Following DNA replication, chromatid pairs are held together by a proteinaceous complex called cohesin until separation during the metaphase-to-anaphase transition. Accurate segregation is achieved by regulation of both sister chromatid cohesion establishment and removal, mediated by post-translational modification of cohesin and interaction with numerous accessory proteins. Recent evidence has led to the conclusion that cohesin is also vitally important in the repair of DNA lesions and control of gene expression. It is now clear that chromosome segregation is not the only important function of cohesin in the maintenance of genome integrity.

Key words: chromosome segregation, cohesion, DNA damage, heterochromatin, transcriptional insulation.

INTRODUCTION

The accurate separation of sister chromatids into daughter cells is critical for maintaining genome integrity. A loss of genome integrity via W-CIN (whole chromosome instability) has been implicated in a variety of pathologies including reproductive failure, and mental and physical abnormalities [1]. However, the link between W-CIN and cancer susceptibility has been difficult to define. Aberrant chromosome numbers, or aneuploidy, is one of the molecular hallmarks of cancer cells but little evidence has been provided to conclude that aneuploidy can lead to cancer rather than it being a consequence of tumorigenesis [2]. Several mouse models of W-CIN have been used to determine whether aneuploidy is a cause or effect of tumorigenesis. These models have provided strong evidence that mutation of several of the genes involved in the regulation of accurate chromosome segregation results in higher cancer susceptibility and thus provides a link between aberrant chromosome segregation and cancer formation [3].

The mechanism by which replicated sister chromatids are accurately segregated into daughter cells initially depends on their physical association with each other, a process termed cohesion. Sister chromatid cohesion facilitates capture by a bipolar mitotic spindle and segregation to opposing poles, resulting in the formation of two genetically identical daughter cells. Without physical association of sister chromatids, the opposing forces required for bipolar separation of chromatids would not exist. In this case premature separation of chromatids could occur before bipolar attachment resulting in inaccurate chromosome segregation and genome instability. It was long thought that sister chromatids were physically associated until the onset of anaphase due to the intertwining or catenation of DNA strands during replication and that DNA topoisomerase II was required for the separation of chromatids at anaphase [4]. Indeed, inhibition of topoisomerase II has shown that DNA catenation between sister chromatids does exist and needs to be resolved to allow accurate sister chromatid separation, but not mitotic exit [5–7]. Although these studies show that DNA decatenation is required for efficient chromatid segregation, cohesin of DNA molecules can, however, be established in the absence of catenation [8]. More compelling evidence for the mechanism of sister chromatid cohesion comes from the isolation of protein complexes essential for cohesion but that play no part in the mechanism by which catenations are removed. These highly conserved protein complexes contain multiple core structural proteins along with several associated regulatory subunits.

MOLECULAR ARCHITECTURE OF THE COHESION COMPLEX

The core members of the protein complex that facilitates chromosomal cohesion during mitosis were first isolated using a yeast screen for temperature-sensitive mutants that lose chromosomes at high frequency. Four genes were isolated using this approach: two known genes belonging to the SMC (structural maintenance of chromosomes) family of ATPases (SMC1 and SMC3), and two novel genes, termed SCC1 and SCC3 due to their involvement in sister chromatid cohesion [9]. A second key finding of that study was that the Scc1 subunit of the cohesin complex was specifically degraded at the metaphase-to-anaphase transition by the APC (anaphase-promoting complex), promoting sister chromatid separation [9]. At the same time,
Table 1  Cohesin subunits and associated proteins

<table>
<thead>
<tr>
<th>Process</th>
<th>S. cerevisiae</th>
<th>S. pombe</th>
<th>D. melanogaster</th>
<th>H. sapiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohesin complex</td>
<td>Smc1</td>
<td>Psrm1</td>
<td>Smc1</td>
<td>Smc1</td>
</tr>
<tr>
<td></td>
<td>Smc3</td>
<td>Psrm3</td>
<td>Smc3 (Cap)</td>
<td>Smc3</td>
</tr>
<tr>
<td>Mccl1 (Scc1)</td>
<td>Rad21</td>
<td>Rad21 (Verhandli)</td>
<td>Rad21</td>
<td>Rad21</td>
</tr>
<tr>
<td>Scc3</td>
<td>Psc3</td>
<td>SA1 (Stromalin)</td>
<td>SA1/SA2/STAG3</td>
<td>SA1/SA2/STAG3</td>
</tr>
<tr>
<td>Cohesin-loading proteins</td>
<td>Scc2</td>
<td>Mis4</td>
<td>Nipped-B</td>
<td>NIPBL</td>
</tr>
<tr>
<td></td>
<td>Scc4</td>
<td>Sst3</td>
<td>Sa21-2 (G4203)</td>
<td>Sa21-2 (G4203)</td>
</tr>
<tr>
<td>Cohesion establishment/anti-establishment, protection</td>
<td>Eco1 (Cit7)</td>
<td>Eco</td>
<td>Eco1/Eco2</td>
<td>Eco1/Eco2</td>
</tr>
<tr>
<td></td>
<td>Pds5</td>
<td>Pds5</td>
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<tr>
<td></td>
<td>Rad51</td>
<td>Wpl1</td>
<td>Wap1</td>
<td>Wap1</td>
</tr>
<tr>
<td>Cohesin cleavage</td>
<td>Pds1</td>
<td>Cdt5</td>
<td>Pimply</td>
<td>Securin</td>
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<tr>
<td></td>
<td>Esp1</td>
<td>Cut1</td>
<td>Separase</td>
<td>Separase</td>
</tr>
</tbody>
</table>

an independent study also identified Scc1 [also known as Mccl1 (mitotic chromosome determinant 1) in *Saccharomyces cerevisiae* (budding yeast) and as Rad21 (radiation-sensitive 21) in *Schizosaccharomyces pombe* (fission yeast) and vertebrates] as a Smc1-associated protein involved in sister chromatin cohesion [10]. It was later shown that the products of these four genes, Smc1, Smc3, Scc1 and Scc3, exist in a complex called cohesin that is central to the mechanism of sister chromatin cohesion. Table 1 gives the names of the cohesin subunits and associated protein in yeasts, *Drosophila* and mammals.

The SMC family of proteins are highly conserved in prokaryotes and eukaryotes and have the same general structure. SMC proteins are large (1000–1300 amino acids) and contain two nucleotide-binding motifs, known as Walker A and Walker B motifs, situated at opposing ends of the polypeptide. A large coiled-coil motif is situated between the Walker A and Walker B motifs and this folds into an antiparallel formation creating an ATP-binding head domain. At the other end of the folded protein is a hinge domain that facilitates either homo- or heterodimerization of SMC molecules creating a V-shaped structure [11,12]. Electron micrographs of SMC dimers show that the coiled-coil arms of each molecule are approx. 50 nm in length and that a high degree of flexibility exists in the hinge, resulting in a wide variability of conformational states between dimers [12,13]. The cohesin complex consists of a heterodimer of the SMC1 and SMC3 subunits, assembled as described above. The kleisin family member Scc1 binds to both the SMC1 and SMC3 head domains creating a closed ring structure that is large enough to encircle chromatin fibres, while the fourth subunit of cohesin, Scc3, associates with the complex via interaction with Scc1 [11] (Figure 1). There are known homologues of Scc3 in vertebrate cells, SA1 and SA2 (stromal antigen 1 and 2; also known as STAG1 and 2; a third homologue STAG3 functions in meiosis and is only expressed in germinal cells). Interestingly, it has been shown that cohesin complexes contain either SA1 or SA2, but not both [14,15], although the function of these distinct protein complexes has not been defined. However, it has been suggested that SA1 and SA2 play a role in the establishment of cohesion at the telomeres and centromeres respectively, potentially revealing distinct roles for SA1- and SA2-associated cohesin [16].

ASSOCIATION OF THE COHESIN RING WITH DNA

The first studies looking at the mechanism by which cohesin associates with DNA showed that the SMC proteins alone can associate with DNA in an ATP-independent manner [17,18]. A further study showed that cohesin can facilitate intermolecular association of DNA molecules rather than the intramolecular knotting directed by the related condensin complex [19]. These results led to the hypothesis that the Smc1–Smc3 heterodimer acts as an intermolecular bridge between two DNA molecules, providing the cohesive force that holds chromosomes together. Initially, this intermolecular bridging was suggested to be regulated or maintained by the non-SMC subunits unique to cohesin, thus explaining the intermolecular bridging facilitated by cohesin rather than the intramolecular interactions facilitated by condensin. However, the bridging hypothesis has been modified further following structural and biochemical studies of the cohesin complex that strongly indicate the formation of a ring-like structure between the V-shaped SMC heterodimer, joined at either end by the cleavable Sccl subunit [11,20,21]. From this it was proposed that the cohesin complex functions by encircling DNA strands, trapping them together rather than forming a molecular bridge between strands.

The requirement for ATP-binding and hydrolysis in cohesin establishment and release has also been a contentious debate.
Although some *in vitro* studies have shown that SMC proteins can associate with DNA via the C-terminal domains in an ATP-independent manner [17,18], *in vivo* studies have shown that ATP hydrolysis is required for the association of cohesin with DNA [22,23]. Evidence suggests that two ATP molecules are held between the SMC heads, which separate and bind a single Scc1 molecule upon ATP hydrolysis [23]. This could be triggered by the presence of DNA and therefore result in the entrapment of DNA strands within the new tripartite ring. Although the cohesion-specific Smc1–Smc3 heterodimer has been shown to bind to DNA in the absence of other proteins and ATP, this association has a very low affinity suggesting that it does not occur *in vivo*, whereas inclusion of the Scc1 subunit in the complex substantially increases binding affinity [24]. In addition, the heterotrimeric complex associates with cruciform DNA with a much higher affinity than unstructured double-stranded DNA, indicating that the presence of secondary structure is important for the association of cohesin with DNA [24]. Indeed, an independent study showed that the cohesin complex bound to chromatin with a much higher affinity than the Smc1–Smc3 heterodimer alone, indicating that the holocomplex requires secondary structure elements to bind to DNA with high affinity [25]. Electron micrographs of SMC heterodimers show that the arms of the molecule are approx. 50–60 nm in length. Inclusion of the Scc1 subunit to form a tripartite ring could allow the encircling of nucleosomal DNA, which is approx. 10 nm in diameter [11]. The topological entrapment of DNA within cohesin rings is supported by studies showing that cleavage of the Scc1 or Smc3 subunits results in dissociation of cohesin from DNA and premature loss of cohesion [26]. Whether one cohesin ring embraces two chromatin fibres to hold them together or each fibre is encircled by a cohesin ring that interconnect holding strands together has been hard to determine (Figure 2).

The ‘ring’ model (Figure 2A) has historically dominated. This model suggests that one single cohesin ring can encircle two DNA strands thus trapping them together [20,27,28] and is supported by experimental results showing that linearization of circular minichromosomes in yeast, by restriction digest, causes dissociation of cohesin from DNA [8]. In addition, interactions between multiple cohesin complexes were undetectable using FRET (fluorescence resonance energy transfer) technology and in co-immunoprecipitation experiments in cells expressing two differently tagged copies of the same cohesin subunit [11,29]. Furthermore, chemical cross-linking of the tripartite cohesin rings produces DNA–cohesin complexes that are insensitive to protein denaturation, thus providing strong evidence that cohesion between DNA molecules is mediated by a single cohesin ring that embraces both sister chromatin [30].

An alternative model of how cohesin physically holds chromatids together is the two-ring ‘handcuff’ model (Figure 2B) in which each sister is encircled by one cohesin ring and two rings then associate with each other or interconnect resulting in cohesion of DNA strands. Although the ring model has been favoured in recent years, new evidence has resurrected the handcuff model. In contrast with the studies described above, self interaction of the Scc1 subunit was detected in a yeast two-hybrid assay and was confirmed in mammalian cells expressing tagged proteins, suggesting the presence of a higher-order cohesin complex [31]. This is consistent with a study showing that cohesin is able to embrace only one sister chromatid at transcriptionally silent heterochromatin regions, which have a larger diameter due to greater chromatin compaction [32]. However, it is possible that this mechanism of cohesion is specific to transcriptionally silent regions and that transcriptionally active regions can be encircled by a single ring.

**Figure 2 Models of sister chromatid cohesion**

(A) Ring model: one cohesin ring encircles two sister chromatids (the DNA is wrapped around nucleosomes and is 10 nm in diameter). (B) Handcuff model: each sister chromatid is encircled by separate cohesin rings and two rings then associate with each other (1,2) or interconnect (3) resulting in cohesion of sister chromatids.

**COHESIN LOADING**

In lower eukaryotes cohesin associates with DNA at the end of G1-phase [9,10,33]. In contrast, cohesin has been shown to assemble on to DNA in mammalian cells following nuclear envelope reformation during telophase [34]. This difference may be due to the different regulatory mechanisms in place to maintain and remove cohesin, or may be due to differences in the functions of cohesin outside of its canonical role in chromosome segregation (discussed below). It has been demonstrated that the interaction of cohesin with DNA in both higher and lower eukaryotes becomes more stable as cells progress from G1 to S to metaphase [35,36] and although the timing differs, cohesin loading in all organisms appears to require ATP hydrolysis [22,23,37] and the Scc2–Scc4 protein complex (Nipped-B in *Drosophila*) [38–41]. Although the specific mechanism of Scc2–Scc4-mediated cohesin loading remains unclear, it has been proposed that the Scc2 protein...
interacts transiently with cohesin to promote ATP hydrolysis by the SMC heads, leading to SMC dimer hinge opening or Scc1 dissociation [22]. Interestingly, the Scc2–Scc4 complex does not play a role in the maintenance of cohesin association with DNA, as cohesin rapidly and independently moves away from chromosomal-loading sites to areas of convergent transcription [33].

Although the loading of cohesin is generally Scc2–Scc4 dependent, there are some instances where cohesin loading appears independent of Scc2–Scc4. In human cells the Scc2–Scc4 complex has been shown to dissociate from chromosomes during mitosis, which is presumed to inhibit the reassociation of cohesin until progression through to telophase, when Scc2–Scc4 and cohesin rebind [34,41]. However, during pre-anaphase centromere ‘breathing’, a process that occurs as sister chromatids transiently split under tension from the mitotic spindle, cohesin is reloaded on to transiently separated chromatids in an Scc2–Scc4-independent manner [42], suggesting that an alternative and as yet undetermined mechanism of cohesin loading may exist at this stage of the cell cycle.

COHESION ESTABLISHMENT DURING DNA REPLICATION

Traditional sister chromatid cohesion can only be established during DNA replication. However, cohesin associates with DNA early in the cell cycle and therefore cohesion establishment is thought not to require new cohesin loading [43]. Cohesion establishment, but not maintenance, has been shown to be dependent on the lysine acetyltransferase Eco1 [establishment of cohesion 1; known as Eso in S. pombe and, Esco1 and Esco2 in humans]]44,45. Eco1 physically associates with numerous members of the DNA replication complex, including two highly homologous RFC (replication factor C) clamp loader proteins, Ctf18 (chromosome transmission fidelity 18) and Elg1 (enhanced level of genomic instability 1), which each play an important role in cohesion establishment [46,47], and PCNA (proliferating-cell nuclear antigen) [48], which genetically associates with Ctf4 and is part of the replication progression complex [49]. Interestingly, Eco1 has also been shown to bind to the XPD DNA-helicase family member, Chl1 [chromosome loss 1; DDX11 (DEAD/H box polypeptide 11) in humans], which plays a role in cohesion establishment [50–53]. Like Eco1, Chl1 associates with members of the DNA replication complex, such as RFC and PCNA [48,52,54]. These results provide strong evidence that cohesion establishment is intimately coupled to PCNA-dependent DNA replication and that Eco1 may function to allow dissociation of cohesin rings, or passage of the replication machinery through cohesin rings, ensuring cohesion establishment and completion of DNA replication. In support of this, it has been shown that acetylation of cohesin is required for replication fork processivity and in the absence of RFC3TFI8 both replication and cohesin acetylation are severely impaired, demonstrating that the acetylation of cohesin is required for replication fork progression [55]. Whether Eco1 alone or in collaboration with Chl1 facilitates opening of a cohesin ring and passage of the replication fork followed by resealing of the ring to encircle both sisters (the ring model), or facilitates the tethering of new cohesin rings deposited in S-phase to pre-loaded rings as the replication machinery passes (the handcuff model) remains to be determined [56]. Interestingly, it has been suggested that although cohesion establishment, or rather re-establishment, during centromere breathing is Scc2–Scc4 independent, Eco1 is required, providing evidence that Eco1-dependent cohesion establishment can also occur in the absence of DNA replication [42].

Eco1 was shown to acetylate components of the cohesin complex in vitro some years ago [57], but the requirement for this activity in cohesion establishment was controversial [58]. However, recent studies have shown that the Smc3 subunit of cohesin is acetylated by Eco1 in vivo at two highly conserved lysine residues [59–61]. Mutation of both of these lysine residues within Smc3 inhibits acetylation and results in a lethal cohesion defect. Conversely, mutation of both lysine residues to asparagine or glutamine, which mimic the acetylated state, abolishes the requirement for Eco1 expression, an otherwise essential protein [61]. Acetylation of Smc3 by Eco1 only occurs at the onset of S-phase and is suppressed during the G1, G2 and mitotic phases [60]. This may provide a mechanism by which cohesin only becomes cohesive as the sister chromatid is synthesized. In addition to Eco1, a second acetyltransferase called San, which exists only in metazoans, has been shown to be important for stabilizing centromeric sister chromatid cohesion during mitosis [62]. Whether this acetyltransferase is important for establishment or maintenance of centromeric cohesion remains unclear. It is also possible that the acetyltransferase activity of San is required for the maintenance of centromeric cohesion during centromere breathing, a hypothesis that should be tested.

The cohesin-associated proteins Wpl1 [wings apart-like 1; Rad61 in S. cerevisiae, Wapl in Drosophila and Wapal in vertebrates] and Pds5 (precocious dissociation of sisters 5) together form a complex [63,64] and play a role in the inhibition of cohesion establishment, so called anti-establishment. In S. cerevisiae, it has been shown that the Wpl1–Pds5 complex associates with cohesin rings until Eco1-induced acetylation of the Smc3 subunit occurs, leading to the hypothesis that the Wpl1–Pds5 complex inhibits establishment when bound to cohesin and that Eco1 functions to inhibit this interaction by acetylating Smc3 during S-phase [65,66]. This function of the Wpl1–Pds5 complex appears conserved in human cells as processive fork movement has been shown to require acetylation-induced dissociation of Pds5 and Wapal [55]. In addition, depletion of Wapal has been shown to prevent removal of cohesin from chromosome arms during prophase, indicating that Wapal also functions to inhibit cohesion re-establishment in higher organisms [63,64]. Studies of Xenopus and human protein complexes have shown that Wapal associates with Pds5 and cohesin via FGF (Phe-Gly-Phe) motifs, which are predicted to bind HEAT [ huntingtin, elongation factor 3, the PR65/A subunit of PP2A (protein phosphatase 2A) and the lipid kinase Tor] repeat motifs found in Pds5 and the SA1/SA2 family of proteins. It is hypothesized that association of Wapal with cohesin in this manner may induce conformation changes in cohesin rings thereby facilitating release from chromatids during prophase, a function that may not exist in yeast [67].

COHESIN REMOVAL DURING MITOSIS

Once all chromatids are captured by the bipolar mitotic spindle and aligned on the metaphase plate, cohesion is dissolved by proteolytic cleavage of Scc1 by the activated cysteine protease separase, in a DNA-dependent manner [26,68]. Activation of separase results from degradation of its inhibitory binding partner securin by the ubiquitin ligase APC/C (APC/cyclosome), which is activated following satisfaction of the spindle assembly checkpoint [69] (see [70] for a review on the spindle assembly checkpoint). Although this has long been the established model of cohesin removal during mitosis, much of the work was performed in lower eukaryotes where evidence suggests that cohesin remains associated with chromatids until anaphase onset. In metazoas, cohesin is removed in a more complex and highly...
regulated two-step process. As cells progress from prophase to metaphase the cohesion on chromosome arms is lost in a pathway that is thought to be independent of Rad21 (Scc1) cleavage by separase. However, centromeric cohesion is protected and remains bound until anaphase onset when it is cleaved by activated separase to allow segregation of chromosomes to opposing spindle poles [34,71]. Loss of arm cohesion in metazoa is dependent on the activity of Plk1 (polo-like kinase 1) [72–74]. Plk1 can phosphorylate both the Rad21 and SA2 (Scc3) subunits of cohesin in vitro [72,73]. In S. cerevisiae it has been shown that phosphorylation of Scc1 by the Plk1 homologue Cdc5 (cell division cycle 5) enhances cleavage of Scc1 by separase [75,76] and hyperphosphorylation of Scc1 at the centromeres of metaphase chromosomes in Xenopus extracts has also been reported [77]. It has also been reported that phosphorylation of SA2 (Scc3) by Plk1 in vertebrate cells is important for the reduced affinity and dissociation of cohesin from chromosome arms during prophase [78].

Although the prophase pathway of cohesin removal was thought absent in lower eukaryotes, a recent study has shown that this may not be the case. Upon entry into mitosis, cohesin dynamics in S. pombe appear to be related to those observed in metazoa as the loss of a fraction of cohesin from chromosomes has been observed as these cells enter mitosis [79]. In higher eukaryotes, cohesin associates with chromatin in two distinct binding modes: one stable, established in S-phase and removed by Scc1 cleavage, and the other highly dynamic and thought to be required for transcription regulation and DNA damage repair, as discussed below [36]. This suggests that the existence of two distinct binding modes of cohesin throughout the cell cycle may be conserved in different model organisms. However, more work needs to be done to determine the role of these binding modes of cohesin in lower eukaryotes and whether similar pathways of cohesin removal during mitosis are in place in these model systems. In vertebrate cells, the cohesion-associated protein sororin may have an important role in regulating the stable association of cohesin and chromatin. Sororin has been shown to be important in sister chromatid cohesion and is degraded by the APC/C in G1 of the cell cycle [80]. Overexpression of sororin prevents resolution of the sister chromatids at anaphase onset and depletion results in precocious sister chromatid separation. In addition, depletion of sororin results in a significant reduction in the stably bound pool of cohesin that exists following cohesin establishment at S-phase [81]. In the light of this result, it has been suggested that sororin may function in the establishment of stably bound cohesin and that degradation as cells enter G1-phase might prevent the re-establishment of this stable pool of cohesin before initiation of DNA replication.

The active protection of centromeric cohesin until anaphase onset requires the recruitment of the ‘guardian spirit’ protein Sgo1 (Shugoshin 1; Mei-S332 (meiotic from Salaria 332) in Drosophila) to the centromeres during prophase [82,83]. Besides its role in the protection of centromeric cohesion, Sgo1 is important for both microtubule stabilization and microtubule-dependent tension between sisters [83] and has been shown to accumulate on kinetochores until anaphase onset when bipolar attachment and inner kinetochore tension is achieved. The role of Sgo1 in tension sensing may be through its ability to recruit Plk1 to the kinetochores as it has recently been shown that depletion of Sgo1 results in reduced accumulation of Plk1 on the kinetochores and a subsequent reduction in the Plk1-dependent 3F3/2 phosphoepitope, important in the tension-sensing mechanism [84]. The recruitment of Sgo1 to centromeres requires both the spindle checkpoint kinase Bub1 (budding uninhibited by benzimidazole 1) and the Aurora B kinase [84–88], which has been shown to phosphorylate Sgo1 in vitro [84]. Interestingly, depletion of Bub1 or Aurora B causes Sgo1 to localize along the length of the chromosomes rather than concentrate at the centromeres, suggesting that these kinases are required for defining the region on each chromosome where cohesion is to persist [84,86]. Interestingly, Aurora B has also been shown to play a role in the recruitment of separase to the chromosomes at early stages of mitosis [89]. Although the exact role of separase recruitment early in mitosis is unclear, the authors of that study suggest that separase may be required for the removal of arm cohesion during the prophase pathway, but until further studies are completed this remains speculative.

Centromeric cohesin is lost following depletion of Sgo1 by RNA interference, resulting in premature chromatid separation. Expression of a non-phosphorylatable SA2 protein in these cells prevents loss of centromeric cohesion, providing evidence that Sgo1 prevents phosphorylation of cohesin situated at the centromere [82]. Sgo1 binds to PP2A with high affinity and it is thought that Sgo1 prevents the phosphorylation of cohesin by recruiting PP2A, rapidly reversing phosphorylation of cohesin by Plk1, thereby inhibiting release [90–92]. Interestingly, prevention of Scc1 phosphorylation by PP2A has been shown to block cleavage of Scc1 only in the presence of Sgo1 [93] and it has been suggested that Sgo1-dependent recruitment of PP2A to unattached kinetochores inhibits separase activity [94]. Sgo1 protein levels peak in mitosis and it undergoes APC/C-dependent degradation at the onset of anaphase; it has been shown that APC/C-mediat ed Sgo1 degradation is necessary for mitotic exit and accurate chromosome segregation [95]. However, a recent study contradicts these findings and suggests that Sgo1 degradation is not crucial for progression through mitosis [96]. Alternative mechanisms to inactivate Sgo1 upon anaphase onset may exist and should be investigated before the true role of Sgo1 degradation in mitotic progression can be elucidated.

COHESIN AND THE CONTROL OF GENE EXPRESSION

In addition to its role in chromosomal segregation cohesin has long been thought to have other cellular functions. This initial hypothesis was sparked from evidence, at least in vertebrate cells, that cohesin re-associates with chromosomes at the end of mitosis, when conventional thinking would assume cohesin is absent from the DNA [34]. This re-association of cohesin occurs well before sister chromatid cohesion is established in the next cell cycle and suggests that cohesin may have a function independent of its role in cohesion. Furthermore, human diseases such as CdLS (Cornelia de Lange syndrome) and Roberts syndrome are associated with mutation of cohesin subunits such as Smc1 and Smc3, or the cohesin loader NIPBL [Nipped-B homologue (Drosophila)] (Scc2) [97–99]. These diseases have phenotypes that include growth and mental retardation, craniofacial anomalies and microcephaly, but surprisingly defects in cell cycling are limited and do not solely account for the severe phenotypes observed, suggesting that other functions of cohesin are disrupted. In addition, these mutations pose no increased predisposition to carcinoma, providing further evidence that cohesin has other cellular functions aside from its role in sister chromatid cohesion and accurate chromosome segregation.

The initial evidence that cohesin is important in the control of gene transcription came from a study showing that the positioning of cohesin on chromatin was dramatically affected by transcriptional activity in S. cerevisiae. ChIP (chromatin immunoprecipitation) experiments have revealed that the initiation of transcription at cohesin-binding sites results
in local disassociation of cohesin from the chromatin and that cohesin subunits relocate from their initial loading sites to places of convergent transcription [33,100]. These results suggest that as the transcription machinery travels along the DNA and encounters bound cohesin rings, the machinery is simply too large to pass through the rings and therefore pushes them until transcriptional termination. This phenomenon has been used to explain why cohesin molecules in yeast accumulate at sites of convergent transcription. An active function for the accumulation of cohesin at sites of convergent transcription was later discovered in S. pombe [101]. This study suggests that the accumulation of cohesin rings may act as a barrier between coding regions to aid efficient transcriptional termination. Convergent genes in S. pombe have been shown to generate overlapping transcripts in G1 due to inefficient transcriptional termination, whereas in G2, when cohesin is present, overlapping transcripts are undetectable. In support of this, loss of the HP1 (heterochromatin protein 1) family member Swi6 (switching deficient 6), required for proper targeting of cohesin to the centromeres, or cohesin itself in G2-phase, results in the presence of overlapping transcripts that are normally absent at this stage of the cell cycle [101]. Transcription termination is particularly important for genes arranged in a convergent orientation because failure of polymerase II complexes to terminate transcription results in inhibition of gene expression due to collision of the elongating complexes [102].

**Chromatin silencing**

Heterochromatin regions are, for the most part, transcriptionally silent and are believed to serve several functions from gene regulation to the protection of the integrity of chromosomes. These areas of DNA are transcriptionally silent because they are inaccessible to DNA-modifying enzymes and therefore transcription elongation is blocked [103]. In S. cerevisiae there are three classes of silent domains: the silent mating-type loci, HMR and HML, telomeres and rDNA (ribosomal DNA). The HMR and HML silent domains contain cryptic copies of the mating locus alleles Matα and Matα respectively. These genes at HML and HMR are not expressed but serve as a repository of genetic information, which upon transposition by the HO endonuclease causes yeast to switch from one mating type to another. The HMR and HML domains remain silent by the action of flanking regulatory elements known as silencers [104]. rDNA consists of a tandem array of 9.1 kb units repeated 100–200 times on chromosome XIII [105]. Such repeated DNA is subject to recombination and excessive recombination of the rDNA domain can be deleterious. Regulation of recombination is thought to involve Sir2 (silencing protein 2) [106]. Four SIR genes exist in yeast and were identified by mutations that activate the silent mating-type genes [107]. Sir2, Sir3 and Sir4 are essential for silencing because of their role as structural components of silenced chromatin [108]. Once these SIR proteins are recruited to a silencer, co-operative interactions enable them to spread throughout the silent locus, with Sir3 and Sir4 binding to the deacetylated tails of histones H3 and H4 [109].

Cohesin-binding sites have been discovered at silent loci in S. cerevisiae, including areas adjacent to telomeres and the HMR locus [110]. Interestingly, cohesin enrichment has also been reported at the LTR (long terminal repeat) of the Ty2 retrotransponson, which has been shown to contribute to boundary element function in the HMR boundaries [110]. The accumulation of cohesin at silent domains has led researchers to hypothesize that cohesin plays an important function in chromatin silencing. Indeed, there is accumulating evidence that cohesin plays a positive role in the formation of silent chromatin in yeast. Initially, it was shown that cohesin is recruited to the HMR locus in a Sir-protein-dependent manner [32]. This is in agreement with results showing that Sir2 mediates the association of Scc1 with rDNA [111]. The genes that encode rRNA are clustered in LTRs and genes with such a repeated structure are, in general, thought to be unstable because of a high frequency of recombination events. Sir2 plays an important role in decreasing the frequency of recombination of rDNA [106]. Interestingly, a decrease in the association of the cohesin subunit Scc1 to rDNA has been reported in Sir2 mutant strains [111]. Therefore it was proposed that Sir2 prevents unequal sister-chromatid recombination, probably by forming DNA structures with the aid of cohesin. Silent chromatin-mediated recruitment of cohesin might serve a similar function at the quiescent mating-type loci where post-replicative recombination between homologous sequences at HMR, HML and MAT could lead to unprogrammed mating-type switching or lethal chromosomal deletions. In support of this, cohesin mutant cells have shown such rearrangements [112].

The idea that cohesin may stabilize silent chromatin and prevent recombination has also been demonstrated in S. pombe [113]. The mating-type region contains three linked loci called MAT1, MAT2 and MAT3, where MAT2 and MAT3 serve as donors for MAT1 switching in a similar mechanism to the HMR and HML loci in S. cerevisiae. It has been shown that a discrete 20 kb region containing MAT2 and MAT3 is assembled into a heterochromatic structure and cohesin is recruited to the region in a Sir6-dependent manner. Although cohesin does not seem to affect Sir6 localization and silencing at the MAT locus, it has been shown that cohesin mutants produce switching-defective colonies at a rate nearly 100-fold that of the wild-type [113]. In addition, defects in the recruitment of cohesin in Sir6 mutants might be partly responsible for the increased rearrangement at the MAT locus. Taken together, these results suggest that cohesin is recruited to silent chromatin to regulate recombination and is essential to prevent unwanted recombination at these unstable regions (Figure 3).

Controversially, cohesin has also been shown to inhibit the establishment of silent chromatin. A study by Lau et al. [114] showed that silencing of the HMR domain is prevented by the presence of Scc1 during G2/M, even in the presence of the Sir proteins. Cleavage or repression of Scc1 during the cell cycle results in silencing of the HMR domain and inhibition of Scc1 cleavage represses silencing [114]. In addition it has been hypothesized that sequences near the border of silent and non-silent chromatin domains would include a boundary element. To test this, a number of target boundary elements were deleted to determine whether neighbouring reporter genes became inactive due of the spread of the silent chromatin from the HMR locus. Of the numerous chromosome structure proteins tested, only mutations in the cohesin subunits Smc1 and Smc3 were found to have significant effects on boundary function [115]. Smc proteins are reported to be part of the nuclear scaffold and are thought to be involved in chromosome loop organization [116], thus linking the insulator function of cohesin at the HMR with models in which insulator element function is dependent on chromosome architecture (Figures 4 and 5). The study by Donze et al. [115] suggests that proteins involved in higher-order chromosome structure might be involved in the functional delineation of the chromosome and that the formation of large multiprotein complexes at the boundary of silent chromatin prevents the spread of heterochromatin (Figure 3).
Cohesin regulation and function

Figure 3  Model for the role of cohesin at silent domains

Top panel: Scc1 associates with the silent domain preventing the establishment of silencing until the creation of cohesin-associated boundary elements. This prevents the unwanted spread of silent heterochromatin. Middle panel: following establishment of the boundary elements, Scc1 is cleaved and the spread of Sir proteins can occur. Bottom panel: once silent heterochromatin is developed, cohesin (orange rings) re-associates to stabilize the domain, preventing unwanted recombination.

Figure 4  Structure of the β-globin locus

Looping of the β-globin locus is created by the interaction of CTCF (green) and cohesin (orange rings), allowing for long-range spatial interactions between the LCR (dark blue) and active genes. The association of cohesin rings at the CTCF-binding sites within the 5’ and 3’ DNase I-hypersensitive regions creates as a barrier, preventing interaction of the β-globin locus and regulatory elements of neighbouring genes.

Control of transcription

In mammalian cells cohesin is not enriched at sites of convergent transcription but at DNase I-hypersensitive sites [117]. These sites have also been shown to contain an over-representation of the consensus sequence CTCF (CCCTC-binding transcriptional insulator protein) [117,118]. CTCF is a ubiquitously expressed protein that contains 11 zinc-finger motifs. Its expression is cell-cycle regulated, peaking at the S- to G2-phase [119]. Interestingly, depletion of CTCF disrupts the specific positioning of the cohesin subunits Rad21 (Scc1) and Smc3, but depletion of cohesin does not affect the positioning of CTCF [117]. CTCF-binding sites often flank groups of genes that are transcriptionally coregulated, suggesting that the majority of CTCF-binding sites function as insulators [120]. Insulators are genetic elements near the chromatin domain boundaries that are involved in the alteration of gene expression. They act as barriers that shield genes from position effects to prevent the spread of repressive heterochromatin from one domain to the next [121]. In addition, they can prevent communication between distant elements, such as enhancers, to influence gene expression, a function known as enhancer blocking [122].

There are a number of genes shown to be insulated by CTCF. The first set of genes that CTCF was found to insulate in an enhancer-blocking fashion was the β-globin gene locus which contains a number of developmentally regulated erythroid-specific genes. CTCF was found at the 5’ and 3’ chromatin boundaries of the chicken β-globin locus [123] and CTCF-binding sites have also been found to flank the equivalent human and mouse gene loci [124]. Enhancer-blocking assays showed that CTCF binding at the boundaries of the human and mouse gene loci results in transcriptional insulation by preventing inappropriate interactions between β-globin regulatory elements and those of neighbouring domains [124]. There is also evidence that CTCF mediates long-range chromatin looping in the β-globin locus (Figure 4) [125]. In mice, three CTCF-binding sites, two upstream and one downstream of the locus, are involved in spatial interactions between the LCR (locus control region) and the active β-globin genes, forming an active chromatin hub [126]. Disruption of CTCF binding at the downstream-binding site results in destabilization of the loop [125]. To determine whether cohesin is important for the insulator function of CTCF, cells were depleted of either CTCF or Rad21 protein and transcriptional changes measured by DNA-chip technology.
DMR resulting in enhancement of IGF2 gene expression from cis-acting enhancer elements. This results in abrogation of CTCF and cohesin binding. This allows interaction of DMR2 and elements resulting in insulation of the gene. (the formation of a loop between DMR1 and DMR, blocking the access of IGF2 to the enhancer association of CTCF (green) and cohesin rings (orange rings) with these domains. This leads to B

IGF2

like growth factor 2)/

The fact that it requires two of the CTCF-dependent insulator association blocks the gene from associated enhancer elements. Interestingly, the expression of genes regulated by CTCF and cohesin are also required for transcriptional insulation of the IL-3 (interleukin 3) and GM-CSF (granulocyte-macrophage colony-stimulating-factor) gene cluster. These genes are responsive to the same signals but are regulated independently by tissue-specific enhancers. Situated between the two genes are CTCF-binding sites that recruit both CTCF and cohesin to block interactions between the various enhancer and promoter elements allowing independent regulation of gene expression [136].

Figure 5 Arrangement of the IGF2/H19 locus and structural rearrangements resulting in maternal and paternal imprinting

(A) Situated between the IGF2 and H19 genes are three DMRs. Each gene is transcribed from a unique promoter (arrows). (B) The maternal locus contains unmethylated DMR allowing association of CTCF (green) and cohesin rings (orange rings) with these domains. This leads to the formation of a loop between DMR1 and DMR, blocking the access of IGF2 to the enhancer elements resulting in insulation of the gene. (C) The paternal locus contains methylated DMRs, which results in abrogation of CTCF and cohesin binding. This allows interaction of DMR2 and DMR resulting in enhancement of IGF2 gene expression from cis-acting enhancer elements. The formation of a loop within the DNA (Figure 4).

Similar transcriptional changes were observed after downregulation of CTCF or Rad21, and genes within 25 kb of cohesin-rich sites had a higher tendency to be up-regulated rather than down-regulated, consistent with the conclusion that cohesin mediates CTCF insulator function [118]. It has also been shown that cohesin plays a role in CTCF insulator function by the insertion of two copies of a known CTCF-dependent insulator between a neomycin gene and an associated SV40 (simian virus 40) enhancer and promoter elements. Depletion of CTCF in cells containing this construct resulted in an enhancement of neomycin expression and surprisingly a similar result was obtained following depletion of Rad21 [117]. This shows that cohesin is involved in CTCF-dependent transcriptional insulation via the creation of a barrier at CTCF sites, where cohesin association blocks the gene from associated enhancer elements. The fact that it requires two of the CTCF-dependent insulator sequences between the gene and enhancer/promoter elements to generate insulation suggests that cohesin creates the insulation by the creation of a loop structure within the DNA (Figure 4).

CTCF is also involved in imprinting of the IGF2 (insulin-like growth factor 2)/H19 (H19 fetal liver mRNA) mouse locus. The IGF2 and H19 genes are located on chromosome 7 within 90 kb of each other. These two genes are reciprocally imprinted in developing embryos with paternal expression of IGF2 and maternal expression of H19 [127]. A set of enhancers downstream of the H19 gene are involved in the regulation of expression of both IGF2 and H19 expression. It is thought that CTCF insulates this locus by binding to DMRs (differentially methylated regions) thus insulating the maternal IGF2 locus [128] (Figure 5B). The DMRs within the IGF2 locus bind to one another via interactions with CTCF and other proteins, creating loops that are involved in the insulation of the locus [129,130]. In support of this, the association of CTCF with DNA has been shown to be methylation-sensitive as methylation of DMR in the paternal locus prevents CTCF binding leading to a bypass of the H19 locus and activation of IGF2 expression (Figure 5C) [131]. To confirm these results, the effect of methylation on the H19/IGF2 locus was explored by Hcy (homocysteine) treatment, which induces hypomethylation of the DNA. Hcy treatment resulted in the hypomethylation of CTCF-binding sites upstream of the H19 locus and an increase in H19 mRNA along with decreased expression of IGF2 mRNA [132].

Cohesin is also involved in the CTCF-dependent insulation of the IGF2/H19 gene locus [133]. Using methylation-sensitive PCR, the cohesin subunit SA1 has been shown to associate with the CTCF-binding sites within the IGF2/H19 gene locus in an allele-specific manner. CTCF has previously been shown to be involved in interchromosomal looping at the β-globin [125] and IGF2/H19 loci [129]. Therefore it is likely that cohesin helps to create these loops by creating a bridge via the handcuff model discussed above (Figures 2, 4 and 5). The creation of interchromosomal loops at the IGF2/H19 locus by cohesin and CTCF has been confirmed using 3C (chromosome conformation capture) assays and RNA interference depletion of cohesin [134]. When cohesin was depleted, the higher-order chromatin conformation at the locus was disrupted, which in turn disrupted insulation of the IGF2 gene [134]. In addition, it has been shown that cohesin is required for long-range CTCF-dependent cis interactions at the developmentally regulated IFNG (interferon γ) cytokine locus [135]. Using 3C-based assays it was shown that the IFGN locus contains topological loops and depletion of either CTCF or cohesin resulted in the loss of these loops, thus demonstrating that CTCF and cohesin are both important for long-range interactions between DNA sites [135]. CTCF and cohesin are also required for transcriptional insulation of the IL-3 (interleukin 3) and GM-CSF (granulocyte-macrophage colony-stimulating-factor) gene cluster. These genes are responsive to the same signals but are regulated independently by tissue-specific enhancers. Situated between the two genes are CTCF-binding sites that recruit both CTCF and cohesin to block interactions between the various enhancer and promoter elements allowing independent regulation of gene expression [136].

Interestingly, a similar CTCF-dependent role for cohesin is seen in KSHV (Kaposi’s sarcoma-associated herpes virus). CTCF-binding sites exist within the major latency control region of KSHV and cohesin co-localizes with CTCF within this region, suggesting that CTCF and cohesin function as insulators in the KSHV genome [137]. Depletion of CTCF results in a loss of cohesin association and inappropriate expression of lytic genes. Interestingly, the expression of genes regulated by CTCF and cohesin is controlled in a cell-cycle-dependent manner and mutation of CTCF-binding sites results in a disruption of phase-dependent gene expression. This suggests that CTCF and the cohesin complex together play a role in regulating the control of cell-cycle-dependent gene expression during latency thus preventing disruption of host cell proliferation [138]. A similar role for CTCF in the prevention of inappropriate expression of
lytic genes has been shown in HSV1 (herpes simplex virus type 1), suggesting this may be a conserved mechanism that DNA viruses use to control gene expression [139,140].

COHESIN AND DNA DAMAGE REPAIR

In recent years it has become apparent that cohesin plays an important role in DNA damage repair. Eukaryotes have evolved two mechanisms for DNA damage repair: NHEJ (non-homologous end-joining) and HR (homologous recombination). HR involves the use of a homologous template to repair the damage. Typically the template is a sister chromatid, therefore HR occurs from S- to G2-phase. NHEJ entails the direct rejoining of DNA ends and is therefore error-prone, unlike HR. It is easy to envisage how cohesin can facilitate HR by keeping the homologous template in close proximity to the damaged DNA strand. Indeed, studies in yeast demonstrated that cohesion established in S-phase is required for efficient DNA damage repair in G2-phase [141]. Furthermore, DNA damage induces post-replicative cohesin loading in the region of a DSB (double-strand break) [142,143]. This was surprising as it had been shown previously that cohesin loading and cohesion establishment occurred in late G1- and S-phases respectively [38,144].

So what are the molecular components required for this process? Perhaps not surprisingly, the cohesin loading protein Scc2 is required for this damage-induced post-replicative cohesin loading [142,143]. In addition, the primary checkpoint kinases Mec1 (mitosis entry checkpoint 1) and Tel1 (telomere maintenance 1) are required, but act in a redundant manner [143]. Phosphorylation of histone H2AX by Mec1/Tel1 generates what is known as γ-H2AX. The γ-H2AX signal extends at least 60 kb from the break, whereas cohesin extends 40–50 kb from the break. Strains expressing a non-phosphorylatable H2AX fail to recruit cohesin thus suggesting that γ-H2AX may act as a signal for cohesin assembly. Mre11 (mitotic recombination 11), a component of the DNA-damage-sensing complex MRX [Mre11/Rad50/Xrs2 (X-ray-sensitive 2)], is also required for cohesin assembly around the DSB but it does not abrogate γ-H2AX formation, indicating that Mre11 is involved in an independent pathway [143]. In addition to cohesin loading to the site of a DSB, DNA damage also induces post-replicative cohesion establishment [142,145] and it has been shown that DNA-damage-induced cohesion is established genome-wide and is not restricted to the break site [146,147]. Curiously, damage-induced cohesion requires Eco1 and Scc2 suggesting that newly loaded cohesin is used to establish damage-induced cohesion.

In S. cerevisiae, mitotic and meiotic cohesin contains different kleisin subunits, Scc1 and Rec8 (recombination 8) respectively. Substitution of Scc1 with Rec8 in mitotic cells results in impaired DSB repair [148]. Crucially Rec8 cohesin fails to become cohesive after DNA damage. Further analysis revealed that Chk1 (checkpoint kinase 1)-mediated phosphorylation of Ser3 of Scc1 is a critical determinant for DSB-induced cohesion. DSB-induced phosphorylation of Ser3 primes Scc1 for acetylation by Eco1 and antagonizes the anti-establishment activity of Wpl1 (see above) [149]. S-phase cohesion, on the other hand, depends on acetylation of Smc3. Thus Eco1-mediated acetylation of distinct targets, depending on the cell cycle phase, facilitates cohesion establishment by antagonizing Wpl1.

During an unperturbed cell cycle, the active site of separate is blocked by the protein securin and the destruction of securin at anaphase facilitates cohesin cleavage [69]. In response to DNA damage the yeast homologue of securin, Pds1, is stabilized, which presumably preserves cohesion until the cell-cycle checkpoint is activated and/or the damage repaired [150]. Pds1 stabilization is mediated by the cell-cycle checkpoint kinases Chk1 and Rad53. Chk1-dependent phosphorylation prevents Pds1 ubiquitination by the APC, whereas Rad53 prevents Pds1 from interacting with the APC-associated protein Cdc20 [151]. It has also been suggested that dephosphorylation of Pds1 occurs following repair of DNA damage [151].

As mentioned previously, DNA damage induces cohesin loading and cohesion establishment. These studies were performed using S. cerevisiae in which a single DSB was generated within the MAT locus on chromosome III by induction of the HO endonuclease [143]. However, a recent study in S. pombe found that an epitope-tagged version of Rad21 (Scc1) was not loaded around the DSB site [152]. Whether this truly reflects divergence in the DNA damage response between fission and S. cerevisiae or if it reflects differences in assay conditions is unclear. It will be interesting to see if DSB-induced cohesion occurs in S. pombe. Adachi et al. [152] identified multiple Rad21 phosphorylation sites in extracts from asynchronous and mitotically arrested cells. One of these phosphorylation sites, Ser553, may have a role in the DNA damage response. Following exposure to UV damage Ser553 was phosphorylated in a Rad3 (which is homologous to Mec1)-dependent manner. Phosphorylation of Ser553 was observed ~2 h later than the γ-H2AX signal, which suggests that phosphorylation of Ser553 may have a role in re-entry into the cell cycle after checkpoint arrest or mitotic exit. Rad3-dependent phosphorylation of Rad21 following UV exposure had been demonstrated previously, although the phosphorylation sites were not determined [153]. Although the signalling pathway may vary, Rad21/Mcd1 phosphorylation following DNA damage occurs in both fission and S. cerevisiae. It remains to be seen if damage-induced Rad21 phosphorylation mediates Ecol acetylation and subsequent cohesion, as has been illustrated for S. cerevisiae [149].

In vertebrate cells there is evidence that cohesin is recruited to DSBs. Remarkably, cohesin recruitment to DSBs is dependent on another SMC complex, Smc5–Smc6 [154]. In S. cerevisiae the Smc5–Smc6 complex has also been shown to co-localize with cohesion on undamaged chromosomes [155]. Furthermore, Smc5–Smc6 is required for DNA damage repair and has a specific role in HR [156]. However, studies using fission and S. cerevisiae present conflicting evidence on the requirement of the Smc5–Smc6 complex for cohesion recruitment to DSBs [146,157]. In S. cerevisiae Smc6 is required for damage-induced genome-wide cohesion, but not for damage-induced cohesion loading, whereas in S. pombe, cohesion is still recruited to DSB regions in the absence of functional Smc6 [146,157]. The interpretation of these studies is further complicated by the finding that depletion of Smc5 [and Mms21 (mitochondrial splicing system 21) a non-SMC subunit of the Smc5–Smc6 complex] results in severe chromosome misalignments, whereas Smc6 depletion does not. This suggests that Smc5 function is not always dependent on its interaction with Smc6 [158]. Further study of this somewhat obscure Smc5–Smc6 complex will help to clarify its role in DSB repair and cohesion dynamics (reviewed in [159]).

There are other lines of evidence that highlight the role of cohesion at DSBs. Cohesin recruitment to DNA damage was illustrated by immunofluorescent staining of Scc1 and SA1 at the site of laser-induced DNA damage [160]. However, in a subsequent report cohesin accumulation could not be detected following DNA damage induced by IR (ionizing radiation), but was only detected when a high-energy laser was used [161]. The authors suggest that such a high laser intensity induces local disruption of the nuclear structure and does not reflect
classical IRIF (IR-induced foci). Indeed, when moderate IR doses (3–10 Gy) were used, focus formation of DNA damage checkpoint proteins [e.g. γ-H2AX, ATM (ataxia telangiectasia mutated), BRCA1 (breast cancer early-onset 1) and 53BP1 (p53-binding protein 1)] was observed, but not cohesin subunits [161]. This seems to contradict the study by Potts et al. [154], which demonstrated Smc5–Smc6-dependent cohesin recruitment to DSBs in an ChIP assay; however, these conflicting results may reflect a difference in sensitivity between the assays used. A recent study has shown that cohesin subunits localize to IR-induced DSBs only during S- and G2-phases and not at G1 [162]. This suggests an active role of the cohesin complex in the repair of DSBs that may be unique to the G2-phase of the cell cycle and hence replicated chromatin [162]. That study may offer some explanation for the discrepancies described above and the cell-cycle-specific recruitment of cohesin to DSBs should be more carefully studied.

Interestingly, it has been shown that Smc1 is phosphorylated at Ser957 and Ser966 following IR and UV damage, mediated by ATM and ATR (ATM- and Rad3-related) respectively [163–166]. Phosphorylated Smc1 localizes to γ-H2AX foci, which mirrors the finding in S. cerevisiae that cohesin is loaded on to DSBs in the same region as the γ-H2AX signal [143,161,167,168]. Phosphorylation of Smc3 also occurs following IR in an ATM-dependent manner [169]. Mutations in Smc1 or Smc3 that prevent phosphorylation result in abrogated DNA damage responses, although the exact mechanism for this remains unclear [167,169]. It is tempting to speculate that ATM-dependent phosphorylation of cohesin subunits generates sister chromatid cohesion; however, evidence of DNA-damage-induced cohesion in mammalian cells has been lacking. A recent study shows that inter-sister chromatid distances were reduced following DSB induction [170]. Although a direct role for cohesin could not be demonstrated, disruption of the ATM locus resulted in inter-sister chromatid distances similar to undamaged cells [170]. This implies that following DNA damage, ATM phosphorylates cohesin leading to cohesion establishment. Further research is required to test this, and indeed whether the proposed DNA-damage-induced cohesion is restricted to the site of the DSB.

Like Pds1, securin is also involved in the DNA damage response. Securin interacts with p53 and represses p53 transcriptional activity [171]. Securin also interacts with the NHEJ proteins Ku70 and Ku80 [172]. In contrast with yeast, DNA damage induces degradation of human securin [173,174]. Given that securin degradation triggers release of separase and subsequent cohesin cleavage it may seem surprising that securin is degraded following DNA damage. However, securins are required for separase activity and localization, suggesting that securin is not merely an inhibitor of separase [175,176]. Indeed, securin has a well-established role in the pathogenesis of pituitary tumours. The involvement of securin in tumour development is perhaps a product of its roles in cohesin dynamics, DNA damage repair and its more recently identified role as a transcription factor which specifically regulates the G1-to-S transition [177].

COHESIN AND HUMAN DISEASES

Two human diseases known as cohesinopathies are characterized by mutations in cohesin subunits and cohesin establishment factors: CdLS and Roberts syndrome. An interesting feature of these diseases is that mutation of the cohesin complex has a limited affect on sister chromatid cohesion and cell cycling and therefore may be caused by changes in gene expression. This hypothesis is supported by a study in S. cerevisiae in which mutations were made that recapitulate the mutations found in cohesinopathies. These mutations had little effect on sister chromatid cohesion or cell viability but led to defects in transcription control, gene silencing and sub-nuclear organization of chromatin [178]. CdLS is caused by mutations in either the NIPBL gene, which, as described above, encodes the human orthologue of Nipped B/ScC2 [98], or in the Smc1- or Smc3-encoding genes [99]. In Drosophila, Nipped B has been shown to alleviate cohesin-mediated blocking of enhancer–promoter communication by assisting in long-range enhancer–promoter interactions. Nipped B and cohesin colocalize to active transcription units and examination of cohesin-binding sites has shown that cohesin preferentially binds to the promoter region of genes [179]. Binding of cohesin to these sites is significantly reduced in NIPBL-mutant CdLS patient samples resulting in up-regulation of cohesin-associated genes [180]. Mapping of the Smc1 and Smc3 mutations found in a number of CdLS patients on to the known structures of the SMC hinge domain from Thermotoga maritima [181] and the S. cerevisiae Smc1 head domain [182] has shown that the mutations predominantly mapped to the coiled-coil and hinge domains of the proteins [97]. These mutations are thought to either cause angulation of the proteins disrupting the approximation of the SMC head domains, or interfere with the binding of accessory proteins to the cohesin ring, such as Scc2, which would abolish loading of cohesin onto chromatin [97].

Roberts syndrome is caused by the mutations in the ESCO2 gene, the human homologue of S. cerevisiae Ecol [183]. Cells derived from Roberts syndrome patients have been shown to have defects in sister chromatid cohesion [184], characterized by heterochromatin repulsion [185], suggesting that Esco2 may be important in the establishment of cohesin at heterochromatic regions. Interestingly, the genetic mutations found in Roberts syndrome patient samples are predominantly associated with loss of the acetyltransferase activity of Esco2 [186], providing evidence that this activity is indeed important for cohesion establishment. Interestingly, replication forks in Roberts syndrome cells are slowed, demonstrating that fork progression is regulated by acetylation of cohesin and loss of this mechanism results in the accumulation of DNA damage, which could contribute to the abnormalities observed in Roberts syndrome [55].

SUMMARY

For a number of years cohesin has been seen as the master regulator of chromosome segregation. This tightly regulated function of cohesin has been explored in depth but there are still many unanswered questions as to how cohesin is loaded on to and associates with DNA. Whether ATP hydrolysis is required for hinge opening, Sec1 binding or Sec1 dissociation needs to be definitively answered. In vitro single molecule studies of fluorescently tagged proteins would enable determination of whether ATP binding and hydrolysis occur simultaneously with hinge opening and/or Sec1 dissociation. In addition, careful analysis of the timing of ATP hydrolysis within the cell cycle would help to determine whether hydrolysis is required for loading on to chromatin prior to S-phase or for replication-coupled cohesion establishment. Determining the stage at which ATP hydrolysis occurs may also shed light on how cohesin holds DNA strands together. If ATP hydrolysis is indeed required for hinge opening and occurs during DNA replication, one could hypothesize that this reflects ring opening as the replication fork progresses and would imply sister chromatid cohesion by single
cohesin rings rather than a higher-order complex as described in the handcuff model. The molecular trigger for ATP hydrolysis is also unclear. Determining the molecular events that lead to ATP binding and hydrolysis will provide new insight into the requirement for ATP in the multiple functions of cohesin. Possible stimuli could include post-translational modification of one of more cohesin complex subunits or interaction with other proteins at specific stages of the cell cycle. Indeed interaction with the cohesin loader Scc2/Nipped-B appears to promote ATP hydrolysis, although the specific mechanism of action remains unknown. It is important to determine whether Scc2 has inherent enzyme activity or whether it functions in the cohesin loading pathway simply by recruiting other proteins to the cohesin complex. It would also be interesting to determine the timing and localization of the Scc2 interaction with cohesin and whether this only occurs during DNA replication.

Whether the handcuff or ring model, or both, accurately reflect the mechanism by which chromatin is captured by cohesin in vivo remains unresolved. FRET and cross-linking studies would suggest the ring model to be most accurate, but such biochemical assays could be misinterpreted in the context of the whole cell. Studies showing self interaction of cohesin subunits suggest that cohesin forms a higher-order complex and thus holds chromatids together via the handcuff model. It is of course possible, and highly likely, that both models are correct and that cohesin can associate with chromatin in multiple ways depending on chromatin structure and specific function (cohesion, transcription control or DNA damage repair). The biochemical assays that have been used to study the molecular architecture of cohesin rings on DNA should be utilized to specifically isolate the structure of cohesin complexes involved in transcriptional control and gene silencing compared with cohesin rings that are involved in sister chromatid cohesion. Any differences observed will help to determine whether cohesin rings do indeed self-associate to hold chromatin together and whether this is a feature of cohesin specifically involved in sister chromatid cohesion. We hypothesize that there are multiple forms of cohesin that each have their own function and differ in their specific mechanism of association with DNA.

In addition to sister chromatid cohesion, cohesin has a major role in DNA damage repair and gene regulation. The mounting evidence shows that cohesin accumulates at sites of damage and mutation of cohesin subunits results in impairment of repair. The majority of these studies have been performed in yeast and work in mammalian cells has produced conflicting results. This is probably due to differing complexity in the pathways involved in DNA damage repair between higher and lower eukaryotes because the cohesin proteins themselves appear highly conserved between yeast and humans. Differences in the mechanism of cohesin-mediated transcriptional insulation in higher and lower eukaryotes have also been highlighted. Whereas in lower eukaryotes cohesin is enriched at sites of convergent transcription, in higher eukaryotes, cohesin appears to be actively recruited to DNAse I-hypersensitive sites by CTCF, a protein that has no known homologue in lower eukaryotes. It is clear that the association of cohesin with chromatin is vitally important in the control of gene transcription has helped explain the defects observed in cohesinopathies, such as CcdLS and Roberts syndrome. As these syndromes are characterized by developmental defects, with little or no association with cancer predisposition, it is hypothesized that the insulator function of cohesin is significantly disturbed, contributing to the phenotypes seen, whereas the impact on sister chromatid cohesion and chromosome segregation is less severe. These newly emerging and novel functions demonstrate that cohesin is not only essential for chromosome segregation, but is the master regulator of multiple aspects of genome integrity in all eukaryotes.

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Cohesin regulation and function


Received 28 January 2010/12 March 2010, accepted 18 March 2010
Published on the Internet 13 May 2010, doi:10.1042/BJ20100151

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