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How Many Roads Lead to Cohesinopathies?

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Genetic mapping studies reveal that mutations in cohesion pathways are responsible for multispectrum developmental abnormalities termed cohesinopathies. These include Roberts syndrome (RBS), Cornelia de Lange Syndrome (CdLS), and Warsaw Breakage Syndrome (WABS). The cohesinopathies are characterized by overlapping phenotypes ranging from craniofacial deformities, limb defects, and mental retardation. Though these syndromes share a similar suite of phenotypes and arise due to mutations in a common cohesion pathway, the underlying mechanisms are currently believed to be distinct. Defects in mitotic failure and apoptosis i.e. *trans* DNA tethering events are believed to be the underlying cause of RBS, whereas the underlying cause of CdLS is largely modeled as occurring through defects in transcriptional processes i.e. *cis* DNA tethering events. Here, we review recent findings described primarily in zebrafish, paired with additional studies in other model systems, including human patient cells, which challenge the notion that cohesinopathies represent separate syndromes. We highlight numerous studies that illustrate the utility of zebrafish to provide novel insights into the phenotypes, genes affected and the possible mechanisms underlying cohesinopathies. We propose that transcriptional deregulation is the predominant mechanism through which cohesinopathies arise. *Developmental Dynamics 000:000–000, 2017*. © 2017 Wiley Periodicals, Inc.

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Part I: Area of Study and Significance

Cohesin-mediated DNA Tetherings Play Multifaceted Roles During Development

The tethering together of two DNA segments, in either intramolecular (cis) or intermolecular (trans) conformations, is a fundamental component of many cellular processes (Zakari et al., 2015; Skibbens, 2016). For instance, DNA-segment cis tethers occur at the loop base of a single DNA molecule and bring into registration regulatory elements (enhancers, promoters, insulators, terminators, etc.) that deploy developmental transcription programs largely thought to occur during the G1 stage of the cell cycle. DNA-segment cis tethers also promote the longitudinal compaction of DNA molecules that is required for regional changes in chromatin structure (including rDNA looping) during G1 and genome-wide chromosome condensation during mitosis. Conversely, DNA-segment trans tethers occur between two separate DNA molecules, such as sister chromatids, and identify the products of chromosome replication as sisters from S phase until anaphase onset. In addition to ensuring high-fidelity chromosome segregation, trans tethers ensure proximity of template DNA required for error-free DNA repair.

Despite the diverse roles of DNA segment tethering across the cell cycle and through distinct cis and trans conformations, a single complex participates in each of these activities. Cohesin complexes are evolutionarily conserved from yeast to humans and are composed of five core subunits: SMC1A, SMC3, RAD21/MCD1/SCC1, SA1,2/STAG1,2/Irr1/Scc3, and PDS5 (Fig. 1). SMC1A and SMC3 are elongated ATPase proteins that together form a ring that is stabilized by RAD21/MCD1/SCC1 (herein RAD21). RAD21 in turn recruits the remaining components (PDS5 and either SA1 or SA2) (Jeppsson et al., 2014; Marston, 2014; Skibbens, 2016). The exact mechanism through which DNA segments become entrapped within this flattened cohesin ring remains enigmatic but likely involves more than one cohesin ring.

Cohesins require a series of auxiliary factors to bind DNA prior to functioning in chromosome segregation, chromatin condensation, DNA repair, and transcriptional regulation (Fig. 1). The NIPBL/SCC2 and MAU-2/SCC4 deposition complex is required to load cohesins onto DNA segments (Ciosk et al., 2000; Seitan et al., 2006; Rollins et al., 1999; Rollins et al., 2004). The rules governing cohesin deposition are poorly understood. Once chromatin-bound, however, cohesins must become activated (promoting cohesin oligomerization and/or stabilizing DNA entrapment). This role is supplied by ESCO2/EFO2/ESCO1/EFO1/Ctf7/Eco1. ESCO/EFO (herein ESCO) proteins are highly conserved acetyltransferases. ESCO activation of SMC3 through acetylation is required for all subsequent cohesin-dependent activities (Skibbens et al., 1999; Toth et al., 1999; Ivanov et al., 2002; Bellows et al., 2003; Hou and Zou, 2005; Rolef Ben-Shahar et al., 2008; Zhang et al., 2008). Cohesins must also be turned off, either to allow for sister

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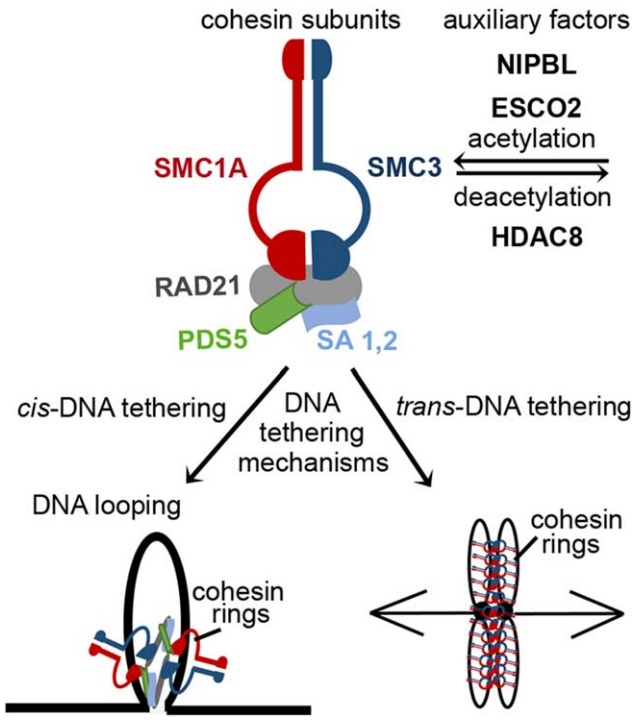

Fig. 1. DNA tethering mechanisms of cohesins and auxiliary factors. Schematic representation of the cohesin ring structure (composed of the five subunits: SMC1A, SMC3, RAD21, SA1,2, and PDS5) and auxiliary factors NIPBL (loader), ESCO2 (acetyltransferase), and HDAC8 (deacetylase). Cohesins and auxiliary factors regulate both cis- and trans-DNA tethering events. Cis-DNA tethering processes include DNA looping mechanisms during transcription, condensation, and ribosome biogenesis. Trans-DNA tethering processes include sister chromatid segregation during S phase of cell cycle and DNA repair events.

chromatid separation at anaphase onset or to respond to transcriptional responses to external cues through tether disassembly/reassembly. Cohesin inactivation at anaphase onset is irreversible and occurs through RAD21 degradation, although a nonproteolytic release mechanism can reduce cohesin binding during prophase (Funabiki et al., 1996; Ciosk et al., 1998; Uhlmann et al., 1999; Shintomi and Hirano, 2010). On the other hand, cohesin inactivation during G1 likely occurs through disassociation of cohesins or cohesin dimers. In both cases, HDAC8/Hos1 deacetylase helps reset the acetylation state of SMC3 (Xiong et al., 2010; Borges et al., 2010; Beckouet et al., 2010; Deardorff et al., 2012a).

Cohesion Pathway Mutations Cause Human Disease

Genetic mapping studies reveal that mutations in cohesion pathways (i.e. including both structural cohesin genes and auxiliary genes) are responsible for multispectrum developmental abnormalities termed "cohesinopathies." These include Warsaw Breakage Syndrome (WABS), Roberts Syndrome (RBS), and Cornelia de Lange Syndrome (CdLS) (Krantz et al., 2004; Tonkin et al., 2004; Schule et al., 2005; Vega et al., 2005; Musio et al., 2006; Deardorff et al., 2007; Gordillo et al., 2008; van der Lelij et al., 2010; Deardorff et al., 2012b; Deardorff et al., 2012a; Yuan et al., 2015). WABS patients exhibit craniofacial abnormalities (severe microcephaly, facial dysmorphia), cognitive impairment, growth retardation (consistent with reduced long-bone growth), deafness, heart defects, and extremity impairments (clinodactyly). WABS arises from mutations in the DNA helicase DDX11/Ch1R (van der Lelij et al., 2010; Capo-Chichi et al., 2013). WABS is considered a cohesinopathy since mutations in ChlR1 family members (including Chl1 in yeast) result in premature sister chromatid separation and significantly reduce cohesin and Scc2 (i.e., NIPBL homolog in yeast) binding to chromatin (Borges et al., 2010; Rudra and Skibbens, 2013; Inoue et al., 2007; Laha et al., 2011). RBS and CdLS patients exhibit significantly overlapping and severe phenotypes that include cleft palate, microcephaly, profound limb reduction, acute mental retardation, and defects in heart, kidney, genital, and gastrointestinal development (Van den Berg and Francke, 1993; Schule et al., 2005; Vega et al., 2005; Liu and Krantz, 2009; Mannini et al., 2010).

RBS is an autosomal recessive disorder arising from mutations in the acetyltransferase ESCO2 (Schule et al., 2005; Vega et al., 2005). Cells from RBS patients exhibit chromosomal segregation defects, premature centromere separation, heterochromatin repulsion (which produces railroad track–like mitotic chromosomes), lagging chromosomes, aneuploidy, and reduced cell proliferation (Schule et al., 2005; Vega et al., 2005; Gordillo et al., 2008). Because of these phenotypes, defects in trans DNA tethering events are believed to be the underlying cause of RBS.

CdLS is an autosomal dominant disorder that arises from either heterozygous or X-linked mutations in the cohesion subunits SMC1A, SMC3, and RAD21, as well as in the auxiliary factors NIPBL and HDAC8 (Krantz et al., 2004; Tonkin et al., 2004; Musio et al., 2006; Deardorff et al., 2007; Deardorff et al., 2012a; Deardorff et al., 2012b). CdLS occurs in approximately 1 in 10,000 live births (Opitz, 1985; Krantz et al., 2004), and around 65% of the reported cases arise from mutations in the *NIPBL* gene (Krantz et al., 2004; Tonkin et al., 2004; Zakari et al., 2015). In contrast to those in RBS patients, cells from CdLS patients do not exhibit premature centromere separation/heterochromatin repulsion or mitotic defects (Krantz et al., 2004). Due to the lack of overt defects in trans DNA tethering and early studies that linked Drosophila Nipped-B (NIPBL homolog) to transcription regulation (Rollins et al., 1999), the underlying cause of CdLS is largely modeled as occurring through defects in cis DNA tethering.

CdLS and RBS comprise overlapping characteristic phenotypes, and the causative genes for both syndromes perform common activities. It is therefore surprising that CdLS and RBS are considered separate syndromes. Rather, perhaps the observed differences between CdLS and RBS phenotypes are based on the effective dosage of the cohesion pathway genes. For example, CdLS is caused by dominant mutations in genes predominantly coding for structural components of the cohesin ring, whereas RBS is caused by recessive mutations in the gene coding for an enzyme that regulates ring closure. In this article, we consider the possibility that the apparent differences in underlying mechanisms are the result of a modest loss of cohesion pathway activity in the CdLS patients (which is sufficient for trans DNA tethering needed during mitosis but insufficient for cis DNA tethering), compared to an almost complete loss of cohesion pathway activity in RBS patients (where defects in trans DNA tethering dominate and therefore mask underlying effects on cis DNA tethering).

Justification of Using Zebrafish for Cohesinopathy Research

Zebrafish is a powerful and penetrant model in which to study human disease, including cohesinopathies (Horsfield et al., 2012).

TABLE 1. Changes in Gene Expression Associated with Cohesinopathy Genes

Gene	Change in gene expression	Pathways	Reference
smc1a	*cyclinD1*	CNS	Fazio et al., 2016
smc3	*cyclinD1, p53, mdm2, bax, p21, myca, ascl1a/b, p53, mdm2*	Tail and notochord morphogenesis	Ghiselli et al., 2006 Muto et al., 2011
rad21	*sox10*	Heart	Schuster et al., 2015
	p53, mdm2, myca		Rhodes et al., 2010
	runx1	Blood cells	Horsfield et al., 2007 Marsman et al., 2014
	myca, ascl1a/b, p53		Muto et al., 2011
nipblb	*wnt, cyclinD2*	CNS	Pistocchi et al., 2013
	cyclinD1		Fazio et al., 2016
nipbla/b	*sox17, sox32, foxa2, gata5, spaw, lefty-2, dnah9, myca, ascl1a/b*	Endoderm differentiation, left-right patterning	Muto et al., 2011
	fgf4, fgf8, fgf16, hoxd, hand2 shha, ptch2	Pectoral fin, limb bud, intestine	Muto et al., 2014
esco2	*myca*	Craniofacial skeleton, pectoral fin	Monnich et al., 2011
	cx43, sema3d, hapln1a	Skeletal regeneration	Banerji et al., 2016

Summary of the significant changes that occur in developmental gene expression associated with the loss of cohesion pathway functions.

For instance, the Zebrafish CNS, heart, gut, cephalic structures, and skeleton appear exquisitely sensitive to cohesins, Nipbl, and Esco2 levels (Muto et al., 2011; Pistocchi et al., 2013; Muto et al., 2014; Banerji et al., 2016; Fazio et al., 2016). Similar to that of humans, the Zebrafish genome harbors orthologues of most cohesion genes (*nipbla* and *nipblb; esco1* and *esco2; smc1a* and *smc1al; smc3; rad21a* and *rad21b; stag1a/b* and *stag2a/b* and *stag3*; *pds5a* and *pds5b*). Gene function may be examined through generation of mutant alleles using genome editing (Hwang et al., 2013; Seruggia and Montoliu, 2014) or by the use of gene-targeting morpholinos (Nasevicius and Ekker, 2000). Below we highlight numerous studies that illustrate the utility of Zebrafish to provide novel insights into the phenotypes and genes effected in cohesinopathies (Table 1).

Zebrafish morphants and mutant strains reveal the high degree of efficacy in recapitulating CdLS and RBS phenotypes, such as cardiac phenotypes (Schuster et al., 2015), which are prevalent in CdLS patients. Because *rad21*^{nz171}-null (*rad21a*) fish die by 72 hours postfertilization (hpf) (Horsfield et al., 2007), a careful examination of associated heart phenotypes is not possible. Using morpholinos, however, Rad21 activity could be titrated to a level where cardiac defects were apparent in the context of normal gross development. The heart structural defects (smaller, looping defects; reduced function) were partly attributed to a failure of cardiac neural crest cells to populate the heart field and partly attributed to apoptosis. Moreover, CdLS and RBS are both multispectrum disorders. Even *nipblb* single morphants exhibit modest developmental defects that include microcephaly, microphthalmy, and short tails (Pistocchi et al., 2013). These phenotypes are enhanced in morphants knocked down for both *nipbla* and *nipblb* (herein *nipbla/b*) (Muto et al., 2011). Further, roughly 50% of *nipbla/b* double-morphant embryos exhibit reduced leftward looping of the heart, cardia bifida, and laterality defects of the gastrointestinal tract (Muto et al., 2011). The combination of *nipblb* and *smc1a* morpholinos, which individually failed to induce developmental effects, succeeded in recapitulating CdLS phenotypes. These findings are consistent with the notion that *nipblb* and *smc1a* operate through a common pathway (Fazio et al., 2016). Equally compelling recapitulation of CdLS phenotypes in Zebrafish comes from studies of the pectoral fin, which is homologous to the mammalian forelimb. Pectoral fin lengths are significantly reduced in *nipbla/b* morphants in the absence of increased apoptosis (Muto et al., 2014). Next, we discuss possible mechanisms underlying cohesinopathy phenotypes.

Part II: State of Understanding—Suggested Mechanisms of Disease

Reduced Cohesion Function as a Model for CdLS

Apoptosis is activated through multiple mechanisms

Apoptosis may be an underappreciated phenotype of CdLS. For example, mitotic defects have been reported in CdLS patient cell lines (Kaur et al., 2005). Moreover, apoptosis is elevated in Zebrafish morphants for *smc3, smc1a, rad21a*, and *nipblb* (Ghiselli, 2006; Fazio et al., 2016, Schuster et al., 2015; Pistocchi et al., 2013). These findings are consistent with the notion that sufficiently decreased dosage of structural components produce trans DNA tethering defects and mitotic failure in cohesinopathies. Knockdown of either *smc3* or *rad21a* exhibits p53-dependent apoptosis, consistent with cell death due to mitotic failure (Ghiselli, 2006; Schuster et al., 2015). Further, *smc3* morphants exhibit genomic instability. In contrast, both *smc1a* and *nipblb* morphants exhibit p53-independent apoptosis, suggesting that cell death occurs independent of mitotic failure. To determine if morphants exhibit cell cycle delays, expression of cyclinD1 was monitored (i.e., cyclinD1 is reduced in CdLS patient cells) (Fazio et al., 2016). Intriguingly, cyclinD1 is reduced in *smc1a, smc3*, and *nipblb* morphants (Pistocchi et al., 2013). Together, these findings demonstrate that cohesin inactivation results in decreased cyclin levels (consistent with cell cycle

delays) and increased apoptosis. However, it remains poorly understood how the observed increase in apoptosis occurs independent of mitotic failure or aneuploidy.

Cohesins regulate gene transcription through multiple mechanisms

It is now well established that cohesins regulate transcription, but the extent to which cohesin and associated cofactors function through a single common mechanism or through multiple mechanisms remains an important developmental issue. One mechanism includes long-distance DNA looping, which plays an important part in cohesin-mediated gene regulation (Ball et al., 2014; Kagey et al., 2010). Cohesin recruitment to these loops involves the CCCTC-binding factor (CTCF) insulator. CTCF binds to specific DNA motifs and in turn recruits cohesins to these specific loci (Wendt and Peters, 2009; Hadjur et al., 2009; Hou et al., 2010; Kagey et al., 2010; Ball et al., 2014; Parelho et al., 2008; Rubio et al., 2008). In fact, studies from numerous model systems suggest that CTCF both precludes enhancer-promoter interactors through DNA looping and recruits cohesins for loop stabilization (Wendt and Peters, 2009; Degner et al., 2011; Nativio et al., 2011; Majumder and Boss, 2011; Guo et al., 2012). In mammalian cells, there is more than 50% overlap between cohesin and CTCF binding sites (Wendt et al., 2008). In Zebrafish, *p53* and *mdm2* were up-regulated in both *rad21*^{nz171} and *ctcf* morphants. Rad21 binds to predicted CTCF binding sites in both genes, suggesting that cohesin and CTCF act together to transcriptionally repress *p53* and *mdm2* (Rhodes et al., 2010). Intriguingly, Drosophila CTCF and cohesin colocalization appear reduced (Misulovin et al., 2008; Pauli et al., 2008; Van Bortle et al., 2014). In combination, these findings suggest that while defects in CTCF recruitment of cohesin may contribute to cohesinopathies, additional mechanisms must be at play (Mourad and Cuvier, 2016).

A comparison between *rad21*^{nz171}-null and *ctcf* Zebrafish morphants reveals only a subset of overlapping misregulated genes (Rhodes et al., 2010). For example, the *myca* gene is strongly down-regulated in *rad21*^{nz171}, but its expression is not affected in *ctcf* morphants. The *myca* promoter contains two predicted CTCF binding sites. Rad21 binds to one of these sites even in *ctcf* morphants, in addition to binding at the *myca* transcriptional start site (i.e., independent of CTCF binding). Therefore, cohesin binding appears to induce *myca* expression independent of CTCF. Transcriptional regulation of *runx1* provides a nuanced view of CTCF and cohesin function. *runx1* mRNA is expressed in hematopoietic precursor cells located in the posterior lateral mesoderm (PLM) in a cohesin-dependent but CTCF-independent manner (Horsfield et al., 2007; Marsman et al., 2014). Moreover, *runx1* becomes ectopically expressed in nearby cells of the tail bud in *ctcf* morphants. This ectopic expression is lost when both CTCF and Rad21 are knocked down, demonstrating that the insulator function of CTCF requires cohesin in cells for ectopic *runx1* expression. Therefore, cohesin both positively (CTCF-independent) and negatively (CTCF-dependent) regulates *runx1* expression in Zebrafish.

In addition to CTCF, the transcription coactivator Mediator is implicated in cohesin-dependent transcription. Mediator is a large complex that recruits RNAPII to the promoter (Kagey et al., 2010; Conaway et al., 2005; Kornberg, 2005; Taatjes, 2010; Malik and Roeder, 2005; Roeder, 1998). Mutations in the Drosophila *NIPBL* ortholog, *Nipped-B*, are linked to long-distance enhancer-mediated transcriptional changes that occur during development (Rollins et al., 1999; Dorsett, 2016). Evidence from embryonic stem cells suggests that Mediator participates in this cohesin-dependent DNA looping (Kagey et al., 2010), consistent with similar findings regarding *hoxd* genes in Zebrafish *nipbla/b* morphants (Muto et al., 2014). Fluorescent in situ hybridizations reveal changes in nuclear architecture in both *nipbla/b* and *med12* single morphants, suggesting that expression of these genes is indeed mediated through chromatin looping. (Muto et al., 2014).

Defective rDNA looping affects ribosome biogenesis and translation

Translational impairment also appears to play a role in CdLS phenotypes (Xu et al., 2014; Zakari et al., 2015). Evaluation of the *rad21*, *smc3*, and *nipbla/b* morphants and CdLS patient cells exhibits reduced phosphorylation of the translational markers RPS6 and 4EBP1, consistent with reduced translation. RPS6 is downstream of mTOR signaling, which is activated in response to reduced protein metabolism. In support of this model, morphant embryo phenotypes are rescued by addition of L-leucine and alpha-KIC (ketoisocaproate). However, considering the role of cohesin function in DNA tethering, cohesins and auxiliary factors may influence rDNA looping and therefore impact the transcription of genes required for ribosome biogenesis and translation.

Reduced Esco2 Function as a Model for RBS

Apoptosis is activated in RBS

To date, *ESCO2* mutations are the only etiologic genetic agent known to produce RBS. Studies using *ESCO2* knockout mice and the RBS patient cells report both cohesion defects and roughly twofold increase in apoptotic cells (Whelan et al., 2012; Gordillo et al., 2008, van der Lelij et al., 2009). Similar findings are observed in studies using both Zebrafish and Medaka embryos, establishing teleosts as a powerful model from which to study the molecular pathologies that underlie RBS (Monnich et al., 2011; Morita et al., 2012). In Zebrafish, *esco2* morpholinos cause lethality by 72 hpf, but reducing the dosage permits bone and cartilage development (Monnich et al., 2011). These *esco2* morphants exhibited craniofacial cartilage defects, underdeveloped jaws, and shorter pectoral fins. Similar findings are observed in *esco2* mutants (Morita et al., 2012; Percival et al., 2015). In Zebrafish *esco2*^{hi2865} mutants, the apoptotic response is p53-dependent, consistent with mitotic failure. However, a subset of mutant cells exhibit normal mitoses, suggesting the potential for multiple mechanisms underlying RBS phenotypes (Percival et al., 2015). Moreover, an apoptotic-independent mechanism underlying bone and tissue growth defects was reported in the regenerating-fin Zebrafish model (Banerji et al., 2016).

A transcriptional mechanism of RBS

Beyond the increase in apoptotic cells, there is now clear evidence that RBS models also exhibit transcriptional deregulation. Fin regeneration is a powerful model system from which to distinguish between different RBS models. For example, morpholino-mediated *esco2* knockdown phenotypes include reduced regenerate length,

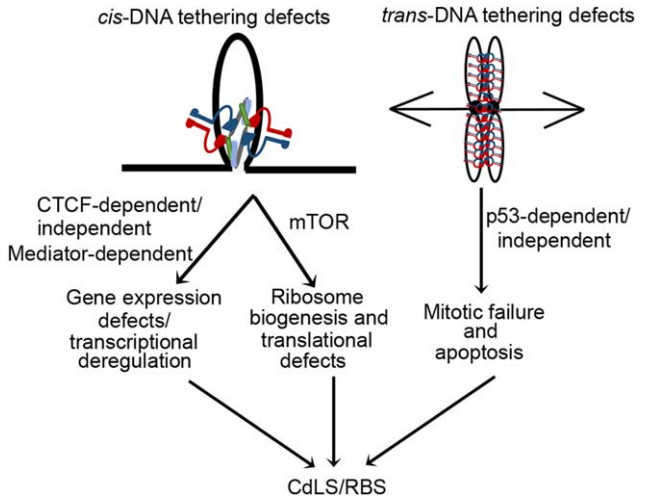

Fig. 2. Mechanisms of cohesinopathies. Transcriptional deregulation caused by defects in cis-DNA tethering events is the predominant mechanism through which both RBS and CdLS arise. The extent to which mitotic failure and apoptosis caused by defects in trans-DNA tethering events contribute to cohesinopathies requires further inquiry.

bone segment length, and cell proliferation (Banerji et al., 2016). It is important to note that apoptosis levels are not increased in *esco2* morphant regenerating tails, in contrast to the embryonic model of RBS (Monnich et al., 2011; Morita et al., 2012; Percival et al., 2015). Intriguingly, Esco2-dependent phenotypes mimic those of the *cx43* mutant, *short fin* (*sof*^{b123}) (Iovine et al., 2005; Hoptak-Solga et al., 2008; Sims et al., 2009), suggesting that Esco2 may directly regulate *cx43* expression. Remarkably, Esco2 knockdown indeed reduces expression of the *cx43* gene as well as *cx43*-dependent genes (Banerji et al., 2016). Overexpression of *cx43* rescues the Esco2-dependent phenotypes, providing compelling support for the hypothesis that *esco2* and *cx43* function in a linear pathway in which *cx43* is downstream of Esco2 function. Since human *CX43* mutations that cause a pleiotropic skeletal disorder oculodentodigital dysplasia (ODDD) overlap with those of both RBS and CdLS (Paznekas et al., 2003), we posit that ODDD is linked to cohesinopathies, and that transcriptional deregulation of developmental genes such as *CX43* contribute to RBS phenotypes.

Additional evidence in support of this model comes from a comparison of gene expression profiles from *rad21*^{nz171}-null embryos and *esco2* embryonic morphants (Rhodes et al., 2010; Monnich et al., 2011). For instance, 24-hpf *rad21*^{nz171}-null embryos and *esco2* morphants contained numerous overlapping genes with altered expression profiles. Intriguingly, this common gene pool exhibited opposing directions in gene expression: Genes up-regulated in the *rad21*^{nz171} mutant were down-regulated in *esco2* morphant embryos, and vice versa. These opposing transcriptional effects likely reflect differences due to the loss of structural components (cohesin) vs. enzymatic activities (Esco2), in which cohesins may remain chromatin-bound and/or CTCF-associated. Alternatively, opposing transcriptional effects between *rad21*^{nz171} mutants and *esco2* morphants may reflect direct vs. indirect roles in transcription. Support for a transcriptional role of ESCO2 also comes from studies using human cell lines, where ESCO2 represses *Notch* transcription for promoting neuronal differentiation (Leem et al., 2011). Additionally, there is evidence that ESCO factors act as a transcription repressor by recruiting chromatin modifiers (Choi et al., 2010; Kim et al., 2008).

Defective rDNA looping affects ribosome biogenesis and translation

Similar to the translational model of CdLS, defects in ribosome biogenesis provide additional mechanisms that may contribute to RBS (Xu et al., 2013). As in the *rad21, smc3*, and *nipbl* morphants, reduced 4EBP1 phosphorylation and mTOR activity are observed in *esco2* morphants. Remarkably, L-leucine ameliorates the developmental defects otherwise present in *esco2* morphants, thus providing support of the role of *esco2* in ribosome biogenesis and translation (Xu et al., 2013; Xu et al., 2014; Xu et al., 2016; Zakari et al., 2015). These studies provide a second example through which mechanism-based boundaries are bridged between CdLS and RBS. We posit that defects in rDNA looping and ribosome biogenesis provide one mechanism through which downstream impairment of translation contributes to both RBS and CdLS.

Part III: Major Questions to be Addressed

Despite the similar manifestations of developmental abnormalities in RBS and CdLS patients, a consensus regarding underlying molecular mechanisms obtained from model systems remains elusive. Analyses of Zebrafish morphants and mutants reveal that multiple mechanisms within each syndrome may be at work. Certainly, the etiology of the cohesinopathies is complex and does not fall into the categories typically defined by the different syndrome names (Fig. 2).

The Role of Transcriptional Deregulation in Cohesinopathy Phenotypes

There is compelling evidence that cohesion pathway factors regulate gene transcription (Dorsett and Krantz, 2009; Cucco and Musio, 2016; Dorsett, 2016). While microarray analyses of individual cohesion pathway gene knockdowns often reveal limited overlap in gene deregulation (Rhodes et al., 2010; Monnich et al., 2011; Muto et al., 2011; Muto et al., 2014), new studies provide a growing body of evidence that RBS models, similar to those of CdLS, exhibit significant changes in gene expression (Banerji et al., 2016; Choi et al., 2010; Leem et al., 2011; Monnich et al., 2011; Xu et al., 2016; Rahman et al., 2015). This unifying model may appear to be challenged by disparate findings regarding NIPBL, cohesin, and CTCF residency on chromatin (Misulovin et al., 2008; Kagey et al., 2010; Muto et al., 2011; Zuin et al., 2014; Minamino et al., 2015; Rahman et al., 2015). It is tempting to speculate that disparities in cohesin recruitment to DNA, as well as identified binding partners, reflect unique cohesin complexes that occur in different tissues and mediate different functions (i.e., transcriptional repression vs. activation). Further efforts in testing a unifying transcriptional basis for all cohesinopathies include identifying the mechanisms through which cohesins are deposited on specific loci, elucidating how chromatin-associated cohesins mediate trans (between different DNA molecules) vs. cis (within a single DNA molecules) conformations, and assessing how those complexes impact gene expression profiles.

Reinterpreting Cohesinopathy Syndromes

Considering the findings described in Zebrafish, paired with additional studies in other model systems, including human patient

cells, the notion that CdLS and RBS represent separate syndromes is challenged. For example, there is evidence that mutations in genes associated with CdLS cause apoptosis through mitotic failure (Kaur et al., 2005; Ghiselli, 2006; Fazio et al., 2016; Schuster et al., 2015; Pistocchi et al., 2013). Furthermore, there is evidence that mutations in *esco2* impact transcription regulation (Leem et al., 2011, Choi et al., 2010, Banerji et al., 2016, Xu et al., 2016). One possibility is that the few observed differences between CdLS and RBS phenotypes are based on the effective dosage of the cohesion pathway genes. RBS patient cells and model systems exhibit significant loss of cohesion function resulting from trans-DNA tethering defects. This mitotic failure, and the subsequent induction of apoptosis, potentially disguises underlying cis-DNA tethering defects. The current focus on apoptotic mechanisms is understandable, given the founding role of ESCO/Eco1/Ctf7 in sister chromatid cohesion (Toth et al., 1999, Skibbens et al., 1999, Bellows et al., 2003, Hou and Zou, 2005, Vega et al., 2005). In reality, more recent findings suggest that a moderate loss of Esco2 function results primarily in cis-DNA tethering defects (Banerji et al., 2016). Moreover, the effective dosage is likely different for structural (i.e., cohesin) and enzymatic (i.e., ESCO2) factors to promote proper development. For instance, in contrast to cohesin, ESCO2 is not required to function in a stoichiometric manner. Therefore, autosomal recessive transmission for RBS is consistent with requiring a greater decrease in ESCO2 to achieve transcriptional defects. At this reduced level of ESCO2 function, chromosome segregation defects also are prominent. Indeed, in budding yeast, modulating levels of cohesin function exert differential effects on cis- vs. trans-DNA tethering (Heidinger-Pauli et al., 2010). On the other hand, autosomal dominant transmission requires only a modest decrease in stoichiometric factors (and including the cohesin loading factor NIPBL), leading to defects in transcription. At this level of function, mitotic defects are not prominent. We therefore propose that transcriptional deregulation is the predominant mechanism through which both RBS and CdLS arise, although we cannot rule out a contributing role for mitotic failure and apoptosis (Fig. 2).

Final Questions

The cohesion pathway is responsible for DNA tethering events that perform a variety of cellular functions. It is essential to understand how the multiple components come together to mediate these functions. For example, do all the cohesion pathway components participate in all its proposed functions? Or are different ring structures formed for different functions? Addressing these questions could provide important insights into the observed differences in microarray profiles of different cohesion pathway morphants, and into the multiple mechanisms underlying apoptosis and changes in gene expression. The challenge will be to identify a standard set of analyses across all cohesion pathway genes (i.e., quantitation of apoptosis levels and quantitative gene expression for a subset of affected genes), and next to determine how many mechanisms in fact underlie the cohesinopathies.

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