Splitting the Nucleus

What's Wrong with the Tripartite Ring Model?

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The segregation of sister DNA molecules at mitosis involves their traction to opposite poles by microtubules attached to kinetochores. By creating tension required to stabilize kinetochore microtubules, sister chromatid cohesion has a key role in ensuring that sister kinetochores attach to microtubules with opposing polarity, a process known as biorientation. Cohesion is mediated by a cohesin complex whose Smc1, Smc3, and kleisin subunits form a tripartite ring thought to hold sister DNAs together by entrapping them (the ring model). Sister chromatid disjunction at the onset of anaphase is triggered by a thiol protease called separase whose activation, only when all chromosomes have bioriented, opens the cohesin ring by cleaving its kleisin subunit. Separase is inhibited by the binding of an inhibitory chaperone called securin whose destruction at the hands of a ubiquitin protein ligase called the anaphase-promoting complex/cyclosome (APC/C) is essential for kleisin cleavage and sister chromatid disjunction. We describe microinjection experiments showing that cohesin cleavage and Cdk1 down-regulation are sufficient to drive formation of daughter nuclei in cells arrested in metaphase due to inactivation of the APC/C and describe chemical cross-linking experiments consistent with the ring model. How sister DNAs enter the cohesin ring and are retained inside for long periods of time after the completion of DNA replication remains poorly understood.

Serious attempts to understand the organization of nuclei must account for how they reproduce, namely how the nuclei of dividing cells give rise to a pair of daughter nuclei shortly before cell division. Possibly the most significant insight into this remarkable process was the realization that unlike cells, nuclei do not undergo a constriction dividing them into two, known as “direct” division. They instead undergo a remarkable metamorphosis involving the appearance of nuclear threads that subsequently split longitudinally with each half moving to opposite poles of the cell before decondensing and nucleating the formation of daughter nuclei. This “indirect” process of nuclear division is now known as mitosis, the nuclear threads as chromosomes, and their progeny as sister chromatids. Although first described in beautiful detail by Flemming (1880), the potential significance of indirect nuclear division was first grasped by Wilhem Roux who suggested that it provided a mechanism for the nucleus to be divided not only quantitatively but also according to the mass and nature of its individual qualities (Roux 1883). We now know that Roux’s “qualities” are conveyed largely by the sequence of nucleotides along the DNA molecules that form the backbone of each chromosome and specify the entire development of the organism.

The realization that DNA is composed of two nucleotide chains wound around each other forming a double helix that is replicated semiconservatively using Watson–Crick base pairing revealed the fundamental principle responsible for chromosome replication (Watson and Crick 1953). It did not, however, explain how the two products of this process, namely sister chromatids, unerringly move to opposite poles of the cell during mitosis, a phenomenon so universal and usually flawless that it has often been

taken for granted and only in recent times acknowledged as a process lacking a satisfactory explanation. Defects in the segregation of chromatids during meiosis are responsible for human aneuploidy and for a large fraction of age-related infertility in females (Hassold and Hunt 2001), whereas defects during mitosis contribute to the genomic instability of tumor cells.

An important but underappreciated step toward understanding how sister chromatids disjoin to opposite poles was the realization that they contain a single, albeit gigantic, DNA molecule. This was so surprising and unexpected that the evidence, in hindsight glaringly unambiguous, was initially not recognized for what it was worth, and a far more improbable, not to mention baroque, alternative favored. The classic experiment (Taylor et al. 1957), somewhat unjustly overshadowed by the work by Meselson and Stahl (1958), involved the use of tritiated thymidine to pulse-label DNA in proliferating bean shoot cells. As predicted by the semiconservative replication intrinsic to the Watson–Crick model, both chromatids of mitotic chromosomes produced after a round of DNA replication in the presence of the tritiated thymidine contained similar levels of the isotope. Remarkably, a subsequent round of replication in the absence of label (the chase) generated mitotic chromosomes in which one chromatid was labeled and one unlabeled, a result that is difficult if not impossible to explain without supposing that each chromatid is composed of a single DNA molecule. This interpretation was confirmed by comparing the kinetics with which the loops and central axes of lampbrush bivalent chromosomes were broken by DNase I, an enzyme that nicks one DNA strand at a time. Breaks in the loops accumulated with double-hit kinetics, consistent with a single double helix, whereas

breaks in the central axis accumulated with quadruple-hit kinetics, consistent with the existence of two double helices (Gall 1963). The problem of chromosome segregation therefore boils down to how sister DNA molecules are pulled toward opposite poles by the microtubules of the spindle apparatus that attach to each molecule via specialized attachment sites known as kinetochores. This is known as the biorientation problem.

SISTER CHROMATID COHESION RESISTS SPINDLE FORCES

Appreciation of the role of sister chromatid cohesion in biorientation stemmed from experiments with meiotic cells in which the participants are not a pair of sister chromatids, as in mitosis, but instead bivalent chromosomes containing four chromatids interconnected by crossing-over between nonsister homologous chromatids (Fig. 1). During meiosis I, sister kinetochores act as a single unit and the name of the game is to pull maternal and paternal sister kinetochore pairs in opposite directions. A beautiful series of experiments with grasshopper spermatocytes demonstrated that attachments leading to the erroneous traction of maternal and paternal kinetochores to the same pole in fact occur with some regularity but are invariably unstable and sooner or later replaced by more stable attachments in which maternal and paternal kinetochores are pulled in opposite directions, which is the desired outcome. This led to the proposal that biorientation, in this case of bivalent chromosomes, comes about by the selective stabilization of attachments that lead to tension. As predicted by this notion, erroneous attachments in which maternal and paternal kinetochores had attached to the same pole were stabilized by pulling the bivalent in the opposite direction with a tiny glass needle (Nicklas 1967).

If microtubules provide the force that pulls chromatids around the cell, the tension required for stable attachments can only be created by forces capable of countering those created by microtubules, namely cohesive forces that hold together sister chromatids and bivalent chromosomes during mitosis and meiosis, respectively. Note that it is not necessary to invoke two different types of cohesiveness during meiosis and mitosis because sister chromatid cohesion would suffice in both cases. What then after replication holds sister DNA molecules together in a manner capable of resisting spindle forces? The first coherent and

serious proposal was the suggestion that cohesion is mediated by the intertwining of sister DNAs created during DNA replication (Murray and Szostak 1985), either by swivelling of replication forks driven by supercoiling ahead of them induced by unwinding the parental DNA strands (Lucas et al. 2001) or by rapid replication fork convergence (Sundin and Varshavsky 1981). Such intertwining or catenation is undoubtedly generated during S phase and its removal requires the action of topoisomerase II (Topo II) (Dinardo et al. 1984; Holm et al. 1985). The model proposed that a level of intertwining sufficient to hold sisters together persists until metaphase, whereupon a sudden burst of Topo II activity would remove the last vestiges and trigger sister chromatid disjunction. To explain the two-step removal of cohesion during meiosis, it was suggested that pericentric DNA was not replicated until meiosis II, whereupon a new wave of intertwining would generate sufficient cohesion at centromeres to drive the second meiotic division (Murray and Szostak 1985). This fascinating proposal had great merits because it provided a clear and testable explanation for the phenomenon of sister chromatid cohesion. It made a very strong prediction, namely that circular minichromosomes in yeast cells arrested in metaphase should be composed of catenated sister DNAs. An investigation revealed this not to be the case, dealing the hypothesis a major, although possibly not mortal, blow (Koshland and Hartwell 1987). The finding that Topo II is still required to decatenate chromosomal DNAs after metaphase (Holm et al. 1989) left open the possibility that catenation, although clearly insufficient to explain minichromosome segregation, might still have an important role in holding real chromatids together during mitosis. As we see below, there are grave doubts as to whether cohesion of this nature can on its own resist spindle forces. However, the catenation hypothesis has another major flaw. Any explanation for sister chromatid cohesion must not only provide a structural explanation for how sister DNAs are held together but must also explain how dissolution of cohesion is delayed until all chromosomes have bioriented (Miyazaki and Orr-Weaver 1994), a process now known as the spindle assembly checkpoint (SAC) (Musacchio and Salmon 2007). If sister chromatid disjunction were triggered by a burst of Topo II activity, the latter must be regulated by the biorientation process. However, to date there is no evidence for a regulatory pathway with these properties.

Figure 1. Meiotic chromosomes. Telocentric meiotic chromosomes from mice. (*Left*) Bivalent chromosomes created by recombination. (*Right*) Dyad chromosomes created by the selective destruction of sister chromatid cohesion along chromosome arms at meiosis I.

If not a burst of Topo II activity, then what triggers sister chromatid disjunction and the onset of anaphase? This has been known to be a sudden and synchronous process ever since its first description. Thus, Flemming rarely saw “images of some of the segments being split while others are not,” leading him to conclude that “the impetus that establishes the tendency for nuclear threads to split longitudinally acts simultaneously throughout the whole nuclear figure” (Flemming 1880). If biorientation during metaphase involves a “tug of war” between forces exerted by kinetochore-associated microtubules and cohesion between sister chromatids, disjunction could be driven by either loss of cohesion or a change in microtubule dynamics. As we see below, both occur at the onset of anaphase. There were two reasons for believing that loss of cohesion might have a key role. In plant cells treated with spindle poisons such as colchicine, all sister chromatids are observed to disjoin suddenly and synchronously in the total absence of microtubules (Mole-Bajer 1958). Needless to say, the chromatids do not subsequently move toward the poles but remain side by side. This process, known as C mitosis and widely used for diploidization of haploid cell lines in plant breeding, comes about because the SAC cannot delay the disjunction process for very long in these cells. A second reason is that when one of the kinetochores of a late-prometaphase chromosome is destroyed by a laser beam, the chromosome moves with the velocity of an anaphase chromosome toward the pole to which the nonirradiated kinetochore is connected (Nicklas 1988; Rieder 1991). If the forces acting on kinetochores do not appear to increase greatly as cells enter anaphase (Miyazaki and Orr-Weaver 1994), then something else, presumably a loss of cohesion, must trigger disjunction.

IDENTIFICATION OF THE ANAPHASE TRIGGER: THE APC/C

What then is Flemming’s “impetus” for sister chromatid disjunction? Our first insight into molecular events at this stage of the cell cycle was the finding that the mitotic cyclin regulatory subunits of the Cdk1 protein kinase responsible for triggering mitotic entry are suddenly degraded at the metaphase-to-anaphase transition (Evans et al. 1983). The finding that expression of nondegradable cyclin variants blocked the metaphase-to-anaphase transition in *Xenopus* extracts suggested that mitotic control might be beguilingly simple, namely Cdk1 activation triggers entry whereas inactivation due to cyclin degradation triggers the metaphase-to-anaphase transition (Murray et al. 1989). This appealing paradigm was subsequently overturned by the findings that unphysiologically high levels of nondegradable cyclins are required to block anaphase in *Xenopus* extracts (Holloway et al. 1993) and more serious, even very high, levels have no effect whatsoever on anaphase in yeast (Surana et al. 1993). That Cdk1 inactivation per se does not trigger anaphase is graphically demonstrated by experiments in which fly embryos arrested in metaphase are injected with a bolus of the potent and highly specific Cdk1-inhibitory protein p27 (Fig. 2A), which triggers mitotic exit but not sister chromatid disjunction (Oliveira et al. 2010).

Although it is not the actual trigger for anaphase, cyclin’s rapid degradation at the metaphase-to-anaphase transition led to attempts to identify the apparatus responsible for this remarkable proteolytic event. Biochemical experiments with extracts from *Xenopus* (Glutzer et al. 1991) and clam (Hershko et al. 1991) suggested that cyclin destruction was accompanied by and dependent on cyclin ubiquitinylation, a process that in addition to ubiquitin activating and conjugating enzymes required a huge 20S ubiquitin protein ligase (King et al. 1995; Sudakin et al. 1995), initially called the cyclosome. Genetic experiments with yeast had meanwhile identified genes specifically required for cyclin degradation (Irniger et al. 1995) that encoded subunits of the ligase (King et al. 1995; Zachariae and Nasmyth 1996; Zachariae et al. 1996). Strikingly, the yeast studies demonstrated that the cyclin ubiquitin protein ligase was not only intimately involved in cyclin degradation but also essential for the metaphase-to-anaphase transition. Temperature-sensitive mutants arrest uniformly in metaphase (Culotti and Hartwell 1971; Irniger et al. 1995), a phenotype that could not be explained by their defective cyclin degradation (Surana et al. 1993). The implication was that the ubiquitin protein ligase responsible for cyclin degradation had other targets whose destruction, unlike that of cyclins, was essential for anaphase. It is for this reason that the ligase, now known to contain 15 or more subunits, is known as the anaphase-promoting complex/cyclosome (APC/C) (Peters 2006).

APC/C TRIGGERS ANAPHASE BY DESTROYING SECURINS

Could anaphase be triggered through destruction by the APC/C of a single key protein besides a cyclin? Candidates for such a protein emerged again from yeast genetic studies. In budding yeast, mutations in a nonessential gene called *PDS1* caused a loss of viability, that was accompanied by a loss of sister chromatid cohesion, in cells arrested in a mitotic state by spindle poisons (Yamamoto et al. 1996b). Like cyclins, the Pds1 protein is dramatically degraded in an APC/C-dependent fashion at the metaphase-to-anaphase transition but, unlike cyclins, expression of a nondegradable version blocks sister chromatid disjunction (Cohen-Fix et al. 1996). More remarkable still, inactivation of Pds1 allows yeast cells lacking APC/C activity to disjoin sister chromatids (Yamamoto et al. 1996b; Ciosk et al. 1998). What turns out to be Pds1’s ortholog in fission yeast is a gene known as *Cut2* that was paradoxically identified not because it inhibits anaphase but because it is required for this process along with a protein called Cut1 with which it was found to coprecipitate (Funabiki et al. 1996a). Like Pds1, destruction of *Cut2* at the hands of the APC/C is essential for sister chromatid separation (Funabiki et al. 1996b). Strangely, Pds1 and *Cut2* share little sequence similarity and it was initially unclear whether they were orthologous proteins with homologous functions. However, this conundrum was resolved when purification of Pds1 revealed that it was bound in a stoichiometric fashion with a protein called Esp1 (Baum et al. 1988) that was clearly homologous to Cut1 (Uzawa et

Figure 2. Artificial mitosis. *Drosophila* embryos expressing solely TEV-sensitive cohesin complexes were arrested in metaphase (by injection of UbcH10^{C114S}) and subsequently injected with the Cdk inhibitor p27 (A), TEV protease (B), or TEV protease together with p27 (C) (for details, see Oliveira et al. 2010).

al. 1990) and, like the latter, essential for sister chromatid separation (McGrew et al. 1992; Ciosk et al. 1998). The picture emerging from these studies was the notion that by destroying Pds1/Cut2-like proteins, now known to be universal and known as securins, the APC/C destroyed an inhibitor of a protein (Esp1/Cut1) that catalyzed sister chromatid disjunction (Ciosk et al. 1998).

COHESINS: CHROMOSOMAL PROTEINS ESSENTIAL FOR SISTER CHROMATID COHESION

What then is the mechanism by which securins inhibit sister chromatid disjunction? Moreover, why, if they are inhibitors, are securins also necessary for sister chromatid disjunction in certain organisms? The observation that securins and their Esp1/Cut1 partners associate with mitotic spindles (Funabiki et al. 1996a) appeared to resurrect the notion that anaphase might after all be triggered by changes in spindle dynamics. As we see below, they do indeed regulate spindle dynamics but in a manner that merely facilitates anaphase chromosome movements but does not trigger sister chromatid disjunction per se. When it was found that DNA catenation could not explain

minichromosome cohesion (Koshland and Hartwell 1987), it was postulated that proteins might perform this function. Might securins be these hitherto elusive proteins? There were two major problems with this. First, in budding yeast and in many other organisms including mammals, securin is not essential (Yamamoto et al. 1996a; Wang et al. 2001). Although not known for sure at the time, it was thought that sister chromatid cohesion would be essential for mitosis. A second problem was that chromosome spreads indicated that securin is not stably associated with chromatin at any stage of the cell cycle, including in postreplicative cells (Ciosk et al. 1998).

The actual function of securins and their Esp1/Cut1 partners only emerged once the proteins that really do hold sisters together had been identified, again through genetic studies. The first identified chromosomal protein required for sister chromatid cohesion was Mei-S332, which is necessary for maintaining cohesion at centromeres following the first meiotic division (Baker et al. 1978; Goldstein 1980; Kerrebrock et al. 1995; Moore et al. 1998). Orthologs of Mei-S332, now known as shugoshins, exist in all eukaryotes (Kitajima et al. 2004; Marston et al. 2004; Rabitsch et al. 2004). It turns out that they are not part of the actual apparatus holding sisters together but rather are regulators of the process by which cohesion is destroyed during mitosis

(Salic et al. 2004; McGuinness et al. 2005) and meiosis (Xu et al. 2009; Ishiguro et al. 2010; Katis et al. 2010).

Proteins more intimately involved in the physical process of holding sisters together were in fact found by yeast genetics. A search for temperature-sensitive mutants that mis-segregated chromosomes at low temperatures but were completely defective in mitosis at high temperatures, and separated sisters without having destroyed securin, led to the identification of four genes essential for sister chromatid cohesion: *SCC1/MCD1*, *SCC2*, *SMC1*, and *SMC3* (Michaelis et al. 1997). *SCC1/MCD1* was simultaneously identified in an independent screen for mutants that lose viability and separate sister chromatids in cells arrested in mitosis by nocodazole (Guacci et al. 1997). A subsequent screen revealed two more, namely *SCC3* and *ECO1* (Toth et al. 1999). Unlike securin, one of the proteins encoded by these genes, Scc1, was found to be stably associated with chromatin from late G₁ until the onset of anaphase, whereupon it disappeared from chromosomes completely (Michaelis et al. 1997). Subsequent biochemical experiments showed that the products of *SCC1*, *SCC3*, *SMC1*, and *SMC3* form a multisubunit complex called cohesin (Losada et al. 1998; Toth et al. 1999) that also contains the Pds5 (Hartman et al. 2000; Panizza et al. 2000) and Wapl/Rad61 proteins (Gandhi et al. 2006; Kueng et al. 2006). Scc2 was meanwhile found to bind the protein Scc4, forming a stoichiometric complex essential for cohesin's stable association with chromatin (Ciosk et al. 2000). The mammalian orthologs of Scc2 and Scc4 are also known as Nipbl (Krantz et al. 2004; Tonkin et al. 2004) and Mau2 (Seitan et al. 2006; Watrin et al. 2006), respectively. Eco1, also identified as Ctf7 (Skibbens et al. 1999), is now known to control cohesion by acetylating cohesin's Smc3 subunit (Ivanov et al. 2002; Ben-Shahar et al. 2008; Unal et al. 2008; Rowland et al. 2009). Eco1 has two mammalian orthologs, namely *ESCO1* and *ESCO2* (Vega et al. 2005). In animal cells, the protein sororin is also required to build cohesion at S phase (Rankin et al. 2005; Schmitz et al. 2007).

CLEAVAGE OF COHESIN'S SCC1 SUBUNIT BY SEPARASE TRIGGERS SISTER SEPARATION

A crucial step in unraveling the mechanism of sister chromatid disjunction was the finding that the dramatic disappearance of Scc1 from chromosomes at the metaphase-to-anaphase transition requires not only the APC/C but also Esp1 (Ciosk et al. 1998), the partner of the APC/C's target securin. This led to the notion that Esp1 and not the APC/C is responsible for removing cohesin from chromosomes. By controlling degradation of securin, the APC/C's role in the process is to regulate Esp1 activity. The real breakthrough was the subsequent discovery that Esp1 is a site-specific protease that removes cohesin from chromosomes by inducing cleavage of Scc1 and that its enzymatic activity is inhibited by securin binding (Uhlmann et al. 1999, 2000). With the realization that all eukaryotes possess such enzymes (Waizenegger et al. 2000), orthologs of Esp1/Cut1 are now known as separases.

Securin is not merely an inhibitor because its prior association with separase appears to be necessary for efficient activation following securin destruction, a phenomenon that is more acute in some organisms such as fission yeast (Funabiki et al. 1996a) and *Drosophila* (Stratmann and Lehner 1996), where, unlike budding yeast and mammals, securin is essential for sister chromatid disjunction. Another important detail that has emerged is that in vertebrates (but not in yeast) separase is inhibited by phosphorylation by cyclin B/Cdk1 (Stemmann et al. 2001) as well as by the binding of securin. In most cell types, neither one nor the other mechanism is essential, but inhibition by Cdk1 is crucial at certain stages of germline development (Huang et al. 2008, 2009), possibly due to lower securin concentrations.

Importantly, both mechanisms regulating separase are controlled by the APC/C whose activation triggers the simultaneous ubiquitinylation of securin and cyclin B. It remains unclear as to exactly how Cdk1 inactivation leads to the rapid dephosphorylation of separase and whether this process involves up-regulation of phosphatases such as PP2A. Crucially, the APC/C and hence separase activity is regulated by the SAC, ensuring that the process of sister chromatid disjunction is not triggered until all chromosomes have bioriented. This occurs through the formation of complexes between Cdc20 and SAC proteins such as Mad2, a process that is under control of kinetochores that have not connected to microtubules in a manner giving rise to tension (Musacchio and Salmon 2007). Thus, the APC/C-separase-cohesin pathway provides a coherent explanation for how cells regulate the disjunction process, in particular Flemming's seminal observation that sister chromatids disjoin synchronously, soon after biorientation of all chromosomes is complete, and not one by one.

ARTIFICIAL MITOSIS

If cohesin alone holds sister chromatids together and if its cleavage is the sole trigger for their disjunction, it should be possible to bypass the APC/C and reproduce at least certain aspects of the metaphase-to-anaphase transition by cleaving Scc1 using a foreign protease. Such experiments initially performed in yeast (Uhlmann et al. 2000) have recently been repeated in early embryos from *Drosophila*, where, unlike yeast, the consequences have been observed using real-time imaging (Oliveira et al. 2010). Embryos whose Scc1 protein (known in the fly as Rad21) contains TEV cleavage sites in addition to those recognized by separase were first arrested in metaphase by injection of a dominant negative version of Ubc10 (UbcH10^{C114S}) the ubiquitin-conjugating enzyme used by the APC/C (Townsend et al. 1997). Subsequent injection of TEV triggered sister chromatid disjunction but not their orderly movement to opposite spindle poles. Indeed, after their initial disjunction, individual chromatids frequently reversed their direction of travel, moving back and forth in an aimless dance on the mitotic spindle (Fig. 2B). Thus, cohesin cleavage alone is sufficient to trigger disjunction but not the orderly segregation of chromatids. Similarly erratic chromosome segregation is observed in embryos

expressing nondegradable cyclin B (Parry and O'Farrell 2001; Parry et al. 2003).

In addition to demonstrating the crucial importance of cohesin in holding sisters together, this experiment reveals that the APC/C has functions in orchestrating chromatid segregation besides inducing cohesin cleavage. What might these be? If kinetochore microtubules are stabilized by tension, what happens when tension is destroyed by cohesin cleavage? The chaotic movement of chromatids following TEV cleavage implies that this class of microtubules is destabilized, with kinetochores reverting to a state in which new attachments are continually made and rebroken. The Aurora B kinase associated with centromeres has a key role in destabilizing kinetochore microtubules during prometaphase and is thought to cease doing so following the creation of tension (Tanaka et al. 2002; Ciferri et al. 2008; Liu et al. 2009). When anaphase is triggered in a physiological manner by the APC/C, one of the many changes that occur besides the loss of cohesion is the translocation of Aurora B from centromeres to the midzone of the mitotic spindle (Adams et al. 2001). This movement may have two or more crucial functions: prevent Aurora B from destabilizing kinetochore microtubules following the loss of tension caused by cohesin cleavage, inactivate the SAC, and stabilize the spindle midzone during the second phase of anaphase when poles themselves move apart. Interestingly, the anaphase-specific translocation of Aurora B fails to take place when cohesin is cleaved in cells lacking APC/C activity (Oliveira et al. 2010) and its persistence at centromeres could be responsible for the destabilization of kinetochore microtubules.

Another crucial function of the APC/C during anaphase may be to regulate, albeit indirectly, the SAC. If, as postulated, the tension that stabilizes kinetochore microtubule interactions also turns off the SAC, then cells must possess a mechanism that prevents SAC reactivation following the sudden loss of tension induced by cohesin cleavage. Such reactivation would enable APC/C targets such as cyclin to reaccumulate and prevent efficient mitotic exit. Silencing of the SAC as chromosomes biorient and come under tension is accompanied by a major reduction in the concentration of SAC proteins such as BubR1 at kinetochores, and there is therefore little BubR1 associated with kinetochores in the embryos arrested in metaphase by injecting UbCH10^{C114S}. However, the loss of cohesion triggered by injection of TEV leads to BubR1's immediate reassociation with kinetochores, suggesting that the sudden loss of tension does indeed reactivate certain aspects of the SAC (Oliveira et al. 2010). A similar conclusion has been drawn from work on yeast (Mirchenko and Uhlmann 2010) and tissue culture cells (Vazquez-Novelle and Petronczki 2010).

The TEV cleavage experiments demonstrate that cohesin cleavage is sufficient to trigger sister chromatid disjunction but not other important properties of normal anaphase cells. The APC/C is known to induce destruction of several proteins at the onset of anaphase besides securin and cyclin B. The polo-like kinases (Shirayama et al. 1998; Lindon and Pines 2004) and Mps1 kinases are good examples (Palframan et al. 2006). Nevertheless, might destruction of just securin and cyclin B be sufficient to orchestrate a normal

anaphase, as suggested by genetic ablation experiments in yeast (Shirayama et al. 1999)? If so, it ought to be possible to mimic the APC/C merely by injecting TEV to cleave cohesin and p27 to inhibit Cdk1. As described above, TEV alone triggers disjunction but not orderly anaphase chromosome movement, whereas p27 alone causes mitotic exit without any semblance of chromosome segregation. What happens when both proteins are coinjected? Remarkably, this induces chromosome segregation and the subsequent formation of daughter nuclei with kinetics and appearance that are almost indistinguishable to normal divisions (Fig. 2C) (Oliveira et al. 2010). Chromosomes segregate to the poles at normal velocities, Aurora B translocates from centromeres to the spindle midzones, and BubR1 remains absent from anaphase kinetochores, all in the complete absence of any APC/C activity. In other words, manipulation of just two key molecules, cohesin and Cdk1, is sufficient for the fission of eukaryotic nuclei. A corollary is that Cdk1 down-regulation at the metaphase-to-anaphase transition does not merely trigger exit from mitosis but alters kinetochore microtubule dynamics and the SAC so that they become refractory to the loss of tension caused by cohesin cleavage, as originally suggested from the results of expressing nondegradable cyclin B (Parry et al. 2003).

The TEV experiments also prove once and for all that it is cohesin and very probably cohesin alone that resists the spindle forces that attempt to disjoin bioriented chromosomes. They prove that in cohesin's absence, DNA catenation (Murray and Szostak 1985; Yanagida 2009), the origin recognition complex (Shimada and Gasser 2007), or condensin cannot perform this function (Lam et al. 2006). They also contradict the mischievous recent suggestion that "the loss of cohesin might not trigger anaphase per se, but that cohesin might be just one of many unwanted components that are removed from mitotic chromosomes to allow the progression of mitosis" (Yanagida 2009). Biology grapples with explaining complex physiological processes in terms of simple physical and chemical principles. An important criterion for evaluating success is whether the process can be reproduced by artificial means. There is a long history of this approach, which now comes under the rubric of synthetic biology. The induction of an event as dramatic as nuclear division merely by cleaving cohesin and down-regulating Cdk1 is surely a good example.

THE COHESIN RING MODEL

How does cohesin hold sister chromatids together and how does cleavage of its Scc1 subunit, either by separase or by TEV protease, destroy the linkage so rapidly? The peculiar quaternary structure adopted by three of its core subunits, namely Smc1, Smc3, and Scc1, has shed important insight. Smc1 and Smc3 fold back on themselves to form 50-nm-long intramolecular antiparallel coiled coils, forming rod-shaped proteins with globular "hinge" domains at one end (composed of sequences from the middle of the proteins) and an ABC-like ATP-binding domain (NBD) at the other end (composed of their amino- and carboxy-terminal globular domains). Heterotypic interac-

tions between Smc1 and Smc3 hinges create stable V-shaped Smc1/Smc3 heterodimers (Melby et al. 1998; Haering et al. 2002; Hirano and Hirano 2002) that are converted to closed rings by the interconnection of their NBDs by cohesin's Scc1 subunit, whose amino- and carboxy-terminal domains bind to Smc3 and Smc1, respectively (Haering et al. 2002; Gruber et al. 2003). Because of their ability to create closed rings, the superfamily of Scc1-like proteins are known as kleisins, after the Greek Κλείσιμο, meaning closure (Schleiffer et al. 2003). Scc1 and its meiotic variant Rec8 belong to the α -kleisin subgroup. Cohesin's ring-shaped structure is shared by the condensin complex, which contains a different pair of Smc proteins, namely Smc2 and Smc4, whose NBDs are connected by β or γ kleisins (Hirano 2005), and homodimeric bacterial complexes whose NBDs are also connected by kleisins at least at certain phases of their nucleotide-binding/hydrolysis cycle (Woo et al. 2009).

In addition to their kleisin-mediated interconnection, Smc1 and Smc3 NBDs can directly engage each other in the presence of ATP in a process involving interaction of phosphates of ATP bound to one head interacting with "signature" motifs on its partner. Only when engaged in this manner can the two ATP molecules sandwiched between Smc1 and Smc3 NBDs be hydrolyzed (Hopfner and Tainer 2003; Arumugam et al. 2006), a process that is essential for cohesin's stable association with chromosomes (Arumugam et al. 2003; Weitzer et al. 2003). Cohesin therefore forms two types of ring: a bipartite ring in which Smc1 and Smc3 NBDs are brought together by ATP, a process that depends on the binding of Scc1's carboxy-terminal winged helical domain to Smc1's NBD (Arumugam et al. 2006), and a tripartite ring in which the Smc1 and Smc3 NBDs are bound by the carboxy- and amino-terminal domains of Scc1, respectively. The intervening central domain contains separase cleavage sites, either toward amino- or carboxy-terminal ends, and residues within the carboxy-terminal half that recruit Scc3 (Haering et al. 2002). How the Pds5/Wapl subcomplex associates with cohesin is poorly understood.

The discovery that cohesin's Smc and kleisin subunits form a tripartite ring led to the proposal that sister chromatid cohesion is conferred by entrapment of sister DNAs within (Fig. 3) (Nasmyth 2001; Haering et al. 2002; Gruber et al. 2003). The fact that cohesion is destroyed within seconds of its kleisin subunit being cleaved, possibly at any point within the latter's central domain, implies that stable entrapment depends on the three sets of interactions that create tripartite rings and not merely on the Smc1/Smc3 hinge and Smc1/Smc3 NBD interactions that create bipartite rings. The suggestion that bipartite ring interactions alone hold sisters together (Weitzer et al. 2003) provides no explanation for how cleavage destroys the linkage instantaneously. Förster resonance energy transfer (FRET) studies suggest that most Smc1 and Smc3 NBDs are in close proximity (Mc Intyre et al. 2007). Because this association occurs throughout the cell cycle, even after Scc1 cleavage during anaphase and in its complete absence during early G₁, the association between NBDs measured by FRET presumably corresponds to a weak one that is inca-

Figure 3. Cohesin ring model shows how cohesin rings could topologically embrace two 10-nm chromatin fibers.

pable of preventing escape of DNAs from the ring following Scc1 cleavage. In other words, the bipartite rings detected by FRET that persist after Scc1 cleavage cannot possibly confer cohesion. If the FRET studies are to be believed, the NBDs even of tripartite rings spend most of their time in close proximity. Nevertheless, unlike the interconnection of Smc NBDs by Scc1, this association is presumably not stable enough to prevent DNAs from escaping.

TESTING THE TRIPARTITE RING MODEL

As predicted by the tripartite ring model, association between cohesin and chromatin is destroyed both in vivo and in vitro not only by cleavage at any position within Scc1's central domain but also by severance of Smc3's coiled coil (Uhlmann et al. 1999; Gruber et al. 2003; Pauli et al. 2008). Equally telling, the association between cohesin and minichromosomes is destroyed in vitro by DNA cleavage, consistent with cohesin sliding off the ends of short linear chromatin fibers (Ivanov and Nasmyth 2005). The most rigorous test of the model has come from chemical cross-linking studies (Haering et al. 2008). If sister DNAs are entrapped inside a single tripartite ring, the generation of cross-links between Smc1 and Smc3 hinges and between amino- and carboxy-terminal domains of Scc1 and Smc3 and Smc1 NBDs, respectively, should entrap sister circular minichromosome DNAs inside a chemically circularized ring in a manner that would resist its denaturation by an ionic detergent such as sodium dodecyl sulfate (SDS). Instead of migrating as monomers in agarose gels, sister DNAs should migrate as an unusual type of dimer, namely one composed of a pair of monomeric DNAs catenated by a chemically circularized cohesin ring.

Three key preconditions were required to be met to perform this experiment. The first was the use of differential sedimentation velocity in sucrose gradients combined with differential agarose gel electrophoresis to separate monomeric from dimeric minichromosomes bound together by cohesin (Ivanov and Nasmyth 2007). The second was the determination by X-ray crystallography of the atomic struc-

ture of two of the tripartite ring's three interfaces, namely the Smc hinge (Haering et al. 2002) and the Smc1NBD/Scc1 complex (Haering et al. 2004). These structures enabled the replacement by cysteine of specific residues on either side of the two interfaces, creating cysteine pairs 5 Å apart that could be cross-linked in a highly specific manner by a bifunctional thiol-specific chemical cross-linker such as dibromobimane. The third precondition was the discovery that cotranslational fusion of Smc3's carboxyl terminus to Scc1's amino terminus does not destroy cohesin function (Gruber et al. 2006), enabling 100% cotranslational cross-linking without having to introduce cysteines into an interface whose atomic structure remains unknown.

By these three means, it was possible to isolate either monomeric or dimeric minichromosomes and then cross-link (with a combined efficiency approximating 35%) the hinge, Smc1-Scc1, and Smc3-Scc1 interfaces of tripartite rings associated with them. In the absence of any introduced cysteines, the DNAs of both monomeric and dimeric minichromosome fractions from sucrose gradients run as monomers in agarose gels after the cross-linking procedure and denaturation in 1% SDS at 65°C. However, when isolated from cells whose cohesin subunits contain all four cysteines, ~40% run somewhat more slowly than do DNA-DNA concatemers, implying that monomeric DNAs have been catenated by the chemically circularized cohesin rings (Fig. 4). Crucially, this only occurs when all four cysteines are present (Haering et al. 2008). It is difficult if not impossible to explain these results without supposing that the cohesion that holds minichromosomes together following their isolation in sucrose gradients is mediated by tripartite cohesin rings that have entrapped DNAs. By far the simplest explanation of these results is that sister DNAs are entrapped within monomeric tripartite rings, very possibly just one per minichromosome (Haering et al. 2008). However, it is just conceivable that they are instead entrapped

by dimeric rings in which Smc NBDs bind to Scc1 molecules whose amino- and carboxy-terminal domains are bound to different Smc1/Smc3 heterodimers or that they are individually entrapped by two separate rings that are themselves catenated. However, in the case of these two more implausible scenarios, the comigration of sister DNAs would require simultaneous cross-linking of all four interfaces containing cysteine pairs as opposed to just two in the simpler case of monomeric rings. Importantly, the fraction of DNAs catenated by circularized cohesin was approximately the same as the predicted degree of circularization of monomeric rings and several-fold higher than the predicted degree of circularization of dimeric rings. Dimeric rings could only have given rise to the observed results if each dimeric minichromosome isolated from sucrose gradients contained several cohesive structures. Crucially, the results are inconsistent with so-called handcuff models, according to which each sister is entrapped by separate rings interacting with each other in a nontopological manner. For example, it has been suggested that individual Scc3 molecules bind simultaneously to two molecules of Scc1, each part of separate tripartite rings that have entrapped different (sister) DNAs (Zhang et al. 2008b). According to this model, cross-linking merely the tripartite ring's three interfaces could not possibly produce DNAs catenated by circularized cohesin rings, but it does!

SO WHAT'S WRONG WITH THE RING MODEL?

Notwithstanding residual doubts as to whether they totally exclude double rings, the cross-linking experiments imply that some if not most minichromosome DNAs isolated as dimers on sucrose gradients are entrapped inside tripartite cohesin rings, raising the possibility that this is the principle governing sister chromatid cohesion. Despite their unequivocal nature, the cross-linking studies have not prevented continued speculation that cohesin holds sisters together by forming unspecified interactions between two or more cohesin complexes (Zhang et al. 2008b; Yanagida 2009; Heidinger-Pauli et al. 2010; Skibbens 2010), even though such models cannot possibly explain the minichromosome observations. The implication implicit in such suggestions is that entrapment of minichromosomes by cohesin rings is either a complete artifact that has no bearing whatsoever on the actual mechanism of cohesion or a property that mediates a form of cohesion responsible for holding minichromosomes together in vitro but not cohesion of real chromosomes in live cells or in organisms other than budding yeast.

Are there in fact reasonable grounds for ignoring the minichromosome cross-linking results? One could argue that cohesion generated by complexes with Smc3 fused to Scc1, a feature essential for the cross-linking experiments, might have very different properties from those of wild-type complexes. Thus, despite the attractive feature of providing an explicit and testable explanation for cohesion, entrapment of chromatin fibers inside cohesin rings might be caused by the protein fusion required to perform the key cross-linking experiments. The flaw in this argument is that

Figure 4. Testing the ring model. Circular DNAs held together by cohesin lose cohesion following protein denaturation. The ring model predicts that they will remain attached if the three interfaces creating the tripartite ring are chemically cross-linked (for details, see Haering et al. 2008).

cells carrying the Smc3-Scc1 fusion are viable, implying that the fusion proteins build “real” cohesion as well as entrap chromatin fibers. It is difficult to imagine that their ability to entrap chromatin arises from properties unique to Smc3-Scc1 fusion proteins and not inherent to wild-type cohesin. To explain away their supposedly artifactual ability to entrap chromatin fibers, one must postulate a strange and, for the unsuspecting investigator, malign coincidence, namely that the fusion just happens to create a complex capable of entrapping chromatin. Despite the implausibility of the criticism of the Smc3-Scc1 fusion protein, it is hoped that future experiments will test whether coentrapment also occurs in cells in which Smc3 is not fused to Scc1 at the time of cohesion establishment. The notion that cohesion that holds minichromosomes together *in vitro* is mediated by coentrapment of sister fibers by cohesin rings, whereas other forms of cohesion are mediated by a very different mechanism, is equally implausible, not to mention *ad hoc*. More serious, it does not explain how minichromosomes possess a totally different mechanism from real chromosomes in the absence of any obvious evolutionary pressure to produce such a complex property.

What else could be so grievously wrong with the tripartite ring model that one would prefer more complicated and still poorly articulated alternatives involving multimeric complexes? It has been suggested (Skibbens 2010) that the proximity of Smc1 and Smc3 NBDs even in the absence of Scc1 is inconsistent with the notion that entrapment depends on bridging of Smc1 and Smc3 NBDs by Scc1, that the model struggles with the reality that phosphorylation of stromal antigen (SA) proteins, the mammalian orthologs of Scc3, drives dissociation of some cohesin from chromosomes during prophase, and most troubling of all that the model provides no role for Scc3 or Pds5. These are possibly all accurate observations not currently explained by the model, but it is hard to see why they are blatantly inconsistent with it. The model is undoubtedly incomplete (see below) but this is not synonymous with its principle being fundamentally flawed. Words and arguments will not kill the model, but hard facts, *i.e.*, killer experiments will.

THE RING MODEL IS INCOMPLETE

The real problems with the ring model are twofold. The first is that loopholes remain in the experimental evidence implying that sister minichromosome DNAs are entrapped inside monomeric tripartite rings as opposed to dimeric variants. Final proof will require either more refined biochemical experiments or possibly visualization of the rings holding minichromosomes together by electron or atomic force microscopy. It would also be comforting to know that the sort of cohesion that holds circular minichromosomes together *in vitro* also holds linear sister chromatids together and that cohesion along chromosome arms obeys the same rules as that conferred by centromeres. The real Achilles heel of the evidence for entrapment is our poor understanding of how Scc1 binds to Smc3 NBDs. Crystal structures exist for the Smc1/Smc3 hinge and the Smc1 NBD-Scc1 interfaces but not for Smc3-Scc1. Scc1 does undoubtedly

associate with Smc3 NBDs, but it has so far proved impossible to reconstruct the association with purified components *in vitro*, which has greatly hindered elucidation of its structure by X-ray crystallography. The reasons for this may have something to do with the possibility that this interface is regulated during the cell cycle. For example, it may be no coincidence that Eco1 promotes the establishment of cohesion by acetylating the Smc3 NBD (Ben-Shahar et al. 2008; Unal et al. 2008; Zhang et al. 2008a; Rowland et al. 2009), an event that might alter the stability or conformation of its association with the amino-terminal domain of Scc1. Structural information about the Smc3NBD-Scc1 interface would not only be revealing about its stability, a crucial feature according to the ring model, but would also enable the cross-linking studies to be repeated with complexes lacking a connection between Smc3 and Scc1. The second problem is potentially more serious and concerns the model’s incompleteness. As it currently stands, the model does not explain how coentrapment of sister DNAs is generated or maintained.

HOW DO DNAs ENTER THE TRIPARTITE RING?

Assuming that cohesion is generated by preassembled rings, how do they open and shut to permit DNA entry? Presumably, one of the three interfaces comprising the ring must transiently open. The finding that cohesin retains function after fusion of Smc1 and Smc3 NBDs to Scc1’s carboxy- and amino-terminal domains, respectively, suggests that the Smc1/Smc3 hinge is the entry gate (Gruber et al. 2006). It is clearly more than just a dimerization domain required to create V-shaped Smc1/Smc3 heterodimers because cohesin rings in which Smc1 and Smc3 hinge domains are replaced by Mp1 and p14, which also form very stable pseudosymmetrical dimers, fail completely to associate with chromatin despite being capable of ATP hydrolysis (Gruber et al. 2006). The mouse Smc1/Smc3 hinge crystal structure closely resembles the homodimeric bacterial *Thermotoga maritima* SMC hinge (Haering et al. 2002; Kurze et al. 2010). In both cases, shallow U-shaped hinge monomers interact to form a pseudo twofold symmetric torus with a small channel in the middle. Remarkably, the channel is positively charged, a feature that, according to modeling, is conserved in SMC hinges from widely different eukaryotic and prokaryotic organisms. A set of amino acid substitutions in budding yeast Smc1 and Smc3 largely eliminate the channel’s positive charge without greatly changing the equilibrium association constant. Although the neutralizing mutations permit formation of cohesin rings that associate stably with the genome, they drastically reduce the establishment of cohesion during S phase (Kurze et al. 2010). These data are difficult to reconcile with the notion that the hinge is merely a dimerization domain and suggest that it undergoes a major conformational change, at least during DNA replication. Probably the strongest evidence that the two hinge halves transiently dissociate is the observation that the artificial connection, by addition of rapamycin, of Smc1 and Smc3 hinge domains containing FKBP12 and Frb dimerization domains, respectively, blocks the establish-

ment but not maintenance of sister chromatid cohesion (Gruber et al. 2006).

Current observations demonstrate that the hinge is not just a dimerization domain and that it has an active role in the establishment of sister chromatid cohesion during S phase, but they do not yet constitute proof that it is a DNA entry gate. This will require either actual observation of opening, which due to its transient nature will be very difficult in live cells with current technologies, or analysis of the effect of site-specific chemical cross-linking of the type used to demonstrate entrapment of minichromosome DNAs inside cohesin rings.

WHAT HAPPENS TO COHESIN RINGS AT S PHASE?

Another crucial gap in our understanding is the state of cohesin's association with chromatin before the passage of replication forks. It is assumed that this is also topological, but there is little direct evidence for this. It is an important issue for two reasons. First, there is increasing evidence that cohesin has important functions regulating transcription both in dividing and nondividing cells (Peters et al. 2008; Nasmyth and Haering 2009). Knowing how it associates with unreplicated chromatin will be essential for elucidating how it affects transcription. Second, knowing more about this state may provide insight into the mechanism by which cohesion is established during DNA replication. The ring model envisages two main scenarios by which this comes about: Either rings that have already entrapped unreplicated chromatin fibers are converted during passage of replication forks to those that entrap sister fibers or the rings that entrap sister fibers are derived from a soluble pool and must reload onto chromatin at the time of replication. In the case of the first scenario, the replication apparatus either passes through rings that remain shut once they have associated with unreplicated chromatin or rings associated with unreplicated chromatin reopen during passage of the fork while remaining associated with the replicating chromatin fiber.

Whichever scenario is correct, stable entrapment of sister fibers is accompanied by and dependent on *de novo* acetylation of a pair of highly conserved lysines (K112 and K113) within Smc3 NBDs by Eco1 (Ben-Shahar et al. 2008; Unal et al. 2008; Zhang et al. 2008a; Rowland et al. 2009). In yeast, Smc3 acetylation is reversed at anaphase by the Hos1 deacetylase as a consequence of Scc1 cleavage (Beckouet et al. 2010). Smc3 molecules that remain acetylated after mitosis due to Hos1 inactivation cannot generate cohesion during the subsequent S phase, implying that cohesion establishment depends on *de novo* acetylation during DNA replication. A key issue surrounding Eco1's function is whether acetylation merely alters the ring to facilitate the establishment of cohesion during S phase and thereafter has no further function, a model suggested by the finding that Eco1 is dispensable for maintaining cohesion after cells have completed DNA replication (Skibbens et al. 1999; Toth et al. 1999). However, the discovery that Hos1 cannot deacetylate Smc3 until Scc1 is cleaved suggests that the acetyl marks at

K112 and K113 are stable and not turned over appreciably between DNA replication and anaphase. Thus, the acetylation mark could indeed have a postreplicative role. What might this be? The observation that overproduction of Hos1 in M-phase cells induces rapid albeit incomplete Smc3 deacetylation that is accompanied by a reduction of sister chromatid cohesion is possibly an important clue that acetylation actively stabilizes cohesion (Beckouet et al. 2010; Borges et al. 2010). The notion that acetylation "locks" cohesin in a cohesive state (Unal et al. 2008) now warrants serious consideration. That we do not understand the role or indeed mechanism by which Smc3 acetylation promotes the establishment or maintenance of cohesion is another demonstration of the paucity of our knowledge. Another is the fact that we have little or no idea how cohesin's Scc3 and Pds5 subunits help to maintain cohesion in postreplicative cells nor how its Wapl subunit (Gandhi et al. 2006; Kueng et al. 2006) together with Scc3 phosphorylation (Hauf et al. 2005) promotes dissociation from chromosome arms in vertebrates of a large fraction of cohesin during prophase and prometaphase in the absence of Scc1 cleavage. We also have little idea as to sororin's role (Rankin et al. 2005; Schmitz et al. 2007). If the ring model is correct, Pds5, Scc3, sororin, and Wapl must somehow regulate, either positively or negatively, the ring opening, an event that would permit DNA escape.

THE SCC2/4 COMPLEX AND THE COHESIN-LOADING REACTION

Yet another area of ignorance concerns the mechanism by which cohesin associates with chromosomes, a process that barring mitosis in organisms other than yeast occurs throughout the cell cycle and is therefore not synonymous with the establishment of cohesion. Association requires a separate loading complex composed of the Scc2 and Scc4 (also known as Nipbl and Mau2 in vertebrates) proteins (Ciosk et al. 2000; Gillespie and Hirano 2004; Takahashi et al. 2004). The molecular mechanism by which the Scc2/4 complex helps cohesin to load onto chromosomes is poorly understood, partly because the reaction occurs rapidly inside cells and has therefore hitherto defied dissection into discrete steps and partly because it is not known where on chromosomes loading takes place. With a diameter of 30 nm or more, cohesin rings should be capable of sliding along one or more 10-nm chromatin fibers, a process that clearly takes place *in vitro*. If this also takes place *in vivo*, the eventual distribution of cohesin as measured by chromatin immunoprecipitation (ChIP) studies may not in fact reflect where loading took place. The suggestion, either from chromosome spreads (Ciosk et al. 2000) or ChIP-Chip studies (Lengronne et al. 2004), that Scc2/4 and cohesin reside at different genomic locations in yeast has been taken to mean that cohesin slides from "loading sites" occupied by Scc2/4 to its final chromosomal destination. However, the conclusion that Scc2/4 and cohesin have different genomic distributions has since been disputed (Kogut et al. 2009). More serious, there is hitherto no evidence that sites occupied by Scc2/4 represent loci at which cohesin is first loaded onto chromosomes.

Cohesin is an ABC-like ATPase. Binding of ATP to Smc1 and Smc3 NBDs is thought to induce their engagement and hydrolysis to drive them apart. We reasoned that blocking the ATP-binding/hydrolysis cycle at a crucial point might cause cohesin to accumulate at an intermediate stage before the chromatin entrapment process. Interestingly, Smc1 or Smc3 variants trapped in a transition state with NBDs engaged (Arumugam et al. 2003, 2006) accumulate to high levels at core centromeres, which are known to promote cohesin's recruitment to a wide swathe of pericentric sequences. The instability of this association and its colocalization with and dependence on Scc2/4 indicates that transition-state cohesin complexes undergo an early step in the loading reaction but fail to entrap chromatin fibers (Hu et al. 2010). The finding that transition-state complexes accumulate at core centromeres but not with pericentric sequences implies that DNA entrapment leads to cohesin's translocation for a very considerable distance along chromatin fibers. If we assume that association with Scc2/4 loading sites of cohesin complexes defective in NBD disengagement corresponds to a state, albeit a transient one, normally entered into by wild-type complexes, investigation of which aspects of cohesin are necessary for entering into this state should be informative about early steps in the cohesin-loading process. Interestingly, the accumulation of transition-state complexes at core centromeres depends on Scc3 but not Pds5 or Wapl. The implication is that Scc3 has key roles not only in maintaining cohesion once established but also during an early step in loading cohesin onto chromosomes, possibly long before cells even enter S phase. The molecular mechanism by which the Scc2/4 complex together with Scc3 promotes cohesin loading remains completely mysterious.

SUMMARY

There is now overwhelming evidence that cohesin alone provides the cohesion between sister chromatids capable of resisting spindle forces and thereby promoting biorientation. We have a working model for how it performs this task, namely coentrapment of sister DNAs inside a tripartite ring made up of cohesin's Scc1, Smc1, and Smc3 subunits. This model has made a number of critical predictions that have been experimentally verified but remains work in progress. Opening of the cohesin ring following cleavage of Scc1 by separase triggers sister chromatid disjunction but is insufficient for other aspects of anaphase chromosome movements that are instead mediated directly or indirectly by Cdk1 down-regulation. Cdk1 down-regulation and separase activation are orchestrated through ubiquitinylation by the APC/C of cyclin B, Cdk1's regulatory subunit, and securin, an inhibitory chaperone of separase. The key role that cohesin cleavage has during Flemming's indirect nuclear division has been highlighted by experiments in which metaphase cells can be induced to produce a pair of daughter nuclei merely by injecting an inhibitor of Cdk1 and a protease capable of cleaving Scc1.

Although first discovered for its role during mitosis, cohesin also has key roles in promoting double-strand break

repair and in regulating transcription during interphase in dividing and nondividing cells. It is important to stress that we still have a very poor grasp of how cohesin builds cohesion, let alone how it regulates transcription during development. Further understanding, which will lay solid foundations for the entire field, will depend on rigorous structural and mechanistic studies, which given cohesin's complicated and flexible structure and the complexities of its association with chromatin are difficult, to say the least. Elucidating how cohesin and its siblings condensin and the Smc5/6 complex actually function will be a wonderful challenge for enzymologists prepared to investigate their properties *in vitro* as well as *in vivo*. Furthermore, understanding the mechanisms by which cohesion is established and maintained may be of great importance to one of the most pressing medical problems of the modern age, namely age-related infertility in women. If cohesion can only be established during DNA replication (Uhlmann and Nasmyth 1998; Haering et al. 2004), the cohesin that holds bivalent chromosomes together in oocytes and necessary for the meiotic divisions that occur in mature adults may have been deposited before birth, which is when meiotic DNA replication in females takes place. The recent finding that bivalents fall apart due possibly to loss of cohesin in oocytes from old mice (Chiang et al. 2010; Lister et al. 2010) suggests that a failure to maintain cohesive structures after several decades could contribute to the chromosome mis-segregation responsible for age-related aneuploidy and infertility.

ACKNOWLEDGMENTS

We thank Christian Hearing (EMBL) for the model presented in Figure 3. Work in the laboratory of K.N. is supported by the Wellcome Trust, Cancer Research UK, and the Medical Research Council (MRC).

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Cold Spring Harb Symp Quant Biol 2010 75: 375-388 originally published online January 5, 2011
Access the most recent version at doi:[10.1101/sqb.2010.75.019](https://doi.org/10.1101/sqb.2010.75.019)

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