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Review

NIPBL and cohesin: new take on a classic tale

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Cohesin folds the genome in dynamic chromatin loops and holds the sister chromatids together. NIPBL^{Scc2} is currently considered the cohesin loader, a role that may need reevaluation. NIPBL activates the cohesin ATPase, which is required for topological entrapment of sister DNAs and to fuel DNA loop extrusion, but is not required for chromatin association. Mechanistic dissection of these processes suggests that both NIPBL and the cohesin STAG subunit bind DNA. NIPBL also regulates conformational switches of the complex. Interactions of NIPBL with chromatin factors, including remodelers, replication proteins, and the transcriptional machinery, affect cohesin loading and distribution. Here, we discuss recent research addressing how NIPBL modulates cohesin activities and how its mutation causes a developmental disorder, Cornelia de Lange Syndrome (CdLS).

Highlights

Cohesin organizes genome topology thanks to its ability to extrude DNA loops and to hold together the sister chromatids.

Genome folding and cohesion establishment by cohesin depend on NIPBL, but its requirement for chromatin association of cohesin is unclear.

The interactions of NIPBL with chromatin remodelers, replication proteins, and transcriptional regulators have important implications for cohesin distribution and function.

Cohesin complexes carrying Stromal Antigen 1 or 2 (STAG1 or STAG2) have different chromatin association dynamics and respond differently to low NIPBL levels.

Altered gene expression in patients with Cornelia de Lange Syndrome with *NIPBL* mutations is likely the consequence of reduced loop extrusion.

NIPBL is essential for cohesin functions

Cohesin is an evolutionarily conserved complex that belongs to the **Structural Maintenance of Chromosomes** (SMC; see Glossary) family. It comprises the heterodimer of SMC1 and SMC3, the kleisin subunit RAD21, and the **HEAT-repeat** subunit Stromal Antigen or SA/STAG (Figure 1A). Following the identification of cohesin in yeast, a separate heterodimer of sister chromatid cohesion (Scc)2 and Scc4 was reported to facilitate binding of cohesin to chromosomes [1]. A few years later, a *Drosophila* homolog of Scc2, Nipped-B, was found in a genetic screen for factors promoting long-range enhancer–promoter communication [2]. Soon after, mutations in human NIPBL (Nipped-B like) were detected in patients with a developmental syndrome known as CdLS [3,4]. These landmark papers recognized the essential contribution of NIPBL to both cohesion and 3D genome organization mediated by cohesin, as well as the deleterious consequences of its malfunction for human development. In this review, we evaluate recent studies addressing all these aspects of cohesin biology to try to understand the exact contribution of NIPBL.

To be or not to be (the cohesin loader), that is the question

NIPBL is a large protein of more than 2500 amino acid residues. In human and mouse cells, two major splicing isoforms exist that differ in their most C-terminal region [3]. The first half of NIPBL comprises unstructured regions, while the second half contains several HEAT repeats arranged in the shape of a hook (Figure 1B) [5]. Its partner, MAU2, is a helical fold encompassing multiple tetratricopeptide repeats (TPRs), which interacts with the N terminus of NIPBL [6]. MAU2 protects NIPBL from degradation and may direct the heterodimer to specific chromosomal regions, as discussed below. Studies in several model organisms and *in vitro* assays have built the case for NIPBL and MAU2 being responsible for cohesin loading on chromatin and for promoting topological DNA entrapment, essential for cohesion establishment [7–11] (Figure 1C). Genome folding by cohesin involves loop extrusion and also requires NIPBL [12–14] (Figure 1D). PDS5 is structurally similar to NIPBL and the two proteins associate with cohesin in a mutually exclusive manner [5,15,16] (Box 1). *In vitro* reconstitution of the loop extrusion reaction has shown that it occurs without topological DNA entrapment [17,18]. This result calls into question the role of NIPBL as

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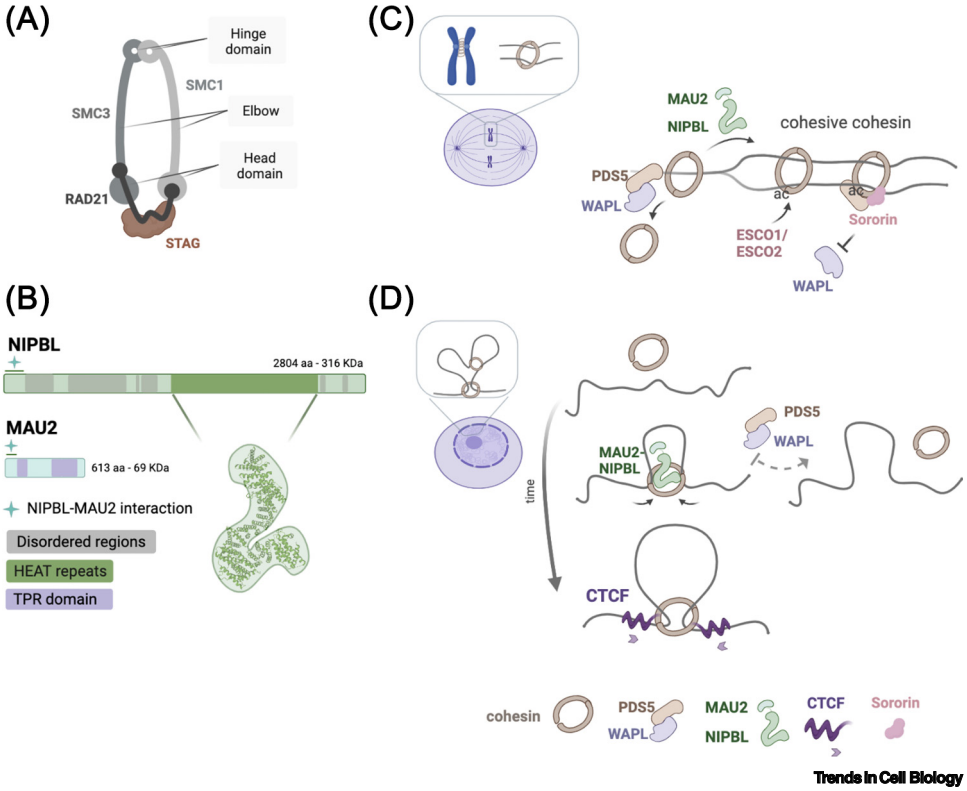

Figure 1. Cohesin functions require NIPBL. (A) Cohesin composition. The Structural Maintenance of Chromosomes (SMC) proteins fold at their hinge domains and are stabilized by intramolecular, antiparallel coiled-coil (CC) interactions. A discontinuity around the middle of the CC allows further folding (elbow). A V-shaped heterodimer is formed through hinge–hinge interactions. At the other end of each protein, globular head domains (hd) associate and form a composite ABC-like ATPase. The N- and C-terminal domains of RAD21 bind to SMC3 and SMC1, respectively, and are linked by a long and unstructured polypeptide. The Stromal Antigen (STAG) subunit binds to the middle of RAD21. (B) Schematic of NIPBL and its binding partner MAU2, indicating major structural features. A model of the C-terminal half of NIPBL based on structural information [Protein Data Bank (PDB) ID: 6WG3.E] is shown on the bottom right. (C) Sister chromatid cohesion mediated by cohesin. A dynamic cohesin complex bound to unreplicated chromatin dissociates by the action of WAPL bound to PDS5. Helped by NIPBL, cohesin embraces the two sister chromatids arising from the replication fork in S phase. To prevent WAPL-mediated release, these cohesive complexes are acetylated on SMC3 by acetyltransferases ESCO1 and ESCO2 and bound by Sororin through PDS5. (D) Role of cohesin in genome folding. The loop extrusion model proposes that cohesin associates with chromatin and extrudes DNA with the help of NIPBL until it is released by PDS5-WAPL, or it becomes stalled by **CCCTC-binding factor (CTCF)** or blocked by other obstacles (not depicted). Stable loops are observed between CTCF sites in **convergent orientation** (purple arrowheads). Figure created with BioRender.com.

cohesin loader, at least before S phase. It could primarily act as a processivity factor that activates cohesin ATPase to fuel loop extrusion and counteract PDS5-WAPL-mediated release [19].

The importance of ATP hydrolysis for cohesin binding to DNA

Early experiments suggested that ATP hydrolysis by SMC heads is required for loading and translocation of cohesin [20]. Topological entrapment of plasmid DNA, which results in salt-resistant cohesin binding, depends on NIPBL and ATP [9], but can be observed in the presence of nonhydrolyzable ATP analogs [21]. Using total internal reflection fluorescence (TIRF) microscopy on DNA molecules tethered to coverslips (DNA curtains), several groups have reported that salt-resistant DNA binding and translocation of cohesin require NIPBL and ATP [11,22,23]. However, cohesin ATPase mutants are able to associate with chromatin both in yeast and human cells, although they cannot move away from their loading sites [24–26]. These findings suggest that ATP

Glossary

BRD4: member of the Bromodomain and Extraterminal (BET) family of chromatin regulators that binds acetylated histones to regulate gene transcription.
CCCTC-binding factor (CTCF): a zinc finger protein with a key role in 3D genome organization together with cohesin.
Chromatin remodeler: protein complexes that modify nucleosomes transiently to increase accessibility of DNA to binding factors.
Convergent orientation (of CTCF): binding motif of CTCF is not palindromic and has an orientation. CTCF-binding sites at the bases of a chromatin loop contain motifs in convergent orientation, that is, facing each other.
Dbf4-dependent kinase (DDK): protein kinase that phosphorylates and activates the MCM helicase to allow DNA unwinding. It comprises a catalytic subunit, CDC7, and a regulatory subunit, DBF4.
HEAT repeat: structural motif comprising two alpha helices linked by a short loop that is found in several proteins, including Huntingtin, Elongation factor 3(EF3), protein phosphatase 2A, and TOR.
Kinetochore: protein assembly built on centromeric DNA that serves as attachment point for spindle microtubules to achieve chromosome segregation. Yeast centromeres are major loading sites for cohesin.
Mediator: multiprotein protein complex that regulates transcription and promotes communication between enhancers and promoters.
Minichromosome maintenance (MCM): complex comprising six subunits, MCM2–7; it is the DNA helicase that separates the two DNA strands at replication forks to allow DNA polymerase progression.
Pre-replication complex (pre-RC): protein assembly, including the MCM helicase, present at replication origins that are ready to be fired to start DNA replication.
Structural Maintenance of Chromosomes (SMC) proteins: in eukaryotes, there are three SMC complexes: cohesin, condensin and the SMC5/6 complex. They comprise an SMC heterodimer, a kleisin subunit, and additional proteins.
***Xenopus* oocyte extracts:** lysates of *Xenopus laevis* oocytes, which store

large quantities of pre-assembled complexes, such as cohesin and condensin. They constitute a powerful cell-free system to recapitulate cell cycle processes, including DNA replication and chromosome segregation.

Box 1. The two alternative configurations of cohesin

In addition to the four core subunits depicted in Figure 1A in the main text, cohesin complexes associate to a second HEAT-repeat protein, NIPBL or PDS5. Both bind to the same region in RAD21, upstream of the region bound by STAG, the other HEAT subunit. This arrangement of SMC dimer, kleisin, and two HEAT proteins is conserved in condensin, another SMC complex that drives mitotic chromosome condensation [94]. Only NIPBL can activate the cohesin ATPase [15]. NIPBL-bound cohesin translocates along DNA forming loops and PDS5-bound cohesin is arrested at CTCF loop anchors [16,95,96] (Figure IA). The binding mode of these complexes to DNA is probably different, because only the latter is sensitive to RAD21 cleavage [89]. The exchange between the two cohesin configurations is regulated by SMC3 acetylation, a modification that strengthens the interaction of cohesin and PDS5 [97,98]. SMC3 acetylation by ESCO1 restricts loop extension and is counteracted by the histone deacetylase HDAC8, which promotes loop extension. After DNA replication, a fraction of cohesin stably bound to DNA holds together the sister chromatids. This cohesive cohesin is bound by PDS5 and Sororin, acetylated, and sensitive to RAD21 cleavage (Figure IB)

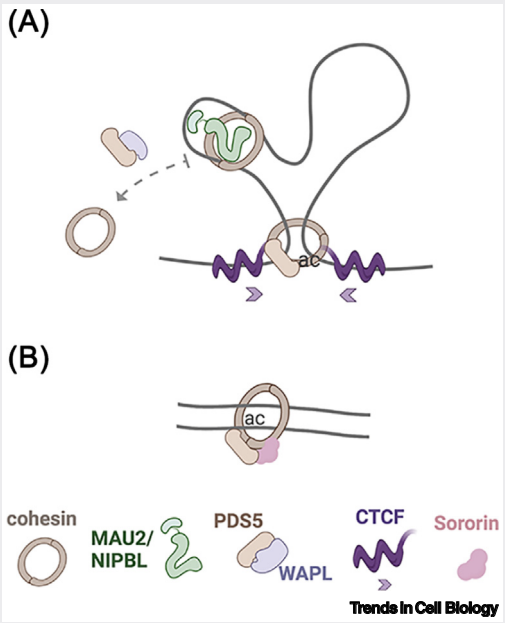

Figure I. Different functions of NIPBL-bound and PDS5-bound cohesin. (A) 3D genome organization. (B) Cohesion. Figure created wih BioRender.com. Abbreviation: CTCF, CCCTC-binding factor.

hydrolysis is not essential for the association of cohesin with DNA. If so, what function of NIPBL other than activation of the cohesin ATPase is important for cohesin loading?

NIPBL as a driver of the interaction of cohesin with DNA

Biochemical and structural evidence indicates that NIPBL promotes the interaction of cohesin with DNA as well as folding of the SMC arms at their elbows [27] (Figure 2A). Two cryo-electron microscopy studies that examined the cohesin loading reaction in the absence of ATP hydrolysis revealed a prominent role of both NIPBL and the STAG subunit in DNA binding [28,29]. The DNA is clamped by NIPBL on top of the SMC heads. The STAG subunit, docked behind NIPBL, interacts with DNA and with the SMC hinge upon folding of the SMC heterodimer (Figure 2B). Building from this 'gripping state', the 'Brownian ratchet' model proposes that ATP hydrolysis leads to head disengagement, with the STAG-hinge DNA-binding module swinging away from the NIPBL-head module and extruding DNA [30] (Figure 2C). A role for the STAG subunit is not described in the alternative 'swing and clamp' model, which instead relies on the mutually exclusive interaction of NIPBL with either the hinge or the SMC3 head [31]. Here, NIPBL binds DNA at the cohesin hinge and a spontaneous swing of the hinge places DNA on top to

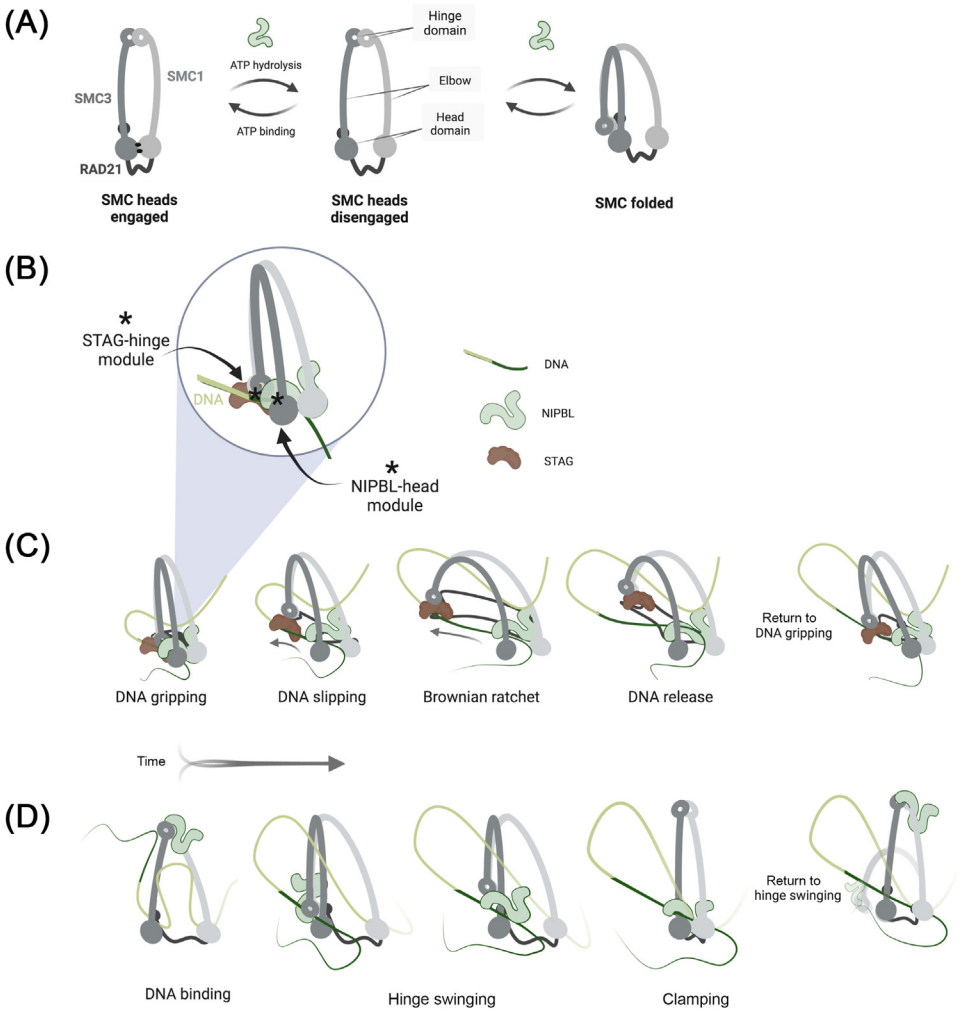

Figure 2. Models of DNA association and loop extrusion by cohesin. (A) Schematic of cohesin conformations. The Stromal Antigen (STAG) subunit is not included for simplicity. (B) Two DNA-binding modules in cohesin. The complex is folded at the Structural Maintenance of Chromosomes (SMC) elbows, and asterisks indicate DNA-binding sites. MAU2 is not depicted. (C) In the 'Brownian ratchet' model, a transitory gripping state is formed upon DNA entry into a folded complex with engaged (ATP-bound) SMC heads and the two DNA-binding modules are next to each other. ATP hydrolysis results in head disengagement and NIPBL binding to DNA is relaxed. The STAG-hinge DNA-binding module swings away from the NIPBL-head module, pulling DNA. Subsequent DNA release allows the STAG-hinge module to return to the gripping state to start a new cycle of DNA extrusion. (D) In the 'swing and clamp' model, NIPBL bound at the cohesin hinge captures DNA. Then, a spontaneous ATP-independent swing, driven by alignment and folding of the SMC arms, places DNA over the SMC3 head. Upon ATP binding, the SMC arms unfold, NIPBL dissociates from the hinge, and instead clamps DNA on top of the SMC heads. Next, ATP hydrolysis disassembles the clamp. NIPBL can interact again with the hinge and search for another DNA segment, while unclamped DNA is pushed beyond the SMC heads leading to loop enlargement. Figure created with BioRender.com.

the SMC3 head (Figure 2D). In both models, NIPBL is essential for DNA binding and loop enlargement. However, the interaction of cohesin with DNA and even DNA entrapment can be observed *in vitro* in the absence of NIPBL when DNA enters through the SMC3-RAD21 interface [32,33]. Conversely, clamping of DNA between NIPBL and the engaged SMC heads can be observed in the absence of the STAG subunit [34]. Whether the two DNA-binding modules described above (NIPBL-head and STAG-hinge) are required for association of cohesin to chromatin *in vivo* remains to be elucidated.

NIPBL recruitment to chromatin

The configuration of soluble cohesin (i.e., before loading onto DNA) is unknown. At least in ***Xenopus* oocyte extracts**, only a small fraction of cohesin can be pulled down with PDS5 or NIPBL antibodies, suggesting that most soluble complexes contain just the four core cohesin subunits [35,36]. One possibility is that NIPBL-bound cohesin scans DNA until finding a site appropriate for extrusion. Another is that NIPBL binds preferentially to certain genomic loci and when cohesin (alone or bound to PDS5) encounters them, entrapment ensues to provide a more stable binding. Consistent with the second hypothesis, experiments in yeast suggest that **chromatin remodelers**, such as Remodels the Structure of Chromatin (RSC), act as chromatin receptors for $Scc2^{NIPBL}$ and $Scc4^{MAU}$ at gene promoters (Figure 3, context 1). The presence of nucleosomes hinders cohesin loading *in vitro* [37]. Therefore, RSC may facilitate DNA entrapment by generating nucleosome-free regions. Other chromatin factors enable recruitment of cohesin-NIPBL to specific genomic loci. One is **Mediator**, which brings yeast $Scc2^{NIPBL}$ to chromatin, particularly at ribosomal small nucleolar RNA (snoRNA) and ribosomal protein genes [38]. Another is the **kinetochore** protein Ctf19, which interacts with $Scc4^{MAU2}$ to promote cohesin loading at budding yeast centromeres, a process that is facilitated by Ctf19 phosphorylation by **Dbf4-dependent kinase** (DDK) [39]. $Scc4^{MAU2}$ may also mediate the interaction with RSC and other chromatin remodelers [40]. While $Scc4^{MAU2}$ is essential in budding yeast, human haploid HAP1 cells can survive without it. These cells present reduced cohesin on chromatin, but co-depletion of WAPL restores the levels of cohesin at most genomic loci [13]. Thus, whether MAU2 has a role in mammalian cells beyond protecting NIPBL from degradation remains unclear.

NIPBL and the replication machinery

The heterodimer $Scc2^{NIPBL}$- $Scc4^{MAU2}$ is stored in *Xenopus* oocytes in a complex with DDK. As a result, cohesin loading is linked to replication origin activation in the transcriptionally inactive nuclei

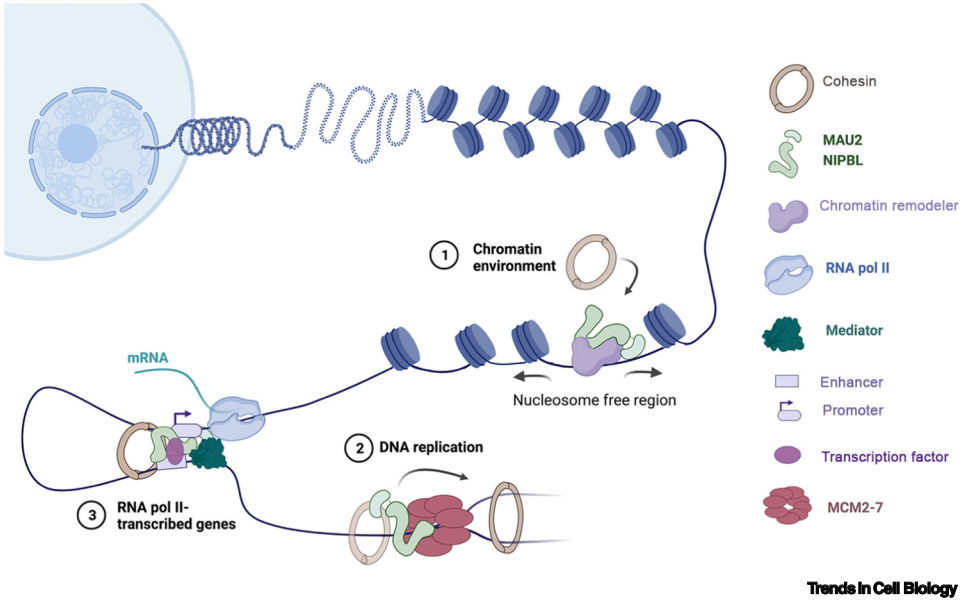

Figure 3. Models of NIPBL recruitment to chromatin in different chromatin contexts. In context 1, NIPBL-MAU2 interact with chromatin remodelers, which in turn generate nucleosome-free regions that facilitate the association of cohesin with DNA. In context 2, NIPBL-MAU2 bound to the minichromosome maintenance (MCM) helicase loads cohesin in the wake of the replication fork to allow cohesion establishment. Alternatively, NIPBL-bound cohesin arrives by loop extrusion at pre-replication complexes (pre-RCs). In context 3, elements of the transcription machinery, such as RNA polymerase (RNA Pol) II, Mediator, or transcription factors, interact with cohesin and/or NIPBL and assist loading and/or provide anchors for loop extrusion. Figure created with BioRender.com.

assembled in oocyte extracts [36]. In budding yeast, cohesin is loaded at the G1/S transition and cohesion establishment during DNA replication can happen by two distinct mechanisms. One, termed 'conversion', is independent of $Scc2^{NIPBL}$ and turns cohesin bound to unreplicated DNA ahead of the replication fork into cohesive cohesin behind the fork. The second one is *de novo* loading mediated by $Scc2^{NIPBL}$ [41]. In human cells, two 'waves' of cohesin loading have been described: one takes place after exit from mitosis and the other in S phase. Both depend on NIPBL, but the latter requires also the replication machinery [42] (Figure 3, context 2). NIPBL-bound cohesin interacts with the **minichromosome maintenance** (MCM) replicative helicase and DDK at **pre-RCs.** Whether pre-RCs actively recruit cohesin bound to NIPBL in human cells, as described in both *Xenopus* [36] and *Drosophila* [43], remains to be elucidated. An alternative proposal comes from the observation that MCM complexes are barriers for loop extrusion [44]. Cohesin transiently arrested at these barriers at the time of replication fork passage could be mobilized and deposited behind the fork to establish cohesion.

NIPBL and the transcriptional machinery

Chromatin immunoprecipitation (ChIP) experiments in mammalian cells place NIPBL at active enhancers and promoters, many of them GC-rich [45–48]. These could be cohesin-loading sites, in which high chromatin accessibility facilitates binding of the complex to DNA (Figure 3, context 3). Consistent with this possibility, acute depletion of RNA polymerase II (RNAPII) at mitotic exit reduces the chromatin-bound levels of NIPBL and cohesin occupancy at **CCCTC-binding factor (CTCF)** sites [49]. Moreover, impaired genome folding observed in these cells can be simulated *in silico* with the assumption that most cohesin is loaded at RNAPII-occupied promoters. Using also computer simulations and a much shorter list of NIPBL positions obtained with degron and HA-tagged versions of NIPBL and MAU2, another report concludes that there is no preferential loading of cohesin at promoters [50]. Detection of NIPBL and cohesin at transcription start sites (TSS) is proposed to be the result of RNAPII acting as a barrier for extrusion.

Additional studies report recruitment of cohesin and NIPBL at enhancers and promoters following transcriptional induction, for example, upon calcium-mediated activation of neutrophils [47] or steroid hormone activation of glucocorticoid receptor (GR) [51]. The GR translocates to the nucleus and acts as a pioneer transcription factor. Chromatin contact maps suggest that enhancer-anchored loop extrusion facilitates the interaction between GR-bound enhancers and distal GR-responsive genes [51]. A similar mechanism could explain the relevance of the physical and functional interaction between NIPBL and the chromatin regulator **BRD4** for neural crest differentiation [52]. Cells acutely depleted of BRD4 or carrying BRD4 point mutants unable to interact with NIPBL show impaired genome folding. WAPL knockdown (KD) rescues differentiation defects caused by BRD4 loss, which supports a direct link between BRD4 and cohesin dynamics. The interaction between BRD4 and NIPBL has also been observed in human cells, and the two factors bind in an interdependent manner and regulate a subset of gene promoters [53]. Moreover, mutations in *BRD4* have been identified in patients with cohesinopathy, as discussed below [54]. Altogether, these studies reveal a clear correlation between NIPBL-cohesin binding and active transcription. NIPBL-bound enhancers and promoters may favor cohesin loading, or be sites in which cohesin binds transcriptional regulators to perform anchored loop extrusion, as suggested for the GR [51]. Alternatively, they could reflect pausing of loop extruding cohesin upon encountering the transcriptional machinery [50].

NIPBL requirement of cohesin-STAG1 and cohesin-STAG2

There are two distinct cohesin complexes in somatic vertebrate cells that carry one of two versions of the STAG subunit, STAG1 or STAG2 [55]. Cohesin-STAG1 is found at CTCF

sites, forms long loops and is more relevant for demarcation of contact domains. Cohesin-STAG2 is detected at CTCF and non-CTCF sites, the latter showing lower cohesin occupancy, and mediates local contacts that regulate tissue-specific transcription [56–59]. Loop extrusion is a highly dynamic process and even CTCF-anchored loops are relatively short-lived [60]. Nevertheless, cohesin-STAG1 displays a longer residence time on chromatin, and is more often acetylated by ESCO1 and bound to CTCF compared with cohesin-STAG2, which associates more frequently with WAPL [16,56,59]. The two complexes also respond in opposite ways to NIPBL KD [61]. Cohesin-STAG2 levels on chromatin decrease as expected, whereas cohesin-STAG1 levels increase. Total cohesin levels are reduced, consistent with previous studies in mammalian cells, and the ratio of the two variant complexes is significantly different in the KD condition [8,13,42,62]. This result supports the possibility that NIPBL is not required for chromatin association of cohesin.

Different loop extrusion strategies for cohesin-STAG1 and cohesin-STAG2?

The STAG subunit does not present the 'safety belt' ability identified in its HEAT protein counterpart in condensin, which provides loop anchoring to ensure unidirectional loop enlargement [63,64]. However, cohesin may perform anchored loop extrusion after interaction with other chromatin-bound proteins, such as CTCF [65]. Loop extrusion is impaired in NIPBL KD cells but STAG1 is still located at CTCF sites, while cohesin-STAG2 decreases genome-wide [61]. Therefore, it is likely that cohesin-STAG1 is loaded preferentially at CTCF-bound sites and performs one-sided loop extrusion (Figure 4, left). Cohesin-STAG2 may associate with chromatin elsewhere and be more dependent on NIPBL to reach and get stabilized at CTCF sites (Figure 4, middle). Cohesin-STAG2 is the preferred variant at non-CTCF cohesin positions detected by ChIP, which correspond largely to enhancers and promoters and, at least in mouse embryo fibroblast and embryonic stem cells, colocalize with NIPBL [16,66]. These could represent loading sites, pausing sites, or sites in which cohesin-STAG2 anchors to BRD4, Mediator, or a transcription factor to perform loop extrusion and facilitate enhancer–promoter interaction, as suggested previously (Figure 4, right). The different behavior of cohesin-STAG1 and cohesin-STAG2 is consistent with the proposal that cohesin-STAG1, which is loaded before cohesin-STAG2 upon exit from mitosis [67], first establishes large loop domains together with CTCF. Then, loading of cohesin-STAG2 promotes intradomain contacts that affect tissue-specific gene expression [68].

NIPBL mutations cause Cornelia de Lange Syndrome

CdLS is the most common cohesinopathy, with an estimated prevalence between 1 in 10 000 and 1 in 30 000 live births [69]. This multisystemic developmental disorder presents with intellectual disability, growth delay, limb malformations, and characteristic facial features. Mutations in genes encoding core cohesin subunits SMC1A, SMC3 or RAD21, or the cohesin deacetylase HDAC8 are found in a small fraction of patients. By contrast, up to 70% of CdLS cases encompass heterozygous mutations in *NIPBL*. Loss-of-function variants cause more severe clinical features compared with missense variants. A deletion of seven amino acids in the N-terminal region of MAU2, which abrogates the interaction with NIPBL, has also been also described [70]. Additional genes found mutated in patients with CdLS lacking those mentioned above are *BRD4*, *ANKRD11*, and *AFF4*, all encoding chromatin regulators [54,71]. ChIP analyses in fibroblasts from patients with CdLS have revealed reduced NIPBL occupancy and redistribution of cohesin. The complex is moderately decreased at CTCF positions and increased at NIPBL sites, a likely consequence of impaired loop extrusion [48]. At least some *NIPBL* mutations identified in CdLS affect its ability to promote loop extrusion *in vitro* [72]. Thus, even when the mutation does not reduce 'total' NIPBL

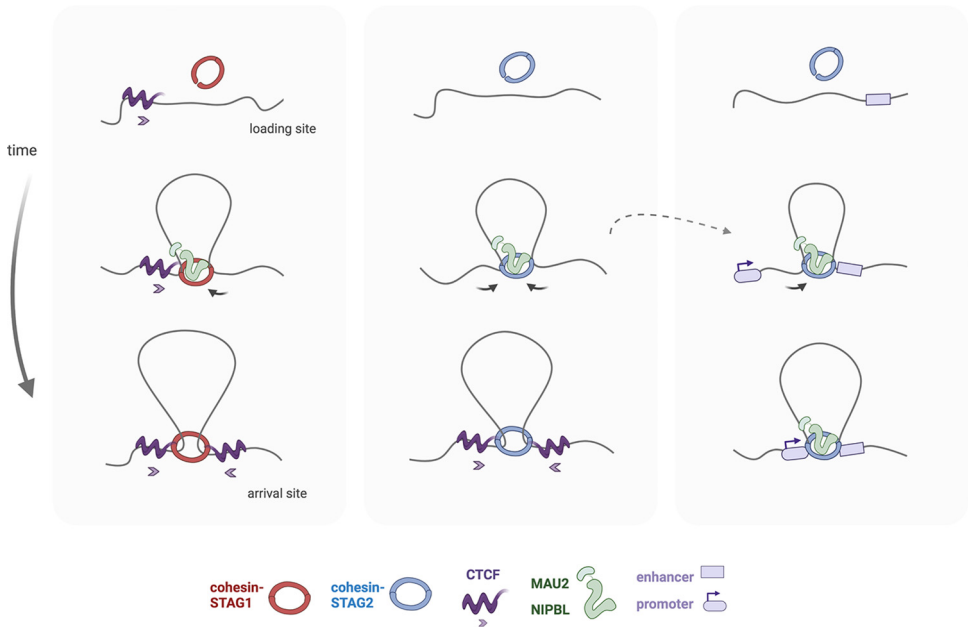

Figure 4. Models of different loop extrusion strategies of cohesin-STAG1 and cohesin-STAG2. Initial association with chromatin of cohesin-Stromal Antigen (STAG)1 and cohesin-STAG2 at different genomic locations affects the mode in which they perform loop extrusion. Left: Cohesin-STAG1, preferentially anchored at CCCTC-binding factor (CTCF) sites, could extrude DNA asymmetrically until reaching a second CTCF site in convergent orientation. Middle: Cohesin-STAG2 associates with chromatin away from CTCF sites and extrudes DNA bidirectionally. Eventually, it reaches one CTCF site and proceeds extruding DNA asymmetrically (not depicted) until reaching the second CTCF site in opposite orientation. Right: Cohesin-STAG2 binds a transcription factor (TF)-bound enhancer and extrudes DNA asymmetrically to facilitate the interaction of the enhancer with its cognate promoter. Alternatively, cohesin-STAG2 performing bidirectional loop extrusion (as in 'middle' panel, dashed arrow) encounters a TF-bound enhancer and switches to asymmetric loop extrusion until it finds the promoter. Figure created with BioRender.com.

levels in the cell, the amount of 'functional' NIPBL may not be sufficient to sustain full loop extrusion by cohesin [48]. We speculate that another set of mutations may decrease the affinity of NIPBL for important interactors beyond cohesin, such as BRD4. Moreover, some mutant NIPBL proteins may behave as dominant negative mutants. Finally, there may be specific moments in the cell cycle and/or in development when even a small drop in functional NIPBL levels is detrimental [68].

Gene deregulation in CdLS and strategies to correct it

Deregulated gene expression, but not cohesion defects, have been observed in cells from patients with CdLS and mouse models of *NIPBL* haploinsufficiency [48,73–77]. To separate the two cohesin functions, Weiss *et al*. analyzed the consequences of cohesin removal by RAD21 cleavage in postmitotic cortical mouse neurons and found disturbed 3D genome organization and gene expression [76]. They observed a significant overlap between these cohesin-dependent genes and genes deregulated in cortical neurons of patients with CdLS and further showed that restoration of cohesin functionality largely rescued the transcription defects. These results indicate that cohesin is required for maintenance of neuronal gene expression programs and open the door to postnatal interventions in patients with CdLS. In this regard, WAPL down-regulation should be considered, at least in NIPBL-deficient cells. As mentioned previously, WAPL depletion rescued impaired smooth muscle differentiation caused by BRD4 loss [52]. A

decrease in *Wapl* gene dosage partially restored transcriptional dysregulation in embryonic brains as well as embryonic growth, although it did not alleviate postnatal lethality of *Nipbl* heterozygous mice [78]. Results in human cells also show that the balance of NIPBL and WAPL levels is important for proper genome folding and gene regulation [13,79]. One intriguing question arising from these reports is the relevance of the dynamic behavior of cohesin in the control of transcription and, in particular, promoter–enhancer interactions [80]. Another is whether cohesin-STAG1 and cohesin-STAG2 could be differentially affected in CdLS, given their different chromatin association dynamics. We hypothesize that partial NIPBL dysfunction affects preferentially the more dynamic cohesin-STAG2 (Figure 5).

Additional pathological mechanisms in CdLS

While the consequences of *NIPBL* mutation in cohesin distribution, genome folding, and, ultimately, gene expression likely account for many adverse defects in development and physiology, some studies point to additional pathological mechanisms that include DNA damage and senescence. Cells from patients harboring *NIPBL* mutations and mouse embryonic stem cells carrying a *Brd4* mutation identified in CdLS present aberrant DNA damage response (DDR) signaling [81]. Additionally, placentas from *Nipbl*-haploinsufficient mice show persistent DNA damage and increased senescence that ultimately impair embryo development and viability [82]. Loop extrusion by cohesin at double-strand breaks has been proposed as the mechanism to establish a repair-prone environment in the form of DDR foci [83]. This could explain some of the defects in DNA repair observed in CdLS cells.

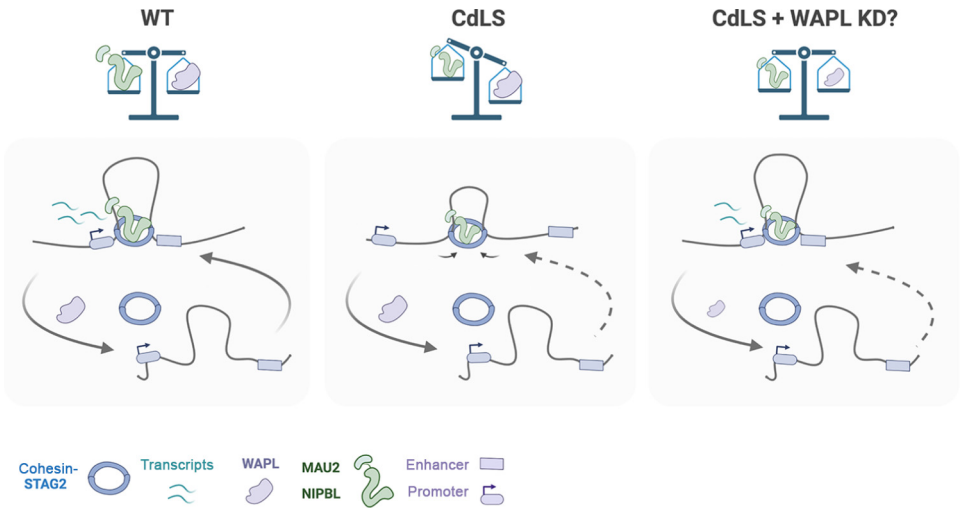

Figure 5. Model of the consequences of *NIPBL* mutation in Cornelia de Lange Syndrome (CdLS) and its reversal by WAPL knockdown (KD). Cohesin-Stromal Antigen (STAG)2 is the more dynamic cohesin and is preferentially found at non-CCCTC-binding factor (CTCF) sites, such as enhancer and promoters. According to the model, the proper balance between loop-extruding cohesin-STAG2, bound by NIPBL, and WAPL-mediated release of this complex is important for transcriptional regulation (wild-type; WT; left). In CdLS, NIPBL function in extrusion is impaired (dashed arrow), while WAPL-mediated release is sustained. As a result, loop enlargement sufficient to reach and facilitate promoter–enhancer interaction will be less probable, decreasing gene expression (middle). Reducing WAPL levels (WAPL KD) in CdLS would reduce release and restore the probability of enhancer–promoter contacts despite lower NIPBL function/levels (right). Figure created with BioRender.com.

It is unclear whether NIPBL has a cohesin-independent role that can contribute to CdLS pathology. Such a role was first suggested when NIPBL KD was found to affect the expression of genes that showed NIPBL, but not cohesin, localized at their TSS [45]. A similar reasoning is behind the proposal that NIPBL regulates expression of neural migration genes together with neural transcription factor Zfp609 and independently of cohesin [84]. However, all subunits of cohesin-STAG2 were identified as interaction partners of Zfp609 in addition to NIPBL [84]. Comparison of the transcriptomes of NIPBL-deficient and cohesin-deficient cortical neural progenitors would help understand whether NIPBL is indeed acting independently of cohesin in these cells [85].

Concluding remarks

The heterodimer of NIPBL^{Scc2} and its partner MAU2^{Scc4} is currently considered the cohesin loader. Structural biology studies and biochemical data, briefly described here, argue that NIPBL is essential for the interaction of cohesin with DNA to establish a 'gripping' or 'clamped' state. Whether that state is mandatory for initial chromatin association of cohesin or it only represents a step in the mechanisms that drive topological entrapment or loop extrusion remains to be elucidated. The contribution of the STAG^{Scc3} subunit as well as the potential differences between STAG1 and STAG2 also need clarification (see Outstanding questions). Separation-of-function mutants for NIPBL and cohesin subunits affecting the three processes differently can shed light on these issues [86].

The mode in which cohesin interacts with chromatin for loop extrusion is different from that required for cohesion, since topological entrapment is only required for the latter [86–89]. Therefore, current *in vitro* assays of DNA entrapment may not recapitulate the full range of possibilities that take place *in vivo* at different times in the cell cycle, upon interaction of cohesin with diverse chromatin-bound factors (CTCF, transcription factors, or replication proteins) or even in the presence of alternative cohesin subunits (STAG1 or STAG2) or regulators (NIPBL or PDS5). More complex reaction mixtures, such as the one devised to reconstitute DNA replication-coupled cohesin acetylation [90], and the use of chromatinized templates will be required to further understand the different modes of cohesin binding to DNA.

Where in the genome cohesin associates with chromatin to start loop extrusion, where and how extrusion pauses for short or long periods of time, the molecular mechanisms governing these arrests, and the different behavior of cohesin-STAG1 and cohesin-STAG2 in all these transactions that involve NIPBL are questions for future research. Identification of critical interaction surfaces between cohesin, NIPBL, and their interactors, followed by functional characterization of the corresponding mutants, both *in vitro* and *in vivo*, will be instrumental to answer these questions [91,92].

Finally, novel cellular models of CdLS are needed to further understand the molecular basis of this disease. Human induced pluripotent stem cells generated from patient fibroblasts or engineered to carry mutations identified in patients offer the possibility to address the consequences of CdLS mutations for differentiation to different cell lineages [93]. The effect of cohesin not only in gene regulation, but also in processes such as DNA repair, is most likely dependent on the particular cellular context.

Outstanding questions

What are the roles of NIPBL and STAG in chromatin association of cohesin?

Is cohesin loaded at specific genomic sites and, if so, what are their features?

What is the NIPBL interactome at the exit from mitosis and at the replication fork? How do these interactors affect the chromatin association of cohesin?

What are the effects of CdLS mutations in different cell types? How do these effects relate to CdLS pathology?

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Declaration of interests

The authors declare no competing interests.

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