Fork it over: the cohesion establishment factor Ctf7p and DNA replication

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Summary

To produce viable progeny, cells must identify the products of chromosome replication as sister chromatids, pair them together and then maintain this cohesion until chromosome segregation. It is well established that cohesin ring-like structures maintain sister chromatid cohesion, but the molecular mechanism by which only sisters become paired (termed establishment) is highly controversial. One of the first establishment models posited in the literature suggested that cohesin complexes associated with each sister become tethered together through an active process that is intimately coupled to progression of the DNA replication fork. A subsequent model posited that the replication fork simply passes through pre-loaded cohesin rings, entrapping within both sister chromatids. The recent findings that the establishment factor Ctf7p/Eco1p is recruited to DNA and binds both a DNA polymerase processivity factor (PCNA) and the cohesin regulator Pds5p test current models of sister chromatid pairing.

Key words: *CTF7/ECO1*, PCNA, DNA helicase, RFC, Sister chromatid cohesion, Cohesion establishment, DNA replication

Introduction

The cell cycle is seductively simple – the overarching principal being that cell division generates two genetically identical daughter cells. Thus, it is often instructive to consider the cell cycle in terms of a chromosome cycle: chromosome replication during S phase produces identical sister chromatids and chromosome segregation during M phase ensures that sisters segregate away from each other and into newly forming daughter cells. Gap phases separate S and M phases, allowing cell growth and maturation. In addition, distinct S and M phases are ensured by DNA replication and DNA damage checkpoints that inhibit cell cycle progression until each chromosome is fully replicated and physically intact. The temporal separation of these two phases requires that cells identify chromatids as sisters during S phase and then maintain this identity over an extended period of time until anaphase onset – a process collectively termed cohesion.

Sister chromatid cohesion requires the coordinated activities of several factors (reviewed in Losada and Hirano, 2005; Nasmyth, 2005; Huang et al., 2005; Skibbens, 2005). In budding yeast, sister chromatid cohesion is maintained by a highly conserved cohesin complex that minimally contains Smc1p, Smc3p, Mcd1p/Scc1p and Irr1p/Scc3p. Cohesin complexes become deposited at discrete sites along the chromosome by a deposition complex that contains an Scc2p-Scc4p heterodimer. Sister chromatids decorated with cohesins become paired by Ctf7p/Eco1p 'establishment activity'. Established cohesion is maintained in part by Pds5p, a cohesin regulator.

Each cohesin SMC protein (Smc1p and Smc3p) contains globular N- and C-termini connected by extended helical domains that are bisected by a hinge region. Hinge folding produces anti-parallel coiled-coil domains and brings N- and C-termini together to form an ATP-binding cassette (ABC) head domain important for Scc2p–Scc4p-dependent loading of cohesin onto DNA. The hinge also contains a dimerization motif that promotes tight Smc1p-Smc3p binding. Mcd1p promotes Smc1p-to-Smc3p head association, which results in formation of a large ring structure ~35 nm in diameter (Haering et al., 2002; Gruber et al., 2003). Topologically closed cohesin rings that 'embrace' chromatin remain the more prominent cohesin structure referred to in the literature, although it is now acknowledged that the current biochemical and EM data cannot distinguish between a single ring, various double ring configurations or filamentous cohesin spirals that wrap around chromatin fibers or even associate laterally (Fig. 1) (reviewed in Losada and Hirano, 2005; Nasmyth, 2005; Huang et al., 2005).

Ultimately, cohesins resist chromatid-to-pole forces produced by the mitotic spindle. However, before the cell can maintain sister chromatid cohesion, it must first select which chromatids to glue together (establishment). Given the presence of homologous chromosomes, conserved gene families and highly repetitive DNA elements within the genomes, cells cannot rely on DNA sequence specificity for establishment. Thus, in addition to cohesins and deposition factors, cells possess a cohesion establishment activity, which is provided by Ctf7p (see below), that is required for sister chromatid pairing reactions and precludes the catastrophic pairing of non-sisters.

Mutations in any cohesin, deposition or cohesion establishment protein result in precocious sister separation, aneuploidy and cell death (Guacci et al., 1997; Michaelis et al., 1997; Skibbens et al., 1999; Toth et al., 1999; Ciosk et al., 2000). As such, elucidating the various activities required for cohesion establishment is likely to have important clinical
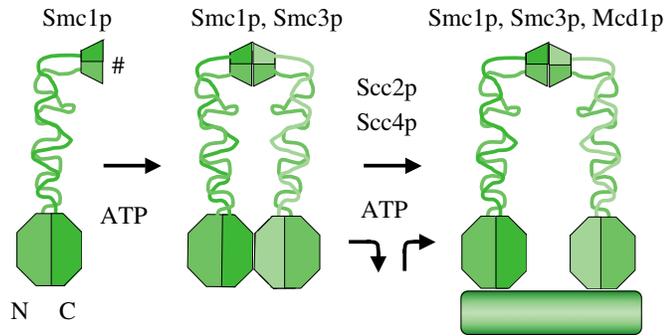
Fig. 1. Cohesin structure and dynamics. Smc1p and Smc3p contain globular N- and C-termini that are aligned by the folding of a centrally positioned hinge (#). This also allows Smc1p-Smc3p binding. Mcd1p associates with SMC head domains, creating an ATP-dependent closed ring structure.

implications. Indeed, mutations in human cohesion factors are known to contribute to cancer progression, premature aging, Cornelia de Lange Syndrome and SC phocomelia/Roberts Syndrome (reviewed in Skibbens, 2005; Dorsett, 2007).

The molecular mechanism through which Ctf7p associates with chromatin and interacts with cohesins to establish sister chromatid cohesion is enigmatic. Recent reports provide critical new tests of long-standing models of cohesion establishment by examining mechanisms of Ctf7p recruitment to chromatin during S phase, comparing Ctf7p dynamics to those of replication fork components and linking Ctf7p function to the cohesin regulator Pds5p (Moldovan et al., 2006; Lengronne et al., 2006; Noble et al., 2006).

Models of cohesion establishment
Studies exploiting the instability of Mcd1p coupled with its inducible expression revealed nearly a decade ago that sister chromatid pairing reactions must occur prior to the end of S phase (Uhlmann and Nasmyth, 1998). However, the molecular mechanism of pairing remained unknown. The first model proposed to solve the sister chromatid pairing problem involved Ctf7p. Ctf7p is essential for cohesion and cell viability: cells lacking Ctf7p contain separated sister chromatids prior to anaphase onset and exhibit massive chromosome mis-segregation, which leads to their death within a single cell cycle. Ctf7p was termed an 'establishment factor' on the basis of the observations that it is not required for cohesin deposition or maintenance of sister chromatid pairing in mitosis (i.e. it is not a cohesin). Instead, Ctf7p performs its essential function in cohesion specifically during S phase (Skibbens et al., 1999; Toth et al., 1999).

Insight into the molecular mechanism of cohesion establishment emanated from the finding that *CTF7* genetically interacts with both *POL30* (which encodes the DNA polymerase processivity factor PCNA) and a subunit of the replication factor C (RFC) complex encoded by *CTF18/CHL12* (Skibbens et al., 1999). RFC complexes hydrolyze ATP to load sliding clamps such as PCNA onto double-stranded DNA to promote processive DNA replication. *CTF7-POL30-CTF18* interactions and the S-phase specificity of Ctf7p function suggested that Ctf7p rides the DNA replication machinery to tether together cohesin complexes associated with each sister

chromatid (reviewed in Skibbens, 2000). Here we term this 'replication-coupled cohesin pairing' (Fig. 2). Multiple DNA replication factors (RFC complexes, RFC-associated proteins, DNA polymerases, DNA helicases and S-phase checkpoint proteins) are now known to promote efficient sister chromatid cohesion, which supports replication-coupled cohesin pairing (Hanna et al., 2001; Mayer et al., 2001; Kenna and Skibbens, 2003; Skibbens, 2004; Mayer et al., 2004; Warren et al., 2004; Petronczki et al., 2004; Edwards et al., 2004). Although this model has been revised to include a cohesin-ring structure (see below), it retains these key elements: (1) cohesins individually associate with each sister chromatid; (2) cohesin deposition is coincident with DNA replication; and (3) separate cohesin complexes become paired by Ctf7p associated with the DNA replication fork (reviewed in Skibbens, 2005).

The two-ring tethering proposed in replication-coupled cohesin pairing models accommodates two key findings. First, two rings explain 'cohesin-without-cohesion' phenotypes that occur in both *ctf7* and *pds5* mutant cells: the rings are on but sisters remain unpaired (Toth et al., 1999; Hartman et al., 2000; Milutinovich et al., 2007). Second, sister chromatid-pairing at silenced loci is not mediated solely by single rings but instead requires cohesins associated with each sister to bind to either opposing sister chromatid cohesins or other higher-order chromatin complexes (Chang et al., 2005).

Biochemical and EM-based analyses of cohesins suggested an alternate model of cohesion establishment (Haering et al., 2002; Gruber et al., 2003; Losada and Hirano, 2005; Nasmyth, 2005; Huang et al., 2005). Smc1p and Smc3p combine to produce a V-shaped heterodimer whose open end is closed by Mcd1p to produce 'a huge triangular ring that could entrap sister chromatids' (Gruber et al., 2003). Such cohesin rings could encircle chromatin fibers. Here, the mystery by which only sister chromatids become paired appears to be elegantly solved: cohesin rings loaded during G_1 allow subsequent passage of the DNA replication fork through the rings, passively trapping the newly formed sister chromatids within (Fig. 2). However, this cohesion establishment model, referred to as 'replication through a ring', disregards prior evidence for Ctf7p-dependent sister pairing activity.

One size may not fit all – issues in establishment
Although both the replication-coupled cohesin pairing model and the replication through a ring model have attractive features, neither escapes unscathed from close scrutiny. *ctf7* mutant cells contain chromosomes properly loaded with cohesins (the timing, level and sites of cohesin deposition are all normal) (Toth et al., 1999; Noble et al., 2006; Milutinovich et al., 2007). Furthermore, they undergo normal DNA replication (DNA replication proceeds with normal kinetics and neither DNA damage nor unreplicated DNA checkpoints become activated) (Skibbens et al., 1999; Toth et al., 1999). These positive outcomes satisfy the key tenets of the replication through a ring model. However, *ctf7* mutant cells contain high levels of precociously separated sister chromatids. This 'cohesin without cohesion' phenotype is recapitulated in cells lacking the cohesin regulator Pds5p (Hartman et al., 2000) (discussed below). Cohesin deposition and DNA replication are thus not sufficient for sister pairing. Further complicating the replication through a ring model is that passage through a cohesin ring is not consistent with all observations. For

instance, the transcription machinery has been reported to push cohesin rings along DNA to position them at intergenic regions whereas leading and lagging replisome complexes supposedly pass inside (Lengronne et al., 2004; Haering et al., 2002; Gruber et al., 2003).

The replication-coupled cohesin pairing model is similarly challenged by a number of findings (reviewed in Skibbens, 2005). First, cohesin ring pairs have yet to be biochemically isolated (Haering et al., 2002; Gruber et al., 2003; Ivanov and Nasmyth, 2005). Second, the model typically envisions cohesin deposition to be tightly coordinated with replication fork progression, but cell cycle mapping of Scc2p-Scc4p function remains controversial (see below). Third, until recently there was no direct test of the model that Ctf7p either is recruited to or translocates with the DNA replication fork, despite the reports that *CTF7* genetically interacts with PCNA-encoding *POL30* and that Ctf7p binds to numerous RFC complexes and a DNA helicase (Skibbens et al., 1999; Kenna and Skibbens, 2003; Skibbens, 2004).

Positioning players within the establishment field: recruitment of Ctf7p to chromatin

Several recent reports examined dynamics of Ctf7p at the DNA replication fork and observed that Ctf7p associates with both replication and cohesin factors (Moldovan et al., 2006; Lengronne et al., 2006; Noble et al., 2006). First, Moldovan and colleagues (Moldovan et al., 2006) found that Ctf7p and PCNA interact directly and that Ctf7p-PCNA binding is conserved through evolution. Chromatin-pelleting assays revealed that Ctf7p becomes chromatin associated in yeast and that this binding peaks during S phase. The PCNA-interacting domain within Ctf7p maps to a highly conserved PIP box (QxxL/I/M) located within the first 33 residues (Majka and Burgers, 2004; Moldovan et al., 2006). Point mutations within the PIP box abolish binding and result in cell death.

These findings support some form of active replication-coupled cohesion establishment, but the role of PCNA in cohesion appears to be much more complicated. At issue is the fate of truncated Ctf7p devoid of a PCNA-binding domain (Ctf7$^{33-281}$). This mutant was reported to exhibit greatly reduced chromatin binding relative to full-length Ctf7p – a conclusion based on comparing the amount of chromatin-bound protein to total protein levels (Moldovan et al., 2006). However, the total protein levels within these experiments were vastly different: the total amount of Ctf7$^{33-281}$ protein was dramatically higher than that of full-length Ctf7p total protein. Thus, comparing chromatin-associated levels to these dramatically different total protein levels of course produced a calculated decrease in chromatin association of Ctf7$^{33-281}$ – but this decrease is likely to be of little physiological relevance. Simply put, sewing more buttons onto a vest will reduce the percent of total buttons used but will not decrease the number of button holes engaged nor diminish their function in fastening. Indeed, independent quantitative measurements reveal that truncated Ctf7p lacking a PCNA-interaction domain associates with chromatin at levels identical to that of full-length Ctf7p and does so specifically during S phase (R.V.S., unpublished).

Although PCNA binding is not required for recruitment of Ctf7p to chromatin, mutations in the Ctf7p PIP box are nevertheless lethal (Moldovan et al., 2006) (but see Antoniacci
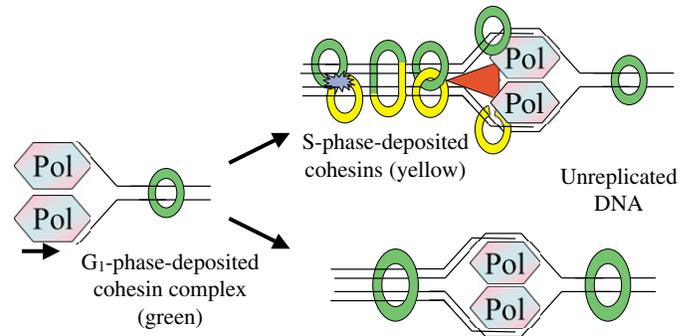

Fig. 2. Early models of sister chromatid pairing reactions. Top: 'replication-coupled cohesin pairing' positions Ctf7p (red triangle) at the replication fork (DNA polymerases as hexagons) to tether together cohesin complexes as nascent sister chromatids emerge. Cohesins are depicted as rings (pre-loaded cohesin rings are shown in green; cohesin loading coincident with replication is shown in yellow) that encircle chromatin (they could also associate laterally; not shown). Several possibilities for cohesion are shown, including various cohesin ring associations or tethering by an additional factor (purple star). Bottom: 'replication through a ring' allows Ctf7p-independent establishment. Here, the replication fork passes through a pre-loaded cohesin ring that entraps both sister chromatids, passively establishing cohesion.

et al., 2004). What then is the role of PCNA in cohesion? The PCNA allele *pol30-104* produces a 20% cohesion defect in cells (Moldovan et al., 2006). A quick comparison illustrates the significance of this value. Mutations in establishment or cohesin factors produce robust cohesion defects in cells that typically range from 50-65% (Guacci et al., 1997; Michaelis et al., 1997; Skibbens et al., 1999; Toth et al., 1999). In contrast, mutations in any one of 20 different replication factors that also function in cohesion produce cohesion defects that range from 14 to 35% (Hanna et al., 2001; Mayer et al., 2001; Kenna and Skibbens, 2003; Mayer et al., 2004; Warren et al., 2004; Skibbens, 2004; Edwards et al., 2004; Petronczki et al., 2004). Even mutations in essential replication factors that function in cohesion exhibit surprisingly low cohesion defects of only 18-22% – identical to *pol30* cells (Mayer et al., 2001; Kenna and Skibbens, 2003; Edwards et al., 2004; Moldovan et al., 2006). Thus most data indicate that replication factors such as PCNA may be peripheral to cohesion establishment activity per se and perform either indirect or redundant roles in cohesion establishment.

How does Ctf7p devoid of its PCNA-binding domain become chromatin associated? Ctf7p binds to RFC subunits including Ctf18p, Rfc1p, Rad24p, Elg1p and Rfc2-Rfc5p (Kenna and Skibbens, 2003; Skibbens, 2005). It also binds to the DNA helicase Chl1p (see below). All of these associations are physiologically relevant in that mutation of RFC subunits (Ctf18p, Rfc4p and Rfc5p), RFC-associated factors (Ctf8p and Dcc1p) or Chl1p produces cohesion defects (Hanna et al., 2001; Kenna and Skibbens, 2003; Mayer et al., 2001; Skibbens, 2004; Petronzcki et al., 2004; Mayer et al., 2004). Ctf7p also contains a zinc finger (a DNA-binding motif) known to function in chromosome segregation (Skibbens, 2004; Brands and Skibbens, 2005). Thus, multiple chromatin-
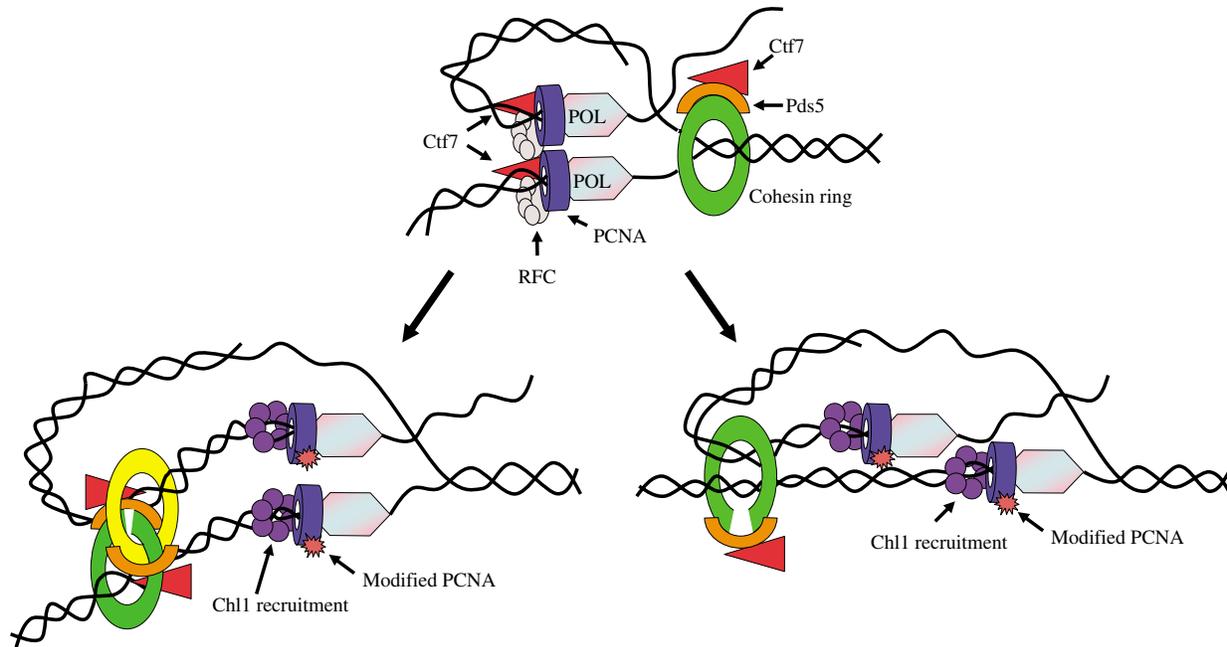
Fig. 3. New models of cohesion establishment. Top: A replication fork moving from left to right encounters a Pds5p-decorated cohesin complex. Ctf7p is recruited to chromatin by binding either to replication fork components (RFCs, PCNA) or to Pds5p, which allows pairing reactions to occur independently of the replication fork. In single-ring models (bottom right), upon interacting with G_1-loaded cohesins, Ctf7p becomes activated and either the replication fork disassembles to pass through the ring or the cohesin ring opens to allow the fork to replicate around the cohesin. Given that Ctf7p associates with chromatin at very low levels and is not restricted to the replication fork, we speculate that PCNA modification (red star) could result in both Chl1p recruitment and altered Ctf7p dynamics (e.g. release from the replication fork). In two-ring models (bottom left), Scc2p-Scc4p (not shown) deposits cohesins both during G_1 phase (green ring) and S phase (yellow ring). In the simplest scenario, Ctf7p (and possibly Pds5p) coordinate cohesin-ring opening/closing reactions and deposition with replication fork progression, leading to ring-ring interactions. Ring catenation is shown for simplicity, but cohesion could involve double rings, filamentous coils or lateral cohesin association. Ctf7p-mediated pairing reactions may occur separately from the replication fork: again PCNA modifications such as SUMOylation (red star) could promote efficient cohesion establishment by recruiting Chl1p and releasing Ctf7p from the replication fork.

recruitment mechanisms, independent of PCNA binding, exist that may recruit Ctf7p to chromatin. Significantly, elevated levels of Ctf7[26-281] support robust cell growth (Antoniacci et al., 2004), which is consistent with the model that multiple mechanisms for Ctf7p chromatin recruitment exist beyond PCNA binding.

Ctf7p and the replication fork

Another critical issue is whether Ctf7p is recruited to and moves with the replication fork. Lengronne and colleagues (Lengronne et al., 2006) used chromatin immunoprecipitation to test for recruitment of Ctf7p to replicating loci (employing BrdU labeling) and included in their analyses two DNA replication factors known to promote efficient cohesion: the RFC subunit Ctf18p and the DNA-polymerase-α-binding protein Ctf4p (Bermudez et al., 2003; Bylund and Burgers, 2005). Both Ctf18p and Ctf4p exhibited chromatin-association dynamics that mirrored DNA replication (Lengronne et al., 2006). Surprisingly, even when Ctf7p resided near Ctf18p, Ctf4p or BrdU, the level of chromatin-associated Ctf7p was remarkably low. We posit that this low level of Ctf7p chromatin association could reflect very high rates of Ctf7p binding and release reactions (as opposed to stable association with replication fork components). More importantly, however, Ctf7p association was not limited to replicating DNA. Instead,

Ctf7p was present at chromatin loci devoid of Ctf4p, Ctf18p and BrdU and also absent from sites containing strong Ctf4p, Ctf18p and BrdU signals. A likely scenario therefore is that Ctf7p can establish sister chromatid cohesion independently of the replication fork.

What replication-independent factors might recruit Ctf7p? Pds5p associates with cohesins and binds chromatin loci decorated by cohesins. Moreover, Pds5p is required to maintain sister chromatid cohesion until anaphase onset (Hartman et al., 2000; Panizza et al., 2000; Tanaka et al., 2001; Stead et al., 2003). SUMOylation of Pds5p promotes cohesin dissolution during mitosis, which suggests that Pds5p is a mitotic cohesin regulator (Tanaka et al., 2001; Stead et al., 2003; Losada et al., 2005). More recent findings revealed that Pds5p also binds to Ctf7p and that elevated Ctf7p levels rescue and exacerbate the temperature sensitivity of *pds5* and *mcd1* mutants, respectively. Thus, Pds5p plays an additional role in cohesion establishment during S phase (Tanaka et al., 2001; Noble et al., 2006). Ctf7p-Pds5p binding allows recruitment of Ctf7p to chromatin independently of the replication fork and potentially places Ctf7p in close proximity to cohesins (Tanaka et al., 2001; Noble et al., 2006). Future studies are required to track Ctf7p-Pds5p binding through the cell cycle and test whether this association is essential for cohesion establishment.

Promoting efficient establishment: SUMO and PCNA

Recent findings portend an exciting regulatory pathway for cohesion establishment. Elevated PCNA levels rescue *ctf7* phenotypes and PCNA is known to become modified (through addition of SUMO, mono-ubiquitin or polyubiquitin) during DNA replication and repair (Skibbens et al., 1999; Hoege et al., 2002). Moldovan and colleagues (Moldovan et al., 2006) therefore asked whether *pol30-104*, which is inviable when combined with *ctf7-203*, exhibits altered levels of SUMOylation. Indeed, the mutant PCNA is hyperSUMOylated. Moreover, elevated expression of unSUMOylatable PCNA mutants (SUMOylated lysines 127 and 164 changed to arginines) partially rescues the temperature sensitivity of *ctf7* mutant strains (Moldovan et al., 2006). This effect is recapitulated by loss of Siz1p, a SUMO ligase that modifies PCNA and other substrates (Hoege et al., 2002; Pfander et al., 2005). PCNA SUMOylation may thus reduce Ctf7p-dependent sister-chromatid-pairing reactions (Moldovan et al., 2006). It is important to note that Ctf7p also binds to Pds5p, which is similarly regulated by SUMOylation (see above) to promote cohesin dissolution (Stead et al., 2003). Thus, cohesion establishment is likely to be coupled to multiple SUMOylation reactions.

PCNA serves as a landing pad for a multitude of proteins, including the DNA helicase Chl1p, which contains a PCNA-binding motif (Bylund and Burgers, 2005; Moldovan et al., 2006). Although the idea is of course speculative, we propose here that specific PCNA modifications, in response to the replication fork encountering cohesins, recruit Chl1p to promote efficient sister chromatid pairing. This mechanism is similar to the recruitment code proposed for binding of Hpr5p/Srs2p to PCNA modified in response to DNA damage (Matunis, 2002; Hoege et al., 2002; Ulrich, 2004; Pfander et al., 2005; Ulrich et al., 2005). Our model is predicated on the findings that SUMOylated PCNA can recruit DNA helicase to the repair fork, that Chl1p binds to both PCNA and Ctf7p and that Chl1p promotes efficient sister chromatid pairing (Hoege et al., 2002; Stelter and Ulrich, 2003; Skibbens, 2004; Mayer et al., 2004; Petronczki et al., 2004; Papouli et al., 2005; Pfander et al., 2005; Moldovan et al., 2006).

Coupling cohesin deposition to establishment

Most replication-coupled cohesin pairing models link cohesin deposition with DNA replication and cohesion establishment (Skibbens, 2000; Skibbens, 2005). The replication through a ring model, by contrast, typically places cohesin deposition prior to DNA replication such that deposition during S phase is inconsequential (Haering et al., 2002; Gruber et al., 2003; Lengronne et al., 2006). Complicating the issue are the observations that cohesin deposition can occur during G_1, S, G_2 and M phase, although cohesins deposited after S phase can participate in sister pairing reactions only in response to DNA damage (Uhlmann and Nasmyth, 1998; Unal et al., 2004; Strom et al., 2004).

Mapping cohesin deposition relative to the replication fork is likely to be a difficult task. Scc2p and Scc4p are certainly at center stage in that cohesins expressed during S phase are not deposited onto cohesin-decorated loci in the absence of Scc2p (Lengronne et al., 2006). The portion of the cell cycle when cohesin deposition is essential remains controversial, but not under debate is the observation that G_1-synchronized *scc2* mutant cells released at the restrictive temperature exhibit a dramatic reduction in viability that strictly parallels S-phase progression (Ciosk et al., 2000; Lengronne et al., 2006). A straightforward interpretation of this is that Scc2p performs its essential function during S phase such that cells progressing into S phase without Scc2p become inviable. An alternative interpretation is that cells become inviable during S phase because they have progressed beyond G_1 phase when cohesin deposition is essential.

New models of cohesion and establishment.

In discussing new models, we start with a replication fork as it encounters a cohesion complex. In such models, Ctf7p is depicted as associated with replication fork components (RFCs and PNCA) and Pds5p-decorated cohesin complexes. Moreover, we can consider both one-ring and two-ring models of establishment.

Single ring models

Upon interacting with G_1-loaded cohesin barriers, the replication fork partially disassembles. This altered fork geometry allows individual replisomes to traverse through the cohesin ring (Bylund and Burgers, 2005; Lengronne et al., 2006). If fork disassembly and cohesion establishment requires Ctf7p, this model would predict that loss of Ctf7p function should result in stalled DNA replication. However, *ctf7* mutant cells completely replicate their DNA without activation of either DNA damage or DNA replication checkpoints and with unaltered kinetics (Skibbens et al., 1999; Toth et al., 1999). Alternatively, replication-fork-coupled establishment factors such as Ctf7p (and Pds5p) could induce opening of the cohesin-ring, which would then reseal immediately after the fork passed (Lengronne et al., 2006). Currently, it is unclear how such a model could account for the cohesin-without-cohesion phenotypes observed in *ctf7* and *pds5* mutant cells (Toth et al., 1999; Hartman et al., 2000; Milutinovich et al., 2007), but one possibility is that Ctf7p is required for cohesin to close around both sisters. The role of replication factor modifications (such as SUMOylation of PCNA) or complex assembly (various RFC complexes) in regulation of Ctf7p function or dynamics clearly warrants further investigation. As we speculate above, PCNA may become modified to recruit Chl1p, and this could alter Ctf7p-fork associations (causing Ctf7p release, for example).

Two-ring models

In the simplest scenario, Ctf7p (and possibly Pds5p) is required to tether together new cohesins deposited in S phase to cohesins loaded during G_1. Similarly to a single-ring model, preloaded cohesin rings that encircle DNA must be dynamic to allow for replication. Thus, Ctf7p and Pds5p may coordinate cohesin-ring opening/closing reactions with replication fork progression and consequently drive ring-ring interactions. Cohesin pairing may occur directly at the replication fork or behind, allowing for transient Ctf7p-fork interactions. Cohesin ring catenations are just one possibility (shown for simplicity in Fig. 3); cohesion could in fact involve double rings, filamentous coils or lateral cohesin associations.

Concluding remarks

Currently, Ctf7p remains the only known essential cohesion establishment factor and appears to couple sister-chromatid-pairing reactions to a cohesin regulator and many components

of the DNA replication machinery. Ctf7p is highly conserved through evolution. Ctf7p orthologs (including *ESO1* in fission yeast, *EFO1* and *EFO2/ESCO2* in humans, *DECO* in *Drosophila*) all function in sister chromatid pairing. Note also that all exhibit acetyltransferase activities, although the role of acetylation in cohesion remains unclear (Tanaka et al., 2001; Ivanov et al., 2002; Williams et al., 2003; Bellows et al., 2003; Brands and Skibbens, 2005; Vega et al., 2005; Hou and Zou, 2005).

The studies discussed here provide positional, binding partner and regulatory information for Ctf7p that is critical to our understanding of cohesion establishment dynamics on and off the replication fork (Moldovan et al., 2006; Lengronne et al., 2006; Noble et al., 2006). As the cohesion field marches forward, it is clear that resolving the structural basis for sister chromatid cohesion will be critical to our understanding of the multi-step process of establishment. For instance, the replication through a ring model will largely become obsolete if cohesins are shown laterally to associate with chromatin and do not topologically encircle DNA (Losada and Hirano, 2005; Nasmyth, 2005; Huang et al., 2005). Moreover, there is now evidence that cohesin rings are much more dynamic than previously envisioned and display alternative opening reactions (Hirano and Hirano, 2006; Gruber et al., 2006; Milutinovich et al., 2007). Similarly, resolving the current controversy regarding when in the cell cycle cohesin deposition is essential will strongly bias for or against models that require only G_1 phase deposition versus S phase deposition. Finally, determining how Ctf7p participates in pairing at the molecular level will profoundly influence models of both cohesion establishment and maintenance.

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