Unzipped and loaded: the role of DNA helicases and RFC clamp-loading complexes in sister chromatid cohesion

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It is well known that the products of chromosome replication are paired to ensure that the sisters segregate away from each other during mitosis. A key issue is how cells pair sister chromatids but preclude the catastrophic pairing of nonsister chromatids. The identification of both replication factor C and DNA helicases as critical for sister chromatid pairing has brought new insights into this fundamental process.

Chromosome segregation and sister chromatid cohesion

In eukaryotes, DNA replication is separated in time from chromosome segregation. For instance, DNA replication and DNA damage checkpoints delay cell cycle progression until each chromosome is fully replicated and physically intact. The G_2 gap phase further separates S-phase from mitosis, allowing for continued cell growth and maturation before cell division. Each chromosome harbors essential genes. Thus, the products of chromosome replication (sister chromatids) generated during S-phase must be identified over time until mitosis when sisters associate with the mitotic spindle and segregate away from each other into the newly forming daughter cells. Identity is achieved by "gluing" sister chromatids together, a process termed sister chromatid cohesion. Beyond identity, cohesion promotes proper chromosome orientation, bipolar spindle formation, plays a critical role in the checkpoint mechanism that regulates the metaphase-to-anaphase transition and is an essential feature of DNA double strand break repair (Skibbens, 2000; Lowndes and Toh, 2005).

At least two classes of factors are critical for sister chromatid pairing: structural cohesins and deposition factors. Enduring and robust, structural cohesins resist poleward-pulling forces produced by the mitotic spindle that act to separate sister chromatids and must often persist for extended periods of time in meiosis. In budding yeast, the cohesin complex is comprised of Smc1p, Smc3p, Mcd1p/Scc1p (herein termed Mcd1p), Irr1p/Scc3p, and Pds5p (Guacci et al., 1997; Michaelis et al., 1997; Toth et al., 1999; Hartman et al., 2000; Panizza et al., 2000). Cohesins associate at discrete sites along the chromosome length, primarily in intergenic regions at roughly 10–15-kb intervals (Blat and Kleckner, 1999; Megee et al., 1999; Tanaka et al., 1999; Laloraya et al., 2000; Lengronne et al., 2004). Early evidence that cohesins form a soluble complex, independent of DNA, suggested that cohesin complexes are loaded, preformed, onto DNA (Losada et al., 1998; Toth et al., 1999; Ciosk et al., 2000). Alternatively, a stepwise assembly model positing that subunits are sequentially recruited is gaining biochemical support (Skibbens, 2000). For instance, SMC proteins (COOH-terminal fragments) are capable of binding DNA in the absence of Mcd1p. In turn, Mcd1p requires SMC proteins and Pds5p requires Mcd1p for chromosomal recruitment. Additionally, Pds5p appears to play a role in stabilizing cohesin's chromatin association and this activity may be regulated by sumoylation (Michaelis et al., 1997; Ahkmedov et al., 1998; Ciosk et al., 2000; Hartman et al., 2000; Panizza et al., 2000; Stead et al., 2003).

Recent studies have focused on cohesin architecture. Biochemical analyses suggested that at least a subset of cohesins (Smc1p, Smc3p, and Mcd1p) associate to form a large but closed ring structure comprised in part of extended coiled coil domains (Melby et al., 1998; Anderson et al., 2002; Haering et al., 2002; Gruber et al., 2003). Although mature cohesin rings appear to be formed via Mcd1p bridging separated Smc1p and Smc3p head domains, more recent data complicates the story in that Smc1p and Smc3p appear to interact directly in the absence Mcd1p. Consistent with a stepwise assembly model, Mcd1p association with SMCs appears ATP-dependent, suggesting a regulated open/shut gating mechanism that conceivably traps DNA within the cohesin ring (Arumugam et al., 2003; Weitzer et al., 2003). Such a gating mechanism, if reversible, might hold the key to unlocking several apparent paradoxes involving cohesin deposition versus sister pairing (see An integrated view of cohesion establishment). Despite these advances, little is understood regarding the structural role of "non-ring" cohesins such as Irr1p/Scc3p and Pds5p in maintaining cohesion, portending that the current view of cohesion may yet undergo additional reincarnations.

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Abbreviations used in this paper: PCNA, proliferating cell nuclear antigen; RFC, replication factor C.

The Journal of Cell Biology, Vol. 169, No. 6, June 20, 2005 841–846
http://www.jcb.org/cgi/doi/10.1083/jcb.200503129

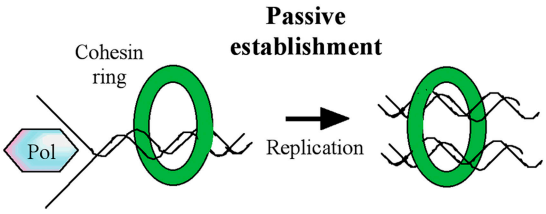

Figure 1. **Replication through a ring model.** DNA replication fork (Pol) passes through a huge cohesin ring (green ring), passively establishing cohesion by entrapping both sisters within a single cohesin ring.

Cohesin deposition requires a separate and highly conserved activity. Cohesins association with chromatin requires deposition factors Scc2p and Scc4p that are essential for cohesion but do not function in cohesion maintenance (Furuya et al., 1998; Toth et al., 1999; Ciosk et al., 2000; Arumugam et al., 2003; Weitzer et al., 2003; Gillespie and Hirano, 2004). The molecular mechanism by which Scc2p and Scc4p promote cohesin deposition remains unknown. One model posited is that Scc2p and Scc4p regulate Smc1p-dependent ATP hydrolysis and that inactivating Scc2p Scc4p after S-phase locks Mcd1p in place to entrap sister chromatids (Arumugam et al., 2003). However, cohesin deposition can occur from late G_1 until anaphase onset, suggesting that Scc2p and Scc4p activity persists beyond cohesion establishment.

Cohesion establishment

How do cells pair sister chromatids but preclude the catastrophic pairing of nonsister chromatids? In humans, sister chromatid pairing reactions must occur in the presence of highly repetitive DNA elements (LINES, SINES, ALUs), homologous chromosomes, and a myriad of gene families, suggesting that cells do not rely on DNA sequence to pair sister chromatids together. Indeed, a comparison of numerous cohesin sites fails to identify a cohesin-binding DNA motif (Megee et al., 1999; Tanaka et al., 1999; Blat and Kleckner, 1999). Based on the observation that Smc1p, Smc3p, and Mcd1p associate to form a large but closed ring structure, it was proposed that a huge cohesin ring, of sufficient size to allow the DNA replication fork to pass through, is loaded onto chromosomes before replication (Haering et al., 2002; Gruber et al., 2003; Nasmyth and Schleiffer, 2004). Passage of the DNA replication machinery through the ring entraps the newly formed sister chromatids within a ring establishing cohesion (Fig. 1). In its simplest form, the "replication through a ring" model asserts that cohesion establishment is a passive process that requires only the loading of cohesin rings and subsequent DNA replication. However, a growing body of evidence suggests that sister pairing is much more complex and that alternate models of cohesion establishment must be considered.

The founding member of a third class of cohesion factors, Ctf7p/Eco1p (herein termed Ctf7p) is unique from structural cohesins in that Ctf7p functions exclusively during S-phase when sister chromatids are first paired but does not function in G_2 or mitosis when cohesion is maintained. Ctf7p is also distinct from the deposition factors Scc2p and Scc4p in that cohesins remain chromatin bound in *ctf7* mutant cells. Despite cohesin deposition and normal DNA replication, *ctf7* mutant cells exhibit dramatic cohesion defects (Skibbens et al., 1999; Toth et al., 1999). In combination, these findings reveal that Ctf7p performs an active pairing function (termed establishment) that is separate and distinct from both cohesin deposition and cohesion maintenance, a finding not predicted by the replication through a ring model. Based on this evidence, we and others proposed various models in which each chromatid associates with individual cohesins that are tethered together (or catenated) (Skibbens, 2000; Campbell and Cohen-Fix, 2002; Milutinovich and Koshland, 2003), possibly by Ctf7p. This two-ring model allows for cohesin deposition, but the absence of cohesin ring pairing, in *ctf7* mutant cells (Fig. 2).

Critical evaluation of chromosome structure in vivo further strengthens the assertion that cohesin deposition and DNA replication does not necessarily equate to loci pairing between sister chromatids. As previously described, numerous labs reported that cohesins are deposited at intervals along the entire length of the chromosome, with the greatest cohesin deposition occurring at centromeres. However, GFP-tagged centromere proteins (Cse4p and Mtw1p) and DNA probes specific to discrete loci along the chromosome arms clearly documents that centromeres are visibly and precociously separated in living yeast cells (Goshima and Yanagida, 2000; He et al., 2000; Pearson et al., 2001). Similarly, high resolution images of living vertebrate newt lung cells reveal that tension produced by the mitotic spindle preciously separates centromeric regions of sister chromatids (Skibbens et al., 1993; Waters et al., 1996).

Cohesion establishment is intimately linked to DNA replication

The above findings make the question of how cells pair sister chromatids all the more intriguing. In part, a molecular basis for establishing cohesion was revealed through interactions between *CTF7*, *POL30* (proliferating cell nuclear antigen [PCNA]), and *CTF18/CHL12* (replication factor C [RFC] homologue; Skibbens et al., 1999). PCNA forms a homotrimeric sliding clamp that associates with DNA polymerases to promote processive DNA replication. *CTF18/CHL12* (herein termed *CTF18*) encodes a RFC subunit (Kouprina et al., 1994). RFC complexes hydrolyze ATP to load sliding clamps, such as

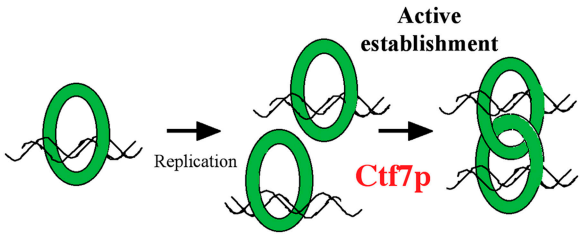

Figure 2. **An active view of cohesion establishment.** Sister chromatids become paired via association of separate cohesin rings *after* DNA replication. This model allows for separation of loci even in the presence of properly deposited cohesins, as observed at centromeres in wild-type cells and throughout chromosomes in *ctf7* mutant cells. For convenience, sister pairing is depicted as ring catenation; numerous alternate ring-bridging structures (and contributions by DNA catenation) are equally plausible (Campbell and Cohen-Fix, 2002; Milutinovich and Koshland, 2003).

PCNA, onto double-stranded DNA (O'Donnell et al., 2001). Recently, much emphasis has been placed on the role of RFC complexes in cohesion. There are four known independent clamp-loading RFC complexes: each comprised of four small subunits (Rfc2p-Rfc5p) and any one of four large unique subunits (Rfc1p, Rad24p, Ctf18p, or Elg1p). RFC complexes perform varying and often overlapping roles in DNA replication, repair and/or checkpoint function depending on the identity of the single large subunit within the complex (Majka and Burgers, 2004). Importantly, mutations in either small or large RFC subunits (Rfc2p, Rfc4p, Rfc5p, and Ctf18p) result in cohesion defects (Skibbens et al., 1999; Mayer et al., 2001; Hanna et al., 2001; Krause et al., 2001; Kenna and Skibbens, 2003; Petronczki et al., 2004). Precocious sister separation in *rfc* mutant cells may have a physical basis: Ctf7p associates with each of the four RFC complexes (Kenna and Skibbens, 2003; unpublished data; B. Satish, personal communication). Although the extent that Ctf7p-RFC associations are required for cohesion remains unknown, a likely model is that each of the four RFC complexes tether Ctf7p to the replication fork. In turn, Ctf7p actively establishes cohesion between nascent sister chromatids as they emerge from behind the replication fork.

In support of the model that cohesion is intimately linked to DNA replication is the multitude of DNA replication factors now shown to participate in cohesion including: (a) RFC-associated factors Ctf8p and Dcc1p; (b) the Polα-binding protein Ctf4p (Okazaki maturation); (c) at least two DNA polymerases, Trf4p (Polσ) and Pol2p; and (d) S-phase checkpoint factors Mre11p, Xrs2p, Mrc1p, Tof1p, and Csm3p (Wang et al., 2000; Edwards et al., 2003; Mayer et al., 2004, Petronczki et al., 2004; Warren et al., 2004; Xu et al., 2004). Beyond replication, DNA repair presents another exciting facet in which DNA metabolism and cohesin subunit dynamics are intimately linked (Lowndes and Toh, 2005).

A new class of cohesion factors: cohesion DNA helicases

New studies reveal that steps critical for cohesion establishment occur even before nascent chromatids are generated. Budding yeast Chl1p is a DNA helicase identified from numerous chromosome loss screens, but little was discovered regarding how Chl1p functions in chromosome segregation (Haber, 1974; Liras et al., 1978; Gerring et al., 1990; Spencer et al., 1990). Paradoxically, *chl1* mutations are lethal when combined with mitotic spindle checkpoint mutations, but not DNA damage checkpoint mutations (Li and Murray, 1991). This apparent paradox was resolved by characterization of Chl1p as a cohesion factor (Mayer et al., 2004; Skibbens, 2004), a role later expanded to include cohesion during meiosis (Petronczki et al., 2004). A previous study of *CTF7* alleles revealed that loss of cohesion results in a cell cycle delay that requires the mitotic spindle checkpoint (Skibbens et al., 1999). Checkpoint activation in the absence of Ctf7p (and probably also for Chl1p) is likely due to a premature loss of tension across centromeres normally produced by poleward-directed spindle forces (Nicklas, 1997).

Around this same time, three other DNA helicases were reported to participate in cohesion: Sgs1p, Rrm3p, and Hpr5p/Srs2p (Warren et al., 2004). Rrm3p enables replication past nonhistone DNA–protein complexes, suggesting that specific DNA helicases such as Rrm3p may be required to unwind cohesin–chromatin complexes. Defects in Rrm3p result in replication fork pausing, although the extent that forks pause at cohesin sites is unknown. Moreover, *rrm3* cells are inviable when combined with alleles of intra–S-phase checkpoint factors, which also function in cohesion (Ivessa et al., 2003; Torres et al., 2004a,b). Significantly, many of the cohesion helicases exhibit similar functions in stalled fork reinitiation and/or fork stabilization, indicating that these activities may be critical components of cohesion establishment (Weitao et al., 2003; O'Neill et al., 2004; Torres et al., 2004b; Xu et al., 2004; Bjergbaek et al., 2005).

Cohesion DNA helicases directly link cohesion establishment to human disease states

Human orthologues of Chl1p, Srs2p, Rrm3p, and Sgs1p include BACH1, BLM, and WRN helicases, all of which, when mutated, contribute to cancer progression or premature aging (Brosh and Bohr, 2002; Wu and Hickson, 2002). For instance, BACH1 (the human orthologue of yeast Chl1p) is a DNA helicase-like protein that associates with the breast cancer tumor suppressor BRCA1 (Cantor et al., 2001; Skibbens, 2004). The association between BACH1 and BRCA1 is physiologically relevant in that BACH1 is required for BRCA1-dependent double strand break repair. Of clinical relevance is the identification of breast cancer patients that harbor mutations in BACH1 but not mutations in either BRCA1 or BRCA2, suggesting that loss of BACH1 helicase activity is itself sufficient to predispose affected individuals to tumorogenesis (Cantor et al., 2004). Importantly, cells harboring mutations in the BRCA1 pathway exhibit gaps between sister chromatids in addition to other chromosome abnormalities (Deming et al., 2001). Similarly, mutations in Werner syndrome helicase (WRN) results in precociously separated sister chromatids, although in both the BRCA1 and WRN studies, these findings were interpreted to involve a "decatenation" checkpoint function (Deming et al., 2001; Franchitto et al., 2003). A more likely model is that these helicases, when mutated, contribute to aneuploidy directly through loss of sister chromatid cohesion. Recent work on the mouse model for Rothmund-Thomson syndrome strongly supports this latter view. Knockout mice homozygous null for RecQL4 (the helicase responsible for Rothmund-Thomson syndrome) recapitulate Rothmund-Thomson syndrome phenotypes including skin abnormalities, skeletal defects, aneuploidy, and a predisposition to cancer. Significantly, cells from recql4$^{-/-}$ mice exhibit dramatic cohesion defects, providing a clear and singular mechanism for aneuploidy and cancer progression (Mann et al., 2005).

An integrated view of cohesion establishment

Models that both acknowledge and account for all of the above findings need not be overly complicated, even when one considers the variety of factors that comprise the replication fork (Fig. 3). DNA helicases that precede the replication fork are

first to encounter sites marked for cohesion, an assertion supported by observations that at least a subset of cohesins remain DNA-associated from the previous cell cycle or are recruited early to chromatin in a step-wise fashion (Skibbens, 2000). As DNA helicases unwind cohesin-decorated DNA: they may either facilitate new cohesin deposition onto separated DNA strands or modify preexisting cohesin complexes. Further inquiries into the replication intersection through which cohesion DNA helicases, RFCs, and Ctf7p establishment factor merge will undoubtedly provide profound insights into the mechanisms by which human disease states progress.

Upon DNA replication initiation and elongation, Ctf7p may be recruited to the DNA replication fork via RFC complexes. At the replication fork, Ctf7p establishes cohesion between nascent sister chromatids, possibly by tethering together cohesin rings that encircle the individual sister chromatids. Despite the highly conserved nature of Ctf7p (human EFO1, ESCO2/EFO2, EFO3, and EFO4, *Drosophila* DECO, and fission yeast ESO1; Tanaka et al., 2000; Bellows et al., 2003; Williams et al., 2003; Vega et al., 2005), the mechanism by which Ctf7p establishes cohesion remains unknown. Possibilities include Ctf7p-dependent pairing functions through catenation of individual cohesin rings (or catenating sister chromatid DNA) or by linking rings via nonring cohesin factors such as Irr1p/Scc3p or Pds5p. Elucidating the molecular mechanism by which cohesion is established will provide new insights into the final structure required to maintain sister pairing.

Any new model must account for the apparent paradoxes that currently plague the literature. The condundrum that centromeres that are heavily laden with cohesins but precociously separate is most easily, but not exclusively, explained by a two-ring model. The two-ring model is supported by observations that cohesins persist but sisters are not paired in mutant *ctf7* cells, revealing the cohesin deposition and cohesion establishment may be distinct processes (Skibbens et al., 1999; Toth et al., 1999; unpublished data). Ctf7p's role in establishment is clearly of clinical relevance: Ctf7p is highly conserved through evolution with at least one human homologue (ESCO2/EFO2) directly linked to Roberts syndrome (Bellows et al., 2003; Williams et al., 2003; Vega et al., 2005).

The remaining issue is to address how centromeres precociously separate despite having elevated levels of cohesin deposition in wild-type cells. Of several possibilities, three are considered. First, centromere pulling forces generate tension that eventually pops open (but not off) cohesin rings. Chromatin stretching dissipates the tension until a threshold point is achieved where cohesin ring structures are able to withstand the separation forces. This model is supported by in vivo observations of chromatin stretching during kinetochore movements and that chromatin elasticity can reduce tension in a distance-dependent fashion (Skibbens et al., 1993; Waters et al., 1996; Goshima and Yanagida, 2000; He et al., 2000; Pearson et al., 2001). The finding that Mcd1p association with SMCs is ATP-dependent provides a unique solution to ring opening and closing (Arumugam et al., 2003; Weitzer et al., 2003). Such a gate-keeping activity provides for a reversible open/shut mechanism that, in this scenario, may be tension sensitive and allow for

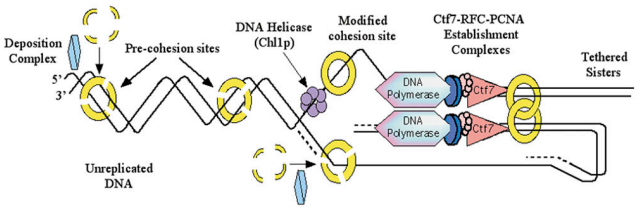

Figure 3. **A multi-step model of cohesion establishment.** (Left) A subset of structural cohesins associated with DNA in G_1 defines precohesion sites. Precohesion sites may be marked by cohesins that remain from the previous cell cycle or by Scc2p–Scc4p-dependent deposition. (Middle) DNA helicases (purple hexameric ring) modify precohesion complexes (broken yellow rings) or promote new cohesin deposition onto single-stranded DNA. (Right) After replication (DNA polymerase), Ct7p-RFC-PCNA (red/pink triangle–ball complex with blue PCNA disc) is posited to pair cohesin rings together, establishing cohesion between sister chromatids (ring catenation shown for simplicity). Black lines represent DNA strands; dashed lines represent RNA primers (primase not depicted) used for Okazaki fragment synthesis during lagging strand synthesis.

ring uncoupling without permanent dissociation. Tension-based responses are already well established in other aspects of chromosome segregation including kinetochore motility and checkpoint activation (Nicklas, 1997; Rieder and Salmon, 1998). Second, an equally likely model is that cohesin rings are recruited to centromeres after both DNA replication and Ctf7p-dependent pairing activity. This model is consistent with previous findings that cohesins spread from preexisting cohesins, occur in clusters and that Scc2/4p can deposit cohesins outside of S-phase (i.e., after establishment; Furuya et al., 1998; Tanaka et al., 1999; Toth et al., 1999; Ciosk et al., 2000; Weitzer et al., 2003). Although it is unclear why cohesins may be recruited so heavily to centromeres after establishment, it is worth speculating as to the role of tension-induced chromatin changes in directing cohesin deposition after establishment. Third, ring structures may slide along chromosome arms to a point where, in reenforcing clusters, cohesion can be maintained. A sliding model is supported by observations that transcription repositions cohesin complexes from initial Scc2p, Scc4p-dependent deposition sites (Lengronne et al., 2004). However, of the three, this model appears less likely to explain cohesin's retention at centromeres where separation forces that might act to slide rings apart are greatest. The goal of future endeavors will likely include elucidating the molecular mechanism of pairing and the resulting structures by which sister chromatids remain paired.

The author thanks Drs. Lynne Cassimeris, Kerry Bloom, Alex Brands, Meg Kenna, Lisa Antoniacci, David Odde, and Skibbens' lab members for stimulating conversation and for providing comments on this manuscript and Basanthi Satish for sharing data before publication.

This report was supported by an award to R.V. Skibbens from the National Science Foundation (MCB-0212323). Any opinions, conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

Submitted: 23 March 2005
Accepted: 13 May 2005

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