Chromosome Research (2009) 17:201–214
DOI 10.1007/s10577-008-9017-7

How cohesin and CTCF cooperate in regulating gene expression

Kerstin S. Wendt · Jan-Michael Peters

Published online: 20 March 2009

Abstract Cohesin is a DNA-binding protein complex that is essential for sister chromatid cohesion and facilitates the repair of damaged DNA. In addition, cohesin has important roles in regulating gene expression, but the molecular mechanisms of this function are poorly understood. Recent experiments have revealed that cohesin binds to the same sites in mammalian genomes as the zinc finger transcription factor CTCF. At a few loci CTCF has been shown to function as an enhancer-blocking transcriptional insulator, and recent observations indicate that this function depends on cohesin. Here we review what is known about the roles of cohesin and CTCF in regulating gene expression in mammalian cells, and we discuss how cohesin might mediate the insulator function of CTCF.

Keywords insulator · chromatin · transcription · cohesin · CTCF

Abbreviations

ATP	adenosin-5′-triphosphate
CdLS	Cornelia de Lange Syndrome
ChIP	chromatin immunoprecipitation
cHS4	5′HS4 chicken β-globin insulator
CTCF	zinc finger transcription factor
EcR-B1	ecdysone receptor B1
ICR	imprinting control region, also called differentially methylated region or domain (DMR/DMD)
KSHV	Karposi sarcoma-associated herpes virus
LCR	locus control region
ncRNA	non-coding RNA
PEV	position-effect-variegation
qPCR	quantitative polymerase chain reaction
RBS/SC	Roberts/SC Phocomelia Syndrome
RNAi	RNA interference
TEV	tobacco etch virus protease

Responsible Editor: Christian Haering.

K. S. Wendt · J.-M. Peters (✉)
Research Institute of Molecular Pathology (IMP),
Dr. Bohr-Gasse 7,
A-1030 Vienna, Austria
e-mail: peters@imp.ac.at

The cohesin complex and its role in sister chromatid cohesion

Cohesin is a protein complex that is essential for cohesion between sister chromatids (reviewed in Peters et al. 2008). Cohesion is required for the biorientation of chromosomes on the mitotic and meiotic spindle. If cohesion is not established properly, sister chromatids can be separated before chromosomes have become attached to both spindle poles. In many species and cell types, this situation causes prolonged activation of a surveillance mechanism, called the spindle checkpoint, which delays chromosome segregation and mitotic exit in the presence of chromosomes that have not been

properly attached to the spindle. Sister chromatid cohesion mediated by cohesin is therefore essential for normal cell proliferation. Cohesin also has important roles in the repair of DNA double strand breaks. During S- and G_2-phases of the cell cycle, such breaks are mostly repaired by homologous recombination, in which the intact sister chromatid serves as a template for repair of the broken chromatid. The cohesion that is mediated by cohesin is thought to facilitate this repair by providing close proximity between sister chromatids, and possibly also by providing physical integrity to the broken chromatid itself.

Cohesin is composed of multiple subunits (Fig. 1). Three of these assemble into an unusual ring-like structure (Anderson et al. 2002; Haering et al. 2002). Two of the ring-forming subunits are ATPases, called Smc1 and Smc3. These proteins are characterized by the presence of a 45 nm-long rod-shaped domain, which is composed of two anti-parallel coiled-coil regions. This domain is flanked by a so-called hinge domain at one end and a globular ATPase domain at the other end. Smc1 and Smc3 heterodimerize via their hinge domains. A third subunit, called Scc1/Mcd1/Rad21, connects the ATPase domains of Smc1 and Smc3 and thereby creates a tripartite ring (Haering et al. 2002). It has been proposed that this ring structure mediates cohesion by topologically embracing two sister chromatids, and a number of experimental tests in the budding yeast *Saccharomyces cerevisiae* have supported this model (Haering et al. 2008 and references therein).

Cohesin is also associated with several other proteins, whose function may be to open, close, or stabilize the cohesin ring. Scc1 is bound to a protein which is also essential for cohesion, called Scc3 in budding yeast and stromal antigen in mammalian cells (SA1, SA2, and SA3). Cohesin complexes also contain the associated proteins Pds5 and Wapl/Rad61 (reviewed in Peters et al. 2008). There are two orthologues of Pds5 in mammalian cells, called Pds5A and Pds5B (Losada et al. 2005). Pds5A and Wapl might form a heterodimer that associates with Scc1 and the SA subunits of cohesin (Gandhi et al. 2006; Kueng et al. 2006). Whereas genetic experiments indicate that Pds5 is required for cohesion (reviewed in Peters et al. 2008), it has been shown that Wapl is needed to remove cohesin from DNA, perhaps by facilitating opening of the cohesin ring (Gandhi et al. 2006; Kueng et al. 2006). In vertebrates, chromatin bound cohesin is also associated with sororin, a small protein that is required for establish-

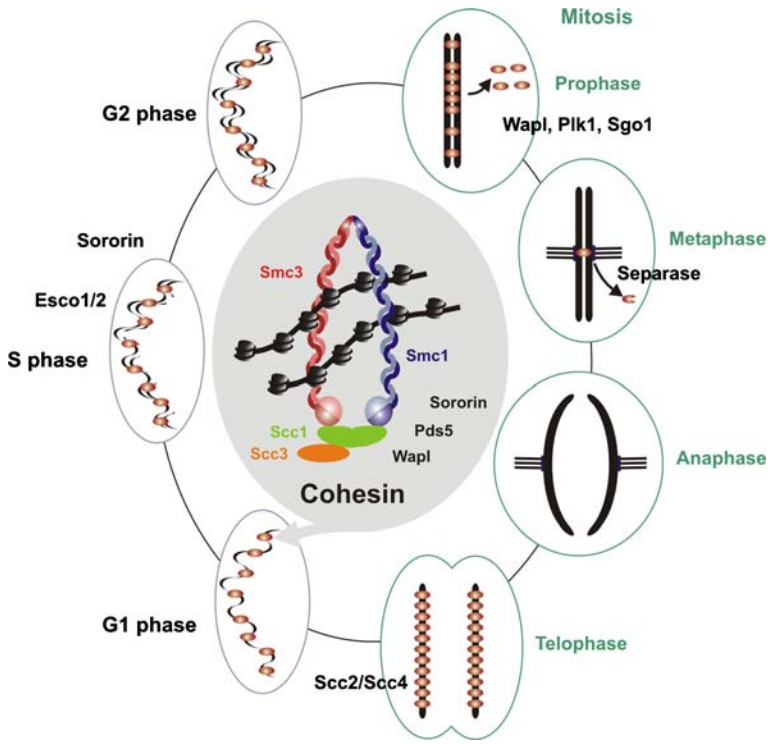

Fig. 1 The cohesin cycle. The cohesin complex consists of the four subunits Smc1, Smc3, Scc1, and Scc3, which form a ring-like structure that was proposed to embrace the two sister DNA strands. The association of cohesin with chromatin is regulated by a number of different proteins that control cohesin loading (Scc2/Scc4), cohesion establishment (Esco1/2, possibly Sororin), cohesion maintenance (Sororin), and removal of cohesin from chromosomes in mitosis (Wapl, Plk1, Sgo1, and Separase)

ment or maintenance of cohesion (Rankin et al. 2005; Schmitz et al. 2007).

The loading of cohesin onto DNA is initiated before DNA replication, and this process depends on a complex of the DNA bound proteins Scc2 and Scc4 (Ciosk et al. 2000). The subsequent establishment of cohesion during S-phase is tightly coupled to DNA replication and requires an acetyltransferase, called Eco1/Ctf7 in *S. cerevisiae* (Toth et al. 1999; Skibbens et al. 2007). Also for this protein there are two orthologues in mammalian cells, Esco1 and Esco2 (Hou and Zou 2005). The key role of Eco1 in cohesion establishment is to acetylate the ATPase domain of Smc3 (Ben-Shahar et al. 2008; Unal et al. 2008; Zhang et al. 2008). How this modification enables cohesin to mediate cohesion is not known, but interestingly Eco1 becomes dispensable for cohesion in the absence of Rad61, the *S. cerevisiae* orthologue of Wapl (Ben-Shahar et al. 2008). It is therefore possible that Smc3 acetylation enables cohesion by protecting cohesin from Rad61/Wapl, which might otherwise dissociate cohesin from DNA again.

Before chromosomes are segregated in anaphase, cohesion between sister chromatids has to be dissolved. In mammalian cells, this is achieved through the cooperation of two distinct mechanisms. In prophase the bulk of cohesin is removed from chromosome arms by a poorly understood mechanism, called the prophase pathway (Losada et al. 1998; Sumara et al. 2000; Waizenegger et al. 2000). This mechanism depends on Wapl and is also facilitated by phosphorylation of SA2 by the mitosis specific Polo-like kinase 1 (Plk1) (Gandhi et al. 2006; Kueng et al. 2006; reviewed in Peters et al. 2008). This process results in partial separation of sister chromatids in chromosome arm regions. Small amounts of cohesin are protected from the prophase pathway, in particular at centromeres, where cohesion is thought to resist the pulling forces of spindle microtubules (Waizenegger et al. 2000). This protection depends on a centromeric protein called Sgo1 in yeasts and vertebrates, and Mei-S332 in the fruit fly *Drosophila melanogaster* (reviewed in Gregan et al. 2008). The remaining cohesin complexes are removed from chromosomes at the metaphase–anaphase transition by the protease separase (Uhlmann et al. 2000; Waizenegger et al. 2000). Separase is activated only once all chromosomes have been bioriented and the spindle checkpoint has become inactive. Separase then cleaves the Scc1 subunit of cohesin and thereby dissolves cohesion completely. This process is thought to initiate chromosome segregation in anaphase.

Evidence that cohesin has functions in gene regulation

Although it is well established that cohesin has an important role in sister chromatid cohesion, it has been suspected since some time that cohesin also performs a distinct function in regulating transcription (Dorsett 2007). The first indications that this may be the case came from genetic experiments in *Drosophila* and budding yeast. In *S. cerevisiae*, some mutations in the cohesin subunits Smc1 and Smc3 were found to compromise the function of boundary elements at the transcriptionally silent *HMR* mating type locus (Donze et al. 1999). These boundary elements are sequences that prevent the spreading of the silencing factors Sir2–4 from the *HMR* locus into transcriptionally active neighbouring regions. In *Drosophila*, the orthologue Nipped-B of the cohesin-loading factor Scc2, was first identified as a protein that controls gene expression. In this study, Dorsett and colleagues used the *gypsy* element, a transcriptional insulator sequence, to search for genes mediating promoter-enhancer interactions. Heterozygous mutations in Nipped-B were found to magnify the enhancer-blocking effect that a weak *gypsy* element causes when inserted between enhancer and promoter of the *cut* gene and of the *Ultrabithorax* (*Ubx*) gene (Rollins et al. 1999). This observation indicated that Nipped-B might facilitate enhancer–promoter interactions. Later experiments showed that homozygous mutation of Nipped-B causes defects in sister chromatid cohesion, suggesting that Nipped-B is required for loading of cohesin onto DNA, as is Scc2 in budding yeast and other species (Rollins et al. 2004). Surprisingly, however, mutation of only one Nipped-B allele is sufficient to affect enhancer–promoter interactions, although Nipped-B mRNA levels are only reduced to 80% of wild-type levels in these heterozygous mutants. The effect of Nipped-B mutations on enhancer–promoter interactions may nevertheless be related to the function of cohesin, because partial depletion of Smc1 and Scc3/SA by RNA interference (RNAi) also affects gene expression, although in a different way. Partial depletion of these cohesin subunits reduces the effect of the *gypsy*

insertion and increases expression of the *cut* gene (Rollins et al. 2004; Dorsett et al. 2005), which is the opposite of what is observed for Nipped-B mutations. Based on these results it has been proposed that dynamic interactions between cohesin and DNA, which depend on Nipped-B, are needed to properly control promoter-enhancer interactions, possibly because cohesin might affect the enhancer blocking activity of the gypsy insulator (for a more detailed discussion see the review by Dale Dorsett 2009).

Another observation that could not be easily explained by cohesin's known role in sister chromatid cohesion has been made in vertebrate cells. In *Xenopus* and human cells, cohesin is already loaded onto unreplicated DNA in telophase, i.e. at a time where sister chromatid cohesion does not yet exist (Losada et al. 1998; Sumara et al. 2000; Waizenegger et al. 2000). Even more surprisingly, cohesin and the cohesin associated protein Pds5B are also expressed in postmitotic cells such as neurons, which normally do not replicate their DNA again and will thus also never establish sister chromatid cohesion (Zhang et al. 2007; Wendt et al. 2008). These observations are thus consistent with the possibility that cohesin performs functions on DNA that are distinct from cohesin's role in sister chromatid cohesion.

More recently, the notion that cohesin may have an important role in gene regulation has further been supported by a number of discoveries in developmental biology and human genetics. These studies revealed that mutations that are predicted to affect cohesin function can lead to defects relatively late in animal and human development. This is not what would be predicted if cohesin's only role was to mediate cohesion, because inactivation of this function would be expected to cause lethality early during the development of multicellular organisms. It was therefore surprising when mutations in cohesin-related genes were linked to developmental abnormalities in a number of different species (Table 1). A particularly striking example is the identification of cohesin-related mutations in patients suffering from Cornelia de Lange Syndrome (CdLS). This rare disorder is characterized clinically by growth and mental retardation, craniofacial anomalies and microcephaly. About half of all studied CdLS cases have now been linked to heterozygous loss-of-function mutations in *NIBPL*, the human orthologue of *S. cerevisae* Scc2 and *Drosophila* Nipped-B (Krantz et al. 2004; Tonkin et al. 2004b). A few mild cases have also been identified that are caused by mutations in *SMC1A* and *SMC3* (Musio et al. 2006; Deardorff et al. 2007). Cells derived from CdLS patients show only mild and in some cases no obvious defects in sister chromatid cohesion, suggesting that the identified mutations in NIBPL and Smc1 do not strongly affect the ability of cohesin to mediate cohesion.

Cohesin binds to CTCF sites in mammalian genomes

Although there are now numerous observations that indicate that cohesin has an important role in

Table 1 Developmental defects that have been linked to cohesin-related mutations

Name[a]	Gene	Species	Relationship to cohesin	Phenotype or disease	Reference
Scc4	*MAU-2*	*C. elegans*	Loading factor	Axon guidance defect	Benard et al. (2004); Takagi et al. (1997)
Wapl	*WAPL*	*D. melanogaster*	Cohesin removal	Heterochromatin defect	Verni et al. (2000)
Scc1	*RAD21*	*D. melanogaster*	Cohesin subunit	Axon pruning defect	Pauli et al. (2008)
Smc1	*SMC1*	*D. melanogaster*	Cohesin subunit	Axon pruning defect	Schuldiner et al. (2008)
Scc1	*RAD21*	*D. rerio*	Cohesin subunit	Haematopoiesis defect	Horsfield et al. (2007)
Smc3	*SMC3*	*D. rerio*	Cohesin subunit	Haematopoiesis defect	Horsfield et al. (2007)
Pds5B	*PDS5B*	*M. musculus*	Cohesin regulator	Defects similar to CdLS	Zhang et al. (2007)
Scc2	*NIBL*	*H. sapiens*	Loading factor	Cornelia de Lange syndrome (CdLS)	Krantz et al. (2004)
Smc3	*SMC3*	*H. sapiens*	Cohesin subunit	Cornelia de Lange syndrome (CdLS)	Deardorff et al. (2007)
Smc1	*SMC1L*	*H. sapiens*	Cohesin subunit	Cornelia de Lange syndrome (CdLS)	Deardorff et al. (2007)
Esco2	*ESCO2*	*H. sapiens*	Cohesion establishment	Roberts SC phocomelia syndrome	Gordillo et al. (2008); Vega et al. (2005)

[a] Generic human protein name.

regulating gene expression, the mechanistic basis of this function is still very poorly understood. An important hint as to how cohesin might mediate this function in mammalian cells has recently come from chromatin immunoprecipitation (ChIP) experiments. In several independent studies, cohesin was found to co-localize on DNA with CCCTC binding factor (CTCF), a protein that has been implicated in transcriptional regulation. Stedman et al. found that cohesin and CTCF co-localize within the control region of the major latency transcript of the Karposi sarcoma-associated herpes virus (KSHV) genome, and at two regulatory sequence elements on human DNA (Stedman et al. 2008). Parelho et al. identified about 1800 cohesin/CTCF sites by using genomic tiling arrays, which represent 3% of the non-repetitive part of the mouse genome (Parelho et al. 2008), and showed that several of these correspond to previously identified DNAse hypersensititve sites that might have a role in gene regulation. Wendt et al. mapped almost 9000 cohesin sites in the entire non-repetitive part of the human genome, and showed that 89% of these sites are identical with CTCF sites (Wendt et al. 2008). More recently, co-localization of cohesin and CTCF at a number of human genomic loci has also been reported by Rubio et al. (2008).

Wendt et al. also identified more than 5000 CTCF sites at which cohesin could not be detected with high statistical significance. However, when a small subset of these 'CTCF only' sites was analyzed by quantitative polymerase chain reactions (qPCR) of ChIP samples, cohesin could also be detected at these sites. Many of the 'CTCF only' sites may therefore also be bound by cohesin. CTCF sites have also been identified in a number of other studies (see below), and one of these reported more than 20 000 binding sites on human DNA (Barski et al. 2007). It is therefore possible that the human genome contains up to 20 000 cohesin binding sites in its non-repetitive sequences. It has so far been impossible to identify cohesin binding sites in repetitive sequences of mammalian genomes, because currently available microarray and sequencing techniques do not allow unambiguous identification of these sequences. Cohesin binding sites must, however, exist in these regions, because cohesin has been detected at centromeric heterochromatin by microscopy, and repetitive sequences of the minor satellite and Alu types have been identified by PCR in cohesin ChIP samples (Hakimi et al. 2002; Koch et al. 2008). It is not known whether cohesin co-localizes with CTCF at these sites.

The function of almost all cohesin/CTCF sites is unknown, largely because the vast majority of them has only been identified very recently. However, the frequency with which cohesin/CTCF sites are located within a few kilobases upstream or downstream of genes is 2- to 3-fold higher than what would be predicted from a random distribution of cohesin sites, consistent with a role of some of these sites in gene regulation (Wendt et al. 2008). Furthermore, there are two loci at which the function of CTCF has previously been studied in detail. These are the imprinting control region of the *H19/IGF2* locus and the locus control region of the β-globin locus. Cohesin was found to co-localize with CTCF also at these sites (Parelho et al. 2008; Rubio et al. 2008; Stedman et al. 2008; Wendt et al. 2008). These loci therefore represent useful models to explore possible roles of cohesin in controlling gene expression (see below).

Surprisingly, the recent identification of cohesin binding sites in mammalian genomes also revealed that the distribution of cohesin differs dramatically between different species. Previous work had shown that most cohesin binding sites in budding yeast are intergenic and are located at sites where opposing transcription units converge (Glynn et al. 2004; Lengronne et al. 2004). Yet a different distribution of cohesin sites has been observed in *Drosophila*, where cohesin was preferentially found associated with actively transcribed genes (Misulovin et al. 2008) and where no co-localization between cohesin and the fly orthologue of CTCF has been detected so far (Holohan et al. 2007; Misulovin et al. 2008; Pauli et al. 2008). In the future it will therefore be important to understand whether cohesin contributes to transcriptional regulation through different mechanisms in different species, or whether perhaps only an unidentified subset of cohesin sites has a role in gene regulation.

Functions of CTCF in gene regulation

CTCF was originally identified as a protein that binds to sequences in the promoter region of the *MYC* oncogene (Lobanenkov et al. 1990) and was also independently discovered as a protein that binds to the human amyloid precursor protein gene

promoter, a chicken lysozyme silencer element, and an enhancer blocking region of the chicken β-globin locus (reviewed in Ohlsson et al. 2001; Gaszner and Felsenfeld 2006; Filippova 2008). Depending on the locus studied, either transcriptional repressor or activator functions have been observed for CTCF. CTCF sites have also been identified at imprinted genes, on the X chromosome and at boundaries between transcriptionally active and inactive chromatin, consistent with regulatory roles of CTCF at these loci (Bell and Felsenfeld 2000; Hark et al. 2000; Kanduri et al. 2000; Chao et al. 2002; Hikichi et al. 2003; Cho et al. 2005; Yoon et al. 2005). More recently, CTCF binding sites have been systematically identified in the human and mouse genome by ChIP-chip, ChIP-sequencing, and bioinformatic approaches (Barski et al. 2007; Kim et al. 2007; Xie et al. 2007; Wendt et al. 2008). These studies indicate that there are between 14 000 and 20 000 CTCF sites in the non-repetitive parts of mammalian genomes. It remains unknown at how many of these sites CTCF binding has effects on gene expression, but recent microarray studies have shown that depletion of CTCF by RNAi misregulates expression of several hundred genes (Wan et al. 2008; Wendt et al. 2008). CTCF may therefore have important functions at many of its binding sites.

CTCF contains 11 zinc-finger domains, which are thought to function in different combinations to mediate binding to diverse DNA sequences (Filippova et al. 1996). These interactions can be controlled by DNA methylation. If cytosine residues in GpC islands are methylated, CTCF binding is inhibited (Bell and Felsenfeld 2000), whereas the association with CTCF can conversely prevent the de-novo methylation of DNA (Pant et al. 2004). CTCF is widely expressed in different vertebrate tissues, whereas a closely related protein, called BORIS or CTCF-like (CTCFL), has only been detected in testis and some human tumours (Loukinov et al. 2002). Because the zinc-finger sequences of BORIS are very similar to those found in CTCF, it is believed that both proteins bind to similar sites in different tissues. It will thus be interesting to test if BORIS also co-localizes with cohesin in male germ cells and tumours.

CTCF binding regions that are located in chromatin insulator sequences have been particularly well studied, and a number of observations indicate that the binding of CTCF to these sequences is important for their insulator function (see below). The presence of insulator sequences can 'protect' genes from either repressive or activating effects that can be caused by neighbouring chromatin regions (reviewed in Wallace and Felsenfeld 2007). To be able to exert these effects, insulators typically have to be located between the 'protected' gene and the sequence elements from which it is to be shielded. 'Barrier' insulators can protect genes from position-effect-variegation (PEV), the transcriptional inactivation that is observed if a gene is placed next to heterochromatic regions, whereas 'enhancer-blocking' insulators prevent activation of genes by a distal enhancer (Fig. 2A). Initial studies using the 5′HS4 chicken β-globin insulator (cHS4) suggested that CTCF is required for both barrier and enhancer-blocking activities that are associated with this sequence element (Chung et al. 1997). A more detailed analysis reported that cHS4 can still act as a barrier insulator when its CTCF binding site is deleted, whereas the enhancer-blocking activity of cHS4 was lost under these conditions (Recillas-Targa et al. 1999). In operational terms, CTCF therefore might function as an enhancer-blocking insulator protein at the β-globin LCR. However, it is less clear how this activity affects the β-globin locus *in vivo*, because recent studies have shown that CTCF mediates long-range chromatin interactions within the locus (Splinter et al. 2006), but that deletion of CTCF binding sites or depletion of CTCF does not change the expression of the β-globin genes (Epner et al. 1998; Bender et al. 2000; Splinter et al. 2006; de Laat et al. 2008).

Another CTCF-dependent and methylation-sensitive enhancer-blocking insulator has been identified at the *H19/IGF2* locus (Fig. 2B and C), which contains a gene for the non-coding RNA (ncRNA) *H19* and one for insulin-like growth factor 2 (*IGF2*). The *IGF2* gene is only expressed on the paternal chromosome, whereas *H19* expression is restricted to the maternal allele (Bartolomei et al. 1991; DeChiara et al. 1991) (Fig. 2B). This reciprocal imprinted gene expression pattern is regulated by enhancers located downstream of *H19* and access to these enhancers is regulated by CTCF binding at the imprinting control region (ICR, also called differentially methylated region or domain, DMR/DMD) that is located 2 kb away from the 5′ end of the *H19* gene (Bell and Felsenfeld 2000; Hark et al. 2000; Kanduri et al. 2000). Only the unmethylated maternal allele can

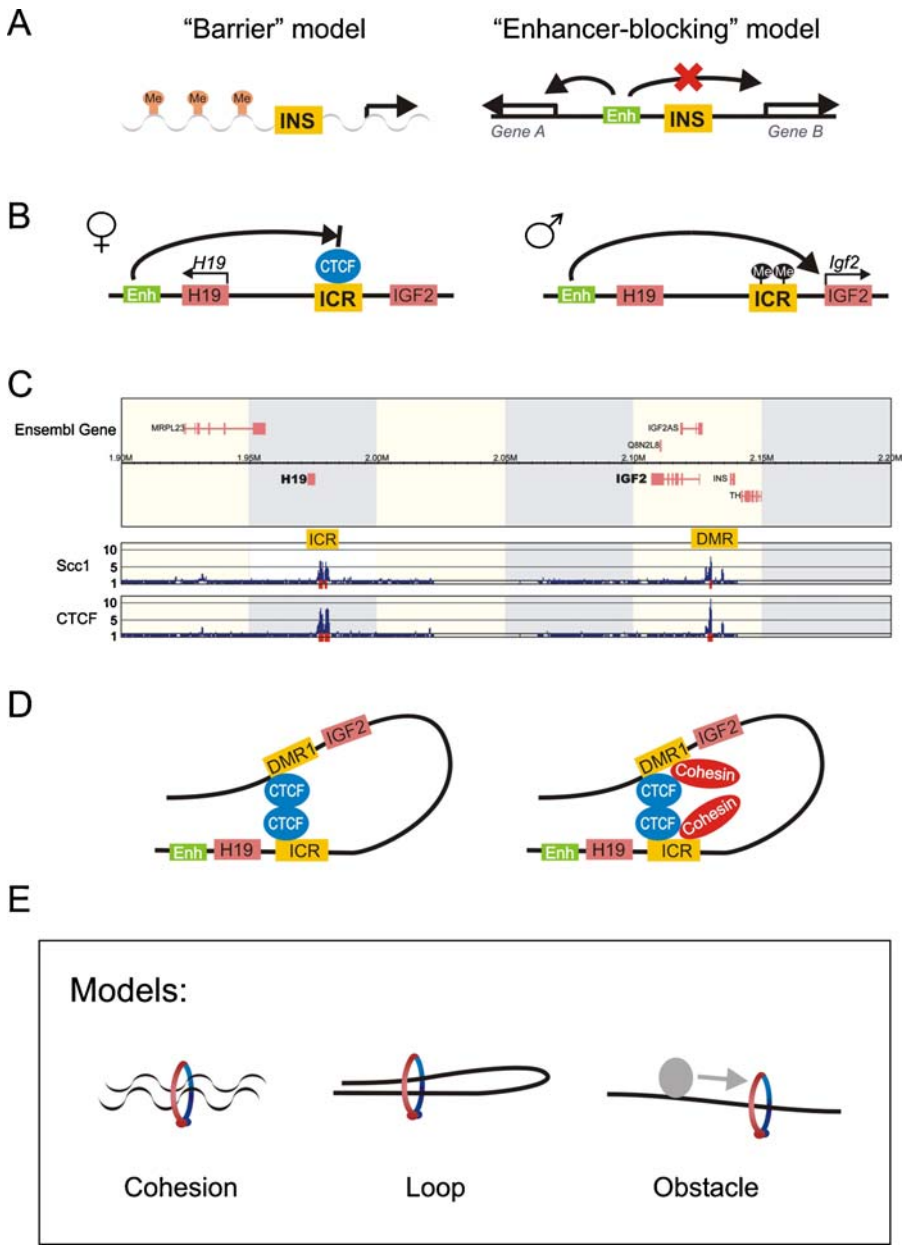

Fig. 2 Cohesin might function as a chromatin insulator by regulating higher-order chromatin structure. **a** Models for chromatin insulator function. Barrier insulators can prevent the spreading of inactive heterochromatin into transcriptionally active regions. Enhancer-blocking insulators can control promoter–enhancer interactions. **b** Schematic representation of the *H19/IGF2* locus and its transcriptional regulation. CTCF is bound to the ICR of the maternal allele, and activation of the *IGF2* gene is blocked while the *H19* gene is transcribed instead. Methylation of the ICR of the paternal allele prevents binding of CTCF, and the enhancer can gain access to the *IGF2* gene. **c** Co-localization of cohesin (Scc1) and CTCF at the human *H19/IGF2* locus. Cohesin and CTCF are located at two differentially methylated regions termed ICR and DMR, which control the imprinted gene expression at this locus. **d** Model for CTCF-dependent chromatin looping at the maternal *H19/IGF2* locus as described by Murrell et al. (2004) and Kurukuti et al. (2006). Cohesin associates with the DMR1 and the ICR in a manner that depends on CTCF and might take part in forming a chromatin loop. **e** Models of how cohesin might organize chromatin structure. The embrace model proposes that cohesin might mediate sister chromatid cohesion by entrapping two sister DNAs (left). Cohesin might stabilize chromatin loops by trapping the loop within the cohesin ring (middle). Cohesin's binding to chromatin might represent a physical obstacle for proteins such as transcription factors that move processively along chromatin (right)

bind CTCF and function as an enhancer-blocking insulator, which precludes the *IGF2* promoters from interacting with the enhancers. The ICR is methylated on the paternal chromosome (Bartolomei et al. 1993; Ferguson-Smith et al. 1993; Tremblay et al. 1995, 1997), which prevents CTCF from binding. This abolishes the enhancer-blocking activity of the ICR and enables the activation of the *IGF2* promoters by the enhancers (Bell and Felsenfeld 2000; Hark et al. 2000; Kanduri et al. 2000) (Fig. 2B).

Results from chromatin conformation capture (3C) experiments in mouse tissues indicate that CTCF regulates allele-specific expression of *H19* and *IGF2* by controlling long-range chromatin interactions (Murrell et al. 2004; Kurukuti et al. 2006; Yoon et al. 2007; Engel et al. 2008). It has been observed that on the maternal allele the ICR interacts preferentially with another differentially methylated region (DMR1) that is located in the 5′ region of the *IGF2* gene (Murrell et al. 2004). In addition, it has been reported that the maternal ICR interacts with *IGF2* promoters and with a matrix attachment region (Murrell et al. 2004; Kurukuti et al. 2006; Yoon et al. 2007). In contrast, these interactions are less frequent on the paternal allele. Instead, an association of the ICR with a differentially methylated region in the 3′ region of the *IGF2* gene (DMR2) has been detected (Murrell et al. 2004). It has been proposed that these differential interactions of the ICR with other regulatory elements may place the *IGF2* gene into a transcriptionally active chromatin domain on the paternal allele, but into an inactive loop on the maternal allele (Fig. 2D). According to this model, DNA loop formation would determine whether or not the *H19* enhancers are located in close proximity to the *IGF2* promoters. Consistent with this possibility, 3C assays have also revealed a striking correlation between enhancer–promoter interactions and transcriptional activity. Physical interactions between *H19* enhancers and *IGF2* promoters were preferentially detected on the paternal chromosome, where *IGF2* is transcribed, but not on the maternal allele, where *IGF2* is silent (Kurukuti et al. 2006; Yoon et al. 2007; Engel et al. 2008). Vice versa, enhancer interactions with the *H19* promoter were detected specifically on the maternal allele, where *H19* is expressed (Engel et al. 2008). Importantly, these allele-specific enhancer–promoter interactions were lost if the ICR was deleted or if CTCF binding sites in the ICR were mutated (Yoon et al. 2007; Engel et al. 2008). These observations indicate that CTCF might control gene expression by mediating local changes in the chromatin structure. These structural changes may then determine which promoters can physically interact with enhancers.

Evidence that cohesin is required for the enhancer-blocking activity of CTCF

The finding that cohesin and CTCF co-localize at thousands of sites in the human and mouse genome immediately raised the question whether these proteins affect each others functions. Cohesin still binds to DNA if CTCF has been depleted by RNAi, and no major cohesion defects can be seen in such cells. This suggests that CTCF is not essential for cohesin's ability to establish sister chromatid cohesion (Wendt et al. 2008). However, when the distribution of cohesin on DNA was analysed by ChIP experiments, much less cohesin was detected at CTCF sites than in control cells (Parelho et al. 2008; Wendt et al. 2008). Because the total amount of cohesin that is bound to DNA in CTCF-depleted cells is not detectably reduced, CTCF may specifically be required to allow enrichment of cohesin at CTCF sites. It remains unknown whether CTCF mediates this effect through direct physical interactions with cohesin, or whether CTCF structures DNA in a way that leads indirectly to accumulation of cohesin. Another implication of these results is that cohesin may not necessarily have to be highly enriched at its normal binding sites to be able to mediate cohesion.

Whether the binding of cohesin to CTCF sites is instead important for gene regulation is not yet known, but several studies of the *H19* ICR and the β-globin cHS4 insulator indicate that this might be the case. At the *H19/IGF2* locus, cohesin is highly enriched at the ICR but, like CTCF, cohesin only binds to the unmethylated maternal allele (Parelho et al. 2008; Rubio et al. 2008; Stedman et al. 2008; Wendt et al. 2008). This finding further supports the notion that the sites of cohesin binding are determined by the presence of CTCF, and it could explain why increased binding of cohesin to Alu repeats was observed in a previous study when DNA methylation was inhibited in cells by 5-azacytidine treatment (Hakimi et al. 2002). More importantly, several observations indicate that the presence of cohesin is

also required for the enhancer-blocking activity of the maternal ICR. Previous studies had shown that this activity can also be detected on plasmids if the ICR is placed between one of the *H19* enhancers and a reporter gene such as luciferase (Ishihara et al. 2006). When such plasmids are used in transient transfection assays, the ICR reduces luciferase expression, and this reduction depends on the presence of CTCF binding sites in the ICR and on the presence of CTCF itself. Cohesin also binds to these ICR-containing plasmids, and remarkably, depletion of cohesin decreases luciferase expression to a similar degree as does CTCF depletion (Wendt et al. 2008). In this assay, cohesin is therefore required for the enhancer-blocking activity of the *H19* ICR.

Is this also the case at the endogenous *H19/IGF2* locus? This question has only been addressed so far in HeLa cells, a highly derived human tumour cell line, but the results from these experiments indicate that cohesin also has an important role in regulating the *H19* and *IGF2* genes in their normal chromosomal context (Wendt et al. 2008). If CTCF is depleted from these cells, the levels of *H19* transcripts decrease and those of *IGF2* transcripts increase. This is consistent with the possibility that the *H19/IGF2* locus is still imprinted in a CTCF-dependent manner in these cells. Cohesin depletion had the same effect as CTCF depletion in this assay, which supports the idea that cohesin is required for the enhancer-blocking activity of the *H19* ICR (Wendt et al. 2008).

Similar results have been obtained for the chicken β-globin 5′ HS4 insulator. In one assay, plasmids were used on which two copies of the insulator were placed between the SV40 enhancer and a neomycin resistance reporter gene (Recillas-Targa et al. 1999; Saitoh et al. 2000). Enhancer-blocking activity was similarly reduced following depletion of either CTCF or cohesin in this system (Parelho et al. 2008). In another assay, two cDNAs encoding fluorescently tagged reporter proteins were stably integrated into the genome of HeLa cells next to each other. It was shown that insertion of cHS4 insulator sequences at both ends of the cDNAs and in between them increased their expression (Yahata et al. 2007). This effect was reverted when CTCF or cohesin were depleted from these cells, further supporting the notion that the insulator activity of cHS4 may be dependent on both proteins (Wendt et al. 2008).

Is cohesin's role in gene regulation independent of its role in cohesion?

To understand how cohesin contributes to transcriptional regulation, it will be important to know whether this function is an indirect consequence of cohesin's role in sister chromatid cohesion. There are several reasons why such indirect effects could exist. For example, it is well established that depletion of cohesin leads to defects in cohesion, which in turn causes delays in mitosis due to defects in chromosome biorientation and continued activation of the spindle checkpoint. Because transcription is largely inhibited during mitosis in vertebrate cells (reviewed in Gottesfeld and Forbes 1997), cohesin depletion could simply reduce transcription by leading to accumulation of cells in mitosis. The effects of cohesin depletion on gene expression can therefore only be interpreted in a meaningful way if these experiments are performed with cells that have been synchronized in the cell cycle or that are postmitotic.

It is also conceivable that the presence of cohesion has some indirect effects on gene expression in cells in G_2-phase, for example by affecting chromatin structure, or by enabling efficient repair of damaged DNA via homologous recombination. In this context it is worth noting that mutations in the *ESCO2* gene can cause Roberts/SC phocomelia syndrome (RBS/SC), a disease whose clinical features are not identical to but similar to those seen in CdLS patients (Schule et al. 2005; Vega et al. 2005). Esco2's budding yeast orthologue Eco1/Ctf7 is specifically required for establishment of cohesion, but there is no indication in either yeast or human cells that this enzyme is needed for the association of cohesin with DNA. A possible implication of these findings is that RBS/SC could be caused by subtle cohesion defects, which have indeed been observed in cells from RBS/SC patients (German 1979; Tomkins et al. 1979). It is possible that these cohesion defects also indirectly affect transcriptional regulation, and thereby lead to developmental defects. However, it still remains to be seen whether cohesin is actually positioned normally at CTCF sites in RBS/SC patients, because defects in cohesion positioning could also affect gene expression.

Although cohesion defects might well affect transcription indirectly, it has been suggested for some time that cohesin also has cohesion-independent functions in gene regulation (Dorsett 2007). Initially,

this hypothesis was based on the observation that cells derived from some CdLS patients do not show defects in sister chromatid cohesion (Krantz et al. 2004; Deardorff et al. 2007). Similarly, it has been reported that mice lacking the cohesin-associated protein Pds5B have developmental defects that are similar to those seen in CdLS patients, but in these mouse cells also no cohesion defects were observed (Zhang et al. 2007). Nevertheless, it remains difficult to completely rule out the possibility that more subtle cohesion defects, which may not be apparent in cultured cells, could contribute to the observed developmental defects.

However, several recent studies have provided strong evidence that at least some of cohesin's roles in gene regulation are independent of cohesin's function in cohesion. When the levels of *H19* and *IGF2* transcripts were analysed in cohesin-depleted HeLa cells, a decrease in *H19* ncRNA and an increase in *IGF2* mRNA were also observed when cells that had been synchronized in G_1-phase were analysed (Wendt et al. 2008). Because cohesion does not exist during this phase of the cell cycle, the observed effects were presumably not indirectly due to defects in cohesion. Clear evidence for a cohesion-independent role of cohesin in transcription has also been obtained in experiments in which cohesin subunits were specifically inactivated in postmitotic neurons in *Drosophila*. This manipulation causes defects in axon pruning, the retraction of axons that naturally occurs during neuronal development (Pauli et al. 2008; Schuldiner et al. 2008). Schuldiner et al. also showed that mutation of the cohesin subunit Smc1 reduces expression of the ecdysone receptor B1 (EcR-B1), which is known to be required for axon pruning (Schuldiner et al. 2008). Importantly, the pruning defects could be partially restored when either wild-type Smc1 or EcR-B1 were specifically expressed in the affected postmitotic neurons. This result indicates that cohesin is needed for pruning in postmitotic cells, possibly to allow EcR-B1 expression, and that the observed pruning phenotype was not an indirect consequence of an earlier cohesion defect.

Pauli et al. used another elegant approach to test whether cohesin has functions specifically in postmitotic cells (Pauli et al. 2008). The authors expressed a version of the cohesin subunit Rad21/Scc1 that can be cleaved by the tobacco etch virus (TEV) protease, and then induced TEV protease expression specifically in postmitotic neurons. Also in this case, pruning defects were seen following proteolytic cleavage of Rad21/Scc1. Because cohesin expression and function remained normal in these experiments until cells stopped proliferating, the observed phenotype must have been caused by inactivating a cohesin function that is distinct from sister chromatid cohesion. At least in some cases, cohesin's role in gene regulation can therefore clearly be separated from its role in cohesion.

How does cohesin mediate gene regulation?

The observations described above suggest that cohesin is as important for the enhancer-blocking activity of the *H19* ICR and the cHS4 insulator as CTCF itself, and that CTCF is needed to recruit cohesin to these sites. Conversely, CTCF is still detected at its normal binding sites if cohesin is depleted (Parelho et al. 2008; Wendt et al. 2008). It is therefore possible that CTCF's primary function is to recognize and bind specific DNA sequences and then to recruit cohesin and other CTCF interacting proteins to these sites. These proteins might then affect DNA in a way that leads to transcriptional insulation, or possibly to other regulatory effects depending on the topology of the locus. How cohesin might mediate these effects is a mystery, but several possibilities can be envisioned.

Since cohesin complexes can physically connect two distinct DNA molecules when they mediate cohesion between sister chromatids, it is tempting to speculate that cohesin might also physically link different sites on one DNA molecule and contribute to the formation of DNA loops. This hypothesis would agree well with the proposal that CTCF is needed for the formation of chromatin loops. It would also be consistent with the observation that cohesin affects the activity of the *Drosophila gypsy* insulator (Rollins et al. 1999), which is also believed to induce DNA loop formation (reviewed in Wallace and Felsenfeld 2007). It could be that cohesin then interacts with DNA in a way that creates the formation of loops. For example, cohesin could bind to two sites at the base of a chromatin loop and stabilize the loop either through physical interactions between two cohesin molecules (Fig. 2D), or help to position another protein that stabilizes the loop. Consistent with this possibility, the two CTCF binding sites that interact on the

maternal *H19/IGF2* allele (ICR and DMR1) are also strong cohesin binding sites (Fig. 2C). Another possibility would be that cohesin could trap the loop within the cohesin ring (Fig. 2E), analogously to how cohesin has been proposed to embrace two sister chromatids. Since cohesin can regulate gene expression independent of cohesion, such chromatin loops might already be established during G_1-phase. If cohesin would create loops by such an embrace mechanism, proteins that might be required for opening and closing of the cohesin ring would also be expected to have an important role in gene regulation, in particular if loops needed to be formed and dissolved in a dynamic fashion during gene regulation. This would be consistent with the observed phenotypes of Nipped-B and Wapl mutants in *Drosophila* (Rollins et al. 1999; Verni et al. 2000), and with identification of *NIPBL* mutations in CdLS patients (Krantz et al. 2004; Tonkin et al. 2004a).

Although a looping model for cohesin's role in gene regulation is attractive, it is not clear whether such a model can explain all experimental observations. The loops formed at the *H19/IGF2* locus are thought to be ~100 kb in size, but the *H19* ICR can also function as a cohesin-dependent insulator on plasmids that are only around 6 kb in total size (Parelho et al. 2008; Wendt et al. 2008). It is unclear whether these plasmids are large enough to allow the formation of chromatin loops. It is therefore also possible that the physical presence of cohesin complexes at particular sites on DNA could simply present a physical barrier for other proteins (Fig. 2E). Such an obstacle could in particular affect the processive movement of RNA polymerases along DNA or the lateral spreading of DNA binding proteins, such as Sir2–4 in budding yeast. It has also been proposed that enhancers move along DNA until they encounter a matching promoter sequence, and that CTCF may mediate transcriptional insulation by blocking this 'tracking' process (Gaszner and Felsenfeld 2006; Filippova 2008). If this is correct, cohesin could have an important role in preventing enhancer tracking.

Models in which cohesin functions as a physical barrier could explain the role of cohesin at the boundary elements of the *HMR* mating type locus (Donze et al. 1999) and would be consistent with several other observations made in yeasts. In *S. cerevisiae*, transcriptional activity can remove cohesin from genes, consistent with the possibility that the presence of cohesin on the active gene would interfere with its transcription (Lengronne et al. 2004; Bausch et al. 2007). In the fission yeast *Schizosaccharomyces pombe*, binding of cohesin to the 3′ regions of active genes is important for efficient termination of transcription, and also in this case it has been speculated that cohesin might function as a physical barrier (Gullerova and Proudfoot 2008). The hypothesis that cohesin functions as a physical barrier is further consistent with findings from a minichromosome model where binding of CTCF to cHS4 can stall the advance of RNA polymerase II and the spreading of histone acetylation from an enhancer to the promoter of a globin gene (Zhao and Dean 2004). In contrast, there is currently no evidence that binding of cohesin to DNA is incompatible with active transcription in *Drosophila* or mammalian cells (Misulovin et al. 2008; Parelho et al. 2008; Wendt et al. 2008). However, these studies have not excluded the possibility that cohesin is removed transiently from active genes and then rebinds rapidly once the transcription machinery has moved through the cohesin binding site.

Outlook

The discovery that cohesin co-localizes with CTCF in mammalian genomes has provided important insight into how cohesin might mediate gene regulation, but it has also raised numerous new questions. How does cohesin mediate the enhancer-blocking activity of CTCF at the *H19/IGF2* and the β-globin locus at the mechanistic level, and is cohesin also needed for this activity *in vivo*, where insulator activity is particularly important during development? What is the function of the thousands of cohesin/CTCF sites that have only been discovered recently? Are these sites also transcriptional insulators, or do they have other gene regulatory functions, and which of these functions are compromised in patients suffering from CdLS? Do cohesin/CTCF sites simultaneously function as sites at which sister chromatid cohesion is established? And finally, why do cohesin and CTCF co-localize so clearly in mammals, but not in *Drosophila*, where CTCF has also been reported to function as an enhancer-blocking insulator (Moon et al. 2005)? Further investigation of how cohesin and CTCF function in gene regulation will require a broad range of approaches from different disciplines. Although the

task may seem daunting, answering these important questions will certainly be worth the effort.

References

Anderson DE, Losada A, Erickson HP, Hirano T (2002) Condensin, cohesin display different arm conformations with characteristic hinge angles. J Cell Biol 156:419–424

Barski A, Cuddapah S, Cui K et al (2007) High-resolution profiling of histone methylations in the human genome. Cell 129:823–837

Bartolomei MS, Zemel S, Tilghman SM (1991) Parental imprinting of the mouse H19 gene. Nature 351:153–155

Bartolomei MS, Webber AL, Brunkow ME, Tilghman SM (1993) Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. Genes Dev 7:1663–1673

Bausch C, Noone S, Henry JM et al (2007) Transcription alters chromosomal locations of cohesin in *Saccharomyces cerevisiae*. Mol Cell Biol 27:8522–8532

Bell AC, Felsenfeld G (2000) Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405:482–485

Benard CY, Kebir H, Takagi S, Hekimi S (2004) mau-2 acts cell-autonomously to guide axonal migrations in *Caenorhabditis elegans*. Development 131:5947–5958

Bender MA, Bulger M, Close J, Groudine M (2000) Beta-globin gene switching, DNase I sensitivity of the endogenous beta-globin locus in mice do not require the locus control region. Mol Cell 5:387–393

Ben-Shahar TR, Heeger S, Lehane C et al (2008) Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. Science 321:563–566

Chao W, Huynh KD, Spencer RJ, Davidow LS, Lee JT (2002) CTCF, a candidate trans-acting factor for X-inactivation choice. Science 295:345–347

Cho DH, Thienes CP, Mahoney SE, Analau E, Filippova GN, Tapscott SJ (2005) Antisense transcription, heterochromatin at the DM1 CTG repeats are constrained by CTCF. Mol Cell 20:483–489

Chung JH, Bell AC, Felsenfeld G (1997) Characterization of the chicken beta-globin insulator. Proc Natl Acad Sci U S A 94:575–580

Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Nasmyth K (2000) Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2, Scc4 proteins. Mol Cell 5:243–254

de Laat W, Klous P, Kooren J et al (2008) Three-dimensional organization of gene expression in erythroid cells. Curr Top Dev Biol 82:117–139

Deardorff MA, Kaur M, Yaeger D et al (2007) Mutations in cohesin complex members SMC3, SMC1A cause a mild variant of Cornelia de Lange syndrome with predominant mental retardation. Am J Hum Genet 80:485–494

DeChiara TM, Robertson EJ, Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor II gene. Cell 64:849–859

Donze D, Adams CR, Rine J, Kamakaka RT (1999) The boundaries of the silenced HMR domain in *Saccharomyces cerevisiae*. Genes Dev 13:698–708

Dorsett D (2007) Roles of the sister chromatid cohesion apparatus in gene expression, development, and human syndromes. Chromosoma 116:1–13

Dorsett D (2009) Cohesin, gene expression and development: lessons from Drosophila. Chomosom Res. doi:10.1007/s10577-009-9022-5

Dorsett D, Eissenberg JC, Misulovin Z, Martens A, Redding B, McKim K (2005) Effects of sister chromatid cohesion proteins on cut gene expression during wing development in *Drosophila*. Development 132:4743–4753

Engel N, Raval AK, Thorvaldsen JL, Bartolomei MS (2008) Three-dimensional conformation at the H19/Igf2 locus supports a model of enhancer tracking. Hum Mol Genet

Epner E, Reik A, Cimbora D et al (1998) The beta-globin LCR is not necessary for an open chromatin structure or developmentally regulated transcription of the native mouse beta-globin locus. Mol Cell 2:447–455

Ferguson-Smith AC, Sasaki H, Cattanach BM, Surani MA (1993) Parental-origin-specific epigenetic modification of the mouse H19 gene. Nature 362:751–755

Filippova GN (2008) Genetics and epigenetics of the multifunctional protein CTCF. Curr Top Dev Biol 80:337–360

Filippova GN, Fagerlie S, Klenova EM et al (1996) An exceptionally conserved transcriptional repressor CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-myc oncogenes. Mol Cell Biol 16:2802–2813

Gandhi R, Gillespie PJ, Hirano T (2006) Human Wapl is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase. Curr Biol 16:2406–2417

Gaszner M, Felsenfeld G (2006) Insulators: exploiting transcriptional and epigenetic mechanisms. Nat Rev Genet 7:703–713

German J (1979) Roberts' syndrome. I. Cytological evidence for a disturbance in chromatid pairing. Clin Genet 16:441–447

Glynn EF, Megee PC, Yu HG et al (2004) Genome-wide mapping of the cohesin complex in the yeast *Saccharomyces cerevisiae*. PLoS Biol 2:E259

Gordillo M, Vega H, Trainer AH et al (2008) The molecular mechanism underlying Roberts syndrome involves loss of ESCO2 acetyltransferase activity. Hum Mol Genet 17:2172–2180

Gottesfeld JM, Forbes DJ (1997) Mitotic repression of the transcriptional machinery. Trends Biochem Sci 22:197–202

Gregan J, Spirek M, Rumpf C (2008) Solving the shugoshin puzzle. Trends Genet 24:205–207

Gullerova M, Proudfoot NJ (2008) Cohesin complex promotes transcriptional termination between convergent genes in *S. pombe*. Cell 132:983–995

Haering CH, Lowe J, Hochwagen A, Nasmyth K (2002) Molecular architecture of SMC proteins and the yeast cohesin complex. Mol Cell 9:773–788

Haering CH, Farcas AM, Arumugam P, Metson J, Nasmyth K (2008) The cohesin ring concatenates sister DNA molecules. Nature

Hakimi MA, Bochar DA, Schmiesing JA et al (2002) A chromatin remodelling complex that loads cohesin onto human chromosomes. Nature 418:994–998

Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM (2000) CTCF mediates methylation-sensitive

enhancer-blocking activity at the H19/Igf2 locus. Nature 405:486–489

Hikichi T, Kohda T, Kaneko-Ishino T, Ishino F (2003) Imprinting regulation of the murine Meg1/Grb10, human GRB10 genes; roles of brain-specific promoters and mouse-specific CTCF-binding sites. Nucleic Acids Res 31:1398–1406

Holohan EE, Kwong C, Adryan B et al (2007) CTCF genomic binding sites in *Drosophila* and the organisation of the bithorax complex. PLoS Genet 3:e112

Horsfield JA, Anagnostou SH, Hu JK et al (2007) Cohesin-dependent regulation of Runx genes. Development 134:2639–2649

Hou F, Zou H (2005) Two human orthologues of Eco1/Ctf7 acetyltransferases are both required for proper sister-chromatid cohesion. Mol Biol Cell 16:3908–3918

Ishihara K, Oshimura M, Nakao M (2006) CTCF-dependent chromatin insulator is linked to epigenetic remodeling. Mol Cell 23:733–742

Kanduri C, Pant V, Loukinov D et al (2000) Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. Curr Biol 10:853–856

Kim TH, Abdullaev ZK, Smith AD et al (2007) Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome. Cell 128:1231–1245

Koch B, Kueng S, Ruckenbauer C, Wendt KS, Peters JM (2008) The Suv39h-HP1 histone methylation pathway is dispensable for enrichment and protection of cohesin at centromeres in mammalian cells. Chromosoma 117:199–210

Krantz ID, McCallum J, DeScipio C et al (2004) Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of *Drosophila melanogaster* Nipped-B. Nat Genet 36:631–635

Kueng S, Hegemann B, Peters BH et al (2006) Wapl controls the dynamic association of cohesin with chromatin. Cell 127:955–967

Kurukuti S, Tiwari VK, Tavoosidana G et al (2006) CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. Proc Natl Acad Sci U S A 103:10684–10689

Lengronne A, Katou Y, Mori S et al (2004) Cohesin relocation from sites of chromosomal loading to places of convergent transcription. Nature 430:573–578

Lobanenkov VV, Nicolas RH, Adler VV et al (1990) A novel sequence-specific DNA binding protein which interacts with three regularly spaced direct repeats of the CCCTC-motif in the 5′-flanking sequence of the chicken c-myc gene. Oncogene 5:1743–1753

Losada A, Hirano M, Hirano T (1998) Identification of *Xenopus* SMC protein complexes required for sister chromatid cohesion. Genes Dev 12:1986–1997

Losada A, Yokochi T, Hirano T (2005) Functional contribution of Pds5 to cohesin-mediated cohesion in human cells and *Xenopus* egg extracts. J Cell Sci 118:2133–2141

Loukinov DI, Pugacheva E, Vatolin S et al (2002) BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma. Proc Natl Acad Sci U S A 99:6806–6811

Misulovin Z, Schwartz YB, Li XY et al (2008) Association of cohesin and Nipped-B with transcriptionally active regions of the *Drosophila melanogaster* genome. Chromosoma 117:89–102

Moon H, Filippova G, Loukinov D et al (2005) CTCF is conserved from *Drosophila* to humans, confers enhancer blocking of the Fab-8 insulator. EMBO Rep 6:165–170

Murrell A, Heeson S, Reik W (2004) Interaction between differentially methylated regions partitions the imprinted genes Igf2, H19 into parent-specific chromatin loops. Nat Genet 36:889–893

Musio A, Selicorni A, Focarelli ML et al (2006) X-linked Cornelia de Lange syndrome owing to SMC1L1 mutations. Nat Genet 38:528–530

Ohlsson R, Renkawitz R, Lobanenkov V (2001) CTCF is a uniquely versatile transcription regulator linked to epigenetics, disease. Trends Genet 17:520–527

Pant V, Kurukuti S, Pugacheva E et al (2004) Mutation of a single CTCF target site within the H19 imprinting control region leads to loss of Igf2 imprinting, complex patterns of de novo methylation upon maternal inheritance. Mol Cell Biol 24:3497–3504

Parelho V, Hadjur S, Spivakov M et al (2008) Cohesins functionally associate with CTCF on mammalian chromosome arms. Cell 132:422–433

Pauli A, Althoff F, Oliveira RA et al (2008) Cell-type-specific TEV protease cleavage reveals cohesin functions in *Drosophila* neurons. Dev Cell 14:239–251

Peters JM, Schmitz J, Tedeschi A (2008) The cohesin complex and its roles in chromosome biology. Genes Dev 22:3089–114

Rankin S, Ayad NG, Kirschner MW (2005) Sororin, a substrate of the anaphase-promoting complex, is required for sister chromatid cohesion in vertebrates. Mol Cell 18:185–200

Recillas-Targa F, Bell AC, Felsenfeld G (1999) Positional enhancer-blocking activity of the chicken beta-globin insulator in transiently transfected cells. Proc Natl Acad Sci U S A 96:14354–14359

Rollins RA, Morcillo P, Dorsett D (1999) Nipped-B, a *Drosophila* homologue of chromosomal adherins, participates in activation by remote enhancers in the cut and Ultrabithorax genes. Genetics 152:577–593

Rollins RA, Korom M, Aulner N, Martens A, Dorsett D (2004) *Drosophila* nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scc3 cohesion factor to facilitate long-range activation of the cut gene. Mol Cell Biol 24:3100–3111

Rubio ED, Reiss DJ, Welcsh PL et al (2008) CTCF physically links cohesin to chromatin. Proc Natl Acad Sci U S A 105:8309–8314

Saitoh N, Bell AC, Recillas-Targa F et al (2000) Structural and functional conservation at the boundaries of the chicken beta-globin domain. EMBO J 19:2315–2322

Schmitz J, Watrin E, Lenart P, Mechtler K, Peters JM (2007) Sororin is required for stable binding of cohesin to chromatin and for sister chromatid cohesion in interphase. Curr Biol 17:630–636

Schuldiner O, Berdnik D, Levy JM et al (2008) piggyBac-based mosaic screen identifies a postmitotic function for

cohesin in regulating developmental axon pruning. Dev Cell 14:227–238

Schule B, Oviedo A, Johnston K, Pai S, Francke U (2005) Inactivating mutations in ESCO2 cause SC phocomelia, Roberts syndrome: no phenotype–genotype correlation. Am J Hum Genet 77:1117–1128

Skibbens RV, Maradeo M, Eastman L (2007) Fork it over: the cohesion establishment factor Ctf7p, DNA replication. J Cell Sci 120:2471–2477

Splinter E, Heath H, Kooren J et al (2006) CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. Genes Dev 20:2349–2354

Stedman W, Kang H, Lin S, Kissil JL, Bartolomei MS, Lieberman PM (2008) Cohesins localize with CTCF at the KSHV latency control region and at cellular c-myc and H19/Igf2 insulators. EMBO J 27:654–666

Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters JM (2000) Characterization of vertebrate cohesin complexes and their regulation in prophase. J Cell Biol 151:749–762

Takagi S, Benard C, Pak J, Livingstone D, Hekimi S (1997) Cellular, axonal migrations are misguided along both body axes in the maternal-effect mau-2 mutants of *Caenorhabditis elegans*. Development 124:5115–5126

Tomkins D, Hunter A, Roberts M (1979) Cytogenetic findings in Roberts-SC phocomelia syndrome(s). Am J Med Genet 4:17–26

Tonkin ET, Wang TJ, Lisgo S, Bamshad MJ, Strachan T (2004a) NIPBL, encoding a homolog of fungal Scc2-type sister chromatid cohesion proteins, fly Nipped-B, is mutated in Cornelia de Lange syndrome. Nat Genet 36:636–641

Tonkin ET, Smith M, Eichhorn P et al (2004b) A giant novel gene undergoing extensive alternative splicing is severed by a Cornelia de Lange-associated translocation breakpoint at 3q263. Hum Genet 115:139–148

Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K (1999) Yeast cohesin complex requires a conserved protein Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev 13:320–333

Tremblay KD, Saam JR, Ingram RS, Tilghman SM, Bartolomei MS (1995) A paternal-specific methylation imprint marks the alleles of the mouse H19 gene. Nat Genet 9:407–413

Tremblay KD, Duran KL, Bartolomei MS (1997) A 5′ 2-kilobase-pair region of the imprinted mouse H19 gene exhibits exclusive paternal methylation throughout development. Mol Cell Biol 17:4322–4329

Uhlmann F, Wernic D, Poupart MA, Koonin EV, Nasmyth K (2000) Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. Cell 103:375–386

Unal E, Heidinger-Pauli JM, Kim W et al (2008) A molecular determinant for the establishment of sister chromatid cohesion. Science 321:566–569

Vega H, Waisfisz Q, Gordillo M et al (2005) Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. Nat Genet 37:468–470

Verni F, Gandhi R, Goldberg ML, Gatti M (2000) Genetic, molecular analysis of wings apart-like (wapl), a gene controlling heterochromatin organization in *Drosophila melanogaster*. Genetics 154:1693–1710

Waizenegger IC, Hauf S, Meinke A, Peters JM (2000) Two distinct pathways remove mammalian cohesin from chromosome arms in prophase and from centromeres in anaphase. Cell 103:399–410

Wallace JA, Felsenfeld G (2007) We gather together: insulators, genome organization. Curr Opin Genet Dev 17:400–407

Wan LB, Pan H, Hannenhalli S et al (2008) Maternal depletion of CTCF reveals multiple functions during oocyte and preimplantation embryo development. Development 135:2729–2738

Wendt KS, Yoshida K, Itoh T et al (2008) Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature 451:796–801

Xie X, Mikkelsen TS, Gnirke A, Lindblad-Toh K, Kellis M, Lander ES (2007) Systematic discovery of regulatory motifs in conserved regions of the human genome, including thousands of CTCF insulator sites. Proc Natl Acad Sci U S A 104:7145–7150

Yahata K, Maeshima K, Sone T et al (2007) cHS4 insulator-mediated alleviation of promoter interference during cell based expression of tandemly associated transgenes. JMB: in press

Yoon B, Herman H, Hu B et al (2005) Rasgrf1 imprinting is regulated by a CTCF-dependent methylation-sensitive enhancer blocker. Mol Cell Biol 25:11184–11190

Yoon YS, Jeong S, Rong Q, Park KY, Chung JH, Pfeifer K (2007) Analysis of the H19ICR insulator. Mol Cell Biol 27:3499–3510

Zhang B, Jain S, Song H et al (2007) Mice lacking sister chromatid cohesion protein PDS5B exhibit developmental abnormalities reminiscent of Cornelia de Lange syndrome. Development 134:3191–3201

Zhang J, Shi X, Li Y et al (2008) Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast. Mol Cell 31:143–151

Zhao H, Dean A (2004) An insulator blocks spreading of histone acetylation, interferes with RNA polymerase II transfer between an enhancer and gene. Nucleic Acids Res 32:4903–4919