PERSPECTIVE

Cohesion by topology: sister chromatids interlocked by DNA

Rodrigo Bermejo, Dana Branzei,[2] and Marco Foiani[1]

Fondazione Italiana per la Ricerca sul Cancro (FIRC) Institute of Molecular Oncology Foundation, 20139 Milan, Italy; and Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, 20133 Milan, Italy

Sister chromatid cohesion is coupled with chromosome replication and influences chromosome segregation and intra-S repair. Specialized proteins, the cohesins, together with other pathways contribute to tether sister chromatids. In this issue of *Genes* & *Development*, Wang and colleagues (pp. 2426–2433) demonstrate that TopoIV, a type II DNA topoisomerase, modulates cohesion in *Escherichia coli*, by removing interlocked DNA junctions between sister chromatids. They propose that DNA precatenanes, arising during replication fork progression, hold sister chromatids together.

Sister chromatid cohesion is crucial for genome integrity as it facilitates replication-coupled DNA repair and is essential for proper chromosome segregation (Nasmyth 2005; Strom and Sjogren 2007). Most of the time we think about cohesion, we tend to imagine the one mediated by a group of proteins, conserved from yeast to humans, called "cohesins," proposed to form a ring that tethers sister duplexes (Nasmyth and Schleiffer 2004; Shintomi and Hirano 2007). However, studies in yeast and other eukaryotes have shown that, in cohesin-defective mutants, loss of cohesion occurs gradually, and can be delayed until anaphase onset, following the completion of replication, thus suggesting that other mechanisms contribute to tether sister chromatids. One of the first mechanisms of cohesion proposed was the one mediated by catenation (Murray and Szostak 1985), which arises concomitantly with chromosome replication. Catenane removal is mainly performed by certain type II topoisomerases, which include TopoIV in *Escherichia coli*, Top2 in budding yeast, TopIIα and TopIIβ in higher eukaryotes (Champoux 2001; Wang 2002). Several studies in eukaryotic cells suggest that both cohesin and catenation contribute to the full establishment of cohesion, and that removal of both is required to achieve full separation of the sister chromatids (Deehan Kenney and Heald 2006; Diaz-Martinez et al. 2006; Toyoda and Yanagida 2006). In this issue of *Genes* & *Development*, Wang et al. (2008) demonstrate that TopoIV acts during S phase to modulate cohesion in *E. coli*, by removing certain topological junctions between sister chromatids (precatenanes) as they form during replication progression.

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Corresponding authors.
[1]E-MAIL marco.foiani@ifom-ieo-campus.it; FAX 39-02-5743-03231.
[2]E-MAIL dana.branzei@ifom-ieo-campus.it; FAX 39-02-574-303-231.
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Precatenanes and DNA replication

The linkage between the two strands of DNA molecules is described by the linking number that measures the times that one strand crosses the other one in the DNA helix and in higher-order superhelical structures. In topologically constrained DNA such as the closed circular chromosome of *E. coli*, or the eukaryotic chromosomes in which torsional stress cannot diffuse by swiveling of the extremities, the linking number has a constant value and can only be changed by mediating the passage of the strands through each other. This process is mediated by specialized DNA nucleases called topoisomerases. During replication, the separation of the parental strands by DNA helicases locally reduces the linking number of DNA molecules (Postow et al. 2001; Wang 2002). This generates a compensatory torsional stress that can take either the form of positive supercoiling ahead of the fork, or of precatenanes that intertwine the two replicated duplexes behind the forks (Fig. 1A; Champoux and Been 1980; Postow et al. 2001; Wang 2002). If not removed at the completion of replication, precatenanes convert into catenanes, which topologically link the two sister chromosomes.

Previous work showed that precatenanes can form during replication in *E. coli*, and work done in *Xenopus* egg extracts demonstrated that TopoII acts behind the fork to eliminate them (Peter et al. 1998; Lucas et al. 2001). A prerequisite for precatenane formation is that the replication fork and the replisome can rotate around the DNA axis. While precatenane formation is easy to envisage if the sister replisomes progress independently of each other (Breier et al. 2005; Reyes-Lamothe et al. 2008), it comes at odds with the fixed double-replisome model (Dingman 1974; Falaschi 2000), in which the two diverging replication machineries remain associated to each other.

In budding yeast, sister replisomes were shown to re-

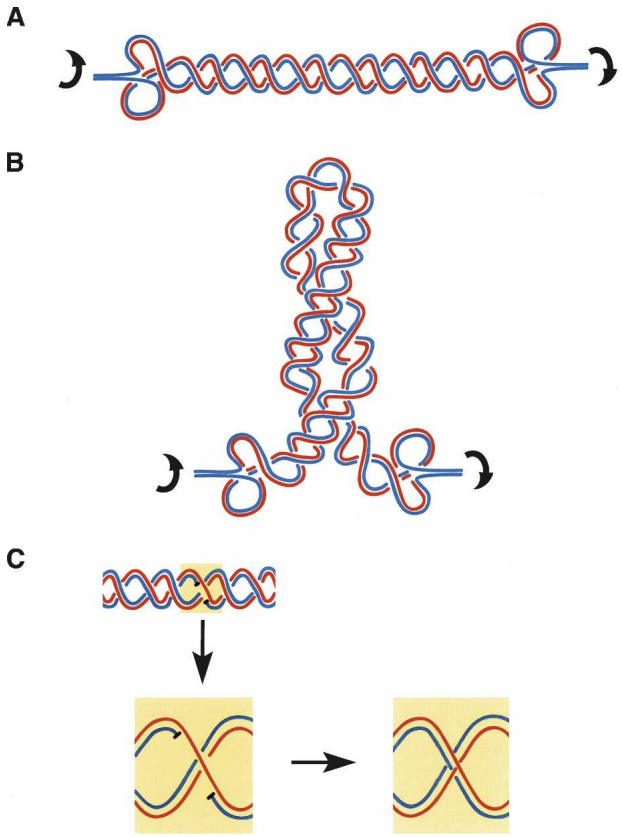

Figure 1. Schematic representation of possible conformations and topological rearrangements of precatenated DNA. (*A*) Precatenane formation during replication: Rotation of the DNA around its axis at the replication fork branching point leads to the intertwining of DNA duplexes behind the fork. Precatenanes topologically link sister chromatids and likely contribute to their cohesion by imposing a physical constrain to their spatial separation. Black arrows indicate the direction of replisome rotation. (*B*) Higher-order organization of replicated DNA into a superhelically coiled precatenated loop. Distribution of torsional stress by the rotation of closely associated replisomes favors the accommodation of precatenates into plectonemic superhelical intertwinings. The formation of "supercoiled" precatenates might influence the compaction of sister chromatids and contribute to modulating chromosome condensation. (*C*) Precatenane conversion into hemicatenanes in the presence of nicks/gaps. Single-strand passage reactions by type I DNA topoisomerases at sites of duplex juxtaposition, most favorably taking place in the presence of nicks of ssDNA gaps, result in the interlocking of sister chromatids and the formation of hemicatenanes. These structures would not be resolved by the relaxation of a topological domain, and would most likely be refractory to resolution by type II enzymes. A likely scenario for this transition is in the proximity of the replication forks where Okazaki fragments form.

main in close proximity during their progression throughout the replicon (Kitamura et al. 2006), but whether they are still able to independently rotate on the DNA axis is not yet known. At least in theory, precatenanes could also form by the rotations of close and coupled sister replisomes, although in this context, precatenanes could be engaged into higher-order loops characterized by plectonemic superhelical windings (Fig. 1B).

The accumulation of positive supercoiling ahead of replication forks is counteracted by the action of DNA gyrase, a type II topoisomerase, unique in its ability to introduce negative supercoils into DNA molecules in prokaryotes (Champoux 2001; Wang 2002). The gyrase function is to keep bacterial DNA underwound (negatively supercoiled), thus absorbing the torsional stress generated during replication. In eukaryotic cells, type IB and type II topoisomerases can both efficiently relax the positive supercoiling generated by fork progression and are thought to cooperate in sustaining the progression of the replication machinery. Catenanes/precatenanes can be resolved by the action of specialized type II topoisomerases (like bacterial TopoIV and eukaryotic TopoII), which mediate the passage of an entire duplex through the other at the sites of sister chromatid juxtaposition. Differently from positive supercoiling, precatenanes do not oppose helicase unwinding, but can counteract the segregation to daughter cells as they physically link the newly replicated DNA molecules together.

Mechanisms of cohesion

The finding that sister chromatids do not separate when the ubiquitin ligase activity of the anaphase promoting complex or cyclosome (APC/C) is mutated, provided an impetus to search for the proteins that mediate cohesion, initially by using genetic studies in yeast. Many proteins required for sister chromatid cohesion have now been identified. Four SMC (structural maintenance of chromosomes) proteins, Smc1, Smc3, Scc1 (Mcd1, Rad21), and Scc3, which form the ring-shaped cohesin complex, play an important role in cohesion in all eukaryotic organisms studied (Losada and Hirano 2005; Nasmyth and Haering 2005; Onn et al. 2008).

Mutations in any of the four subunits of cohesin leads to cohesion defects, measured as failure to hold sister chromatids together. Furthermore, the proteolytic cleavage of the Scc1 subunit of cohesin by a protease, called separase, following activation of the APC/C, triggers the disjunction of the sister chromatids at the onset of anaphase (Uhlmann et al. 1999, 2000).

Although cohesin plays an important role in promoting cohesion, it should be noted that its contribution to chromosome cohesion in budding yeast varies significantly depending on the different genomic loci analyzed. The loss of cohesion in cohesin mutants varies from the 100% loci separation of telomeric regions to 40%–75% in chromosome arms and pericentromeric regions (Diaz-Martinez et al. 2008). Although in certain vertebrate cells noncohesin factors were shown to be recruited to the chromosomes in prometaphase and support chromosome cohesion until the onset of anaphase, it is enticing to speculate that the remaining of cohesion on chromosome arms and at centromeric loci is generally mediated by catenation. The hypothesis that topological interlocking mediates, at least in part, sister chromatid cohesion has been investigated by several laboratories (Murray et

al. 1986; Koshland and Hartwell 1987). Supportive of this view, it has been recently shown that centromeric catenations and cohesion are actively maintained until anaphase, in a process that appears to be regulated by SUMO post-translational modifications. In yeasts, mice, humans, and metazoans it has been shown that sumoylation of TopoII is required for its enrichment at centromeres, and defects in TopoII recruitment or its sumoylation are associated with failure to segregate chromosomes (Bachant et al. 2002; Takahashi et al. 2006).

Mechanisms of sister chromatid separation relate to different types of cohesion

The advantage of using a proteinaceous structure (the one of cohesin), instead of relying solely on chromatid intertwinings was proposed to ensure the efficiency and easiness of sister chromatid segregation (Haering and Nasmyth 2003). If only catenation held sisters together, then a sudden increase in TopoII activity should be expected once all chromosomes had bioriented, and failure to resolve the linkage would lead to catastrophic chromosome breakage and aneuploidy. As the chromosome itself is likely organized into topological domains with barriers that impede the free diffusion of supercoils (Postow et al. 2004), it is envisaged that the action of topoisomerases should be regulated not only temporally, but also spatially. Conversely, failure to degrade the cohesin complex could still be resolved by disengagement of noncovalent protein–subunit interactions, which are presumably much weaker than the forces holding together a catenane structure.

While many lines of evidence suggest that in eukaryotic cells a large amount of cohesion is mediated by cohesin (Michaelis et al. 1997), the situation in *E. coli* seems different. A cohesin-related protein, MukBEF, is found also in *E. coli*, but it does not appear to be required for sister locus cohesion in bacteria (Danilova et al. 2007). Furthermore, Wang et al. (2008) demonstrate in this issue that TopoIV is crucial for resolving the topological structures, which play a major role in mediating sister chromatid cohesion in *E. coli*.

It is important to note that the function of cohesion in bacteria may be different from the one in eukaryotes, as is the timing of sister chromatid segregation. In eukaryotes, the cohesin SMC proteins prevent separation of the sisters until they achieve biorientation on the microtubule spindle, and a main purpose of cohesion is therefore to ensure equipartition of the replicated genome between the daughter cells. In contrast, in bacteria, a large body of evidence supports the view that the newly replicated loci segregate during replication fork progression (Viollier et al. 2004; Bates and Kleckner 2005; Nielsen et al. 2006; Reyes-Lamothe et al. 2008), and nucleoid splitting is initiated midway through S phase (Bates and Kleckner 2005; Reyes-Lamothe et al. 2008). Thus, in *E. coli,* the period of cohesion may simply mean the interval between locus replication and its separation into the splitting sister chromosomes.

By using fluorescence microscopy experiments, Wang et al. (2008) show that separation of sister genetic loci requires TopoIV activity: Impairment in TopoIV activity impedes the separation of the sister loci and the bulk of chromosome segregation, while small increases in TopoIV levels decrease the cohesion time substantially.

Recently, Sherratt's laboratory (Reyes-Lamothe et al. 2008) reported that the sister replisomes originating from a single initiation event at *oriC* separate into opposite cell halves soon after replication initiation, and suggested that this event is a reflection of the fact that the sister replisomes track along DNA. This interpretation is also supported by previous DNA combing and microarray studies (Breier et al. 2005). In this issue, Wang et al. (2008) further find that TopoIV impairment does not interfere with sister replisome replication, but affects chromosome segregation. Thus, according to their previous interpretation (Reyes-Lamothe et al. 2008), it is unlikely that the separation of the sister replisomes is a consequence of chromosome segregation, which is blocked when TopoIV is impaired (Wang et al. 2008). This result confirms and strengthens previous observations that impairment of TopoIV does not interfere with replication fork progression (Grainge et al. 2007), and suggests that TopoIV action is needed for chromosome segregation throughout replication. Given that TopoIV is a good decatenase, but removes positive supercoils inefficiently (Champoux 2001), Wang et al. (2008) argue that TopoIV supports chromosome segregation by removing precatenanes, which in turn act to "cohese" sister chromosomes in *E. coli*.

Interestingly, also in yeast it has been shown recently that topological entanglement might mediate cohesion at least at certain genomic locations (Diaz-Martinez et al. 2008). While most of the genomic DNA in yeast cells is segregated upon cohesin cleavage during anaphase (Koshland and Guacci 2000), rDNA arrays segregate later on, in a fashion dependent on the function of the condensin complex (D'Amours et al. 2004; Sullivan et al. 2004). Recently, it has been shown that ectopic overexpression of a type II topoisomerase in yeast condensin mutants rescues the defect in rDNA segregation (D'Ambrosio et al. 2008). These observations implicate that there is a crosstalk between cohesion and condensation, and that the role of topological interlockings in mediating sister chromatid cohesion has been evolutionary conserved but, perhaps confined to certain chromosomal domains.

Precatenane versus sister chromatid-mediated DNA repair

The results of Wang et al. (2008) provide a clear indication for the in vivo generation of precatenanes during replication, and for a biological role for such precatenanes in chromosome cohesion. However, these exciting results also highlight new questions. For instance, what biological processes may depend on the cohesion induced by precatenation? Are these precatenanes stable

or are they converted into other topological structures such as hemicatenanes, which have been also implicated in sister chromatid cohesion (Lopes et al. 2003; Robinson et al. 2007)?

Yeast cells replicating with attenuated Top2 activity, and thus theoretically defective in precatenane removal, were shown to accumulate cruciform replication intermediates, likely representing precatenane derivatives (Bermejo et al. 2007). At least in theory, precatenanes could be converted into hemicatenanes (Fig. 1C) perhaps through the action of type I topoisomerases that could catalyze strand passage reactions in the presence of nicks/gaps (Zechiedrich and Osheroff 1990). In this view, an ideal context for converting precatenanes into hemicatenanes would be the one at replication forks where the Okazaki fragments are generated.

Moreover, as precatenane formation is coordinated with replication, and homologous recombination plays an important role in intra-S repair, one question that comes to the mind is whether the precatenanes may facilitate recombination repair between the newly replicated sisters. Indeed, precatenanes could facilitate the strand invasion event triggering the bypass of DNA damage by using the newly synthesized sister chromatid as a template, in a process referred to as template switching (Branzei and Foiani 2008).

Another key point that needs to be addressed is whether precatenane formation can indeed occur if the sister replisomes remain in close proximity. If that is the case, one expectation would be that the higher-order structures adopted by precatenanes might also affect the chromosome consensation process (Fig. 1B).

In conclusion, the recent breakthroughs in the study of precatenane formation in both prokaryotes and eukaryotes foreshadows an exciting time for the unraveling of their connection with sister chromatid cohesion and segregation as well as of their implications for sister chromatid-mediated DNA repair and recombination events.

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