Sister Chromatid Cohesion: A Simple Concept with a Complex Reality

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Abstract

In eukaryotes, the process of sister chromatid cohesion holds the two sister chromatids (the replicated chromosomes) together from DNA replication to the onset of chromosome segregation. Cohesion is mediated by cohesin, a four-subunit SMC (structural maintenance of chromosome) complex. Cohesin and cohesion are required for proper chromosome segregation, DNA repair, and gene expression. To carry out these functions, cohesion is regulated by elaborate mechanisms involving a growing list of cohesin auxiliary factors. These factors control the timing and position of cohesin binding to chromatin, activate chromatin-bound cohesin to become cohesive, and orchestrate the orderly dissolution of cohesion. The 45-nm ringlike architecture of soluble cohesin is compatible with dramatically different mechanisms for both chromatin binding and cohesion generation. Solving the mechanism of cohesion and its complex regulation presents significant challenges but offers the potential to provide important insights into higher-order chromosome organization and chromosome biology.
Sister chromatids:
the two copies of each chromosome generated by DNA replication
Sister chromatid cohesion: the tethering together of sister chromatids
Cohesin regulatory factors: facilitate cohesin loading on chromatin, convert cohesin to the cohesive state, or control the maintenance or dissolution of cohesion
Cohesin: four-subunit complex consisting of Smc1, Smc3, Sec3, and Mcd1 (also named Rad21 or Scc1) that mediates tethering of sister chromatids

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INTRODUCTION
During a eukaryotic cell division, the two sister chromatids (the newly replicated chromosomes) are held together from the time of their synthesis in S phase through metaphase of mitosis. At the onset of anaphase this cohesion between sister chromatids is dissolved, allowing them to segregate to the opposite poles of the mitotic spindle. Initially, the mechanism of sister chromatid cohesion was thought to be a passive process mediated by the inherent stickiness of chromatin or by topological interwines between sister DNA molecules. However, the characterization of two mutants in flies with specific defects in cohesion provided the first evidence that this process was mediated by dedicated cohesion factors (Kerrebrock et al. 1992, Miyazaki & Orr-Weaver 1992). Indeed, these fly mutants were the tip of the iceberg. Studies during the past decade and a half have revealed that sister chromatid cohesion is a complicated molecular process involving numerous cohesion proteins. Cohesion and its factors are important for diverse biological processes including chromosome segregation, DNA repair, gene expression, and development.

The recent surge in the understanding of sister chromatid cohesion was initiated by the convergence of three different conceptual approaches. The visualization of sister chromatids by fluorescent in situ hybridization (FISH) in the simple budding yeast enabled a systematic cytogenetic approach to identify factors that mediate and regulate cohesion (Guacci et al. 1993, 1994, 1997; Yamamoto et al. 1996b). This approach was greatly augmented by the subsequent development of green fluorescent protein (GFP) tagging of chromosomes, which provided a direct visualization of the cohesion state of yeast sister chromatids (Straight et al. 1996). At the same time, extracts of Xenopus laevis egg were used to develop a systematic biochemical approach to identify and characterize components of mitotic chromosomes necessary for sister chromatid cohesion and condensation (Hirano & Mitchison 1994, Losada et al. 1998). Finally, these two bottom-up approaches were complemented by a top-down approach to understand the role of cohesion in the cell cycle, in particular the commitment to chromosome segregation at the metaphase-to-anaphase transition in mitosis (Irniger et al. 1995, Michaelis et al. 1997). These three approaches rapidly merged and were augmented by other approaches, leading to an explosion of observations from many different systems.

Summarizing these observations is a challenge. In principle, protein-based sister chromatid cohesion could be achieved by a very simple biochemical mechanism like the dimerization of two subunits, each with a canonical DNA binding domain. Instead, cohesion is mediated predominantly by cohesin, a four-subunit protein complex with complicated architecture and function. Despite great progress since the discovery of cohesin, many fundamental questions remain unanswered. How cohesin binds chromatin and how cohesin tethers two sister chromatids together remain controversial. Furthermore, cohesin is subjected to complex temporal and spatial regulation to ensure the proper establishment, maintenance, and...
dissolution of cohesion. Only partial skeletons of these regulatory pathways are known. Finally, elucidating the biological role of cohesion is complicated by the fact that cohesin factors are important for several distinct chromosomal processes. As a result of these complications, the field of cohesion is very much a work in progress and still evolving rapidly.

Because details are likely to change, this review emphasizes emerging concepts and outstanding questions in the field. We summarize the current understanding of the molecular mechanism of cohesion and its regulation. We then assess the biological function of cohesion in chromosome segregation and its emerging roles in other diverse biological processes. To limit the scope of the review, we present our analyses primarily in the context of mitotic cells but draw upon relevant meiotic results when pertinent. We also limit discussion of cohesin architecture, cohesion dissolution, and cohesion regulation in meiosis. For further information about these important and interesting topics, the reader should see other excellent reviews (Nasmyth & Haering 2005, Watanabe 2005).

**MOLECULAR MECHANISM OF SISTER CHROMATID COHESION**

**Cohesin Structure**

Early studies of cohesin structure have had a tremendous impact on the field of cohesion and provide a framework for our ensuing discussion of the molecular and biological functions of cohesin. Cohesin is composed of four evolutionarily conserved subunits, a pair of SMC (structural maintenance of chromosomes) proteins called Smc1 and Smc3, a kleisin subunit called Mcd1 (also Scc1 or Rad21), and Sc3 (Guacci et al. 1997, Losada et al. 1998, Michaelis et al. 1997, Nasmyth & Haering 2005). Unfortunately, the conservation of the subunits did not manifest itself with conserved nomenclature (Table 1).

### Table 1 Cohesin subunits and regulatory proteins nomenclature

<table>
<thead>
<tr>
<th></th>
<th><em>Saccharomyces cerevisiae</em></th>
<th><em>Schizosaccharomyces pombe</em></th>
<th><em>Drosophila melanogaster</em></th>
<th><em>Xenopus laevis</em></th>
<th>Human</th>
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<tbody>
<tr>
<td><strong>Cohesin subunits</strong></td>
<td>SMC1</td>
<td>PSM1</td>
<td>SMC1</td>
<td>SMC1</td>
<td>SMC1</td>
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<td>SMC3</td>
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<td>SMC3</td>
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<tr>
<td></td>
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<td>RAD21</td>
<td>RAD21</td>
<td>RAD21 (SCC1)</td>
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<tr>
<td></td>
<td>IRR1 (SCC3)</td>
<td>PSC3</td>
<td>SA</td>
<td>SA1, SA2</td>
<td>SA1 (STAG1), (STAG2)</td>
</tr>
<tr>
<td><strong>Loading</strong></td>
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<td>MIS4</td>
<td>NIPBL</td>
<td>SCC2</td>
<td>NIPBL</td>
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<tr>
<td></td>
<td>SCC4</td>
<td>SSL3</td>
<td>N/C</td>
<td>xSCC4</td>
<td>MAU2 (hSCC4)</td>
</tr>
<tr>
<td><strong>Establishment</strong></td>
<td>ECO1 (CTF7)</td>
<td>ESO1</td>
<td>San, Deco</td>
<td>XECO1, XECO2</td>
<td>EFO1 (ESCO1), EFO2 (ESCO2)</td>
</tr>
<tr>
<td><strong>Maintenance</strong></td>
<td>PDS5</td>
<td>PDS5</td>
<td>PDS5</td>
<td>PDS5A, PDS5B</td>
<td>PDS5A, PDS5B</td>
</tr>
<tr>
<td></td>
<td>RAD61*</td>
<td>WPL1*</td>
<td>WAPL</td>
<td>N/C</td>
<td>WAPL</td>
</tr>
<tr>
<td><strong>Dissolution</strong></td>
<td>PDS1</td>
<td>CUT2</td>
<td>PIM</td>
<td>Securin</td>
<td>Securin (PTTG)</td>
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<tr>
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<td>ESP1</td>
<td>CUT1</td>
<td>Separase (SSE)</td>
<td>Separin</td>
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<td></td>
<td>CDC5</td>
<td>PLO1</td>
<td>POLO</td>
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<td>PLK1</td>
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<tr>
<td></td>
<td>SGO1*</td>
<td>SGO1, SGO2</td>
<td>SGO1 (Mei-S332)</td>
<td>Shugoshin-like 1 (xSGO1)</td>
<td>Shugoshin (hSGO1)</td>
</tr>
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*N/C denotes that a homologous gene has not been identified or annotated in this organism. Asterisks indicate that homology has been inferred from sequence similarity but that either genes have not been characterized yet or function is not fully overlapping.
For simplicity we use the official nomenclature in budding yeast for describing generic observations.

At the heart of the cohesin complex is a dimer of Smc1 and Smc3 (Figure 1). Smc1 and Smc3 share similarities in protein sequence and architecture with each other and with other SMC and Smc-like proteins conserved from bacteria to humans (Hirano 2005). Like Smc1 and Smc3, these other SMC proteins dimerize and associate with additional subunits to form structurally related Smc complexes. These Smc complexes also mediate higher-order changes in chromosome structure, presumably by tethering chromatin (for reviews, see Hirano 2006, Nasmyth & Haering 2005). Given these similarities, we use relevant knowledge from other SMC complexes to supplement our discussion of cohesin structure.

The remarkable architecture of Smc proteins provided an early indication that the process of cohesion is likely to be complicated. All Smc proteins contain globular domains at the N- and C-terminal ends that are connected by a long alpha helical structure. This alpha helical structure is broken in the middle by a globular hinge domain (Figure 1). Folding at the hinge domain brings together the two halves of the alpha helix to form a long, antiparallel coiled-coil domain. Folding at the hinge also brings together the globular domains at the N and C termini to form the head domain (Figure 1, middle) (Haering et al. 2002, 2004; 108 Onn et al. 2008). The head domain shares striking similarity with the half site of the ATP binding cassette (ABC), the ATPase domain found in ABC transporters and the Smc-like Rad50 protein (Haering et al. 2004, Hopfner et al. 2000). These ATPase domains contain three small highly conserved motifs called Walker A, Walker B, and signature motif (Hopfner et al. 2000, Saitoh et al. 1994, Walker et al. 1982). The final folded Smc forms an extended dumbbell with globular hinge and head domains separated by ~45 nm of coiled coil.

Soluble chromatin-free SMC proteins dimerize by a number of mechanisms (Figure 1). The most stable association occurs through the hydrophobic interactions of the hinge domain (Haering et al. 2002, 2004;
Hirano et al. 2001). This dimerization generates large V-shaped structures visualized in the electron microscope (Anderson et al. 2002, Haering et al. 2002). Smc dimers also form closed rings through the additional association of their head domains (Figure 1). This association brings together the two half sites of the ABC-like ATPase domains, generating two functional ATPases. The association of the head domains depends in part on ATP binding because the ATP is coordinated simultaneously by the Walker A motif of one head and the signature motif of the other head (Haering et al. 2004, Jones & Sgouros 2001, Lowe et al. 2001). However, ATP-independent determinants for association of the heads apparently exist as well (Haering et al. 2002). Finally, the coiled coils of Smc1 and Smc3 provide another interface for potential interaction between Smc molecules. In some electron microscopy (EM) images the coiled-coil regions of the dimer are separate, whereas in others they are together (Anderson et al. 2002, Haering et al. 2002). In summary, the structure of the Smc dimer reveals multiple interaction surfaces and two ATPases, providing a potential energy-dependent mechanism to regulate those interactions.

Soluble chromatin-free cohesin complexes resemble the Smc dimers with the addition of the non-Smc subunits bound to the head domain (Figure 1). Of the non-Smc subunits, only the structure of the kleisin subunit, Mccl, has been studied extensively. The N terminus of Mccl interacts with the Smc3 head domain, whereas the C terminus is the docking site for the Smc1 head domain (Haering et al. 2002). These two interactions allow the kleisin subunit to cross-link the head domains (Figure 1). Although both interactions occur at the bottom of the head near the ATP binding pockets of the Smc heads, only the Smc1-Mccl interaction is ATP dependent (Gruber et al. 2003; Haering et al. 2002, 2004). The proximity of Mccl to the ATPase active site and the ability of Mccl to cross-link heads make Mccl ideally suited to be a central regulator of cohesin function.

Independently of its interaction with the SMCs, Mccl also binds the fourth cohesin subunit, Scc3 (Haering et al. 2002). The sequence of this subunit lacks any defined motif other than HEAT repeats, loosely defined protein-protein interaction motifs. HEAT repeats are found in the cohesin-associated factor Pds5 (see subsection on Cohesion Maintenance and Dissolution, below), and the cohesin loading factor Scc2 (see subsection on Cohesin Binding to Chromatin, below). The structural/functional roles of Scc3 and its HEAT domains in cohesin remain a mystery.

The structure of the soluble chromatin-free cohesin inspired a number of models for the mechanism of cohesion (Figure 2). One of the first models proposed was the embrace model (Gruber et al. 2003). In this model the soluble chromatin-free ring structure is also the relevant structure for both chromatin binding and tethering. As originally postulated, this model proposed that ATP hydrolysis by the cohesin heads causes them to dissociate and the ring to open. Opening of the ring allows the two sister chromatids to enter the interior of the ring, either simultaneously or sequentially. The sister chromatids are entrapped when the ring reforms by the reassociation of the head domains through the binding of ATP and the kleisin subunit.

Alternative models for cohesin function begin with the premise that the single-ring structure of soluble cohesin is not the active form on chromatin (Huang et al. 2005, Milutinovich & Kosland 2003). Rather, the structure changes when cohesin binds to chromatin and actually tethers sister chromatids together (the cohesive state). One suggestion is that cohesins bind to each sister chromatid and then oligomerize to generate cohesion (Figure 2). In these models, oligomerization is achieved by exploiting one of the many interaction surfaces, like the coiled coils or hinge, to promote inter- rather than intracomplex association. Experiments to test the validity of the embrace and oligomerization models have resulted in important insights. However, active cohesin likely must undergo structural changes not anticipated by any model. These are discussed in detail below. Clearly, crystallographic and EM structures of
Figure 2
Models for cohesin chromosome tethering. In the ring model (a) soluble chromatin-free single cohesin topologically entraps sister chromatids inside the ring-shaped structure. Alternatively, the snap (b) and bracelet (c) models suggest that chromatin-bound cohesins form oligomers mediated by the coiled coils or hinges, respectively. In these models chromatids are held by a specific DNA-protein interaction inside or outside the complex. The actual molecular mechanism for chromosome tethering most likely is a hybrid of the models presented.

Cohesin, both bound to chromatin and in the cohesive state, are critical steps toward unraveling the molecular basis of cohesion.

Cohesin Binding to Chromatin
Early studies revealed that the binding of cohesin to chromatin is highly regulated both spatially and temporally (Figure 3a). Cohesins bind initially to DNA during the G1/S phase transition in budding yeast and during telophase of the preceding cell division in vertebrates. Cohesins then continue to bind until anaphase onset (Guacci et al. 1997, Losada et al. 2000, Michaelis et al. 1997, Sumara et al. 2000). This cell-cycle-regulated binding of cohesin occurs at high density in a large domain around each centromere (pericentric) and at lower density along chromosome arms. In budding and fission yeast, the low-density binding occurs on average at 15-kb intervals called cohesin-associated regions (CARs) (Blat & Kleckner 1999, Glynn et al. 2004, Laloraya et al. 2000, Lengronne et al. 2004). CARs tend to lie in AT-rich intergenic regions between divergently transcribed genes, but many exceptions to this distribution are found (Glynn et al. 2004, Laloraya et al. 2000, Lengronne et al. 2004). CARs span ~1 kb of DNA (Laloraya et al. 2000), but no specific sequence within any
CAR has been demonstrated to be essential for cohesin binding. The large size of the CAR may reflect multiple interactions between a cohesin and the CAR, the binding of multiple cohesins to the CAR, or variable binding of a cohesin within the CAR.

Subsequent studies of cohesin revealed even greater regulation of its binding to chromatin. Cohesin binding is enriched at silent chromatin (Bernard et al. 2001, Chang et al. 2005, Nonaka et al. 2002). Cohesin also binds extensively to chromatin surrounding a DNA double-strand break (DSB) to form a large cohesin domain (Figure 3b) (J.S. Kim et al. 2002, Strom et al. 2004, ¨Unal et al. 2004). Finally, cohesins are even targeted to extrachromosomal elements like the "selfish" 2-μ plasmid in budding yeast (Ghosh et al. 2006, Mehta et al. 2002). These diverse examples of cohesin binding to chromatin raise two questions. What is the underlying mechanism that allows cohesin to bind chromatin,
and how is this binding controlled spatially and temporarily?

A simple idea is that cohesin binds chromatin by binding DNA. In vitro biochemical studies indicate that the purified SMC dimers contain an intrinsic DNA binding activity and that cohesin can weakly bind to double-stranded and single-stranded DNA (Losada & Hirano 2001). Under these in vitro conditions, cohesin does not exhibit any preference to bind a specific DNA sequence but does exhibit some preference for secondary structure. However, the biological significance of these activities has not been established by mutations that block both DNA binding in vitro and chromatin binding in vivo.

The absence of a recognizable DNA binding motif in any of the cohesin subunits suggests a more complex method of binding. Indeed, genetic studies show that chromatin binding in vivo requires all the subunits of cohesin (Michaelis et al. 1997, Tóth et al. 1999). Moreover, cohesin binding to chromatin in vivo is abrogated by natural cleavage of the kleisin subunit or engineered cleavage of Smc3 (Hornig & Uhlmann 2004, Ivanov & Nasmyth 2005). These in vivo studies imply that the assembly of the entire cohesin complex is needed for its stable binding to chromatin.

More detailed structure/function studies reveal that cohesin association with chromatin requires motifs within Smc heads and the head-proximal region of the coiled coils. Mutations have been made in the Walker A and B motifs as well as in the signature motifs of the Smc1 and Smc3 heads to perturb ATP binding and hydrolysis, respectively (Arumugam et al. 2003, Weitzer et al. 2003). These mutations also block cohesin binding to chromatin. These ATP-dependent functions in cohesin binding are not to promote head dissociation, as postulated in the original embrace model, because cohesin binding and cohesion generation still occur when the head domains of Smc1 and Smc3 are tethered in vivo by artificial protein cross-links (Gruber et al. 2006). Intriguingly, an unbiased mutagenesis of Smc1 identified residues near the Loop1 region of the coiled coil of Smc1 as being critical for efficient cohesin binding (Milutinovich et al. 2007). Loop1 is defined as a predicted break in the coiled coil that is proximal to the head. These observations are consistent with a model in which the Smc head domains alter the coiled coil to facilitate chromatin binding.

The hinge also functions in cohesin binding to chromatin. Chromatin binding appears reduced when the hinge domains of Smc1 and Smc3 are tethered in vivo by artificial protein cross-links (see below) (Gruber et al. 2006). Furthermore, five-amino-acid insertions in the hinge are compatible with general chromatin binding but inhibit binding to CARs (Milutinovich et al. 2007). This observation extends the role of the hinge beyond general chromatin binding to the modulation of specific binding. The requirement for specific residues in the hinge, the head, and the head-proximal coiled coil for chromatin binding is fascinating given that the head and hinge lie at opposite ends of the cohesin complex, some 45 nm apart in the soluble structure. This requirement suggests that chromatin binding is achieved by a large energy-dependent change in cohesin conformation.

These observations are consistent with a modification of the original embrace model. In the revised model, cycles of ATP binding and hydrolysis of the head cause conformational changes in the Smc1 and Smc3 hinges, allowing sister chromatin strands to enter inside the ring and be entrapped (Gruber et al. 2006). The head may cause the dissociation of the hinge domains through a direct interaction. Indeed, several independent studies suggest head-hinge interactions for cohesin. A direct head-hinge interaction was implicated by FRET analysis of cohesin subunit interactions (Mc Intyre et al. 2007). In addition, atomic force microscopy of condensin, another Smc complex, reveals folded structures with the head and hinge in close proximity (Yoshimura et al. 2002). Finally, in the bacterial SMC complex, binding of DNA to the hinge stimulates the ATPase of the head in vitro (Hirano & Hirano 2006, Onn et al. 2007).
However, all these observations are also consistent with nontopological modes of DNA binding, like the DNA binding of histones or Mu transposase, in which DNA makes multiple contacts with multiple subunits (Chaconas 1999). DNA binding would be blocked by any mutation that prevents cohesin from assembling into the final active conformation. Indeed, tethering of the hinge domains has been interpreted solely to prevent hinge dissociation, but this tethering may also block any hinge structural change needed for nontopological chromatin binding. Furthermore, the bulky cross-linking method (engineered protein dimerization motifs) may simply preclude the hinge domains from binding another cohesin factor. Precedent that other proteins can bind to the hinge exists for Smc2 of condensin (Chen et al. 2004; Patel & Ghiselli 2005a,b).

The most compelling argument that cohesins binds chromatin by topologically entrapping DNA comes from studies of cohesin bound to purified circular minichromosomes. Cohesin can be released from the minichromosome by a single cleavage of the minichromosome DNA (Ivanov & Nasmyth 2005, 2007). However, this linearization also seems to perturb DNA binding of the centromere-specific nucleosome to a lesser but still significant extent (Ivanov & Nasmyth 2005). Perturbing the robust nucleosome/DNA interaction suggests that the linearization conditions may alter the minichromosome in additional ways beyond just modifying topology. Thus, although the current observations are consistent with a topological mechanism of cohesin binding to chromatin, its definitive demonstration awaits additional approaches, most importantly reconstitution in vitro by a biologically relevant assay.

The Scc2/4 complex is an evolutionarily conserved factor required for cohesin association with chromatin in vivo in all contexts (heterochromatin, CARs, centromeres, etc.) (Ciok et al. 2000, Furuya et al. 1998, Tomonaga et al. 2000, Watrin et al. 2006). Virtually nothing is known about how Scc2/4 loads cohesin onto DNA. Studies of Scc2/Scc4 on the binding of cohesin to DNA in vitro have not been reported. An early bioinformatic study suggested that the Scc2 protein may be a kinase (Jones & Sgouros 2001), and mutants in the Scc2 homolog in fission yeast have greatly reduced Mccl1 (Rad21) phosphorylation (Tomonaga et al. 2000). Yet, to our knowledge, the Scc2/4 complex has not been tested for kinase activity either. Even more enigmatic is the role of Scc4, which is only loosely conserved among eukaryotes. Given our current poor understanding of cohesin binding to chromatin, alternative functions for Scc2/4 may include an activator of Smc ATPase, a facilitator of hinge dimerization, a chromatin binding protein, or a chromatin remodeler.

However cohesin binds chromatin, this binding must be regulated to achieve the complicated spatial and temporal patterns of cohesin-chromosome association. Early studies suggested that the spatial pattern of cohesin binding could be attributed to a passive response to general chromosome properties and activities. As stated above, cohesins do not appear to recognize any specific DNA sequence. In addition, cohesin enrichment at CARs has been postulated to occur by a nonspecific process in which the cohesin ring is pushed along the chromatin by the transcription machinery (Lengronne et al. 2004). However, the notion of cohesin localization by such passive processes is becoming less and less attractive with the discovery of elaborate mechanisms to target cohesin to specific regions of chromosomes.

Underlying chromatin marks are required to target cohesin to many of its known chromosomal binding sites/regions. In fission yeast, histone H3 K9 methylation-dependent recruitment of the heterochromatin-associated protein Swi6 (HP1) directs the assembly of pericentromeric cohesin domain (Bernard et al. 2001, Hall et al. 2003, Nonaka et al. 2002, Partridge et al. 2002). Sir2, the histone deacetylase, is required for cohesin binding at HMR (Chang et al. 2005). Furthermore, damage-induced phosphorylation of the histone variant H2AX proximal to the DSB is important for the formation of damage-induced cohesin domains.
Cohesion maintenance: the persistence of sister chromatid cohesion from its establishment in S phase until its dissolution at anaphase onset

(Figure 3b) (Unal et al. 2004). In humans, cohesin is recruited to Alu repeats, and binding is influenced by DNA methylation (Hakimi et al. 2002). Finally, both Rsc and ISWI chromatin remodelers have been implicated in cohesin loading (Baetz et al. 2004, Hakimi et al. 2002, Huang et al. 2004, Yang et al. 2004). Thus, cohesin appears to be targeted to specific chromosomal regions by a code of evolutionarily conserved histone modifications and local chromosome structure. With this in mind, it will be interesting to revisit the mechanism that targets cohesin to CARs.

Cohesins also are recruited to specific chromosome addresses by distinct protein complexes. The cohesin domain assembled around a DSB requires the MRX complex bound to the broken end as well as the large domain of H2AX phosphorylation (Unal et al. 2004). In budding yeast, conserved centromere proteins assemble on a 300-bp CEN sequence and then nucleate cohesin binding over a large pericentric domain (Weber et al. 2004). Thus, in these two contexts the MRX and CEN complexes nucleate cohesin binding at both proximal and distal chromatin sites. Another example is the Rep proteins, which recruit cohesin to the STB locus of the extrachromosomal 2-μ plasmid in yeast (Yang et al. 2004). Rep recruitment of cohesin may foreshadow how cohesin is recruited by other site-specific chromatin-binding proteins like transcription factors. This notion is attractive given the emerging role of cohesin in transcription regulation (Dorsett 2007). In summary, cells have elaborate mechanisms specifically dedicated to the temporal and spatial regulation of chromatin binding of cohesin. This complexity enables cohesin to perform diverse biological functions.

Generation of Cohesion: Making Chromatin-Bound Cohesins Become Cohesive

Once cohesin is chromatin bound, it must become cohesive, tethering together only sister chromatids. Numerous observations support the idea that cohesin can exist in two chromatin-bound states, cohesive and noncohesive. In most eukaryotes, cohesins bind to chromosomes prior to DNA replication at the G1/S transition or in telophase of the previous cell cycle (Guacci et al. 1997, Losada et al. 1998, Michaelis et al. 1997, Sumara et al. 2000, Tomonaga et al. 2000). Because in these phases of the cell cycle there is no sister chromatid, cohesins must be able to bind chromatin independently of being cohesive. Also, although cohesins generate cohesion when bound to CARs during DNA replication, they fail to generate cohesion when loaded onto CARs in G2/M (Figure 3a) (Haering et al. 2004, Lengronne et al. 2006, Strom et al. 2004, Uhlmann & Nasmyth 1998). Thus, cohesin binding to chromatin is not sufficient to generate cohesion even when sister chromatids are in close proximity. However, cohesins bound to chromatin in G2/M become cohesive when the cell suffers a DSB in one of its chromosomes (Figure 3b) (Strom et al. 2007, Unal et al. 2007). These results suggest that cohesion requires two distinct steps. Cohesin first binds chromatin in a noncohesive state and then matures to a cohesive state. We define this second post-chromatin-binding step as cohesion generation. Cohesion generation is regulated by cell cycle progression and DNA damage.

Perhaps the most compelling evidence for a distinct step for cohesion generation came with the discovery and characterization of the evolutionarily conserved protein Eco1/Ctf7. Eco1 is not needed for chromatin binding of cohesin in S phase at pericentric regions, at CARs, or around a DSB (Milutinovich et al. 2007, Noble et al. 2006, Skibbens et al. 1999, Tóth et al. 1999). Eco1 is critical to generate cohesion in all cases (Figure 3), and inactivation of Eco1 in G2 does not abrogate cohesion once it is formed (Skibbens et al. 1999, Tóth et al. 1999). Thus, Eco1 acts after chromatin binding to help cohesin become cohesive but is dispensable for maintaining cohesion.

The activity of Eco1 responsible for cohesion generation remains to be elucidated. Eco1 contains a noncanonical C2H2 zinc finger (Ivanov et al. 2002). Eco1 also contains
acetyl transferase activity. In vitro it can acetylate itself as well as Med1, Scc3, and Pds5, but not histones or PCNA (Ivanov et al. 2002).

To date, mutations in these two domains of Eco1 are consistent with a model in which the acetyl transferase performs a regulatory function whereas the zinc-finger domain is responsible for cohesion generation, perhaps through direct interaction with cohesin (Brands & Skibbens 2005, Ivanov et al. 2002, Unal et al. 2007).

However, this conclusion needs to be substantiated. First, Eco1 has not been localized to CARs or shown to bind to cohesin, as might be expected of a structural component. The evidence that the acetyl transferase has a regulatory function is based on phenotypic analysis of mutations that significantly reduce acetyl transferase activity of the recombinant protein in vitro (Ivanov et al. 2002). These eco1 mutants do not impair the generation of cohesion in vivo during S phase but do block cohesion induction by a DSB (Unal et al. 2007). However, if these acetyl transferase mutants retain some activity in vivo, then these results will not eliminate a critical role for acetylation in cohesion generation during S phase. This supports a model in which different levels of acetyl transferase activity are required for S phase and DSB-induced cohesion. If it turns out that the acetyl transferase activity is required for cohesion generation, then identifying the critical Eco1-acetylated residues of cohesin should be particularly insightful in understanding cohesion generation.

How might an activity(s) of Eco1 promote chromatin-bound cohesin to become cohesive? We can envision two types of models. In one, the initial chromatin-bound form of cohesin is primarily in the noncohesive state, and Eco1 helps promote its transition to the cohesive state. In the second, chromatin-bound cohesin reversibly switches between its noncohesive state and its cohesive state. Thus, cohesion is established but is maintained too transiently to be effective. Eco1 activity would change cohesin so that it is trapped in its cohesive state. The value of these models is that they make testable predictions and provide explanations to apparent dilemmas in the field.

One of the attractions of the embrace model is its inherent simplicity (Figure 2). Cohesion occurs by a single embrace of both sisters or by sequential embrace of first one and then the other sister chromatid. In either case, cohesion is the result of a single biochemical activity, chromatin binding by opening and closing the ring. However, the embrace model provides no explanation for either the existence or necessity of a second Eco1-dependent step after cohesin binds chromatin. In addition, the embrace model predicts that cohesin binding to chromatin in G2/M should topologically trap both sister chromatids (held in juxtaposition by cohesion generated in S phase) and generate additional cohesion. Yet cohesin binding in G2/M in an unperturbed cell fails to generate additional cohesion (Haering et al. 2004, Lengronne et al. 2006, Strom et al. 2004, Uhlmann & Nasmyth 1998), again implying the existence of an extra step not in the embrace model.

The essence of the embrace model can be salvaged if cohesin readily switches between embracing chromatids (the cohesive chromatin-bound state) and releasing chromatids (the noncohesive chromatin-free state). Eco1 would modify cohesin after embracing the two sister chromatids to lock it in the chromatin-bound cohesive state. If Eco1 activity were limiting in G2/M, cohesins would bind chromatin but be unable to be locked in the cohesive state. Indeed, Eco1 does appear to be limiting because its overexpression in G2/M allows cohesion even without a DSB (Unal et al. 2007). This modified embrace model predicts that manipulating Eco1 activity should alter the off rate of cohesin from chromatin.

Oligomerization models posit that cohesion is generated when two or more chromatin-bound cohesins associate. Hence these models have an intrinsic requirement for a second step for cohesion after chromatin binding of cohesin that may be facilitated by Eco1 activity. A simple idea is that the coiled coils of cohesin undergo a conformation change to present interaction surfaces like the hinge or
the coiled coils themselves for intercomplex association (Figure 2). Studies of other Smc and SMC-like complexes provide precedent for conformational changes in the coiled coil. In the Smc-like Rad50 complex, coiled coils undergo a dramatic conformation change upon binding to DNA, converting from a floppy rod to a rigid rod (Moreno-Herrero et al. 2005). Condensin may undergo significant changes in conformation, as evidenced by increased susceptibility of its Smc2 subunit to in vitro proteases upon ATP binding (Onn et al. 2007). In principle, Eco1 may either facilitate a conformational change in cohesin structure or stabilize dimerization after the conformational change has occurred.

The main criticism of oligomerization models for cohesion is the absence of evidence for biologically relevant cohesin oligomers. Although rare oligomers of purified Smc complexes can be observed either by microscopy or by biochemical methods (Haering et al. 2002, Hirano et al. 2001, Yoshimura et al. 2002), these oligomers can be attributed to artificial aggregation. However, the failure to recover soluble cohesin oligomers can easily be explained if they form only on chromatin as a way to ensure that cohesion occurs in the proper temporal/spatial context (see below). Chromatin binding may be a prerequisite for becoming cohesive by restricting Eco1 activity to chromatin-bound cohesin. If this is the case, manipulating Eco1 activity to make it active on the soluble complex may reveal changes in cohesin oligomerization that are otherwise difficult to detect.

Understanding the mechanism of cohesion generation does not explain how this cohesion is restricted to the relevant sister chromatid. In other words, given the global genome binding of cohesin, cohesins should tether any nearby chromatin, including unrelated chromosomes, and distant positions on the same chromosome. In the original version of the embrace model, cohesin embraces chromatin in G1, and DNA replication passes through the cohesin ring, ensuring the tethering of only sister chromatids (Haering et al. 2002). However, this replication-dependent model for specificity now seems unlikely because a DSB in G2/M induces sister chromatid cohesion without DNA replication (Strom et al. 2007, Ünal et al. 2007).

Alternatively, specificity can be achieved in S phase by the activation of chromatin-bound cohesin to become cohesive only near the replication fork (Milutinovich et al. 2007, Moldovan et al. 2006, Skibbens et al. 1999). The chromatin-bound cohesin(s) will preferentially tether the other sister chromatid by virtue of its proximity. In oligomerization models upon DNA replication, both newly synthesized CARs bind a cohesin(s). Fork-dependent activation of these cohesins would favor their oligomerization. Indeed, mutations in a number of replication factors exhibit partial cohesion defects, and direct physical and functional interactions between PCNA and Eco1 have been established (Bermudez et al. 2003; Kenna & Skibbens 2003; Moldovan et al. 2006; Skibbens et al. 1999, 2007). Whether Eco1 itself actually travels with the fork or might be activated by its passage is unclear. In a proximity model, DSB-induced cohesion in G2/M occurs between sister chromatids because their proximity is ensured by the preexisting cohesion established in S phase.

The mechanisms for generating sister chromatid cohesion remain major unsolved questions in the field. Above, we discuss observations in the context of the embrace and oligomerization models. However, these models are not mutually exclusive, and the real mechanism may well be a hybrid of the two (Figure 2). The ability to dissect cohesion generation would be greatly facilitated by the reconstitution of cohesion in vitro with purified components. With the knowledge that in vivo cohesion can be generated independently of DNA replication (Strom et al. 2007, Ünal et al. 2007), reconstitution of in vitro cohesion no longer requires in vitro DNA replication. Thus, developing cohesion in vitro should be feasible and represent an important future direction.

Although the cohesin-dependent mechanism is a major contributor to sister chromatid cohesion, it does not appear to be the sole source of cohesion. Inactivation of cohesin
never causes complete dissolution of chromatid pairing, suggesting the existence of alternative cohesion pathways. Recent evidence suggests that condensin complexes, ORCs (origin recognition complexes), and centromere complexes mediate cohesion through cohesin-independent mechanisms (Lam et al. 2006, Monje-Casas et al. 2007, Shimada & Gasser 2007, Suter et al. 2004). In addition, DNA catenation may contribute to cohesion (Díaz-Martínez et al. 2006, Uemura et al. 1987). However, it is important to remember that topoisomerase II will catenate as well as decatenate any DNA molecules held in close juxtaposition in vitro. Therefore, the persistence of catenation until anaphase may be a consequence of cohesin-mediated cohesion rather than a mechanism to generate cohesion. Insight into the establishment, maintenance, and dissolution of cohesion by these alternative pathways would make important contributions to our understanding of chromosome transmission.

**Cohesion Maintenance and Dissolution**

The cohesion established in S phase must be maintained until anaphase onset. After every pair of sister chromatids achieves bipolar attachment, cohesion is dissolved, and sisters segregate. In principle, the maintenance of cohesion should require only that the cohesins remain stably bound and cohesive after S phase and that the trigger for cohesion dissolution be inactive until anaphase onset. However, studies in vertebrates reveal a process called prophase removal, exposing another layer of regulation (Figure 4) (Giménez-Abián et al. 2004, Losada et al. 2000, Sumara et al. 2000, Waizenegger et al. 2000). In early prophase, each pair of sister chromatids initially appears as a single unresolved rod, consistent with the extensive cohesion along the arms and around the centromere. During prophase, cohesins begin to disassociate from the arms so that by metaphase only a small fraction of cohesin remains bound. Concomitant with cohesin loss, the cohesion

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**Figure 4**

Early prophase chromosomes with cohesin in its cohesive state. For simplicity, we omit any chromatin-bound cohesins in the noncohesive state at early prophase, as depicted in Figure 3. During late prophase, Polo phosphorylation of SA2, the vertebrate isoform of Scc3, along with the action of Wapl convert most of the arm cohesins to the noncohesive form. These noncohesive cohesins ultimately dissociate from chromosomes by metaphase. Sgo and protein phosphatase 2A (PP2A) oppose Polo/Wapl, primarily at centromeric loci, to preserve the cohesive state and maintain cohesion at the centromeric loci. Complete dissolution of cohesion at anaphase onset is triggered by separase-mediated cleavage of the cohesin subunit Mcd1p in a subset of cohesins and potentially by other cleavage-independent mechanisms.
between the arms is reduced but not eliminated, as they now appear as closely associated parallel rods. The pericentric cohesin is protected from prophase removal, and cohesion around the centromere remains robust, maintaining the single-rod appearance. The spatial and temporal changes in cohesin/cohesion during prophase removal were the first hints that the maintenance of cohesion was a complex and highly regulated process.

Significant progress has been made in understanding the regulation of prophase removal from sister chromatid arms. Initial studies showed that vertebrate Polo-like kinase 1 (Plk1) is the primary kinase required for prophase removal (Losada et al. 2002, Sumara et al. 2002). Two vertebrate cohesin subunits, SA2 (a mammalian Scc3 isoform; see Table 1) and RAD21/MCD1, are Plk1 substrates, but SA2 is the key target for prophase removal (Hauf et al. 2005). SA2 phosphorylation occurs during mitosis, and cohesin driven from chromosomes during prophase removal contains phosphorylated SA2 (Hauf et al. 2005, Kueng et al. 2006). More definitively, cells expressing SA2-12xA, an allele that lacks Plk1 phosphorylation sites, are inhibited for prophase removal (Hauf et al. 2005). Consequently, these metaphase chromosomes now contain high levels of cohesin on the arms as well as pericentric regions and maintain arm cohesion after prolonged metaphase arrest. Prophase removal also requires an evolutionarily conserved protein called Wap1. In Wap1-depleted cells, prophase removal is blocked, and arm cohesion is stabilized (Gandhi et al. 2006, Kueng et al. 2006). In these cells phosphorylated SA2 remains bound to metaphase chromosomes (Kueng et al. 2006). Thus, SA2 phosphorylation is not sufficient for prophase removal but rather works either in conjunction with or upstream of Wap1.

If cohesins bound to pericentric regions and along the arms are structurally equivalent, why are pericentric cohesins not inactivated by prophase removal? The initial breakthrough came from Drosophila and then subsequent elegant meiotic studies in fission and budding yeast (Kerrebrock et al. 1995, Watanabe 2005). The centromeric MEIS-322/Sgo1 family of proteins is required for centromeric cohesion during meiosis I and for cohesion in metaphase-arrested cells. Depletion of human SGO1 leads to the complete loss of both cohesin and cohesion along the entire length of the chromosomes during early mitosis (McGuinness et al. 2005, Salic et al. 2004). Sgo1 also localizes predominantly to centromeric regions, and when Sgo1 is mislocalized to chromosome arms by depletion of the Bub1p kinase, arm cohesion becomes more robust (Kitajima et al. 2005, Salic et al. 2004, Tàng et al. 2004). Therefore, human Sgo1p preserves cohesin binding to chromosomes and sister cohesion wherever it localizes. Because the S42-12xA allele suppresses the effects of SGO1 depletion, Sgo1 appears to act upstream of this phosphorylation (McGuinness et al. 2005).

On the basis of these results and additional meiotic studies, a simple model is emerging for the protection of pericentric cohesion in mitosis (Figure 4). Enrichment of Sgo1 and an associated phosphatase at the pericentric regions allows local removal of Polo-dependent phosphorylation of SA2, protecting cohesin from Wap1-dependent removal (Clarke & Orr-Weaver 2006, Kitajima et al. 2006, Riedel et al. 2006). This simple model will likely need additional modification to account for newly identified components like sororin, which appears to be important to protect prophase cohesion (Díaz-Martínez et al. 2007, Rankin et al. 2005, Schmitz et al. 2007). Moreover, recent evidence suggests that Wap1 contributes to the turnover of cohesin binding in interphase, indicating that this pathway may function outside the context of prophase (Kueng et al. 2006). Finally, budding and fission yeast cells defective for Sgo have only minor defects in cohesin maintenance during mitosis (Katis et al. 2004, Kitajima et al. 2004). This difference between yeasts and other eukaryotes underscores that it may not be possible to generate simple models that are valid for all organisms.

Although our understanding of prophase removal regulation has advanced significantly, the actual mechanism of prophase removal remains
A mystery. A key to unlocking this mystery may be Pds5p, a protein conserved from yeast to humans (Hartman et al. 2000, Sumara et al. 2000, Tanaka et al. 2001, van Heemst et al. 1999). When Pds5p function is abrogated, cohesion is still established but cannot be maintained, despite the fact that cohesins remain chromatin bound (Dorsett et al. 2005, Hartman et al. 2000, Losada et al. 2005, Stead et al. 2003, Tanaka et al. 2001). Pds5p associates with cohesin, Wapl, Eco1, and sororin (Gandhi et al. 2006, Kueng et al. 2006, Noble et al. 2006, Rankin et al. 2005, Sumara et al. 2000). One possibility is that the common interaction of these factors with Pds5 coordinates their functions to toggle cohesin between cohesive (Eco1 and sororin) and noncohesive (Wapl) states. Validating these and other mechanisms for prophase removal will require a better understanding of the functional significance of the interactions between Pds5, Wapl, and sororin.

The cohesion protected from prophase removal must be completely dissolved at anaphase onset to enable sisters to segregate to opposite poles (Figure 4). An early insight into cohesion dissolution at anaphase came with the identification of Pds1p and Cut2, founding members of the securin family (Funabiki et al. 1996a, Yamamoto et al. 1996a). Securins inhibit cohesion dissolution and the metaphase-to-anaphase transition (Funabiki et al. 1996b, Yamamoto et al. 1996b). To initiate anaphase, the anaphase-promoting complex triggers the degradation of securin, releasing its binding partner separase, a caspase-like protease (Ciosk et al. 1998, Cohen-Fix et al. 1996, Funabiki et al. 1996b). Separase cleaves some fraction of Mcd1, which facilitates the inactivation of cohesin and dissolution of cohesion (Hauf et al. 2001, Tomonaga et al. 2000, Uhlmann et al. 1999, Waizenegger et al. 2000). Although separase is essential for cohesion inactivation, a number of observations suggest that separase is unlikely to act alone (Guacci 2007). The efficiency of these putative alternative pathways may explain the range of sister chromatid separation observed upon separase depletion in different organisms (Ciosk et al. 1998, Funabiki et al. 1993, Giménez-Abian et al. 2005, Wirth et al. 2006). Indeed, given that the persistence of even a little cohesion causes defects in chromosome segregation, it is likely that the cell uses multiple mechanisms to ensure the complete inactivation of all cohesion (Guacci 2007).

THE BIOLOGICAL FUNCTION OF COHESION AND COHESIN

Chromosome Segregation

Early cytological observations suggested that sister chromatid cohesion was critical for proper chromosome segregation in mitosis. As new genetic and cell biological tools became available, this function of cohesion was the first to be tested and dissected. Indeed, the precise separation of sister chromatids is induced by mutations that inactivate cohesin, cohesin regulators, or cohesion auxiliary factors (Guacci et al. 1997, Michaelis et al. 1997, Yamamoto et al. 1996a). In all cases, chromosome nondisjunction and aneuploidy ensue, consistent with a failure to achieve proper bipolar attachment of sister kinetochores to the mitotic spindle. Subsequently, a defect in bipolar attachment was demonstrated visually in cohesin mutants of budding yeast and Xenopus egg extracts depleted for cohesin function (Dewar et al. 2004, Tanaka et al. 2000). Taken together, these observations demonstrate an evolutionarily conserved role for cohesion in the bipolar attachment of sister kinetochores.

It is intriguing that chromosomes have a large pericentromeric domain with high-density cohesin binding, and mutations that lower this density cause elevated errors in chromosome disjunction (Bernard et al. 2001, Eckert et al. 2007). Pericentric cohesion appears to facilitate bipolar attachment of sister kinetochores, in part by sterically constraining the orientation of the sister kinetochores (Eckert et al. 2007). In addition, bipolar attachment of paired sister chromatids generates tension when microtubule-based forces move the sister kinetochores poleward.
Tension triggers a signal that stabilizes the microtubule-kinetochore interaction, locking in the bipolar attachment (Nicklas & Koch 1969, Pinsky & Biggins 2005). The pericentric cohesion may contribute to tension sensing of the kinetochore by increasing the resistance to poleward stretching.

Organisms with longer chromosomes have larger pericentric cohesin domains and tend to have kinetochores with increased numbers of microtubule attachments. The increase in pericentric cohesion is likely a necessity to respond to increases in tension or steric complexities associated with larger chromosomes. Even within an organism, individual chromosomes exhibit significant differences in the size of the pericentric cohesin domains, which may change the efficiency of generating a bipolar attachment and the spontaneous rate of chromosome aneuploidy (Weber et al. 2004). Thus, the size of the pericentric domain may contribute to the elevated frequency of aneuploidy for specific chromosomes in birth defects and cancer. With these intra- and interspecies differences in the size of pericentric cohesion, it will be interesting to use emerging tools to modulate systematically the size of the domain on an individual chromosome and monitor changes in chromosome transmission (Eckert et al. 2007).

Changes in cohesin and cohesion in meiosis I play a critical role in altering the pattern of chromosome segregation (Chelysheva et al. 2005, Klein et al. 1999, Parra et al. 2004, Watanabe & Nurse 1999). Meiotic cells express an additional cohesin complex in which Rec8, a meiotic-specific isoform of the kleisin subunit, replaces most, but not all, of the Mcd1. Meiotic (Rec8) cohesin along with other factors alter sister kinetochores such that they co-orient to form a monopolar rather than a bipolar attachment to the meiosis I spindle (Monje-Casas et al. 2007, Tóth et al. 2000, Watanabe & Nurse 1999). An intriguing idea is that in at least some organisms, meiotic cohesion causes a unique temporal or spatial juxtaposition of sister kinetochores, which promotes their maturation into a single unified structure (Yokobayashi & Watanabe 2005).

Changes in the dissolution of meiotic cohesion are also critical to ensure proper sister chromatid segregation in meiosis II. Unlike mitosis, in which all cohesion is dissolved in anaphase, pericentric cohesion is not dissolved in anaphase I, allowing such cohesion to persist until anaphase II (Klein et al. 1999, Watanabe & Nurse 1999). This cohesion ensures that sister chromatids make a bipolar attachment and segregate to opposite poles in the second meiotic division. MeiS322/Sgo1, which protects pericentric cohesion from the anaphase I dissolution machinery, mediates the persistence of pericentric cohesion (Katis et al. 2004, Kerrebrock et al. 1995, Watanabe 2005). Interestingly, the same master kinase, Aurora, controls these critical changes in meiotic cohesion (Monje-Casas et al. 2007, Yu & Koshland 2007).

DNA Repair

In principle, the segregation function of cohesion in mitosis could be mediated solely by the pericentric cohesion. Thus, the existence of arm cohesion led to the supposition that cohesion must be seen in processes other than chromosome segregation (Koshland & Guacci 2000). Indeed, additional functions for arm cohesion have been identified by the use of genetic and cell biological tools derived from cohesins and their regulators. Cohesin mutants are sensitive to different DNA-damaging agents, including gamma irradiation, camptothecin, and hydroxyurea (Birkenbihl & Subramani 1992, S.T. Kim et al. 2002, Schar et al. 2004). This sensitivity supported the idea that sister chromatids improve the efficiency of DNA repair by ensuring the close proximity of a template for homologous recombination (Kadyk & Hartwell 1992, Sjögren & Nasmyth 2001). It was initially thought that this proximity was generated by the repair-independent cohesion generated during S phase, the same cohesion that is critical for chromosome segregation.

However, it is now clear that at least the efficient postreplicative repair of DSBs requires additional cohesion generated via a specific and
complex cellular response to damage (J.S. Kim et al. 2002, Strom et al. 2004, Ünal et al. 2004). In yeast, the master DNA damage regulators, Mec1 (ATM) and Tel1 (ATR), phosphorylate H2AX over a large domain around the DSB, making it permissive for cohesin loading (Ünal et al. 2004). MRX bound at the break site stimulates cohesin loading on the domain of H2AX phosphorylation. In G2/M, Mec1 and MRX act in a distinct process to promote cohesion generation around the break site and genome wide through the activation of Eco1 in G2 (Strom et al. 2007, Ünal et al. 2007). Why Eco1 is unable to generate cohesion in G2/M in undamaged cells remains to be elucidated. Cohesion around the break site apparently promotes efficient repair from the sister chromatid (Strom et al. 2004, Ünal et al. 2004, Xie et al. 2004). This restriction suppresses ectopic repair through recombination with the homolog or disperse repetitive sequences elsewhere in the genome. Importantly, suppression of ectopic recombination reduces loss of heterozygosity, translocation, and internal deletions.

Chromosome Morphogenesis

Phenotypes of cohesin mutants have also implicated cohesins in mitotic and meiotic chromosome morphogenesis. In budding and fission yeast, changing the levels of cohesins causes hypo- or hypercondensation (Ding et al. 2006, Guacci et al. 1997, Hartman et al. 2000). This has led to the idea that cohesin binding may define boundaries for the condensation machinery. Perturbing cohesin function in higher eukaryotic cells or Xenopus egg extracts appears to have subtler effects on chromosome condensation (Kuang et al. 2006, Losada et al. 2002, Sonoda et al. 2001). Thus, cohesin may not be an absolute requirement for condensation in larger eukaryotes. Indeed, cohesin-independent mechanisms for condensation have now been revealed in budding yeast as well (Lavoie et al. 2004). However, no experiment in any organism has yet shown that cohesin-independent condensation can support chromosome segregation. The amount of compaction in the absence of cohesins may be unchanged, but the quality of compaction may be less ordered, compromising segregation. Ultimately testing the contribution of cohesion to functional condensation in vivo awaits the identification of cohesin alleles that are defective only for condensation but not for cohesion.

In meiosis, the presence of chromatin-bound cohesins is essential to the assembly of the synaptonemal complex, a protein structure that forms between homologs and is critical for meiotic chromosome pairing, synapsis, and recombination (Klein et al. 1999). This observation sets a precedent for cohesins as a chromosomal platform for the assembly of other macromolecular complexes. In this light the large domain of cohesins bound around a DSB may be a platform for the assembly of an as-yet-undiscovered macro complex that modulates DNA repair.

Transcription

The functions of cohesins, as potential boundary elements and platforms for complex assembly, may be used to modulate transcription as well. The first studies to link a cohesin factor with gene expression came from studies in flies and yeast. The Drosophila Scc2 homolog, Nipped-B, is required for long-range activation of the homeobox genes (Rollins et al. 2004). In Saccharomyces, cohesins act as boundary elements to limit the spreading of transcriptional silencing at the silent mating cassette (Donze et al. 1999). In zebrafish, the functions of Rad21 and Smc3 are needed for proper expression of runx3 runx1 genes in early embryonic development (Horsfield et al. 2007). In humans, the Scc3 homolog SA2 activates a multimeric NF-kappaB reporter construct and enhances the activity of the transactivation domain of p65/RelA (Lara-Pezzi et al. 2004). Elucidating the molecular mechanism for cohesin modulation of transcription is a critical new direction in cohesin biology. In particular, it will be important to determine whether the transcription functions of cohesin reflect novel activities distinct from its ability to tether chromatin.
CONCLUSION
The field of sister chromatid cohesion has exploded, revealing remarkable complexity in the mechanism and regulation of cohesion and in the range of biological functions for cohesin. At first blush this complexity is intimidating. Solving how cohesins bind chromatin or generate cohesion has already seemed daunting because of the sophisticated architecture of cohesins and their chromatin substrate. Answering these questions seems even more difficult now because the kleisin subunit and the domains of the Smc proteins each are likely to have multiple biochemical/structural activities. Furthermore, chromatin binding and/or cohesion generation are likely to involve a large change in the conformation of the complex. This structural change mandates that even previously simple hypotheses like the embrace or oligomerization models become more complex. Similarly, the complexity of the regulation and the diverse role of cohesion in chromosome biology complicate the interpretation of in vivo analyses. For example, a number of exciting studies have begun to associate defects in cohesion factors with human genetic disorders, including cancer and developmental diseases like Cornelia de Lange syndrome (Barber et al. 2008, Dorsett 2007). It is unclear whether these disorders arise from perturbing the transcription, chromatid cohesion, chromosome segregation, or DNA repair functions of cohesin.

However, the studies that revealed the mechanistic complexity of cohesion have also provided novel technical tools. For example, the fields of DNA replication and transcription were greatly advanced when they were studied in the specialized context of viruses. Similarly, studying the biochemical activities of cohesin in the specialized context of a DSB, on the 2-μ extrachromosomal element, or at homeobox promoters may provide easier readouts than does chromosome segregation to address mechanistic questions using both in vivo and in vitro approaches.

The emerging biological complexity may also inspire researchers to entertain new concepts to address previously perplexing observations. For example, initially it was thought that prophase removal in mitotic cells might act as a preemptive strike, reducing the amount of cohesin that must be inactivated at anaphase. However, mutant cells incapable of prophase removal undergo apparently normal anaphase (Gandhi et al. 2006, Hauf et al. 2005, Kueng et al. 2006). In the absence of a mitotic segregation function, the reason for prophase removal has been a mystery. The solution to this mystery may come from the newly discovered roles for cohesin in transcription and DNA repair. These processes may be inhibited by cohesion and may hence need cohesin removal to inactivate cohesion. Alternatively, these processes may need to generate cohesion at a new location in the genome. This new cohesion may form from a soluble cohesin pool that is generated by cohesin removal. The idea that cohesion is dynamic, remodeling to accommodate different chromosome biology, is consistent with the recent observation that cohesin removal is not limited to prophase (Kueng et al. 2006). Thus, in the end, the current biological and mechanistic complexities offer tremendous technical and conceptual tools that will lead to rapid clarification rather than confusion—so much so that much in this review will be soon obsolete.

SUMMARY POINTS
1. Sister chromatid cohesion is mediated by cohesin, a four-subunit SMC (structural maintenance of chromosome) complex, and a growing list of cohesin auxiliary factors.
2. Cohesion and its factors are essential for diverse biological processes including chromosome segregation, DNA repair, gene expression, and development.
3. Cohesin subunits can associate through multiple surfaces in the Smc subunits and non-Smc subunits that are potentially regulated through the Smc ATPase domains.

4. The complex architecture of cohesin has been used to generate dramatically different mechanisms for both chromatin binding and the generation of cohesion.

5. The chromatin binding of cohesin is regulated temporally and spatially by elaborate mechanisms to enable cohesin to perform diverse biological functions.

6. The chromatin-bound cohesin matures from a noncohesive state to a cohesive state. This conversion is regulated both during the cell cycle and in response to DNA damage.

7. The maintenance of cohesion is a dynamic process involving restricted dissolution along chromosome arms during prophase as well as de novo cohesion generation after DNA damage in G2/M.

8. Solving the mechanism of cohesion and its complex regulation presents significant challenges but offers the potential to provide important insights into higher-order chromosome organization and chromosome biology.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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