Chromosome cohesion – rings, knots, orcs and fellowship

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Summary

Sister-chromatid cohesion is essential for accurate chromosome segregation. A key discovery towards our understanding of sister-chromatid cohesion was made 10 years ago with the identification of cohesins. Since then, cohesins have been shown to be involved in cohesion in numerous organisms, from yeast to mammals. Studies of the composition, regulation and structure of the cohesin complex led to a model in which cohesin loading during S-phase establishes cohesion, and cohesin cleavage at the onset of anaphase allows sister-chromatid separation. However, recent studies have revealed activities that provide cohesion in the absence of cohesin. Here we review these advances and propose an integrative model in which chromatid cohesion is a result of the combined activities of multiple cohesion mechanisms.

Key words: Cohesin, Chromosome cohesion, Catenations

Introduction

Accurate chromosome segregation is the crux of mitosis – failure causes genetic disorders, spontaneous abortions and cancer (Kops et al., 2005; Rubio et al., 2005; Shah et al., 2003; Weaver and Cleveland, 2006; Weaver et al., 2007). To allow accurate segregation, sister chromatids must remain cohered from their inception, which occurs in S-phase, until anaphase (Fig. 1). Sister-chromatid cohesion has been proposed to depend on a group of proteins called cohesins, which were first identified in yeast (Guacci et al., 1997; Michaelis et al., 1997), that form a tetra-subunit complex of Mcd1 (also known as Rad21 in Schizosaccharomyces pombe and Sec1 in Saccharomyces cerevisiae), Smc1, Smc3 and Sec3 (also known as SA1 and SA2 in mammals). This ‘cohesin complex’ can form a 35-nm ring that tethers sister duplexes. Two main models have been proposed to explain how this ring provides cohesion: the ring might entrap both duplexes (Haering et al., 2002; Nasmith and Haering, 2005) or two rings might interact, each one entrapping a duplex (Anderson et al., 2002; Guacci, 2007; Huang et al., 2005). Conserved orthologs of the cohesins are found in metazoans (Darwiche et al., 1999; Losada et al., 1998; Rollins et al., 1999; Sumara et al., 2000), indicating that cohesin might be the universal mechanism of sister-chromatid cohesion. Accessory proteins regulate the loading of cohesin onto chromosomes (reviewed in Huang and Laurent, 2004; Lee and Orr-Weaver, 2001; Nasmith and Schleiffer, 2004; Riedel et al., 2004; Skibbens, 2005; Uhlmann, 2004) and a protease, separase, cleaves Mcd1, presumably leading to sister-chromatid separation (Ciosk et al., 1998; Uhlmann et al., 1999; Uhlmann et al., 2000). Thus, it is thought that cohesin-loading factors establish cohesion and separase triggers anaphase.

The first evidence that protein complexes provide cohesion came from studies of circular minichromosomes in budding yeast; these minichromosomes remain cohered in the absence of DNA intertwinnings (Guacci et al., 1994; Koshland and Hartwell, 1987). Two lines of investigation have indicated that cohesin provides this activity. First, mutant cohesins have been found to cause an increased distance between sister loci (Guacci et al., 1997; Michaelis et al., 1997), as measured using the LacO/LacI-GFP system (Straight et al., 1996). A cohered locus is normally visualized as a discrete fluorescent spot in early mitosis, but this was observed to prematurely separate into two discrete spots in cohesin mutants. Second, the association of cohesin with DNA mirrors the cohesion cycle: cohesin is bound to chromosomes from S-phase, when cohesion is established, until anaphase, when cohesion is abolished (Michaelis et al., 1997). Other experiments indicate that cleavage of Mcd1 is necessary for loss of cohesion. Sister chromatids cannot separate in yeast cells that express non-cleavable Mcd1 that is resistant to Esp1 (separase) (Uhlmann et al., 1999). Conversely, artificial Mcd1 cleavage (not by Esp1) leads to premature sister-chromatid separation (Uhlmann et al., 2000). Together, these studies form the foundation of a compelling model in which cohesion depends on the cohesin complex and cleavage of the ring results in loss of cohesion.

Here we review the role of cohesin in sister-chromatid cohesion and discuss recent discoveries of cohesin-independent mechanisms of cohesion. We will not, however, discuss recent evidence indicating that the regulation of the onset of anaphase by the spindle-assembly checkpoint is more complex than had been previously appreciated (Chestukhin et al., 2003; Gimenez-Abian et al., 2005a; Gimenez-Abian et al., 2005b; Papi et al., 2005; Zur and Brandeis, 2001) because this topic has been reviewed in detail elsewhere (Clarke et al., 2005). In this Commentary, we propose a model in which cohesion depends on multiple mechanisms that collaborate to ensure cohesion along the length of the whole chromosome.

Cohesion without cohesin in yeast

Although it has been widely documented in budding yeast that cohesins can provide cohesion, the penetrance of the loss-of-cohesion phenotype in cohesin mutants depends on the locus that is observed (Antonacci and Skibbens, 2006; Baetz et al., 2004; Ciosk et al., 2000; D’Amours et al., 2004; Guacci et al., 1997; Lam et al., 2006; Mayer et al., 2001; Michaelis et al., 1997; Strom et al., 2007; Sullivan et al., 2004; Suter et al., 2004; Toth et al., 2006). The first evidence that protein complexes provide cohesion came from studies of circular minichromosomes in budding yeast; these minichromosomes remain cohered in the absence of DNA intertwinnings (Guacci et al., 1994; Koshland and Hartwell, 1987). Two lines of investigation have indicated that cohesin provides this activity. First, mutant cohesins have been found to cause an increased distance between sister loci (Guacci et al., 1997; Michaelis et al., 1997), as measured using the LacO/LacI-GFP system (Straight et al., 1996). A cohered locus is normally visualized as a discrete fluorescent spot in early mitosis, but this was observed to prematurely separate into two discrete spots in cohesin mutants. Second, the association of cohesin with DNA mirrors the cohesion cycle: cohesin is bound to chromosomes from S-phase, when cohesion is established, until anaphase, when cohesion is abolished (Michaelis et al., 1997). Other experiments indicate that cleavage of Mcd1 is necessary for loss of cohesion. Sister chromatids cannot separate in yeast cells that express non-cleavable Mcd1 that is resistant to Esp1 (separase) (Uhlmann et al., 1999). Conversely, artificial Mcd1 cleavage (not by Esp1) leads to premature sister-chromatid separation (Uhlmann et al., 2000). Together, these studies form the foundation of a compelling model in which cohesion depends on the cohesin complex and cleavage of the ring results in loss of cohesion.

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Providing cohesion, non-functional cohesin or the absence of an independent mechanism might contribute to cohesion at other loci. To provide cohesion in yeast, because it seems to be the only one and only copy of each chromosome to each daughter cell. When sister-chromatid cohesion is defective, mitotic processes such as chromosome biorientation and chromosome segregation are disrupted, resulting in aneuploidy, a hallmark of most cancers.

Fig. 1. Sister-chromatid cohesion is required for accurate chromosome segregation. During a mitotic cell cycle, the genome is duplicated in S phase and each identical copy is then segregated into the daughter cells. In eukaryotes, this process is complex owing to the fragmentation of the genome in several chromosomes. Eukaryotic cells have evolved a mechanism, termed sister-chromatid cohesion, that keeps the two copies of a chromosome (sister chromatids) together from the moment of duplication to the onset of anaphase. This mechanism ensures the accurate segregation of one and only one copy of each chromosome to each daughter cell. When sister-chromatid cohesion is defective, mitotic processes such as chromosome biorientation and chromosome segregation are disrupted, resulting in aneuploidy, a hallmark of most cancers.

Cohesin in other eukaryotes

Cohesins are conserved from yeast to humans, which suggests that their role in cohesion is conserved. Efforts to directly assess the role of cohesin in sister-chromatid cohesion in higher eukaryotes include the depletion of cohesins from Xenopus laevis egg extracts (Kenney and Heald, 2006; Losada et al., 1999) (reviewed in Koshland and Guacci, 2000; Nasmyth and Schleiffer, 2004; Skibbens, 2000). Only telomeres lose cohesion in 100% of cohesin-mutant cells, whereas loci at the chromosome arms and pericentromere remain cohered in 40-75% of cells and the ribosomal DNA (rDNA) locus remains cohered in the majority of cohesin-mutant cells (Fig. 2). This indicates that cohesion is sufficient to provide cohesion in yeast, because it seems to be the only mechanism that operates at telomeress, but also that cohesin-independent mechanisms might contribute to cohesion at other loci.

In a scenario in which cohesins were the sole mechanism providing cohesion, non-functional cohesin or the absence of a cohesin subunit should lead to loss of cohesion upon replication of any locus. In studies that timed the loss of cohesion in mcd1 mutants, some cells did lose cohesion as soon as DNA replication was observed (Noble et al., 2006). However, despite synchronous DNA replication within populations of yeast cells in such experiments, loss of cohesion typically occurred asynchronously in mcd1 mutants after the release from G1 arrest. The maximum percentage of mcd1 cells that displayed a loss-of-cohesion phenotype was only achieved after all the cells reached G2 (Michaelis et al., 1997; Noble et al., 2006). The fact that loss of cohesion in cohesin-defective cells can be delayed for some time after replication suggests that other mechanisms of cohesion exist.

The key issues thus become identifying the source of these arrested mitotic cells that have separated sister chromatids and determining at which point cohesion was lost. By examining complete cell cycles in synchronized cells, we observed that ~100% of cohesin-depleted cells reach mitosis with normally cohered sister chromatids and that all of these are able to form a metaphase plate (Diaz-Martinez et al., 2007). These arrested cells presumably account for the previous observations of mitotic cells with separated sister chromatids that...
are seen after depletion of cohesin (Losada et al., 2005; Rankin et al., 2005; Toyoda and Yanagida, 2006; Waterman et al., 2006). However, because such cells first form metaphase plates before sister-chromatid separation (Diaz-Martinez et al., 2007), we suggest that this phenotype is not the result of a complete failure in the establishment of cohesion, but is due to uncoordinated loss of cohesion during anaphase. We propose that cohesin accounts for synchronous sister-chromatid separation at anaphase but is not required for centromere cohesion in early mitosis. The mechanism by which cohesin promotes synchronous sister-chromatid separation remains unclear, but one possibility is that cleavage of Rad21 has a signaling role that serves to inactivate the spindle-assembly checkpoint, triggering coordinated and efficient loss of cohesion. Upon checkpoint silencing, multiple pathways that drive synchronous anaphase might become active.

Consistent with the observation that most cohesin-depleted human cells retain centromere cohesion until metaphase or anaphase, the frequency of cells with separated sister chromatids following RNAi of cohesin dramatically diminishes to less than 20% of mitotic cells after 3 hours in colcemid (Inoue et al., 2007) and 4% after 16 hours (Diaz-Martinez et al., 2007). By contrast, the percentage of cells with separated sister chromatids that is observed after a long mitotic arrest in the presence of nocodazole remains high (>65% after 16 hours) in cells that have been depleted of the centromere-guardian Shugoshin (Sgo1). Therefore, Sgo1, a protein that is thought to protect centromeric cohesin from removal, might regulate cohesin and additional cohesion factors at the centromere. Together, these results indicate that sister-chromatid cohesion can be maintained in a manner that is independent of cohesin, but that cohesin is needed for faithful anaphase. Thus, studies in yeast cells and higher eukaryotes have left open the possibility that cohesin and additional factors collaborate to ensure the association between sister chromatids until anaphase.

**Cohesin-independent cohesion**

**DNA catenations**

DNA catenation (the physical intertwining of sister chromatids) was the first mechanism of cohesion to be formally proposed (Murray and Szostak, 1985). Catenations are a byproduct of semi-conservative replication (Sundin and Varshavsky, 1980) and physically couple replication to cohesion – one being the inevitable consequence of the other (Fig. 3). By contrast, cohesin-mediated cohesion is biochemically coupled to replication, because genomic DNA can be replicated to completion in the absence of cohesin. Logically, cells probably co-evolved their mechanisms of genome replication and sister-chromatid cohesion. As a mechanism of cohesion, catenation fulfills the expected physical dependency on replication, whereas cohesin-mediated cohesion does not.

The removal of catenations is performed by topoisomerase type II enzymes (Top2 in budding yeast and topoisomerase I in II in higher eukaryotes), the activity of which is required for chromosome individualization (Gimenez-Arbi, 2000), sister-chromatid resolution (Gimenez-Arbi et al., 1995) and chromosome segregation (Clarke et al., 1990; Holm et al., 1985; Holm et al., 1989; Sakaguchi and Kikuchi, 2004; Uemura et al., 1987; Uemura and Yanagida, 1984). That catenations can maintain an association between chromatids is evident from the failed sister-chromatid separation, which leads to a cut (cells untiemn turn) phenotype in the absence of Top2 (Holm et al., 1985; Uemura et al., 1987). What appear to be catenations that link sister-chromatid centromeres are present in human cells that are arrested in mitosis by microtubule poisons (Bickmore and Oghene, 1996). Furthermore, some evidence indicates that catenation-mediated cohesion is sufficient for cohesion independent of cohesin (Toyoda and Yanagida, 2006; Vagnarelli et al., 2004; Diaz-Martinez et al., 2006), and it has been suggested that the removal of both cohesin and catenations contributes to regulate sister-chromatid separation (Diaz-Martinez et al., 2006; Kenney and Heald, 2006; Toyoda and Yanagida, 2006).

The requirement of topoisomerase II at different stages of the chromosome cycle is presumably why it has been an effective target of anti-cancer drugs (Nelson et al., 1984; Tewey et al., 1984).
Fig. 3. The formation of catenations and their resolution by topoisomerase II. Catenations are a byproduct of DNA replication and they form at the points at which two replication forks collide. Removal of catenations is performed by type-II topoisomerases, which produce a double-strand break in one of the chromatids and pass the other through the break. Two resolved sister chromatids are the result of the strand-passing process and re-ligation reaction.

However, the idea that there is a progressive removal of catenations during the cell cycle led to the idea that, although catenations remain at centromeres until anaphase, they are maintained as a consequence of cohesion and their removal is not regulated. This hypothesis is supported by the dispensability of catenations for minichromosome segregation in yeast (Guacci et al., 1994; Koshland and Hartwell, 1987). However, as pointed out by Guacci et al. (Guacci et al., 1994), these data do not rule out a function of catenation in the establishment of cohesion. Moreover, minichromosomes are segregated 100-fold less efficiently than endogenous yeast chromosomes (10^{-3} versus 10^{-5} errors per cell division) (Koshland and Hartwell, 1987). Whether this decrease in the faithfulness of chromosome segregation is due to reduced cohesion in the absence of catenations remains unknown.

The view that decatenation is unregulated has begun to change in light of studies indicating that topoisomerase II\(\alpha\) is regulated by sumoylation during mitosis in yeast (Bachant et al., 2002; Takahashi et al., 2006) (reviewed in Porter and Farr, 2004), Xenopus (Azuma et al., 2005; Azuma et al., 2003), mice (Dawlaty et al., 2008) and human (Diaz-Martinez et al., 2006) cells. In budding yeast, mutation of the SUMO-isopeptidase Smt4 leads to premature sister-chromatid separation. This cohesion defect is probably caused by defects in the regulation of decatenation, because Top2 sumoylation was increased in smt4 mutants, a non-sumoylatable top2-SNM mutant rescued the cohesion defect of smt4 mutants (Bachant et al., 2002) and constitutive Top2 sumoylation induced enrichment of Top2 at pericentromeric regions (Takahashi et al., 2006). These results suggest that sumoylation increases Top2 activity at the centromeres by promoting its recruitment to these loci, which leads to premature decatenation.

That Smt4-dependent regulation of cohesion is independent of cohesin is indicated by the unchanged distribution of cohesin in smt4 mutants and the sister-chromatid separation observed in these mutants in the absence of separase (Bachant et al., 2002). Despite these intriguing data, other facts indicate that Smt4 is unlikely to regulate cohesion solely via Top2. Smt4 is required for cohesion in the context of a circular minichromosome that lacks catenations, indicating that there is another Smt4 target that contributes to cohesion.

Interestingly, Smt4-dependent cohesion shares three important features with cohesin-mediated cohesion. First, as observed in cohesin mutants, cohesion is lost only in a fraction of smt4 cells. Second, similar to cohesin-mediated cohesion, Smt4-dependent cohesion varies from locus to locus (Bachant et al., 2002). Consistent with a sufficiency for cohesin-dependent cohesion at telomeres (Antoniacci and Skibbens, 2006), smt4 mutants do not show loss of cohesion at telomeres (Bachant et al., 2002), whereas cohesion at the URA3 locus is partially lost both in smt4 mutants (Bachant et al., 2002) and in cohesin mutants (Weitzer et al., 2003). These results suggest that these two mechanisms of cohesion have additive roles in sister-chromatid cohesion. A systematic analysis to understand the specific contributions of each cohesion mechanism at different loci is needed. Third, the separation that is observed after smt4 or cohesin inactivation occurs after the S-phase is completed, at least in a subset of cells. These results suggest that sister-chromatid cohesion at a specific locus is the result of a complex balance between different cohesion mechanisms – absence of any one of these mechanisms might lead to weakened cohesion that, although able to initially provide association and perhaps provide cohesion in most of the cells during an unperturbed mitosis, is not sufficient to maintain pairing after a prolonged mitotic arrest. Studies to determine whether this is true, and to test the specific contribution of each cohesion mechanism at different loci, are needed.

A cohesion-related role for sumoylation of topoisomerase II has also been revealed in higher eukaryotes. Depletion of the SUMO E3-ligase PIAS\(\gamma\) from human cells leads to prolonged metaphase arrest and lack of enrichment of topoisomerase II\(\alpha\) at centromeres (Diaz-Martinez et al., 2006). Xenopus PIAS\(\gamma\) is required for the formation of a unique SUMO focus at the inner centromere (Azuma et al., 2005) and is responsible for topoisomerase-II\(\alpha\) sumoylation. Disrupting PIAS\(\gamma\) activity in egg extracts leads to failed chromosome segregation (Azuma et al., 2005). Topoisomerase II\(\alpha\) is sumoylated in human cells (Mao et al., 2003) and evidence suggests that PIAS\(\gamma\) is the relevant E3 ligase (Diaz-Martinez et al., 2006; Agostinho et al., 2008). Possible redundancy or cooperation between SUMO ligases is suggested by the dispensability of PIAS\(\gamma\) in mice (Wong et al., 2004), which in turn rely on the SUMO-ligase activity of RanBP2 for topoisomerase-II enrichment at the centromere (Dawlaty et al., 2008). Interestingly, regardless of the particular SUMO ligase that is involved, sumoylation of topoisomerase II is required for its enrichment at the centromere and defects in this process can result in failure of sister-chromatid segregation (Azuma et al., 2005; Diaz-Martinez et al., 2006; Dawlaty et al., 2008). Furthermore, PIAS\(\gamma\) most probably regulates a cohesin-independent form of cohesion, because the centromeres of human cells simultaneously depleted of PIAS\(\gamma\) and the cohesin-protector Sgo1 remain cohered but lack cohesion at the centromere (Diaz-Martinez et al., 2006).

These data have revealed a mechanism that specifically regulates topoisomerase II\(\alpha\) at the centromere during mitosis in metazoans. Thus, centromeric catenations might be actively maintained until anaphase, in a checkpoint-dependent manner, by modulation of the activities of SUMO ligases. However, in addition to topoisomerase II, other proteins that have cohesion-related functions, such as Pds5 (Stead et al., 2003) and Ycs4 (D’Amours et al., 2004), are known to be sumoylated. Therefore, the SUMO
ligases have the potential to drive different aspects of sister-chromatid separation and perhaps direct the concerted dissolution of different cohesion mechanisms (Fig. 4). Further understanding of these enzymes will clarify their roles in sister-chromatid cohesion.

**ORC and condensin**

During DNA replication, the cohesin loading factors interact with components of the replication machinery such as PCNA and RFC (reviewed in Guacci, 2007; Skibbens et al., 2007); thus, components of the replication fork might activate these loading mechanisms. In addition, replication proteins might have a direct role in cohesion. The origin recognition complex (ORC) comprises six subunits, marks replication origins in eukaryotic cells and facilitates the binding of other replication proteins (Quintana and Dutta, 1999). ORC mutants in budding yeast arrest in mitosis with a normal 2C binding of other replication proteins (Quintana and Dutta, 1999).

ORC mutants in budding yeast arrest in mitosis with a normal 2C binding of other replication proteins (Quintana and Dutta, 1999). The contribution of each mechanism at a specific locus might be influenced by factors such as the spacing of catenations, the location of cohesin-binding regions, chromatin structure and changes in chromatin cohesion that are induced after DNA replication (e.g. DNA damage or de novo cohesin loading) (Kim et al., 2002; Nagao et al., 2004; Potts et al., 2006; Sjogren and Nasmyth, 2001; Strom et al., 2004; Strom et al., 2007; Unal et al., 2007). The existence of different cohesion mechanisms is advantageous because it allows differential regulation of cohesion at specific chromosome regions. Both cohesin and catenations are subject to complex regulatory mechanisms and have to be concertedly removed during anaphase, possibly by post-translational modifications such as phosphorylation and sumoylation. Some of these regulatory mechanisms are depicted here.

A role of ORC in mitosis seems to be conserved in metazoans, because Orc2 and Orc5 mutants in Drosophila cells (Pflumm and Botchan, 2001), as well as Orc2- or Orc6-depleted human cells (Prasanth et al., 2004; Prasanth et al., 2002), have mitotic defects that increase ploidy, induce mitotic arrest and spindle abnormalities, and affect chromosome congression and condensation (Pflumm and Botchan, 2001; Prasanth et al., 2004; Prasanth et al., 2002), which is consistent with the localization of ORC to centromeres and centrosomes during mitosis (Prasanth et al., 2004; Prasanth et al., 2002). However, none of these studies has yet described defects in cohesion (Pflumm and Botchan, 2001; Prasanth et al., 2004; Prasanth et al., 2002).

Recent studies have also linked the condensin complex with cohesion. Drosophila mutants of a condensin subunit (Cap-G) have cohesion defects at centromeres, whereas the chromosome arms remain cohered even in anaphase, which causes chromosome missegregation (Dej et al., 2004). In yeast, mutations in condensin subunits have been associated with loss...
of cohesion (Lam et al., 2006; Vas et al., 2007) as well as with lack of separation at the rDNA locus (D’Amours et al., 2004; Freeman et al., 2000; Strunnikov et al., 1995). Whether this is due to independent roles of condensin, namely cohesion and condensation, or two manifestations of the same activity that lead to opposite effects, perhaps owing to the special structure of the rDNA locus, remains to be tested. The contribution of condensin to cohesion, similar to the contribution of cohesin, varies from locus to locus and the effects are additive in a condensin-cohesin double mutant (Lam et al., 2006). The possibility that condensin maintains catenation-mediated cohesion remains to be tested, but condensin has been shown to be involved in recruiting topoisomerase II to the chromosome scaffold (Coelho et al., 2003). This suggests a link between condensin and catenations. Alternatively, the role of condensin in cohesion might be a secondary consequence of its role in condensation. This might be particularly important in regions that have complex chromatin structures such as centromeres and rDNA.

Multiple mechanisms of cohesion

An integrative model of cohesion

Based on the data discussed above, we propose a model in which cohesion is the result of a complex balance and coordination between several mechanisms of cohesion. Two main mechanisms, provided by DNA catenations and cohesin, result in cohesion that is distributed along the whole chromosome in all eukaryotes that have been studied (Fig. 4). However, these mechanisms are subject to differential regulation at centromeres, arms, telomeres and perhaps other loci. Both centromeric cohesin and catenations are maintained in a spindle-checkpoint-dependent manner in higher eukaryotes. Similarly, cohesin and catenations are removed from euchromosomes coordinately in anaphase – complete and efficient removal of both of these cohesion systems is required for the accurate segregation of chromosomes.

We propose that catenations are the default or primary mechanism of cohesion because they are physically coupled to replication. Cohesin is then loaded onto replicated (already catenated) sister chromatids. This process occurs concurrently with replication, because cohesin-loading mechanisms are active during S phase. But, we argue that the establishment of cohesin-mediated cohesion in S phase might be a dynamic process. The observation that activation of cohesin-loading mechanisms during G2-M after a single double-strand break leads to genome-wide de novo establishment of cohesion (Strom et al., 2007; Unal et al., 2007) raises the possibility that a similar mechanism operates during S phase. In this scenario, activation of cohesin loading would lead to loading of cohesin at all loci. Some of these cohesin complexes will land in unreplicated regions and will later be removed to allow passage of the replication machinery. Once that region is replicated and held close to its sister chromatid (perhaps aided by catenations), cohesin can be loaded again, this time providing cohesion. It can be envisioned that this genome-wide loading of cohesin is a dynamic event that occurs throughout S phase. The alternative model is that cohesin loading is restricted to the replication forks.

In addition to catenations and cohesin, other mechanisms of cohesion that are contributed to by ORC and condensin might exist. However, further research is needed to determine whether these are bona fide cohesion factors or whether they are regulators of cohesin and catenations. ORC and condensin could also act by affecting cohesion indirectly – by providing specific chromatin structures. As is evident from the yeast data, the contribution of different cohesion mechanisms varies from locus to locus, perhaps being influenced by the locations in which catenations arise during replication, and the sites of cohesin, condensin and ORC binding, as well as the surrounding chromatin environment.

Different needs, different solutions

The existence of collaborative mechanisms of cohesion makes the need for multiple pathways that inactivate cohesion in anaphase obligatory. Two events that are necessary for sister-chromatid separation, namely cohesion removal (promoted by separase and the kinases of the prophase pathway) and decatenation (promoted by topoisomerase II), have been widely documented, but further research is needed to understand the mechanism that regulates their concerted activity in anaphase. Two types of mechanism for the concerted removal of multiple cohesion devices can be envisioned: a parallel and independent activation of all cohesion-removal mechanisms upon the generation of a single ‘go’ signal, or a network-type regulation in which activation of one separation mechanism influences the activity of the others. It is also possible that a mixture of these two mechanisms exists so that a single ‘go’ signal serves as the initial activator of some or all of these pathways, which, in turn, regulate the other pathways. Interestingly, recent research has shown that some cohesion mechanisms might share a common regulatory theme: sumoylation of both topoisomerase II and the cohesin-associated protein Pds5 have been shown to regulate cohesion (Azuma et al., 2005; Azuma et al., 2003; Bachant et al., 2002; Díaz-Martínez et al., 2006; Stead et al., 2003). These results suggest that SUMO-related processes might be responsible for tipping the balance in favor of loss of cohesion during anaphase.

The existence of multiple mechanisms of cohesion creates the difficulty of ensuring their concerted deactivation to allow complete sister-chromatid segregation during mitosis. However, it can also explain the ability of cells to provide differential cohesion dynamics within a chromosome. Examples of the locus-specific timing of separation exist in nature, such as the centromere-breathing phenomenon (the brief and reversible separation of centromeres upon attachment to the spindle) and the late separation of the rDNA locus in budding yeast. Separation of the rDNA locus seems to be a clear example of specialized cohesion because cohesion at this locus is independent of cohesin and its separation depends on a functional condensin complex (D’Amours et al., 2004). Although parallel separation (at centromeres and chromosome arms) of the sister chromatids during anaphase is the norm in human cells during undisturbed mitoses (Gimenez-Abian et al., 2005b), ~4% of HeLa cells separate the centromeres before the chromosome arms (Gimenez-Abian et al., 2005b). This suggests that the mechanisms of chromosome-arm and centromere separation, although normally acting in concert, can be uncoupled. This differential regulation is perhaps more dramatically revealed by the separation of chromosome arms but not centromeres during prolonged arrest in the presence of microtubule poisons (Gimenez-Abian et al., 2004).

The observations that expression of non-cleavable cohesin or depletion of separase results in failure to separate the chromosome arms but that centromeres can separate (Díaz-Martínez et al., 2007; Gimenez-Abian et al., 2005a; Gimenez-Abian et al., 2005b; Papi et al., 2005; Yalon et al., 2004) suggest that cohesin-mediated cohesion, although present at the centromere, might be primarily responsible for the cohesion of chromosome arms, whereas centromeric cohesion could be mediated by a cohesin-independent mechanism, both factors being synchronously removed in anaphase.
It is noteworthy that a step-wise loss of cohesion is observed during meiosis, with cohesion at the chromosome arms being lost during anaphase I, allowing the separation of homologous chromosomes, followed by the dissolution of centromeric cohesion during anaphase II to separate sister chromatids. The existence of multiple mechanisms of cohesion that are differentially regulated at the arms and centromeres could account for this step-wise regulation of cohesion during meiosis.

Conclusions
A complex picture has emerged in which diverse mechanisms collaborate to provide cohesion along the chromosome. These mechanisms of cohesion include, but might not be limited to, cohesion and DNA catenations. Renewed effort to understand the nature, regulation and complex interactions of the multiple mechanisms of cohesion is needed to attain a comprehensive understanding.

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