Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Cell Biology

Cohesin and human disease: lessons from mouse models

Vijay Pratap Singh[1] and Jennifer L Gerton[1,2]

Cohesin is an evolutionarily conserved large ring-like multi-subunit protein structure that can encircle DNA. Cohesin affects many processes that occur on chromosomes such as segregation, DNA replication, double-strand break repair, condensation, chromosome organization, and gene expression. Mutations in the genes that encode cohesin and its regulators cause human developmental disorders and cancer. Several mouse models have been established with the aim of understanding the cohesin mediated processes that are disrupted in these diseases. Mouse models support the idea that cohesin is essential for cell division, but partial loss of function can alter gene expression, DNA replication and repair, gametogenesis, and nuclear organization.

Addresses
[1] Stowers Institute for Medical Research, Kansas City, MO 64110, United States
[2] Department of Biochemistry and Molecular Biology, University of Kansas School of Medicine, Kansas City, KS 66160, United States

Corresponding author: Gerton, Jennifer L (jeg@stowers.org)

Current Opinion in Structural Biology 2015, **37**:9–17

This review comes from a themed issue on **Differentiation and disease**

Edited by **Scott A Armstrong** and **Michael Rape**

For a complete overview see the Issue and the Editorial

Available online 11th September 2015

http://dx.doi.org/10.1016/j.ceb.2015.08.003

Introduction: cohesin is regulated over the cell cycle

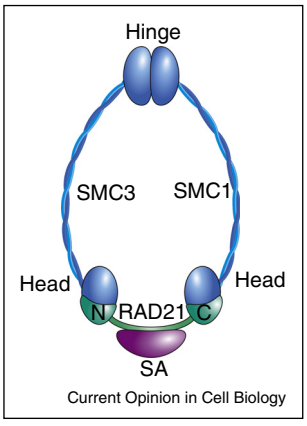
Chromosomes have to duplicate and segregate each cell cycle. Accurate segregation requires opposition to the separation force of the microtubules. This cohesion between sister chromatids is provided by the cohesin complex. Genes encoding the subunits of this eukaryotic complex were originally discovered in yeast mutants that displayed premature separation of sister chromatids. However, cohesin is evolutionarily related to the bacterial structural maintenance of chromosomes (SMC) complex, which is also important for genome transmission. SMC complexes exist in all eukaryotes with an increasing number of possible complexes and specializations over evolutionary time [1]. Cohesin is important for many chromosomal processes, including chromosome segregation, DNA replication, double-strand break repair, condensation, chromosome organization, and gene expression [2–4]. Mutations in the genes that encode the subunits of the cohesin complex and associated factors cause human developmental disorders such as Cornelia de Lange syndrome (CdLS) [5,6] and Roberts syndrome (RBS) [7] as well as cancer [8–10].

The cohesin complex consists of four subunits that are arranged in a ring shaped structure. Two of the subunits are SMC proteins, SMC1 and SMC3; one is a kleisin (closure) subunit, RAD21; and one is stromal antigen (SA; also known as STAG) (Figure 1). With the exception of SMC3, all other subunits exist in at least two forms, with one or more being meiosis specific (Table 1). The SMC proteins contain globular domains at their N and C termini and in the middle. In between globular domains there is a long coiled-coil region. The N and C termini come together to form a 'head' domain, which contains a ABC-type ATPase [11]. The middle globular domain forms a 'hinge' domain, which interacts with the hinge domain of the other SMC protein. The N terminus of the kleisin subunit binds to the SMC3 head domain and the C terminus binds to the SMC1 head domain [12]. The SA subunit associates with the complex via the kleisin subunit. The integrity of the ring structure is important for the stable association of cohesin with chromatin.

The association of the cohesin complex with chromatin is regulated over the cell cycle. Cohesin loading occurs in G1 phase (yeast) or telophase (vertebrates) with the help of the NIPBL-MAU2 heterodimer (Figure 2) and ATP hydrolysis [13]. Recent biochemical reconstitution of the loading reaction showed that cohesin has an intrinsic ability to load onto naked DNA but loading is more efficient in the presence of NIPBL [14]. MAU-2 is not required *in vitro* for cohesin loading but is essential *in vivo*. Cohesin encircles two sister chromatids during S phase, but the details leading to this state are murky. This cohesion 'establishment' requires acetylation of the SMC3 head domain by acetyl transferases ESCO1 and ESCO2 [15]. Two proteins, wings apart-like protein (WAPL) and PDS5 (PDS5A and PDS5B) bind with chromatin associated cohesin and promote cohesin unloading, especially in the absence of acetylation [16]. In vertebrates binding of sororin to PDS5 displaces WAPL and prevents cohesin unloading [17].

Cohesion destruction occurs in at least two cell cycle coupled waves. Cohesin is first removed from chromosome arms during prophase and then from the centromeric regions to enable the transition from metaphase to ana-

Figure 1



Structure of the cohesin complex. In somatic cells, the cohesin ring is composed of SMC1α, SMC3, RAD21, and SA1 or SA2. In germ cells, the cohesin ring can also contain substitutions with SMC1β, REC8, RAD21L, and SA3. The number of complexes that exist during mammalian meiosis is currently unclear. SMC proteins are long polypeptides that fold back on themselves to form a coiled-coil domain with a hinge domain at one end and an ATPase domain at the other. SMC1 and SMC3 (blue) together form a V-shaped structure by interaction of their hinge domains. The N-terminus and C-terminus of RAD21 (green) interacts with SMC3 and SMC1 respectively. The SA subunit (purple) interacts with RAD21.*Source*: Adapted from Ref. [74•].

phase [18]. In the prophase pathway, CDK1 and AURKB phosphorylate sororin which leads to its dissociation from PDS5 [19] so that WAPL can unload cohesin. PLK1 can phosphorylate the SA2 subunit of cohesin, which also facilitates cohesin removal [20]. In prophase, centromeric cohesin is protected by shugoshin 1 (SGO1) and protein phosphatase 2A (PP2A). SGO1-PP2A antagonises sororin phosphorylation and protects it from removal [21]. To initiate anaphase, activation of the anaphase promoting complex/cyclosome (APC/C) leads to securin degradation and separase activation. The activated separase cleaves the kleisin subunit and allows for separation of the sister chromatids [22].

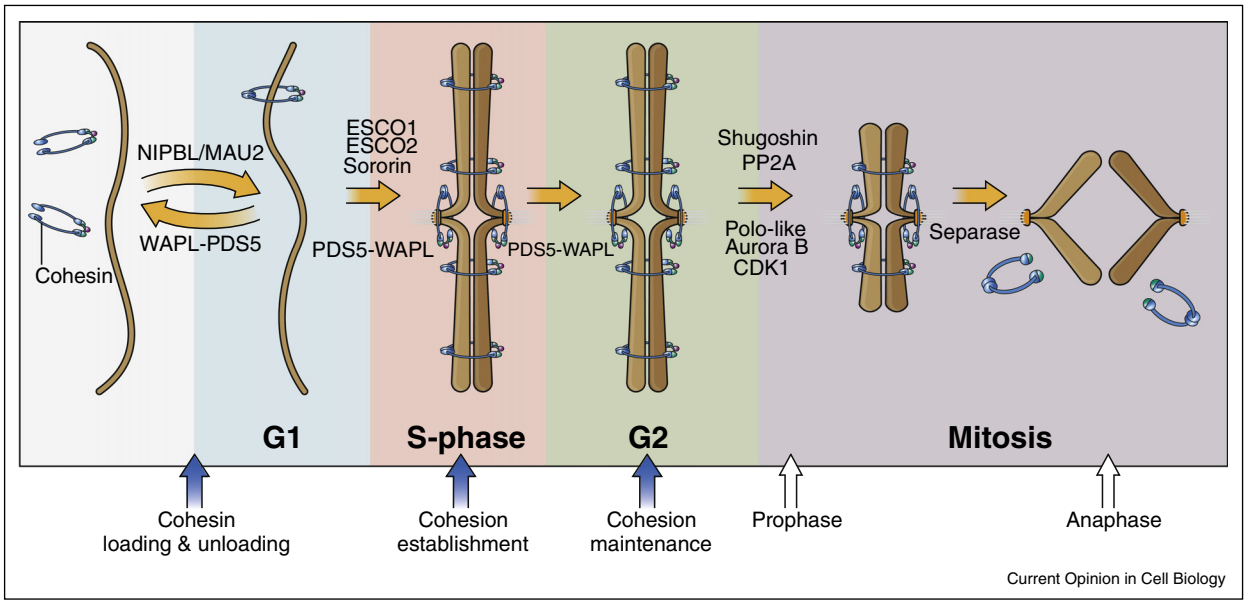
In this article we will (1) summarize the phenotypes of mice with mutations that affect cohesin and its regulators and (2) examine what is known about how mutations in the genes that encode these proteins cause human developmental disorders and cancer. Many of these diseases appear to be caused by partial loss of function; complete loss of function is not compatible with life. With partial loss of function, there is evidence that DNA repair and replication, chromosome organization, and gene expression are disrupted. Many reviews have been written describing the regulation and structure of the cohesin

Table 1

Cohesin subunits and regulators

Subunits	*S. cerevisiae*	Mammals	Null phenotype in mouse	Human syndromes
Structural maintenance of chromosomes (SMC)	Smc1	Smc1α		CdLS, Cancer
	Smc3	Smc3	Embryonic lethal (E14.5) [25]	CdLS, Cancer
		Smc1β	Male and female sterile [33,34]	
α-Kleisin	Scc1	Rad21	Early embryonic lethal [26]	CdLS, Cancer
		Rad21L	Male sterile [35]	
	Rec8	Rec8	Male and female sterile [36]	
Stromalin	Scc3	SA1	Embryonic lethal (E11.5) [30]	Cancer
		SA2		Cancer
		SA3		
Loading	Scc2	Nipbl	Early embryonic lethal [38]	CdLS
	Scc4	Mau2	Early embryonic lethal [40]	
Establishment	Eco1	Esco1		
		Esco2	Lethal at 8 cell stage [43•]	RBS
Maintenance	Pds5	Pds5A	Growth retardation [44]	
		Pds5B	Growth retardation [45]	Cancer
	Wpl1	Wapl	Embryonic lethal [16]	
		Sororin		
	Pp2A	Pp2A	Embryonic lethal [47]	
		Sgo1	Embryonic lethal [46]	Cancer
	Sgo1	Sgo2	Male and female sterile [48]	
Dissolution	Esp1	Separase	Cell cycle arrest at blastocyst [53]	
	Pds1	Securin	Viable [55]	Cancer
	Cdc5	Plk1	Lethal at 8 cell stage [52]	Cancer
		AuroraB	Embryonic lethal [49,50]	
		Cdk1	Embryonic lethal [51]	
	Hos1	Hdac8	Perinatal lethal [58]	CdLS

Figure 2



Cell cycle dependent cohesin loading and unloading. Cohesin (blue) is loaded onto the chromosome in late telophase by the NIPBL-MAU2 heterodimer. Binding of PDS5-WAPL through RAD21 and SA1/2 promotes cohesin unloading. ESCO1 and ESCO2 acetylate SMC3 to stabilize the cohesin–DNA association. Sororin is recruited to PDS5 to antagonise WAPL function. During prophase, Aurora kinase B and CDK1 phosphorylates sororin and facilitate its removal. Polo-like kinase 1 phosphorylates the SA subunit to facilitate its removal cohesin from chromosome arms. During prophase, centromeric cohesin is protected by the binding of shugoshin and PP2A. During anaphase, the APC/C complex activates separase which cleaves RAD21 to release centromeric cohesin to allow sister chromatid separation. SMC3 is recycled by deacetylation with HDAC8. *Source*: Adapted from Ref. [74•].

complex. For more information on these topics the reader is referred to Nasmyth *et al.* and Xiong *et al.* [1,23•].

Phenotypes of mice with mutations in cohesin and associated factors are consistent with defects in many different cellular processes

With the discovery of mouse embryonic stem cells and the ease of gene manipulation in these cells, mouse has developed into a premier mammalian model system for genetic research in the last few decades [24]. Yeasts, *C. elegans* and Drosophila are excellent models for cell cycle regulation and many developmental processes. But mice are often a better model for understanding reproductive and cell type specific processes because of the close genetic and physiological similarities to humans. Mice can be maintained at relatively low cost and multiply quickly. Many different cohesin-associated genes have been mutated in mice. The phenotypes are summarized in Table 1.

Cohesin subunits are indispensable for early embryonic development

Homozygous deletions of genes encoding the mitotic cohesin subunits are early embryonic lethal while heterozygous knockout mice have variable phenotypes. Mutations in one copy of *RAD21*, *SMC1a* or *SMC3* have all been associated with human developmental syndromes known as cohesinopathies. *Smc3* homozygous mutant mice die prior to E14.5 [25]. *Smc1α* deficient mice have not been reported but would also presumably show embryonic lethality. Some lethality was also observed in *Smc3* heterozygous mice. The live heterozygous mice showed reduced body weight, some craniofacial defects, and an increased number of helper and cytotoxic T-cells, suggesting cell and tissue specific developmental processes are being affected with haploinsufficiency.

Rad21 homozygous knockout mice die by E8.5 [26]. *Rad21* heterozygous cells are impaired in homologous recombination and heterozygous mice are more sensitive to whole body irradiation suggesting DNA repair is compromised. Heterozygous *Rad21* MEFs show increased aneuploidy in culture. The conditional knockout of *Rad21* in non-cycling thymoctes suggests a clear role of cohesin in gene expression regulation [27].

The fourth cohesin subunit, SA, has three version; SA1, SA2, and SA3 [28,29]. Two different cohesin complexes can co-exist in somatic vertebrate cells each with SMC1α, SMC3 and RAD21 and either SA1 or SA2. *SA1* null mice die by E11.5 while heterozygous mice have a shortened life span and early onset of tumorigenesis [30••]. *SA1* null embryonic fibroblasts show reduced cell proliferation and enhanced aneuploidy. The aneuploidy is not due to a

defect in centromeric cohesion, but instead is attributed to defective telomere replication. Cohesin is also lost from promoters and CTCF sites, consistent with a role for SA1-cohesin in developmental gene expression programs [31]. The late lethality of *SA1* null mice and establishment of mouse embryonic fibroblasts (MEFs) from null embryos suggests that there is a compensatory response from SA2. Mice lacking SA2 have not been reported, although cohesin complexes with this subunit may be particularly important for centromeric cohesion [32], suggesting *SA2* deletion would be lethal once maternal load is expended.

In summary, homozygous knockout of a gene encoding a unique somatic cohesin subunit is lethal in mice. Presumably this would lead to significant defects in chromosome segregation and rampant aneuploidy that would not be compatible with life. In the heterozygous state many different processes appear to be affected, including gene expression in developmental programs, DNA repair and replication.

Meiosis-specific cohesin genes are essential for fertility

There are meiosis specific versions of several cohesin subunits, including REC8 (RAD21), RAD21L, SA3, and SMC1β (SMC1). Homozygous deletion of the genes encoding these proteins reduces fertility. *Smc1β* deficient mice are healthy with normal mating behaviour but are sterile [33,34]. Male meiosis is blocked at the pachytene stage with unstable crossovers, whereas female meiosis continues up to metaphase II with a high rate of chromosome mis-segregation due to premature loss of cohesin. Two paralogs of *Rad21*, *Rec8* and *Rad21L*, are meiosis specific in mammals. The knockout of *Rad21L*, like *Smc1β*, shows gender specific phenotypes. Males are sterile due to defective synapsis of homologous chromosome at meiotic prophase I which leads to arrest at zygotene. However, females are fertile and develop age dependent sterility due to a reduction in the initial number of oocytes [35]. SA3 is another meiosis-specific subunit, but a knockout has not been reported. Unlike the somatic cell specific cohesin gene knockouts, meiosis-specific cohesin gene knockouts do not display haploinsufficiency but sometimes show gender specific phenotypes.

The other meiosis specific kleisin, REC8, is essential for mammalian meiosis and null mice are sterile [36]. In the absence of REC8, pairing of homologous chromosome occurs normally but synapsis occurs between sister chromatids. Therefore, REC8 normally guides synapsis between homologous chromosomes. Interestingly, *Rec8* null mice displayed *in utero* and postnatal growth retardation, suggesting a role for REC8 in somatic cell development also [37]. All of the meiosis specific cohesin gene knockout mice have defects in fertility, consistent with critical roles for these protein complexes in the meiotic chromosome divisions.

Cohesion is established is S phase, and S phase in mammalian oocytes occurs *in utero*. Meiotic cohesion therefore has to be maintained until the oocyte completes meiotic divisions which could be months later in mice and decades later in humans. Loss of meiotic cohesion is thought to contribute to the increase in aneuploidy associated with oocytes from older mice and humans. However, given the fact that other chromosomal processes depend on cohesin, in the future it will be interesting to explore whether age-dependent loss of cohesion could compromise additional processes in oocytes.

Cohesin loading and establishment is essential for mammalian cell viability

The human developmental disorder CdLS is most often caused by a mutation in one copy of *NIPBL*. Cohesin is loaded onto chromatin by the NIPBL-MAU2 heterodimer during telophase. Homozygous knockout of *Nipbl* is lethal early in mouse embryogenesis [38]. Heterozygous mice show defects characteristic of CdLS, including small body size, craniofacial anomalies, heart defects, microbrachycephaly, hearing abnormities and delayed bone formation. In fact, these phenotypes arise due to a 30% reduction in mRNA levels of *Nipbl*, demonstrating that developmental processes are extremely sensitive to the dosage. Heterozygous MEFs have robust cohesion all along chromosomes, with effective DNA repair and DNA replication [39•], suggesting that gene expression is the main process affected.

Mau2 homozygous knockout is also embryonic lethal [40]. However heterozygous mice are indistinguishable from wild type mice suggesting either a difference in the role of NIPBL and MAU2 in gene regulation or a difference in the importance of dosage. Neural crest specific knockouts of both *Nipbl* and *Mau2* show defects in craniofacial development. Interestingly, neural crest specific *Mau2* mutants display more severe phenotypes than *Nipbl* mutants, suggesting that MAU2 is more essential in these cells [40], although mutations in *Mau2* have not been associated with CdLS. The developmental abnormalities that occur with heterozygous deletions in these genes are best explained by defects in gene expression, consistent with the proposal that the developmental defects in CdLS arise from changes in gene expression. These changes may be due to the disruption of chromatin, chromatin looping, transcriptional elongation, and other processes related to gene expression [41].

During DNA replication, the establishment of cohesion requires acetylation of the SMC3 head domain by ESCO1 and ESCO2 at K105 and K106 [42]. Both copies of *ESCO2* are non-functional in the human developmental disorder RBS. *Esco1* knockout mice have not been reported. *Esco2* knockout mice die at the eight cell stage before implantation [43•]. Deletion of *Esco2* reduces SMC3 acetylation and decreases chromatin associated sororin throughout the genome. ESCO2 is essential for cohesin acetylation in

pericentric heterochromatin and this modification is necessary for proper distribution of cohesin on mitotic chromosomes. The early lethality in the *Esco2* knockout mice suggests that there is no compensation from ESCO1 (or other acetyltransferases) in mice, as there might be in humans. The very early lethality precludes the use of this mouse model for understanding the developmental phenotypes associated with RBS, but a heterozygous or conditional allele could be useful.

Cohesin maintenance factors are crucial for embryonic development

Consistent with PDS5, WAPL, PP2A and shugoshin acting as maintenance factors for cohesion, complete loss of function is not tolerated. Because there are two *Pds5* genes in mammals and cohesion relies on both *Pds5A* and *Pds5B*, each one can be deleted separately, but the double knockout is early embryonic lethal [44], suggesting that PDS5A and B have partially overlapping functions. Single *Pds5* knockout mice show phenotypes resembling some of the developmental defects associated with CdLS, such as growth retardation, congenital heart defects, cleft palate, and skeletal patterning defects [44,45]. However, mutations in *PDS5* have not been reported to cause CdLS. Interestingly, similar to *Nipbl* heterozygous MEFs, *Pds5B* null MEFs do not show cohesion defects. The developmental abnormalities associated with *Pds5A* and *Pds5B* null mice suggest an important role in gene expression while the nucleolar localization of PDS5B suggests an additional role in nucleolar function.

Homozygous knockout of the gene encoding the PDS5 interacting protein WAPL is embryonic lethal [16]. Deletion of *Wapl* causes premature condensation of chromosomes during interphase due to increased stability of the cohesin-chromosome association. WAPL is essential for the proper release of cohesin from DNA. The action of WAPL is antagonised by sororin, which has not been mutated in mice.

SGO1 and SGO2, known as shugoshins, help to protect centromeric cohesion during the prophase dissociation of arm cohesin. Complete loss of SGO1 leads to early embryonic lethality [46•]. *Sgo1* heterozygous mice are viable and fertile. Interestingly, after exposure to a colon carcinogen, these mice have accelerated tumorigenesis and chromosome instability, suggesting a defect in DNA damage repair. The heterozygous *Sgo1* MEFs show missegregation of chromosomes and formation of extra centrosomal foci that contribute to genome instability. Along with shugoshin, PP2A protects centromeric cohesin. Knockout of the catalytic subunit is embryonic lethal after implantation [47]; chromosomal phenotypes were not reported.

In contrast to *Sgo1*, homozygous knockout of *Sgo2* results in a viable mouse [48]. *Sgo2* deletion did not cause any chromosome missegregation in MEFs or other somatic tissues. However, both male and female *Sgo2* null mice are sterile. Thus, SGO2 appears to play a meiosis specific role in protecting centromeric cohesion; loss of SGO2 promotes premature release of the meiosis-specific REC8 cohesin complex prior to anaphase I. The *Sgo2* mutant demonstrates the essential role for meiosis-specific cohesin regulators in the meiotic chromosome divisions.

Cohesin dissolution factors are essential for early embryonic development

Dissolution of cohesion occurs first via the prophase pathway at chromosome arms and then second at centromeres to initiate anaphase. During prophase, Aurora B and CDK1 phosphorylate sororin to release cohesin. Deletion of *Aurkb* leads to embryonic lethality and MEFs show delayed G1/S phase transition and premature mitotic exit [49,50]. *Cdk1* null mice are embryonic lethal at the blastocyst stage [51] and CDK1 is essential for cell proliferation. *Plk1* encodes a kinase that phosphorylates SA2 for cohesin release during prophase dissociation. Similar to the *Esco2* mutant mice, *Plk1* homozygous deletion is lethal at the 8-cell stage [52]; in both cases this is likely due to the end of maternal load. Interestingly, in contrast to human tumors - where PLK1 was overexpressed, heterozygous *Plk1* mice have a higher incidence of tumor formation.

To initiate anaphase, separase cleaves centromeric RAD21 to allow chromosome segregation. Separase deletion is lethal at the blastocyst stage [53]. Separase deficient MEFs show growth retardation and increased ploidy. During anaphase, APC/C degrades securin to activate separase and in yeast securin inhibits separase activity [54]. Surprisingly, securin null mice are viable and fertile with no overt phenotype [55], suggesting additional mechanisms must regulate separase. In vertebrates inhibitory phosphorylation on serine 1121 of separase provides another level of regulation. Interestingly, mice carrying mutations in separase (S1121A) are infertile [56]. Although these two mechanisms of separase regulation are redundant in somatic cells, the inhibitory phosphorylation of separase may uniquely affect germ cells.

Following anaphase, cohesin dissociates from chromatin. Histone deacetylase 8 (HDAC8) deacetylates SMC3 to allow it to be recycled for the next cell cycle. Mutations in *HDAC8* cause CdLS. Without HDAC8 activity, SMC3 acetylation and chromatin association increases [57••]. Homozygous deletion of *Hdac8* causes perinatal lethality in mice due in part to skull instability [58]. Neural crest cell specific inactivation of *Hdac8* leads to CdLS phenotypes in mice [58]. Both the *Hdac8* and *Nipbl* mutant mice recapitulate the craniofacial defects associated with CdLS, likely due in part to defects in neural crest cells. Future experiments aimed at understanding how cohesin mutations specifically affect mouse neural crest cells

should help shed light on the role of cohesin in this important cell type.

Human developmental syndromes and cancers are associated with mutations in cohesin

Cornelia de Lange syndrome is caused by mutations in several different cohesin genes

Cohesinopathies are human developmental syndromes caused by mutations in cohesin and its regulators. Mutations in cohesin subunits and regulatory factors lead to developmental disorders such as CdLS [59••] and RBS [7] as well as cancer [8,60]. CdLS affects one individual in every 10,000 live births while RBS and other cohesinopathies are extremely rare. The affected individuals have craniofacial and limb deformities, hirsutism, gastroesophageal dysfunction, neurodevelopmental delay [61], and mild to severe intellectual disability [5]. The majority of CdLS mutations have been reported in *NIPBL* (~65%), *SMC1α* (~5%), *SMC3* (1-2%), and *HDAC8* (~5%) [59,62]. Mutations in *RAD21* cause a CdLS-like disorder [63]. Together mutations in these five genes account for ~75% of patients with CdLS or CdLS-like phenotypes. In most cases, the reported mutations are predicted to cause a partial loss of function or haploinsufficiency. However, the mutations in the SMC subunits may instead cause disease by virtue of dominant negative effects on the cohesin complex.

Mutations in *NIPBL* associated with severe CdLS are found throughout the gene and consist mainly of nonsense, splice site, and frame shift mutations which produce haploinsufficiency. Missense mutations are more often associated with a mild CdLS phenotype. The idea that loss of NIPBL function causes CdLS is supported by *Nipbl*$^{+/-}$ mice with CdLS phenotypes. This is noteworthy because many cases of haploinsufficiency in humans are not well phenocopied in mice. For example, mutation in one copy of *RAD21* causes a mild CdLS-like phenotype [63] but *Rad21*$^{+/-}$ mice have no overt developmental phenotypes.

Recently mutations in *HDAC8* have been reported in CdLS patients and the majority of these mutations cause loss of HDAC8 activity [6]. *Hdac8* homozygous knockout mice have severe craniofacial defects and small size, consistent with phenotypes in CdLS. Heterozygous female mice are born at sub-Mendelian ratios due to random inactivation of one allele (*Hdac8* is encoded on the X chromosome), again suggesting haploinsufficiency. Poor growth is a feature shared between *Nipbl* and *Hdac8* mice and CdLS patients. Since this phenotype is clearly modelled in the mice, the mouse models could be used to unravel the molecular origins of this phenotype.

All the mutations reported in *SMC1α* (X-chromosome encoded) in CdLS are either missense or small in-frame deletions, suggesting nonsense mutations are not tolerated [64]. These mutations have been reported as hemizygous males and heterozygous females, but most of the cases are females, suggesting that these mutations in *SMC1α* could act in a dominant negative manner [64]. Mutations in *SMC3* associated with CdLS also suggest a possible dominant negative mode of action [65]. *Smc3*$^{+/-}$ mice show a higher mortality rate. Biochemical analysis of these mutant proteins may provide insight into how they affect the activity of the complex.

Roberts syndrome is caused by mutations in a cohesin acetyltransferase

RBS is an autosomal recessive disorder caused by mutation in both copies of *ESCO2*, resulting in complete loss of either protein or enzyme activity [66]. The complete loss of ESCO2 function may be possible since humans have ESCO1. The affected individuals have pre-natal and postnatal growth retardation, limb defects, and craniofacial anomalies [67]. *ESCO1* mutations are probably not compatible with life. RBS cells from patients display aneuploidy, micronuclei, and heterochromatin repulsion [68].

Results using various model systems suggest ESCO2-mediated cohesion is important for nucleolar organization, rRNA production, and ribosome biogenesis [2,69]. Loss of ESCO2 in mice is lethal in an early stage of embryo development [43•] suggesting it is essential for cell survival. The difference between human and mice could be due to (1) the existence of other isoforms of ESCO2 in humans or (2) the fact that all mouse chromosomes are acrocentric. The mouse genome may be more susceptible to *Esco2* mutation since ESCO2 function appears to be most significant for heterochromatin cohesion on acrocentric chromosomes [67]. ESCO1 and ESCO2 probably have both overlapping and specific roles. Their specificity is still not well understood, but they are differentially regulated [70]. ESCO2, in particular, is nucleolar [71], consistent with data showing severe nucleolar dysfunction associated with cells derived from RBS patients [69,72]. Nucleolar dysfunction appears to contribute significantly to this disease.

Several types of cancer are caused by mutations in different cohesin genes

Mutations in *NIPBL* were initially reported in human colorectal cancer [8] and later mutations in *SA2* were reported in glioblastoma, melanoma, and acute myeloid leukemia (AML) [9]. Mutations in other cohesin subunits such as SA1, SA2, SMC3, RAD21, and SMC1α have been reported as driver mutations in AML [73••]. Interestingly, the majority of these cancers have normal karyotypes, suggesting chromosome segregation and aneuploidy are not driving the cancer. Instead, these results suggest that partial loss of function affects other chromosomal processes, such as gene expression, chromosome organization, and DNA

Box 1 Cohesin, mediator (a transcriptional coactivator), and Nipbl have been shown to interact in mouse embryonic stem cells [76]. This interaction may facilitate gene looping at active genes [41,74]. Furthermore, cohesin has been proposed to maintain embryonic stem cell pluripotency, although this finding has been debated [77].

replication. Cohesin binds to the promoters and enhancers of highly transcribed genes where it has the possibility to affect gene expression and chromosome organization [74•] (Box 1), as well as other processes. Glioblastoma cells with mutations in *SA2* are sensitive to PARP (poly-ADP ribose polymerase) inhibitors, suggesting DNA repair may be an important process to target in *SA2*-driven cancers [75••]. In the future, it will be interesting to determine which cohesin-regulated chromosomal processes drive specific cancers in order to predict and test potential therapies.

Summary

In the last decade tremendous effort has been made to understand how cohesin contributes to chromosome function using mouse models. Many of the homozygous deletions in genes encoding subunits or regulators of the somatic complex in mice are lethal, but some heterozygous or homozygous mutant mice display features consistent with human CdLS. Deletion of genes with different molecular functions produces some overlapping developmental phenotypes, but also some distinct phenotypes, which will be interesting to untangle in future studies. While the underlying molecular mechanisms have yet to be fully determined, gene expression and DNA repair are processes that can clearly be affected. These findings have already provided useful insights into the complex molecular etiology of cohesinopathies. Furthermore, meiosis-specific subunits and regulators are clearly essential for gametogenesis. Future studies taking advantage of conditional knockouts will provide insights into the tissue specific roles of cohesin and its regulators while gene editing will allow for the construction of mice with specific disease alleles. The development of mouse models for cohesin-driven cancers will aid our understanding of this infant field.

Acknowledgements

This work was funded by SIMR.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- • of special interest

1. Xiong B, Gerton JL: **Regulators of the cohesin network**. *Annu Rev Biochem* 2010, **79**:131-153.
2. Harris B, Bose T, Lee KK, Wang F, Lu S, Ross RT, Zhang Y, French SL, Beyer AL, Slaughter BD *et al.*: **Cohesion promotes nucleolar structure and function**. *Mol Biol Cell* 2014, **25**:337-346.
3. Dorsett D, Strom L: **The ancient and evolving roles of cohesin in gene expression and DNA repair**. *Curr Biol* 2012, **22**:R240-R250.
4. Bose T, Gerton JL: **Cohesinopathies, gene expression, and chromatin organization**. *J Cell Biol* 2010, **189**:201-210.
5. Liu J, Krantz ID: **Cornelia de Lange syndrome, cohesin, and beyond**. *Clin Genet* 2009, **76**:303-314.
6. Kaiser FJ, Ansari M, Braunholz D, Concepcion Gil-Rodriguez M, Decroos C, Wilde JJ, Fincher CT, Kaur M, Bando M, Amor DJ *et al.*: **Loss-of-function HDAC8 mutations cause a phenotypic spectrum of Cornelia de Lange syndrome-like features, ocular hypertelorism, large fontanelle and X-linked inheritance**. *Hum Mol Genet* 2014, **23**:2888-2900.
7. Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, van Gosliga D, Kayserili H, Xu C, Ozono K *et al.*: **Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion**. *Nat Genet* 2005, **37**:468-470.
8. Barber TD, McManus K, Yuen KW, Reis M, Parmigiani G, Shen D, Barrett I, Nouhi Y, Spencer F, Markowitz S *et al.*: **Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers**. *Proc Natl Acad Sci U S A* 2008, **105**:3443-3448.
9. Solomon DA, Kim T, Diaz-Martinez LA, Fair J, Elkahloun AG, Harris BT, Toretsky JA, Rosenberg SA, Shukla N, Ladanyi M *et al.*: **Mutational inactivation of STAG2 causes aneuploidy in human cancer**. *Science* 2011, **333**:1039-1043.
10. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, Wartman LD, Lamprecht TL, Liu F, Xia J *et al.*: **The origin and evolution of mutations in acute myeloid leukemia**. *Cell* 2012, **150**:264-278.
11. Jessberger R: **The many functions of SMC proteins in chromosome dynamics**. *Nat Rev Mol Cell Biol* 2002, **3**:767-778.
12. Haering CH, Lowe J, Hochwagen A, Nasmyth K: **Molecular architecture of SMC proteins and the yeast cohesin complex**. *Mol Cell* 2002, **9**:773-788.
13. Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Nasmyth K: **Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins**. *Mol Cell* 2000, **5**:243-254.
14. Murayama Y, Uhlmann F: **Biochemical reconstitution of topological DNA binding by the cohesin ring**. *Nature* 2014, **505**:367-371.
15. Hou F, Zou H: **Two human orthologues of Eco1/Ctf7 acetyltransferases are both required for proper sister-chromatid cohesion**. *Mol Biol Cell* 2005, **16**:3908-3918.
16. Tedeschi A, Wutz G, Huet S, Jaritz M, Wuensche A, Schirghuber E, Davidson IF, Tang W, Cisneros DA, Bhaskara V *et al.*: **Wapl is an essential regulator of chromatin structure and chromosome segregation**. *Nature* 2013, **501**:564-568.
17. Nishiyama T, Ladurner R, Schmitz J, Kreidl E, Schleiffer A, Bhaskara V, Bando M, Shirahige K, Hyman AA, Mechtler K *et al.*: **Sororin mediates sister chromatid cohesion by antagonizing Wapl**. *Cell* 2010, **143**:737-749.
18. Waizenegger IC, Hauf S, Meinke A, Peters JM: **Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase**. *Cell* 2000, **103**:399-410.
19. Nishiyama T, Sykora MM, Huis in 't Veld PJ, Mechtler K, Peters JM: **Aurora B and Cdk1 mediate Wapl activation and release of acetylated cohesin from chromosomes by phosphorylating Sororin**. *Proc Natl Acad Sci U S A* 2013, **110**:13404-13409.
20. Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM: **Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2**. *PLoS Biol* 2005, **3**:e69.
21. Liu H, Rankin S, Yu H: **Phosphorylation-enabled binding of SGO1-PP2A to cohesin protects sororin and centromeric cohesion during mitosis**. *Nat Cell Biol* 2013, **15**:40-49.
22. Waizenegger I, Gimenez-Abian JF, Wernic D, Peters JM: **Regulation of human separase by securin binding and autocleavage**. *Curr Biol* 2002, **12**:1368-1378.

23. Nasmyth K, Haering CH: **Cohesin: its roles and mechanisms**. • *Annu Rev Genet* 2009, **43**:525-558.
This review discusses the molecular mechanism of cohesin function and summarizes various existing models for the cohesin ring structure.

24. Hall B, Limaye A, Kulkarni AB: **Overview: generation of gene knockout mice**. *Curr Protoc Cell Biol* 2009:11-17. (Chapter 19: Unit 19 12 19 12).

25. White JK, Gerdin AK, Karp NA, Ryder E, Buljan M, Bussell JN, Salisbury J, Clare S, Ingham NJ, Podrini C *et al.*: **Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes**. *Cell* 2013, **154**:452-464.

26. Xu H, Balakrishnan K, Malaterre J, Beasley M, Yan Y, Essers J, Appeldoorn E, Tomaszewski JM, Vazquez M, Verschoor S *et al.*: **Rad21-cohesin haploinsufficiency impedes DNA repair and enhances gastrointestinal radiosensitivity in mice**. *PLoS One* 2010, **5**:e12112.

27. Seitan VC, Hao B, Tachibana-Konwalski K, Lavagnolli T, Mira-Bontenbal H, Brown KE, Teng G, Carroll T, Terry A, Horan K *et al.*: **A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation**. *Nature* 2011, **476**:467-471.

28. Losada A, Yokochi T, Kobayashi R, Hirano T: **Identification and characterization of SA/Scc3p subunits in the Xenopus and human cohesin complexes**. *J Cell Biol* 2000, **150**:405-416.

29. Carramolino L, Lee BC, Zaballos A, Peled A, Barthelemy I, Shav-Tal Y, Prieto I, Carmi P, Gothelf Y, Gonzalez de Buitrago G *et al.*: **SA-1, a nuclear protein encoded by one member of a novel gene family: molecular cloning and detection in hemopoietic organs**. *Gene* 1997, **195**:151-159.

30. Remeseiro S, Cuadrado A, Carretero M, Martinez P, •• Drosopoulos WC, Canamero M, Schildkraut CL, Blasco MA, Losada A: **Cohesin-SA1 deficiency drives aneuploidy and tumourigenesis in mice due to impaired replication of telomeres**. *EMBO J* 2012, **31**:2076-2089.
The authors in this paper report a role for SA2 in telomere replication and as a novel cause for aneuploidy and tumorigenesis in mice.

31. Remeseiro S, Cuadrado A, Gomez-Lopez G, Pisano DG, Losada A: **A unique role of cohesin-SA1 in gene regulation and development**. *EMBO J* 2012, **31**:2090-2102.

32. Canudas S, Smith S: **Differential regulation of telomere and centromere cohesion by the Scc3 homologues SA1 and SA2, respectively, in human cells**. *J Cell Biol* 2009, **187**:165-173.

33. Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA: **SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction**. *Nat Genet* 2005, **37**:1351-1355.

34. Revenkova E, Eijpe M, Heyting C, Hodges CA, Hunt PA, Liebe B, Scherthan H, Jessberger R: **Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination**. *Nat Cell Biol* 2004, **6**:555-562.

35. Herran Y, Gutierrez-Caballero C, Sanchez-Martin M, Hernandez T, Viera A, Barbero JL, de Alava E, de Rooij DG, Suja JA, Llano E *et al.*: **The cohesin subunit RAD21L functions in meiotic synapsis and exhibits sexual dimorphism in fertility**. *EMBO J* 2011, **30**:3091-3105.

36. Xu H, Beasley MD, Warren WD, van der Horst GT, McKay MJ: **Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis**. *Dev Cell* 2005, **8**:949-961.

37. Tachibana-Konwalski K, Godwin J, Borsos M, Rattani A, Adams DJ, Nasmyth K: **Spindle assembly checkpoint of oocytes depends on a kinetochore structure determined by cohesin in meiosis I**. *Curr Biol* 2013, **23**:2534-2539.

38. Kawauchi S, Calof AL, Santos R, Lopez-Burks ME, Young CM, Hoang MP, Chua A, Lao T, Lechner MS, Daniel JA *et al.*: **Multiple organ system defects and transcriptional dysregulation in the Nipbl(+/−) mouse, a model of Cornelia de Lange Syndrome**. *PLoS Genet* 2009, **5**:e1000650.

39. Remeseiro S, Cuadrado A, Kawauchi S, Calof AL, Lander AD, • Losada A: **Reduction of Nipbl impairs cohesin loading locally and affects transcription but not cohesion-dependent functions in a mouse model of Cornelia de Lange Syndrome**. *Biochim Biophys Acta* 2013, **1832**:2097-2102.
This paper describes the role of cohesin in gene expression regulation in the *Nipbl* mouse model. This paper supports the idea that developmental defects in CdLS are caused by dysregulated gene expression, not other cohesin mediated processes.

40. Smith TG, Laval S, Chen F, Rock MJ, Strachan T, Peters H: **Neural crest cell-specific inactivation of Nipbl or Mau2 during mouse development results in a late onset of craniofacial defects**. *Genesis* 2014, **52**:687-694.

41. Dorsett D, Merkenschlager M: **Cohesin at active genes: a unifying theme for cohesin and gene expression from model organisms to humans**. *Curr Opin Cell Biol* 2013, **25**:327-333.

42. Zhang J, Shi X, Li Y, Kim BJ, Jia J, Huang Z, Yang T, Fu X, Jung SY, Wang Y *et al.*: **Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast**. *Mol Cell* 2008, **31**:143-151.

43. Whelan G, Kreidl E, Wutz G, Egner A, Peters JM, Eichele G: • **Cohesin acetyltransferase Esco2 is a cell viability factor and is required for cohesion in pericentric heterochromatin**. *EMBO J* 2012, **31**:71-82.
This study shows the essential role of ESCO2 for viability in mice. The study suggests that there is no compensatory response from ESCO1 and that ESCO2 is essential for cohesin acetylation in pericentric heterochromatin.

44. Zhang B, Chang J, Fu M, Huang J, Kashyap R, Salavaggione E, Jain S, Kulkarni S, Deardorff MA, Uzielli ML *et al.*: **Dosage effects of cohesin regulatory factor PDS5 on mammalian development: implications for cohesinopathies**. *PLoS One* 2009, **4**:e5232.

45. Zhang B, Jain S, Song H, Fu M, Heuckeroth RO, Erlich JM, Jay PY, Milbrandt J: **Mice lacking sister chromatid cohesion protein PDS5B exhibit developmental abnormalities reminiscent of Cornelia de Lange syndrome**. *Development* 2007, **134**:3191-3201.

46. Yamada HY, Yao Y, Wang X, Zhang Y, Huang Y, Dai W, Rao CV: • **Haploinsufficiency of SGO1 results in deregulated centrosome dynamics, enhanced chromosomal instability and colon tumorigenesis**. *Cell Cycle* 2012, **11**:479-488.
This study shows for the first time the role of SGO1 in centrosome dynamics and that haploinsufficiency for *Sgo1* causes genomic instability and tumorigenesis.

47. Gotz J, Probst A, Ehler E, Hemmings B, Kues W: **Delayed embryonic lethality in mice lacking protein phosphatase 2A catalytic subunit Calpha**. *Proc Natl Acad Sci U S A* 1998, **95**:12370-12375.

48. Llano E, Gomez R, Gutierrez-Caballero C, Herran Y, Sanchez-Martin M, Vazquez-Quinones L, Hernandez T, de Alava E, Cuadrado A, Barbero JL *et al.*: **Shugoshin-2 is essential for the completion of meiosis but not for mitotic cell division in mice**. *Genes Dev* 2008, **22**:2400-2413.

49. Trakala M, Fernandez-Miranda G, Perez de Castro I, Heeschen C, Malumbres M: Aurora B: **prevents delayed DNA replication and premature mitotic exit by repressing p21(Cip1)**. *Cell Cycle* 2013, **12**:1030-1041.

50. Fernandez-Miranda G, Trakala M, Martin J, Escobar B, Gonzalez A, Ghyselinck NB, Ortega S, Canamero M, Perez de Castro I, Malumbres M: **Genetic disruption of aurora B uncovers an essential role for aurora C during early mammalian development**. *Development* 2011, **138**:2661-2672.

51. Diril MK, Ratnacaram CK, Padmakumar VC, Du T, Wasser M, Coppola V, Tessarollo L, Kaldis P: **Cyclin-dependent kinase 1 (Cdk1) is essential for cell division and suppression of DNA re-replication but not for liver regeneration**. *Proc Natl Acad Sci U S A* 2012, **109**:3826-3831.

52. Lu LY, Wood JL, Minter-Dykhouse K, Ye L, Saunders TL, Yu X, Chen J: **Polo-like kinase 1 is essential for early embryonic development and tumor suppression**. *Mol Cell Biol* 2008, **28**:6870-6876.

53. Kumada K, Yao R, Kawaguchi T, Karasawa M, Hoshikawa Y, Ichikawa K, Sugitani Y, Imoto I, Inazawa J, Sugawara M *et al.*: **The selective continued linkage of centromeres from mitosis to**

interphase in the absence of mammalian separase. *J Cell Biol* 2006, **172**:835-846.

54. Yamamoto A, Guacci V, Koshland D: **Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae***. *J Cell Biol* 1996, **133**:85-97.

55. Mei J, Huang X, Zhang P: **Securin is not required for cellular viability, but is required for normal growth of mouse embryonic fibroblasts**. *Curr Biol* 2001, **11**:1197-1201.

56. Huang X, Andreu-Vieyra CV, York JP, Hatcher R, Lu T, Matzuk MM, Zhang P: **Inhibitory phosphorylation of separase is essential for genome stability and viability of murine embryonic germ cells**. *PLoS Biol* 2008, **6**:e15.

57. •• Deardorff MA, Bando M, Nakato R, Watrin E, Itoh T, Minamino M, Saitoh K, Komata M, Katou Y, Clark D *et al.*: **HDAC8 mutations in Cornelia de Lange syndrome affect the cohesin acetylation cycle**. *Nature* 2012, **489**:313-317.
This paper is the first report of *HDAC8* mutations in CdLS. This study shows that loss of HDAC8 activity leads to increased SMC3 acetylation and chromatin association.

58. Haberland M, Mokalled MH, Montgomery RL, Olson EN: **Epigenetic control of skull morphogenesis by histone deacetylase 8**. *Genes Dev* 2009, **23**:1625-1630.

59. •• Mannini L, Cucco F, Quarantotti V, Krantz ID, Musio A: **Mutation spectrum and genotype-phenotype correlation in Cornelia de Lange syndrome**. *Hum Mutat* 2013, **34**:1589-1596.
This paper summarizes the nature of various human mutations in cohesin genes and correlates them with the severity of CdLS phenotype. Translocations, nonsense, and frameshift mutations result in severe phenotypes while missense mutations have milder phenotypes.

60. Rocquain J, Gelsi-Boyer V, Adelaide J, Murati A, Carbuccia N, Vey N, Birnbaum D, Mozziconacci MJ, Chaffanet M: **Alteration of cohesin genes in myeloid diseases**. *Am J Hematol* 2010, **85**:717-719.

61. Dorsett D, Krantz ID: **On the molecular etiology of Cornelia de Lange syndrome**. *Ann N Y Acad Sci* 2009, **1151**:22-37.

62. Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodriguez C, Arnedo M, Loeys B, Kline AD *et al.*: **Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation**. *Am J Hum Genet* 2007, **80**:485-494.

63. Deardorff MA, Wilde JJ, Albrecht M, Dickinson E, Tennstedt S, Braunholz D, Monnich M, Yan Y, Xu W, Gil-Rodriguez MC *et al.*: **RAD21 mutations cause a human cohesinopathy**. *Am J Hum Genet* 2012, **90**:1014-1027.

64. Mannini L, Liu J, Krantz ID, Musio A: **Spectrum and consequences of SMC1A mutations: the unexpected involvement of a core component of cohesin in human disease**. *Hum Mutat* 2010, **31**:5-10.

65. Gil-Rodriguez MC, Deardorff MA, Ansari M, Tan CA, Parenti I, Baquero-Montoya C, Ousager LB, Puisac B, Hernandez-Marcos M, Teresa-Rodrigo ME *et al.*: **De novo heterozygous mutations in SMC3 cause a range of Cornelia de Lange syndrome-overlapping phenotypes**. *Hum Mutat* 2015, **36**:454-462.

66. Gordillo M, Vega H, Trainer AH, Hou F, Sakai N, Luque R, Kayserili H, Basaran S, Skovby F, Hennekam RC *et al.*: **The molecular mechanism underlying Roberts syndrome involves loss of ESCO2 acetyltransferase activity**. *Hum Mol Genet* 2008, **17**:2172-2180.

67. Van Den Berg DJ, Francke U: **Roberts syndrome: a review of 100 cases and a new rating system for severity**. *Am J Med Genet* 1993, **47**:1104-1123.

68. Xu B, Lu S, Gerton JL: **Roberts syndrome: a deficit in acetylated cohesin leads to nucleolar dysfunction**. *Rare Dis* 2014, **2**:e27743.

69. Xu B, Lee KK, Zhang L, Gerton JL: **Stimulation of mTORC1 with L-leucine rescues defects associated with Roberts syndrome**. *PLoS Genet* 2013, **9**:e1003857.

70. Minamino M, Ishibashi M, Nakato R, Akiyama K, Tanaka H, Kato Y, Negishi L, Hirota T, Sutani T, Bando M *et al.*: **Esco1 acetylates cohesin via a mechanism different from that of Esco2**. *Curr Biol* 2015, **25**:1694-1706.

71. van der Lelij P, Godthelp BC, van Zon W, van Gosliga D, Oostra AB, Steltenpool J, de Groot J, Scheper RJ, Wolthuis RM, Waisfisz Q *et al.*: **The cellular phenotype of Roberts syndrome fibroblasts as revealed by ectopic expression of ESCO2**. *PLoS One* 2009, **4**:e6936.

72. Bose T, Lee KK, Lu S, Xu B, Harris B, Slaughter B, Unruh J, Garrett A, McDowell W, Box A *et al.*: **Cohesin proteins promote ribosomal RNA production and protein translation in yeast and human cells**. *PLoS Genet* 2012, **8**:e1002749.

73. •• Thol F, Bollin R, Gehlhaar M, Walter C, Dugas M, Suchanek KJ, Kirchner A, Huang L, Chaturvedi A, Wichmann M *et al.*: **Mutations in the cohesin complex in acute myeloid leukemia: clinical and prognostic implications**. *Blood* 2014, **123**:914-920.
The authors report various cohesin subunit mutations in AML. This study suggests that the mutations cause AML due to changes in gene expression.

74. • Zakari M, Yuen K, Gerton JL: **Etiology and pathogenesis of the cohesinopathies**. *Wiley Interdiscip Rev Dev Biol* 2015, **4**:489-504.
This review article integrates data from several genomics studies and outlines many of the mechanisms by which cohesin may influence gene expression.

75. •• Bailey ML, O'Neil NJ, van Pel DM, Solomon DA, Waldman T, Hieter P: **Glioblastoma cells containing mutations in the cohesin component STAG2 are sensitive to PARP inhibition**. *Mol Cancer Ther* 2014, **13**:724-732.
The authors demonstrate that DNA repair is compromised in glioblastoma cells with mutations in SA2, and are hypersentive to treatment with PARP inhibitors, presenting a possible therapeutic strategy.

76. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC, Goossens J, Rahl PB, Levine SS *et al.*: **Mediator and cohesin connect gene expression and chromatin architecture**. *Nature* 2010, **467**:430-435.

77. Lavagnolli T, Gupta P, Hormanseder E, Mira-Bontenbal H, Dharmalingam G, Carroll T, Gurdon JB, Fisher AG, Merkenschlager M: **Initiation and maintenance of pluripotency gene expression in the absence of cohesin**. *Genes Dev* 2015, **29**:23-38.