

Cohesin and chromatin organization

Vlad C. Seitan and Matthias Merkschlager

Lymphocyte Development Group, MRC Clinical Sciences Centre, Imperial College London,
Du Cane Road, London W12 0NN, UK

Address correspondence to vlad.seitan@csc.mrc.ac.uk or
matthias.merkschlager@csc.mrc.ac.uk

Abstract.

Cohesin defines the topology of chromosomes in mitosis and meiosis by holding sister chromatids together, more recently a role for cohesin in chromatin organization and gene expression in interphase has emerged

Overview.

Cohesin belongs to an ancient family of protein complexes that are dedicated to chromosome biology. Aptly named 'SMC' proteins for 'structural maintenance of chromosomes' cohesin, condensin and Smc5/6 complexes maintain the integrity of genetic information by enabling post-replicative DNA repair, shaping chromosomes in preparation for cell division, and holding sister chromatids together to ensure that daughter cells receive a full complement of chromosomes. Cohesin is essential for DNA repair and chromosome segregation [1,2]. Heterozygous cohesin mutations are the cause of multi-systems developmental disorder such as Cornelia de Lange syndrome [3–5], and cohesin mutations have also been reported in cancer [6–8]. However, it has been difficult to disentangle cell cycle-related cohesin functions in DNA repair and chromosome segregation from a potential role in interphase. Here we discuss recent progress towards understanding the role of cohesin in chromatin organisation and gene regulation. In particular, cohesin is recruited to specific sites on mammalian chromosome arms by mechanisms that include binding to the insulator protein CTCF [9–11], tissue-specific transcription factors [12] or the mediator complex [13], which bridges tissue-specific transcription factors with RNA polymerase. Once positioned, cohesin facilitates long-range chromosomal interactions between its binding sites [13–18]. Finally, cohesin is required for correct gene expression in non-dividing cells in *Drosophila* [19–21] and in mammalian cells, as illustrated by the impact of cohesin deletion on T lymphocyte differentiation [16].

The cohesin complex.

Cohesin is a multi-subunit complex formed of a heterodimer of SMC proteins, SMC1 and SMC3,

and two non-SMC proteins Rad21 (also known as Scc1) and STAG (also known as Scc3). The SMC proteins each consist of a large coiled-coil domain flanked by a hinge domain that mediates SMC dimer formation and ATPase 'head' that contacts Rad21 to form a topologically closed tripartite structure - commonly referred to as the cohesin ring. STAG interacts with Rad21 and is essential for the functional integrity of the complex (Figure 1). In mammalian cells cohesin is loaded onto chromosomes at the end of mitosis by a separate complex consisting of Nipbl (also known as Nipped-B or Scc2) and Mau-2 (also known as Scc4) [22–26]. Although there are dissenting views, cohesin is thought to associate with chromosomes by 'topological embrace' [1] or, more prosaically, by trapping chromosomes inside its ring-like structure. The stability of cohesin binding is regulated by additional proteins in a cell cycle-dependent manner. In the G1 phase of the cell cycle, cohesin is in a dynamic equilibrium of association and dissociation with a half-life of approximately 25 minutes [27]. This turnover requires the cohesin-interacting proteins Wapl and Pds5, which unload cohesin from chromatin. During S-phase this equilibrium shifts towards chromatin-association, and the half-life of cohesin binding increases considerably [27]. Stable cohesin binding requires the acetylation of Smc3 by the acetyltransferases Esco1 and -2 (also known as Eco1 and -2) and helps to establish cohesion between sister chromatids as they are formed in S-phase [28–31]. Mechanistically, Smc3 acetylation allows the Sororin protein to counteract the chromatin-dissociation activity of Wapl and Pds5 throughout S-phase and G2 [32]. Once established, sister chromatid cohesion assists the repair of any DNA double strand breaks that occur during DNA replication: additional cohesin is loaded to the break sites and genome-wide [33–35]. Just like the establishment of S-phase cohesion, cohesin association with DNA double strand breaks requires Esco, but current evidence suggests that distinct modifications mark cohesin complexes that mediate sister chromatid cohesion in S-phase and cohesin complexes that facilitate post-replicative DNA repair [36,37]. At the beginning of mitosis, vertebrate Sororin is phosphorylated, which allows Wapl and Pds5 to evict the majority of cohesin from chromosome arms. This coincides with the increased binding of another SMC complex, condensin, which promotes the packaging of chromatin into the highly condensed mitotic chromatids. A limited amount of cohesin persists at the centromeres under the protection of Shugoshin-PP2A [38–41]. These residual cohesin complexes are critical for holding sister chromatids together at their centromeres, giving mitotic chromosomes their iconic shape. At anaphase the ubiquitin-ligase anaphase-promoting complex (APC) induces the degradation of Securin, an inhibitor of the protease Separase, which in turn cleaves the Rad21 subunit of cohesin [42–44]. Sister chromatids are now free to follow the pull of the mitotic spindles and to segregate towards the newly forming daughter cells. This spells the end of mitosis, telophase, and the re-loading of cohesin onto chromosomes in preparation for a new cell cycle. While it has been clear for some time that cohesin orchestrates proper chromosome segregation and facilitates post-replicative DNA repair by constraining the topology of sister chromatids, recent work has shed light on the contribution of cohesin to chromatin organisation and gene regulation from the time of its loading in G1 until

cohesion establishment in S-phase.

Cohesin and transcription – friend or foe?

The relationship between cohesin and transcription is of interest because cohesin complexes occupy chromosome arms throughout the G1 phase of the cell cycle, when gene expression is in full swing. This raises the question how RNA polymerases get on with these large, ring-shaped objects. At first sight, the relationship between transcription and cohesin appears diametrically opposite in different organisms. However, as explained below, emerging data begin to explain these differences and suggest that the loading of cohesin onto chromatin generally occurs at sites of active transcription, while differences between species may be due to the redistribution of cohesin subsequent to loading.

In *Drosophila*, for example, cohesin is enriched at actively transcribed genes together with RNA polymerase II [45], the polymerase responsible for the transcription of most protein-coding genes and many non-coding RNAs. Cohesin is largely excluded from repressed regions of the *Drosophila* genome that are marked by Polycomb protein binding and trimethylation of lysine 27 of histone H3 (H3K27me3) [46]. In stark contrast to *Drosophila*, yeast cohesin is excluded from actively transcribed genes and instead accumulates between convergently transcribed genes [47,48]. In the fission yeast *S. Pombe*, cohesin even functions as a terminator of RNA polymerase progression, and is recruited to sites of convergent transcription by the RNA interference (RNAi) transcriptional gene-silencing pathway [49]. In mammalian cells, cohesin accumulates at the binding sites of CTCF, a Zn-finger DNA-binding protein and transcriptional regulator [9–11]. CTCF can function as an insulator (by blocking enhancer-promoter interactions) and as a boundary element (by marking the transition between active and repressive chromatin modifications), and at least some of these functions may be mediated by cohesin [11]. The relationship between cohesin and CTCF appears to be vertebrate-specific: *Drosophila* CTCF does not bind or co-localise with cohesin, and CTCF is not conserved in yeast. Vertebrate cohesin directly interacts with CTCF via its STAG subunit [10,50] interestingly the same subunit that mediates the interaction with the gene-silencing factor Swi6 in *S. pombe* [51].

Cohesin recruitment to non-CTCF sites has been reported at tissue-specific genes in human MCF-7 breast cancer cells and in liver cells [12]. A role for CTCF in recruiting cohesin to these sites is unlikely, since CTCF consensus motifs were absent. A contribution of tissue-specific transcription factors to the recruitment of cohesin to these sites has been suggested [12], but no mechanism has been defined.

Recent genome-wide mapping studies in mouse ES cells show that the mammalian cohesin-loading factor Nipbl co-localises genome-wide with mediator, a complex that bridges between cell type-specific transcription factors and RNA Polymerase II at active promoters and enhancers [13]. In yeast, the cohesin-loading factors Scc2 and Scc4 are enriched at sites of active transcription not only by RNA Pol II, but also RNA Pol I (which transcribes rDNA genes) and RNA Pol III (which

transcribes tRNA genes) [52]. So why do we find cohesin loading factors but not cohesin itself at sites of active transcription in yeast? A possible solution to this conundrum comes from studies with ATPase-deficient cohesin, which binds chromosomes only very transiently. Due to its instability, only trace amounts of ATPase-deficient cohesin are present on chromosomes. Interestingly, however, these short-lived cohesin complexes are found close to Scc2 at active genes [52], perhaps because they have no time to re-distribute before they dissociate from chromatin. Similarly, it is likely that mammalian cohesin is loaded at active promoters and enhancers and subsequently relocated to CTCF sites, consistent with the observation that knockdown of CTCF affects the distribution of cohesin, but not its association with chromatin or the ability to support sister chromatid cohesion [9,11].

Hence, it appears that a link between the cohesin loading machinery and the transcriptional machinery is well conserved, while the subsequent distribution of cohesin has significantly diverged during evolution. An important remaining question concerns the mechanisms that re-position cohesin from its loading sites to its final destination [53]. Transcription appears to drive cohesin repositioning in the budding yeast *S. cerevisiae*, since cohesin is only excluded from genes as long as they are transcribed. This led to the suggestion that the transcription machinery might clear cohesin complexes that stand in its way. However, the positive correlation between cohesin and RNA polymerase in *Drosophila* suggests that cohesin and RNA polymerase progression are fundamentally compatible.

Cohesin and DNA replication

As discussed in the previous paragraph, the relationship between RNA polymerases and cohesin plays out differently in various organisms, but transcription is of course not the only occasion where a processive enzyme has to deal with a chromatin substrate populated with SMC complexes. DNA polymerases also face cohesin complexes as they work to replicate the genome in S-phase. In contrast to RNA polymerases in species such as yeast (see above), DNA polymerases may not have the option of banishing cohesin complexes - after all the mission is to establish cohesion between the newly replicated sister chromatids.

In *Xenopus* egg extracts, cohesin is loaded at pre-replication complexes [54,55] and enrichment of mammalian cohesin at replication origins has also been reported [56]. Replication origins showed larger than usual spacing in cohesin-depleted mammalian cells [56]. The replication factor RFC-CTF18 promotes the acetylation of the SMC3 cohesin subunit and therefore the establishment of cohesion during S-phase. Interestingly, cohesin acetylation in turn facilitates the processivity of the replication fork [57].

A role for cohesin in gene expression and development: Disentangling cohesin functions in cell division from cohesin functions in interphase.

As discussed above, transcription shapes the landscape of chromatin-bound cohesin, but does

cohesin in return influence gene expression during its residence on chromosomes in G1? Previous evidence for such a role came from studies in model organisms [5,23,45,58] and cells from patients with cohesin mutations [3,4,59]. However, these systems were based on dividing cells and it therefore remained possible that defective development and gene expression could have been triggered by inefficient DNA repair, chromosome segregation defects or prolonged checkpoint activation. Premature sister chromatid separation occurs in Roberts syndrome and DNA double strand breaks are frequent in Nijmegen breakage syndrome, no clear evidence for overt chromosome segregation defects was found in cells from Cornelia de Lange Syndrome patients [60] and in some of the animal models [5,23,58,61]. While it is likely that different cellular functions require different amounts of cohesin (evidence for this idea has recently been provided in yeast [62]), it is difficult to test whether the amounts of cohesin available in Cornelia de Lange Syndrome and related animal models are sufficient for post-replicative DNA repair and chromosome segregation at all stages of development.

The difficulties in attributing cell division-independent functions to cohesin are illustrated by its proposed involvement in embryonic stem cell self-renewal and pluripotency (the ability to differentiate into multiple lineages). Several genome-wide RNAi screens have identified factors required for self-renewal, and cohesin and condensin have shown up time and time again [13,63–66]. Since self-renewal implies proliferation, these screens will identify a large fraction of genes required for DNA replication, repair and cell division. It is therefore important to experimentally separate mitotic and interphase functions of cohesin. This was initially achieved in *Drosophila* where inactivation of cohesin in post-mitotic cells caused abnormal gene expression together with developmental defects in axonal pruning of gamma neurons from the mushroom body due to reduced expression of ecdysone receptor [19,21]. However, cohesin is recruited to specific sites by different mechanisms in flies and mammals (see above), so it was critical to experimentally test whether cohesin regulates gene expression in mammalian cells. This was achieved recently by selectively inactivating cohesin in a sub-population of non-dividing thymocytes that are arrested in G1 as part of their normal developmental program. Defective transcription of the T cell receptor alpha chain locus led to inefficient T cell receptor rearrangement, which in turn impaired the differentiation of cohesin-deficient thymocytes [16]. This provides an example for a role of cohesin in the differentiation of mammalian cells and at the same time establishes the developing thymocytes as a system for studying the mammalian-specific mechanisms by which cohesin regulates gene expression in interphase. Similar studies are certain to follow in other developmental systems.

Cohesin shapes interphase chromatin by forming long-range interactions between distant chromosomal locations

High-resolution chromatin conformation studies have revealed that cohesin forms long-range interactions between its binding sites (**Figure 2**) [13–18,67–70]. Current models support the idea

that cohesin controls gene expression by contributing to the organisation of higher-order chromatin structure. Communication between gene regulatory elements is believed to be a major mechanism for transcriptional control [71] and it is conceivable that interactions between cohesin binding sites that involve gene regulatory elements such as enhancers, promoters or insulators, would favour or inhibit gene expression [12–18,67–69]. These studies only survey a tiny proportion of the genome (**Figure 2**), and attempts to visualise cohesin effects on global chromatin organisation have to date been at low resolution. For example, hypotonic lysis of cohesin-depleted cells show chromatin loops that appear to be larger than in control cells, consistent with a change in the spacing of replication origins discussed above [56]. When introduced into budding yeast, human cohesin molecules isolated from patients with cohesin mutations cause the dispersal of normally clustered tRNA genes and alter the nuclear position and the activity of specific genes [72]. Elegant experiments from the lab of Jan-Michael Peters show that the loss of Wapl from non-dividing cells reduces cohesin turnover, increases the association of cohesin with chromatin, and dramatically alters the organisation of interphase chromatin (J-M Peters, personal communication). Technologies are now available to combine a global approach with high resolution, as recently demonstrated for an interaction network based on the cohesin-interacting factor CTCF [73].

Outlook

Even though cohesin may facilitate long-range interactions, vexing questions remain about how these interactions are regulated, and how they contribute to regulating gene expression. First, as discussed above, the two canonical functions of cohesin, the establishment of sister chromatid cohesion and DSB-induced DNA repair involve different modifications of cohesin complexes [36,37]. This raises the question whether cohesin complexes engaged in the regulation of gene expression have specific modifications or a specific composition. Second, the idea that cohesin facilitates long-range interactions in G1 phase of the cell cycle, including in non-dividing cells, is attractive due to cohesin's proven ability to constrain the topology of chromosomes during DNA repair and in preparation for cell division. But how can it be reconciled with the dynamic nature of cohesin's association with the chromatin at this phase of the cell cycle [27]? As discussed above, sister chromatid cohesion and DNA repair functions involve specific mechanisms that stabilise the binding of cohesin complexes to chromosomes. Does this imply that cohesin-mediated long-range interactions in G1 are short-lived, maintained by the simultaneous presence of multiple cohesin complexes, or does it suggest an involvement of additional factors or modifications? Interestingly, the extended residence time of cohesin in Wapl-deficient fibroblasts mentioned above distorts both chromatin organization and gene expression (J-M Peters, personal communication), indicating that the dynamic nature of cohesin association in interphase cells is essential. Finally, cohesin's association with mediator components of the basal transcriptional machinery and its loading on the chromatin at active promoters and enhancers might indicate that -in addition to long-range interactions - cohesin could have additional functions

in the regulation of gene expression.

Figure 1. Structure and regulation of the cohesin complex.

A. Cohesin structure and topology. Smc1 and Smc3 heterodimerise via their hinge domains and their ATPase heads associate with the kleisin subunit Rad21 to form a tripartite ring structure. The STAG subunit interacts with Rad21. Key sites in the complex are numbered (1-5), and their involvement in cohesin's functions during the cell cycle is summarised on the left. **B.** The cohesin cycle: dynamics and regulatory factors. The association of cohesin with chromatin is closely linked to cell cycle progression (stages indicated at the bottom) and is regulated by multiple factors (listed above and below the transition arrows). In G1, cohesin binds to chromatin in a dynamic equilibrium of association and dissociation. During S-phase sister chromatid cohesion is established, and cohesive complexes (represented in bold) become more stably bound to chromosomes. Cohesion is maintained genome-wide throughout G2 when it assists DNA double-strand break repair. During prophase, cohesin is unloaded from chromosome arms, the chromosomes condense, and cohesion is only maintained at the centromeres until it is cleaved at anaphase, which allows chromosome segregation to take place.

Figure 2. Cohesin contributes to the architecture of interphase chromatin by mediating long-range interactions.

High-resolution chromatin conformation studies have revealed that cohesin contributes to long-range interactions between genomic sites. Although these assays can track cohesin-mediated loops that span hundreds of kilobases, they capture only a minute fraction of the entire genome, which is measured in millions of kilobases. A genome-wide approach will be required to define cohesin's global contribution to chromatin architecture in interphase.

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References

1. Nasmyth K, Haering CH: **Cohesin: its roles and mechanisms**. *Annu. Rev. Genet.* 2009, **43**:525-558.
2. Covo S, Westmoreland JW, Gordenin DA, Resnick MA: **Cohesin Is Limiting for the Suppression of DNA Damage–Induced Recombination between Homologous Chromosomes**. *PLoS Genet* 2010, **6**:e1001006.
3. Tonkin ET, Wang T-J, Lisgo S, Bamshad MJ, Strachan T: **NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins and fly Nipped-B, is mutated in Cornelia de Lange syndrome**. *Nat. Genet.* 2004, **36**:636-641.

4. Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, et al.: **Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of Drosophila melanogaster Nipped-B.** *Nat. Genet.* 2004, **36**:631-635.
5. Kawauchi S, Calof AL, Santos R, Lopez-Burks ME, Young CM, Hoang MP, Chua A, Lao T, Lechner MS, Daniel JA, et al.: **Multiple Organ System Defects and Transcriptional Dysregulation in the Nipbl+/- Mouse, a Model of Cornelia de Lange Syndrome.** *PLoS Genet* 2009, **5**:e1000650.
- *6. Barber TD, McManus K, Yuen KKY, Reis M, Parmigiani G, Shen D, Barrett I, Nouhi Y, Spencer F, Markowitz S, et al.: **Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers.** *Proc. Natl. Acad. Sci. U.S.A.* 2008, **105**:3443-3448.
This study reports colorectal cancer-associated somatic mutations in several genes that regulate sister chromatid cohesion.
- **7. Solomon DA, Kim T, Diaz-Martinez LA, Fair J, Elkahoul AG, Harris BT, Toretsky JA, Rosenberg SA, Shukla N, Ladanyi M, et al.: **Mutational Inactivation of STAG2 Causes Aneuploidy in Human Cancer.** *Science* 2011, **333**:1039 -1043.
This study provides evidence for a causal link between STAG2 mutations and malignant transformation.
8. Xu H, Tomaszewski JM, McKay MJ: **Can corruption of chromosome cohesion create a conduit to cancer?** *Nat Rev Cancer* 2011, **11**:199-210.
- *9. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, Gregson HC, Jarmuz A, Canzonetta C, Webster Z, Nesterova T, et al.: **Cohesins functionally associate with CTCF on mammalian chromosome arms.** *Cell* 2008, **132**:422-433.
See reference 11
- *10. Rubio ED, Reiss DJ, Welch PL, Distèche CM, Filippova GN, Baliga NS, Aebbersold R, Ranish JA, Krumm A: **CTCF physically links cohesin to chromatin.** *Proceedings of the National Academy of Sciences* 2008, **105**:8309 -8314.
See reference 11
- *11. Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, Schirghuber E, Tsutsumi S, Nagae G, Ishihara K, Mishiro T, et al.: **Cohesin mediates transcriptional insulation by CCCTC-binding factor.** *Nature* 2008, **451**:796-801.
Together with reference 9, 10, and other papers that could not be cited here due to space constraints this study shows that mammalian cohesin is recruited by CTCF.
- *12. Schmidt D, Schwalie PC, Ross-Innes CS, Hurtado A, Brown GD, Carroll JS, Flicek P, Odom DT: **A CTCF-independent role for cohesin in tissue-specific transcription.** *Genome Research* 2010, **20**:578 -588.
This is the first identification of cell type-specific cohesin binding to sites that lack detectable CTCF protein and CTCF consensus sequences.
- **13. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, Ebmeier CC,

Goossens J, Rahl PB, Levine SS, et al.: **Mediator and cohesin connect gene expression and chromatin architecture**. *Nature* 2010, **467**:430–435.

This study maps cohesin loading factors to sites of active transcription in mouse ES cells.

*14. Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, Fraser P, Fisher AG, Merkenschlager M: **Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus**. *Nature* 2009, **460**:410-413.

See reference 15

*15. Nativio R, Wendt KS, Ito Y, Huddleston JE, Uribe-Lewis S, Woodfine K, Krueger C, Reik W, Peters J-M, Murrell A: **Cohesin Is Required for Higher-Order Chromatin Conformation at the Imprinted IGF2-H19 Locus**. *PLoS Genet* 2009, **5**:e1000739.

Together with reference 14 and other papers that could not be cited here due to space constraints, this study shows that cohesin complexes mediate long-range chromosomal interactions.

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

This study provides a clear dissociation between cell cycle-related and interphase functions in mammalian cells and shows how the differentiation of non-dividing mammalian cells can depend on cohesin-mediated gene regulation.

17. Majumder P, Boss JM: **Cohesin Regulates MHC Class II Genes through Interactions with MHC Class II Insulators [Internet]**. *The Journal of Immunology* 2011, doi:10.4049/jimmunol.1100688.

18. Kim YJ, Cecchini KR, Kim TH: **Conserved, developmentally regulated mechanism couples chromosomal looping and heterochromatin barrier activity at the homeobox gene A locus**. *Proceedings of the National Academy of Sciences* 2011, **108**:7391 -7396.

*19. Pauli A, Althoff F, Oliveira RA, Heidmann S, Schuldiner O, Lehner CF, Dickson BJ, Nasmyth K: **Cell-type-specific TEV protease cleavage reveals cohesin functions in Drosophila neurons**. *Dev. Cell* 2008, **14**:239-251.

See reference 21

20. Pauli A, van Bommel JG, Oliveira RA, Itoh T, Shirahige K, van Steensel B, Nasmyth K: **A Direct Role for Cohesin in Gene Regulation and Ecdysone Response in Drosophila Salivary Glands**. *Current Biology* 2010, **20**:1787-1798.

*21. Schuldiner O, Berdnik D, Levy JM, Wu JS, Luginbuhl D, Gontang AC, Luo L: **piggyBac-Based Mosaic Screen Identifies a Postmitotic Function for Cohesin in Regulating Developmental Axon Pruning**. *Developmental Cell* 2008, **14**:227-238.

Together with reference 19 and 20 this study demonstrates a role for cohesin in regulating gene expression in non-dividing *Drosophila* cells.

22. Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Shevchenko A, Nasmyth K: **Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and**

Scs4 proteins. *Mol. Cell* 2000, **5**:243-254.

23. Rollins RA, Korom M, Aulner N, Martens A, Dorsett D: **Drosophila nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scs3 cohesion factor to facilitate long-range activation of the cut gene.** *Mol. Cell. Biol.* 2004, **24**:3100-3111.
 24. Seitan VC, Banks P, Laval S, Majid NA, Dorsett D, Rana A, Smith J, Bateman A, Krpic S, Hostert A, et al.: **Metazoan Scs4 homologs link sister chromatid cohesion to cell and axon migration guidance.** *PLoS Biol.* 2006, **4**:e242.
 25. Watrin E, Schleiffer A, Tanaka K, Eisenhaber F, Nasmyth K, Peters J-M: **Human Scs4 is required for cohesin binding to chromatin, sister-chromatid cohesion, and mitotic progression.** *Curr. Biol.* 2006, **16**:863-874.
 26. Bernard P, Drogat J, Maure J-F, Dheur S, Vaur S, Genier S, Javerzat J-P: **A screen for cohesion mutants uncovers Ssl3, the fission yeast counterpart of the cohesin loading factor Scs4.** *Curr. Biol.* 2006, **16**:875-881.
 27. Gerlich D, Koch B, Dupeux F, Peters J-M, Ellenberg J: **Live-Cell Imaging Reveals a Stable Cohesin-Chromatin Interaction after but Not before DNA Replication.** *Current Biology* 2006, **16**:1571-1578.
 28. Rowland BD, Roig MB, Nishino T, Kurze A, Uluocak P, Mishra A, Beckouët F, Underwood P, Metson J, Imre R, et al.: **Building sister chromatid cohesion: smc3 acetylation counteracts an antiestablishment activity.** *Mol. Cell* 2009, **33**:763-774.
 29. Unal E, Heidinger-Pauli JM, Kim W, Guacci V, Onn I, Gygi SP, Koshland DE: **A molecular determinant for the establishment of sister chromatid cohesion.** *Science* 2008, **321**:566-569.
 30. Rolef Ben-Shahar T, Heeger S, Lehane C, East P, Flynn H, Skehel M, Uhlmann F: **Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion.** *Science* 2008, **321**:563-566.
 31. Zhang J, Shi X, Li Y, Kim B-J, Jia J, Huang Z, Yang T, Fu X, Jung SY, Wang Y, et al.: **Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast.** *Mol. Cell* 2008, **31**:143-151.
 - *32. Nishiyama T, Ladurner R, Schmitz J, Kreidl E, Schleiffer A, Bhaskara V, Bando M, Shirahige K, Hyman AA, Mechtler K, et al.: **Sororin Mediates Sister Chromatid Cohesion by Antagonizing Wapl.** *Cell* 2010, **143**:737-749.
- This study shows that the acetylation of Smc3 during S-phase promotes the association of Sororin with cohesin and antagonizes the ability of Wapl and Pds5 to dissociate cohesin from chromatin.
33. Sjögren C, Nasmyth K: **Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*.** *Curr. Biol.* 2001, **11**:991-995.
 34. Unal E, Arbel-Eden A, Sattler U, Shroff R, Lichten M, Haber JE, Koshland D: **DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain.** *Mol. Cell* 2004, **16**:991-1002.
 35. Ström L, Lindroos HB, Shirahige K, Sjögren C: **Postreplicative recruitment of cohesin to**

double-strand breaks is required for DNA repair. *Mol. Cell* 2004, **16**:1003-1015.

36. Heidinger-Pauli JM, Unal E, Guacci V, Koshland D: **The kleisin subunit of cohesin dictates damage-induced cohesion.** *Mol. Cell* 2008, **31**:47-56.

*37. Heidinger-Pauli JM, Unal E, Koshland D: **Distinct targets of the Eco1 acetyltransferase modulate cohesion in S phase and in response to DNA damage.** *Mol. Cell* 2009, **34**:311-321.

This study shows that both S-phase and DNA damage-induced sister chromatid cohesion require Eco1. However, while S-phase cohesion requires Smc3 acetylation, post-replicative cohesion involves specific amino acids in Mcd1, the yeast homolog of Rad21.

38. Kitajima TS, Sakuno T, Ishiguro K-ichiro, Iemura S-ichiro, Natsume T, Kawashima SA, Watanabe Y: **Shugoshin collaborates with protein phosphatase 2A to protect cohesin.** *Nature* 2006, **441**:46-52.

39. Riedel CG, Katis VL, Katou Y, Mori S, Itoh T, Helmhart W, Gálová M, Petronczki M, Gregan J, Cetin B, et al.: **Protein phosphatase 2A protects centromeric sister chromatid cohesion during meiosis I.** *Nature* 2006, **441**:53-61.

40. Kitajima TS, Kawashima SA, Watanabe Y: **The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis.** *Nature* 2004, **427**:510-517.

41. McGuinness BE, Hirota T, Kudo NR, Peters J-M, Nasmyth K: **Shugoshin prevents dissociation of cohesin from centromeres during mitosis in vertebrate cells.** *PLoS Biol.* 2005, **3**:e86.

42. Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K: **An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast.** *Cell* 1998, **93**:1067-1076.

43. Uhlmann F, Lottspeich F, Nasmyth K: **Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1.** *Nature* 1999, **400**:37-42.

44. Zou H, McGarry TJ, Bernal T, Kirschner MW: **Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis.** *Science* 1999, **285**:418-422.

*45. Misulovin Z, Schwartz YB, Li X-Y, Kahn TG, Gause M, MacArthur S, Fay JC, Eisen MB, Pirrotta V, Biggin MD, et al.: **Association of cohesin and Nipped-B with transcriptionally active regions of the Drosophila melanogaster genome.** *Chromosoma* 2008, **117**:89-102.

This genome-wide study maps Drosophila cohesin complexes and cohesin loading factors to active genes.

46. Schaaf CA, Misulovin Z, Sahota G, Siddiqui AM, Schwartz YB, Kahn TG, Pirrotta V, Gause M, Dorsett D: **Regulation of the Drosophila Enhancer of split and invected-engrailed Gene Complexes by Sister Chromatid Cohesion Proteins.** *PLoS ONE* 2009, **4**:e6202.

47. Lengronne A, Katou Y, Mori S, Yokobayashi S, Kelly GP, Itoh T, Watanabe Y, Shirahige K, Uhlmann F: **Cohesin relocation from sites of chromosomal loading to places of convergent transcription.** *Nature* 2004, **430**:573-578.

48. Glynn EF, Megee PC, Yu H-G, Mistrot C, Unal E, Koshland DE, DeRisi JL, Gerton JL: **Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*.** *PLoS Biol.* 2004, **2**:E259.

*49. Gullerova M, Proudfoot NJ: **Cohesin Complex Promotes Transcriptional Termination between Convergent Genes in *S. pombe*.** *Cell* 2008, **132**:983-995.

This paper demonstrates an involvement of the RNAi machinery in recruiting cohesin to sites of bi-directional transcription in fission yeast and suggests a role for cohesin in defining transcription termination sites.

*50. Xiao T, Wallace J, Felsenfeld G: **Specific Sites in the C Terminus of CTCF Interact with the SA2 Subunit of the Cohesin Complex and Are Required for Cohesin-Dependent Insulation Activity.** *Mol. Cell. Biol.* 2011, **31**:2174-2183.

This paper details the sites of interaction between cohesin and CTCF.

51. Nonaka N, Kitajima T, Yokobayashi S, Xiao G, Yamamoto M, Grewal SIS, Watanabe Y: **Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast.** *Nat Cell Biol* 2002, **4**:89-93.

*52. Hu B, Itoh T, Mishra A, Katoh Y, Chan K-L, Upcher W, Godlee C, Roig MB, Shirahige K, Nasmyth K: **ATP Hydrolysis Is Required for Relocating Cohesin from Sites Occupied by Its Scc2/4 Loading Complex.** *Current Biology* 2011, **21**:12-24.

This paper demonstrates a positive association between RNA polymerases and cohesin loading factors in budding yeast.

53. Ocampo-Hafalla MT, Uhlmann F: **Cohesin loading and sliding.** *Journal of Cell Science* 2011, **124**:685 -691.

54. Takahashi TS, Yiu P, Chou MF, Gygi S, Walter JC: **Recruitment of *Xenopus* Scc2 and cohesin to chromatin requires the pre-replication complex.** *Nat. Cell Biol.* 2004, **6**:991-996.

55. Gillespie PJ, Hirano T: **Scc2 couples replication licensing to sister chromatid cohesion in *Xenopus* egg extracts.** *Curr. Biol.* 2004, **14**:1598-1603.

*56. Guillou E, Ibarra A, Coulon V, Casado-Vela J, Rico D, Casal I, Schwob E, Losada A, Méndez J: **Cohesin organizes chromatin loops at DNA replication factories.** *Genes & Development* 2010, **24**:2812 -2822.

This study reports that cohesin interacts with proteins at replication origins and is required for efficient origin firing.

*57. Terret M-E, Sherwood R, Rahman S, Qin J, Jallepalli PV: **Cohesin acetylation speeds the replication fork.** *Nature* 2009, **462**:231-234.

This paper shows that RFC-CTF18, a factor involved in DNA replication, stimulates the acetylation of Smc3 and the progression of the replication fork.

58. Horsfield JA, Anagnostou SH, Hu JK-H, Cho KHY, Geisler R, Lieschke G, Crosier KE, Crosier PS: **Cohesin-dependent regulation of Runx genes.** *Development* 2007, **134**:2639-2649.

59. Liu J, Zhang Z, Bando M, Itoh T, Deardorff MA, Clark D, Kaur M, Tandy S, Kondoh T,

- Rappaport E, et al.: **Transcriptional dysregulation in NIPBL and cohesin mutant human cells.** *PLoS Biol.* 2009, **7**:e1000119.
60. Kaur M, DeScipio C, McCallum J, Yaeger D, Devoto M, Jackson LG, Spinner NB, Krantz ID: **Precocious sister chromatid separation (PSCS) in Cornelia de Lange syndrome.** *Am. J. Med. Genet. A* 2005, **138**:27-31.
61. Zhang B, Jain S, Song H, Fu M, Heuckeroth RO, Erlich JM, Jay PY, Milbrandt J: **Mice lacking sister chromatid cohesion protein PDS5B exhibit developmental abnormalities reminiscent of Cornelia de Lange syndrome.** *Development* 2007, **134**:3191-3201.
62. Heidinger-Pauli JM, Mert O, Davenport C, Guacci V, Koshland D: **Systematic reduction of cohesin differentially affects chromosome segregation, condensation, and DNA repair.** *Curr. Biol.* 2010, **20**:957-963.
63. Fazio TG, Huff JT, Panning B: **An RNAi screen of chromatin proteins identifies Tip60-p400 as a regulator of embryonic stem cell identity.** *Cell* 2008, **134**:162-174.
64. Hu G, Kim J, Xu Q, Leng Y, Orkin SH, Elledge SJ: **A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal.** *Genes Dev.* 2009, **23**:837-848.
65. Ding L, Paszkowski-Rogacz M, Nitzsche A, Slabicki MM, Heninger A-K, de Vries I, Kittler R, Junqueira M, Shevchenko A, Schulz H, et al.: **A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity.** *Cell Stem Cell* 2009, **4**:403-415.
66. Nitzsche A, Paszkowski-Rogacz M, Matarese F, Janssen-Megens EM, Hubner NC, Schulz H, de Vries I, Ding L, Huebner N, Mann M, et al.: **RAD21 Cooperates with Pluripotency Transcription Factors in the Maintenance of Embryonic Stem Cell Identity.** *PLoS ONE* 2011, **6**:e19470.
67. Mishiro T, Ishihara K, Hino S, Tsutsumi S, Aburatani H, Shirahige K, Kinoshita Y, Nakao M: **Architectural roles of multiple chromatin insulators at the human apolipoprotein gene cluster.** *EMBO J.* 2009, **28**:1234-1245.
68. Hou C, Dale R, Dean A: **Cell type specificity of chromatin organization mediated by CTCF and cohesin.** *Proceedings of the National Academy of Sciences* 2010, **107**:3651 -3656.
69. Chien R, Zeng W, Kawauchi S, Bender MA, Santos R, Gregson HC, Schmiesing JA, Newkirk DA, Kong X, Ball AR, et al.: **Cohesin Mediates Chromatin Interactions That Regulate Mammalian β -globin Expression.** *Journal of Biological Chemistry* 2011, **286**:17870 -17878.
70. Degner SC, Verma-Gaur J, Wong TP, Bossen C, Iverson GM, Torkamani A, Vettermann C, Lin YC, Ju Z, Schulz D, et al.: **CCCTC-binding factor (CTCF) and cohesin influence the genomic architecture of the IgH locus and antisense transcription in pro-B cells.** *Proceedings of the National Academy of Sciences* 2011, **108**:9566 -9571.
71. Amit R, Garcia HG, Phillips R, Fraser SE: **Building enhancers from the ground up: a synthetic biology approach.** *Cell* 2011, **146**:105-118.
72. Gard S, Light W, Xiong B, Bose T, McNairn AJ, Harris B, Fleharty B, Seidel C, Brickner JH,

Gerton JL: **Cohesinopathy mutations disrupt the subnuclear organization of chromatin.** *The Journal of Cell Biology* 2009, **187**:455 -462.

*73. Handoko L, Xu H, Li G, Ngan CY, Chew E, Schnapp M, Lee CWH, Ye C, Ping JLH, Mulawadi F, et al.: **CTCF-mediated functional chromatin interactome in pluripotent cells.** *Nat Genet* 2011, **43**:630-638.

This study uses a genome-wide approach to generate a high-resolution map of long-range interactions involving CTCF.

Figure 1

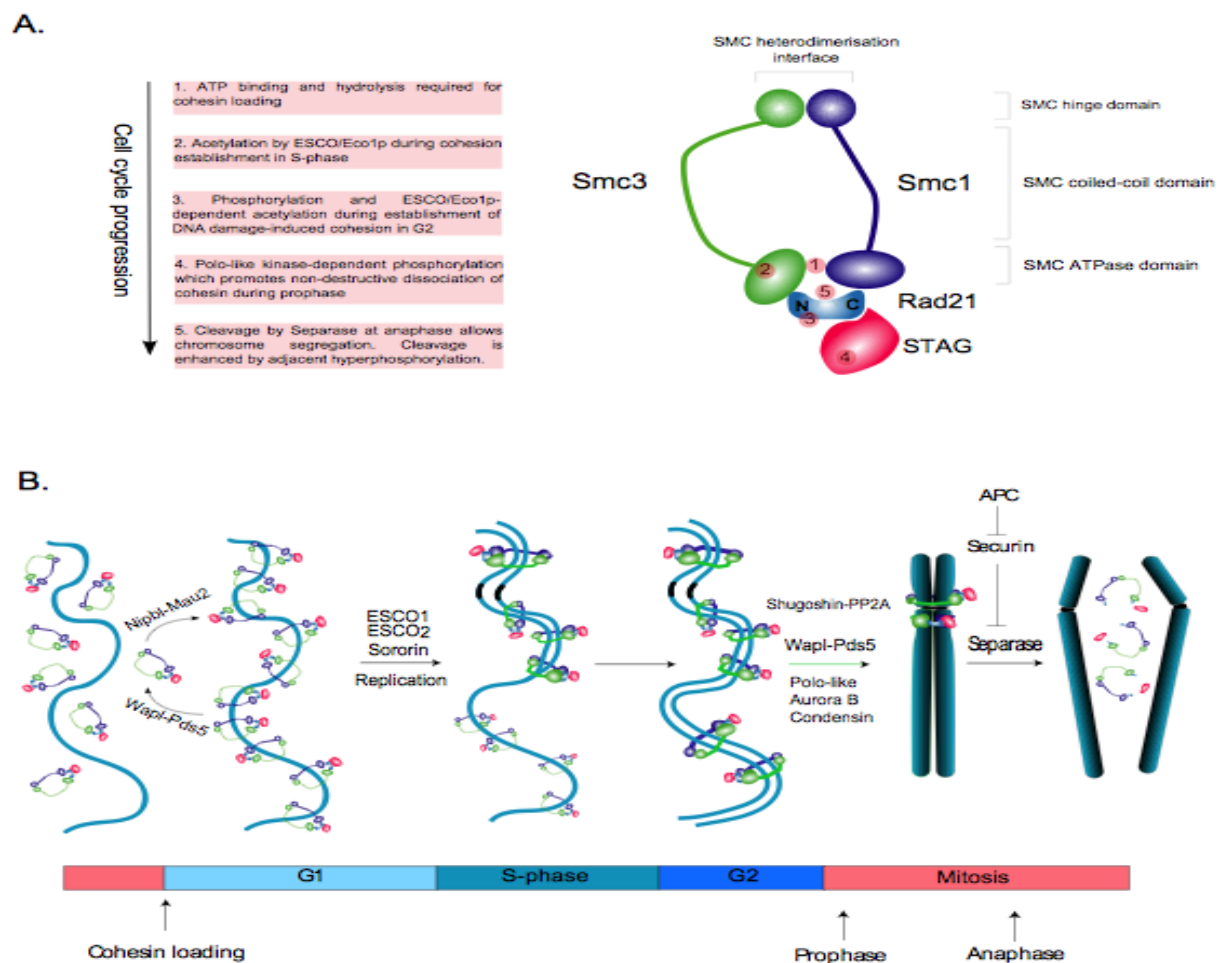


Figure 2

