REVIEW

Sister acts: coordinating DNA replication and cohesion establishment

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The ring-shaped cohesin complex links sister chromatids and plays crucial roles in homologous recombination and mitotic chromosome segregation. In cycling cells, cohesin's ability to generate cohesive linkages is restricted to S phase and depends on loading and establishment factors that are intimately connected to DNA replication. Here we review how cohesin is regulated by the replication machinery, as well as recent evidence that cohesin itself influences how chromosomes are replicated.

Physical pairing between sister chromatids plays a vital role in the faithful transmission of genetic information during cell division. In eukaryotes, this pairing is mediated primarily by cohesin, a ring-shaped multiprotein complex that appears to catenate DNA molecules by topological means (Nasmyth and Haering 2009). An important feature of cohesin is that its ability to form such linkages is tightly regulated and, under normal conditions, occurs only during S phase, when new sister DNAs are synthesized. In this review, we discuss how DNA replication and cohesion establishment are coordinated, with specific emphasis on how factors at licensed origins and replication forks modify cohesin's architecture and association with chromatin, and how this in turn affects the dynamics of DNA replication. Highlighting the importance of such regulation, germline mutations that compromise cohesin function are responsible for a constellation of human developmental disorders that have been collectively termed "cohesinopathies" (Dorsett 2007; Liu and Krantz 2008).

Cohesin loading prior to S phase

Cohesin is a member of a large family of DNA-associated complexes based on SMC (structural maintenance of chromosome) proteins, whose evolutionary emergence predates the trifurcation of eukaryotes, archaea, and prokaryotes ~3.5 billion years ago (Woese et al. 1990). All SMC proteins use intramolecular coiled-coil interactions

to fold on themselves, producing rod-shaped molecules with an ATPase "head" domain and dimerizing "hinge" at either end (Hirano 2006). In the case of cohesin, the ATPase heads of the Smc1–Smc3 heterodimer are bridged by the non-SMC subunit Scc1 to form a closed ring of 45 nm (Haering et al. 2002; Gruber et al. 2003; Arumugam et al. 2006), while a fourth non-SMC subunit (Scc3 in yeast or stromalin antigen isoforms 1 and 2 [SA1 and SA2] in vertebrates) (Losada et al. 2000; Sumara et al. 2000) interacts directly with Scc1. Considerable evidence supports the notion that the cohesin ring holds sister DNAs together topologically (Nasmyth and Haering 2009), with DNA entering the ring via ATP-dependent opening of the Smc1–Smc3 hinge interface (Arumugam et al. 2003; Weitzer et al. 2003; Gruber et al. 2006).

In yeast, a separate complex consisting of the Scc2 and Scc4 proteins is necessary to load cohesin onto chromatin in G1 phase (Ciosk et al. 2000; Tomonaga et al. 2000; Bernard et al. 2006). Both Scc2 and Scc4 are widely conserved and necessary for cohesin loading in higher eukaryotes that occurs during telophase (Gillespie and Hirano 2004; Rollins et al. 2004; Takahashi et al. 2004; Seitan et al. 2006; Watrin et al. 2006). Notably, the yeast Scc2–Scc4 complex also regulates the deposition of condensin (D'Ambrosio et al. 2008) and the Smc5–Smc6 repair complex at some chromosomal loci (Lindroos et al. 2006), suggesting that it can function generally as an "SMC loader." Whether a similar dependency exists between these complexes in multicellular organisms remains to be determined. Molecularly, all Scc2 orthologs are typified by large arrays of HEAT repeats at their C termini (Neuwald and Hirano 2000), whereas their N termini show minimal sequence conservation. Somewhat surprisingly, the latter are crucial for Scc4's interaction with Scc2 (Seitan et al. 2006; Takahashi et al. 2008). How the Scc2–Scc4 complex loads cohesin and other proteinaceous rings onto chromatin is currently unclear, but experiments in *Xenopus* egg extracts indicate that Scc2's HEAT repeats are essential (Takahashi et al. 2008). One attractive hypothesis is that this portion of Scc2 modulates the ATPase activity of the Smc1–Smc3 heads and/or the opening of the hinge region. Of note, HEAT repeats are also present within Pds5, a cohesin-binding partner with complex and paradoxical roles in cohesion establishment and maintenance, as discussed below.

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In metazoans, the Scc2–Scc4 complex also plays a substantial role in gene expression. For instance, the *Drosophila* Scc2 ortholog Nipped-B influences the transcription of several developmentally important genes, including *cut* and *Ubx* (Rollins et al. 1999; Dorsett et al. 2005). Interestingly, cohesin both enhances and represses transcription of distinct sets of Nipped-B targets (Rollins et al. 2004; Horsfield et al. 2007; Gause et al. 2008; Hallson et al. 2008; Pauli et al. 2008; Schuldiner et al. 2008). Based on these complex phenotypes, the Scc2–Scc4 complex has been proposed to load and unload cohesins dynamically in order to regulate transcription (Dorsett 2009). In humans, mutations in the Scc2 ortholog *NIPBL* give rise to Cornelia de Lange syndrome (CdLS), a hereditary disorder associated with developmental anomalies across multiple organ systems. Moreover, cell lines established from CdLS patients exhibit abnormalities in gene expression that correlate with diminished levels of cohesin binding at transcription start sites of specific promoters (Liu et al. 2009). Subtle but widespread transcriptional changes have also been detected in *Nipbl*^{+/−} mice, which display gross developmental defects characteristic of CdLS (Kawauchi et al. 2009).

How cohesin itself regulates gene expression has also been clarified recently. In budding yeast, cohesin preferentially accumulates between convergently transcribed genes and limits spreading of silent chromatin (Donze et al. 1999; Lengronne et al. 2004). A similar pattern of cohesin enrichment is also found in fission yeast and is important for proper termination of convergently synthesized mRNAs (Gullerova and Proudfoot 2008). By comparison, the major binding sites of mammalian cohesins overlap strongly with CTCF, a critical *trans*-acting factor at chromatin insulators and boundary elements (Parelho et al. 2008; Rubio et al. 2008; Stedman et al. 2008; Wendt et al. 2008). Recent chromosome conformation capture (3C) studies suggest that cohesin and CTCF both contribute to the formation of intrachromosomal loops at specific genes, including the imprinted *IGF2/H19* region, the interferon- γ locus, and the apolipoprotein gene cluster (Hadjuri et al. 2009; Mishiro et al. 2009; Nativio et al. 2009). At present, the physiologic relevance of such loops remains poorly understood, as current methods do not allow for their detection and tracking over time in intact cells. Regardless, both cohesin and CTCF associate with their preferred binding sites throughout the cell cycle, including in G1 phase, when sister chromatids are absent. This observation implies that cohesin's role in regulating gene expression is likely to be orthogonal to its function in pairing sister chromatids.

A link between cohesin loading and prereplication complexes (pre-RCs) in vertebrates

Timed temperature shift experiments in yeast suggest that the Scc2–Scc4 complex becomes dispensable for cohesion as cells enter S phase (Furuya et al. 1998; Ciosk et al. 2000; Bernard et al. 2006; Lengronne et al. 2006). With the caveat that some residual Scc2–Scc4 activity could have escaped these manipulations, one plausible

interpretation is that cohesin rings must be loaded onto chromatin by the time DNA replication begins, or else they lose eligibility to pair sister chromatids. Consistent with this view, metazoans have evolved additional mechanisms that sequence cohesin loading upstream of DNA synthesis. In *Xenopus* egg extracts, loading of the Scc2–Scc4 complex onto chromatin itself depends on pre-RCs (Gillespie and Hirano 2004; Takahashi et al. 2004). Pre-RCs are formed through the sequential assembly of origin recognition complex (ORC), Cdc6, and Cdt1 at future sites of initiation, termed replication origins (Bell and Dutta 2002), and in turn load the Mcm2–7 helicase onto DNA in an inactive form (Randell et al. 2006; Evrin et al. 2009; Remus et al. 2009). Subsequent activation of the helicase at the G1/S transition requires further steps, including phosphorylation by the S-phase kinase Cdc7 (Masai et al. 2006; Sheu and Stillman 2006) and interaction with Cdc45 and GINS (Gambus et al. 2006; Moyer et al. 2006; Pacek et al. 2006; Aparicio et al. 2009; Ilves et al. 2010). Interestingly, Cdc7 interacts with the Scc2–Scc4 complex, and its kinase activity is essential for loading the complex onto chromatin in *Xenopus* egg extracts (Fig. 1; Takahashi et al. 2008). Because Cdc7 associates with pre-RCs during G1 phase and is crucial for their subsequent activation at the start of S phase, its involvement might be useful in targeting cohesin to sites of imminent replisome assembly, thereby facilitating cohesin's subsequent interaction with fork-associated regulators (see below).

Unlike in *Xenopus*, the association of budding yeast cohesin with chromatin is not affected when pre-RC

Figure 1. The Scc2–Scc4 cohesin-loading complex is targeted to chromosomes via both replication-dependent and replication-independent mechanisms. In *Xenopus* egg extracts, the Scc2–Scc4 complex binds chromatin through specific interactions with pre-RCs via the S-phase kinase Cdc7. In contrast, the yeast Scc2–Scc4 complex associates with chromatin and loads cohesin even when pre-RC assembly is prevented. Whether yeast Scc2–Scc4 binds chromosomes directly or through an alternative targeting factor (denoted here by the question mark) is not known.

assembly is blocked by depleting Cdc6 (Uhlmann and Nasmyth 1998). Conversely, DNA replication is grossly normal when cohesin or the Scc2–Scc4 complex is inactivated (Guacci et al. 1997; Michaelis et al. 1997; Furuya et al. 1998; Ciosk et al. 2000). Thus, the *Saccharomyces cerevisiae* Scc2–Scc4 complex can be recruited onto chromatin either autonomously or through a replication-independent mechanism. What about the somatic cells of higher eukaryotes? While this issue awaits further investigation, fragments of the human Scc2–Scc4 complex bind chromatin in a Cdc7-dependent manner in *Xenopus* egg extracts, indicating that such a mechanism is tenable (Takahashi et al. 2008). Equally suggestive is the recent finding that the *Drosophila* ORC preferentially colocalizes with cohesin, as judged from genome-wide chromatin immunoprecipitation studies (MacAlpine et al. 2010). Nevertheless, whether replication origins (and pre-RCs specifically) regulate cohesin in either flies or mammals remains uncertain. Indeed, as discussed above, cohesin loading is also required to control gene expression, including in terminally differentiated cell types such as neurons (Pauli et al. 2008; Schuldiner et al. 2008). In such compartments, Scc2–Scc4's chromatin association might be dictated by alternative transcription-linked mechanisms, rather than the replication-coupled pathway operating in early *Xenopus* embryos, which cycle in the absence of zygotic transcription. Clearly, more work is needed to understand in detail how the Scc2–Scc4 complex is targeted to chromatin and then loads cohesin onto DNA.

Establishing cohesion in S phase via Eco1-mediated acetylation

The prereplicative loading of cohesin is necessary but not sufficient to ensure that sister chromatids are ultimately linked to one another. Rather, this requires both passage through S phase (Uhlmann and Nasmyth 1998) and the action of so-called cohesion establishment factors. Paramount among these is the Eco1/Ctf7 acetyltransferase (Skibbens et al. 1999; Toth et al. 1999), which is conserved throughout the eukaryotic kingdom (Tanaka et al. 2000; Williams et al. 2003). Vertebrate genomes encode two Eco1 orthologs, called Esco1 and Esco2, that jointly contribute to cohesion (Hou and Zou 2005). Clinically, biallelic mutations in the *ESCO2* gene are responsible for Roberts syndrome (RBS), a hereditary developmental disorder typified at the cellular level by weakened centromere cohesion and hypersensitivity to certain genotoxic drugs (e.g., mitomycin C, camptothecin, and etoposide) that target S phase (Vega et al. 2005; van der Lelij et al. 2009).

Early studies in budding and fission yeast demonstrated that Eco1 executes its cohesion-promoting role during S phase, but is not required for cohesin loading in G1 phase or maintenance of cohesion in G2 and M phases (Skibbens et al. 1999; Toth et al. 1999; Tanaka et al. 2000), thus distinguishing it from both the Scc2–Scc4 complex and cohesin itself. Subsequent biochemical analysis revealed Eco1 to be a highly diverged but bona fide protein

acetyltransferase (Ivanov et al. 2002), but how this activity contributes to sister chromatid cohesion remained obscure until recently, when two conserved lysines in Smc3's ATPase domain were found to be Eco1's critical *in vivo* targets (Rolef Ben-Shahar et al. 2008; Unal et al. 2008; Zhang et al. 2008; Rowland et al. 2009). Acetylation at these sites peaks in S phase and defends cohesin against an “anti-establishment” activity associated with Pds5 and Wapl (Tanaka et al. 2001; Rowland et al. 2009; Sutani et al. 2009), two proteins that interact with cohesin's Scc1 and Scc3 subunits as well as each other (Gandhi et al. 2006; Kueng et al. 2006). A similar regulatory framework also exists in mammals, as depletion of Wapl or Pds5a rescues the phenotypic effects of Esco1 or Esco2 deficiency or mutagenesis of Smc3's acetylation sites in human cells (Gandhi et al. 2006; Terret et al. 2009).

What is the nature of Wapl and Pds5's anti-establishment activity? Previous work uncovered key roles for both proteins in cohesion maintenance, albeit with dramatically different effects, depending on the model system. For example, human Wapl RNAi cells and *Xenopus* egg extracts depleted of either Wapl or Pds5 fail to remove cohesin from G2 and mitotic chromosomes, resulting in “hypercohesed” sister chromatids that cannot be resolved from one another (Gandhi et al. 2006; Kueng et al. 2006; Shintomi and Hirano 2009). In contrast, yeast *wpl1* and *pds5* mutants have less (rather than more) cohesin bound to their chromosomes, resulting in subnormal and unstable sister chromatid cohesion (Panizza et al. 2000; Tanaka et al. 2001; Stead et al. 2003; Rowland et al. 2009; Sutani et al. 2009). Mild cohesion defects are also evident in *Drosophila wapl* mutant embryos (Verni et al. 2000) and in human cells acutely depleted of Pds5a or Pds5b via RNAi (Losada et al. 2005), but not in *Pds5a*^{−/−} or *Pds5b*^{−/−} mouse embryonic fibroblasts (Zhang et al. 2009), suggesting functional compensation during chronic growth conditions.

One clue for resolving this apparent paradox may reside in the fact that Pds5 itself is a large HEAT repeat protein, akin to the Scc2 subunit of the cohesin loader complex. It is tempting to speculate that the Wapl–Pds5 complex regulates cohesion via a conceptually similar process, involving the stepwise opening and closing of the cohesin ring. If so, then the net effect of Wapl and Pds5 inactivation in a given organism may simply reflect which step of the reaction (ring opening or ring closing) becomes rate-limiting. Regardless, these data also indicate that the anti-establishment activity of the Wapl–Pds5 complex cannot be neatly equated with its effect on cohesion maintenance in G2 or M phase, but might arise only in the specific context of DNA replication. Consistent with this view, recent work indicates that human Wapl and Pds5 slow replication forks unless neutralized by Esco1- and Esco2-mediated acetylation, which weakens cohesin's interaction with the Wapl–Pds5 complex (Terret et al. 2009). It remains to be determined if cohesin acetylation has other consequences that are important for establishment, such as a direct effect on cohesin's ability to bind or hydrolyze ATP. Also unclear at the moment is

the role of sororin, a vertebrate-specific protein that binds to and stabilizes cohesin on chromatin (Rankin et al. 2005; Schmitz et al. 2007). In particular, whether sororin participates directly in cohesion establishment during S phase or simply maintains cohesion post-replicatively remains to be elucidated.

Building cohesion at the replication fork

Sister chromatid cohesion requires the identification and pairing of two identical copies of each chromosome, rather than two unrelated chromosomes or homologs. A natural context for these transactions exists at the replication fork, which provides a temporary bridge between sister DNAs. Indeed, considerable physical and functional evidence links both Eco1-related acetyltransferases and other cohesion establishment factors to replication forks. For example, early studies indicated that yeast with thermolabile versions of Eco1 could be at least partially rescued through overexpression of PCNA (Skibbens et al. 1999), the homotrimeric sliding clamp that tethers DNA polymerases and other replication proteins to the fork (Moldovan et al. 2007). One such factor is Eco1 itself, which contains a conserved PCNA-binding motif that is essential for its cohesive activity (Lengronne et al. 2006; Moldovan et al. 2006). Consistently, cohesin acetylation depends on DNA synthesis per se, rather than cell cycle timing (Rolef Ben-Shahar et al. 2008), and, at least in human cells, is supported by the alternative replication factor C-Ctf18 (RFC^{Ctf18}) complex, which loads PCNA onto forks both in vitro and in vivo (Bermudez et al. 2003; Lengronne et al. 2006; Terret et al. 2009). Finally, multiple components of the replisome progression complex (RPC), which couples the activated form of the Mcm2–7 helicase to the leading and lagging strand machinery (Gambus et al. 2006), are also important for cohesion establishment. This group includes the Ctf4/AND-1 protein, which tethers and activates DNA polymerase α on the lagging strand (Zhu et al. 2007; Gambus et al. 2009; Tanaka et al. 2009; Bermudez et al. 2010); the Mrc1/claspin, Tof1/timeless, and Csm3/tipin proteins, which stabilize stalled replication forks and transduce checkpoint signals in concert with DNA polymerase ϵ on the leading strand (Chini and Chen 2003; Katou et al. 2003; Lee et al. 2003; Chou and Elledge 2006; Errico et al. 2007; Unsal-Kacmaz et al. 2007; Yoshizawa-Sugata and Masai 2007; Lou et al. 2008; Leman et al. 2010); and Chl1/CHLR1, a 5'–3' DNA helicase thought to be involved in processing Okazaki fragments (Petronczki et al. 2004; Farina et al. 2008). Recently, biallelic mutations in human *CHLR1* were linked to Warsaw breakage syndrome, a rare hereditary disorder associated with both cohesion loss and chromosome breakage (van der Lelij et al. 2010).

The involvement of these replisome-associated factors is especially fascinating in light of current ideas for how replication forks and cohesin rings interact during the establishment process. One proposal is that either replication forks must be smaller than cohesin's central aperture, in which case they could simply slide through the ring, or else cohesin must be transiently enlarged to

accommodate the fork without dissociating from chromatin entirely. Based on concepts derived from prokaryotic DNA replication—such as the formation of large DNA loops that enable coordinated leading and lagging strand synthesis (Alberts et al. 1983), efficient recycling of the lagging strand polymerase (Hamdan et al. 2009), and inclusion of a third clamp–polymerase module in the replisome (McInerney et al. 2007; Reyes-Lamothe et al. 2010)—and considering the greater number of fork-associated proteins in eukaryotes (Stillman 2008), eukaryal replisomes may experience significant challenges in traversing cohesin-bound regions of the genome (Fig. 2). Such encounters are expected to affect nearly all replication forks, given the high density of cohesin-binding sites across the genome (approximately one per 20 kb) (Parelho et al. 2008; Wendt et al. 2008) as compared with the ~100-kb interval between replication origins (Huberman and Riggs 1968). Furthermore, atomic force microscopy and in vivo Förster resonance energy transfer (FRET) experiments suggest that cohesin's coiled coils are remarkably flexible, and that Pds5 contacts not only Scc1, but also the Smc1–Smc3 hinge on the opposite side of the ring (Sakai et al. 2003; McIntyre et al. 2007), which would tend to reduce cohesin's cross-sectional area. Smc3 acetylation might be required to counteract this tendency as replisomes pass through the ring, thereby facilitating replication fork progression and sister chromatid entrapment in one step. Once formed, such entrapment may also stabilize replication forks and/or enable sister chromatid-dependent restart pathways (Branzei and Foiani 2010).

In addition to the preceding model, other scenarios can be envisioned, whereby cohesin's interaction with

Figure 2. Building cohesion at the replication fork. At S-phase onset, replication forks encounter cohesin rings that are unacetylated and tightly bound to Wapl and Pds5, which antagonize cohesion establishment and replication fork progression. Through recruitment and activation of Eco1-related acetyltransferases, replication forks modify cohesin's Smc3 subunit to weaken its interaction with the Wapl–Pds5 complex. This may put cohesin in an “open” conformation that allows the replication fork to progress while trapping the nascent sister chromatids inside the ring. Alternatively, it is possible that cohesin rings are displaced by advancing replication forks, but then are subjected to a second round of loading behind the fork. However generated, entrapment of the nascent sister DNAs is likely to enhance fork stability by enabling homology-dependent pathways of fork repair and restart. Further discussion of these points can be found in the text.

unreplicated chromosomes is nontopological or otherwise sensitive to displacement by the replisome, but is then reconstituted (and converted into a topological interaction that catenates both sister DNAs) via a second round of cohesin loading behind the fork (Milutinovich et al. 2007). Attempts to detect such reloading via timed inactivation of the Scc2–Scc4 complex have thus far proven unsuccessful (Lengronne et al. 2006). Nevertheless, it cannot be excluded that such reactions, mediated by trace levels of Scc2–Scc4 activity or an entirely separate cohesin-loading system, still transpired at replication forks. Clearly, more work is needed to understand how cohesin interacts with DNA templates before, during, and after their transit through the replication machinery.

Regardless of whether replication forks slide through or displace and reload cohesin rings, the notion that fork progression itself is tied to cohesin acetylation may shed light on why all known cohesion establishment factors are also implicated in maintaining the processivity and stability of replication forks. For example, the inactivation of RPC components causes significant slowing of replication forks at baseline, as well as high rates of fork collapse in the presence of chemical inhibitors of DNA synthesis (Szyjka et al. 2005; Tourriere et al. 2005; Errico et al. 2007; Unsal-Kacmaz et al. 2007; Petermann et al. 2008; Gambus et al. 2009; Tanaka et al. 2009; Bermudez et al. 2010). Both effects are thought to arise from uncoupling of the replicative helicase from the leading and/or lagging strand polymerases (Katou et al. 2003; Gambus et al. 2009). One possibility is that these replisome components are needed primarily to recruit or activate Eco1-related acetyltransferases and counteract Wapl and Pds5's tendency to uncouple replisomes. This scenario explains the role of the RFC^{Ctf18} clamp loader in human cells, as its absence causes Smc3 hypoacetylation and a marked decrease in fork speeds that can be reversed by codepleting Pds5a (Terret et al. 2009). Alternatively, replisomes missing these accessory factors might be intrinsically prone to uncoupling regardless of cohesin's acetylation status, in which case ectopic inactivation of Wapl or Pds5 should have little or no ability to restore normal fork speeds or stability. In conclusion, it will be important to understand whether other establishment factors build cohesion primarily by promoting cohesin's timely modification by Eco1-related enzymes, or through collateral activities that impinge directly on leading or lagging strand synthesis.

Relevance of cohesion establishment to human disease

As noted above, biallelic mutations in the human *ESCO2* locus are responsible for RBS. The vast majority of these mutations truncate *Esco2* upstream of its C-terminal acetyltransferase domain, while the remainder directly target its enzymatic activity (Gordillo et al. 2008). The manifestations of *Esco2* deficiency in RBS patients partially overlap with those of CdLS, as both syndromes are associated with symmetrical limb defects, mental retardation, and delayed intrauterine and postnatal growth (Dorsett 2007; Liu and Krantz 2008). However, a unique

and diagnostically important feature of RBS is the presence of centromere cohesion defects, which manifest as a parallel (rather than centrally constricted) configuration of sister chromatids on metaphase spreads (German 1979). Nevertheless, RBS patient cell lines do not accumulate in mitosis (Gordillo et al. 2008), as would be expected if centromere cohesion was weakened to levels that were inadequate to satisfy the spindle assembly checkpoint (Kops et al. 2005). Consequently, it has been proposed that the nonmitotic effects of *Esco2* deficiency might be more relevant (Gordillo et al. 2008). Cells from RBS patients exhibit both increased sensitivity to genotoxic drugs that target S phase (van der Lelij et al. 2009) and diminished replication fork speeds, similar to *Esco2* RNAi cells (Terret et al. 2009). Could such defects contribute to the pathogenesis of RBS? While a definitive answer will require the generation of *Esco2* knockout mice as in vivo models, there are intriguing reasons to think so. DNA replication in metazoan organisms is remarkably plastic, as early embryos activate ~10 times as many origins as somatic cells, resulting in an average replicon size of ~10 kb (Arias and Walter 2007). How 90% of these initially active origins become dormant as development proceeds is a fascinating unsolved problem. Nevertheless, this arrangement provides somatic cells with a crucial defense against stalled or collapsed replication forks, as conditions that slow DNA synthesis rates also trigger the reactivation of dormant origins (Anglana et al. 2003). This shift in replication dynamics is also accompanied by a significant change in nuclear architecture, as chromatin loops (operationally defined by their attachment to an insoluble nuclear scaffold) are reset to a smaller size (Courbet et al. 2008). Conversely, down-regulating cohesin increases the size of such loops, while diminishing their density of active replication origins (Guillou et al. 2010). Thus, *Esco2* deficiency might cause not only replication stress in developmentally sensitive progenitor cells, but also more sweeping changes in chromosome structure. Regardless of the exact route by which *Esco2* sustains embryogenesis, it will be important to know if *Esco2*-deficient mice develop normally when Wapl, Pds5a, or Pds5b levels are partially reduced, as it would validate these cohesin regulators as pathological effectors and therapeutic targets in human RBS.

Outlook

Considerable progress has been made toward understanding how sister chromatids become connected to one another during S phase. However, major questions remain about the mechanism of cohesin's initial loading onto chromatin and subsequent entrapment of nascent sister chromatids at replication forks, which have yet to be visualized directly. Doing so will require adaptation of in vitro DNA replication systems, based on DNA tumor viruses or *Xenopus* egg extracts, to model these reactions in ensemble or single-molecule forms. A clearer view of how cohesin is regulated by post-translational modifications and interactions with its regulatory partners is also needed, which will depend on the systematic

reconstitution of holo and apo complexes for enzymatic and structural studies. Complementing this effort, it will be useful to determine how the cohesin loading and establishment machinery are directed to chromatin, especially in higher eukaryotes, where cohesin has a prominent noncohesive function in gene expression. Lastly, it will be important to generate accurate models of human cohesinopathies in animals in order to understand how alterations in cohesin regulation give rise to such profound and even life-threatening consequences for the whole organism.

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