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Roles of the sister chromatid cohesion apparatus in gene expression, development, and human syndromes

Dale Dorsett

D. Dorsett, Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, Saint Louis, MO 63104, USA

Abstract

The sister chromatid cohesion apparatus mediates physical pairing of duplicated chromosomes. This pairing is essential for appropriate distribution of chromosomes into the daughter cells upon cell division. Recent evidence shows that the cohesion apparatus, which is a significant structural component of chromosomes during interphase, also affects gene expression and development. The Cornelia de Lange (CdLS) and Roberts/SC phocomelia (RBS/SC) genetic syndromes in humans are caused by mutations affecting components of the cohesion apparatus. Studies in *Drosophila* suggest that effects on gene expression are most likely responsible for developmental alterations in CdLS. Effects on chromatid cohesion are apparent in RBS/SC syndrome, but data from yeast and *Drosophila* point to the likelihood that changes in expression of genes located in heterochromatin could contribute to the developmental deficits.

Introduction

During the mitotic cell cycle, the sister chromatid products of DNA replication are held together until anaphase, when the sisters segregate to the two daughter cells. In recent years, many of the proteins that mediate sister chromatid cohesion and its dissolution have been discovered and characterized. There are recent reviews that cover the structure and function of the sister chromatid cohesion apparatus in chromosome segregation (Nasmyth 2005; Nasmyth and Haering 2005; Losada and Hirano 2005; Yanagida 2005; Huang et al. 2005; Hirano 2005), and on its potential roles in Cornelia de Lange syndrome (Dorsett 2004; Strachan 2005). In this paper, I review certain key components of the cohesion apparatus, discuss their roles in gene expression and development in model organisms, and then the implications of the model organism studies for Cornelia de Lange and Roberts-SC phocomelia human genetic syndromes.

The sister chromatid cohesion factors considered here are the cohesin protein complex that holds sister chromatids together, the Scc2 (Mis4, Nipped-B, delangin/NIPBL) adherin factor required for binding of cohesin to chromosomes, the Pds5 (Spo76, BimD) protein that associates with cohesin on chromosomes, and the Ctf7/Eco1 (Deco, Escol1/Efo1, Escol2/Efo2) family of protein acetyltransferase enzymes (Table 1).

e-mail: dorsettd@slu.edu.

Note added in proof While this review was in proof, papers describing *S. pombe* and human homologues of the Scc4 adherin subunit were published:

Bernard P, Drogat J, Maure JF, Dheur S, Vaur S, Genier S, Javerzat JP (2006) A screen for cohesion mutants uncovers Ssl3, the fission yeast counterpart of the cohesin loading factor Scc4. *Curr Biol* 16:875–881.

Watrin E, Schleiffer A, Tanaka K, Eisenhaber F, Nasmyth K, Peters JM (2006) Human Scc4 is required for cohesin binding to chromatin, sister-chromatid cohesion, and mitotic progression. *Curr Biol* 16: 863–874.

Cohesin

Cohesin belongs to an ancient family of chromosome structural components, known collectively as SMC (structural maintenance of chromosome) complexes (Losada and Hirano 2005; Nasmyth and Haering 2005). These complexes, which include the condensins required for chromosome condensation in eukaryotes, and complexes required for chromosome partitioning in bacteria, contain SMC protein homo- or heterodimers. Cohesin consists of an Smc1 and Smc3 heterodimer, the Rad21 (Mcd1, Scc1) kleisin family protein, and Stromalin (SA, Scc3) (Fig. 1a). Like other SMC proteins, Smc1 and Smc3 fold back on themselves to form antiparallel coiled coils, with their N and C termini forming ATPase head domains. Their hinge regions interact to form the SMC heterodimer. The N and C termini of Rad21 interact with the head domains of Smc3, and Smc1, respectively, to form a ring-like structure. The Smc3 arm has a kink, which forms a more open structure than seen in other SMC complexes (Anderson et al. 2002).

Current evidence strongly favors the idea that the cohesin complex holds sister chromatids together, but the mechanism by which it performs this task is not completely established. The large ring-like structure of cohesin, and several other lines of evidence, including topological linkage of cohesin to circular DNA molecules (Ivanov and Nasmyth 2005) supports the attractive hypothesis that cohesin mediates cohesion by encircling both sister chromatids (Nasmyth 2005; Fig. 1b). Other models suggest that two cohesin rings bound via direct DNA-protein contacts to the two sisters interlock to establish cohesion (Huang et al. 2005; Milutinovich and Koshland 2003; Nasmyth 2005). Another idea is that a cohesin ring encircling one sister can form protein-protein contacts with the other sister (Chang et al. 2005). It is possible that cohesin could mediate cohesion by multiple mechanisms, and that the mechanisms may depend on chromosome location and/or other chromosomal proteins.

In *Saccharomyces cerevisiae* cohesin is loaded near the G1-to-S-phase transition (Michaelis et al. 1997), but in most organisms, cohesin binds to chromosomes in telophase. During interphase, cohesin binds many sites along chromosome arms, about once every 10 kbp or so in *S. cerevisiae* (Blat and Kleckner 1999; Laloraya et al. 2000; Glynn et al. 2004; Lengronne et al. 2004; Tanaka et al. 1999), and estimated to be about once every 20 kbp or so in higher organisms (Dorsett et al. 2005; Losada et al. 1998). Cohesin is more concentrated around centromeres, and interactions with heterochromatin proteins appear to be important (Megee et al. 1999; Bernard et al. 2001; Nonaka et al. 2002; Partridge et al. 2002). For the purposes of this review, the key point is that cohesin is a major structural component of both euchromatin and heterochromatin during interphase.

It is unclear how cohesion between sister chromatids is established during or after DNA replication, but certain replication factors, including a replication factor C (RFC) complex that loads PCNA-type DNA clamps, are required to establish cohesion (reviewed by Skibbens 2005). Most of the cohesin bound to chromosome arms is removed during mitotic prophase (Losada et al. 1998; Sumara et al. 2000; Waizenegger et al. 2000), while the shugosin protein protects the cohesin at the centromeres (Salic et al. 2004; McGuinness et al. 2005; reviewed by Watanabe 2005). The Rad21 (Mcd1/Scc1) subunit of the centromeric cohesin is proteolytically cleaved at the metaphase to anaphase transition to dissolve cohesion and permit chromosome segregation (Hauf et al. 2001; Tomonaga et al. 2000; Uhlmann et al. 1999; Uhlmann et al. 2000; Waizenegger et al. 2000).

Adherin (Scc2, Nipped-B, NIPBL/delangin)

The Scc2 factor of *S. cerevisiae* and its orthologs (Mis4, Nipped-B, delangin, Xscc2) in other organisms, known collectively as adherins, are required for sister chromatid cohesion (Furuya et al. 1998; Gillespie and Hirano 2004; Kaur et al. 2005; Michaelis et al. 1997; Rollins et al.

2004; Seitan et al. 2006). Scc2 and its orthologs are required for cohesin to bind to chromosomes (Arumugam et al. 2003; Ciosk et al. 2000; Gillespie and Hirano 2004; Seitan et al. 2006; Takahashi et al. 2004; Tomonaga et al. 2000). The loss of cohesin binding is likely responsible for the cohesion defects in adherin mutants, but a more direct role for adherins in cohesion cannot be ruled out at this time. Scc2 forms a complex with the Scc4 protein, which is also required for cohesin chromosome binding (Ciosk et al. 2000). Recently, proteins that are distantly related to Scc4 have been shown to interact with Scc2 orthologs in *Drosophila*, *C. elegans*, and human cells (Seitan et al. 2006). The *C. elegans* factor, Mau-2, was originally discovered as a factor required for axon migration (Bénard et al. 2004; Takagi et al. 1997). Knockdown of human Mau-2 in cultured cells causes sister chromatid cohesion defects (Seitan et al. 2006). Thus, the Mau-2 proteins of higher organisms appear to be functional homologs of Scc4, although they may have acquired additional developmental functions.

In a *Xenopus* cell-free system, licensing of the replication origins is required for the Xscc2 adherin to bind to chromatin, which is then followed by binding of cohesin (Gillespie and Hirano 2004; Takahashi et al. 2004). This suggests that early in the cell cycle, long before replication occurs, adherin plays a critical role in coordination of chromatid cohesion with DNA replication.

The adherins are large proteins with several HEAT repeats (Neuwald and Hirano 2000). HEAT repeats are bihelical folds that are likely to be sites of interaction with other proteins. One thought is that the adherins might stimulate ATP hydrolysis by the SMC head domains and help open the cohesin ring to allow passage of the chromosome into the ring (Fig. 1c; Arumugam et al. 2003; Weitzer et al. 2003). It is also possible that the adherins facilitate assembly of cohesin on chromosomes.

Pds5 (Spo76, BimD)

Like the Scc2/Nipped-B adherin family, the Pds5 proteins are large HEAT repeat proteins conserved from fungi to vertebrates (Neuwald and Hirano 2000). In yeasts and higher organisms, Pds5 is not required for cohesin to bind to chromosomes, but is required for cohesion (Dorsett et al. 2005; Hartman et al. 2000; Losada et al. 2005; Stead et al. 2003). Pds5 is not a stable subunit of cohesin, but colocalizes with cohesin on chromosomes in fungi and *Drosophila* (Hartman et al. 2000; Panizza et al. 2000; Tanaka et al. 2001; Z. Misulovin, J.C. Eissenberg, D. Dorsett, unpublished). Association of Pds5 with chromatin depends on cohesin (Hartman et al. 2000; Panizza et al. 2000; Losada et al. 2005) and human Pds5 interacts with cohesin (Sumara et al. 2000). The *S. pombe* Pds5 protein also interacts with the Eso1 (Ctf7/Eco1) cohesin factor discussed below (Tanaka et al. 2001).

A special *Drosophila pds5* mutation that encodes an N-terminally-truncated protein provides an intriguing clue regarding its function. In contrast to a null mutant, this mutant reduces binding of cohesin to chromosomes (Dorsett et al. 2005). This and the structural similarities with Nipped-B suggest that Pds5 may function in a manner similar to that proposed for Nipped-B. For instance, Pds5 could open and reclose the cohesin ring after DNA replication to allow cohesin to encircle both chromatids and establish cohesion (Fig. 1d). The mutant protein might fail to complete this process, effectively removing cohesin from the chromosome.

Ctf7/Eco1 (Esco2/Efo2, Deco, Eso1)

Similar to Pds5, the Ctf7/Eco1 protein of *S. cerevisiae* and the *S. pombe* Eso1 ortholog are not required for binding of cohesin to chromosomes, but are needed to establish cohesion during S phase (Skibbens et al. 1999; Tanaka et al. 2000; Toth et al. 1999). Ctf7/Eco1 associates with a replication factor C complex, a PCNA sliding clamp loader, suggesting that it has a critical role during DNA replication (Kenna and Skibbens 2003). As mentioned above, Eso1 also

interacts with Pds5, and genetic evidence suggests that Pds5 inhibits establishment of cohesion in the absence of Eso1 (Tanaka et al. 2001). The Ctf7/Eco1 orthologs in *Drosophila* (Deco) and humans (Esco1/Efo1, Esco2/Efo2) are also required for cohesion (Bellows et al. 2003; Hou and Zou 2005; Williams et al. 2003). *Drosophila deco* mutations affect cohesion primarily at centromeres (Williams et al. 2003). Although these proteins are acetyltransferase enzymes (Bellows et al. 2003; Ivanov et al. 2002), their in vivo substrates are unknown, and it has recently been shown that the acetyltransferase activity is not required for Ctf7/Eco1 to establish cohesion in yeast (Brands and Skibbens 2005).

Effects of sister chromatid cohesion factors on gene expression and development

The first evidence that the sister chromatid cohesion apparatus plays a role in gene expression arose from studies on gene silencing in yeast, and on long-range gene activation in *Drosophila* (Donze et al. 1999; Rollins et al. 1999). The yeast data indicated that cohesin helps establish the boundaries that define the ends of a silenced chromatin domain, while the *Drosophila* studies showed that the Nipped-B cohesin-loading adherin is required for long-range activation of two homeobox protein genes that play critical roles in development. I will first consider the current understanding of the roles of cohesin in yeast gene expression, which has implications regarding the roles of the cohesion apparatus on gene expression and development in higher organisms.

Cohesin and yeast gene silencing

The silent-mating-type loci of *S. cerevisiae* are surrounded by boundary elements that block the spread of the silent chromatin established by chromatin-bound SIR (silent information regulator) protein complexes (Fig. 2). Deletion of the boundaries surrounding the *HMR* silent-mating-type locus leads to spreading of the SIR complexes into neighboring regions (Donze et al. 1999). A search for factors that establish these boundaries revealed that some mutations affecting the Smc1 subunit of cohesin cause drastic loss of boundary function and spread of silencing (Donze et al. 1999).

The Mcd1/Sccl cohesin subunit binds to the *HMR* boundary elements, and within the *HMR* locus itself, consistent with the idea that cohesin functions directly in controlling the spread of silencing complexes (Chang et al. 2005; Glynn et al. 2004; Laloraya et al. 2000; Lengronne et al. 2004). Cohesin also binds to a non-transcribed region contained in each ribosomal RNA gene repeat, and in the subtelomeric repeats of chromosome III. Binding of cohesin to sites within *HMR* depends on Sir3 protein, and the binding to rDNA repeats requires Sir2, indicating that cohesin binding to these loci requires silencing (Chang et al. 2005; Kobayashi et al. 2004). The presence of cohesin in rDNA repeats and subtelomeric repeats, where SIR proteins are also involved in silencing, raises the interesting possibility that cohesin might also inhibit spreading of SIR complexes at these locations.

In addition to the evidence that cohesin can inhibit the spread of silencing protein complexes beyond *HMR*, there is also evidence that cohesin can inhibit establishment of silencing. Loss of cohesion is essential to establish silencing at *HMR*, while loss of the Mcd1/Sccl cohesin subunit allows silencing to be established prematurely (Lau et al. 2002). In contrast, it was also observed that DNA replication factors needed to establish cohesion are also required for silencing at telomeres and the mating-type loci (Suter et al. 2004). In these cases, it is unclear if the effects of cohesin are as direct as they appear to be in the case of its function at the *HMR* boundaries. For example, it is possible that generalized loss of boundary function that occurs when cohesin function is compromised could lead to reduced overall silencing because extensive spreading of the SIR protein complexes dilutes their density in silenced regions.

Recent studies have revealed that in addition to the effects cohesin has on silencing, silencing has reciprocal effects on cohesin binding and cohesion (Chang et al. 2005). In these experiments, the *HMR* silent-mating-type locus was excised from the chromosome in circular DNA molecules, and the circles were assayed for cohesin binding and cohesion. Remarkably, cohesion between sister circles required both cohesin and silencing. Binding of cohesin to *HMR* is SIR-dependent, and loss of silencing leads to loss of cohesin from a chromosomal copy of *HMR*, but not from *HMR* in an extrachromosomal circle. The retention of cohesin but loss of cohesion in the extrachromosomal circles led to the suggestion that the mode of cohesin binding at the silent loci may differ from other locations—it is proposed that a cohesin ring encircles one sister and interacts with silencing factors such as SIR proteins on the other sister to maintain cohesion (Chang et al. 2005). Interactions between SIR complexes and cohesin could be involved in the recruitment and retention of cohesin at the *HMR* locus.

Studies in *S. pombe* are consistent with the idea that silenced chromatin recruits and retain cohesin. Unlike *S. cerevisiae*, and similar to higher eukaryotes, the centromeric regions of *S. pombe* form heterochromatin. The proteins associated with the centromeric regions of *S. pombe* are related to those of higher organisms, and include Swi6, the ortholog of HP1 (heterochromatin protein 1) proteins in higher organisms. Swi6 is required for association of cohesin with heterochromatic regions, and interacts directly with cohesin (Bernard et al. 2001; Nonaka et al. 2002; Partridge et al. 2002). Such interactions between heterochromatin proteins and cohesion factors could be important for the high density of cohesin around the centromere, and retention of cohesion at metaphase. A key question that remains unanswered is whether or not the cohesin recruited by silenced chromatin is required for gene silencing. As discussed below, this may be relevant for the molecular etiology of Roberts-SC phocomelia syndrome in humans.

Another model for the effects of silenced chromatin on cohesin binding arises from studies on the silenced rDNA repeats in *S. cerevisiae*. Association of cohesin with silenced rDNA repeats is Sir2-dependent (Kobayashi et al. 2004; Kobayashi and Ganley 2005). In this case, it is proposed that loss of silencing leads to transcription that actively displaces cohesin from the locus. It is possible that this mechanism also contributes to reduced cohesin binding at the chromosomal *HMR* locus when silencing is reduced.

Genome-wide mapping of cohesin in yeast supports the idea that transcription helps determine the location of cohesin binding sites. This mapping revealed a strong tendency of cohesin to bind in non-transcribed spacer regions between convergent transcription units, as if cohesin was pushed to these locations by RNA polymerase (Glynn et al. 2004; Lengronne et al. 2004). In those rare cases where cohesin binds to a regulatory or transcribed region, gene activation shifts the cohesin-binding site in a manner consistent with the idea that RNA polymerase pushes cohesin along the chromosome.

In recent years, it has been noted that tRNA genes provide boundary functions in both *S. pombe* and *S. cerevisiae* (reviewed by Haldar and Kamakaka 2006), and it is tempting to ask if this is because they effectively localize cohesin through transcription. For instance, the cohesin binding site in the right domain boundary of *HMR* (Fig. 2) is positioned between convergent *tRNA^{Thr}* and *Git1* genes. It is currently unknown if cohesin relocalization requires factors other than the transcriptional machinery. As discussed below, this question could be significant with regards to effects of Nipped-B/delangin and cohesin on gene activation in *Drosophila* and Cornelia de Lange syndrome.

Effects of sister chromatid cohesion factors on long-range gene activation in *Drosophila*

The first mutations in the *Drosophila* *Nipped-B* gene, which encodes an ortholog of the yeast Scc2 cohesin-loading factor, were recovered in a genetic screen for factors that facilitate long-

range activation of the *cut* and *Ultrabithorax* homeobox genes by distant transcriptional enhancers (Rollins et al. 1999). Subsequent work revealed that, as expected, Nipped-B is also required for sister chromatid cohesion (Rollins et al. 2004).

Nipped-B is essential for viability, so effects on gene expression were detected by examining the effects of partially reducing Nipped-B activity or dosage on weak *cut* and *Ultrabithorax* mutants (Rollins et al. 1999). These studies revealed that Nipped-B is particularly limiting for *cut* activation when communication between a remote enhancer and the *cut* promoter is partially compromised by a weak insulator insertion. In contrast, other factors required for *cut* activation are more limiting when the enhancer itself is mutated (Morcillo et al. 1996; Rollins et al. 1999). These *cut* allele-specific effects led to the proposal that the role of Nipped-B in gene expression is to facilitate long-range enhancer–promoter communication (Dorsett 1999).

The finding that Nipped-B, a cohesin-loading factor, is required for long-range gene activation initially suggested that cohesin itself might facilitate activation by, for example, stabilizing "pairing" interactions between distant enhancers and promoters in a manner similar to the way it holds sister chromatids together (Dorsett 1999; Hagstrom and Meyer 2003). This hypothesis was negated, however, when it was discovered that reducing the levels of cohesin subunits by RNAi or mutation increased *cut* expression, which is opposite to the effect of *Nipped-B* mutations (Rollins et al. 2004; Dorsett et al. 2005).

The opposite effects of cohesin and the Nipped-B cohesin-loading factor on *cut* gene expression suggests certain possibilities. It could be that cohesin acts as an insulator that blocks enhancer–promoter communication (Fig. 3; Rollins et al. 2004), similar to the manner in which cohesin blocks spreading of SIR protein complexes at the *HMR* locus in yeast (Fig. 2). In this scenario, in addition to loading cohesin onto chromosomes, Nipped-B could also remove or relocalize cohesin to permit gene activation, similar to the manner in which cohesin relocates upon gene activation in yeast. Reducing Nipped-B dosage would slow the rate of cohesin mobilization, thus hindering activation. It is unknown how cohesin would hinder enhancer–promoter communication, but one possibility is similar to that proposed for the gypsy transposon insulator, in which the insulator blocks a spread of homeoprotein binding between the enhancer and promoter (Dorsett 1999; Gause et al. 2001; Torigoi et al. 2000).

It appears unlikely that sister chromatid cohesion per se inhibits *cut* gene activation by locking the enhancers and promoters of the sisters together and thereby reducing the ability of the enhancer and promoter on each sister to come together. This is because reducing the dosage of Pds5, which is required for cohesion, but not cohesin binding, does not improve gene expression as does reducing the dosage of cohesin subunits (Dorsett et al. 2005).

Another possibility is that cohesin inhibits gene activation indirectly, by interacting with Nipped-B, and preventing it from performing another task that supports gene activation. Current evidence, however, favors the cohesin insulator model over this idea. Although it is not technically feasible to measure binding of cohesin to *cut* in the developing wing margin cells, cohesin binds to *cut* in the polytene chromosomes in salivary glands, and to multiple sites between the wing margin enhancer and promoter in cultured cells of embryonic origin (Dorsett et al. 2005; Fig. 3). Thus, cohesin is positioned to block enhancer–promoter communication. Moreover, a special *pds5* mutation that reduces cohesin binding to chromosomes when homozygous, dominantly increases *cut* expression. A null *pds5* mutation, which causes loss of sister chromatid cohesion, but does not reduce cohesin binding when homozygous, does not dominantly increase gene activation. These results are concordant with the idea that the effect of Nipped-B on gene expression involves its role in regulating binding of cohesin to chromosomes.

In yeast, the Scc2 homolog of Nipped-B does not co-localize with cohesin on chromosomes. The evidence suggests that cohesin loads at Scc2 binding sites and translocates away (Lengronne et al. 2004). In contrast, immunostaining of *Drosophila* polytene chromosomes, and chromatin immunoprecipitation experiments with the *cut* locus indicate that Nipped-B and cohesin bind to the same sites (Z. Misulovin, J.C. Eissenberg, D. Dorsett, unpublished). The basis for this difference between yeast and *Drosophila* is unknown, but co-localization of Nipped-B with cohesin may be critical for cohesin relocation to permit gene activation by remote enhancers. Such relocation may be less critical in yeast, which do not have long-range gene activation.

Scc2 interacts with the Scc4 protein, which is also essential for sister chromatid cohesion. Nipped-B interacts with the *Drosophila* homolog (CG4203) of the *C. elegans* Mau-2 protein, which is only very distantly related to Scc4 (Giot et al. 2003; Seitan et al. 2006). Knockdown of human Mau-2 (KIAA0892) protein in cultured cells causes cohesion deficits, but *C. elegans mau-2* mutants do not display obvious cohesion defects (Seitan et al. 2006). *C. elegans mau-2* mutants do, however, display defects in cell and axonal migration (Bénard et al. 2004; Takagi et al. 1997). This provides an additional indication that the sister chromatid cohesion apparatus can influence development under conditions that do not have an overt effect on cohesion. One possibility is that *mau-2* mutations only partially reduce activity of the *C. elegans* Scc2 homolog (Pqn-85), resulting in changes in gene expression without an effect on cohesion, similar to the way heterozygous *Drosophila Nipped-B* mutations reduce gene expression without obvious effects on cohesion (Rollins et al. 1999; Rollins et al. 2004). The threshold for effects on both gene expression and cohesion may be higher with Mau-2 than with Nipped-B, as partial knockdown of *Drosophila mau-2* (CG4203) expression by RNAi did not have detectable effects on either *cut* gene expression or sister chromatid cohesion (Rollins et al. 2004; Seitan et al. 2006).

Sister chromatid cohesion factors in human syndromes

It was recently discovered that certain human syndromes are caused by mutations in genes encoding components of the sister chromatid cohesion apparatus. Cornelia de Lange syndrome is caused by mutations in *Nipped-B-Like* (*NIPBL*), which encodes the human ortholog of Nipped-B and Scc2 (Krantz et al. 2004; Tonkin et al. 2004), and in *Smc1L1*, which encodes the human Smc1 cohesin subunit (Musio et al. 2006). The Roberts-SC phocomelia syndrome is caused by mutations in *Esco2*, one of the human orthologs of *Ctf7/Eco1* (Vega et al. 2005; Schüle et al. 2005). Although the Cornelia de Lange and Roberts-SC phocomelia syndromes display similar developmental deficits, the molecular etiologies may differ significantly.

Cornelia de Lange syndrome (CdLS)

CdLS (OMIM #122470, also known as Brachmann-de Lange syndrome) is estimated to occur at a frequency of 1 per 10,000 to 30,000 births and displays significant deficits in both physical and mental development (de Lange 1933; Ireland et al. 1993; Jackson et al. 1993; Opitz 1985). The deficits begin prenatally and continue after birth. A remarkable feature is the diversity of developmental effects, and thus, CdLS may provide clues regarding the mechanisms of other developmental disorders that display subsets of these features. There is also considerable variation in the severity of the syndrome. Table 2 lists some features of CdLS, which include diagnostic facial characteristics, upper limb abnormalities, esophageal defects, cardiac malformations, and mental retardation. CdLS patients display slow growth and are small.

Although genetic data and samples were accumulated over several years, mapping of the gene(s) responsible for CdLS proved challenging because it is genetically dominant, and the vast majority of cases are spontaneous. There are rare examples of a parent having more than one

CdLS child, which is most likely caused by germline mosaicism, and a few cases in which a mildly affected CdLS patient passed on the disorder to their offspring. The first CdLS gene was mapped by linkage exclusion analysis and a de novo balanced translocation affecting the 5p13.1 region, which is one of the five non-excluded regions (Krantz et al. 2004; Tonkin et al. 2004). Subsequent analysis revealed mutations in the *NIPBL* gene in 5p13.1 in many of the patients, and now several studies have identified a variety of *NIPBL* mutations in CdLS patients (Borck et al. 2004; Bhuiyan et al. 2005; Gillis et al. 2004; Krantz et al. 2004; Miyake et al. 2005; Tonkin et al. 2004).

NIPBL mutations have been identified in about half of the CdLS patients. Recently, two mutations in the *Smc1L1* gene (Xp11.21) were identified in certain relatively mild cases of CdLS that lacked *NIPBL* mutations (Musio et al. 2006). *Smc1L1*, which encodes the Smc1 cohesin subunit, is X-linked, and the affected individuals are male. This indicates that the mutations are unlikely to be strong loss-of-function alleles, which would likely be lethal. The *Smc1L1* gene escapes X-inactivation (Brown et al. 1995), and of the three female carriers that passed on an *Smc1L1* mutation, one displayed very mild characteristics consistent with CdLS. It is currently unknown how many of cases of CdLS are caused by *Smc1L1* mutations.

It remains possible that mutations in other genes encoding components of the sister chromatid cohesion apparatus could cause CdLS. However, none of the other potential candidates, such as the human *Mau-2* (KIAA0892) or *Pds5* genes, are located in the five candidate regions identified by linkage exclusion (Krantz et al. 2004). It is also likely that some CdLS-causing mutations occur in non-transcribed regulatory regions of the *NIPBL* gene, or have been missed because the large gene size and complex splicing patterns currently preclude sequencing of the entire *NIPBL* transcription unit. Improvements in screening for *NIPBL* mutations, and large scale screening of patients for *Smc1L1* mutations will resolve some of these questions in the near future.

The known *NIPBL* mutations indicate that reduced levels or activity of the encoded delangin protein cause CdLS. Many *NIPBL* mutations predict protein truncations, although it is currently unknown whether or not the encoded mutant proteins are expressed. A deletion removing the *NIPBL* region, however, was seen in a severe case of CdLS, supporting the idea that loss-of-function mutations cause CdLS (Hulinsky et al. 2003). Many of the less severe cases of CdLS are associated with mutations that cause amino acid substitutions, although the general correlation of milder CdLS cases with missense mutations and more severe forms with truncations has exceptions (Bhuiyan et al. 2005; Gillis et al. 2004). Many polymorphisms are found in unaffected parents, and in addition to numerous other genetic differences between individuals, these polymorphisms could contribute to the phenotypic variability of the syndrome.

An intriguing finding from molecular analysis of *NIPBL* mutations in CdLS patients is that a central conserved arginine residue (R2298) in one of the HEAT repeats has been altered by missense mutations in multiple independent cases, suggesting that it is a critical residue. Another important finding is that with only a few exceptions, the *NIPBL* missense mutations associated with CdLS affect residues conserved in *Drosophila Nipped-B*. Thus, it appears likely that determination of the molecular functions of Nipped-B in *Drosophila* and other model organisms will illuminate the molecular etiology of CdLS.

As expected, the two *Smc1L1* mutations causing CdLS are not severe. A spontaneous missense mutation found in one patient converts a glutamic acid residue to alanine, and an inherited 3-bp deletion found in three patients and three female carriers simultaneously makes a conservative aspartic acid to a glutamic acid change and deletes a neighboring glutamine residue. In both cases, full-length Smc1 is detected at apparently normal levels in peripheral

blood lymphocytes (Musio et al. 2006). The glutamic acid residue affected by the spontaneous mutation is conserved in *Drosophila*, and based on the partial crystal structure of a bacterial SMC protein (Haering et al. 2002), is positioned precisely at the junction of the N-terminal helix of the antiparallel coiled coil and the hinge domain. The inherited mutation affects a conserved region in the C-terminal helix of the coiled coil.

Evidence from *Drosophila* favors the idea that the developmental disorders in CdLS are caused by altered expression of genes that control development. Although effects on sister chromatid cohesion are seen in homozygous *Nipped-B* mutants, no effects on cohesion are observed in heterozygotes, which show measurable effects on gene expression. Even partial reduction of *Nipped-B* by RNAi sufficient to cause lethality had no detectable effect on cohesion (Rollins et al. 2004).

If the cohesin insulator mechanism (Fig. 3) proposed to explain the opposite effects of *Nipped-B* and cohesin on gene expression in *Drosophila* is correct, then one would predict that several genes that control development will be affected in CdLS. Cohesin likely binds every 20 kb or so, and many mammalian genes rely on distant control elements for appropriate temporal and spatial expression. Many of these could explain some of the developmental deficits in CdLS. For example, some of the genes in the *HoxD* locus are critical for proximal-distal limb patterning, and this locus is regulated by transcriptional control elements located more than 200 kb away (Spitz et al. 2003). Reduced *HoxD* expression could easily be responsible for some of limb defects seen in CdLS. Similar effects on other homeobox genes could cause the other physical birth defects, and effects of gene expression on neural development could contribute to mental retardation.

The *Drosophila* data also suggest that the *Smc1L1* mutations associated with milder forms of CdLS likely cause alterations in gene expression. A null allele of *Smc1* in *Drosophila* dominantly increases *cut* gene expression (Dorsett et al. 2005), as does RNAi knockdown of the Stromalin and Rad21 cohesin subunits (Rollins et al. 2004). These effects are opposite to those of *Nipped-B* mutations, and, thus, it might be surprising at first that human *Smc1L1* mutations cause similar developmental problems as *NIPBL* mutations. There are multiple possible explanations that could resolve this apparent paradox. The CdLS patients with *Smc1L1* mutations display relatively mild symptoms, and it is possible that overexpression or inappropriate expression of certain genes could have similar, but not identical, effects on development as their underexpression.

An intriguing possibility is that the identified *Smc1L1* mutations are not null alleles, but slow down cohesin-binding dynamics as is proposed above in the case of reduced *NIPBL*/delangin levels. As noted above, one *Smc1L1* mutation alters a conserved residue at the junction of the N-terminal helix and the hinge domain that dimerizes with Smc3 hinge. It has been shown for a prokaryotic SMC protein that the hinge is critical for DNA-binding dynamics, and that hinge-DNA interactions stimulate the ATPase activity of the head domain (Hirano and Hirano 2002; Hirano and Hirano 2006). The ATPase activity, along with Scc2/*Nipped-B*/delangin, is essential for the binding of cohesin to chromosomes (Arumugam et al. 2003; Weitzer et al. 2003). Stimulation of the head ATPase activity by the hinge domain may involve direct interactions, as atomic force microscopy has revealed hinge-head contacts in fission yeast Smc1-Smc3 heterodimers (Sakai et al. 2003). These contacts could be affected by changes in the flexibility of the coiled-coil domain. Thus, it remains a possibility that both *Smc1L1* mutations could slow the kinetics of cohesin binding and unbinding, and thereby alter gene expression in manner similar to that proposed for *NIPBL* loss-of-function mutations.

Another possibility is that a subset of the CdLS deficits, including those found in patients with *Smc1L1* mutations, reflect changes in sister chromatid cohesion, as opposed to changes in gene

expression. Using immortalized cell lines, a recent study revealed mild cohesion defects in 40% of CdLS patients (Kaur et al. 2005). The procedures used to prepare metaphase spreads enhance chromatid separation, so it is unclear if such cohesion defects occur *in vivo*, or if the analysis reveals a subtle defect that has little role in the etiology of CdLS.

The current challenge is to determine the relative contributions of changes in gene expression and sister chromatid cohesion in CdLS, and to identify the critical target genes responsible for the known developmental effects. This will be aided by studies on the molecular mechanisms by which *Nipped-B* and cohesin subunit mutations affect gene expression, cohesion, and development in *Drosophila*, but a mammalian model is essential to identify target genes relevant to specific CdLS birth defects.

Roberts and SC phocomelia syndromes (RBS/SC)

Roberts (OMIM #268300) and SC phocomelia (OMIM #269000) are genetically recessive syndromes caused by mutations in the *Esco2/Efo2* homolog of *Ctf7/Eco1* located at 8p21.1 (Vega et al. 2005; Schüle et al. 2005). RBS/SC and CdLS display similar characteristics, which include, but are not limited to, slow growth, mental retardation, and limb defects (Table 2; Roberts 1919; Herrmann and Opitz 1977; Van den Berg and Francke 1993). RBS/SC limb defects are symmetrical, which is not the case in CdLS. In CdLS, most of the defects occur in the upper limbs (Jackson et al. 1993). While all four limbs are usually affected in RBS/SC, the upper limbs are affected more frequently (Van den Berg and Francke 1993).

Patients diagnosed with SC phocomelia display less severe physical defects and milder mental retardation than those diagnosed with Roberts (Herrmann et al. 1969), although it has long been suspected that RBS and SC are the same syndrome. Some of the more compelling clues include siblings that were diagnosed individually as RBS and SC (Romke et al. 1987), the presence of chromosomal abnormalities in metaphase cells from both RBS and SC patients (Freeman et al. 1974; Judge 1973; Tomkins et al. 1979), and cell hybrid and chromosome transfer complementation studies indicating that they are caused by mutations in the same gene (McDaniel et al. 2000; McDaniel et al. 2005). Identification of *Esco2/Efo2* mutations in both RBS and SC patients confirms their long-suspected relationship (Vega et al. 2005; Schüle et al. 2005).

The Roberts syndrome gene was mapped using consanguineous families from Colombia, leading to the discovery of mutations in the *Esco2/Efo2* gene (Vega et al. 2005). Analysis of SC phocomelia families also uncovered novel *Esco2* mutations (Schüle et al. 2005). Although the difference in severities of the RBS and SC characteristics might predict that the SC-causing mutations would be less severe, in fact, all the SC phocomelia mutations appear to be truncating, loss-of-function alleles, similar to most of the Roberts mutations (Schüle et al. 2005). Thus, the reason for the variability in phenotypic severity remains unknown, although it is easy to speculate that genetic background could play an important role. Indeed, the one mutation that was found twice was present in an adult with SC phocomelia and a fetus with severe Roberts (Schüle et al. 2005). In both cases, this allele was heterozygous with similar truncating alleles.

A key distinguishing feature of Roberts and SC phocomelia is the presence of chromosomal and mitotic defects (German 1979; Louie and German 1981; Tomkins and Siskin 1984; Tomkins et al. 1979; Jabs et al. 1991). Although there are reports of RBS patients in which chromosomal defects were not apparent, reexamination revealed that none of the cases lacking defects could be confirmed and, in at least one case, might have involved misdiagnosis of a CdLS patient as Roberts syndrome (Schüle et al. 2005).

The chromosomal phenotype of Roberts patients has been termed "heterochromatin repulsion" (HR). Constitutive heterochromatic regions (C bands) appear puffy, and there is separation or "splaying" of these regions (German 1979; Louie and German 1981; Tomkins et al. 1979). Because centromeres are located in heterochromatin, there is separation of centromeres, but all heterochromatic regions are affected, including the nucleolar organizer regions and the long arm of the Y chromosome. This is similar to what is seen in *Drosophila deco* mutants, in which cohesion is affected at centromeres, but not along the euchromatic arms (Williams et al. 2003). Similar to RBS/ SC patient cells, *Drosophila deco* mutants also show occasional anaphase defects such as lagging chromosomes.

An intriguing question is why RBS/SC patients and *Drosophila deco* mutants show effects on cohesion specifically in heterochromatic but not euchromatic regions. As discussed above, yeast orthologs of Esco2 interact with Pds5 and with specific DNA replication factors, and thus, might be expected to affect establishment of cohesion along the length of the chromosome during S phase. In *S. pombe*, Pds5 appears to inhibit establishment of cohesion in the absence of the Eso1 ortholog of Esco2, although cohesion is not completely lost (Tanaka et al. 2001). It is possible that other factors can compensate for loss of Deco and Esco2 in euchromatic regions, where the cohesin density is lower. It is also possible, as suggested by the requirement for both heterochromatin proteins and cohesin at the yeast *HMR* locus for cohesion, that the mode of cohesin binding is different in heterochromatic regions in a way that makes establishment of cohesion more dependent on Esco2 and Deco.

It has long been postulated that the growth defects in RBS/SC patients result from an abnormal mitotic cycle in which the metaphase is extended, and there is an increase in abnormal anaphases (Tomkins and Siskin 1984). Indeed, the delayed mitotic cycle appears likely to contribute to the growth and developmental deficits. The similarity of RBS/ SC to CdLS, and data from *Drosophila* and yeast, however, raise an additional possibility. CdLS patients show developmental deficits similar to those in RBS without the same chromosomal defects. Moreover, association of the Rad21 cohesin subunit with centromeric regions is reduced in *Drosophila deco* mutants. The reduced binding of cohesin to centromeric regions in *deco* mutants, together with the roles of cohesin in gene silencing in yeast discussed above, suggests that effects on gene expression could occur in *deco* mutants and RBS/SC. Studies in *Drosophila* have revealed that some genes that normally reside in heterochromatin require heterochromatic proteins for normal expression, while others are silenced (reviewed by Dimitri et al. 2005). Thus, it is possible that changes in the level or mode of cohesin binding in heterochromatic regions could increase or decrease expression of heterochromatic genes. It is also feasible that changes in cohesin binding could increase the spread of heterochromatin, similar to the manner in which cohesin mutations allow spreading of SIR complexes from the yeast *HMR* locus as discussed above. Such a spread could also alter expression of genes that reside near the borders of centromeric heterochromatin. Thus, it is possible that both changes in the mitotic cell cycle and effects on gene expression could be critical to the etiology of the RBS/SC syndrome.

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Fig. 1.

Proposed functions for sister chromatid cohesion proteins. **a** Structure of cohesin; **b** topological linkage of sister chromatids by a cohesin ring. Other models for how cohesin mediates cohesion are discussed in the text. **c** Speculative model for how the Nipped-B/Mau-2 (Sccl/Sccl4) adherin complex facilitates binding of cohesin to chromosomes. It is proposed that adherin stimulates opening of the cohesin ring, which requires ATP hydrolysis by the SMC dimer, allowing the chromosome to enter the ring. **d** Speculative models for the functions of Pds5 and Ctf7/Eco1 (Eso1, Esco2, Deco) in establishing cohesion during DNA replication. Ctf7/Eco1 interacts with certain DNA replication factors and with Pds5. Pds5 also binds to cohesin and inhibits establishment of cohesion in the absence of Ctf7/Eco1. Based on structural similarities between Pds5 and Nipped-B, and the ability of a mutant *Drosophila* Pds5 protein to reduce cohesin binding, it is proposed that Pds5 opens cohesin after DNA replication to allow both sisters to enter the ring.

Fig. 2.

Cohesin functions at the boundaries surrounding the yeast *HMR* silent mating-type locus. *HMR* silencing is mediated by binding of SIR (silent information regulator) protein complexes (red circles) to the *a2* and *a1* genes. The E and I sequence elements are needed to establish silencing. Cohesin binds to the boundary elements that block the spread of SIR protein binding beyond the *HMR* locus, and boundary function is compromised in *smc1* mutants, indicating that cohesin blocks the spread of SIR complexes

Fig. 3.

Model for the effects of Nipped-B and cohesin on long-range activation of the *Drosophila cut* gene. A wing margin-specific transcriptional enhancer is located more than 80 kbp upstream of the promoter. Cohesin binds to four regions between the enhancer and the promoter and inhibits gene expression. Nipped-B facilitates gene activation, and it is proposed that Nipped-B facilitates a dynamic equilibrium between bound and unbound cohesin. Removal of cohesin facilitates enhancer–promoter contact and gene activation. Reduction of Nipped-B activity slows the rate of cohesin removal and the efficiency of gene activation

Table 1

Major components of the mitotic sister chromatid cohesion apparatus

	<i>S. cerevisiae</i>	<i>S. pombe</i>	<i>C. elegans</i>	<i>Drosophila</i>	Human
<i>Cohesin</i>					
<i>Smc1</i>	Smc1	Psm1	Him-1	Smc1	Smc1/Smc1L1
<i>Smc3</i>	Smc3	Psm3	Smc-3	Smc3/Cap Smc3	
<i>Rad21</i>	Med1/Sec1	Rad21	Coh-2/Sec-1	Rad21	Rad21
<i>Stromalin</i>	Sec3	Psc3	Sec-3	Stromalin/SA	Stag1, Stag2
<i>Adh1er1</i>					
<i>Sec2</i>	Sec2	Mis4	Pqn-85	Nipped-B	de1angin/NIPBL
<i>Sec4</i>	Sec4	Ssl3	Mau-2	Mau-2	Mau-2/Sec4
<i>Pds5</i>	Pds5	Pds5	Evl-14/Pds-5	Pds5	Pds5A, Pds5B
<i>Eco1</i>	Ctf7/Eco1	Eso1		Eco/Deco	Eso1, Eco2

Table 2
Occurrence of clinical features in Cornelia de Lange and Roberts-SC phocomelia syndromes

Feature	CdLS (%) ^a	RBS/SC (%) ^b
Growth retardation (pre- and postnatal)	~100	98
Mental retardation	(mean IQ 53, range<30 to 102)	79
Facies		
Microcephaly (small head)	93	80
Low posterior hairline	92	—
Bushy eyebrows, synophrys (unibrow)	98	—
Ocular abnormalities (ptosis, myopia, nystagmus)	57	—
Prominent eyes	—	69
Widely spaced eyes	+	87
Cloudy cornea	—	68
Long curly eyelashes	99	—
Depressed/wide nasal bridge	83	+
Anteverted nares (upturned nostrils)	85	—
Hypoplastic nasal alae	—	+
Long philtrum, thin upper lip, downturned mouth	94	—
High arched palate	86	+
Late eruption and widely-spaced teeth	86	—
Micrognathia (small jaws/mandible)	84	+
Prominent maxilla	—	+
Cleft palate	+	61
Cleft lip	—	49
Skin	—	—
Hirsutism (excessive hair)	78	—
Cutis marmorata (marble skin)	56	—
Hypoplastic nipples/navel	50	—
Facial hemangiomas	—	+
Extremities		
Micromelia (small hands and feet)	93	—
Reduction defects/oligodactyly	27	+
Phocomelia	—	98 ^c
Clinodactyly (deflection)	74 (5th finger)	+
Radioulnar synostosis (fusion)	+	+
Leg bone synostosis	—	+
Simian crease (single palm crease)	51	—
Proximally placed thumbs	72	—
Flexion contractures	64 (elbows)	+
Syndactyly	86 (toes 2 and 3)	43
Genitalia		
Hypoplasia (arrested development)	57	—
Undescended testes (cryptorchidism)	73	51
Hypospadias (urethra anomalies)	33	—
Enlarged phallus	—	53
Gastrointestinal		
GE reflux	+	—
Gut duplication, malrotation	+	—
Volvulus (intestinal twisting)	+	—
Pyloric stenosis (narrow pylorus)	4	—
Diaphragmatic hernia	+	—
Other		
Hearing loss	60	—
Heart defects	14	48
Kidney/urinary tract defects	36	+
Thrombocytopenia (low blood platelets)	+	—

^a Data are from Jackson et al. 1993, L. Jackson, personal communication, and Selicorni et al. 2005. When reported, the frequency of occurrence is indicated. Otherwise, "+" indicates that the feature has been reported for multiple patients and "—" indicates that feature has not been reported

^b Data are from Van den Berg and Francke 1993

^c 87% show tetrachomelia