From a single double helix to paired double helices and back

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The propagation of our genomes during cell proliferation depends on the movement of sister DNA molecules produced by DNA replication to opposite sides of the cell before it divides. This feat is achieved by microtubules in eukaryotic cells but it has long remained a mystery how cells ensure that sister DNAs attach to microtubules with opposite orientations, known as amphitelic attachment. It is currently thought that sister chromatid cohesion has a crucial role. By resisting the forces exerted by microtubules, sister chromatid cohesion gives rise to tension that is thought essential for stabilizing kinetochore–microtubule attachments. Efficient amphitelic attachment is therefore achieved by an error correction mechanism that selectively eliminates connections that do not give rise to tension. Cohesion between sister chromatids is mediated by a multisubunit complex called cohesin which forms a gigantic ring structure. It has been proposed that sister DNAs are held together owing to their becoming entrapped within a single cohesin ring. Cohesion between sister chromatids is destroyed at the metaphase to anaphase transition by proteolytic cleavage of cohesin's Sccl subunit by a thiol protease called separase, which severs the ring and thereby releases sister DNAs.

**Keywords:** mitosis; sister chromatid cohesion; cohesin; separase

1. THE GENOME MUST BE INHERITED

The year 2003 is not only the fiftieth anniversary of the double helix but it is also *ca.* 100 years since the genome was first defined by Theodore Boveri (1902). Although central to all existing living organisms, genomes presumably did not exist in our earliest ancestors, whose enzymes were presumably largely RNA based and responsible for their own propagation. The evolution of DNA-based genomes that possessed no enzymatic activity but merely encoded information for enzyme production must have gone hand-in-hand with the evolution of mechanisms responsible for ensuring that daughter cells inherited a complete set of their DNA code from their ancestors.

In all living organisms, the two sister double helices produced by DNA replication are moved to opposite poles of the cell prior to its division by highly accurate, albeit potentially diverse, mechanisms. The mitotic mechanism, involving traction of sister DNAs to opposite sides of the cell by microtubules, appears to be universal in eukaryotic cells. Because the mechanisms by which prokaryotic and archaeabacterial chromosomes (nucleoids) are segregated remain somewhat mysterious, it is still not possible to say whether they differ fundamentally from mitosis. The recent discovery that bacteria possess microtubule-like structures composed of the tubulin-like FtsZ protamer (Lowe & Amos 1998) and actin-like structures composed of the actin-like protamer like MreB (Van den Ent et al. 2001) raise the possibility that their chromosomes might also be segregated using cytoskeletal proteins, as has been found for the *Escherichia coli* R1 plasmid (Van den Ent et al. 2002).

Given that genomes are in a sense ‘there to be inherited’, i.e. segregated, it is only appropriate that the original concept of a genome was derived from the observation of cells that indulged in systematic chromosome mis-segregation. This was Boveri’s analysis of development after sea urchin eggs had been fertilized by two sperm (Boveri 1902). Owing to the accident that Mendel’s pioneering work had been completely ignored (until its rediscovery in 1900), the notion that chromosomes might carry the information passed down from parent to offspring stemmed from cytological observations; namely, that the sole but clearly not insignificant contribution of the father to the zygote was the contents of the male pronucleus, which metamorphoses into chromosomes resembling those inherited from the mother at the onset of the first cleavage division (Beneden 1883). Underlying this notion was of course the belief that chromosomes remained intact throughout the cell division cycle despite seeming to disappear during interphase (Boveri 1888). It was known that the number of chromosomes per cell varied enormously between organisms despite fundamental similarities in their physiology and design. This presumably contributed to the erroneous belief that each chromosome carried the entire hereditary material and that the extra chromosomes were merely back-ups (Baltzer 1967). This notion was only dispelled by Boveri’s remarkable experiments with sea urchin eggs fertilized with two sperm, from which he rightly concluded that different chromosomes possessed different properties and that normal development depended on the inheritance of a combination of chromosomes (Boveri 1902).
2. THE LOGIC OF MITOTIC CHROMOSOME SEGREGATION

To appreciate Boveri’s dispermic experiment best, it is necessary to consider the fundamental logic underlying chromosome segregation in eukaryotic cells. The traction of sister chromatids to opposite poles of the cell depends on sister chromatids becoming attached (via their kinetochores) to microtubules with opposite orientations. In most but not all cells, microtubules grow and shrink from defined MTOCs (Mitchison & Salmon 2001). Growth involves addition of new subunits at the plus ends distal from these centres. Such growing microtubules have three possible fates. They can suffer a ‘catastrophe’, which causes them to switch from a growing mode to a shrinking one in which microtubules de-polymerize from the plus ends. Alternatively, their plus ends can be captured by kinetochores in which case de-polymerization at the plus ends while still attached to kinetochores will tend to move the latter towards MTOCs, assuming that the latter remain fixed in space. The third fate is for special proteins to cross-link the plus ends of microtubules that have grown out from different poles. This cross-linking creates a continuous pole-to-pole spindle that enables MTOCs to resist being pulled together upon the de-polymerization of kinetochore microtubules (Mitchison & Salmon 2001). One of the most enduring mysteries about mitosis is how cells ensure that sister kinetochores attach to microtubules with opposite orientations, which is known as amphitelic attachment. Were sister kinetochores to attach to microtubules with the same orientation, which is known as syntenic attachment, then both chromatids would be dragged to the same pole, which would lead to one daughter cell inheriting both chromatids while the other inherited neither.

It is currently thought that amphitelic attachment is achieved by a process of trial and error (Nicklas & Ward 1994). Activity of the Ipl1/Aurora B kinase appears to de-stabilize syntenic kinetochore–microtubule attachments, which enables kinetochores to capture a new microtubule (Biggins et al. 1999; Tanaka et al. 2002; Hauf et al. 2003; figure 1). If this new attachment happens to be made to microtubules emanating from a different pole from those attached to its sister, then sister kinetochores will be pulled in opposite directions, a process known as bi-orientation. This does not, however, immediately lead to the migration of sister chromatids to opposite poles because they remain connected to each other after DNA replication is complete. This sister chromatid ‘cohesion’ (Miyazaki & Orr-Weaver 1994) resists the disjunction of sisters and as a result creates a state of tension. It is currently thought that the tension created as a consequence of amphitelic attachment stabilizes the connection between kinetochores and microtubules (Nicklas & Ward 1994). The Ipl1/Aurora B kinase de-stabilizes syntenic but not, apparently, amphitelic kinetochore–microtubule attachments, and this then is thought to be the mechanism by which eukaryotic cells ensure bi-orientation. It is not yet known how the kinase de-stabilizes syntenic attachments or how tension suppresses the disconnection process. The Ipl1/Aurora B kinase is associated with centromeres (the DNA associated with kinetochores) (Adams et al. 2000; Buvelot et al. 2003) and its activity might conceivably be downregulated when centromeric chromatin comes under tension. Alternatively, tension may alter kinetochore–microtubule

Figure 1. A role for the Ipl1/Aurora B protein kinase in correcting false (e.g. syntenic) microtubule–kinetochore attachments. Discs, sister kinetochores; lines, microtubules with opposite orientation; arrows, pulling force.
attachments in a manner that renders them refractory to the action of the Ipl1/Aurora B kinase.

In summary, three fundamental processes are required for establishing the bi-orientation of sister kinetochores: (i) microtubules with different orientations whose interdigitation creates a symmetrical bipolar spindle and whose capture by kinetochores causes chromatids to be dragged towards the minus ends of microtubules; (ii) sister chromatid cohesion whose resistance to spindle forces generates tension at the kinetochore–microtubule interface; and (iii) a system that de-stabilises the connections between microtubules and kinetochores in the absence of tension. Only when every single chromosome has bi-oriented (a period known as metaphase) are the microtubule forces allowed to ‘win the day’ and eventually overpower sister chromatid cohesion, which triggers the segregation of sister chromatids to opposite poles of the cell (a period known as anaphase). Recent work implies that a catastrophic loss of sister chromatid cohesion rather than any huge increase in traction mediated by microtubules may be the primary trigger for chromatid disjunction at the metaphase to anaphase transition (Uhlmann et al. 2000).

3. MIS-SEGREGATION AND DISCOVERY OF THE GENOME

In most but not all cells, the bipolar spindle that creates the axis along which chromatids are pulled is produced by MTOCs. However, many meiotic cells and in particular oocytes produce bipolar spindles without these organelles. In oocytes, microtubules appear to be seeded by factors associated with chromatin and the spindle poles that define the axis of the bipolar spindle are created by factors that selectively cross-link the minus ends of microtubules (Heald et al. 1996). This MTOC-independent mechanism is nevertheless rapidly supplanted by the arrival of an MTOC supplied by the sperm, which duplicates soon after fertilization and organizes the bipolar spindle of the first and all subsequent divisions of the zygote. Sea urchin eggs, like those of all other species, are normally fertilized by a single sperm but in rare cases two different sperm manage to clamber on board and the resulting zygote therefore possesses three sets of chromosomes, one maternal and two paternal, as well as two MTOCs. The latter both duplicate before the first cleavage division and the zygote therefore enters mitosis with a quadri-polar spindle. Individual chromosomes still bi-orient but do so along four instead of two axes, which are defined by the four sides of the square whose corners each contain a MTOC. Chromatid disjunction and cell cleavage perpendicular to each of the four spindle axes results in the three genomes being distributed between four and not two cells.

The four blastomeres produced by two successive divisions of a normal sea urchin zygote can be teased apart and each gives rise to normal-looking albeit small embryos. However, the four blastomeres produced by the first quadripolar division of dispermic eggs only rarely give rise to normal embryos. Many die rapidly while others develop in highly abnormal ways. Boveri’s key insight (Boveri 1902) was the realization that each of the 18 chromosomes supplied by the mother (and father) made unique contributions to development, that they were not merely back-ups of each other (though homologous chromosomes from the other parent were), and that the inheritance of at least one complete set of chromosomes (a haploid complement) was needed for normal embryogenesis. If so, the abnormal development of the four-celled blastomeres produced from dispermic eggs must be due to their failure to inherit a complete set of chromosomes. By assuming that the axis along which particular chromosomes disjoined was random, he showed that the incidence of producing a normal-looking embryo was the same as the chances of a blastomere inheriting at least one of each of the eighteen unique chromosomes from the chaotic division during which three complete chromosome sets distributed chromatids at random along four axes. It turned out that sometimes one of the two MTOCs introduced by the two different sperm failed to duplicate before the first division with the result that chromosome segregation occurred along three and not four different axes. This greatly increased the chances of one of the three blastomeres inheriting a complete haploid set of chromosomes. The incidence with which blastomeres produced by a tripolar division underwent normal embryogenesis increased accordingly. Boveri’s dispermic experiments were completed around the same time as the re-discovery of Mendel’s work, and both Boveri and in particular Sutton (1903) realized that chromosomes might therefore be the carriers of Mendel’s genetic determinants. This then must be considered the point when the genome was first defined. The double-helical structure of DNA was proposed 50 years later (Watson & Crick 1953a) while the complete sequence of the human genome was completed 100 years later.

4. SISTER CHROMATID COHESION

While the double helix model immediately suggested a mechanism by which the information encoded by chromosomes was encoded and also the principle by which it was duplicated (Watson & Crick 1953a), it shed little or no light on the mechanisms by which it might be segregated. The nature of sister chromatid cohesion remained particularly elusive, even long after the discovery of microtubules and the sites of their attachment to chromosomes. It was suggested that intercatenation of sister DNA molecules produced by the collision of converging replication forks might be responsible for holding sisters together until the metaphase to anaphase transition (Murray & Szostak 1985). This intertwining of chromatids undoubtedly exists and the cell requires a special enzyme, namely topoisomerase II, to de-catenate sisters after replication and during mitosis (Dinardo et al. 1984). Topoisomerase II acts by introducing a double-strand break into one sister molecule and passing the other sister between the broken ends before resealing the broken strand (Wang 2002). It is an essential enzyme without which chromatids fail to disengage properly either during the process of chromatid individualization in prophase (Gimenez-Abian et al. 2000) or during chromatid disjunction in anaphase (Dinardo et al. 1984). However, there is no evidence that a delay in de-catenating sister DNA molecules is responsible for preserving the cohesion needed to resist microtubule-induced splitting during mitosis (Koshland & Hartwell 1987).
It seems increasingly likely that the force that resists chromatid splitting is supplied by a multi-subunit complex called cohesin whose constituents were first identified in the yeast Saccharomyces cerevisiae by the isolation of mutants incapable of holding sisters together during metaphase (Gucci et al. 1997; Michaelis et al. 1997; Toth et al. 1999). Cohesin consists of four proteins: Scc1 (also known as Mcd1 and Rad21), Scc3 (also known as SA1 and SA2), Smc1 and Smc3 (Hirano 2000; Nasmyth 2001). The genomes of all eukaryotic cells encode homologues of all four of these proteins. In cells lacking any one of cohesin’s subunits, sister chromatids separate precociously, which leads to inefficient bi-orientation and to catastrophic chromosome mis-segregation (Tanaka et al. 2000; Sonoda et al. 2001; Hoque & Ishikawa 2002). Partial cohesin defects that are still compatible with cell proliferation lead to very high levels of aneuploidy (Michaelis et al. 1997).

The four core subunits of cohesin are by no means all of the proteins implicated in sister chromatid cohesion by genetic studies. Cohesion in yeast also depends on a protein loosely associated with cohesin called Pds5 (Hartman et al. 2000; Panizza et al. 2000), on a partly independent complex composed of the Scc2 and Scc4 proteins (Furuya et al. 1998; Ciosk et al. 2000), on an acetyl transferase called Eco1 (Toth et al. 1999; Ivanov et al. 2002) or Ctf7 (Skibbens et al. 1999), and at least partly on a version of the RFC complex (involved in loading DNA polymerase sliding clamps) whose Rfc1 subunit has been replaced by a protein called Ctf18 (Mayer et al. 2001). Given the plethora of proteins implicated in establishing or maintaining sister chromatid cohesion, are there any grounds for believing that cohesin and not one of these other proteins forms the bridges between chromatids? Analysis of the genome of the microsporidian Encephalitozoon cuniculi is very revealing in this regard. Encephalitozoon cuniculi is a parasite of fungal origin with a 2.9 Mb genome encoding ca. 2000 proteins. A systematic search for homologues of all four cohesin subunits as well as Ipl1, separase and Rfc1 but failed to find homologues of Pds5, Eco1, Scc2 or Ctf18 (table 1). Homologues of four of these proteins can, by contrast, readily be found in all fully sequenced genomes of plants and animals. This suggests that cohesin in E. cuniculi may be able to establish and maintain sister chromatid cohesion in the complete absence of Pds5, Eco1, Scc2 and Ctf18 proteins. Though highly conserved in most eukaryotic lineages, these proteins cannot therefore constitute the bridges holding sisters together. The finding that E. cuniculi lacks proteins homologous to Pds5 and Eco1 is consistent with the finding that Schizosaccharomyces pombe can proliferate when the genes for these two proteins are simultaneously deleted (Tanaka et al. 2001).

Cohesin’s localization both spatially and temporally also makes it a good candidate to be directly involved in holding sister DNA molecules together. In budding yeast, where chromatids are very closely held together along their entire length until the onset of anaphase, cohesin is tightly associated with chromosomes until the metaphase to anaphase transition (Michaelis et al. 1997), whereasupon proteolytic cleavage of its Scc1 subunit, by a cysteine protease called separate, triggers its dissociation from chromatin and the destruction of cohesion (Uhlmann et al. 1999, 2000). In mammalian cells, the bulk of cohesin associated with chromosome arms dissociates at the time of their individualization during prophase (Losada et al. 1998; Sumara et al. 2000; Waizenegger et al. 2000) but much remains at centromeres, where cohesion between chromatids is tightest. A small but significant amount remaining along the inter-chromatid axes of chromosome arms is responsible for holding chromatid arms together during undisturbed metaphases. However, the eventual disappearance of cohesin from chromosome arms causes them to separate when cells are arrested in a mitotic state for substantial periods owing to incubation in the presence of spindle poisons, thereby generating the canonical cross-shaped image of chromosomes. Dissociation of cohesin from chromosome arms, initiated during prophase, is thought to be driven by its phosphorylation by Polo-like kinases (Losada et al. 2002; Sumara et al. 2002), while that of centromeric cohesin at the onset of anaphase is mediated by cleavage of Scc1 by separase (Waizenegger et al. 2000).

The distribution of cohesin during meiosis is particularly revealing. Unlike mitotic and meiosis II cells whose chromosome arms are poorly if not at all cohesed (and contain little or no cohesin), meiosis I cells actually require sister chromatid cohesion along chromosome arms if chiasmata are to hold homologues together while they (and not sister chromatids) are bi-oriented by meiosis I spindles (Petronczki et al. 2003). Cohesion between sister chromatids in meiosis is established during pre-meiotic DNA replication and is mediated by a meiosis-specific form of the cohesin complex (Watanabe & Nurse 1999; Klein et al. 1999; Kitajima et al. 2003). Meiotic cohesin differs from its mitotic equivalent, partly because it has to participate in the recombination process and partly because cohesin holding sister centromeres must survive the first meiotic division, so that it can direct the second one. In budding yeast, it appears that meiotic and mitotic cohesin differ only in the replacement of Scc1 by the meiosis-specific variant Rec8 (Klein et al. 1999). In mammalian cells, however, Scc1 is replaced by a meiosis-specific variant called Smc1β (Revenkova et al. 2001), the Scc3 subunits SA1 and SA2 at least partly by a meiosis-specific variant called STAG3 (Prieto et al. 2001), and Scc1 by meiosis-specific Rec8 (Eijpe et al. 2003; N. Kudo, personal communication). During meiosis I, be it in yeast, Caenorhabditis elegans (Pasierbek et al. 2001) or mice, considerable amounts of Rec8 persist along chromosome arms, both proximal and distal to chiasmata as well as at centromeres, right up until metaphase I. At the onset of anaphase I, Rec8 disappears suddenly and completely from chromosome arms; that is, precisely at the point when loss of arm cohesion permits the resolution of chiasmata and disjunction of maternal from paternal centromeres. Sister centromeres remain closely juxtaposed as they are dragged to the same pole during meiosis I and this ‘centromeric cohesion’ enables their bi-orientation by meiosis II spindles. Remarkably, Rec8 persists in the vicinity of centromeres throughout anaphase I (i.e. when it simultaneously disappears from chromosome arms), remaining at this location until sister centromere disjunction at the onset of anaphase II. Thus, cohesin is not only essential for sister chromatid cohesion but is invariably
Table 1. The presence or absence of cohesion proteins in *Sacccharomyces cerevisiae*, *Encephalitozoon cuniculi* and *Homo sapiens*. (The *E. cuniculi* proteome (Katinka et al. 2001) was screened with NCBI-blastp (Altschul et al. 1997) and profile-hidden Markov model (HMM) searches (Eddy 1998) using standard statistical parameters. In the case of Ipl1, Chl1 and Ctf18, a phylogenetic analysis of the closest kinases, helicases and ATPases families, respectively, was applied for classification. Although Ctf18, Pds5, Eco1 and Scs2 proteins contain regions conserved between yeast and mammals, no orthologues could be assigned to *E. cuniculi*. The *E. cuniculi* accession numbers are derived from the NCBI-nr database (Wheeler et al. 2003.).

<table>
<thead>
<tr>
<th>biological process</th>
<th><em>S. cerevisiae</em></th>
<th><em>H. sapiens</em></th>
<th><em>E. cuniculi</em></th>
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found within regions of the chromosome where sisters are known to be cohesed and disappears from such sites when cohesion is lost.

5. MIGHT COHESIN EMBRACE DNA?

Whether cohesin really forms the tether that holds sister DNAs together and if so, how, is not fully understood. There is, nevertheless, mounting evidence that cohesin forms a proteinaceous loop or ring within which DNA strands might be entrapped (Haering et al. 2002; Gruber et al. 2003). Cohesin’s structure is therefore consistent with it being the tether itself. Smc1 and Smc3 form long rod-shaped proteins whose N- and C-terminal halves fold back on themselves to form long, up to 50 nm, stretches of intramolecular (Haering et al. 2002) and anti-parallel (Melby et al. 1998) coiled coils. This brings N- and C-terminal domains together to form one half of an ABC-like ATPase (Hopfner et al. 2000; Lowe et al. 2001). At the centre of their folding axis lies a domain whose dimerization joins two Smc proteins to form a V-shaped molecule with ATPases at the apex of each arm and a doughnut-shaped dimeric structure at its base (Haering et al. 2002). Most, if not all, bacteria produce Smc homodimers (Melby et al. 1998) created by homotypic interactions between their dimerization domains. Cohesin instead forms heterodimers through a heterotypic interaction between the dimerization domains of Smc1 and Smc3.

The binding of ATP to the heads of Smc1 and Smc3 is thought to cause their association (Hopfner et al. 2000; Hirano et al. 2001), but this is not the only conformation in which cohesin might form closed rings. In both soluble and chromatin-bound forms of cohesin, its Scc1 subunit bridges the Smc1 and Smc3 heads, which do not appear to be otherwise intimately associated except for their peripheral connection via the distant dimerization domains of Smc1 and Smc3 (Haering et al. 2002; Gruber et al. 2003). Scc1’s N- and C-terminal domains bind to the heads of Smc3 and Smc1, respectively. Significantly, separate cleaves Scs1 in the region that connects its N- and C-terminal domains (Uhlmann et al. 1999), which opens the ring and causes cohesin to fall off chromosomes. These observations have led to the notion that cohesin might associate with DNA using a topological principle, namely trapping of DNA strands inside cohesin’s ring (figure 2). If so, then trapping of sister DNA molecules within a single cohesin ring could provide the mechanism that holds sister chromatids together until separase is activated at the onset of anaphase (figure 3). According to this model, the ‘arms’ of the Smc1/3 heterodimer hold DNA in an embrace that is locked by the binding of Scs1 to their ‘hands’, i.e. ATPase domains.

Cohesin binds to chromosomes prior to DNA replication but it builds connections between chromatids only during DNA replication (Uhlmann & Nasmyth 1998). If cohesin is expressed only after DNA replication, it still associates with chromatin but is unable to hold sisters together, demonstrating that the bridges built by cohesin must be established during S-phase. The close physical proximity of newly born double-stranded sister DNAs, a situation occurring exclusively following the passage of the replication fork, presumably facilitates the establishment of cohesion (figure 3). If the ring model is correct, then one way of ensuring that sister DNAs end up being embraced by a single Smc1/3 heterodimer would be for replisomes to pass through a cohesin ring that had already embraced DNA ahead of the replication fork. The very large diameter of cohesin rings (30–40 nm) means that passage of enormous complexes is not inconceivable.

There is no direct evidence that DNA really does pass through cohesin’s ring. Nevertheless, the finding that
proteolytic cleavage either of Scc1 (Uhlmann et al. 2000) or of Smc3’s coiled coil (Gruber et al. 2003) is sufficient to release cohesin from chromosomes is certainly consistent with this notion. If cohesin’s association with DNA is largely topological, cleavage of DNA as well as cleavage of the ring should be sufficient to release cohesin from circular mini-chromosomes. Another important prediction of the topological model is that it should be possible to prevent binding of cohesin to chromosomes as well as the establishment of sister chromatid cohesion by preventing the opening of cohesin’s two Smc arms.

If correct, the ring model raises all sorts of questions: namely, how does DNA gain entry into the ring or, rather, how are rings formed around DNA and how do sister DNA strands end up trapped inside the same ring? It is of course conceivable that sister DNAs are trapped by different rings whose interaction holds them together. This notion predicts that it should be possible to measure stable interactions between two different molecules of any cohesin subunit extracted from chromosomes. No such interactions could be detected between cohesin subunits released from chromatin by micrococcal nuclease digestion (Haering et al. 2002), which is a very gentle procedure. If most cohesin complexes extracted by this means had been participating in cohesion while bound to chromosomes, which is an important but possibly unjustified assumption, then the failure to detect interactions between different cohesin complexes implies that cohesion is mediated by individual monomeric cohesin complexes. In summary, four key postulates underpin the ring-embrace model, namely, that cohesin functions as a monomer, that DNA is trapped inside cohesin rings, that trapping is essential for cohesin’s stable association with DNA, and that DNA trapping is essential for cohesion. There is some, but not yet overwhelming, evidence for all four. The model is therefore still at the stage of a good working hypothesis.

It is important to appreciate that cohesin is just one of a family of complexes with similar architectures. Most bacteria also possess Smc proteins (Soppa 2001), which form homodimers not heterodimers and are associated with ScpA-like and ScpB-like proteins (Mascarenhas et al. 2002). Bacterial Smc-ScpA-ScpB complexes like the related MukBEF complex in *E. coli* (Melby et al. 1998; Yamazoe et al. 1999) are essential for accurate chromosome (nucleoid) partition. There is no evidence that persistent cohesion between sister DNAs mediated by Smc proteins has any role in bacterial chromosome segregation and it is currently thought that bacterial Smc and MukB proteins are instead involved in the packaging or condensation of chromosomes.

The eukaryotic condensin complex (Hirano et al. 1997), which is composed of an Smc2/Smc4 heterodimer and three non-Smc subunits including a protein called Barren, also has a role in chromosome packaging that is essential for the efficient disjunction of sister chromatids during anaphase. Condensin is largely absent from chromosomes until pro-metaphase, when its deposition on chromosomes forms a spiral axis along the length of each chromatid (Maeshima & Laemmli 2003). This location differs from

![Figure 2.](image-url) Figure 2. The structure of cohesin and a model for how it might interact with DNA. Cohesin’s kleisin subunit Scc1 (green) is shown connecting the two ATPase heads of Smc1 (red) and Smc3 (blue). It is not known whether Scc1 connects heads that are otherwise apart (as shown) or are themselves connected by ATP molecules bound to each head (not shown). DNA is shown passing once or more through the cohesin ring.

![Figure 3.](image-url) Figure 3. A model for how cohesin associates with DNA in front of and behind a replication fork. Polymerases associated with clamps are shown on both the leading and lagging strand.
that of cohesin at this stage, which is found along the axis between chromatids (Losada et al. 2002; Sumara et al.
2002). The recent finding that Scc1 and Rec8 are distantly related to ScpA and Barren proteins (forming a superfamily called kleisins), suggests that the principles by which cohesin’s kleisin subunit helps tether sister chromatids may underlie the mechanism by which bacterial and eukaryotic condensins organize individual chromatid fibres (Schleiffer et al. 2003). It would seem likely that all Smc–kleisin complexes operate using a similar mechanistic principle. If cohesin acts by trapping double helices, then should not also condensin? It is possible that while cohesin cross-links sister DNA molecules, condensin cross-links different stretches of the same DNA molecule. How the latter would promote efficient chromatid disjunction during anaphase is a key problem for the future.

6. DISSOLUTION OF SISTER CHROMATID COHESION

The ‘tug of war’ between cohesin and microtubules that gives rise to bi-orientation during metaphase is finally resolved by cleavage of cohesin’s Scc1 subunit by separase (Uhlmann et al. 1999, 2000), which is a cysteine protease distantly related to the caspases involved in programmed cell death. Proteolysis of Scc1 by separase triggers the dissociation of cohesin from chromosomes, the destruction of cohesion and the sudden segregation of sister chromatids to opposite poles of the cell (figure 4). Separase is kept inactive for most of the cell cycle by binding to an inhibitory chaperone called securin (Ciosk et al. 1998; Zou et al. 1999; Hornig et al. 2002), which is only removed through proteolysis (Cohen-Fix et al. 1996; Funabiki et al. 1996) mediated by a ubiquitin protein ligase called the APC/C (reviewed in Zachariae & Nasmyth 1999). Activation of the APC/C by a WD40 protein, called Cdc20 (Visintin et al. 1997), causes the ubiquitination and hence destruction not only of securin but also of mitotic cyclins which, when complexed with the protein kinase Cdk1, drive G2 cells into mitosis.

Because separase appears to act globally within the cell, it must not be activated while chromosomes still exist that have not yet attached to microtubules in an amphitelic manner. Kinetochores of such ‘lagging’ chromosomes bind and thereby activate an inhibitor of the APC/C called Mad2 (reviewed in Shah & Cleveland 2000). Mad2 exerts a veto, called the mitotic checkpoint, on the destruction of securin and, hence, also on separase activation. Only once every chromosome has bi-oriented is this inhibitory signal shut off. In budding yeast, lagging chromosomes halt separase activation solely through the control of securin proteolysis by the APC/C (Yamamoto et al. 1996; Alexandru et al. 1999). Additional mechanisms controlling separase activity and, hence, anaphase must exist in mammals as cell lines lacking securin still arrest the onset of anaphase in response to spindle poisons (Jallepalli et al. 2001). Recent work indicates that separase in vertebrate cells is inhibited through its phosphorylation by Cdk1 (Stemmann et al. 2001). Thus, cyclin destruction as well as that of securin by the APC/C may be important for separase activation. Phosphorylation of Scc1 by Cdc5/Polo kinase, which enhances Scc1’s ability to act as a separase substrate also regulates the Scc1 cleavage reaction, at least in budding yeast (Alexandru et al. 2001).

7. WHY USE COHESIN AND NOT, SAY, CATENATION TO HOLD SISTERS TOGETHER DURING BI-ORIENTATION?

If generation of tension is the key to producing kinetochore–microtubule attachments that are refractory to destabilization mediated by the Ipl1/Aurora protein kinase, then any form of connection that holds sister chromatids together should be sufficient for the bi-orientation process. Cells appear to use cohesin for this purpose but there may be no intrinsic reason why they could not equally well use the intertwining (catenation) of sister DNA double helices that is a legacy of DNA replication (Sundin & Varshavsky 1981). One potential disadvantage of using catenation is that removal of most intercatenation of sister DNAs may...
be necessary for chromatic individualization during prophase and pro-metaphase (Gimenez-Abian et al. 2000). The extraordinary metamorphosis of interphase chromatin into paired individualized chromatids presumably requires much disentangling and hence de-catenation. Preservation of some intertwining to hold sister chromatids together until the onset of anaphase would require that some but not other inter-sister catenations would have to be refractory to topoisomerase II activity until this point but thereafter become exquisitely sensitive to the enzyme. There is no fundamental problem with arranging this. It is, for instance, believed that most cohesin is removed from chromosome arms during prophase while a subpopulation remains on chromosomes and is responsible for holding sisters together during the bi-orientation process. If cohesin can behave in this heterogenous manner then so too presumably could a subpopulation of intertwinnings between sisters. A real problem with using catenation is the potential danger of failing to convert such stable catenations that had persisted up to metaphase into unstable ones at the onset of anaphase. Any single instance of a failure to achieve this along an entire chromosome would lead to anaphase bridges and hence to chromatic breakage, which is disastrous at this stage of the cell cycle. Occasional cohesin-mediated sister-sister bridges may in an analogous fashion survive separate during anaphase but this need not pose any grave danger to the cell as long as the tensile strength of cohesin’s ring is substantially less than that of double-stranded DNA. If so, the cohesin ring will always break before the DNA. We do not yet have any idea of the ring’s exact strength but its non-covalent nature suggests that that it must be well below that of phosphodiester bonds. There is in fact some evidence that the connections mediated by cohesin are breakable even by the splitting forces mediated by a single pair of microtubules. Circular minichromosomes in yeast, which are clearly held together by cohesin both before and during bi-orientation (Megee et al. 1999), appear to segregate to opposite spindle poles before separate is activated by the APC/C (Tanaka et al. 1999). The simplest explanation for this phenomenon is that the limited number of cohesin bridges on mini-chromosomes can literally be ripped apart by the force of a single microtubule pair. One presumes that this would be prevented by inactivation of Topo II, in which case one would have direct proof that cohesin bridges are weaker than the double helix.

8. SUMMARY

In eukaryotic cells, replication of the double helix does not merely produce two identical helices from a single one but two identical helices that are tethered together in a fashion that is sufficiently robust to withstand their being split apart by kinetochore-associated microtubules. This sister chromatic cohesion can endure not only for hours but also for decades and permits cells to bi-orient and segregate chromatids long after DNA replication has been completed. The failure of bacteria to develop such a mechanism may have contributed to their persistent use of replication origins in the process of chromosome segregation, a consequence of which is that their chromosomes must be replicated from a single origin, which places an upper limit on their size. There is mounting but not yet incontrovertible evidence that eukaryotic sister chromatid cohesion may be mediated by the entrapping of sister DNAs inside the large proteinaceous rings of a cohesin complex. Severance of cohesin rings through cleavage of their Scc1 kleisin subunit by the cytessine protease separate releases sisters from each other and permits their traction to opposite poles of the cell during anaphase. The genome was defined by Boveri’s dispersive experiments ca. 100 years ago. Not only has the sequence of the human genome just been deciphered but we are starting to understand how it has been propagated for the last two to three billion years.

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GLOSSARY

**APC/C**: anaphase-promoting complex or cyclosome

**MTOC**: microtubule organizing centre

**RFC**: replication factor C