

# Cohesinopathies, gene expression, and chromatin organization

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The cohesin protein complex is best known for its role in sister chromatid cohesion, which is crucial for accurate chromosome segregation. Mutations in cohesin proteins or their regulators have been associated with human diseases (termed cohesinopathies). The developmental defects observed in these diseases indicate a role for cohesin in gene regulation distinct from its role in chromosome segregation. In mammalian cells, cohesin stably interacts with specific chromosomal sites and colocalizes with CTCF, a protein that promotes long-range DNA interactions, implying a role for cohesin in genome organization. Moreover, cohesin defects compromise the subnuclear position of chromatin. Therefore, defects in the cohesin network that alter gene expression and genome organization may underlie cohesinopathies.

## Introduction

During the cell cycle, sister chromatids are held together from the time of their replication until the time of their separation, which occurs at the metaphase to anaphase transition. This cohesion, especially at centromeric regions, is essential for chromosome segregation and is mediated by the cohesin protein complex, which is composed of four subunits, Smc1, Smc3, Scc3, and Mcd1/Scc1/Rad21 (Guacci et al., 1997; Michaelis et al., 1997). Smc1 and Smc3 are part of a large family of proteins known as the structural maintenance of chromosomes superfamily that form long antiparallel intramolecular coiled coils. Smc1 and Smc3 fold back on themselves at the hinge domain. The hinge regions of Smc1 and Smc3 interact to form a heterodimer (Fig. 1 A). The N and C termini of each of these protein subunits form an ATPase head domain. Mcd1/Scc1/Rad21 (hereafter Rad21) binds to these head domains to complete the cohesin ring structure. Scc3 binds to the Rad21 subunit of the cohesin ring complex. Based on measurements of the length of the Smc1-Smc3 heterodimer by electron microscopy, the

maximum diameter of the ring is  $\sim 38$  nm (Anderson et al., 2002; Haering et al., 2002).

There are various views as to how the cohesin ring interacts with the chromosomes (Onn et al., 2008). One model, known as the embrace model, predicts that the cohesin ring encircles two sister chromatids (Fig. 1 B). In this case, two 10-nm chromatin fibers could be accommodated in a single ring, which is the form of chromatin in which DNA is wrapped into nucleosomes (Gruber et al., 2003). Another model, known as the handcuff model, dictates that each cohesin ring encircles one sister chromatid, and the interaction between two rings via the Scc3 and Rad21 subunits holds the sister chromatids together (Fig. 1 C; Zhang et al., 2008b). In this case, the chromatin could be in the form of the 30-nm fiber. This latter model would allow for two rings to modulate the interaction of two distant DNA sequences.

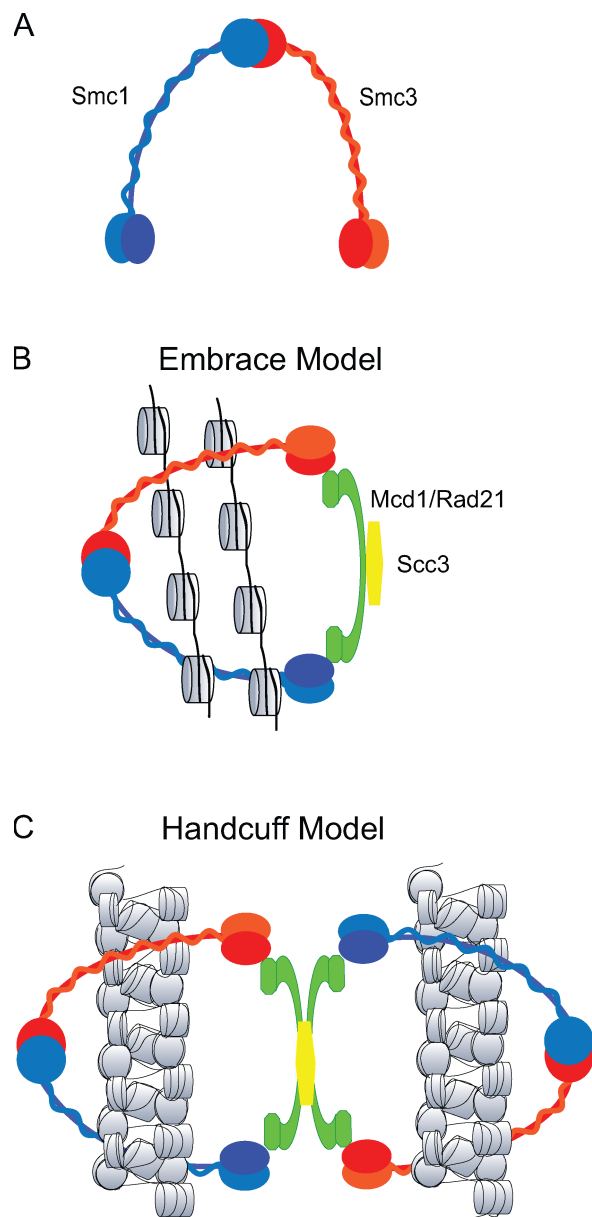
Sister chromatid cohesion is regulated by the activity of different proteins over the course of the cell cycle. Hereafter, we will refer to the cohesin subunits and additional regulators collectively as the cohesin network (Tables I and II). Cohesin is loaded onto chromosomes by the Scc2–Scc4 protein complex (Ciosk et al., 2000; Gillespie and Hirano, 2004; Takahashi et al., 2004) before cohesion is established. In budding yeast, this occurs in G1, whereas in vertebrate cells, it occurs in telophase (Ciosk et al., 2000; Gillespie and Hirano, 2004; Takahashi et al., 2004). Although an initial report in budding yeast suggested that Scc2 and cohesin do not colocalize, more recent reports in budding yeast, fission yeast, and flies suggest that Scc2-binding sites do in fact substantially overlap with cohesin-binding sites (Misulovin et al., 2008; Kogut et al., 2009; Schmidt et al., 2009). Scc2 is also required in G2/M to establish cohesion in response to a DNA double-strand break (Ström et al., 2007).

The Scc2–Scc4 complex loads cohesin onto chromosomes before cohesion is established. However, Eco1 is required to establish sister chromatid cohesion during DNA replication (Skibbens et al., 1999; Tóth et al., 1999). Eco1 is an acetyltransferase (Ivanov et al., 2002), and the acetylation of Smc3 by Eco1 appears to be critical for establishing cohesion (Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a; Rowland et al., 2009) and promoting replication fork progression

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Abbreviation used in this paper: 3C, chromosome conformation capture.

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**Figure 1. The four subunits of the cohesin complex form a ring structure.** The manner in which this ring interacts with DNA is a matter of debate. (A) Both Smc1 and Smc3 fold back on themselves to form intramolecular interactions. They associate to form a heterodimer via their hinge domains. (B) The Smc1-Smc3 heterodimer binds to Rad21 and Scc3, forming a ring. The cohesin ring is shown embracing two sister chromatids, drawn as 10-nm fibers, to mediate cohesion. (C) The cohesin ring may encircle a single sister chromatid and interact with a second ring containing the other sister to mediate cohesion. In this model, the DNA could be accommodated as a 30-nm fiber. The interaction between rings could be mediated through Rad21 and Scc3, a model known as the handcuff model.

(Terret et al., 2009). Eco1, like Scc2, is also required in G2/M to establish cohesion in response to a DNA double-strand break (Ström et al., 2007; Unal et al., 2007).

A different protein, Pds5, helps maintain sister chromatin cohesion. This protein appears to associate with the cohesin complex during the G2/M phase of the cell cycle and coimmunoprecipitates with cohesin subunits in vertebrates (Sumara et al., 2000; Losada et al., 2005). However, it is not considered a

bonafide subunit of the cohesin complex in yeast because of the salt-sensitive nature of the association (Panizza et al., 2000). Although Pds5 is necessary to maintain cohesion, it has more recently been shown to exist in protein complexes with Wpl1, which can negatively regulate cohesion (Kuong et al., 2006; Rowland et al., 2009; Shintomi and Hirano, 2009; Sutani et al., 2009), suggesting that Pds5 has the ability to both promote and dissolve cohesion. Many additional proteins are part of the cohesin network (for review see Xiong and Gerton, 2010).

### Cohesinopathies

Human diseases caused by mutations in genes associated with the cohesin network are termed cohesinopathies. Roberts/SC phocomelia syndrome is caused by mutation of both alleles of *ESCO2* (Vega et al., 2005), the human orthologue of yeast *ECO1*. In most cases, the mutations are truncating, but at least two mutations that disrupt the acetyltransferase activity of the protein have been identified (Vega et al., 2010). Cornelia de Lange syndrome is caused by point mutations or small deletions/insertions in one of the two alleles of *SMC1*, *SMC3*, or most commonly, *NIPBL*, the human orthologue of *SCC2* (Krantz et al., 2004; Tonkin et al., 2004; Musio et al., 2006; Deardorff et al., 2007). Although mutations in the cohesin network might be expected to generate defects in chromosome segregation and/or the ability to repair DNA, mutations that affect these functions of the network are probably lethal and have not been reported. Instead, the cohesinopathies are characterized by a variety of developmental defects, including growth and mental retardation, limb deformities, and craniofacial anomalies (Liu and Krantz, 2008). These phenotypes are taken as an indication that the cohesin network is involved in gene expression during embryogenesis.

Patients with Roberts syndrome tend to have both upper and lower limb defects, mental retardation, and craniofacial defects that include microcephaly, ear malformation, cleft lip and palate, and an undersized jaw (micrognathia; Vega et al., 2010). One unique and invariant feature of the chromosomes in cells from Roberts/SC phocomelia patients is a characteristic puffing or repulsion of heterochromatic chromatin regions (Tomkins et al., 1979). Although this resembles a cohesion defect, it does not appear to translate into increased chromosome segregation defects. *ECO1* is essential in yeast; however, the presence of two orthologues in humans, *ESCO1* and *ESCO2*, both of which appear to contribute to chromosome cohesion (Hou and Zou, 2005), may allow mutations in *ESCO2* to be compatible with life. *ESCO2* is expressed in human embryonic tissues in a pattern that is consistent with the systems and organs affected in individuals with Roberts syndrome (Vega et al., 2010). Cells from these patients are hypersensitive to DNA damage caused by mitomycin C, camptothecin, and etoposide, but not UV, ionizing radiation, hydroxyurea, or aphidicolin (Gordillo et al., 2008; van der Lelij et al., 2009), which is consistent with *ESCO2* promoting genome stability. Analysis of *ECO1* mutations in budding yeast suggests a role for *ESCO2* in replication-coupled cohesion, and in fact, processivity of DNA replication forks in cells from Roberts syndrome patients is reduced (Terret et al., 2009). How replication defects might

Table I. Cohesin complex subunits

Species	Gene			
<i>S. cerevisiae</i>	Smc1	Smc3	Mcd1/Sccl Rec8 <sup>a</sup>	Sccl/lrr1
<i>S. pombe</i>	Psm1	Psm3	Rad21 Rec8 <sup>a</sup>	Psc3 Rec11 <sup>a</sup>
<i>C. elegans</i>	Him-1	Smc-3	Coh-1/Sccl Rec8/Coh-3 <sup>a</sup>	Sccl-3
<i>D. melanogaster</i>	Smc1	Cap	Rad21 c(2)M <sup>a</sup>	SA
<i>X. laevis</i>	Smc1	Smc3	Rad21 Rec8 <sup>a</sup>	Stag1, Stag2
<i>H. sapiens</i>	Smc1 $\alpha$ Smc1 $\beta$ <sup>a</sup>	Smc3	Rad21 Rec8 <sup>a</sup>	Stag1, Stag2 Stag3 <sup>a</sup>

SA, stromal antigen.

<sup>a</sup>Meiosis-specific function.

contribute to the disease etiology is a question that needs to be addressed by future studies.

Cornelia de Lange syndrome manifests with variable degrees of severity, with mild cases caused by mutations in *SMC1* and *SMC3* and more severe cases caused by mutations in *NIPBL* (Krantz et al., 2004; Tonkin et al., 2004; Musio et al., 2006; Deardorff et al., 2007). Behavioral and cognitive defects display a wide range of severity, as do limb malformations, which can range from small digits to both upper and lower limb malformations. The characteristic facial features include synophrys, long eyelashes, depressed nasal root with upturned nose, and a thin upper lip. Gastroesophageal dysfunction is common. Sister chromatid cohesion has been reported to be mildly affected in cell lines derived from individuals with mutations in *NIPBL* (Kaur et al., 2005), although no defects in precocious sister chromatid separation were observed in cells bearing mutation in *SMC1* or *SMC3* (Revenkova et al., 2009). Another group failed to find any defect in centromere separation in *NIPBL* cell lines (Tonkin et al., 2004). *NIPBL* expression in human embryonic tissue sections is largely reflective of where developmental defects are observed in patients (Tonkin et al., 2004). Cells derived from Cornelia de Lange syndrome patients are sensitive to mitomycin C, a DNA-damaging agent (Vrouwe et al., 2007). X rays also boost the number of chromosomal aberrations observed, but only when the damage was inflicted during the G2 phase of the cell cycle, presumably related to the role of cohesion in homologous recombination (Vrouwe et al., 2007). Thus, although Cornelia de Lange syndrome and Roberts syndrome are both caused by mutations in the cohesin network, the two syndromes are quite distinct cellularly and clinically, and it is

quite possible that the molecular mechanisms that cause the defects could also be distinct.

The molecular mechanisms behind the changes in gene expression that lead to the cohesinopathies are elusive. Several mechanisms have been proposed, including that cohesin may act in transcriptional activation, transcriptional repression, transcript termination, and long-distance enhancer–promoter interactions, with none of these possibilities being mutually exclusive. One possible etiology for the cohesinopathies that we will discuss in this article is that the organization of the genome is altered at particular critical developmental time points in these individuals. In this model, the changes in developmental gene expression programs that lead to the cohesinopathies are a result of disruption of chromatin organization within the nucleus. This would lead to the misregulation of developmental gene expression programs without significantly affecting chromosome segregation. In this article, the term “genome organization” refers to the idea that DNA sequences are positioned in the nucleus; this position can affect their behavior with respect to expression and stability (Misteli, 2007).

### Colocalization of cohesin with CTCF

Cohesin reproducibly associates with the same regions of a given genome. In budding yeast and fission yeast, there are a few hundred binding sites, with one every ~10–15 kb and a large domain of binding surrounding centromere regions (Glynn et al., 2004; Lengronne et al., 2004; Schmidt et al., 2009). In the mouse and human genomes, cohesin also binds to the genome at fairly frequent intervals, with a binding site on average every 22 and 340 kb, respectively (Parelho et al., 2008;

Table II. Accessory factors for cohesion

Species	Gene						
<i>S. cerevisiae</i>	Sccl2	Sccl4	Pds5	Rad61	Eco1	Esp1	Pds1
<i>S. pombe</i>	Mis4	Ssl3	Pds5	Wpl1	Eso1	Cut1	Cut2
<i>C. elegans</i>	Pqn-85	Mau-2	Evl-14	Wapl-1	F08F8.4	Sep-1	Ify-1
<i>D. melanogaster</i>	Nipped-B	Sccl4	Pds5	Wapl	Deco	Sse/Thr	Pim
<i>X. laevis</i>	Nipbl/Sccl2	Kiaa0892	Pds5a, Pds5b	Wapl	Eco1, Eco2	Espl1	Securin
<i>H. sapiens</i>	Nipbl	Sccl4/Kiaa0892	Pds5a, Pds5b	Wapl	Esco1, Esco2	Espl1	Pttg1

Wendt et al., 2008). In metazoans, a big breakthrough in understanding alternative functions of cohesin came with the realization that cohesin binds to the same parts of the genome as CTCF, with many binding sites being transcription start sites (Parelho et al., 2008; Rubio et al., 2008; Stedman et al., 2008; Wendt et al., 2008). Cohesin is speculated to function in collaboration with CTCF, which is a protein thought to mediate long-range DNA contacts that influence chromatin structure, promoter activity, and transcription (Wallace and Felsenfeld, 2007). Although CTCF binds to a particular sequence, the association of cohesin with the CTCF-binding motif is dependent on CTCF because upon depletion of CTCF, cohesin no longer localizes to the CTCF sequence but still appears to associate with DNA (Wendt et al., 2008).

### Gene regulation depends on cohesin

There are many examples of gene regulation being affected by cohesin, although in most cases studied, the effect on steady-state transcription is somewhat subtle, usually twofold or less. However, in a few cases in flies and yeast in which the inducibility of a locus has been studied, the effect is stronger, suggesting that there may be a subset of genes whose transcription is hypersensitive to cohesin defects (Gard et al., 2009; Schaaf et al., 2009). In some cases, the evidence for cohesin involvement in gene regulation stems from genome-wide expression studies performed in cell culture, in which cohesin binding is correlated with expression (Liu et al., 2009; Schaaf et al., 2009). Genetic screens designed to find mutants with particular development phenotypes eventually led to the discovery that the developmental defects were caused by mutations in the cohesin network (Rollins et al., 1999, 2004; Horsfield et al., 2007; Hallson et al., 2008; Schuldiner et al., 2008). Reverse genetics has also revealed developmental defects associated with defects in cohesin, presumably resulting from altered gene expression programs (Zhang et al., 2007, 2009; Kawauchi et al., 2009). The colocalization of cohesin and CTCF has been shown to correlate with lytic gene repression and the maintenance of latency for a herpesvirus (Stedman et al., 2008). Finally, in a clever study in which the cohesin ring was cleaved in postmitotic fly neurons, gene expression was clearly affected (Pauli et al., 2008). From these studies and many others, a picture is emerging in which the necessity of cohesin for chromosome segregation can be separated from its role in gene regulation.

Forward genetic screens in flies and zebrafish identified mutations in the cohesin network that led to particular developmental phenotypes. Although homozygous Nipped-B (the fly orthologue of *SCC2*) mutations are lethal and have defects in sister chromatid cohesion (Rollins et al., 2004), heterozygous Nipped-B mutants do not have cohesion defects and thus can be used to study gene expression (Rollins et al., 1999, 2004; Dorsett et al., 2005). Reduced expression of Nipped-B was found to reduce expression of *Cut* and *Ultrabithorax*, which results from a defect in the interaction between an enhancer and a promoter element of the *Cut* gene (Rollins et al., 1999, 2004; Dorsett et al., 2005; Gause et al., 2008). In contrast to the Nipped-B results, reduction of the level of cohesin subunits Rad21 or Smc1 or mutation of *Pds5* increased the expression of *Cut* (Rollins et al., 2004; Dorsett et al., 2005). Nipped-B was proposed to function in the

removal of the cohesin complex, which could normally block the interaction of the enhancer and the promoter (Rollins et al., 2004). The conclusion of these studies is that Nipped-B and cohesin may oppositely modulate enhancer–promoter communication. In zebrafish reduction in Rad21 levels correlated with reduced expression of *Runx* genes, the net result being arrested development because the *runx* gene family is critical for embryogenesis (Horsfield et al., 2007). Thus, reducing the dosage of the cohesin subunits has been shown to lead to both increases and decreases in gene expression, enforcing the idea that cohesin may not act as a simple activator or repressor.

Recently, it was discovered that the *verthandi* mutation in flies, which is considered a member of the trithorax group of proteins implicated in transcriptional regulation, was in the cohesin subunit Rad21 (Hallson et al., 2008). Trithorax genes were initially characterized as regulators of homeotic genes and are required to maintain their activation and oppose the action of polycomb group genes that repress homeotic genes. In the rare cases in the genome in which cohesin and polycomb colocalize at the enhancer of split and invected–engrailed gene complexes, the effects on gene expression after depletion of cohesin subunits or Nipped-B are dramatic, up to 100-fold (Schaaf et al., 2009), suggesting that a few particular genomic regions may be directly and strongly controlled by cohesin levels.

Gene expression profiling has been performed on 16 lymphoblastoid cell lines derived from Cornelia de Lange syndrome patients (Liu et al., 2009). A group of ~420 dysregulated genes was identified in the mutant cells, with about two thirds of genes being down-regulated and one third being up-regulated. Although the changes in expression from mutant to wild type were typically twofold or less, the degree of dysregulation correlated with the severity of the disease. Cohesin was found to bind to many active transcription start sites, which is similar to the observations in flies (Misulovin et al., 2008), and to a lesser extent, termination sites. Furthermore, there was a correlation with reduced cohesin binding around the misregulated genes in the mutant cells, suggesting that cohesin binding may directly control the expression of some genes. It is currently unclear whether the cohesinopathies might be caused by an accumulation of many small effects on gene regulation, such as those observed in the Cornelia de Lange syndrome lymphoblasts (Liu et al., 2009), or a few large changes occurring early in development, such as those observed at the enhancer of split and invected–engrailed gene complexes in flies (Schaaf et al., 2009).

A mouse model of Cornelia de Lange syndrome has been developed using reverse genetics. Specifically, mice have been made that are heterozygous null for *Nipbl* (Kawauchi et al., 2009). *Nipbl*<sup>+/-</sup> mice exhibited defects characteristic of the syndrome, including small size, craniofacial anomalies, microbrachycephaly, heart defects, hearing abnormalities, delayed bone maturation, reduced body fat, behavioral disturbances, and high mortality (75–80%) during the first weeks of life. Gene expression profiling demonstrated that *Nipbl* deficiency leads to modest but significant transcriptional dysregulation of many genes. Expression changes at the protocadherin  $\beta$  locus, as well as at other loci, support the view that *NIPBL* influences long-range chromosomal regulatory interactions.



In addition to the colocalization of cohesin and CTCF in the mammalian genome, the colocalization has been observed at Kaposi's sarcoma-associated herpesvirus latency control regions during S phase (Stedman et al., 2008). In an interesting example of a virus using host factors, cohesin and CTCF are required to maintain cell cycle-regulated expression of lytic genes in Kaposi's sarcoma-associated herpesvirus. Colocalization correlates with repressed transcription and localization at the nuclear interior during S phase. However, in G2/M phase, CTCF, but not cohesin, accumulates at the nuclear periphery, and this correlates with renewed transcription (Stedman et al., 2008; Kang and Lieberman, 2009). In this case, CTCF interaction with cohesin varies over the course of the cell cycle, and CTCF appears to have a repressive function that is alleviated when CTCF is relocated to the nuclear periphery (Kang and Lieberman, 2009).

Metazoans contain two copies of the Pds5 gene, Pds5A and Pds5B, which differ in their expression (Losada et al., 2005). Both Pds5A- and Pds5B-deficient mice die at birth (Zhang et al., 2007, 2009). Pds5B<sup>-/-</sup> and Pds5A<sup>-/-</sup> mice have multiple congenital abnormalities, including cleft palate, skeletal patterning defects, growth retardation, congenital heart defects, and delayed migration of enteric neuron precursors, many of which are similar to abnormalities found in humans with Cornelia de Lange syndrome. Surprisingly, Pds5B<sup>-/-</sup> mouse embryonic fibroblasts lack sister chromatid cohesion defects (Zhang et al., 2007). Pds5B expression was detected in postmitotic neurons in the mouse brain (Zhang et al., 2007; Wendt et al., 2008), which is reminiscent of the expression pattern of Smc1, Rad21, Pds5B, and Smc3 in zebrafish (Mönnich et al., 2009) and the expression of Rad21 in fly  $\gamma$ -neurons (Pauli et al., 2008). The expression patterns, coupled with the neurological phenotypes of the mutants, suggest a critical role for cohesin in the development and migration of neurons.

A major factor that has complicated the study of cohesin in gene regulation is that cohesin is essential for chromosome segregation and therefore cell division. Although many studies have overcome this obstacle by reducing the dosage of the proteins, Pauli et al. (2008) were able to overcome this obstacle by specifically cleaving the kleisin component of the complex in postmitotic neurons. This cleavage is lethal, causing defects in axon pruning in mushroom body neurons and defects in locomotion when cleaved in cholinergic neurons. The ecdysone receptor EcR-B1, which is a key regulator in axon pruning, is down-regulated in *SMC1*<sup>-/-</sup> fly clones, which also show axon-pruning defects and mistargeting of olfactory projection neurons (Schuldiner et al., 2008). Axon migration is also affected in *Caenorhabditis elegans* with mutations in Mau-2, the worm homologue of *SCC4* (Takagi et al., 1997; Bénard et al., 2004; Seitan et al., 2006). Results in these two model organisms strongly suggest a role for cohesin in neural development.

One unifying theme to date for the role of cohesin in gene regulation that can be found in studies of metazoans is that mutations in the cohesin network affect neuronal development. This is particularly interesting in light of the cognitive defects associated with the cohesinopathies. If the neuronal defects in model organisms are found to sufficiently imitate those associated with the human cohesinopathies, then model organisms may

prove to be a good starting point for the identification of treatments that might ameliorate the neurological deficiencies.

### Distant DNA-DNA interactions may depend on cohesin

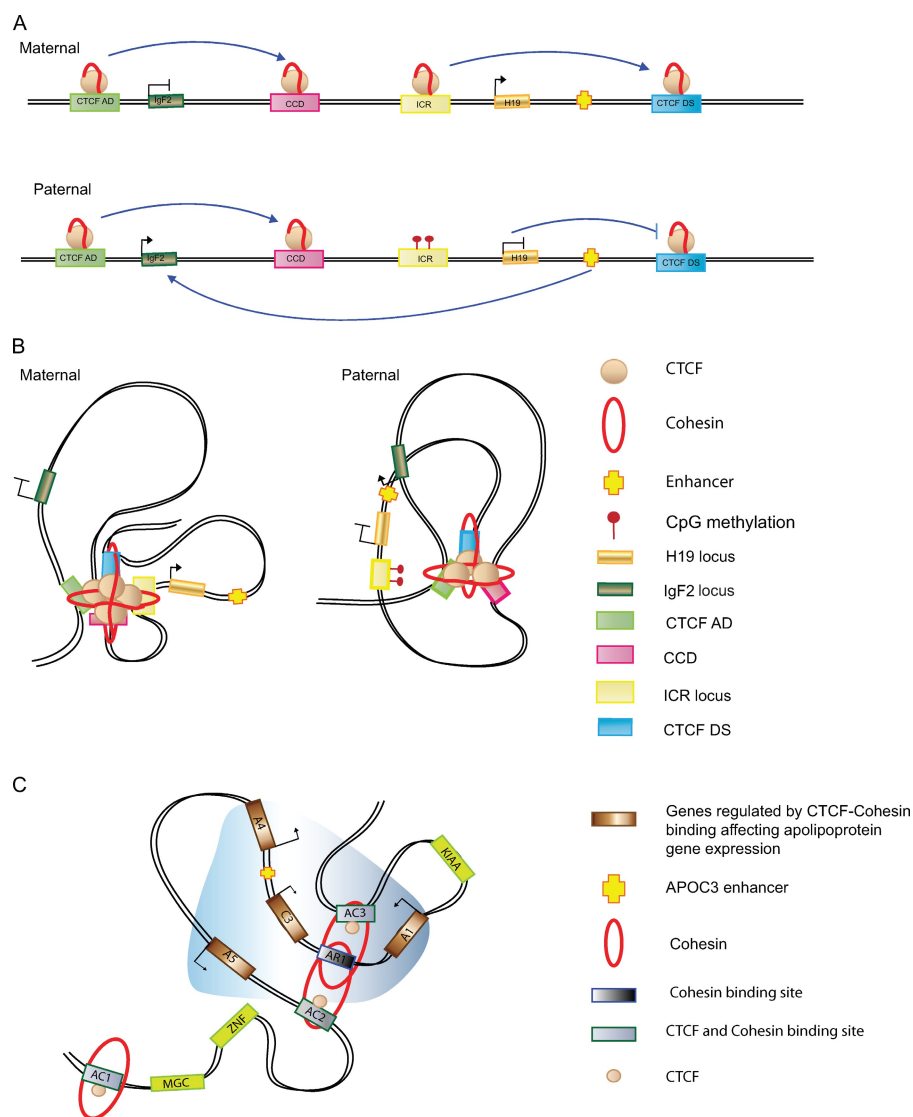
It has been proposed that cohesin might organize chromatin through the creation or stabilization of chromatin loops. Cohesin could either encircle two different DNA sequences, creating the base of a loop, or two distant cohesin rings could interact. Such loops would constrain chromatin folding and might alter gene regulation through an effect on the interaction between the promoter and distant regulatory sequences. Disruption of looped or clustered structures could result in increased transcription for some genes and decreased transcription for others. For the purposes of chromosome segregation, it is generally presumed that identical sequences are held together by cohesin, but in chromatin loops, nonidentical sequences could be held together.

Recently, evidence for the involvement of cohesin in the long-distance tethering of sequences in mammalian cells has come to light, mostly thanks to chromosome conformation capture (3C) technology. 3C was developed to determine which noncolinear sequences are near each other (Dekker et al., 2002). Cells are treated with a cross-linking agent such as formaldehyde. The DNA is digested with a restriction enzyme, and the chromatin is isolated and ligated under dilute conditions to promote intramolecular ligation. PCR is performed on the ligated products with one primer at a constant location and a second primer whose distance from the first site varies. Normally, the PCR signal will fall off with distance, but some distant sites may show a spike in enrichment because of cross-linking, indicating an interaction between the anchor sequence and a distant site.

CTCF and cohesin bind to regions within the developmentally regulated cytokine locus IFN- $\gamma$ . Long-range chromosomal cis-interactions at IFN- $\gamma$  are dependent on both cohesin and CTCF (Hadjur et al., 2009), as determined by a combination of 3C and RNAi. These interactions are specific to T<sub>H</sub>1 cells and were not detected in T<sub>H</sub>2 or nonpolarized CD4 T cells. Basal levels and inducibility of IFN- $\gamma$  were both significantly decreased when RNAi was used to target Rad21, but the inducibility of a control locus, IL-2, was unaffected. These results demonstrate that cohesin can contribute to the dynamic regulation of this particular locus in a cell type-specific manner.

At the imprinted H19/Igf2 locus, depletion of either cohesin or CTCF resulted in reduced transcription of H19 and increased transcription of Igf2, implying a role for these proteins in regulation (Stedman et al., 2008; Wendt et al., 2008). The putative looped structure at H19/Igf2 that brings the promoter and enhancer together in a parental allele-specific manner is dependent on a differentially methylated sequence (the imprinting control region), and both cohesin and CTCF bind to several regions within the locus (Fig. 2 A; Nativio et al., 2009). CTCF binding is sensitive to DNA methylation, and cohesin localization is dependent on CTCF, allowing for the integration of DNA binding with epigenetic state (Parelho et al., 2008). 3C assays in combination with cell cycle staged RNAi experiments show that cohesin is necessary to maintain one set of contacts at the maternal allele and a different set of contacts at the paternal

**Figure 2. The formation of chromatin loops is dependent on cohesin.** (A) Schematic representation of long-distance interactions at the Igf2/H19 locus. Some interactions (blue arrows) occur on both alleles (biallelic), and some interactions are specific to the maternal or paternal allele. Specific CTCF cohesin-binding sites have been identified by 3C. Upstream of the Igf2 locus lies the differentially methylated region (DMR0), which includes a CTCF-binding site (CTCF AD). The centrally conserved DNase I hypersensitive site (CCD) lies between Igf2 and H19. A CTCF-binding region downstream of the H19 locus is denoted as CTCF DS. CTCF AD and CCD interactions occur on both alleles. (B) Looping may differ at the maternal and paternal Igf2/H19 locus. Cohesin and CTCF bind to the imprinting control region (ICR) when it is not methylated. CTCF AD and CCD interactions occur on both alleles. In the paternal allele, CTCF cohesin colocalization results in looping together the CTCF AD, CCD, and CTCF DS. Methylation of ICR causes CTCF DS to remain out of the loop, which in turn may cause activation of the Igf2 locus via interaction with the enhancer. The maternal allele carries an unmethylated copy of ICR that results in interaction between ICR and CTCF DS, which stops activation of Igf2 by the enhancer. (C) Schematic representation of the apolipoprotein locus. The AC2, AR1, and AC3 elements are bound to cohesin as measured by chromatin immunoprecipitation, but CTCF is only found associated with AC2 and AC3. 3C data indicate that the AC2, AR1, and AC3 elements interact with an enhancer in Hep3B cells, and the interaction is dependent on Rad21 and CTCF (Mishiro et al., 2009). The zone of influence of the enhancer element (yellow) is depicted in blue.



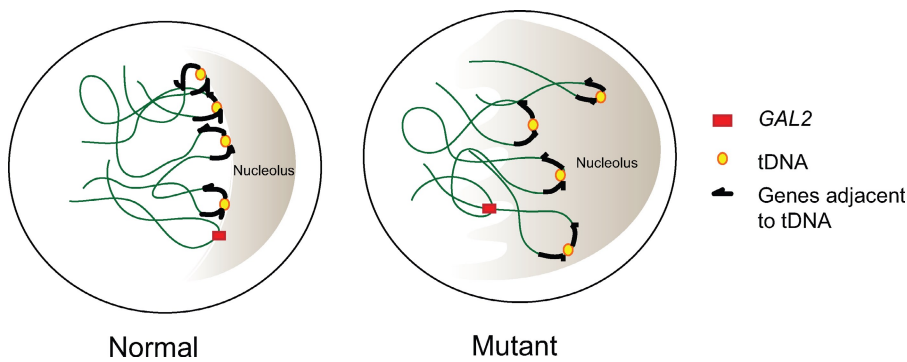
allele (Fig. 2 B). The putative looped structure is present in both G1 and G2 cells, arguing for a role for cohesin in distant DNA–DNA interactions that is independent of its role in sister cohesion (Nativio et al., 2009).

The  $\beta$ -globin locus is another locus that demonstrates cell type specificity of chromatin organization mediated by CTCF and cohesin (Hou et al., 2010). At the  $\beta$ -globin locus, a series of different genes are activated over the course of development. CTCF has been shown to mediate the interaction between the locus control region and active genes in a developmentally controlled fashion (Tolhuis et al., 2002; Palstra et al., 2003; Splinter et al., 2006). Insertion of another copy of the CTCF-dependent enhancer within (but not outside) the locus creates an alternative chromatin loop that nullifies locus control region function (Hou et al., 2008). Although CTCF and cohesin bind to several sites in the region containing the  $\beta$ -globin locus and flanking olfactory genes, these sites show more densely clustered organization in a cell line lacking  $\beta$ -globin gene activity (Hou et al., 2010). Although depletion of CTCF was previously shown to have no effect on transcription at the  $\beta$ -globin locus (Splinter et al., 2006), a new study shows that knockdown of CTCF results

in the acquisition of repressive histone marks in the globin locus and reduced expression, whereas the flanking olfactory receptor genes were unaffected (Hou et al., 2010). These results suggest a cell type- and locus-specific chromatin organization supported by cohesin and CTCF at the globin locus.

In another example of cohesin influencing distant DNA–DNA interactions, depletion of either CTCF or Rad21 resulted in a disruption of the putative chromatin loop structure present at the apolipoprotein gene cluster and changed the expression of the genes in the cluster in a complex way (Fig. 2 C; Mishiro et al., 2009). The apolipoprotein gene cluster is proposed to form two loops, which encompass seven genes and an enhancer. There are three CTCF-binding sites and three Rad21 sites, with two overlapping the CTCF sites. Disrupting the proposed looped structure could change the proximity of the promoter and the enhancer or other regulatory sequences and result in an increase or decrease in transcription, depending on the new structure of the region.

To date, there are four examples in which cohesin has been suggested to control distant DNA–DNA interactions. At this point, it is unclear how widespread this type of regulation might



**Figure 3. Cohesin may contribute to subcellular localization of DNA sequences.** tDNAs (yellow) cluster near the nucleolus (gray) in a budding yeast nucleus (Thompson et al., 2003). Genes (black) located adjacent to tDNAs can be silenced, and this depends on the proximity to the nucleolus (Wang et al., 2005). Recently, it was shown that strains bearing cohesinopathy mutations in either *Eco1* (*eco1-W216G*) or *Scc2* (*scc2-D730V*) lose tDNA clustering and tRNA gene-mediated silencing (Gard et al., 2009). *GAL2* (red) is normally tethered to the nucleolus, but nucleolar morphology and *GAL2* tethering is disrupted in the mutant backgrounds, and the induction of *GAL2* is increased. Cohesin may contribute to tethering of tDNAs at a particular subcellular location, and this may affect the regulation of neighboring genes. The *eco1-W216G* mutation also causes defects in telomere clustering.

be, and furthermore, if the magnitude of the effect would be sufficient to cause serious biological consequences. Future experiments aimed at better understanding cohesin-mediated chromatin looping and gene expression should shed light on whether this is an important mechanism by which cohesin exerts its influence on gene regulation. With the presence of cohesin at active transcription start sites, it is tempting to speculate the cohesin might control the interaction of promoters with enhancers or other regulatory sequences to influence gene regulation.

#### The subnuclear position of sequences may depend on cohesin

The position of a gene within the nucleus can be both a cause and consequence of its transcriptional regulation. However, the expression of some genes may be influenced by their subnuclear position (Sexton et al., 2007). In mammalian cells, heterochromatin tends to be located at the nuclear periphery, and transcriptionally active chromatin tends to be located at the nuclear interior, although exceptions do exist. In yeast cells, at least two zones at the nuclear periphery have been proposed: transcriptionally active genes would be located in one type of zone near nuclear pores, and transcriptionally silent chromatin, such as telomeres, would be located at a second, repressive zone lacking pores (Akhtar and Gasser, 2007). There is some evidence that the cohesin network might influence the position of DNA sequences within the nucleus.

Telomeres are known to cluster at the nuclear periphery. Telomeres in budding yeast cluster into four to five foci at the nuclear periphery, which can be visualized using Rap1-GFP (Gotta et al., 1996). A subset of telomeres in budding yeast is associated with cohesin (Glynn et al., 2004). Interestingly, yeast carrying a mutation in *ECO1* meant to mimic a mutation associated with Roberts syndrome (Gordillo et al., 2008) have dispersed Rap1 signal throughout the cell cycle (Gard et al., 2009). However, the molecular defect underlying the putative telomere dispersion is unknown. In meiosis, telomeres associate with the nuclear envelope and transiently cluster into a structure known as a bouquet, which is thought to facilitate chromosome organization. Cohesin complexes containing the meiosis-specific subunit Smc1 $\beta$  are required for complete telomere attachment to the nuclear envelope in meiosis (Adelfalk et al., 2009). Without

Smc1 $\beta$ , Smc3 and Smc1 $\alpha$  subunits are not present at telomeres, demonstrating that cohesin is not present. In addition to the defects in peripheral attachment, the absence of cohesin correlates with defects in meiotic telomere integrity in Smc1 $\beta^{-/-}$  meocytes, including shortening, formation of large telomeric protein–DNA extensions, extended telomeric bridges, ring-like chromosomes, and intrachromosomal telomeric repeats (Adelfalk et al., 2009). Thus, cohesin may facilitate positioning of telomeres, and furthermore, cohesin might contribute to a structure that protects their integrity.

In budding yeast, ~274 tRNA genes are positioned throughout the 16 chromosomes but cluster near the nucleolus (Thompson et al., 2003). Their location adjacent to the nucleolus is important for their ability to silence neighboring genes (Wang et al., 2005). Interestingly, mutations in *ECO1*, *SCC2*, and condensin, a complex evolutionarily related to cohesin, all cause tDNAs to become dispersed (Fig. 3; Haeusler et al., 2008; Gard et al., 2009). Furthermore, the *GAL2* gene, which is bound by cohesin when inactive (Glynn et al., 2004; Bausch et al., 2007) and colocalizes with the nucleolus (Berger et al., 2008), no longer colocalizes with the nucleolus in the same *ECO1* and *SCC2* mutants that disrupt tRNA gene-mediated silencing (Gard et al., 2009). The induction of *GAL2* is increased in the mutants, suggesting that proximity to the nucleolar silencing factors may normally suppress its induction. It remains to be determined whether *GAL2* is an isolated case or whether other genes are regulated by similar mechanisms in budding yeast. And although tRNA gene-mediated silencing may be a yeast-specific phenomenon, it may provide a valuable model for how the position of sequences adjacent to other sequences and subcellular locations may control their regulation.

#### Conclusions

In summary, many developmental processes, particularly neural development, appear to be affected by mutations in cohesin and cohesin-associated genes. To date, there is tantalizing evidence that cohesin is involved in the positioning of DNA sequences with the potential to affect gene expression. It is also possible that cohesin may directly regulate transcription. At this point, it is unclear how many genes might be directly regulated by cohesin-dependent mechanisms. It is also unclear whether



the mode of association of cohesin with the regions being regulated is the same as the association for the purposes of chromosome segregation. Answers to these questions will depend on being able to separate the essential role of cohesin in chromosome segregation from its role in gene regulation. Although 3C has proven useful, the field would greatly benefit from additional methods to study the three-dimensional structures of chromosomes and genomes. Given the colocalization of cohesin with the well-studied CTCF protein, it will be important to elucidate the molecular contribution of each to interactions between promoters and regulatory sequences. Finally, the three-dimensional position of chromatin as controlled by cohesin is likely to be dynamic and controlled by local chromatin features; these aspects of the process have yet to be understood in molecular detail.

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