Evidence that replication fork components catalyze establishment of cohesion between sister chromatids

Dena R. Carson and Michael F. Christman*

Department of Microbiology, Box 800734, Jordan Hall, 1300 Jefferson Park Avenue, University of Virginia, Charlottesville, VA 22908

Accurate chromosome segregation requires that replicated sister chromatids are held together until anaphase, when their ''cohesion'' is dissolved, and they are pulled to opposite spindle poles by microtubules. Establishment of new cohesion between sister chromatids in the next cell cycle is coincident with replication fork passage. Emerging evidence suggests that this temporal coupling is not just a coincident timing of independent events, but rather that the establishment of cohesion is likely to involve the active participation of replication-related activities. These include PCNA, a processivity clamp for some DNA polymerases, Trf4/Pol σ (formerly Trf4/Polκ), a novel and essential DNA polymerase, and a modified Replication Factor C clamp–loader complex. Here we describe recent advances in how cohesion establishment is linked to replication, highlight important unanswered questions in this new field, and describe a ''polymerase switch'' model for how cohesion establishment is coupled to replication fork progression. Building the bridges between newly synthesized sister chromatids appears to be a fundamental but previously unrecognized function of the eukaryotic replication machinery.

Cohesion and the Chromosome Cycle

The chromosome cycle is a complex series of events that begins with both the duplication of DNA sequences and the rebuilding of an equal mass of chromatin around the duplicated DNA molecules. The duplicated chromosomes are called sister chromatids. A pair of sister chromatids must remain associated with each other from the time of their formation in S-phase (1) until they are separated at anaphase (Fig. 1). This association is termed sister chromatid cohesion and occurs at discrete sites along the entire length of the chromosome (2–4). Without cohesion, the cell would have no means to ensure that the chromosome complement of a daughter cell remains identical to that of its mother cell. Thus, cohesion must be established, maintained, and then dissolved in every cell cycle. Until very recently, little was known about the molecular basis of any of these key events in the chromosome cycle.

Early observations of chromosome dynamics suggested that cohesion constrains the geometry of sister chromosome pairs so that sister kinetochores face in opposite directions, which presumably facilitates their attachment to spindle microtubules emanating from opposite spindle poles (5, 6). The poleward forces of the microtubules produce tension when both sisters are properly attached to a bipolar spindle, and this tension is opposed by the cohesive bond (7–9). Cohesion occurs before chromosome condensation; therefore, sites of cohesion may influence chromosome architecture during condensation. Indeed, condensed sister pairs display a mirror symmetry (10) that may arise because of their cohesion at specific sequences. Furthermore, proper cohesion is required for subsequent condensation in *Saccharomyces cerevisiae* (11). Cohesed sister pairs exist for a substantial part of the chromosome cycle and may define topological domains that influence other aspects of chromosome dynamics.

When stable bipolar attachment of microtubules to all sister kinetochore pairs is established at metaphase, a signal is generated that results in the rapid, simultaneous dissolution of all cohesion. This event defines the metaphase-to-anaphase transition. The result is even segregation of all chromosome pairs to opposite spindle poles, because cohesion no longer opposes the microtubule pulling forces. A single sister pair that is not attached to opposing kinetochore microtubules prevents the generation of the signal for dissolution of cohesion (12) through a complex signaling mechanism (13, 14). This checkpoint mechanism ensures that cohesion is not dissolved until all sister pairs are oriented for equal distribution, allowing the spindle to sense tension (15) and/or microtubule occupancy (reviewed in ref. 16). Cytokinesis and chromosome decondensation rapidly follow to form the resulting mother and daughter cells. To date, most fundamental aspects of this process appear to work the same way in yeast and animal cells.

The last few years have seen prodigious advances in knowledge of the molecular events required to maintain and then dissolve cohesion between sister chromatids (reviewed in refs. 17–19). Our goal in this review is to give a brief introduction to the genes involved in maintaining and dissolving sister chromatid cohesion and then to focus on the emerging, but still poorly understood, events involved in establishment of cohesion. The focus will be on work from budding yeast, but most of the cohesion proteins identified to date are evolutionarily conserved throughout eukaryotes. The regulation of cohesion dissolution (18) and meiotic cohesion (20) has been reviewed recently and will not be discussed here.

Historical Observations

Replicated linear chromosomes are intertwined after DNA synthesis because of the helical structure of the DNA molecule and the incomplete removal of positive supercoils by DNA topoisomerases during replication (21). The intertwined sister chromatids must be untangled by DNA topoisomerase II before segregation of chromatids to mother and daughter cells (22, 23). In principle, this physical intertwining could be the basis of maintaining chromatid cohesion.

An early experiment using circular yeast minichromosomes correctly predicted that this was unlikely to be the case (24). Yeast cells containing the circular chromosome were arrested after DNA synthesis, but before chromatid separation, by using

This paper results from the National Academy of Sciences colloquium, ''Links Between Recombination and Replication: Vital Roles of Recombination,'' held November 10–12, 2000, in Irvine, CA.

Abbreviations: CAR, cohesion attachment region; RFC, Replication Factor C.

*To whom reprint requests should be addressed. E-mail: mfc3f@virginia.edu.

 www.pnas.org/cgi/doi/10.1073/pnas.131022798

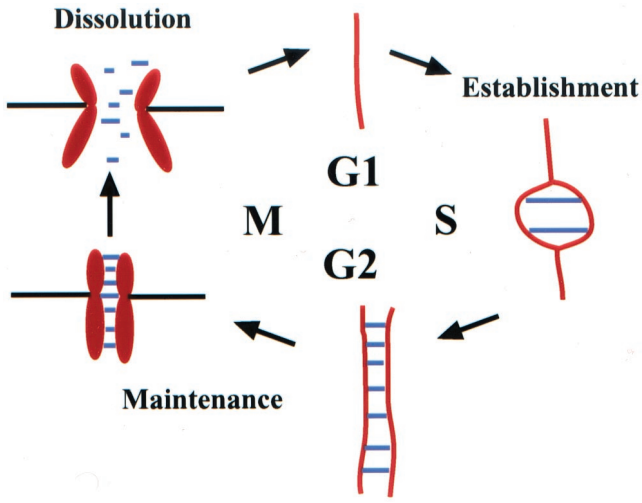

Fig. 1. Steps in sister chromatid cohesion during the chromosome cycle. Red lines represent a single double-stranded chromosome, and blue lines represent cohesive bonds between chromatids.

a microtubule poison. If cohesion were mediated by intertwining of linear chromosomes, it would be reflected by catenation of duplicated circular sister minichromosomes. Examination of the topological status of the replicated sister circles revealed that the majority were not catenated (24). This observation suggested that a protein bridge of some sort was more likely to mediate cohesion. Whether topological intertwining of chromosomes influences the establishment of cohesion remains an open question.

Consistent with cohesion resulting from a protein bridge between chromatids, the dissolution of cohesion was found to require ubiquitin-dependent proteolysis (25, 26) through a ubiquitin ligase termed the anaphase-promoting complex (27, 28). These important findings prompted a search for a ubiquitin-dependent proteolytic target protein that was required to maintain cohesion.

Identification of genes necessary to maintain cohesion through classical genetics required the development of methods to examine the status of sister cohesion in yeast cells, because the *S. cerevisiae* chromosomes are too small to be visualized directly by light microscopy. The advent of sensitive fluorescence *in situ* hybridization methods for yeast nuclei partly solved this problem (4). Properly paired chromatids are held together so closely that a single hybridization signal is observed for a given chromatid pair during mitosis in haploid cells, whereas cohesion failure is manifest as two spots. Subsequent development of green fluorescence protein-marked chromosomes provided an analogous but simpler means to assay the status of sister chromatid cohesion as well as the ability to monitor chromosome movement in live cells (29). These technical advances set the stage for identification of the genes required for this fundamental but poorly understood aspect of the cell cycle.

Genes Required to Maintain Sister Chromatid Cohesion

To identify genes required to maintain cohesion, a genetic screen was performed by using the assays described above. The *PDS1* gene (for Premature Dissociation of Sisters) was the first cohesion-defective mutant described (30). Indeed, Pds1 is targeted for ubiquitin-dependent degradation by the anaphase-promoting complex ubiquitin ligase at the appropriate time in the cell cycle, as is its fission yeast homolog Cut2 (31). Furthermore, its degradation is required to trigger dissolution of cohesion and the metaphase-to-anaphase transition (32, 33). This nicely explains the requirement for ubiquitin-dependent proteolysis in dissolving cohesion. However, Pds1 does not bind to chromosomes and has subsequently proved to be a key negative regulator of the dissolution of cohesion (31, 34) rather than a protein that might directly maintain chromatid cohesion.

Control of cohesion dissolution is a critical feedback point in the cell cycle for the maintenance of genome integrity (35), because accurate repair of errors in DNA replication and realignment of misaligned chromosomes are no longer possible when anaphase begins. In budding yeast, checkpoints cause cell cycle arrest at the metaphase-to-anaphase transition rather than at the G_2/M boundary. Indeed, blockage of Pds1 degradation is a target of both DNA damage (35) and spindle assembly checkpoint pathways (36). Nonetheless, the gene products that might directly mediate cohesion remained elusive.

Major insight resulted from a genetic screen to identify genes required for cohesion, even in the presence of Pds1 (11, 37). Although mutants lacking the anaphase-promoting complex components are unable to dissolve cohesion because of the persistence of the negative regulator of anaphase, Pds1, it was reasoned that a mutation in a chromosomal protein that directly mediated cohesion would cause a structural failure in cohesion even in the presence of negative regulators of cohesion dissolution. This approach resulted in the identification of several genes required for cohesion, including the *S*tructural *M*aintenance of *C*hromosome genes, *SMC1* and *SMC3*, notable as members of a conserved family of chromosomal ATPases, some of which had been implicated in chromosome condensation (38, 39) (reviewed in ref. 19). Smc1 and Smc3 form a complex that also includes two non-SMC proteins, Scc1 and Scc3, in *Xenopus* (40), yeast cells (41, 42), and human cells (43–45). The complex is collectively referred to as "cohesin."

The Smc1/Smc3 "Cohesin" Complex

The cohesin complex is an appealing candidate for the glue that directly mediates chromatid cohesion (reviewed in refs. 19 and 46). This conclusion stems from the obvious fact that it is a chromosome-associated protein complex required for cohesion but also from an interesting prediction of its Smc subunit structure. An important finding from electron microscopy studies by Harold Erickson's group was that bacterial Smc proteins (Bacillus subtilis BsSMC and *Escherichia coli* MukB), which form homodimers rather than heterodimers, form long antiparallel coiled coils with a highly flexible internal hinge region (47). Another Smc-related protein, Rad50, forms homodimers with a similar structure (48). By analogy with the bacterial proteins and Rad50 family members, eukaryotic cohesin is likely to bind directly to DNA with the two Smc subunits forming a long antiparallel coiled-coil structure. This structure would mean that there is a symmetry to the ends of the cohesin Smc heterodimer whereby each end could bind one sister chromatid and hold the sisters together (47) (Fig. 2*A*). Consistent with this prediction, the C-terminal region of Smc proteins is required for DNA binding (49). In addition, biochemical studies of human cohesin show that it can stimulate the ability of DNA topoisomerase II to catenate DNA circles in an *in vitro* reaction, as well as promote the ligation of linear DNAs by DNA ligase (50), properties that presumably result from its ability to simultaneously bind to two different DNA molecules.

At the precise time of cohesion dissolution, the Scc1 subunit is removed from chromatids through a site-specific proteolysis event that is required for dissolution of cohesion (51). This proteolysis is mediated by the action of the caspase-like Esp1 (separin) protease that acts directly on Scc1 (52). A cell with a mutant Scc1 that cannot be proteolyzed by Esp1 is unable to dissolve cohesion and dies as a result (51). That Scc1 is targeted for dissolution may indicate that it regulates the chromosome-binding activity of the Smc heterodimer. Alternatively, a pair of Smc heterodimers may be bridged by the Scc1 subunit (Fig. 2*B*),

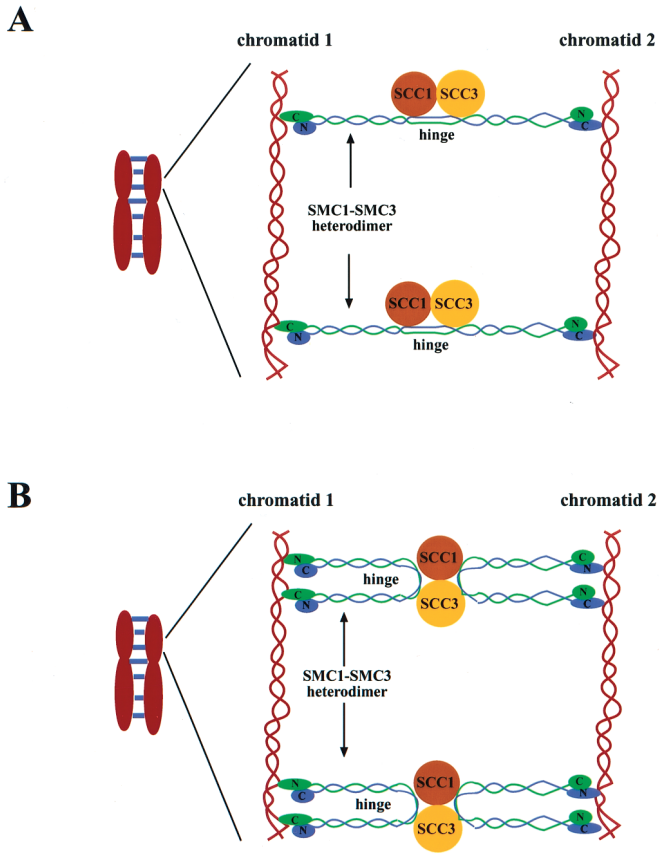

Fig. 2. The "Cohesin" complex is a key component of the chromatid glue. (*A*) SMC heterodimers are shown in an extended conformation, which may allow simultaneous binding of two sister chromatids. (*B*) SMC heterodimers are shown with the hinge region flexed, so that a pair of heterodimers could be bridged by non-SMC subunits.

a model that is also compatible with what is known about Smc dimer structure. These data provide compelling evidence that cohesin is a crucial part of the chromatid glue and explain how the glue is dissolved at the metaphase-to-anaphase transition.

Recently another protein essential to maintain cohesion has been described, the *PDS5* gene product. These authors report that *PDS5* is essential to maintain cohesion, evolutionarily conserved, and removed from chromosomes with kinetics identical to Scc1 (53). Furthermore, localization of Pds5 to chromosomes requires the Scc1 cohesin subunit. *PDS5* and its homologs in *Sordaria macrospora* (Spo76) (54) and *Aspergillus nidulans* (BimD6) (55) are essential for mitotic chromosome segregation. Thus, Pds5 is an essential chromosome-associated and evolutionarily conserved protein required to maintain cohesion. The role of Pds5 adds further complexity to attempts to understand how cohesion is mediated and underscores the fact that the nature of the cohesive bond is unknown at present. The cohesin complex alone, although necessary, is clearly not sufficient to maintain cohesion.

Sites of Cohesion

An *S. cerevisiae* centromere was the first cis-acting sequence shown to be capable of mediating cohesion (56). The cohesin complex associates with chromosomes at discrete sites both at centromeres (56) and along chromosome arms (57–59). Centromeric regions are a major site of cohesin binding, with association extending several kilobases in each direction (56), whereas sites on arms occur approximately every 9 kb, with a strong preference for AT-rich intergenic regions in *S. cerevisiae* (59). These sites, typically 500–800 base pairs, are referred to as "CARs," for cohesion attachment regions. Cohesin subunits bind CARs from late G_1 until anaphase. Pds5 also binds to CAR regions specifically (53). The cohesin complex is loaded onto chromosomes at these "precohesion" sites in late G_1 in a reaction that requires the Scc2/Scc4 protein complex (60). Some CAR sites have been mapped with high precision, and they are clearly distinct from replication origins, which occur about every 45–90 kb in *S. cerevisiae*.

Building Chromatid Bridges Occurs During S-Phase

The least understood aspect of cohesion is the establishment step. It had been suggested that cohesion was likely established during S-phase, on the basis of the fact that separated sister chromatids were never detectable as cells progressed from G_1 through S to G_2 (3, 4). This idea was supported by data showing that the Scc1 cohesin subunit must be present during S-phase for cohesion to be effective in G_2 cells (61). If *SCC1* is expressed after S-phase is completed, it is able to bind to chromosomes, but cohesion fails nonetheless.

Further evidence that cohesive bridges are built during S-phase came from the identification of a new kind of cohesion molecule. The *CTF7/ECO1* gene is required only to establish cohesion in S-phase but is not needed in G_2/M to maintain cohesion (41, 62). In contrast, the cohesin complex is required to establish cohesion in S-phase and to maintain cohesion in G_2/M. The biochemical function of *CTF7/ECO1* is unknown, but formally it can be thought of as a tool required to build the cohesive bridge. The lethal defect in the *ctf7–203-ts* mutant can be suppressed by overexpression of PCNA (62), a processivity factor for some DNA polymerases (63). This important observation suggested that building cohesion not only occurred during S-phase but might actually be mediated by replication fork components [(62); reviewed in ref. 64].

Trf4p/DNA Polymerase σ Links Replication and Cohesion

Recently, we described a DNA polymerase, Trf4/Pol σ, that is required to establish cohesion during S-phase (1) (note that Trf4/Pol σ is the new name for Trf4/Pol κ), whereas human dinB1 is now referred to as Pol κ (65). Trf4/Pol κ may be a key link between the replication machinery and the cohesion machinery. This finding strongly supports the idea that fork components play an active role in establishing cohesion and provides, to our knowledge, the first biochemical activity other than DNA binding ascribed to any protein required to establish cohesion. Intriguingly, the *ECO1/CTF7* gene in *Schizosaccharomyces pombe* exists as a gene fusion of the *ECO1/CTF7* domain to a DNA polymerase η-like domain (66), further suggesting that it, too, functions in the environment of the replication fork.

In *S. cerevisiae*, Pol σ is encoded by two redundant homologs, *TRF4* and *TRF5* (67–69). A conditional *trf4 trf5* double mutant, when shifted to the nonpermissive temperature, is unable to complete DNA synthesis, whereas a *trf4* single mutant undergoes an enfeebled S-phase in which there is a failure to establish cohesion. Partial loss-of-function mutations in *SCC1* or *SMC1*, encoding the cohesin complex subunits, are lethal if Pol σ levels are diminished because of a single *trf4* mutation (1, 69).

We have identified two human Pol σ/*TRF4* genes, *hTRF4–1* on 5p15 and *hTRF4–2* on chromosome 16 (70). The *hTRF4–1* product also encodes a DNA polymerase (Zhenghe Wang and M.F.C., unpublished observations). DNA Pol σ/*TRF4* is the fourth essential nuclear DNA polymerase in yeast, and probably in all eukaryotes, in addition to polymerases α, δ, and ε.

Together, these observations suggest an attractive model whereby sister chromatid cohesion is actively established at the replication fork. In this model, sister chromatids are never apart after replication, and cells avoid the difficult task of pairing sister chromatids in G_2 (Fig. 3). The fork components must somehow

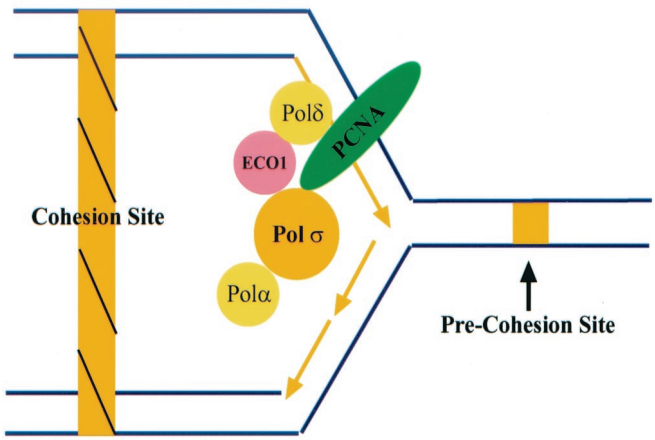

Fig. 3. Model for cohesion establishment by replication-related activities. Precohesion site is meant to show the cohesin complex, because it is known to bind to G_1 chromosomes. A cohesion site is a functional protein bridge formed after fork passage. The cartoon is not meant to imply the existence of direct protein–protein interactions, because such interactions have not been demonstrated to date.

convert a precohesion site into a bona fide cohesion site. Replication in the absence of *ECO1* or with diminished levels of Trf4/Pol σ results in a failure to establish cohesion. The molecular difference between a precohesion site and an established site is unknown. Note that the cartoon in Fig. 3 is not meant to imply the existence of direct protein–protein interactions, because such interactions have not been demonstrated to date.

A Polymerase Switch Model for Cohesion Establishment

We have proposed that Trf4/Pol σ may function to establish cohesion through a polymerase switching mechanism in which the core replicative polymerases are used to replicate from an origin up to a cohesion site, but that when the replication fork encounters a cohesion site, there is a switch to DNA pol σ (1). In this view, DNA pol σ and associated fork components then catalyze formation of the cohesive bond between sister chromatids in some as-yet-unknown fashion as the cohesion site region (CAR) is replicated. In this model, cohesion establishment would occur through a replication-coupled remodeling of chromatin, analogous to the establishment of silencing at *HM* loci in yeast, which requires both specific cis-acting sequences and DNA replication (71).

The cohesin complex resides at CAR sites on late G_1 chromosomes, and therefore would indeed be encountered by progressing replication forks. Cohesins are tightly bound to chromatin and could represent a barrier to fork passage that requires the action of a special polymerase. A similar polymerase switch is postulated to occur during the bypass of otherwise lethal DNA damage (65, 72). Alternatively, the encounter of cohesins could simply be a signal to fork components to initiate a polymerase switch.

This model predicts that replication through CAR sites specifically should be defective in the absence of Trf4/Pol σ. We are in the process of examining this using the powerful two-dimensional gel method to analyze replication intermediates (73). If a block is observed at cohesion sites in the absence of Pol κ, it may be relieved by the absence of the cohesin complex. A replication fork pause is known to occur at yeast centromeres (74), the major site of cohesin association.

A polymerase switch is thought to occur during the synthesis of every Okazaki fragment (75), and this event may provide a paradigm for the postulated cohesion polymerase switch. During Okazaki fragment synthesis, the DNA Pol α/primase first makes

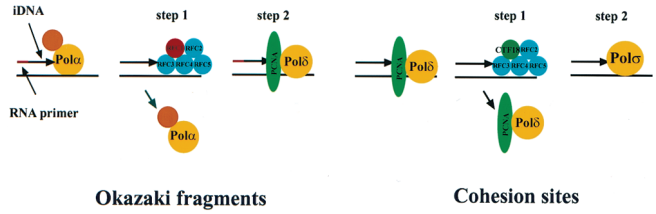

Fig. 4. Polymerase switching on Okazaki fragments may be analogous to what happens at cohesion sites.

a short RNA primer and about 40 nucleotides of iDNA (initiator DNA). After that, the remaining 180 base pairs of the typical Okazaki fragment is synthesized by the highly processive PCNA/Pol δ complex (75). The key event in the switch from Pol α to Pol δ is the competition between Pol α/primase and the Replication Factor C (RFC) clamp–loader complex (Fig. 4). RFC consists of a five-protein complex (Rfc1–Rfc5) that is evolutionarily conserved (63). Pol α/primase is a relatively distributive polymerase and frequently dissociates from the primer/template terminus, whereas RFC has a very high affinity for a vacant primer/terminus. RFC binds the iDNA primer/terminus when Pol α falls off. RFC then opens the PCNA ring and topologically links PCNA/Pol δ to the DNA backbone at the primer terminus, completing the switch. Thus, the polymerase switch during Okazaki fragment synthesis is mediated by the RFC complex, at least *in vitro* (Fig. 4 *Left*).

Does the RFC complex somehow also mediate a switch to Trf4/Pol σ at cohesion sites? A polymerase switch at cohesion sites may require clamp unloading rather than loading. The *E. coli* RFC equivalent is indeed capable of removing as well as loading the processivity clamp (76). Exciting new data from several groups indicate that a novel RFC-like protein, Ctf18, is required for cohesion. Ctf18 is related to all five canonical Rfc subunits in primary sequence but most closely to Rfc1. A protein complex has been identified that contains Ctf18, Rfc3, and Rfc4, but not Rfc1 (refs. 77 and 86). This arrangement is strikingly similar to the novel Rfc complex recently implicated in the DNA damage checkpoint, in which Rfc1 is replaced by Rad24 (78). A Ctf18 homolog is present in all eukaryotes examined thus far (77).

These data add to the growing collection of replication-like molecules involved in cohesion and provide a plausible mechanism through which a polymerase switch might occur at CARs. We suggest that the modified RFC complex may be required to facilitate a switch to Trf4/Pol σ at CAR sites. The modified RFC complex could either remove PCNA-Pol δ from the boundary of CAR sites or load PCNA-Trf4/Pol σ and thereby facilitate Trf4/Pol σ action (Fig. 4). That *CTF18* is not essential means that its role is likely to be functionally redundant with another gene. We have observed genetic interactions between *TRF4* and *CTF18* (unpublished observations) supporting the notion that they function in a common pathway.

The *CTF4* gene product is also physically associated with Ctf18, although it is not RFC-related, and a null mutant in *CTF4* is also cohesion defective (refs. 77 and 86). *CTF4* was independently identified on the basis of the biochemical property of associating with DNA polymerase α [also known as *POB1* for Polymerase One Binding (79)], making it a likely replication fork protein as well. The existence of a modified and evolutionarily conserved version of the canonical RFC complex indicates that cohesion establishment may in fact be the devoted task of a subset of replication components.

Cohesion and Cancer

Seminal work from the Vogelstein group has shown that many tumors acquire the property of missegregating their chromo-

somes very early in tumorigenesis and do so regardless of ploidy (80). This finding suggests that the missegregation phenotype (termed "CIN" for chromosomal instability) is likely to drive tumorigenesis rather than being a secondary effect of the transformed state.

On the basis of work in model organisms, many different molecular events are necessary to ensure proper chromosome segregation. Some of the genes identified in these studies have subsequently been shown to affect tumorigenesis in animals, including yeast *BUB1* (13)–human *BUB1* (81) and yeast *MEC1* (82)–human *ATM* (83). For the vast majority of CIN tumors, the molecular defect is not yet known. The process of sister chromatid cohesion requires many conserved genes and may prove to be defective in some tumors.

Indeed, the human biochemical equivalent of the cohesion regulator *PDS1*, called PTTG, is capable of transforming NIH 3T3 cells in culture (84). PTTG stands for pituitary tumor-transforming gene (84) and is overexpressed in some tumors. In addition, the *SMC3* gene can cause transformation when overexpressed in normal fibroblasts (85). Whether alterations in cohesion molecules contribute to the genomic instability observed in tumors will be important to determine.

Some Unanswered Questions

An abundance of major questions remain regarding how cohesion is established and how this is coupled to replication. Among the biggest are simply: (*i*) what is the nature of the cohesive bond between chromatids? and (*ii*) how is it built by the replication fork? These are daunting questions, likely to require some time to answer. However, the combination of work in budding and fission yeasts to identify all relevant factors in cohesion establishment, together with the ability to examine the biochemical properties of these factors during replication-coupled cohesion *in vitro* by using *Xenopus* egg extracts (40), should eventually yield answers. Some more specific questions that are likely be answered sooner include:

What does Eco1 do? New evidence indicates that DNA replication in the absence of the establishment factor Eco1 results in effective association of cohesin with CARs but a failure of cohesion nonetheless (Robert Skibbens and Doug Koshland, personal communication). This observation formally demonstrates that the establishment of cohesion is not as simple as binding cohesin to CAR sites on replicated chromatids. Thus, Eco1 is not required for replication-coupled deposition of cohesin, nor is the presence of cohesin at CARs sufficient to establish cohesion. Does Eco1 modify a cohesin subunit or Pds5 during replication?

Is the function of Trf4/Pol σ devoted to cohesion establishment? If its role in replication is limited to CAR regions, this would support a devoted role. Does Trf4/Pol σ associate with Eco1 and is it stimulated by PCNA? Are the cohesins and Pds5 deposited appropriately in its absence? If cohesion establishment proves half as complex as DNA replication itself, a full picture of the events will be years in the making.

In summary, a recent spate of data suggests that the replication fork is required not only to duplicate chromosomes but also to glue them together. Building the bridges between the newly synthesized sister chromatids appears to be a fundamental but previously unrecognized function of the eukaryotic replication fork. Understanding how this is accomplished at the molecular level is a central question in biology.

We are grateful to Phil Hieter, Tatsuya Hirano, Doug Koshland, Robert Skibbens, and Forrest Spencer, for communicating results before publication, to Tatsuya Hirano, Nikki Levin, David Pellman, M. Mitchell Smith, and P. Todd Stukenberg for comments that improved the manuscript, and the to the National Institutes of Health and Human Frontiers Science Program for supporting work in the lab.

1. Wang, Z., Castano, I. B., De Las Penas, A., Adams, C. & Christman, M. F. (2000) *Science* **289,** 774–779.
2. Sumner, A. T. (1991) *Chromosoma* **100,** 410–418.
3. Selig, S., Okumura, K., Ward, D. C. & Cedar, H. (1992) *EMBO J.* **11,** 1217–1225.
4. Guacci, V., Hogan, E. & Koshland, D. (1994) *J. Cell Biol.* **125,** 517–530.
5. Rieder, C. L. (1982) *Int. Rev. Cytol.* **79,** 1–58.
6. Tanaka, T., Fuchs, J., Loidl, J. & Nasmyth, K. (2000) *Nat. Cell Biol.* **2,** 492–499.
7. McNeill, P. A. & Berns, M. W. (1981) *J. Cell Biol.* **88,** 543–553.
8. Rieder, C. L., Davison, E. A., Jensen, L. C., Cassimeris, L. & Salmon, E. D. (1986) *J. Cell Biol.* **103,** 581–591.
9. Skibbens, R. V., Rieder, C. L. & Salmon, E. D. (1995) *J. Cell Sci.* **108,** 2537–2548.
10. Boy de la Tour, E. & Laemmli, U. K. (1988) *Cell* **55,** 937–944.
11. Guacci, V., Koshland, D. & Strunnikov, A. (1997) *Cell* **91,** 47–57.
12. Rieder, C. L., Cole, R. W., Khodjakov, A. & Sluder, G. (1995) *J. Cell Biol.* **130,** 941–948.
13. Hoyt, M. A., Trotis, L. & Roberts, B. T. (1991) *Cell* **66,** 507–517.
14. Li, R. & Murray, A. W. (1991) *Cell* **66,** 519–531.
15. Nicklas, R. B., Ward, S. C. & Gorbsky, G. J. (1995) *J. Cell Biol.* **130,** 929–939.
16. Gardner, R. D. & Burke, D. J. (2000) *Trends Cell Biol.* **10,** 154–158.
17. Koshland, D. E. & Guacci, V. (2000) *Curr. Opin. Cell Biol.* **12,** 297–301.
18. Nasmyth, K., Peters, J. M. & Uhlmann, F. (2000) *Science* **288,** 1379–1385.
19. Hirano, T. (2000) *Annu. Rev. Biochem.* **69,** 115–144.
20. Dej, K. J. & Orr-Weaver, T. L. (2000) *Trends Cell Biol.* **10,** 392–399.
21. Cozzarelli, N. R. & Wang, J. C. (1990) *DNA Topology and Its Biological Effects* (Cold Spring Harbor Lab. Press, Plainview, NY).
22. DiNardo, S., Voelkel, K. & Sternglanz, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2616–2620.
23. Holm, C., Goto, T., Wang, J. C. & Botstein, D. (1985) *Cell* **41,** 553–563.
24. Koshland, D. & Hartwell, L. H. (1987) *Science* **238,** 1713–1716.
25. Holloway, S. L., Glotzer, M., King, R. W. & Murray, A. W. (1993) *Cell* **73,** 1393–1402.
26. Surana, U., Amon, A., Dowzer, C., McGrew, J., Byers, B. & Nasmyth, K. (1993) *EMBO J.* **12,** 1969–1978.
27. Irniger, S., Piatti, S., Michaelis, C. & Nasmyth, K. (1995) *Cell* **81,** 269–278.
28. King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Hieter, P. & Kirschner, M. W. (1995) *Cell* **81,** 279–288.
29. Straight, A. F., Belmont, A. S., Robinett, C. C. & Murray, A. W. (1996) *Curr. Biol.* **6,** 1599–1608.
30. Yamamoto, A., Guacci, V. & Koshland, D. (1996) *J. Cell Biol.* **133,** 85–97.
31. Funabiki, H., Kumada, K. & Yanagida, M. (1996) *EMBO J.* **15,** 6617–6628.
32. Cohen-Fix, O., Peters, J. M., Kirschner, M. W. & Koshland, D. (1996) *Genes Dev.* **10,** 3081–3093.
33. Funabiki, H., Yamano, H., Nagao, K., Tanaka, H., Yasuda, H., Hunt, T. & Yanagida, M. (1997) *EMBO J.* **16,** 5977–5987.
34. Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M. & Nasmyth, K. (1998) *Cell* **93,** 1067–1076.
35. Yamamoto, A., Guacci, V. & Koshland, D. (1996) *J. Cell Biol.* **133,** 99–110.
36. Hwang, L. H., Lau, L. F., Smith, D. L., Mistrot, C. A., Hardwick, K. G., Hwang, E. S., Amon, A. & Murray, A. W. (1998) *Science* **279,** 1041–1044.
37. Michaelis, C., Ciosk, R. & Nasmyth, K. (1997) *Cell* **91,** 35–45.
38. Hirano, T. & Mitchison, T. J. (1994) *Cell* **79,** 449–458.
39. Strunnikov, A. V., Hogan, E. & Koshland, D. (1995) *Genes Dev.* **9,** 587–599.
40. Losada, A., Hirano, M. & Hirano, T. (1998) *Genes Dev.* **12,** 1986–1997.
41. Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A. & Nasmyth, K. (1999) *Genes Dev.* **13,** 320–333.
42. Tomonaga, T., Nagao, K., Kawasaki, Y., Furuya, K., Murakami, A., Morishita, J., Yuasa, T., Sutani, T., Kearsey, S. E., Uhlmann, F., *et al.* (2000) *Genes Dev.* **14,** 2757–2770.
43. Losada, A., Yokochi, T., Kobayashi, R. & Hirano, T. (2000) *J. Cell Biol.* **150,** 405–416.
44. Waizenegger, I. C., Hauf, S., Meinke, A. & Peters, J. M. (2000) *Cell* **103,** 399–410.
45. Sumara, I., Vorlaufer, E., Gieffers, C., Peters, B. H. & Peters, J. M. (2000) *J. Cell Biol.* **151,** 749–762.
46. Jessberger, R., Frei, C. & Gasser, S. M. (1998) *Curr. Opin. Genet. Dev.* **8,** 254–259.
47. Melby, T. E., Ciampaglio, C. N., Briscoe, G. & Erickson, H. P. (1998) *J. Cell Biol.* **142,** 1595–1604.
48. Hopfner, K. P., Karcher, A., Shin, D. S., Craig, L., Arthur, L. M., Carney, J. P. & Tainer, J. A. (2000) *Cell* **101,** 789–800.
49. Akhmedov, A. T., Frei, C., Tsai-Pflugfelder, M., Kemper, B., Gasser, S. M. & Jessberger, R. (1998) *J. Biol. Chem.* **273,** 24088–24094.
50. Losada, A. & Hirano, T. (2001) *Curr. Biol.* **11,** 268–272.
51. Uhlmann, F., Lottspeich, F. & Nasmyth, K. (1999) *Nature (London)* **400,** 37–42.

52. Uhlmann, F., Wernic, D., Poupart, M. A., Koonin, E. V. & Nasmyth, K. (2000) *Cell* **103,** 375–386.
53. Hartman, T., Stead, K., Koshland, D. & Guacci, V. (2000) *J. Cell Biol.* **151,** 613–626.
54. van Heemst, D., James, F., Poggeler, S., Berteaux-Lecellier, V. & Zickler, D. (1999) *Cell* **98,** 261–271.
55. Denison, S. H., Kafer, E. & May, G. S. (1993) *Genetics* **134,** 1085–1096.
56. Megee, P. C., Mistrot, C., Guacci, V. & Koshland, D. (1999) *Mol. Cell.* **4,** 445–450.
57. Blat, Y. & Kleckner, N. (1999) *Cell* **98,** 249–259.
58. Tanaka, T., Cosma, M. P., Wirth, K. & Nasmyth, K. (1999) *Cell* **98,** 847–858.
59. Laloraya, S., Guacci, V. & Koshland, D. (2000) *J. Cell Biol.* **151,** 1047–1056.
60. Ciosk, R., Shirayama, M., Shevchenko, A., Tanaka, T., Toth, A., Shevchenko, A. & Nasmyth, K. (2000) *Mol. Cell* **5,** 243–254.
61. Uhlmann, F. & Nasmyth, K. (1998) *Curr. Biol.* **8,** 1095–1101.
62. Skibbens, R. V., Corson, L. B., Koshland, D. & Hieter, P. (1999) *Genes Dev.* **13,** 307–319.
63. Waga, S. & Stillman, B. (1998) *Annu. Rev. Biochem.* **67,** 721–751.
64. Skibbens, R. V. (2000) *Genome Res.* **10,** 1664–1671.
65. Gerlach, V. L., Aravind, L., Gotway, G., Schultz, R. A., Koonin, E. V. & Friedberg, E. C. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 11922–11927.
66. Tanaka, K., Yonekawa, T., Kawasaki, Y., Kai, M., Furuya, K., Iwasaki, M., Murakami, H., Yanagida, M. & Okayama, H. (2000) *Mol. Cell. Biol.* **20,** 3459–3469.
67. Sadoff, B. U., Heath-Pagliuso, S., Castaño, I. B., Yingfang, Z., Kieff, F. S. & Christman, M. F. (1995) *Genetics* **141,** 465–479.
68. Castaño, I. B., Heath-Pagliuso, S., Sadoff, B. U., Fitzhugh, D. J. & Christman, M. F. (1996) *Nucleic Acids Res.* **24,** 2404–2410.
69. Castaño, I. B., Brzoska, P. M., Sadoff, B. U., Chen, H. Y. & Christman, M. F. (1996B) *Genes Dev.* **10,** 2564–2576.
70. Walowsky, C., Fitzhugh, D. J., Castano, I. B., Ju, J. Y., Levin, N. A. & Christman, M. F. (1999) *J. Biol. Chem.* **274,** 7302–7308.
71. Miller, A. M. & Nasmyth, K. A. (1984) *Nature (London)* **312,** 247–251.
72. Johnson, R. E., Prakash, S. & Prakash, L. (1999) *Science* **283,** 1001–1004.
73. Brewer, B. J. & Fangman, W. L. (1987) *Cell* **51,** 463–471.
74. Greenfeder, S. A. & Newlon, C. S. (1992) *Mol. Cell. Biol.* **12,** 4056–4066.
75. Waga, S. & Stillman, B. (1994) *Nature (London)* **369,** 207–212.
76. Stukenberg, P. T., Turner, J. & O'Donnell, M. (1994) *Cell* **78,** 877–887.
77. Hanna, J. S., Kroll, E. S., Lundblad, V. & Spencer, F. A. (2001) *Mol. Cell. Biol.* **21,** 3144–3158.
78. Green, C. M., Erdjument-Bromage, H., Tempst, P. & Lowndes, N. F. (2000) *Curr. Biol.* **10,** 39–42.
79. Miles, J. & Formosa, T. (1992) *Mol. Cell. Biol.* **12,** 5724–5735.
80. Lengauer, C., Kinzler, K. W. & Vogelstein, B. (1997) *Nature (London)* **386,** 623–627.
81. Cahill, D. P., Lengauer, C., Yu, J., Riggins, G. J., Willson, J. K., Markowitz, S. D., Kinzler, K. W. & Vogelstein, B. (1998) *Nature (London)* **392,** 300–303.
82. Weinert, T. A. (1992) *Radiat. Res.* **132,** 141–143.
83. Canman, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella, E., Kastan, M. B. & Siliciano, J. D. (1998) *Science* **281,** 1677–1679.
84. Zou, H., McGarry, T. J., Bernal, T. & Kirschner, M. W. (1999) *Science* **285,** 418–422.
85. Ghiselli, G. & Iozzo, R. V. (2000) *J. Biol. Chem.* **275,** 20235–20238.
86. Mayer, M. L., Gygi, S. P., Aebersold, R. & Hieter, P. (2001) *Mol. Cell.* **7,** 959–970.