DNA binding to SMC ATPases—trapped for release

Herwig Schüler¹ & Camilla Sjögren²

The SMC/Rad50/RecN proteins are universal DNA-associated ABC-type ATPases with crucial functions in genome maintenance. New insights into Rad50–DNA complex structure and cohesin regulation inspire a speculative look at the entire superfamily. Identification of a continuous DNA binding site across the Rad50 dimer interface (Liu *et al*, 2016; Seifert *et al*, 2016) suggests a similar site in cohesin. The localization of this site hints a DNA-activated mechanism for cohesin removal from chromosomes.

See also: Y Liu *et al* (April 2016) and
FU Seifert *et al* (April 2016)

The Smc proteins form the core of multi-subunit complexes that are essential for genome stability. The cohesin complex is needed for sister chromatid cohesion and is built around heterodimers of Smc1 and Smc3, while the MRN (Mre11–Rad50–Nbs1) DNA repair complex contains homodimers of the Smc protein Rad50. The large and elongated Smc proteins have Walker A and B motifs at their globular ends, and are folded on themselves at a central hinge domain (called hook in Rad50). This leads to the formation of an ATPase-containing head which is connected to the hinge through an antiparallel coiled coil. Within the complex, the Smc proteins bind each other at the hinge/hook, and two ATP molecules are sandwiched between their heads. The heads are also sites for interactions with additional subunits of the complexes, such as the nuclease Mre11 in MRN, and Scc1 in cohesin (Fig 1A and B).

Compelling evidence indicates that cohesin creates cohesion through topological

entrapment of the chromatid pair within the complex's ringlike structure. It is loaded onto chromosomes by the Scc2–Scc4 complex in a reaction that requires the ATPase activity of Smc1 and Smc3. Cohesin then interacts with chromosomes in a dynamic manner until two conserved lysines in the Smc3 head get acetylated by the acetyltransferase Eco1. This prevents unloading of the complex by the regulatory protein Wapl, leading to stable entrapment of DNA, which is essential for establishment of cohesion. Four recent studies published in *eLife*, *Cell*, and *Molecular Cell* describe a role for the ATPase activities of Smc1 and Smc3 in unloading, highlighting how cohesin could be stabilized on chromosomes (Camdere *et al*, 2015; Murayama & Uhlmann, 2015; Beckouet *et al*, 2016; Elbatsh *et al*, 2016). In parallel, two articles in *The EMBO Journal* describe the atomic details of the interaction of Rad50–Mre11 with double-stranded DNA (Liu *et al*, 2016; Seifert *et al*, 2016).

The new structures of the Rad50–DNA complexes from two thermophiles, an archaeon and a eukaryote respectively, reveal similar modes for DNA binding (Fig 1A; Liu *et al*, 2016; Seifert *et al*, 2016). The DNA interaction sites are located in regions of the Rad50 head domains on the inside of the ring structure. Basic surface patches contribute to binding, but the DNA-interacting residues are not strictly conserved among Rad50 orthologs, and DNA binding is sequence-independent. Both Rad50 heads are engaged in DNA association, although the binding is of an asymmetric nature. Moreover, both groups show that some of the DNA–protein interactions can only form

in the ATP-bound state of Rad50, and association of a non-hydrolyzable nucleotide analog was required to obtain both DNA complex structures. Providing further insights into the first step of DNA repair by MRN, Seifert *et al* (2016) also show that Rad50 binds tightest to a continuous duplex or DNA with complementary 3' overhangs, and Liu *et al* (2016) propose a model for DNA duplex unwinding by Rad50:ATP.

It is known that DNA activates SMC ATPases, and this activation likely requires direct DNA–protein interaction. The analyses performed by the groups of Hopfner and Cho suggest that ATP hydrolysis will disrupt the DNA binding site in Rad50 and allow access of the Mre11 subunits, located at the base of the heads, to DNA (Liu *et al*, 2016; Seifert *et al*, 2016). Similarly, a first step in the unloading of cohesin could be DNA-activated ATP hydrolysis, and subsequent release of DNA through disengaged Smc1–Smc3 heads.

When superimposing the crystal structures of the Smc1 and Smc3 head domains (Haering *et al*, 2004; Gligoris *et al*, 2014) onto the Rad50–DNA complexes (Fig 1B), the DNA binding sites of the two Rad50 head domains coincide with two basic patches on Smc1 and Smc3. Interestingly, the putative Smc3 DNA binding site contains the two lysines that are targets for the Eco1 acetyltransferase (Fig 1C). Acetylation of the lysines in the basic patch is expected to prevent DNA binding, which in turn could preclude ATPase activation and DNA release. Such a mechanism gains support from *in vitro* analysis of cohesin which indicates a role for ATP hydrolysis in cohesin unloading (Murayama & Uhlmann, 2015). Using Smc3 mutants suggested to

¹ Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

² Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden. E-mail: camilla.sjogren@ki.se
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Figure 1. DNA binding in SMC ring complexes.

(A) Simplified schematic model of the MRN-DNA complex based on a superposition of the structures of *Chaetomium thermophilum* Rad50 (PDB entry 5DAC; Seifert *et al.*, 2016) and the *Methanocaldococcus jannaschii* Rad50-Mre11 complex (PDB entry 5DNY; Liu *et al.*, 2016). (B) Simplified schematic model of the cohesin complex based on a superposition of the individual structures of budding yeast SMC1/Scc1 (1W1W; Haering *et al.*, 2004) and SMC3/Scc1 (4UX3; Gligoris *et al.*, 2014) onto the *Mj*Rad50-DNA complex. The center section of Scc1 (4PK7; Hara *et al.*, 2014) was added solely to indicate Scc1 bridging of the two head domains. The DALI server (Holm & Rosenström, 2010) aligned 318 SMC1 residues and 355 SMC3 residues to *Mj*Rad50 with root-mean-square differences (α -positions) of 3.6 Å and 6.3 Å, respectively. (C) Electrostatic surface rendering of the putative DNA binding site of cohesin. As in the Rad50 structures, the number of DNA-interacting side chains appears rather limited. A basic patch in SMC3 that is in contact with DNA contains Eco1 target residues K112, K113 (arrows). The coiled-coil regions of SMC1 and SMC3 were omitted for clarity. The bound nucleotides are shown (with green carbons for SMC1-bound ATP γ S, and yellow carbons for SMC3-bound ATP γ S) to indicate the position of the SMC1-SMC3 interface.

mimic lysine acetylation at the Eco1 site, Murayama and Uhlmann also provide supportive evidence for the idea that acetylation of these residues inhibits DNA-activated ATP hydrolysis by cohesin. Similarly, work from the three other groups indicates that mutations interfering with cohesin's ATPase activity create Wapl-resistant cohesin complexes (Camdere *et al.*, 2015; Beckouet *et al.*, 2016; Elbatsh *et al.*, 2016). Their analyses also suggest that the structural asymmetry of SMC1 and SMC3 heads leads to functional differences between these two ATPases. Based on the structure of DNA in complex with Rad50, it could be that the asymmetric DNA binding to SMC1 and SMC3 also contributes to these differences.

The Uhlmann and Nasmyth groups have also investigated the interaction between SMC3 and the N-terminal part of Scc1, the so-called kleisin subunit, which bridges SMC1 and SMC3 heads. This interaction creates an extra barrier, apart from the interacting SMC1-SMC3 heads, that needs to be opened for release of DNA from cohesin. This step is stimulated by Wapl (Murayama & Uhlmann, 2015; Beckouet *et al.*, 2016), and *in vitro* analysis suggests that dissociation of Scc1 from

SMC3 requires ATP, but not its hydrolysis (Murayama & Uhlmann, 2015). These findings could suggest that the heads need to re-associate in order to allow release. Investigations *in vivo* reveal a more complicated situation; some, but not all, cohesin mutants that are proficient for ATP binding, but not hydrolysis, stabilize the interaction between Scc1 and SMC3 (Beckouet *et al.*, 2016; Elbatsh *et al.*, 2016). The emerging picture indicates that a specific conformational state, dependent on the SMC head interaction surface, is responsible for DNA release (Beckouet *et al.*, 2016). With this in mind, and considering the many regulators of cohesin not mentioned here, it is clear that there is still much to explore to reach full understanding of the complex's association with chromosomes.

In the future, the endeavor to understand SMC/Rad50/RecN protein mechanisms will involve continued mapping of large-scale structural rearrangements and their regulation. But atomic-resolution freeze frames of these processes are painstakingly difficult to obtain. Thus, the new Rad50 structures (Liu *et al.*, 2016; Seifert *et al.*, 2016) provide another welcome landmark with bearing on the entire superfamily.

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