 3D), either topologically (the rings are catenated) or by a hitherto unknown factor. However, the only indication so far that SMC dimers might interact on DNA comes from *in vitro* cross-linking studies of bacterial SMC homodimers [24]. Experiments on yeast argue against stable interactions between two or more cohesin rings in vivo as well as formation of multimeric rings. When cohesin is released from a chromosomal pellet by digestion of DNA with micrococcal nuclease, differently tagged versions of the same cohesin subunit cannot be co-immunoprecipitated [16], implying that most if not all chromosomal cohesin is monomeric. Chromatids might be wrapped around cohesin's ring instead of simply passing through it, as suggested by electron spectroscopic images of DNA associated with the head domains of Smc2/Smc4 heterodimers in condensin [46]. Clearly, the next important step in understanding cohesin's association with chromosomal DNA will be to establish whether chromatids do indeed run through cohesin's ring.

The ring model suggests that cohesin might possess considerable mobility when associated with chromatin and need not be associated with particular sequences. However, chromatin immunoprecipitation studies suggest that cohesin is in fact not found at random positions and has been mapped to specific loci on chromosome arms called Cohesin Associated Regions (CARs) [47-50]. CARs are spaced roughly every 5-10 kbp and most are situated in intergenic and transcriptionally inactive sequences. Apart from the fact that they are rich in AT content, CARs do not seem to contain any obvious consensus sequence. Cohesin is highly enriched at active centromeric regions, which is where cohesin must most actively counteract splitting forces exerted by microtubules. In fission yeast, cohesin's enrichment at centromeres depends on the heterochromatin protein Swi6 (HP1) [51, 52], which binds to nucleosomes whose histone H3 subunits have been methylated on lysine 9. The recent finding that during the first meiotic division in fission yeast, cohesin complexes on chromosome arms contain the Scc3 homolog Rec11 while those bound to centromeric regions contain Psc3 [53] suggests that Scc3-like subunits may have a role in targeting cohesin to the distinct chromosome regions. An association between Swi6 (HP1) and Psc3 might target cohesin not only to centromeres but also to other so-called heterochromatic regions such as silent mating loci and



telomeres [52]. The finding that cohesin accumulates at specific loci is in fact not inconsistent with the notion that its primary mode of association with chromatin is topological. Weaker interactions between cohesin subunits like Scc3 and specific chromatin proteins might constrain one-dimensional diffusion of cohesin rings along chromatin fibers.

How cohesin is loaded onto chromosomes is still poorly understood. In budding yeast, a protein complex composed of Scc2 and Scc4 proteins is required for cohesin's localization both to centromeres and to the arms of chromosomes [45]. The Scc2 fission yeast homolog (Mis4) also has been implicated both in the formation of sister chromatid cohesion and in loading cohesin onto chromosomes[54]. Homologs of Scc2 also exist in Drosophila (Nipped-B) [55] and most but possibly not all eukaryotic species [56]. The mechanism by which the Scc2/Scc4 complex promotes association of cohesin with chromosomes is unknown. Cohesin has recently been reported to co-purify with a SWI/SNF containing chromatin remodeling complex [57], which might have a role in cohesin loading, because expression of an inactive mutant form of SNF2 reduced the levels of cohesin bound to chromosomal regions containing Alu short interspersed DNA elements.

Another player with functions in loading cohesin onto chromosomes has recently been described in *C. elegans* where a protein called TIM-1 co-immunoprecipitates with cohesin [9]. TIM-1 is homologous to the *Drosophila* TIMELESS protein, which is essential for circadian rhythm regulation in the fly. A reduction in TIM-1 levels by RNA interference (RNAi) causes embryonic lethality. Its inactivation in the germ cell lineage using a temperature sensitive allele causes meiotic defects: homologous chromosomes fail to synapse properly and neither homologs nor sister chromatids remain paired after the first meiotic prophase. This phenotype resembles that caused by the abolition of REC-8 protein (Scc1's replacement in meiotic cells) [31]. Interestingly, neither REC-8 nor SCC-3 associate with meiotic chromosomes in the absence of TIM-1 function but their customary SMC partners continue to do so, at least during the early stages of

Figure 4 Hypothetical role of Smc1's and Smc3's ATPase domains in cohesin loading onto chromosomes. (A) In the context of the ring model, two possible conformations for chromosomal bound cohesin can be envisaged. Scc1 might either connect Smc1/Smc3 head domains which do not directly interact with each other (left), or Scc1 might connect Smc1/Smc3 head domains which themselves dimerize by sandwiching two ATP molecules at their interface (right). SMC head dimerization might be analogous to the dimerization of the head domains of the SMC-related Rad50 protein, which bind ATP via the Walker A (P-loop) motif in the amino-terminal domain of one head and the signature motif in the carboxy-terminal domain of the other head. Side chains of the Walker B motif position a Mg^{2+} ion coordinating the terminal ATP phosphate groups and a residue of the D-loop is thought to be involved in catalyzing ATP hydrolysis. Such a dimerization is illustrated using the crystal structures of *T. maritima* SMC head domains with exit and entry sites of the coiled coils modeled (top). **(B)** Scc1 also very likely connects the head domains of Smc1/Smc3 dimers off chromatin. Two scenarios can be envisaged how a chromatin fiber could enter such rings: **(C)** Scc1 might dissociate from the SMC head domains. Free Smc1/Smc3 dimers might bind to chromatin and binding of Scc1 then closes the ring shut. **(D)** One or both of the interactions of Scc1 with the Smc1/Smc3 head domains of pre-assembled ring disengage to allow the passage of a chromatin fiber.

meiosis. TIM-1 appears therefore to be required for inducing or maintaining the association of Rec8 with chromosomal Smc1/Smc3 proteins. This raises the interesting possibility that Smc1/Smc3 heterodimers might under some circumstances bind stably to chromatin without the participation of kleisin subunits (see below). It is however conceivable that kleisins other than REC-8 associate with Smc1/Smc3 complexes in a manner that does not depend on TIM-1.

What then is the molecular mechanism by which cohesin associates with chromosomes? ABC-like ATPases are involved in transporting substances across membranes. Might therefore the ATPase domains of SMC proteins have a role in transporting DNA into the space between their coiled coil arms? A crystal structure of the head domains of the SMC-like protein Rad50 shows that two Rad50 head domains can dimerize by sandwiching a pair of ATP molecules at their interface [58]. It seems likely that Smc1/Smc3's head domains can dimerize in an analogous manner (Fig. 4A, top). Upon ATP binding and dimerization, Rad50 ATPase heads undergo a conformational change. A similar conformational change in the head domains of Smc1/Smc3 might regulate their interaction with Scc1. A key question is therefore whether the cohesin monomers that hold sister chromatids together contain Scc1 bound to Smc heads in a 'heads open' (ATP hydrolyzed) (Fig. 4A, left) or in a 'heads closed' (ATP bound) state (Fig. 4A, right).

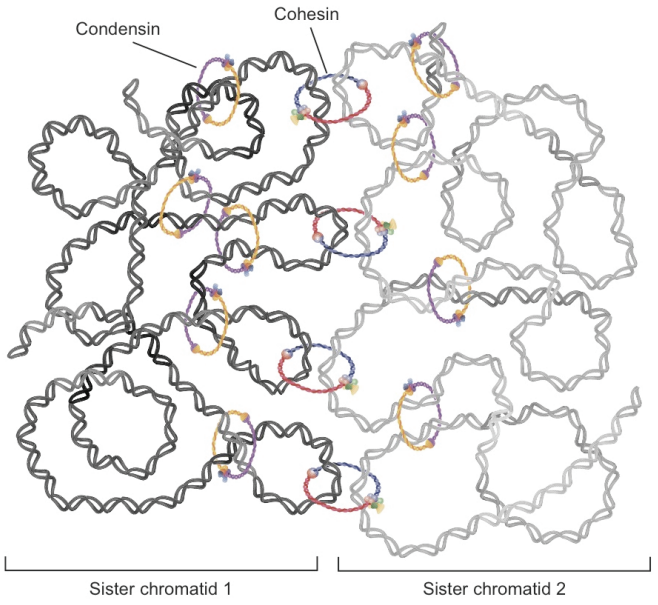


Figure 5 A model how condensin promotes coherence of chromatids. While the passage of two sister chromatid fibers through the same cohesin ring might promote inter-chromatid cohesion, the passage of different segments of the same chromatid through the same condensin ring may promote intra-chromatid cohesion. Such intra-chromatid cohesion may be required to confer coherence to each chromatid fiber, preventing that chromatids get tangled up.

In such a two-state model, a separate question then concerns the pathway by which chromosomal cohesin rings are formed. Dependent whether Scc1 initially bind to a 'heads open' or 'heads closed' Smc1/Smc3 heterodimer, one can imagine two possible scenarios.

In the first scenario, chromatin might first engage with free Smc1/Smc3 heterodimers. If so, ATP binding and hydrolysis cycles might drive the opening and shutting of Smc1/Smc3 heterodimers independently of Scc1 binding. DNA may enter between the arms of free Smc1/Smc3 heterodimers in the 'heads open' state (Fig. 4C). Switching to the 'heads closed' state would initially trap DNA within bipartite Smc1/Smc3 rings. These would then be converted to tripartite Smc1/Smc3/Scc1 rings by the binding of Scc1 to the dimerized SMC heads. The SMC heads might remain dimerized or be dissociated due to ATP hydrolysis triggered by Scc1 binding (Fig. 4A). The finding that stable association of Scc1 cleavage fragments depends on the integrity of SMC coiled coils [32] (head-head interactions appear insufficient) suggests that conversion of bipartite to tripartite rings on chromatin might involve replacement of head-head by head-Scc1 interactions. Thus, once locked onto chromosomes by the binding of Scc1, cohesin rings may be in an obligatory 'heads open' state (Fig. 4A). The idea that Smc1/Smc3 might first bind to chromosomal DNA before Scc1 connects their head domains is consistent with the observations that Smc1 and Smc3 are present in excess over Scc1 and that SMC-1's chromosomal localization appears normal in *tim-1* mutant worms in which neither REC-8 nor SCC-3 can be detected on chromosomes [9]. However, a significant fraction of soluble Smc1/Smc3 dimers are associated with Scc1 in soluble fractions from frog [23] and yeast cell extracts. These soluble cohesin complexes presumably also exist as rings (Fig. 4B) [32]. To allow entry of a chromatin fiber into these rings, at least one of their subunit interactions would need to be at least temporarily broken – the ring must be opened. According to the above scenario, Scc1 must first disengage from Smc1/Smc3 heterodimers completely before they can trap DNA and soluble Smc1/Smc3 heterodimers must presumably rapidly exchange between Scc1-bound and free states. This raises a conundrum. Such exchange must be rapid for soluble Smc1/Smc3 heterodimers but be slow (or even non-existent) for those participating in cohesion.

In a second scenario, Smc1/Smc3 heterodimers only join chromosomes after they have been complexed with Scc1. In this case, transient disengagement of one of the two SMC head domains from Scc1 would be required for entry of DNA into the ring (Fig. 4D). Such a transient opening would have to be restricted to when cohesin is loaded onto chromatin, since the integrity of the ring presumably must

be maintained when sister chromatids are held together. For both scenarios, factors required for cohesin's loading onto DNA (e.g. Scc2/Scc4, TIM-1) could play a role in regulating the disengagement of Scc1's association with one or both SMC head domains.

ESTABLISHMENT OF COHESION DURING DNA REPLICATION

There are two states of cohesin bound to chromosomes, one where cohesin does confer sister chromatid cohesion and one where it does not (see Fig. 1B, before and after replication). What is the evidence for this? When expression of Scc1 in yeast is delayed until S-phase had been completed, cohesin is still loaded onto chromatin but it cannot generate sister chromatid cohesion [59]. The establishment of cohesive structures clearly depends on cohesin's presence during DNA replication. In most eukaryotic cells, unlike yeast, cohesin is abundant throughout the cell cycle and a sizeable fraction of it is associated with chromatin throughout G1 phase where it obviously cannot confer cohesion. Cohesin can clearly bind to chromosomes without holding sister DNAs together.

Interestingly, special factors are required during DNA replication for the establishment of cohesive structures. For example, cohesin complexes are formed and loaded onto chromatin normally but are incapable of generating sister chromatid cohesion during S phase in *eco1* (*ctf7*) mutant yeast cells [7]. Though required for generating cohesive structures, the Eco1 protein is not required to maintain cohesion after replication has been completed. Sequence analysis suggests that Eco1's carboxyterminus is homologous to acetyl-transferases. Eco1 can acetylate recombinant cohesin subunits but there currently is no evidence that this acetylation occurs *in vivo* [60]. Surprisingly, Eco1's fission yeast homolog Eso1 is not essential in fission yeast cells lacking Pds5 [61]. It has therefore been suggested that Pds5 might inhibit the formation of cohesion until counteracted by Eso1. Pds5's role is clearly complex, because (in contrast to Eco1) it is essential for maintaining cohesion in budding yeast as well as in fission yeast cells arrested in G2.

Another factor possibly important for generating cohesive structures is an alternative version of Replication Factor C (RFC) [62, 63] in which Rfc1 has been replaced by the Ctf8, Ctf18, and Dcc1 proteins. Despite normal amounts of cohesin associated with their chromosomes, *ctf8*, *ctf18* and *dcc1* mutants have reduced but still significant (Ctf8 etc are not essential proteins) sister chromatid cohesion. It is suspected but has never been proven that the alternative RFC complex acts like Eco1 only during S phase. The canonical RFC complex is required to load the polymerase processivity factor PCNA (Proliferating Cell Nuclear Antigen) onto DNA. It is therefore likely that the alternative RFC containing Ctf8/Ctf18/Dcc1 loads PCNA-like protein clamps or possibly PCNA itself. These clamps might conceivably be used to recruit a special DNA polymerase required to replicate through cohesin sites [62]. It has been suggested that Trf4, a protein that possesses nucleotide polymerase activity and has been implicated in forming cohesin, might be such a polymerase [64]. However, the finding that Trf4's closest homolog in fission yeast (called Cid1) has poly(A) polymerase activity instead of DNA polymerase activity [65] is inconsistent with this notion. Bearing in mind that PCNA recruits not only DNA polymerase but also other chromosomal proteins (e.g. those involved in DNA damage responses such as Gadd45 and p21), the clamps loaded by the Ctf8/Cft18/Dcc1 containing RFC might be used to recruit proteins required for the formation of cohesion (such as Eco1) rather than a DNA polymerase.

If cohesin's ring model proves correct, then one of the real mysteries about sister chromatid cohesion is how sister DNA molecules are entrapped within the same ring. One possibility is that cohesin rings are pre-assembled around chromosomes before replication, in which case passage of the replication machinery through these rings would assure that both sisters are trapped inside the same one. However, it is hard to imagine how passage of a replication fork through a cohesin ring could be accomplished if the DNA polymerases and their associated factors were assembled into stationary 'replication factories' [66]. A second possibility is that cohesin rings encircle the newly generated sister chromatids while they are held together in special structures in the wake of replication forks.

REMOVING COHESIN FROM AND BINDING OF CONDENSIN TO CHROMOSOME ARMS IN PROPHASE

In most eukaryotic cells including vertebrates, the bulk of cohesin dissociates from chromosome arms during prophase (Fig. 1B). It is at this stage of mitosis that sister chromatid arms resolve into two individual, coherent, rod-shaped structures which are primarily connected at their centromeres. Cohesin's dissociation during prophase appears to require neither cleavage of Scc1 nor activation of the APC/C [10, 15, 67]. Whatever the process, it appears to continue unabated when cells are arrested in metaphase and is thought to be responsible for the total loss of arm cohesion and for endowing mitotic chromosomes their X-like shape in cells treated with spindle poisons. The dissociation of cohesin from chromosome arms depends on the activity of Polo-like (PLK) [68] and Aurora B mitotic kinases [69] but is independent of Cdk1[27, 70]. How do PLK and Aurora B promote cohesin's release? Affinity-purified PLK can phosphorylate cohesin's

Scc1 and Scc3 (SA) subunits in vitro. Furthermore, phosphorylation of both Scc1 and Scc3 is reduced in extracts from which PLK has been depleted. When *Xenopus* extracts containing either mitotically phosphorylated or non-phosphorylated interphase cohesin are incubated with sperm chromatin, the phosphorylated form associates less efficiently with the chromatin [68]. Cohesin's release might therefore be triggered by the phosphorylation of its subunits by PLK. But how would phosphorylation cause cohesin's release from chromosomes? One possibility is that it promotes disengagement of Scc1 from either one or both SMC head domains. The role of Aurora B is less clear because there is no evidence that cohesin itself is its target. One of the mysteries of this so-called prophase pathway is the apparent immunity of cohesin in the vicinity of centromeres, which might conceivably be conferred through a potential association with HP1.

What is the purpose of loading cohesin onto chromosome arms and then removing it during prophase before it can be used to resist spindle forces and promote bi-orientation? As long as sisters remain paired, it is possible to repair DNA damage (such as a double-strand break) by homologous recombination, using the other sister DNA strand as template. Cohesin might therefore keep sister chromatid arm regions connected until prophase to allow efficient DNA damage repair. Indeed, yeast mutants defective in cohesion are impaired in postreplicative double-strand break repair [71]. The reason for cohesin's release during prophase is less clear. Initially, it was though that cohesin's dissociation might be required for the binding of condensin to chromosomes. However, when cohesin's dissociation from chromosome arms was inhibited in *Xenopus* extracts by depleting PLK (and Aurora B), the association of condensin with chromatin and the compaction of mitotic chromosomes appeared to be normal [68, 69]. It nevertheless seems likely that the prophase pathway facilitates the extensive separation of sister DNA sequences that proceeds their final parting during anaphase and is driven by forces that do not involve microtubules. This pre-separation process appears to be totally absent in *S. cerevisiae*. Its actual role in animal cells will require analysis of the consequences of replacing cohesin subunits by versions that can no longer be phosphorylated by PLK.

If cohesin operates using a topological principle, then one might expect other SMC/kleisin containing complexes such as condensin to do so as well. What then do we know about the function of cohesin's brother condensin? In some experimental systems such as *Xenopus* egg extracts and to a lesser extent fission yeast cells, depletion of condensin subunits leads to a defect in compacting chromosomes upon entry into mitosis [72-74]. In other organisms such as *Drosophila* or *C. elegans*, where condensin has been inactivated either by mutation or by RNAi-mediated depletion of one of its subunits, chromosomes clearly undergo extensive compaction in the apparent absence of condensin activity [75, 76]. This makes it unlikely that condensin's function is simply chromosome compaction. A phenotype that is common to all systems is the failure of sister chromatids to disjoin efficiently during anaphase. It is as if condensin has a crucial role in preventing chromatids from getting tangled up. Is condensin therefore essential for promoting the resolution of sister chromatid arms into cytologically distinguishable rod-like structures? The finding that distinct sister chromatid axes decorated by topo II appear to form before condensin arrives on chromosomes during prophase suggests that at least some aspects of chromatid individualization can take place in the absence of condensin [77, 78]. If condensin has a role in chromatid individualization, then it must augment other processes.

In vitro, purified condensin introduces positive supercoils into circular DNA in an ATP-dependent manner [79] and it has been suggested that this might facilitate and organize chromosome coiling *in vivo*. It is however very unclear whether the amount of supercoiling imparted by condensin could have much influence on chromosome structure, given the ubiquity of eukaryotic topoisomerases. Indeed, the supercoiling observed *in vitro* might be an epi-phenomenon; namely, a consequence of condensin's mode of binding to DNA, which has been proposed to involve wrapping DNA around its SMC head domains [46]. Another possibility is that condensin's primary function is to promote the chiral trapping of chromatin fibers, which would subdivide the chromosome into a series of loops [80, 81]. A third possibility is that condensin might help to cross-link chromatin fibers belonging to the same chromatid, thereby promoting a coherence and continuity to that chromatid. Without some kind of cross-linking between DNA sequences belonging to the same DNA molecule, chromatids would be floppy and therefore have a tendency to tangle up with other DNA molecules (Fig. 5). All three of these types of activity could in principle be mediated or at least facilitated by the trapping of DNA strands inside ring-like structures analogous to but not identical to those produced by cohesin. If cohesin's role is to promote inter-chromatid cohesion, then condensin's may be to promote intra-chromatid cohesion. One of the most fascinating issues for future research will be to identify not only cohesin and condensin's functional similarities but also the crucial differences which ensure that condensin only organizes DNAs from the same chromatid while cohesin is able to connect sister DNAs and be capable of holding them together for very extended periods of time.

DISSOLVING COHESION AT THE METAPHASE TO ANAPHASE TRANSITION

Once the kinetochores of all sister chromatid pairs have attached to opposite spindle poles in metaphase, it is time to destroy the bridges that hold sisters together and allow microtubules to pull them apart in anaphase. Work on budding yeast has shown that loss of cohesion coincides with the cleavage of cohesin's Scc1 subunit into two fragments, and that this cleavage depends on the Esp1 protein [13]. It turned out that Esp1 is the protease that cleaves Scc1 [12]. Esp1 also triggers the segregation of homologs during anaphase I of meiosis by cleaving Scc1's meiotic homolog Rec8 along chromosome arms [82, 83]. Homologs of Esp1 have been found in all organisms studied so far and are now called 'separases'. Separase proteases are related to caspases, containing a cysteine residue close to their carboxytermini, which is activated for nucleophilic attack by a histidine residue in a catalytic dyad [12]. Separase target sites have been identified in Scc1 and Rec8 proteins from various species, but their low consensus sequence has made the search for further separase substrates difficult. The only other substrate known to be cleaved by separase is a protein called Slk19, but the biological significance of Slk19 cleavage is unclear [84].

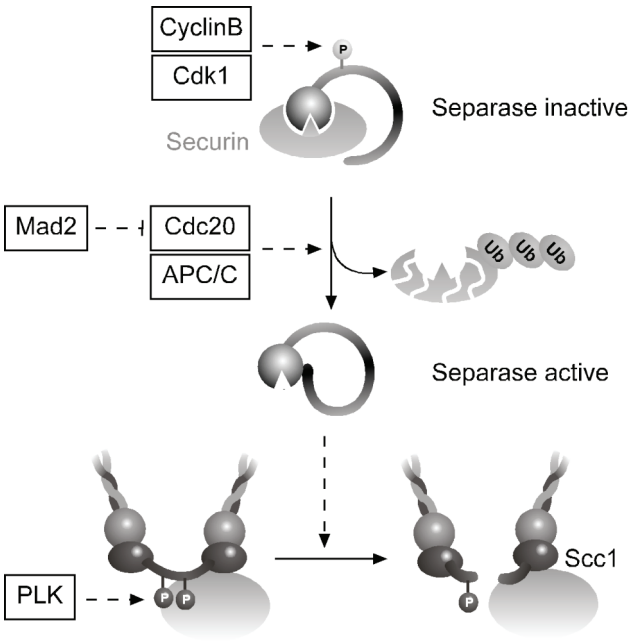


Figure 6 Regulation of Scc1's cleavage by separase. Separase is initially held inactive by its association with securin and, in the case of vertebrates, an inhibitory phosphorylation by Cdk1/cyclinB. Ubiquitination of securin by the APC/C and its activator Cdc20 targets securin for destruction by the 26S proteasome. Upon liberation from securin, amino- and carboxy-terminal domains of separase are thought to interact to fully activate separase. Separase cleaves cohesin's Scc1 subunit and thereby resolves cohesion. Scc1's susceptibility to separase is augmented when it is phosphorylated by PLK. Unattached kinetochores are sensed by the spindle checkpoint and cause Mad2-dependent inhibition of APC/C.

Separase is also involved in a network regulating exit from mitosis and meiosis by promoting the release of the Cdc14 phosphatase from the nucleolus [85, 86]. The release of Cdc14 by separase is required for nucleolar fission (that is, the separation of sister and homologous ribosomal DNA sequences). Interestingly, this aspect of separase does not involve its proteolytic activity [85].

Cleavage of Scc1 by separase is irreversible. Before unleashing separase, cells must ensure that chromosomes are not damaged and that every single one has bi-oriented on the mitotic spindle. Separase is held inactive by its association with a protein called 'securin' [87, 88], which is thought to block access to separase's active site at its carboxyterminus [89, 90] (Fig. 6). Polyubiquitination of securin by the APC/C in conjunction with APC/C's mitotic activator Cdc20 targets securin for destruction by the 26S proteasome [91] and thereby liberates separase [92]. The aminoterminus of separase can now bind to its catalytic carboxy-terminal domain, and this is thought to fully activate the protease (Fig.6) [89, 90]. Surprisingly, human separase remains inactive after its release from securin in extracts containing high concentrations of undegradeable cyclin B [93]. The inhibition of separase was found to depend on its phosphorylation at a single serine residue. The finding that this serine residue is also phosphorylated in metaphase cells *in vivo* implies that vertebrate cells use a second, separase independent mechanism to regulate the activation of separase. Destruction of both securin and B-type cyclins by the APC/C might therefore be essential for separase activation in vertebrates.

Is activation of separase the only mechanism that controls the cleavage of cohesin?

Unexpectedly, yeast and human cells lacking securin still undergo cell cycle regulated separation of sister chromatids [94, 95]. In yeast, the observation that Scc1 is phosphorylated shortly before its cleavage suggests that phosphorylation might enhance its susceptibility to separase. Scc1 cleavage is less efficient in cells lacking the Polo-like kinase Cdc5 and separation of sister chromatid arms but not centromeres is significantly delayed [96]. Cdc5 phosphorylates Scc1 at several serine residues *in vitro*, one of which is situated within one separase cleavage site. A serine residue at this position is conserved in separase targets sites of budding yeast Scc1 and Rec8 and the fission yeast homolog of Scc1 (Rad21). Mutation of this serine within both of Scc1's separase cleavage sites abolishes sister chromatid separation in the absence of securin [96]. In most vertebrates, the equivalent residue is replaced by aspartate, implying that a negative charge at this position within a separase target site is required for efficient cleavage at this site. It is interesting that the same kinase, which is required for

cohesin's dissociation from chromosome arms during prophase in vertebrates, enhances cohesin's cleavage at the metaphase to anaphase transition in yeast.

Cohesin cleavage must be delayed until all chromosomes have bi-oriented on the mitotic spindle. Kinetochores that have not attached to microtubules or have failed to come under tension due to bi-orientation activate the Mad2 protein, which binds and inhibits the APC/C activator Cdc20, reviewed by Yu [97] (Fig. 6). Neither securin nor cyclins are destroyed and separase can therefore not be activated until all chromosomes have bi-oriented.

CONCLUSIONS

Cohesin and condensin are key regulators of chromosome morphology. Both are crucial for ensuring that daughter cells receive complete sets of chromosomes during each cell division cycle. New insights into cohesin's and condensin's molecular architectures have opened new ways of thinking how they could fulfill their functions. Cohesin might hold sister chromatids together by trapping them inside a proteinaceous ring. Separase triggers sister chromatid disjunction at the metaphase to anaphase transition by cleaving the ring open. It is not inconceivable that condensin operates according to a similar albeit different topological principle. Both cohesin and condensin may be molecular clamping devices, similar to carabiners used to hold ropes together. ATP binding and hydrolysis cycles might be used to open and fasten the SMC clamps by regulating their association with their non-SMC partners. A similar principle might also be used by prokaryotic SMC protein complexes. At first sight the clamping of chromatid fibers has little resemblance to a sophisticated machine. But how for instance does the cell ensure that SMC complexes trap the correct DNA molecules? How for instance does cohesin specifically trap sister DNAs and how is this coordinated with DNA replication? If cohesin and condensin really act as clamps, then these clamps must be very sophisticated ones and much future research will be needed to unravel their secrets.

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