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Review

Cohesin: Functions beyond sister chromatid cohesion

Gunjan D. Mehta, Ravinder Kumar, Sanjeeva Srivastava, Santanu Kumar Ghosh *

Department of Biosciences and Bioengineering, Wadhwani Research Centre for Biosciences and Bioengineering, Indian Institute of Technology-Bombay, Mumbai 400076, India

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ABSTRACT

Faithful segregation of chromosomes during mitosis and meiosis is the cornerstone process of life. Cohesin, a multi-protein complex conserved from yeast to human, plays a crucial role in this process by keeping the sister chromatids together from S-phase to anaphase onset during mitosis and meiosis. Technological advancements have discovered myriad functions of cohesin beyond its role in sister chromatid cohesion (SCC), such as transcription regulation, DNA repair, chromosome condensation, homolog pairing, monoorientation of sister kinetochore, etc. Here, we have focused on such functions of cohesin that are either independent of or dependent on its canonical role of sister chromatid cohesion. At the end, human diseases associated with malfunctioning of cohesin, albeit with mostly unperturbed sister chromatid cohesion, have been discussed.
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1. Introduction

DNA replication at the beginning of mitotic and meiotic cell cycle results in duplication of each of the chromosomes, producing sister chromatids. The memory of which one is whose sister, from the time when the chromatids are generated until they separate, is provided by cohesin, a molecular glue, that keeps the sisters together [reviewed in 1]. Cohesin is a multi-protein complex, made up of four subunits Smc1, Smc3, an α-kleisin subunit – Mcd1/Scc1 (mitosis)/Rec8 (meiosis), and Irr1/Scc3, which is conserved from yeast to human (Table 1, Fig. 1). Three of these subunits (Smc1, Smc3, and α-kleisin) are believed to form a tripartite ring structure in which the sister chromatids are topologically entrapped during/after replication (Fig. 1, [reviewed in 1]). Several accessory proteins (listed in Table 1) play regulatory roles in timely loading, maintenance and removal of cohesin from the chromatin. How interplay of these proteins along with the four core cohesin subunits engages in generation of sister chromatid cohesion (SCC) has been described elaborately elsewhere [reviewed in 1,2]. However, intense research on cohesin for the last decade has revealed that cohesin is involved in many other aspects of cellular processes apart from its major function in SCC. These cellular processes include transcriptional regulation, DNA repair, chromosome condensation and morphogenesis, centrosome-related functions, etc. [3–12, listed in Tables 2 and 3]. It is important to note that SCC per se may or may not be directly involved in these non-canonical roles of cohesin. In fact, some of the studies indicate that severe reduction in the level of chromatin bound cohesin does not affect its function of holding the sister chromatids together, but drastically affects the non-canonical functions [reviewed in 13–15]. How the stoichiometry of cohesin affects different functions in the cell is poorly understood. From the structure of cohesin as known to date, it is apparent that the ability of cohesin to tether distant chromosomal loci may be the key event to manifest its function in different cellular activities. As expected from cohesin's involvement in diverse cellular activities, loss of or compromised function of cohesin in humans leads to several diseases collectively termed as "cohesinopathies" [reviewed in 16,17].

In order to point out directions for future research, in this review we have compiled in a coherent way the available information about cohesin's functions beyond SCC. Due to space constraints, we could not accommodate all the works in the main text. We therefore present a table (Table 2) that summarizes such novel functions of cohesin that are either dependent of or independent

Abbreviations: DSB, double-strand break; SPB, spindle pole body; SCC, sister chromatid cohesion; SC, synaptonemal complex; AE, axial element; LE, lateral element; CE, central element; CdLS, Cornelia de Lange syndrome; RBS, Roberts syndrome; CTCF, CCCTC binding factor; MEF, mouse embryonic fibroblasts; OMIM, online Mendelian inheritance in men

* Corresponding author. Address: Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai 400076, India. Fax: +91 22 2576 4770.
E-mail address: santanughosh@iitb.ac.in (S.K. Ghosh).

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Table 1
Cohesin subunits and regulatory proteins.

S. cerevisiae	Human	*Drosophila*	*S. pombe*	Function
Smc1	Smc1a	Smc1	Psm1	Cohesin subunit
	Smc1b			Cohesin subunit (meiosis)
Smc3	Smc3	Smc3	Psm3	Cohesin subunit
Mcd1/Scc1	Rad21/Scc1	Rad21	Rad21	Cohesin subunit (α-kleisin)
Rec8	Rad21L/Rec8	C(2)M	Rec8	Cohesin subunit (meiotic α-kleisin)
Scc3/Irr1	SA1	SA	Psc3	Cohesin subunit
	SA2	SA2		
	STAG3		Rec11	Cohesin subunit (meiosis)
Pds5	Pds5a	Pds5	Pds5	Cohesion maintenance
	Pds5b			
Wpl1/Rad61	Wap1	Wap1	Wap1	Cohesin dissociation
Sororin	CDCA5			Cohesion establishment/maintenance
Scc2	Nipbl/Scc2	*Nipped-B*	Mis4	Cohesin loading
Scc4	Mau2/Scc4	Scc4	Ssl3	Cohesin loading
Eco1/Ctf7	Esco1	Deco	Eso1	Cohesion establishment
	Esco2	San		
Esp1	Espl1	Sse	Cut1	Separase
		Thr		
Pds1	Pttg1	Pim	Cut2	Securin
Hos1	HDAC8	?	?	Smc3 deacetylase
Sgo1	Sgo1	MEI-S332	Sgo1	Protection of centromeric cohesin
	Sgo2		Sgo2	

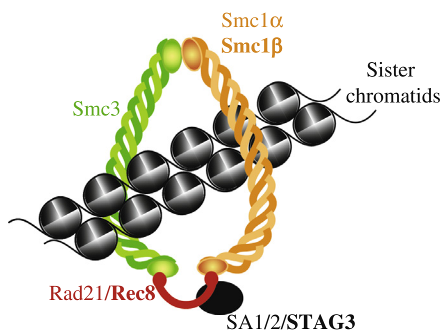

Fig. 1. Entrapment of sister chromatids by the cohesin ring in vertebrates: mitotic cohesin ring is made up of four subunits: Smc1α, Smc3, Rad21 and SA1/2. Meiotic counterparts of these subunits are shown in bold letters. Smc1 and Smc3 form a "V" shaped dimer, which is enclosed by Rad21/Rec8. SA1/2/STAG3 binds to the C-terminal domain of Rad21/Rec8.

of its canonical function of SCC to provide a ready reference. Additionally, how these different functions of cohesin are conserved across eukaryotes is shown in Table 3. Towards the end of this text we discuss the human diseases (cohesinopathies) that occur as a result of malfunctioning of cohesin without much observable disruption of SCC.

2. Functions of cohesin beyond sister chromatid cohesion

Extensive research on the structure and function of cohesin for the last decade has not only delineated the mechanism of SCC to some extent, but also identified other biological functions of cohesin where the act of cohesion is either indirectly involved or dispensable. In the following sections, we review some of such functions of cohesin with plausible mechanisms. Due to space constraints, we have not discussed works from several groups, but have listed them in Table 2.

2.1. Transcriptional control

The cohesin complex has recently emerged as a key regulator of eukaryotic gene expression, although the mechanism of this regulation is poorly understood. Some elegant experiments, as mentioned below, have provided evidence that cohesin is a transcriptional regulator. The first evidence for this came when, in *Drosophila*, a mutant carrying a mutation in *Nipped-B* (ortholog of budding yeast cohesin loader, Scc2) was found to be deficient in activation of homeobox genes [18]. A similar observation was made in patients suffering from Cornelia de Lange syndrome (CdLS), a human disease that mainly leads to upper limb malformation and mental retardation. CdLS has been shown to be primarily associated with mutations in *NIPBL*, a mammalian ortholog of *Nipped-B* [19,20]. Interestingly, further experiments also demonstrated the role of cohesin in regulation of gene expression even in cells not undergoing mitosis, indicating a functionally separate role of cohesin in gene regulation and in the cell cycle. Such non-mitotic function of cohesin came to light from the discovery that cohesin resides within the nuclei of most postmitotic cells, including neurons [11], and that inactivation of cohesin results in axon pruning defects in postmitotic mushroom body γ-neurons in *Drosophila*. These defects happen partially due to a lack of expression of the ecdysone receptor, which demonstrates the role of cohesin as a transcriptional regulator of the receptor gene [21,22]. The involvement of cohesin loader and the cohesin subunits in transcription regulation have also been demonstrated in other model organisms, such as zebrafish, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Trypanosomes*, mice, etc. [7,23–26; Table 2 and 3]. Apart from transcription, even translation has been shown to be influenced by cohesin, although indirectly. In budding yeast and humans, cohesin has been shown capable of augmenting translational capacity by increasing transcription of rRNA [27]. Overall these studies conclude that accumulation of functional cohesin complex on chromatin can regulate the expression of genes, and that this is true for many species (Table 3).

The mechanism by which cohesin influences transcription is not clear. However, genome-wide mapping of the localization of cohesin and its loading factors in different model organisms has thrown light on probable modes of action of cohesin with respect to transcription. In *Drosophila*, there is complete overlapping of cohesin and Nipped-B localization, peaking at the transcriptional start sites of active genes [28], whereas cohesin in mammals also accumulates at sites where transcription factors CCCTC-binding factors (CTCFs) are localized, apart from the sites where cohesin loader Nipbl is found [11]. This co-localization of cohesin with CTCF appears to be related to cohesin's role in transcription, as

Table 2
Functions of cohesin beyond sister chromatid cohesion.

Organism	Cohesion (subunits*)	Function	Ref.
Arabidopsis	Syn1 (Rec8)	Pairing of homologous chromosomes	[86]
Arabidopsis	AtREC8 and AtSCC3	Monoorientation of the sister kinetochores during meiosis I. AtREC8 is involved in chromosome axis formation in an AtSPO11-1-independent manner	[3]
Arabidopsis	SYN3	Homologous chromosome synapsis and synaptonemal complex (SC) formation during male and female meiosis	[141]
C. elegence	Smc3	Homologous recombination	[142]
C. elegence	Scc3	Meiotic synapsis and proper chromosome disjunction in mitosis and meiosis	[143]
Daphnia	Rec8	Conversion of meiotically reproducing lineages into obligate asexuals	[144]
Drosophila	Smc1, SA	Expression of the Ecdysone-receptor (EcR) gene, absence of which disrupts axon pruning at a postmitotic stage in development of the nervous system	[21,22]
Drosophila	Rad21	Represses the expression of genes by hindering the transition of paused RNA polymerase to elongation	[39]
Drosophila	Cohesin	Polycomb dependent silencing of a transgenic reporter depends on cohesin function	[145]
Drosophila	Rad21	Mutations in Rad21 and Nipped B act as dominant suppressors of Polycomb and hedgehog	[146]
Human	Cohesin	In association with CTCF, it co-ordinates expression of latent and lytic genes of Herpes virus	[147]
Human	STAG1/2 (Scc3)	Transcriptional co-activator for promoters with NF-kappaB element	[12]
Human	Cohesin	Protects genes against γH2AX induced by DNA DSBs which leads to their reduced expression	[148]
Human	Cohesin	Regulates repression of MHC class II genes through interactions with MHC class II insulators	[149]
Human	Rad21	Cleaved fragment of Rad21 (c-terminal) and its translocation from nucleus to cytoplasm plays a role in early signaling of apoptosis	[150]
Human	Cohesin (Smc1, SA1)	Cell cycle (S/G2) specific DNA DSB repair by recruitment of cohesin at the DNA damage site with the help of Mre11/Rad50	[63]
Human	Scc1	Aki1 associates with Scc1 to recruit Scc1 at the centrosomes during mitosis to mediate centriole cohesion to prevent premature centriole splitting	[70]
Human	Smc3	Acetylation of Smc3 maintains processivity of replication forks and speeds up the movement of replication forks	[151]
Human	Cohesin	Along with CTCF, cohesin is involved in interchromatin interaction between Bcl11b and Arhgap6 loci in a cell-type specific manner	[152]
Human	Cohesin	Required for DNA replication. Cohesin is particularly enriched at the replication origins and interacts with prereplication complex proteins. Down-regulation of cohesin slows down S-phase progression by limiting the number of active origins and increasing the length of chromatin loops that correspond to each replicon unit	[153]
Human	Rad21	Maintenance of telomeres	[154]
Human	Rad21	Maintains the integrity of centrosomes independently from its role in chromosomal cohesion. Depletion of Rad21 leads to extra gamma tubulin and separation of centrioles	[155]
Human	Smc1	Over-expression of Smc1 leads to formation of multi polar spindles in 25% of cells	[156]
Human	Smc1, Smc3	Smc1 and Smc3 become phosphorylated by Atm and Nbs1 in response to DNA damage to activate Intra-S checkpoint	[65,157,64]
Human	Cohesin (Smc3, Scc1)	Require for G1, Intra-S and G2/M DNA damage checkpoints	[66]
Human	SA2	Provides insulation activity with CTCF, which is required to act as boundary element	[158]
Human	SA2	Along with CTCF, it is required for X chromosome inactivation	[159]
Human	Cohesin	Along with CTCF, plays a critical role in regulating the cell cycle control of viral gene expression during latency, failure of which inhibits host cell proliferation and survival	[160]
Human, Mouse	Cohesin	Regulation of mammalian β-globin expression	[161]
Human, *S. cerevisiae*	Smc1	Cohesin proteins promote ribosomal RNA production and protein translation	[27]
Mouse	Cohesin	Along with CTCF, regulation of V(D)J gene rearrangement to generate diversity of lymphocyte receptors and antibodies	[37,164]
Mouse	Rad21	Cooperates with pluripotency transcription factors in the maintenance of embryonic stem cell identity	[162]
Mouse	Rad21	Along with Scp3 and Scp2, Rad21 is involved in monoorientation of sister knietochores during meiosis I	[104]
Mouse	Rad21	T-cell-receptor rearrangement and thymocyte differentiation	[163]
Mouse	Rad21L	Full synapsis of homologous chromosomes at meiotic prophase I	[91]
Mouse	Smc1b	Mutation in Smc1b results in absence of spermatids and spermatozoa in male mice and reduction in the number of oocytes with age in female mice, and hence sterility in both	[95]
Mouse	SA1	Preferentially accummulates at promoter regions and determines the distribution of cohesin on the genome to regulate gene expression	[111]
Mouse	Rec8, Rad21L	Meiotic cohesin complexes are essential for the formation of the axial element	[95]
Mouse	Rad21	Along with CTCF, required for the generation of single-cell diversity of protocadherin-α gene expression	[166]
Rice	Rec8	Monoorientation of the sister kinetochores during meiosis I	[101]
S. pombe	Rec8	Monoorientation of the sister kinetochores during meiosis I	[167]
S. pombe	Cohesin (Rad21)	Promotes transcriptional termination between convergent genes	[24]
S. pombe	Rec8	Chromosome compaction during meiotic prophase	[5]
S. pombe	Rec8, Rec11	During meiosis, both are required for meiotic recombination and DNA breakage in a region-specific manner	[6]
S. pombe	Rad21	Formation of heterochromatic domain at subtelomeres and control of gene expression within that domain	[23]
S. cerevisiae	Cohesin	Full activation of the transcription of Rec8 during meiosis	[25]
S.cerevisiae	Cohesin (Smc3, Mcd1)	Repair of replication-born DSB by sister chromatid exchange, but not for other recombinational repair events	[4]
S.cerevisiae	Cohesin	With Eco1, cohesin is required for the post replicative DSB repair	[10]
S.cerevisiae	Rec8	Required for the distribution of Spo11 along yeast meiotic chromosomes	[59]
S.cerevisiae	Smc3, Rec8	Formation of synaptonemal complex and axial element and recombination during meiosis	[8]
S.cerevisiae	Rec8	Homolog pairing, recombination, chromosome axis and SC assembly function	[61]
Trypanosomes	Cohesin	Differential allelic expression of gene coding for variant surface glycoprotein	[165]
Xenopus	Cohesin	Proper attachment of kinetochore with the spindle, hence chromosome alignment during metaphase	[168]
Zebrafish	Rad21	Regulates expression of myca, p53 and mdm2 gene expression	[139]
Zebrafish	Smc3, Rad21	Regulates Runx gene expression during Zebrafish development	[7]

Cohesin's functions beyond sister chromatid cohesion in various model systems have been briefly described in this table.
* Studies mentioned in the references involved these subunits.

Table 3
Functional conservation of cohesin.

Transcription regulation	DSB repair	Chromosome condensation	Function at centrosomes	Homologoue pairing/ homologous recombination	Non-homologous centromere coupling	Monoorientation	DNA replication	Chromosome architecture/ chromosome rearrangement
S. cerevisiae [25,27,33,34]	*S. cerevisiae* [4]	*S. cerevisiae* [14,78,83,84]	*S. cerevisiae* [75]	*S. cerevisiae* [8,61]	*S. cerevisiae* [98]	*S. pombe* [9,100,102,167]	Human [151,153]	Human, [147]
S. pombe [23,24]	*S. pombe* [6,48]	*S. pombe* [5]	Human [68–71,155,156]	*S. pombe* [6,87]		*A. thaliana* [3]		Mouse [34,37,38,163]
Drosophila [18,21,22,28,39,46,145]	Human [63–66]	*Xenopus* [79]		Mouse [58,91,93]		Mouse [104,163]		
Mouse [25,32,109,159,162]				*A. thaliana* [6,86,141]		Rice [101]		
Human [11,12,19,20,149,158,160,161]				*C. elegans* [88,142,170]				
Trypanosoma brucei [165]				Maize [169]				
Zebrafish [7,139]								

Functional conservation of cohesin across the species. Transcription regulation is the most conserved function of cohesin from lower to higher eukaryotes, whereas functions such as chromosome condensation, non-homologous centromere coupling, DNA replication, chromosome architecture and rearrangement are least conserved as per the current literature. Functions such as monoorientation of the sister kinetochores, homolog pairing and recombination are quite conserved among eukaryotes.

CTCF is believed to regulate gene expression by insulating interaction of a gene promoter with that of an enhancer/silencer [reviewed in 29]. However, Nipbl was not found at the CTCF sites. Thus it is not clear whether the cohesin observed at the CTCF sites might have been loaded directly through a distinct Nipbl-independent mechanism, or might have come to these sites simply by sliding. Regardless of the mechanism, the loading of cohesin at these CTCF-binding sites depends on CTCF itself, whereas cohesin is dispensable for CTCF binding at these locales [11,30,31]. Thus, it is possible that the regulation of transcription by CTCF is probably mediated through its ability to recruit cohesins at the promoters. The advanced sequencing technique associated with chromatin immunoprecipitation (ChIP-seq) has identified a CTCF-independent pathway of cohesin mediated gene regulation. Higher resolution of the binding sites using ChIP-seq has revealed that in mouse embryonic stem cells, cohesin loader (Nipbl), cohesin, and mediator (transcriptional co-activator) co-localize with each other at many sites other than CTCF-binding sites [32]. Depletion of cohesin, Nipbl, or mediator leads to changes in expression of the genes whose *cis*-acting regulatory elements show peaks of cohesin or mediator accumulation. To explain how the transcription is regulated, it has been shown that the mediator forms a complex with cohesin, and the resulting complex is believed to form rings that connect two DNA segments to bring enhancer and promoter in close proximity (discussed further below).

The regulatory role of cohesin in transcription of genes appears to be conserved across eukaryotes (Table 3). In budding yeast, cohesin is believed to form boundary elements to restrict the spreading of transcriptional silencing at the silent mating type loci [33], and, in fission yeast cohesin was shown to regulate transcriptional termination [24]. Recently, it has been reported that the cohesin loader Scc2 recruits cohesin to activate the transcription of *REC8* (which encodes the meiotic subunit of cohesin) during meiosis in budding yeast. Rec8-cohesin further activates the promoter of *REC8* to the full extent, and thus meiotic cohesin exhibits a positive feedback on its own transcription [25]. Additionally, mere binding of other cohesin subunits Smc1 and Scc3 on the chromosome were found to be also essential for the expression of *REC8* in budding yeast [34].

How cohesin fulfills its role in the regulation of transcription can be viewed in light of its role in SCC. During SCC, cohesin holds two sister chromatids through topological entrapment of two DNA helices within a ring-like structure [reviewed in 2]. Likewise, tethering together of distant chromosomal loci by the cohesin ring may be the key event in regulation of transcription. According to this theme, the proposed model is that cohesin and its loader, Nipped-B/Nipbl/Scc2, regulates gene expression by causing physical interactions among long-range chromosomal loci, presumably by forming long-distance DNA loops [18, Fig. 2A]. The cohesin ring is believed to stabilize these DNA–DNA interactions by establishing physical linkage between neighboring intra- or inter-chromosomal cohesin binding sites. In mouse embryonic stem cells, according to this model, mediator and other transcription factors in association with Nipbl bring enhancer and promoter together, forming a DNA loop that is reinforced by cohesin to promote transcription [32]. The loop model can also be true in cases where cohesin in association with CTCF regulate gene expression (Fig. 2B). The fact that CTCF functions through formation of a chromatin loop has already been demonstrated at different loci through chromosome conformation capture (3C) assay [35,36]. CTCF is believed to insulate transcription of genes by sequestering the enhancer within the loop. Cohesin knock-down experiments have suggested that cohesin is required for maintaining the insulating function of CTCF [11,30]. This suggests that the chromosomal looping formed by CTCF can be maintained by cohesin, although cohesin itself is recruited by CTCF. Apart from its role in promoting enhancer-promoter interaction, CTCF has been shown to regulate compaction of chromatin and thus helps in recombination [37], presumably through similar cohesin-assisted reinforcement of chromatin loops. A recent study using ChIP-seq has demonstrated that the cohesin binding sites are co-localized with majority of the CTCF binding sites throughout the ~2.5 Mb-long immunoglobulin heavy chain locus, and thus cohesin is believed to help in generation of multi-loop rosette structure at that locus essential for producing immunoglobulin heavy chain diversity [38].

It is to be noted that cohesin can induce or repress gene transcription depending upon whether the enhancer or silencer, respectively, is sequestered within the loop, putting them close to or further apart from the gene promoter (Fig. 2). For example, if an enhancer or silencer cannot interact with the promoter due to cohesin-assisted loop formation, the transcription will be repressed or activated, respectively (Fig. 2B). Interestingly, in the *Drosophila* central nervous system, genes that are activated upon Nipped-B or cohesin knock-down are more enriched in cohesin than the genes that are deactivated. This result suggests that cohesin has more repressive effects than activating effects, at least in this system [15]. In support of this notion, recent studies on *Drosophila* cells have shown that cohesin selectively binds to genes on which RNA polymerase II pauses just downstream of the transcription start site [39]. Further analysis revealed that cohesin does not inhi-

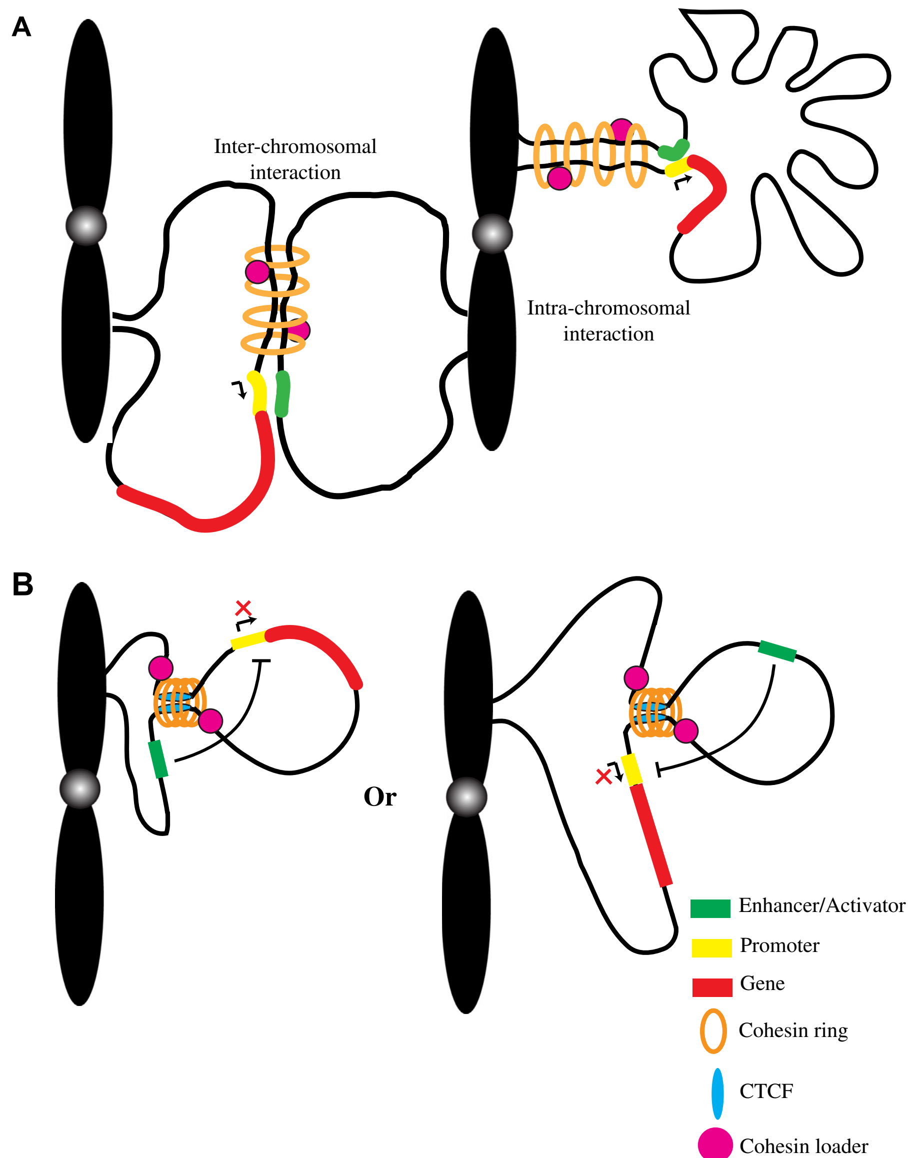

Fig. 2. (A) Mechanism of cohesin-mediated transcription activation: cohesin entraps intra/inter chromosomal DNA duplexes in such a way that otherwise remote chromosomal loci can be brought into proximity. In this diagram, cis-acting enhancers come into close proximity to the promoters through cohesin to switch on the transcription of a gene (as shown by the arrow). Similarly, entrapment of a cis-acting silencer close to a promoter can impose transcriptional suppression of the corresponding gene (not shown). (B) Mechanism of CTCF and cohesin mediated transcription repression: cohesin stabilizes the chromatin loops formed by CTCF. Formation of the loops blocks the interaction between the enhancers/activators and the promoters, leading to suppression of transcription. Similarly, entrapment of silencer sequences (just like enhancer/activator) within the loop can activate the transcription of a gene (not shown).

bit binding of polymerase to the promoters, does not physically block transcription elongation, and does not cause polymerase to pause. Actually it is believed to be the transition of a paused polymerase state to a transcription elongation state which is hindered by cohesin [39].

Interestingly, in most of the experiments where gene expression is altered due to compromised cohesin function, SCC remains largely unaffected [40–43]. Furthermore, it has been noticed consistently that a mild decrease in Nipped-B/Nipbl/Scc2 or cohesin activity is associated with alteration in gene expression without affecting SCC or chromosome segregation. This argues that a higher level of cohesin activity may be required for transcriptional control than is required for SCC [reviewed in 13]. In accordance with this, ablation of ~80% of cohesin activity in *Drosophila* indeed does not lead to cohesion defects [15]. Similarly, Zhang, et al., have shown that in mice the absence of Pds5B, one of the homolog of Pds5 in mammals, does not affect chromosomal cohesion, although it generates severe developmental abnormalities presumably due to altered gene expression [44]. Additionally, different dosages of Pds5A and Pds5B have been implicated in regulation of gene expression and thus embryonic development [45]. These results suggest that the effect of PDS5 on regulation of cohesin function is more crucial for transcription than for SCC. In the context of regulation of transcription through cohesin-associated proteins, presumably through their interactions with core cohesin subunits, it can be hypothesized that such regulation may be achieved in a manner distinct from that observed in the case of loop-mediated long range DNA-DNA interactions.

To accommodate functional differences (gene expression vs. SCC) exhibited by the same cohesin complex, a binary mode of cohesin binding can be considered. This stems from the observation in *Drosophila* of a dual mode of cohesin binding to the chromatin in the form of strong and weak interactions [46]. The same group has shown that the pool of cohesin that binds chromatin

strongly is reduced in *Nipped-B* mutants that exhibit altered gene expression, suggesting that strong binding may be essential for regulating transcription [46]. A similar dual mode of cohesin binding has also been demonstrated in yeast using an in vitro system [47], which attests to a unified theme of cohesin binding across eukaryotes.

2.2. DNA double-strand break repair

The fact that a cohesin mutant is sensitive to γ-irradiation due to a defect in DNA double-strand break (DSB) repair in *S. pombe* was the earliest evidence for a role of cohesin in maintaining the integrity of the genome against DNA damage [48]. Since then, several groups have confirmed the role of cohesin in repair of DSBs in mitosis [4,10] and in meiosis [6] in *S. cerevisiae, S. pombe*, and humans (Table 3). The cohesion generated at the time of DNA replication is required for postreplicative DSB repair, and in budding yeast it has been demonstrated that the cohesion establishment factor Eco1/Ctf7, which has acetylation activity, is instrumental in DSB repair [10]. In unperturbed cells, cohesin loaded following S phase is not capable of generating cohesion, presumably due to limiting Eco1/Ctf7 concentration. However, it has been demonstrated that cohesin can be loaded and generate SCC even at the postreplicative phase if there is DNA damage [49], which supports the belief that the presence of DNA lesions somehow augments Eco1/Ctf7 activity beyond S phase. Interestingly, this damage-induced cohesion (DI-cohesion) is generated not only at the site of damage, but also globally on all the chromosomes in a Eco1/Ctf7-dependent manner [50]. Regardless of the timing of cohesion (S-phase or post S-phase), the essential job of cohesin in DNA repair is to bring the two sister chromatids together so that DSB on one sister can be repaired using the unscathed sister as a template for homologous recombination. Addressing the requirement for DI-cohesion has clearly identified Scc2 as a factor required for fresh loading of cohesin in response to DSB [49]. This suggests that postreplicative recruitment of cohesin to DSBs is required for DNA repair. To explain the targeting of cohesin at the damage site in budding yeast, it has been demonstrated that phosphorylation of histone H2AX (γ-H2AX) by checkpoint kinases Mec1 and Tel1 at least 60 kb from the DSB site may act as an epigenetic mark for this purpose [51]. Additionally, the MRX (Mre11/Rad50/Xrs2) complex, which recognizes a DSB site, also independently promotes cohesin assembly at the break site [51]. Further studies in budding yeast comparing the ability to generate DI-cohesion by Scc1 (mitotic cohesin) vs. its meiotic version, Rec8, have revealed that Scc1 phosphorylation by checkpoint kinase1 (Chk1) at Ser83 is essential for DI-cohesion, but replacement of Scc1 by Rec8 cannot facilitate DI-cohesion [52]. Since damage-induced augmentation of Eco1/Ctf7 activity is key to generation of DI-cohesion, investigations carried out to find the target of Eco1/Ctf7 have revealed that acetylation of Scc1 at K84 and K210 by Eco1/Ctf7 is important for DI-cohesion, and that Ser83 phosphorylation of Scc1 augments its acetylation [53]. Although several factors have been identified as responsible for DI-cohesion and hence for DSB repair, it has been observed that in many cases such factors become dispensable for DSB repair, which suggests that repair can take place in redundant ways and perhaps can occur in such cases in spite of a lack of DI-cohesion [51,54,55]. On the other hand, evidence for DI-cohesion without DSB repair has supported the argument that DI-cohesion is not sufficient for DSB repair [56]. These results indicate that cohesin may initiate the process of DSB repair by bringing damaged and intact strands in close vicinity, but becomes dispensable for the subsequent repair process. Importantly, it is to be noted that although Eco1/Ctf7 acts as general cohesion establishment factor both during replicative and postreplicative (DI-cohesion) cohesion pathways, functions of Eco1/Ctf7 in these two processes may differ. In support of this, Lu et al., have recently observed in budding yeast that an *eco1* mutant, carrying a disease mutation that causes a developmental disease in human called Robert syndrome, fails to provide DSB repair without any perturbation in SCC [57]. On the basis of the above observations it has been proposed that Eco1/Ctf7 might have additional yet to be discovered target(s) that link(s) activity of Eco1/Ctf7 directly to DSB repair.

In meiosis, cohesin is involved in the programmed double-strand-break (PDSB) repair required for the generation of chiasmata, a structure developed due to physical exchange of non-sister chromatids in unperturbed cells. This differs from mitosis, where repair is done in a different context to safeguard the integrity of the genome against perturbation. Furthermore, in meiosis, mostly non-sister strands are used for recombination mediated repair, whereas sister strands are used in mitosis. These functional differences might have evolved through a recruitment of a different α-kleisin subunit within the cohesin complex during meiosis (Rec8) vs. mitosis (Scc1/Mcd1/Rad21). Indeed, replacement of Scc1 with Rec8 renders mitotic cells impaired in DSB repair, and Rec8 fails to provide cohesion following DNA damage [52]. Similarly, replacing Rec8 with cohesion proficient Scc1/Mcd1/Rad21 cannot provide PDSB repair and subsequent formation of chiasmata [58]. Apart from its role in repair of PDSB, cohesin may also be even involved indirectly in the formation of these PDSBs. In support of this, it has been demonstrated in budding yeast that Spo11, the enzyme responsible for making PDSBs, is not only localized at the expected break sites but also at the Rec8 binding sites with a peak at the centromeres [59]. Consequently, *rec8* deletion has been shown to reduce Spo11 binding on the chromosome. Therefore, it has been proposed that Rec8 might recruit Spo11 to the chromosome sites from which Spo11 moves to actual break sites [59]. Although localization of break sites away from the stable Rec8 binding sites excludes any possibility of Rec8's being involved in formation of PDSB per se [60], deletion of *REC8* in budding yeast leads to a significant decrease in the rate of recombinant product formation, suggesting its role in PDSB processing [8,61]. Furthermore, co-purification from different organisms of Rec8 with Rad51/Dmc1, which coats ssDNA required for strand invasion during PDSB repair, also argues for involvement of Rec8 in events downstream of PDSB formation [62].

Apart from budding yeast, experimental evidence has shown the involvement of cohesin in DSB formation and repair in other systems such as in *S. pombe* [6,48] and in humans [63–66]. In humans, cohesin has also been found to be important for the activation of DNA damage-induced intra S phase and G2-M checkpoints [63–66].

The above results are indicative of cohesin as a responsible factor for DSB formation and repair. Interestingly, as mentioned above it has been shown that an over 80% reduction in cohesin activity leads to normal cohesion, albeit causing a defect in DNA repair and chromosome condensation in budding yeast [6]. These data again reinforce, as mentioned in Section 2.1, the importance of cohesin stoichiometry in regulating different cellular activities, and it appears that a low level of weakly bound cohesin may be sufficient to provide canonical SCC function whereas non-canonical functions require high levels of strongly bound cohesin.

2.3. Function at centrosomes

The microtubule-organizing center, known as the centrosome in animal cells, is referred to as the spindle pole body (SPB) in budding yeast. During DNA replication, centrosomes/SPBs are duplicated to form two sister centrosomes/SPBs. Interestingly, several studies indicate that cohesin may be involved in faithful centrosome/SPB duplication. The first clue came from a study in which it was shown that separase, required for cohesin cleavage, is also

essential for centriole disengagement and for licensing of centrosome duplication [67]. Subsequently, other groups were able to detect the presence of cohesin subunits at the spindle pole [68] and centrosomes [69]. More direct evidence came from HeLa cells, as si-RNA mediated depletion of cohesin subunit Rad21 was shown to cause premature separation of paired centrioles, the core of the centrosome [70]. In accord with this, a smaller spliced variant of shugoshin (sSgo1), a regulator of SCC, has also been found to be required for the protection of centriole cohesion in mammalian cells [71]. Recently, Stemmann and colleagues have shown that ectopic activation of separase or depletion of Sgo1 results in premature sister chromatid separation as well as centriole disengagement in human cells, and thus the chromosome and centrosome cycles exhibit extensive similarities and appear to be coordinated with each other through the dual roles played by cohesin at the chromosome and at the centrosome [72]. Similarly, Clarke and co-workers have reported the involvement of chromosomal cohesin regulators such as Aurora B/Plk1 kinases (phosphorylates cohesin subunits Scc1/SA2 for its removal during prophase) and separase in centrosomal localization of cohesin subunit Rad21 [73]. The same group has also shown the importance of cohesin (Rad21, Smc1 and Smc3) for execution of bipolar mitosis in human [74]. The above data suggest that the cohesin ring is required for maintaining spindle pole integrity, and thus is the key determinant of bipolar anaphase, independently of its role in SCC. A recent study by Jin et al., has shown the potential role of cohesin in SPB cohesion during yeast meiosis [75]. In this study, the appearance in a cohesin mutant of supernumerary GFP foci of tagged Spc42, an SPB marker, possibly indicates defective SPB cohesion. Given the fact that SCC and natural separation of duplicated cohesed SPB/centrosome occur almost at the same time, more experimental support is required to conclude that the observed separation of SPB/centromsome markers are really due to a cohesin defect. Identification of interactions of cohesin of SPB/centrosome components will elucidate the mechanism of how cohesin becomes targeted and performs its role at the SPB/centrosome.

2.4. Chromosome condensation

Chromosome condensation is one of the key events of chromosome morphogenesis required for faithful chromosome segregation. The chromosome compaction to a large extent is mediated by, at least in yeasts and *Xenopus laevis*, a multi-protein evolutionarily conserved cohesin-like complex called condensin [76,77]. In the case of budding and fission yeast, changes in the level of cohesin lead to hypo- or hyper-condensation of the chromosomes presumably due to altered targeting of condensin to the chromosome, although the condensation of chromosomes in higher eukaryotes showed only subtle effects of the level of cohesin [5,78–80]. This is consistent with the fact that in higher systems condensin is not directly involved in chromosome compaction [81,82]. These studies suggest that cohesin can somehow influence condensin localization on the chromosome, at least in yeasts. In support of this, it has been demonstrated in budding yeast that chromosome condensation occurs in two steps, and that the first step of condensation that occurs between G2 to metaphase depends on cohesin. This function of cohesin is in concordance with its retention time on the chromatin [83]. Interestingly, in a more recent study using the ChIP technique followed by hybridization with oligonucleotide tiling arrays, it has been demonstrated that the DNA binding sites of condensin overlap with the sites of occupancy of the Scc2/Scc4 complex, which acts as a cohesin loader to the chromatin [84]. This implies that at least in budding yeast condensin is loaded at the sites where cohesin is also recruited. Nevertheless, the mechanism of how cohesin might interact with condensin is poorly understood. Whether the formation of the DNA loop through trapping of two DNA helices by the cohesin ring eventually reinforces condensin-mediated chromosome compaction, or if it is some other structural features of cohesin that promotes condensation, is yet to be resolved. Isolation of a cohesin mutant unable to support chromosome condensation without any perturbation in SCC would be a tempting step to resolve this issue. As mentioned earlier, the cellular stoichiometry of cohesin may be instrumental in determining whether to support SCC alone or also condensation [14]. It would be interesting to know how the concentration of cohesin influences SCC and chromosome condensation differently.

2.5. Meiosis specific roles of cohesin

During meiosis, the mitosis-specific α-kleisin subunit of a cohesin complex (Scc1/Rad21) is universally replaced by a meiotic version called Rec8 in *S. cerevisiae*, *S. pombe*, and *Caenorhabditis elegans*, Rad21L (recently identified α-kleisin subunit having sequence similarity to Rec8 and Rad21) in mammals, and C(2)M in *Drosophila* (Table 1). It has been observed that meiotic cohesin is involved in other cellular functions that are not directly linked to SCC. Such functions of meiotic cohesin have been addressed more elaborately elsewhere [reviewed in 85]. Below, we have addressed a few of them, discussing the recent findings.

2.5.1. Pairing of homologous chromosomes during meiosis

Homologous chromosomes of paternal and maternal origin physically link to each other, which is known as the pairing of homologs, and move to opposite spindle poles during the reductional division during meiosis I. The function of meiotic cohesin in pairing of homologous chromosomes during meiosis has been demonstrated in budding yeast, fission yeast, *Arabidopsis thaliana*, *C. elegans*, and mice [8,58,61,86–88]. In most of the organisms, the homologous pairing or synapsis is reinforced by a tripartite, robust, cytologically visible structure called a synaptonemal complex (SC). The assembly of the SC starts with pairing of proteinacious structures, known as axial elements (AEs), from two homologous chromosomes. The AEs eventually mature into lateral elements (LEs). The SC is formed when two LEs are linked to each other through formation of a central element (CE) by virtue of tetramerization of a protein called Zip1 [89]. Assembly and/or recruitment of all these structural components of SC have been shown to be impaired to different extents in cells from various organisms compromised in the function of Rec8 or its orthologs [8,61,86,88]. An extreme case of cohesin's role in supporting homolog synapsis is revealed when it was demonstrated during mammalian meiosis that DNA recombination proteins such as Dmc1 and Msh4 can be recruited to the chromosomes by cohesin complex proteins (Smc1, Smc3 and STAG3) and can promote homolog synapsis, even in the absence of an AE protein (Scp1, Scp2 and Scp3) and proper SC assembly [90]. Similarly, Rad21L, a meiosis-specific cohesin variant in mammals, has been shown to be responsible for initiating synapsis and crossover recombination between the homologs [91,92]. Consequently, absence of Rad21L in male mice leads to failure in completing synapsis of homologs, resulting in total azoospermia and infertility, whereas in female mice absence of Rad21L showed age-dependent sterility [91]. Further similar studies have shown that mice spermatocytes lacking two meiosis-specific cohesin subunits, RAD21L and REC8, were unable to assemble their AEs and arrested at the leptotene stage of prophase I, demonstrating that cohesin plays an essential role in AE/SC assembly [93]. Apart from the core cohesin subunits, cohesin accessory proteins have also been shown to be involved in SC assembly and homolog pairing. Disruption of cohesin function through depletion of Pds5, a cohesin associated protein required for maintenance of cohesion, did not affect SCC significantly, but led to the failure of homolog pairing and presumably SC formation [94]. Given the above results,

it would be interesting to know whether meiotic cohesin influences the assembly of these SC-related structures through its canonical function of SCC, or independently of SCC function. It would be useful for further study if careful analysis of different alleles of *REC8*/*RAD21L* could reveal different mutant forms of meiotic cohesin capable of performing one function but not the other.

Besides the kleisin subunit, other core cohesin components also appear to have a role in meiosis. Studies in mice have demonstrated that a spontaneous frame-shift mutation in the *Smc1B* gene, encoding a non-kleisin component of a cohesin complex, can cause cohesin protection dysfunction and sterility in both the sexes [95].

2.5.2. Non-homologous centromere coupling

Pairing of homologous chromosomes is an outcome of both recombination-dependent and recombination-independent mechanisms [96]. It is believed that the pairing process starts initially through coupling of centromeres in a recombination-independent, non-homologous manner. In support to this, it has been demonstrated that blocking of homologous recombination by deletion of *SPO11* cannot block the pairing of non-homologous centromeres [97]. This pairing appears to be mediated through localization of Zip1 at the centromere. Experimental evidence in budding yeast has shown that absence of Rec8 can cause reduced localization of Zip1 at the centromeres that can lead to failure of non-homologous centromere coupling [98], although the same study has also shown Rec8 is dispensable for general association of Zip1 with the chromosomes. Therefore, it is presently unclear whether Rec8 per se is required for non-homologous centromere coupling or rather that it merely facilitates this task through recruitment of Zip1 at the centromeres, perhaps with the help of proteins at the kinetochore. It would be interesting to test if this function of meiotic cohesin also requires higher stoichiometry of cohesin than it is required for SCC function.

2.5.3. Monoorientation of sister kinetochores during meiosis I

One of the unique features of meiosis I is that the sister kinetochores of each homolog pair are attached to the same spindle pole (monooriented) [99] on the metaphase I spindle. It has been observed in *S. pombe*, *Arabidopsis* and *Oryza sativa* (rice) that mutated/null Rec8 subunit of cohesin complex leads to the biorientation of the sister kinetochores as well as to SCC defects during meiosis I [3,100,101]. These results suggest the possibility that in these organisms cohesin may connect sister kinetochores to orient them side-by-side during meiosis I in order to promote their monoorientation. In support of the role of cohesin as an orientation determining factor for the kinetochores, it has been demonstrated in fission yeast that the cohesin at the core centromere is responsible for the monoorientation of the kinetochores, whereas cohesin at the pericentromeric region is responsible for the bi-orientation of the kinetochores [9,102]. However, in budding yeast cohesin doesn't appear to play any role in side-by-side orientation of the sister kinetochores, since replacement of Rec8 with the mitotic version Scc1 did not abrogate sister kinetochore monoorientation [103]. Interestingly, in higher eukaryotes (mice), mitotic cohesin Rad21 is also expressed during meiosis and has been found to have a role in monopolar attachment of the sister kinetochores during meiosis I, but not for the maintenance of SCC during meiosis I or meiosis II [104]. Consistent with this observation, Rad21, along with shugoshin (Sgo2), a protector of centromeric cohesin at meiosis I, has been shown to be involved in sister kinetochore association during meiosis I in mouse spermatocytes [105]. Since both Rad21 and STAG3 become phosphorylated, which may facilitates their dissolution from the chromatin [106,107], it needs to be tested whether this sister kinetochore co-orientation function of Sgo2 is indirectly executed through retention of Rad21 and/or STAG3 at the centromere through an association with protein phosphatase 2A (PP2A). It is true that the cross-linking of the sister kinetochores is required for achieving side-by-side orientation over back-to-back orientation (observed when sister kinetochores are bioriented). Whether this cross-linking is facilitated due to certain structural features of cohesin per se or due to generation of SCC in combination with some meiotic factors is not known.

3. Impairment of the cohesin network leads to development of cohesinopathies in human

The human diseases that occur due to defects in cohesin function have been termed collectively "cohesinopathies", and comprise developmental disorders such as Cornelia de Lange Syndrome (CdLS, OMIM#122470), Roberts Syndrome (RBS, OMIM#268300)/SC phocomelia (SC, OMIM#269000) [reviewed in 16,17,108], or human malignancies [reviewed in 109]. Importantly, recent literature suggests that defects in cohesin-assisted transcriptional regulation, rather than defects in cohesin-mediated SCC, play significant roles in the etiology of these diseases.

3.1. Cornelia de Lange syndrome

About 60% of probands of CdLS possess a heterozygous mutation in the *NIPBL* gene whereas approximately 5% possess a mutation in *SMC1A* and <1% a mutation in *SMC3* [reviewed in 109]. The molecular etiology of the remaining 35% of probands is unknown at this time, but it is possible that mutations in the cohesin core and other associated/regulatory proteins such as Rad21, SA1/2/STAG3, Pds5, HDAC8 etc. may contribute to this. In support of this idea, mutation in Rad21 has been shown to cause developmental abnormalities similar to CdLS in humans [110]. Furthermore, analysis of SA1 $-/-$ mice revealed that ablation of SA1 subunit can also lead to CdLS-type phenotypes [111]. Direct evidence that the role of cohesin-mediated gene expression is altered in CdLS came from the studies of transcriptional regulation of the mammalian *HOXD* locus, which is critical for proximal-distal limb patterning. A reduced *HOXD* expression has been observed in a CdLS patient. The transcriptional regulation of the *HOXD* locus is believed to be controlled by cohesin bringing the transcriptional control element, located more than 200 kb away, close to the transcription start site [reviewed in 112,113]. Similarly in *Drosophila*, heterozygous mutation in *Nipped-B* (cohesin loader) shows no defects in SCC, but exhibits measurable defects on expression of the *cut* gene, required for development of bristle forming cells around the adult wing margin, resulted in deformed wing margins. A zebrafish model of *nipbl* (cohesin loader) deficiency showed specific heart and gut/visceral organ defects similar to those observed in CdLS, due to altered expression of genes involved in endodermal differentiation and left–right patterning [114]. Experiments with a mouse model of CdLS have also revealed that reduction in *Nipbl* transcript level by 30%, and hence attenuation of cohesin activity, can lead to developmental defects characteristic of CdLS [115]. Gene expression profiling from the same study has demonstrated that *Nipbl* deficiency causes modest but significant transcriptional dysregulation of many genes, including the protocadherin beta (*Pcdhb*) genes required for cell surface diversity generation in nervous system. Whether partial reduction of Nipbl activity causes global reduction of cohesion level across the chromosomes or the reduction is restricted to certain promoters of the genes, inactivation of which creates CdLS, remains to be tested. Nevertheless, the above results suggest that cohesin loaders in different organisms influence long range chromosomal regulatory interactions.

As mentioned above, besides involvement of the impaired cohesin loader NIPBL, the mutated core component of cohesin and other cohesion regulatory proteins have also been implicated in CdLS. For example, a mutation in SMC1L1, an X-linked gene, is

responsible for the milder form of CdLS [116]. To explain this phenomenon, it is proposed that the Smc1L1 mutation may cause alterations in gene expression presumably because of slow turnover of cohesin on the chromatin. The neurodevelopmental, gastrointestinal and skeletal abnormalities of CdLS patients depend on the improper expression of Runx transcription factors [117]. Consequently, Horsfield et al. have shown that mutation in Rad21 leads to reduced expression of *runx1* and *runx3* genes, resulting in developmental delay in zebrafish embryo [7]. Other core cohesin subunits also appear to be involved in regulation of gene expression. Genome wide ChIP-seq analysis in mice has demonstrated that cohesin-SA1 is enriched at the promoter regions of certain genes and at CTCF sites [111]. Global gene expression profiling of the wild-type and cohesin-SA1 null mice has revealed down-regulation of the genes associated with limb and skeletal system morphogenesis, heart and lung development and lipid metabolism in cohesin-SA1 null mice. Moreover, significant overlap between the transcriptional changes linked to the loss of cohesin-SA1 and those reported for Nipbl-heterozygous mice were observed [111], suggesting that both cohesin-SA1 $-/-$ and Nipbl $+/-$ conditions alter transcription of certain genes responsible for CdLS pathogenesis. As mentioned in the earlier section, cohesin-associated proteins also influence transcription, so it is expected that loss of their functions can also lead to CdLS. In support of this, Zhang et al. have shown that absence of Pds5B leads to developmental disorders characteristics of CdLS in mice [44]. A recent study by Deardorff et al. has identified an epigenetic determinant of cohesin malfunction and CdLS pathogenesis in several CdLS patients where the disease was attributed to mutations in HDAC8, a vertebrate SMC3 deacetylase [118]. Smc3 acetylation is a key event to establish SCC [reviewed in 1]. However, following release from chromatin in prophase and anaphase, the 'used' acetylated cohesin should be deacytylated before it can be freshly loaded on the chromatin in the next cycle. Loss of HDAC activity results in accumulation of chromatin-released acetylated cohesin, which is loaded on the chromatin at a reduced rate. The resulting dysregulation of transcription then leads to cellular and clinical features of CdLS [118].

The above results indicate that although cohesin loaders (Nipbl/NIPPED-B) play pivotal role in CdLS development through transcriptional dysregulation, the core cohesin components and other cohesin regulators also contribute. However, one interesting revelation came from the study of zebrafish deficient in *nipbl* and other cohesin subunits, where it is proposed that transcription regulation by Nipbl may not be executed merely through its cohesin loading function. This is because different phenotypes and gene expression profiles have been observed in the organism harboring mutations in *nipbl* or depleted for Nipbl or other cohesin subunits. In several of the cases, the CdLS phenotype has occurred as a consequence of additive effects of gene expression changes [114]. Nevertheless, the above data suggest that impairment in the cohesin network causes a transcription dysregulation that leads to CdLS pathogenesis in human and in other model organisms. Therefore, the current challenge would be to identify whether changes in cohesin-mediated gene expression or changes in SCC or both are responsible for the developmental defects observed in CdLS. It would be interesting to address whether unified mechanism of transcription regulation has been followed in all the CdLS cases where the disease has occurred due to dysfunction of cohesin loader, cohesin core or associated proteins.

3.2. Roberts syndrome and SC phecomelia

RBS and SC phecomelia are autosomal recessive genetic disorders caused by homozygous or compound heterozygous mutations in the *ESCO2* gene, a human homolog of the yeast *ECO1*/*CTF7* genes which is essential for the establishment of SCC and codes for an acetyltransferase enzyme [119,120]. SC phecomelia is a milder form of RBS in terms of physical defects and mental retardation. Most of the RBS probands harbor mutation in the *ESCO2* gene, specifically disrupting the acetyltransferase domain. These results indicate that acetyltransferase activity is essential for the development of the major organ systems affected in RBS [121]. To address whether Esco2 and Nipbl/Rad21 influence different sets of genes to cause the RBS and CdLS phenotypes, respectively, the transcription profiles of Rad21- (cohesin subunit) or Esco2-depleted zebrafish embryos were analysed. The expression of genes changed in the absence of Rad21 or Esco2 were indeed non-overlapping [122]. In a study to address how Esco2 brings about RBS, it was found, in contrast to human patients with RBS who can survive until adulthood, early embryonic lethality was obtained in Esco2 deficient mice [123]. The reason behind this disparity has not been resolved. However, deletion of both copies of *Esco2* in mouse embryonic fibroblasts (MEFs) revealed that Esco2 is required for cell survival, and that its absence results in severe chromosome segregation defects as well as apoptosis [123]. Although in mammals both ESCO1 and ESCO2 are responsible for cohesion establishment and Sororin loading on the chromatin, emergence of Esco2 as cell viability factor suggests that this protein functions non-redundantly with Esco1. Using a mouse system, Whelan et al. [123] have shown that Esco2 is involved in cohesion of centromeric heterochromatin, a function that has also been shown to be compromised in RBS patients [119]. Although heterochromatin is perceived as a transcriptionally silent chromosomal domain, paradoxically gene expression can take place within certain heterochromatin regions in a heterochromatin protein 1 (HP1)-dependent way [reviewed in 124,125]. Interaction between cohesin and heterochromatin has been demonstrated in *S. pombe*, where Swi6 (HP1 ortholog) directly interacts with cohesin and is involved in physical association of cohesin with the heterochromatin [126–128]. This leads one to speculate that the level of cohesin accumulated at the heterochromatin region can influence the expression of genes at these loci. In support of this, studies in yeast and *Drosophila* have indeed demonstrated that the expression of genes located in the heterochromatin region can be influenced by cohesin binding at this region [33,129,130]. Therefore, in collating these results, it has been proposed that the developmental defects observed in RBS/SC syndrome may be a consequence of cohesin-dependent gene expression changes at the heterochromatin regions [reviewed in 108]. Alternatively, though not mutually exclusive, it is also possible that changes in cohesin binding could increase the spreading of heterochromatin to the adjacent euchromatin region. For example, cohesin mutations allow spreading of *SIR* (Silent Information Regulator) complexes from the yeast *HMR* (Homeobox corepressor) locus [33]. Such a spread could potentially alter expression of the genes near the borders of heterochromatin, and that could be critical to the etiology of the RBS/SC syndrome. Interestingly, a recent study by Bose et al., with budding yeast strains bearing mutations analogous to those causing the human cohesinopathy diseases (*eco1*-W216G and *smc1*-Q843Δ) showed no significant defect in chromosome segregation but did exhibit defects in ribosome biogenesis and a deficit in protein translation [27]. Detailed analysis revealed that both the mutants produce less ribosomal RNA, which is expected to constrain ribosome biogenesis. Similar defects in rRNA production and protein translation were observed in a human RBS cell line. Although chromosome segregation is not affected, cohesion was found to be defective specifically at the rDNA locus in *eco1*-W216G mutant [27], and a similar cohesion defect at the rDNA locus has been previously reported in an RBS

patient [131]. Thus, these studies demonstrate that cohesin can regulate gene expression and cause disease etiology by altering general translational efficiency.

3.3. Human malignancies

Consistent with roles in chromosome segregation and regulation of gene expression, it is expected that cohesin and its associated proteins will be an important factor for cancer development. Increasing evidence has revealed a link between malfunctioning of the cohesin network with different forms of human cancers. Overexpression of WAPL, a cohesin-binding protein that facilitates timely release of cohesin from chromosome arms during prophase, has been observed in cervical cancers, and down-regulation of WAPL inhibits the growth of tumors derived from cervical cancer cell lines [132,133]. Separase, the molecular scissor that cleaves cohesin during the metaphase to anaphase transition [reviewed in 2], has also been implicated in tumorigenesis. Heterozygous mutants for separase contribute to the initiation and progression of epithelial tumors partially due to their ability to generate genome instability in zebrafish, and thus separase has been suggested to function as a tumor suppressor gene in this organism [82]. In contrast, overexpression of separase in mammary epithelial cells of mouse in a p53 mutant background induces aneuploidy and tumorigenesis, and thus here separase behaves as an oncogene [134]. Cohesin establishment factor Esco2 has also been implicated in human cancer, and an elevated level of this protein has been observed in human melanoma cells [135]. Furthermore, human cells bearing mutations either in *BRCA1* (tumor suppressor gene) or the *ESCO*-related pathway exhibit the same chromosomal abnormalities, including cohesion defects [136,137]. Brca1 interacts with many proteins that play essential function in the SCC pathway, indicating a common mechanism between cohesin functions and BRCA1-tumorigenesis. Recent studies have identified mutations in cohesin network proteins (*SMC1A, NIPBL, SMC3, STAG3*) in cases of colorectal cancers [138]. These mutations may lead to the chromosome instability observed in colorectal cancers. The important point that needs further attention is whether the aneuploidy and tumorigenesis observed in the cohesin function impaired cells are due to altered gene expression or due to chromosome missegregation or both. Measuring the cohesin level and the localization of cohesin at the cancer critical genes that show altered level of transcription in normal and tumor cells will be a step towards addressing this issue. Experiments on zebrafish have suggested a role of cohesin (Rad21) in regulating transcription of genes such as *myca*, *p53* and *mdm2*, whose transcriptional dysregulation can lead to cancers as well as developmental defects observed in CdLS [139].

An important point to be noted here is that when different components of the cohesin network are compromised, different subsets of genes are misregulated, leading to various forms of cohesinopathies [reviewed in 140]. This has been manifested in diseases like CdLS and RBS, where distinct developmental outcomes have been observed due to mutations in different subunits of the cohesin apparatus. For example, ESCO2, a component of the cohesin network, is associated with RBS, whereas other components of the same network (Nipbl/Rad21) have been linked to a different disease, CdLS. This is somewhat puzzling because all the components of the cohesin network are expected to perform a similar role in cell division and thus to affect similar sets of genes. Clearly apart from cohesin core subunits, there are two other groups of cohesin associated proteins – those who load and retain cohesin on the chromatin and those who generate a cohesive state of cohesin. Careful analysis of the mutant forms of these proteins have revealed that the mutants compromised in cohesin loading/retentions exhibit transcriptional misregulation (as in CdLS), suggesting involvement of the cohesin loader/retention in regulating gene expression. On the other hand, the mutants that fail to generate cohesion exhibit chromosome segregational defect, mitotic retardation and apoptosis (as in RBS), suggesting that the cohesion generators are involved in protecting cells from death pathways. However, the apoptotic functions can also be ascribed to transcriptional regulation of genes involved in apoptosis. Nevertheless, some commonalities in gene expression changes and disease phenotypes have been observed among different cohesinopathy diseases, which are likely due to deficiency of core cohesin subunits that are required for all the processes in the cohesin pathway.

4. Future directions

The rapidly emerging field of cohesin biology has now established itself as an inevitable platform to enrich our understanding of complex biological interdependencies. From the discovery a decade ago of cohesin in yeast and *Xenopus* for keeping the sister chromatids together, different aspects of cohesin's functions have emerged exponentially, ranging from gene expression to chromosome condensation, DNA damage repair, centrosome/microtubule organization, and many more. The amount of evidence demonstrating cohesin's involvement in myriad cases of transcriptional regulation is increasing so rapidly that cohesin may soon be recognized as an important epigenetic factor to control gene expression. All these functions show the versatile nature of the cohesin complex and its subunits, which allow it to interact with a wide variety of cellular targets for performing various functions. Obviously, it would be exciting to know how a single protein complex can perform so many types of functions. The majority of these functions may be manifested through entrapment of the two DNA helices inside the cohesin ring, as also occurs in the tethering of two sister chromatids. The evidence available for the role of cohesin in controlling gene expression is circumstantial. Development of an in vitro system would address whether cohesin is directly involved in the process. Therefore, the major challenge ahead is to delineate the mechanism by which cohesin is switching on or switching off the transcription of certain genes. Is there a unified theme, or does the mechanism vary with the context? If binary modes of cohesin binding, strong and weak, are indeed involved in gene expression and SCC, respectively, understanding the nature of these bindings at the molecular level will be really informative. This will address why high level and strong binding of cohesin is crucial for regulation of transcription but not so for generation of SCC. On the other hand, it would be interesting to know the biological cues that allow cohesin to repress or activate transcription. Also, how does cohesin interact with other intrinsic and extrinsic molecular signals for this purpose? How, from the same cohesin apparatus, one set of components can influence transcription of developmental genes whereas another set is involved in cell death pathways, is also an important aspect to address. Although the structure of cohesin is well conserved across eukaryotes, significant variations in the life cycle of cohesin from yeast to humans makes it difficult to find out a generalized mechanism through which cohesin performs its other functions besides SCC. Nevertheless, as shown in Table 3, it is also interesting to know whether these functions are conserved across different eukaryotic species.

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