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Establishing and dissolving cohesion during the vertebrate cell cycle

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Replicated chromatids are held together from the time they emerge from the replication fork until their separation in anaphase. This process, known as cohesion, promotes faithful DNA repair by homologous recombination in interphase and ensures accurate chromosome segregation in mitosis. Identification of cohesin thirty years ago solved a long-standing question about the nature of the linkage keeping together the sister chromatids. Cohesin is an evolutionarily conserved complex composed of a heterodimer of the Structural Maintenance of Chromosomes (SMC) family of ATPases, Smc1 and Smc3, the kleisin subunit Rad21 and a Huntingtin/EF3/PP2A/Tor1 (HEAT) repeat domain-containing subunit named SA/STAG. In addition to mediating cohesion, cohesin plays a major role in genome organization. Cohesin functions rely on the ability of the complex to entrap DNA topologically and in a dynamic manner. Establishment of cohesion during S phase requires coordination with the DNA replication machinery and restricts the dynamic behaviour of at least a fraction of cohesin. Dissolution of cohesion in subsequent mitosis is regulated by multiple mechanisms that ensure that daughter cells receive the correct number of intact chromosomes. We here review recent progress on our understanding of how these processes are regulated in somatic vertebrate cells.

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Introduction

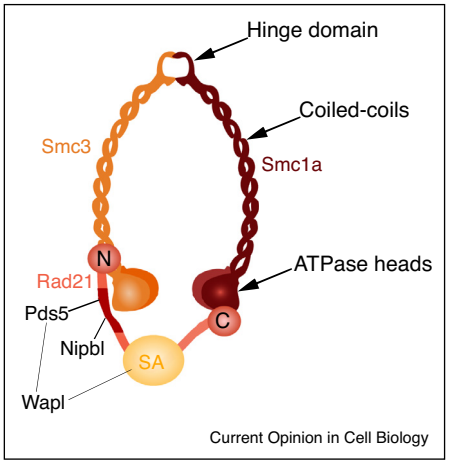
Cohesin was identified as the key mediator of sister chromatid cohesion in yeast [1,2] and vertebrate cells [3]. It consists of Smc1 and Smc3, Rad21 and a SA/STAG. Smc1 and Smc3 belong to the SMC family of chromosomal ATPases, conserved in all kingdoms of life. SMCs are rod-like proteins with a hinge domain on one end and an ATPase head domain on the other, separated by long anti-parallel coiled coils (Figure 1). Hinge–hinge interactions lead to the formation of a V-shaped SMC heterodimer. The C-terminal and N-terminal domains of the kleisin subunit Rad21 bind near the Smc1 and Smc3 head domains, respectively, to create a tripartite ring that can entrap the DNA topologically while the SA subunit associates with the middle region of Rad21 [4–6]. The ring-shaped structure of cohesin complexes purified from cell extracts or assembled from recombinant proteins has been observed by electron microscopy [7,8]. Somatic cells contain two versions of cohesin that carry Smc1a, Smc3, Rad21 and either SA1 or SA2 [9]. Two HEAT repeat domain-containing proteins associate with cohesin during most of the cell cycle to modulate its association with chromatin, Pds5 and Wapl [10–12]. A third HEAT protein, Nipbl, loads cohesin on chromatin and may be also required for its translocation along DNA, as discussed below. In this way, cohesin resembles its related complex, condensin, formed by a heterodimer of SMC subunits (Smc2 and Smc4), a kleisin (CAP-H/H2) and two HEAT repeat proteins (CAP-D2/D3 and CAP-G/G2) [13].

Cohesion mediated by cohesin is important for faithful chromosome segregation and contributes to homologous recombination (HR)-mediated DNA repair after DNA replication. Cohesin has additional functions in chromosome organization and for this reason the complex is present in all cells, including those that do not proliferate. Together with CTCF, Mediator and other transcriptional regulators, cohesin plays a major role in genome folding and gene regulation [14]. In this review, however, we focus on recent progress in our understanding of how cohesin mediates sister chromatid cohesion in vertebrate cells. Although many key aspects of the process are conserved in yeast and other eukaryotes, regulation of cohesion in vertebrates presents also important singularities.

Cohesin loading and unloading

Cohesin is loaded on chromatin as vertebrate cells exit from mitosis in a reaction facilitated by the heterodimer formed by Nipbl and Mau2 (Scc2 and Scc4, respectively, in budding yeast), which promotes ATP hydrolysis at the Smc heads [15–17]. *In vitro*, cohesin can associate topologically with DNA without the loader, although rather inefficiently [18]. Entrapment of the DNA fiber has been proposed to occur through an 'entry gate' formed by the Smc1/3 hinges, at least *in vivo* [19 ("a" in Figure 2, left). The SMC coiled coils may transmit conformational

Figure 1



Ring-shaped structure of cohesin complex in vertebrates. Smc proteins fold back on themselves to form a rod-shape molecule with the hinge domain on one side and the ATPase domain on the other connected by the coiled-coil domain. Smc1 and Smc3 interact through their hinges while the kleisin subunit Rad21 bridges the two head domains. The SA subunit associates with the middle region of the kleisin. Main interaction sites for regulatory factors Nipbl, Pds5 and Wapl are indicated. Nipbl and Pds5 bind the same region in Rad21 (darker tone).

changes caused by ATP hydrolysis at the head domains to promote hinge opening, as preventing post-translational modification of Lysine residues present along the coiled coils affects both association of the SMC heterodimer with the kleisin subunit and cohesin loading on DNA [20].

Pds5 and Wapl are not stoichiometric components of the cohesin complex. Wapl promotes cohesin unloading [10,11], which requires opening of an 'exit gate' located in the interface between Smc3 head domain and the N terminus of Rad21 [5,8,21,22] ("c" in Figure 2, left). Pds5 also participates in this reaction [23], which has been reconstituted *in vitro* [24••]. In addition to the two aforementioned 'gates', a third 'inner gate' is created by the interaction of Smc1 and Smc3 head domains, and is regulated by ATP binding and hydrolysis ("b" in Figure 2, left). In this way, DNA can be present in two different locations inside ring, between the coiled coils or between the closed inner gate and the kleisin [24••,25•]. It has been proposed that DNA entrapped between the coiled coils could be sensed by two conserved Lysines located on the Smc3 head domain unless modified by acetylation and would promote ATP hydrolysis and nucleotide release, driving the two Smc heads apart to allow passage of the DNA [25•,26,27•]. Next, dissociation of the Smc3–Rad21 interaction driven by Wapl–Pds5 would occur after new ATP molecules bind the Smc heads to close the inner gate and DNA would be released from the cohesin ring. A more thorough description of this model can be found in [28•].

Before DNA replication, cohesin is constantly being loaded by Nipbl–Mau2 and released by Wapl–Pds5. Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) analyses in mammalian cells show the presence of Nipbl around promoters of actively transcribed genes [29]. The little colocalization of Nipbl and cohesin along the genome suggests that cohesin moves away from its loading sites either by itself or pushed by other translocases such as RNA polymerase II [29]. A recent hypothesis proposes that cohesin — and other SMC complexes — can extrude DNA and thereby generate DNA loops that promote long-range chromatin contacts [30,31]. Movement of cohesin along DNA by passive diffusion has been recently observed *in vitro* using single molecule imaging [32•,33•]. How well these assays reflect cohesin dynamics *in vivo* is uncertain since they are performed on naked DNA and do not require ATP or the cohesin loader. A third study showed cohesin complexes loaded by Nipbl–Mau2 and moving along DNA in an ATP-dependent manner [34••]. Addition of Wapl–Pds5 reduced translocation but the underlying reason remains unclear. It is possible that binding of these two proteins promotes a conformation of cohesin that inhibits translocation while favouring cohesin release, for example, with the inner gate closed.

If cohesin translocation and/or loop extrusion requires the energy of ATP hydrolysis, Nipbl could be important to enhance the inefficient cohesin ATPase. Consistent with this possibility, Nipbl–Mau2 are required for loop extension [35] and imaging experiments suggest that Nipbl binds to cohesin as it translocates along DNA, 'hopping' from one complex to another [36]. Structural data indicate that Pds5 and Nipbl bind the same region of Rad21 [37•] (highlighted in Figure 1). Thus, interaction of Nipbl with cohesin could promote translocation also indirectly by displacing Pds5 and preventing in so doing cohesin release. This hypothesis is consistent with fluorescent recovery after photobleaching (FRAP) data showing that Pds5 and Wapl bind dynamically to cohesin [38].

Cohesion establishment

Cohesion establishment takes place during DNA replication in a process that requires acetylation of Lysines K105 and K106 in the Smc3 head domain and interaction of cohesin with a protein known as sororin [39] (Figure 2, middle). These two events are coupled and must take place in the context of DNA replication in order to achieve cohesion [38,40]. Sororin binds Pds5 and functions to counteract Wapl–Pds5 releasing activity [12,39]. In this way, a fraction of cohesin tethering the two sister chromatids is stabilized [41]. Since the binding of sororin to cohesin is highly dynamic — as that of Pds5 — it is likely that the requirement of DNA replication for sororin recruitment depends on a property that cohesin acquires during cohesion establishment, for example, a conformational change that allows the complex to embrace two