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Architecture of SMC–kleisin complexes

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Summary

In all cellular systems, the transmission of bulk genetic information during proliferation occurs in the form of chromosomes. The segregation of these entities upon cell division is of pivotal importance for all forms of life. Structural maintenance of chromosomes (SMC)–kleisin complexes are ubiquitous and essential factors that ensure proper organisation and segregation of the genetic material. Aim of this work was to elucidate evolutionary conserved features in the architecture of SMC–kleisin complexes, and to probe these features for functional relevance. We find that two major architectural themes have been constrained by evolution: (I) SMC–kleisin complexes form asymmetric assemblies with a ring-like topology, whereby a kleisin monomer bridges two different binding sites on a SMC dimer, (II) SMC–kleisin complexes form rod-like structures, whereby the SMC proteins of a given dimer are closely juxtaposed in a well-defined manner. Based on these findings, we propose that SMC–kleisin complexes from all domains of life act by a unifying mechanism.

Zusammenfassung

Die universellen Träger genetischer Information bei der Vermehrung zellulären Lebens sind die Chromosomen. Die Segregation dieser Einheiten während der Zellteilung ist für alle Organismen unabdingbar. Structural Maintenance of Chromosomes (SMC)–Kleisin-Komplexe sind universelle und essentielle Faktoren, welche die korrekte Organisation und Segregation des Erbmaterials sicherstellen. Ziel dieser Arbeit war es, evolutionär konservierte Erscheinungsmerkmale von SMC–Kleisin-Komplexen aufzudecken, und diese Merkmale auf funktionelle Relevanz zu testen. Wir haben zwei evolutionär invariante Leitmotive der Architektur von SMC–Kleisin-Komplexen identifiziert: (I) SMC–Kleisin-Komplexe haben eine asymmetrische Konfiguration, in der ein Kleisin-Monomer zwei unterschiedliche Bindungsstellen eines SMC-Dimers miteinander verbindet, (II) SMC–Kleisin-Komplexe bilden stäbchenförmige Strukturen, in denen die SMC-Proteine eines Dimers in einer wohl definierten Art eng aneinander liegen. Basierend auf diesen Resultaten schlagen wir vor, dass sämtliche SMC–Kleisin-Komplexe aus allen phylogenetischen Domänen einen gemeinsamen Funktionsmechanismus haben.

Contents

1 Introduction

1.1 Preamble

Chromosomes are the universal carriers of genetic information in cellular systems. They are formed as huge macromolecular assemblies of chromosomal DNA and associated proteins. During cell division, the accurate distribution of these entities to daughter cells, called chromosome segregation, is of fundamental importance. In all domains of life, structural maintenance of chromosomes (SMC)–kleisin complexes are critical factors for this process.

The projects that are presented in this doctoral thesis focus on the architecture of SMC–kleisin complexes, and their structural transitions. The first chapter serves as an introduction to the field, summarises important concepts and is intended to frame the scientific motivation for the study. The author has contributed to three peer-reviewed publications, which are included as chapters 2, 3 and 4. Important findings from these papers are consolidated in the discussion at the end of the thesis.

1.2 Principles of chromosome segregation

1.2.1 Chromosome segregation in eukaryotes

The cycle of genome duplication, chromosome segregation and cell division is the fundamental cytological process that drives the reproduction of life and the generation and maintenance of multi-cellular organisms. In eukaryotes, two different types of chromosome-segregation patterns occur which are called mitosis and meiosis (reviewed in Duro and Marston, 2015). Mitosis is fundamental for vegetative reproduction and tissue formation, whereas meiosis is the basis for sexual reproduction. In mitosis, daughter cells inherit a full copy of their mother cell's genetic content. Meiosis, on the other hand, generates cells that contain their mother cell's genetic content reduced by half, which ultimately leads to the production of gametes. These cells either arise immediately after meiosis or after intermediary mitotic cell divisions. Gametes can fuse to combine their genetic contents in a zygote, thereby closing the sexual reproduction cycle.

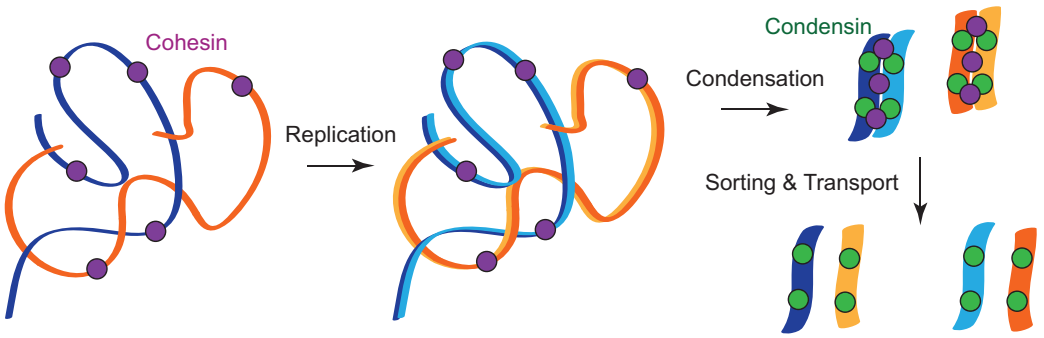

Figure 1.1: Shaping eukaryotic chromosomes for transport. Replicated sister chromatids are held together by cohesin complexes (purple). Chromosomes are then compacted and individualised in a process dependent on condensin complexes (green). Sorting and transport processes segregate individualised chromatids to different daughter cells.

To meet the requirements for transport through the cell, chromosomes are converted to a compact shape after DNA replication. This process is known as chromosome condensation (**Figure 1.1**). Condensation probably promotes separation of chromosomes on small length scales (chromosome demixing) and confers mechanical stability for transport on larger length scales. It depends on type II topoisomerases and condensin complexes, which are associated with the longitudinal axis of the chromosome (Hirano and Mitchison, 1993, 1994; Maeshima and Laemmli, 2003; Coelho et al., 2003). Along this chromosome axis chromatin may be organised in loops, however, the detailed molecular structure of condensed chromosomes remains largely obscure (Naumova et al., 2013; Hirano, 2014).

In both mitosis and meiosis, chromosomes are transported to emerging daughter cells by a microtubule-based apparatus called the spindle (reviewed in Walczak et al., 2010). The spindle is generated by microtubules radiating from two microtubule-organising centres (MTOCs) called centrosomes or spindle-pole bodies. The growing ends of the microtubules eventually dock at chromosomal structures called kinetochores, elaborate macromolecular assemblies that are tightly associated with centromeric chromatin. Ultimately, kinetochore-docked microtubules shrink and drag the attached chromosomes toward their MTOCs, each of which will be positioned in a daughter-cell-to-be.

To ensure that chromosomes are not distributed randomly to daughter cells but are segregated in a mitotic or meiotic pattern, cells initially keep the genetic units to be separated in tight association. Mitosis segregates sister chromatids, exact duplicates arising during DNA replication, which are initially paired in “dyads”. A prerequisite for successful mitotic segregation is that each chromatid of a dyad becomes connected to a different MTOC. Because sister chromatids are tightly held together by cohesin complexes, a process called sister-chromatid cohesion, productive attachment of microtubules generates tension between sisters due to spindle pulling-forces (reviewed in Kotwaliwale and Biggins,

2006; Foley and Kapoor, 2013). Once all dyads are under tension, cells dissolve sister-chromatid cohesion and proceed with chromosome segregation. In contrast to mitosis, meiotic sister chromatid segregation is preceded by an additional chromosome segregation event, whereby homologous dyads are partitioned to different daughter cells. These two distinct meiotic chromosome segregation events are called meiosis I and meiosis II, respectively. To segregate homologous chromosomes in meiosis I, cells initially pair homologous dyads into "tetrads", i.e. chromosomes containing four chromatids. During this process, the dyads become transiently connected by a protein assembly called the synaptonemal complex and, eventually, covalently exchange material by homologous DNA recombination (reviewed in Page and Hawley, 2004). Because sister chromatids are held together by cohesin complexes, any DNA exchange between non-sister chromatids converts sister-chromatid cohesion into homologue cohesion. Consequently, homologue segregation in meiosis can use similar tension-based supervision as sister segregation in mitosis. To achieve this, sister kinetochores attach to microtubules from the same MTOC during meiosis I, a process called "mono-orientation", which in many species depends on the cohesin complex (Parra et al., 2004; Chelysheva et al., 2005; Yokobayashi and Watanabe, 2005; Sakuno et al., 2009; Severson et al., 2009), and possibly also condensin (Brito et al., 2010; Lee et al., 2011).

1.2.2 Chromosome segregation in prokaryotes

It is a commonplace that proper sister chromosome segregation is as essential in prokaryotes as it is in eukaryotes (reviewed in Reyes-Lamothe et al., 2012; Lindås and Bernander, 2013). Bacteria segregate their chromosomes co-replicationally, whereas many archaea have been reported to perform replication and segregation in separate steps. However, neither bacteria nor archaea rely on the spindle apparatus. In fact, no universally required prokaryotic segregation machinery has been identified to date. Inspired by ideas from the field of polymer physics, this has led to the proposal that prokaryotic chromosome segregation might directly result from first principles, whereby an entropy-driven DNA-fibre demixing process is merely enhanced by protein factors (Jun and Wright, 2010). In a generalist view, prokaryotic chromosome segregation might correspond to eukaryotic chromosome individualisation in prophase, which also occurs on a similar length scale (**Figure 1.1**) (Nasmyth, 2002; Jun and Wright, 2010).

Although chromosome segregation over short distances might be an immediate consequence of the fibrous structure of DNA, proteins promoting this process do exist in prokaryotes (reviewed in Gruber, 2014). Widely distributed segregation factors are ParAB–*parS*, FtsK–XerCD, type II topoisomerases and SMC–kleisin complexes (introduced below). The type II topoisomerase Topo IV probably resolves sister-chromosome

intertwinings arising during DNA replication, similar to the activity of eukaryotic topoisomerase II (Topo II) (Kato et al., 1990; Wang et al., 2008; Lesterlin et al., 2012). Chromosomal ParAB–*parS* systems are related to plasmid partitioning factors and have been proposed to mediate the active segregation of replication origins (Ptacin et al., 2010). In many species, these systems are also used to orient the chromosome with respect to the cell envelope (Bowman et al., 2008; Ebersbach et al., 2008; Donovan et al., 2012; Ditkowski et al., 2013; Ginda et al., 2013). FtsK, in contrast, promotes the segregation of the replication terminus region. FtsK proteins are sequence-guided directional DNA pumps, which associate with the division septum and actively translocate DNA into daughter cells. This activity is used to drive chromosome segregation during sporulation of *Bacillus subtilis* cells, but also plays a role during vegetative growth in both firmicutes and proteobacteria (Wu and Errington, 1994; Bath et al., 2000; Kaimer and Graumann, 2011; Stouf et al., 2013). Furthermore, FtsK helps to resolve covalent chromosome dimers, which are produced by homologous recombination between sister chromosomes due to the circular structure of most prokaryotic chromosomes. For segregation to complete, chromosome dimers have to be converted into monomers, and this is usually catalysed by the XerCD tyrosine recombinase. XerCD operates in collaboration with FtsK, at least in many bacteria, to find its *dif* recognition site near the replication terminus (reviewed in Reyes-Lamothe et al., 2012).

1.3 SMC–kleisin complexes

As stated above, mechanisms of chromosome segregation seem quite diverse in prokaryotes. Hallmark macromolecular assemblies required for the segregation of genetic material in eukaryotes, i.e. the kinetochore complex and microtubules, are not found in bacteria and archaea. Quite the contrary, a conserved and generally essential prokaryotic chromosome segregation machinery has not been found to date. Although this does not necessarily mean that such machinery does not exist, a dedicated chromosome transport mechanism might only be essential if, as in eukaryotes, long distance movement of chromosomes is required. However, there are two known chromosome-segregation factors which are conserved in all domains of life: Type II topoisomerases and SMC–kleisin complexes. These factors might promote DNA separation processes on short length scales, which is conceivably relevant for prokaryotes. Type II topoisomerases support chromosome segregation by decatenation of entangled DNA. Molecular mechanisms of SMC–kleisin complexes, however, are not well understood and their elucidation remains a formidable challenge.

The most conserved part of SMC–kleisin assemblies is the SMC protein dimer. SMC proteins fold into long rods that contain a dimerisation domain (“hinge”) at one end and

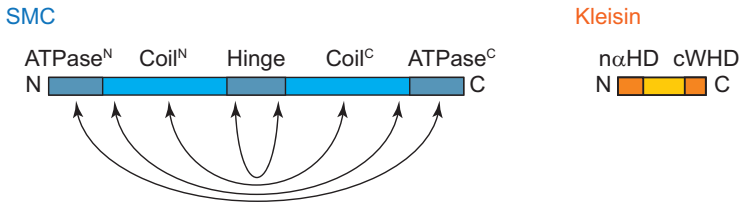

Figure 1.2: Core subunits of SMC–kleisin complexes. The linear organisation of SMC proteins (left) and kleisins (right) is shown. SMC proteins fold back on themselves (visualised by double-headed arrows), whereby the very N- and C-terminal regions constitute the ATPase head domain, and central regions form an antiparallel, intramolecular coiled coil and the hinge domain. Kleisin proteins comprise an N-terminal α-helical domain (nαHD), a middle region and a C-terminal winged-helix domain (cWHD). The length of the middle region (shown in yellow) is variable.

an ATP-binding cassette (ABC)-type ATPase domain ("head") at the other (**Figure 1.2**). Head and hinge domains are separated by a long stretch of antiparallel, intramolecular coiled coil. SMC dimers associate with less well conserved members of the kleisin family to constitute the SMC–kleisin core complexes. These assemblies bind additional accessory proteins that are not conserved between different classes of SMC–kleisin complexes.

1.3.1 Prokaryotic condensins

In prokaryotes, three classes of SMC complexes have been identified to date. They are termed prokaryotic condensins. Smc–ScpAB complexes are widely distributed in bacteria and archaea and are most similar to eukaryotic SMC–kleisin complexes. In contrast, the distantly related MukBEF complexes are found exclusively in some lineages of γ-proteobacteria. Recently, a third class of prokaryotic condensins has been identified: MksBEF. This class is related to MukBEF, exclusively found in bacteria, and scattered over the phylogenetic tree (Petrushenko et al., 2011; Gruber, 2011).

Smc–ScpAB contains the SMC protein Smc and the kleisin ScpA. MukBEF/MksBEF contain the SMC proteins MukB/MksB, but lack a *bona fide* kleisin subunit. The MukF subunit, however, shares some structural homology with kleisin proteins (see below). Phenotypes associated with mutations in all classes of prokaryotic condensins are similar. Disruption of Smc–ScpAB, MukBEF or MksBEF causes aberrations in chromosome compaction and segregation, whereby the severity ranges from mild to lethal depending on species and growth conditions (Niki et al., 1991; Britton et al., 1998; Jensen and Shapiro, 1999; Güthlein et al., 2008; Bouthier de la Tour et al., 2009; Yu et al., 2010; Minnen et al., 2011; Petrushenko et al., 2011). More specifically, the observed phenotypes have been linked to a role of Smc–ScpAB in the segregation of the *oriC* region (Gruber et al., 2014; Wang et al., 2014). Consistent with this model, Smc–ScpAB is recruited

to origin-proximal DNA by the ParB protein (Gruber and Errington, 2009; Sullivan et al., 2009; Minnen et al., 2011). Moreover, MukBEF also localises to the *oriC* region as judged by fluorescence microscopy (Danilova et al., 2007), indicating that it might act by a similar biological mechanism. For both Smc and the distantly related MukB, ATPase activity and binding of the respective non-SMC subunits is essential for function and chromosomal targeting (Mascarenhas et al., 2005; Shin et al., 2009; Schwartz and Shapiro, 2011; Badrinarayanan et al., 2012a). In good agreement with its importance for chromosome segregation, the SMC protein MukB interacts with the bacterial decatenase enzyme Topo IV (Li et al., 2010b; Hayama and Marians, 2010; Vos et al., 2013; Nicolas et al., 2014). Possibly, this interaction facilitates chromosome segregation by recruiting Topo IV to sister-chromosome intertwinings, interwound DNA structures that arise during replication.

1.3.2 Cohesin

The SMC–kleisin assembly that has been most thoroughly studied is the cohesin complex (reviewed in Nasmyth and Haering, 2009). Cohesin is essential for sister-chromatid cohesion in eukaryotes and has additional functions in DNA double-strand break (DSB) repair and gene regulation. It is formed by a Smc1–3 heterodimer that associates with the Scc1/Rad21 kleisin. Scc1 binds Smc3 as well as Smc1 to form a tripartite ring (Haering et al., 2002; Gruber et al., 2003). In addition, the HEAT-repeat containing proteins Scc3/SA, Pds5 and Wpl1/Wapl are peripheral subunits of cohesin that associate primarily with Scc1 (Rowland et al., 2009; Tóth et al., 1999; Panizza et al., 2000; Haering et al., 2002; Kueng et al., 2006).

The widely accepted model of cohesin function in chromosome segregation states that cohesin is the direct mediator of sister-chromatid cohesion (**Figure 1.3A**) (reviewed in Nasmyth, 2011). Evidence suggests that the complex entraps sister chromatids within its ring structure, thereby forming a molecular tether that can resist spindle pulling forces (Haering et al., 2008). In yeast, cohesin is loaded onto chromosomes via the Scc2–4 complex during late G1-phase of the cell cycle, and quickly redistributes from its loading sites to pericentromeres and regions of convergent transcription (Ciosk et al., 2000; Lengronne et al., 2004). However, chromatin-associated cohesin initially turns over with a half-life of minutes (Chan et al., 2012). Transport of DNA into the cohesin ring has been proposed to involve dissociation of the SMC hinge interface in a process powered by the SMC ATPase heads (Gruber et al., 2006), whereas unloading presumably occurs via Wpl1/Wapl dependent dissociation of the Smc3–Scc1 interface (Chan et al., 2012; Eichinger et al., 2013; Buheitel and Stemmann, 2013). Cohesin becomes stably bound to chromosomes only after acetylation of Smc3 by Eco1 during S-phase, the time

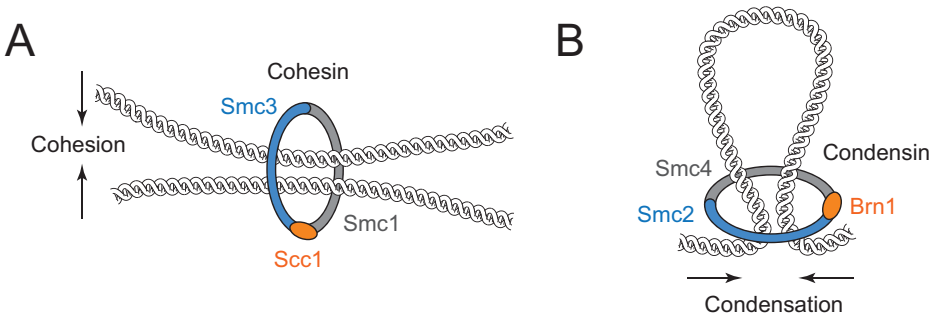

Figure 1.3: Models for SMC–kleisin function. Core subunits of yeast cohesin and condensin are shown. (**A**) The ring model for sister-chromatid cohesion. Sister chromatids are entrapped within cohesin complexes, which form a molecular tether. (**B**) Loop model of DNA condensation. DNA loops are trapped inside condensin complexes, resulting in length-wise DNA compaction.

when sister-chromatid cohesion is being established (Uhlmann and Nasmyth, 1998; Unal et al., 2008; Rolef Ben-Shahar et al., 2008; Rowland et al., 2009; Chan et al., 2012). In mammals, Smc3 acetylation promotes recruitment of Sororin to the complex, which prevents unloading by blocking cohesin's Wapl binding-site (Nishiyama et al., 2010). Smc3 acetylation also blocks Wpl1-dependent unloading in yeast, but a Sororin homologue has not been identified to date (Chan et al., 2012).

Cohesed chromatids build up tension due to spindle pulling forces in metaphase and enable inactivation of the spindle-assembly checkpoint. As a consequence, cells initiate anaphase by activating the anaphase promoting complex (APC) ubiquitin ligase. APC mediates proteasomal degradation of securin, an inhibitor of the cystein protease separase (Cohen-Fix et al., 1996; Ciosk et al., 1998). Activated separase in turn cleaves the Scc1 kleisin subunit of cohesin, thereby liberating sister chromatids and enabling their segregation by the spindle (Uhlmann et al., 1999, 2000). Interestingly, higher eukaryotes remove the bulk of cohesin on chromosome arms already during prophase by a non-proteolytic Wapl-dependent pathway (Waizenegger et al., 2000; Sumara et al., 2002; Kueng et al., 2006; Nishiyama et al., 2010). This triggers the individualisation of chromosome arms, causing the transition to the characteristic X-shape of metaphase chromosomes. Cohesin removal on chromosome arms is regulated by cohesin phosphorylation by polo-like kinase, whereas centromeric cohesin is protected by the shugoshin protein Sgo1 in complex with the protein phosphatase PP2A (Hauf et al., 2005; McGuinness et al., 2005; Kitajima et al., 2006; Liu et al., 2012; Hara et al., 2014).

In higher eukaryotes, chromosomal cohesin is replenished already in telophase and also expressed in post-replicative cells (Gerlich et al., 2006b; Wendt et al., 2008). This suggests that cohesin has functions apart from mediating sister-chromatid cohesion (reviewed in Mehta et al., 2013). Indeed, cohesin colocalises with the insulator protein CTCF and

the mediator complex, respectively, which regulate interactions of promoters with their enhancers (Wendt et al., 2008; Parelho et al., 2008; Kagey et al., 2010). Concomitantly, cohesin has been proposed to mediate DNA looping and control transcription and recombination at the IFNG and TCR loci, respectively (Hadjur et al., 2009; Seitan et al., 2011). In addition, the complex might also promote transcription termination at converging genes (Gullerova and Proudfoot, 2008). In support of a regulatory role in gene expression, hypomorphic mutations in human cohesin, its loading factor Scc2/NIPBL and its deacetylase HDAC8 are the genetic basis for the developmental disorder Cornelia de Lange syndrome (Tonkin et al., 2004; Deardorff et al., 2007, 2012).

In addition to its role in mitosis, cohesin is indispensable for normal progression of meiosis. Cohesin is required for homologue pairing, synaptonemal-complex assembly, recombination and chromosome segregation (reviewed in McNicoll et al., 2013). In yeast, the mitotic kleisin Scc1 is largely replaced by Rec8 during meiosis (Klein et al., 1999; Watanabe and Nurse, 1999). In higher eukaryotes, other meiosis-specific cohesin subunits such as Smc1β, the kleisins Rad21L and Coh-3/-4, and the Scc3 homologue SA3 can be found, depending on the species under investigation (Prieto et al., 2001; Revenkova et al., 2001; Severson et al., 2009; Gutiérrez-Caballero et al., 2011; Lee and Hirano, 2011; Ishiguro et al., 2011). These subunits participate in the formation of distinct meiotic cohesin complexes, which can differ in chromosomal localisation in both space and time (Kitajima et al., 2003b; Lee and Hirano, 2011; Ishiguro et al., 2011).

In mammals, oocytes undergo S-phase already before birth and arrest in prophase I until just before ovulation. During this time, which takes decades in humans, sister-chromatid cohesion needs to be faithfully maintained. The time-dependent loss of cohesion, called cohesion fatigue, might be responsible for age-related loss of fertility in females (Hodges et al., 2005; Chiang et al., 2010; Lister et al., 2010). Sister-chromatid cohesion during meiosis I also has special regulatory requirements. Initially, cohesion will have been established within the two pairs of sister chromatids in a tetrad, but due to cross-over recombination between homologues a situation is created where all four chromatids are directly or indirectly interlinked via cohesin complexes. Therefore, cohesin needs to be specifically removed from chromosome arms to permit homologue segregation in anaphase I. However, it must be maintained at centromeres to enable tension generation in metaphase II. In yeast, cohesin complexes at chromosome arms are proteolytically removed by separase in anaphase I, an activity that requires phosphorylation of the Rec8 kleisin (Brar et al., 2006). Cohesin at centromeres, however, is preserved due to shielding of Rec8 by the Sgo1–PP2A complex (Kitajima et al., 2003a, 2004, 2006; Riedel et al., 2006; Ishiguro et al., 2010; Katis et al., 2010).

In addition to its functions in chromosome segregation and gene expression, the cohesin complex is recruited to DSBs in an Scc2–4 dependent manner. It is essential for post-

replicative DSB repair by homologous recombination (Kim et al., 2002; Unal et al., 2004; Ström et al., 2004). Interestingly, the presence of post-replicative DSBs can induce genome wide sister-chromatid cohesion, suggesting that cohesin might operate by keeping the damaged chromosome region and its repair template in close proximity (Unal et al., 2007; Ström et al., 2007).

1.3.3 Condensin

The condensin complex was initially discovered as a factor required for chromosome condensation in egg extracts from *Xenopus laevis* (Hirano and Mitchison, 1994; Hirano et al., 1997). It was soon recognised as an essential mediator of chromosome condensation and segregation *in vivo* (Sutani et al., 1999; Freeman et al., 2000; Ono et al., 2003). Condensins are based on the Smc2–4 heterodimer and come in two major variants termed condensin I and II, respectively, each of which contains a distinct set of non-SMC subunits comprising the Cap-H/-H2 kleisins, and the Cap-G/-G2 and Cap-D2/-D3 HEAT-repeat containing proteins (Ono et al., 2003; Schleiffer et al., 2003). Condensin I is ubiquitously found in eukaryotes, whereas condensin II has been lost from some genomes during evolution, for example in yeast (Hirano, 2012; Thadani et al., 2012). A variant of condensin I, termed "dosage-compensation complex", contains the alternate Smc4 subunit Dpy-27 and mediates X-chromosome gene-dosage compensation in *Caenorhabditis elegans* hermaphrodites (Chuang et al., 1994; Lieb et al., 1996; Csankovszki et al., 2009; Hirano, 2012; Thadani et al., 2012).

Budding yeast condensin (corresponding to condensin I of non-fungal eukaryotes) resides in the nucleus throughout the cell cycle and is enriched at centromeric, intergenic and subtelomeric regions as well as at the repetitive ribosomal DNA (rDNA) locus (D'Ambrosio et al., 2008; Bhalla et al., 2002; Wang et al., 2005). Concomitantly, it promotes rDNA segregation and biorientation of kinetochores (Freeman et al., 2000; Verzijlbergen et al., 2014; Peplowska et al., 2014). The localisation of fission yeast condensin, however, appears more dynamic as it translocates into the nucleus in a cell-cycle specific manner (Sutani et al., 1999). In higher eukaryotes, condensin localisation is even more complex due to the presence of condensin II. Human condensin II is found on chromosomes throughout the cell cycle, whereas condensin I localises on chromatin only after nuclear envelope breakdown in prometaphase (Ono et al., 2004). Both condensins have distinct localisation patterns and turnover kinetics on chromatin, and have been proposed to differentially affect mitotic chromosome structure (Ono et al., 2004; Gerlich et al., 2006a; Shintomi and Hirano, 2011).

Several pathways have been proposed for targeting condensins to the chromosome. In budding yeast, condensin recruitment is mediated by the RNA polymerase III transcription

factor TFIIIC and possibly by the Scc2–4 complex, the latter of which being also essential for loading of cohesin (D'Ambrosio et al., 2008). TFIIIC also recruits condensin to chromosome arms in fission yeast (Iwasaki et al., 2010; Tada et al., 2011). In addition, the monopolin complex has been suggested to target condensin to rDNA in budding yeast and to kinetochores in fission yeast (Johzuka and Horiuchi, 2009; Tada et al., 2011). Furthermore, the shugoshin protein Sgo1 mediates recruitment of condensin to the pericentromere in budding yeast (Verzijlbergen et al., 2014; Peplowska et al., 2014). Condensin I is targeted to chromatin via binding of histone H2A and H2A.Z in fission yeast and human cells, an interaction that is regulated by phosphorylation of the kleisin subunit by the aurora B kinase (Tada et al., 2011).

The molecular mechanisms by which condensins support chromosome segregation remain somewhat enigmatic (Cuylen and Haering, 2011). Condensin can interact with DNA in a similar manner as the cohesin complex, i.e. by entrapping DNA within its ring structure (Cuylen et al., 2011). This binding mode could create DNA loops and/or cross-links that help to collapse and organise chromosomes for transport (**Figure 1.3B**) (Renshaw et al., 2010; Cuylen et al., 2011). A proposition that is not mutually exclusive with loop formation is that condensin might constrain a supercoiled DNA conformation with positive writhe. This could, by some means, facilitate chromosome segregation by promoting DNA packaging, cohesin removal or sister chromatid decatenation (Kimura and Hirano, 1997; Kimura et al., 1999; Strick et al., 2004; Yu and Koshland, 2005; Baxter et al., 2011).

1.3.4 Smc5–6 complex

The third type of SMC–kleisin assembly found in eukaryotes is the Smc5–6 complex. Its structural core is formed by a Smc5–6 heterodimer in complex with the kleisin Nse4 (Palecek et al., 2006). Other conserved subunits are Nse1 and Nse3, which bind the kleisin, and the SUMO ligase Mms21, which binds the Smc5 coiled coil (Sergeant et al., 2005; Zhao and Blobel, 2005; Palecek et al., 2006; Duan et al., 2009a).

The importance of Smc5–6 for DNA repair is well established (reviewed in De Piccoli et al., 2009) and involves SUMO-ligase activity of its Mms21 subunit (Zhao and Blobel, 2005; Andrews et al., 2005; Duan et al., 2009a). Concordantly, Mms21 dependent sumoylation of cohesin's kleisin subunit Scc1 has been proposed to regulate sister-chromatid cohesion during recombinational DSB repair in both yeast and human cells (McAleenan et al., 2012; Wu et al., 2012). However, protein sumoylation at DNA lesions is probably not the most important function of Smc5–6, because sumoylation activity of Mms21 is dispensable in yeast (Zhao and Blobel, 2005; Andrews et al., 2005; Duan et al., 2009a), whereas presence of all conserved subunits in the complex (including Mms21) is essential

(Lehmann et al., 1995; Fujioka et al., 2002; McDonald et al., 2003; Harvey et al., 2004; Morikawa et al., 2004; Pebernard et al., 2004).

Notably, depletion of Smc5–6 subunits from otherwise unchallenged human cells does severely affect mitotic chromosome structure and segregation, suggesting an important function independent from DNA repair (Gallego-Paez et al., 2014). This is supported by the finding that the cellular localisation of human Smc5–6 is cell-cycle regulated and mirrors that of cohesin: It is largely removed from chromatin during prophase, and re-associates with chromosomes during telophase/early G1 (Gallego-Paez et al., 2014). In addition, recruitment of budding yeast Smc5–6 to chromosome arms during S-phase depends on cohesin, and both complexes co-localise at centromeric regions of mitotic chromosomes (Lindroos et al., 2006; Jeppsson et al., 2014). Interestingly, Smc5–6 levels on chromatin positively correlate with chromosome length and are exacerbated by inhibition of Topo II or by chromosome circularisation (Kegel et al., 2011; Lindroos et al., 2006; Jeppsson et al., 2014). Possibly, Smc5–6 is attracted to sister-chromatid intertwinings (SCI), DNA structures that can arise upon rotation of the replication fork. SCI might accumulate when their removal by swivelling of chromosome arms is inefficient or impossible. However, the role of Smc5–6 at SCI remains to be established.

In addition to its functions in DNA repair and mitotic chromosome segregation, Smc5–6 is indispensable for progression of meiosis in both budding and fission yeast (Pebernard et al., 2004; Copsey et al., 2013; Lilienthal et al., 2013; Xaver et al., 2013). In budding yeast, the complex is required for the resolution of meiotic recombination intermediates, and mutations of Smc5–6 genes result in near complete chromosome-segregation failure during meiosis I.

1.3.5 The DNA-entrapment model

How do SMC–kleisin complexes interact with chromosomes? It has been unequivocally demonstrated that cohesin forms complexes with a ring-like topology that can entrap chromatin fibres (Gruber et al., 2003; Haering et al., 2008). In addition, it is very likely that condensin binds DNA in a similar manner (Cuylen et al., 2011). Clearly, the DNA entrapment model is an attractive framework for interpreting SMC–kleisin function, because it can readily explain chromosome condensation and cohesion phenomena in terms of DNA looping and tethering (**Figure 1.3**).

The entrapment model raises one immediate question: How many DNA stretches thread through a fully loaded SMC–kleisin complex? For cohesin, simultaneous covalent cross-linking of all three ring interfaces efficiently generates sister DNA cohesion that is resistant to denaturing conditions (Haering et al., 2008). With respect to the reported cross-linking efficiencies and the fact that higher-order assemblies of endogenous cohesin

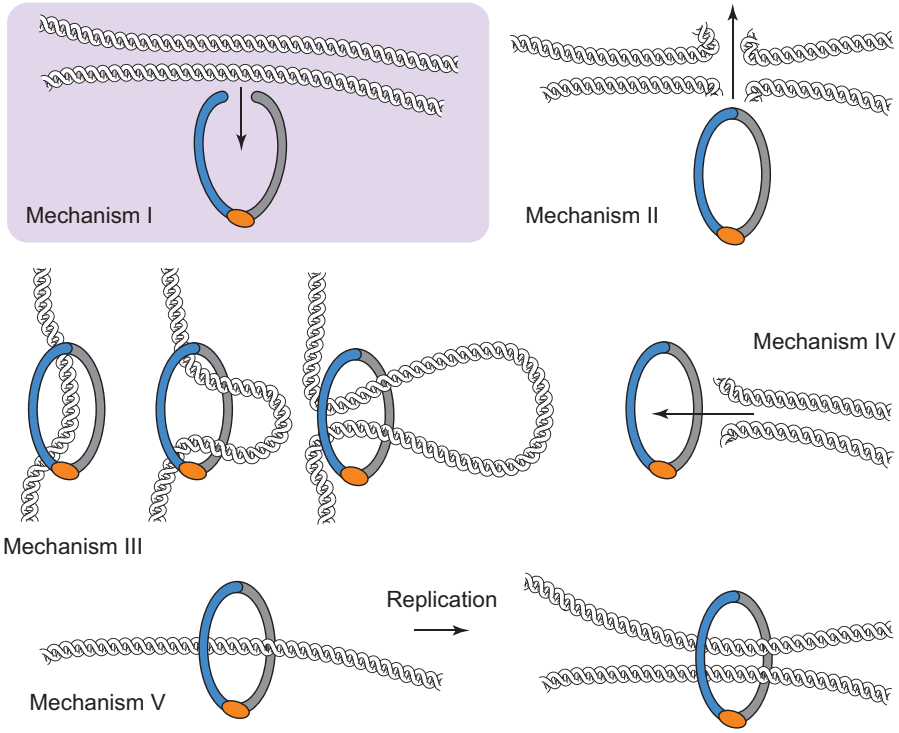

Figure 1.4: Possible mechanisms for achieving DNA double-entrapment. Mechanisms I-V are described in the text. The model with the highest likelihood for correctly unifying cohesin and condensin functions is highlighted.

have not been detected to date, the most likely explanation is that DNA passes the cohesin ring at least twice. The simplest and most general – but not necessarily correct – inference is that *all* loaded SMC–kleisin complexes, not only cohesin, are passed by exactly two stretches of DNA. Clearly, sister chromatid cohesion as well as DNA condensation can be intuitively derived from this double-entrapment model (**Figure 1.3**). What are possible mechanisms that could achieve such double entrapment?

Theoretically, a protein ring which is passed by two stretches of DNA can be established in five principal ways (**Figure 1.4**): (**I**) Two stretches of DNA are passed through a transient opening in the protein ring, (**II**) two stretches of DNA are transiently cleaved, the protein ring is passed through the cleavage sites, which are then ligated inside the ring, (**III**) a DNA loop is extruded through the ring, (**IV**) two linear DNA molecules are threaded through the protein ring ends on, (**V**) a single DNA stretch that has been loaded by mechanisms I, II or IV is replicated within the ring.

Mechanism II seems rather obscure because it would require an elaborate topoisomerase-like activity that can transport protein stretches through DNA breaks. Mechanism III seems useful for establishing large DNA loops, because it would ensure that both DNA stretches are part of the same DNA molecule. It is more difficult, though, to envision

it working in cohesion, especially if cohesion maintenance should be resistant to type II topoisomerase activity. Moreover, this mechanism would need to be realised in a way that it can handle the presence of nucleosomes in eukaryotes. Mechanism IV would obviously not work for circular chromosomes found in prokaryotes and, especially in the light of telomere structure and chromosome size, seems highly unlikely to operate on eukaryotic chromosomes. Mechanism V has been put forward for cohesion establishment during S-phase, although it is clearly inconsistent with *de novo* establishment of cohesion during DSB repair, interphase functions of cohesin and functions of most other SMC–kleisin complexes, which are largely independent from DNA replication.

Mechanism I would be useful for establishing or stabilising DNA loops if both transport steps could be targeted to the same DNA molecule. Possibly, a bias towards loop formation by an SMC–kleisin complex would exist naturally after entrapment of the first DNA stretch due to increased local concentration of DNA from the same fibre. Loop extension could then be achieved for example by directional loading of a second complex at the root of the loop, and recycling of the founder complex. Adapting mechanism I for a cohesive function would require that transport steps for single DNA stretches alternate between sister chromatids. In both loop formation and cohesion establishment by mechanism I, geometrical cues might be essential for directing the loading of the correct DNA stretches. These cues could be provided for example by the replication fork, by DNA loop associated proteins (like CTCF and possibly ParB) or, in the case of *de novo* cohesion establishment during post-replicative DSB repair, by pre-existing sister chromatid cohesion. Mechanism I, although clearly speculative at this stage, appears by far best suited to explain both loop formation and cohesion phenomena by a single model.

1.3.6 Structure of SMC–kleisin complexes

To understand the molecular mechanism of SMC–kleisin complexes and their interaction with DNA, it is undoubtedly necessary to understand their structure. As judged by electron and atomic-force microscopy, SMC complexes from various organisms form rod- or ring-like assemblies with maximum dimensions of 30-50 nm (Anderson et al., 2002; Yoshimura et al., 2002; Matoba et al., 2005; Fuentes-Perez et al., 2012). Higher order multimers have been reported for the MukBEF complex and the Smc–ScpAB complex (Matoba et al., 2005; Fuentes-Perez et al., 2012).

At the heart of SMC–kleisin complexes are the SMC proteins. SMCs are rod-shaped molecules ($\approx$ 50 nm in length) that consist of a long intramolecular, antiparallel coiled coil separating the hinge domain from the head domain (**Figure 1.5**) (Melby et al., 1998; Anderson et al., 2002; Haering et al., 2002). The hinge is formed by the central part of the SMC polypeptide and contains two dimerisation surfaces, which together mediate

the formation of a toroidal dimer around a twofold symmetry axis (Haering et al., 2002; Griese and Hopfner, 2010; Griese et al., 2010; Kurze et al., 2011). Hinge structures of cohesin, condensin and Smc–ScpAB are very similar. The hinge of the divergent MukB protein, however, differs in size and structural details (**Figure 1.5**) (Li et al., 2010a; Ku et al., 2010).

At the opposite end of the SMC rod, the head domain is constituted by the protein's very N- and C-terminal residues. The head adopts an ABC-type ATPase fold that can dimerise upon binding two molecules of ATP (**Figure 1.6**) (Hopfner et al., 2000; Löwe et al., 2001; Lammens et al., 2004; Haering et al., 2004). Head dimerisation reconstitutes two active sites: In each site, one head contributes catalytic and substrate-coordinating residues from Walker A and B motives, and the other head contributes residues from its ABC signature-motive (Hopfner et al., 2000; Lammens et al., 2004). After ATP hydrolysis has occurred, heads are thought to dissociate, release the nucleotide and restart the ATPase cycle. In analogy to ABC-type transporters, ATP-driven cycling between head engagement and disengagement likely powers a conformational change in the SMC–kleisin holocomplex. This conformational change is, however, poorly understood. Possibly, the structural transition involves disruption of the hinge dimerisation interface for loading of DNA into the complex (Gruber et al., 2006).

SMC proteins are bound by kleisins to constitute the SMC–kleisin core complexes. In cohesin, the kleisin Scc1 binds the heads of Smc1 and Smc3, thereby creating a tripartite core complex with a ring-like topology (**Figure 1.5A**) (Gruber et al., 2003). In this complex, the C-terminal winged-helix domain (cWHD) of Scc1 binds the bottom surface of the Smc1 head domain (**Figure 1.5A**) (Haering et al., 2004). The N-terminal domain of Scc1, on the other hand, binds Smc3 (Gruber et al., 2003). However, it has remained elusive whether this interaction is similar to the Smc1–Scc1 interface.

Core-complex interactions mapped in condensin and the Smc5–6 complex indicate that these assemblies likely have the same tripartite topology as cohesin (Onn et al., 2007; Sergeant et al., 2005; Palecek et al., 2006; Duan et al., 2009b). In striking contrast to the eukaryotic SMC–kleisin complexes, however, MukBEF probably forms tetrapartite rings (**Figure 1.5B**), or even large multimeric assemblies (Matoba et al., 2005; Woo et al., 2009; Badrinarayanan et al., 2012b). Although not homologous to Scc1 on the sequence level, the MukBEF kleisin-analogue MukF also contains a WHD at its C-terminus, which binds the MukB head (Schleiffer et al., 2003; Woo et al., 2009). This interface is structurally similar to the Smc1–Scc1 interaction (**Figure 1.5B**) (Woo et al., 2009). Bridging of two MukF-bound MukB heads is mediated by extensive homodimerisation of the N-terminal part of MukF, which folds into a helical bundle and another WHD (Fennell-Fezzie et al., 2005; Woo et al., 2009). MukF is bound by dimeric MukE to constitute the (MukF–$(MukE)_2$)$_2$ subcomplex (Gloyd et al., 2007; Woo et al., 2009). MukE contains

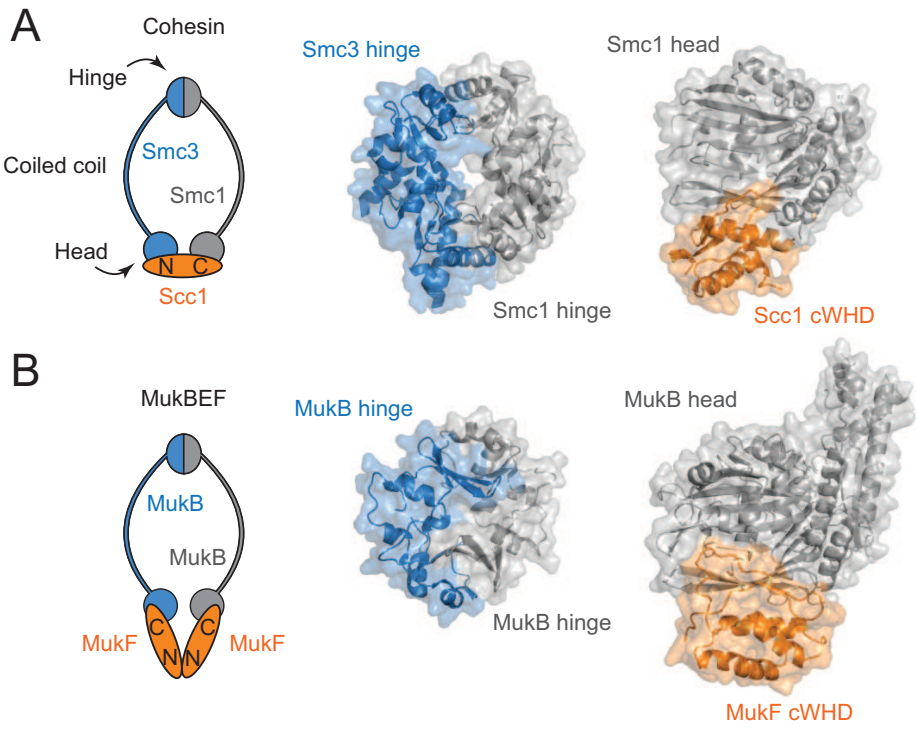

Figure 1.5: Subunit interactions in SMC complexes. (**A**) Composition of the mitotic cohesin core complex is shown on the left. Structures of the *Mus musculus* cohesin hinge dimer (PDB: 2WD5) and the *Saccharomyces cerevisiae* Smc1–Scc1 interface are shown (PDB: 1W1W). (**B**) Composition of the MukBEF core complex is shown on the left. Structures of the MukB hinge dimer from *Escherichia coli* (PDB: 2WMM) and the MukB–MukF interface from *Haemophilus ducreyi* are shown (PDB: 3EUK).

two WHDs, whereby the N-terminal WHD mediates stable homodimerisation (Woo et al., 2009). The ScpB subunit of prokaryotic condensin Smc–ScpAB, although unrelated to MukE on the sequence level, adopts a similar structure (Kim et al., 2006, 2008). In contrast, the HEAT-repeat containing proteins Scc3 and Wapl, which bind cohesin's kleisin Scc1, do not share structural similarity with ScpB or MukE (Chatterjee et al., 2013; Ouyang et al., 2013; Hara et al., 2014; Roig et al., 2014).

Based on the finding that the kleisin ScpA can allegedly form MukF-like homodimers in size-exclusion chromatography, it has become a widespread notion that Smc–ScpAB, which is probably the most broadly distributed SMC–kleisin complex, adopts a MukBEF-like architecture (Volkov et al., 2003; Hirano and Hirano, 2004; Nasmyth and Haering, 2005; Mascarenhas et al., 2005; Cuylen and Haering, 2011). In other words, eukaryotic and prokaryotic SMC–kleisin complexes might be structurally quite divergent and, in consequence, might perform their biological function by non-related biochemical mechanisms. However, reports addressing the stoichiometry of Smc–ScpAB have yielded somewhat unclear results, and were recently challenged by analytical sedimentation-velocity centrifu-

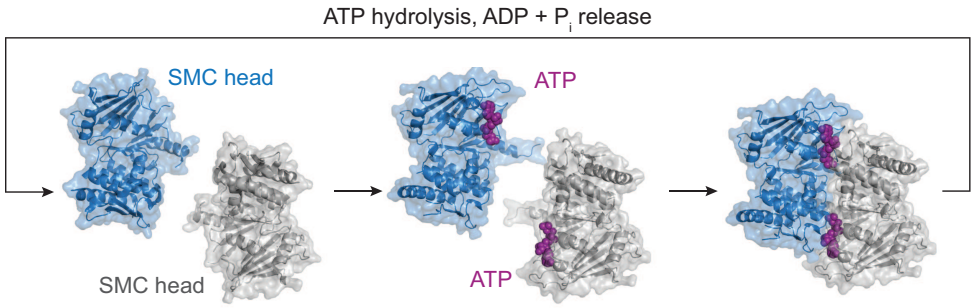

Figure 1.6: ATP-hydrolysis cycle of SMC proteins. Two monomeric ABC-type SMC head domains bind a molecule of ATP each, engage in an "ATP sandwich" dimer, hydrolyse the nucleotides, release reaction products and dissociate. Based on *Pyrococcus furiosus* Smc heads in nucleotide free (PDB: 1XEW) and ATP-bound dimeric form (PDB: 1XEX).

gation experiments, which indicated that isolated ScpA might form monomers exclusively (Volkov et al., 2003; Hirano and Hirano, 2004; Mascarenhas et al., 2005; Fuentes-Perez et al., 2012).

1.4 Aim of the study

SMC proteins are highly conserved factors in the biology of chromosomes. However, they form complexes with other proteins which are poorly conserved or not related at all. Peculiarly, these subunits are virtually always essential for SMC function. Put differently, SMC proteins apparently cannot exert their biological activity without their respective binding partners. Therefore, an important question is whether the molecular mechanism of SMC–kleisin complexes has been conserved during evolution, or if it is as divergent as their non-SMC subunits.

To start answering this question, we were guided by the rational that if the physical architecture of SMC–kleisin complexes was similar, then this should also be true for their molecular mechanism. Therefore, a comparative biological strategy has been chosen that focusses on the architecture of prokaryotic Smc–ScpAB, which is probably most similar to the most recent common ancestor of all SMC–kleisin complexes. The biological mechanism and structure of Smc–ScpAB are poorly understood. Important findings for Smc–ScpAB were then translated to yeast condensin and cohesin. In case a unifying model for SMC–kleisin function exists, it might be most readily derived by differential analysis of the evolutionary archetype together with its more specialised variants.

The projects that are presented here have – in a collaborative effort – applied structure-guided functional analysis to the investigation of SMC–kleisin complexes. This methodology, often in combination with unbiased genetic screening, is an approach that is quite successful in obtaining biological insights with high explanatory and predictive power.

2 Publication I: An asymmetric SMC–kleisin bridge in prokaryotic condensin

Bürmann, F., Shin, H.C., Basquin, J., Soh, Y.M., Giménez-Oya, V., Kim, Y.G., Oh, B.H., and Gruber, S. (**2013**). An asymmetric SMC-kleisin bridge in prokaryotic condensin. *Nat Struct Mol Biol*, 20(3):371–379

Author contribution

The author constructed *B. subtilis* strains, conceived and performed *in vivo* cross-linking experiments, genetic experiments, biochemical experiments, and significantly contributed to manuscript preparation.

Original publication: DOI:10.1038/nsmb.2488

3 Publication II: Closing the cohesin ring: Structure and function of its Smc3–kleisin interface

Gligoris, T.G., Scheinost, J.C., Bürmann, F., Petela, N., Chan, K.L., Uluocak, P., Beckouët, F., Gruber, S., Nasmyth, K., and Löwe, J. (**2014**). Closing the cohesin ring: structure and function of its Smc3-kleisin interface. *Science*, 346(6212):963–967

Author contribution

The author designed and established cysteine-specific cross-linking of *S. cerevisiae* Smc3–Scc1 and performed the Smc–ScpA experiment.

Original publication: DOI:10.1126/science.1256917

4 Publication III: Molecular basis for SMC rod formation and its dissolution upon DNA binding

Soh, Y.M., Bürmann, F., Shin, H.C., Oda, T., Jin, K.S., Toseland, C.P., Kim, C., Lee, H., Kim, S.J., Kong, M.S., Durand-Diebold, M.L., Kim, Y.G., Kim, H.M., Lee, N.K., Sato, M., Oh, B.H., and Gruber, S. (**2015**). Molecular basis for SMC rod formation and its dissolution upon DNA binding. *Mol Cell*, 57(2):290–303

Author contribution

The author constructed *B. subtilis* and *S. cerevisiae* strains, conceived and performed cross-linking experiments, genetic experiments, biochemical experiments, and significantly contributed to manuscript preparation.

Original publication: DOI:10.1016/j.molcel.2014.11.023

5 Discussion

5.1 Smc–ScpAB as the archetype of SMC–kleisin complexes

Although most prokaryotes might lack essential chromosome segregation machineries, the physical problem of spatially sorting long DNA fibres at sub-micrometre length scales is likely the same in all domains of life. In prokaryotes, kinetics and efficiency of segregation might be dominated by physical properties of the chromosome such as stiffness and self-adherence. Cells might fine-tune these properties biochemically to support proper inheritance of genetic material (Jun and Wright, 2010). Concomitantly, the very same biochemical processes that promote demixing and disentangling of eukaryotic DNA molecules on small length scales, which leads to chromosome individualisation and chromatid resolution, could mediate bulk DNA segregation in prokaryotes (Nasmyth, 2002; Jun and Wright, 2010). This is unequivocally the case for the action of type II topoisomerases, which help disentangling chromosomes in probably all domains of life by providing strand-passage activity. Do SMC–kleisin complexes, which are the only conserved segregation factors alongside type II topoisomerases, operate by a common mechanism as well?

Phenotypes associated with mutations in both eu- and prokaryotic condensins suggest that at least these classes of SMC–kleisin complexes might share the same *modus operandi*. Loss of condensins in eukaryotes affects chromosome segregation, probably by perturbing chromosome structure (Hirano and Mitchison, 1994). However, this causality is less clear in prokaryotes where the aberrant chromosome structure of condensin mutants might also be indirectly caused by clustering of non-segregated material (Gruber et al., 2014). Furthermore, scepticism towards the existence of a unifying mechanism was fuelled by the assumption that Smc–ScpAB, in contrast to eukaryotic SMC–kleisins, might form polymeric chains or adopt a symmetric architecture similar to that of the deviant MukBEF (Hirano, 2006; Graumann and Knust, 2009; Cuylen and Haering, 2011). These assumptions, however, turned out to be wrong. Unexpectedly, the SMC–kleisin core of Smc–ScpAB has a ring-like topology with the same asymmetric subunit arrangement as its eukaryotic cousins, although it comprises a homodimeric (supposedly symmetric)

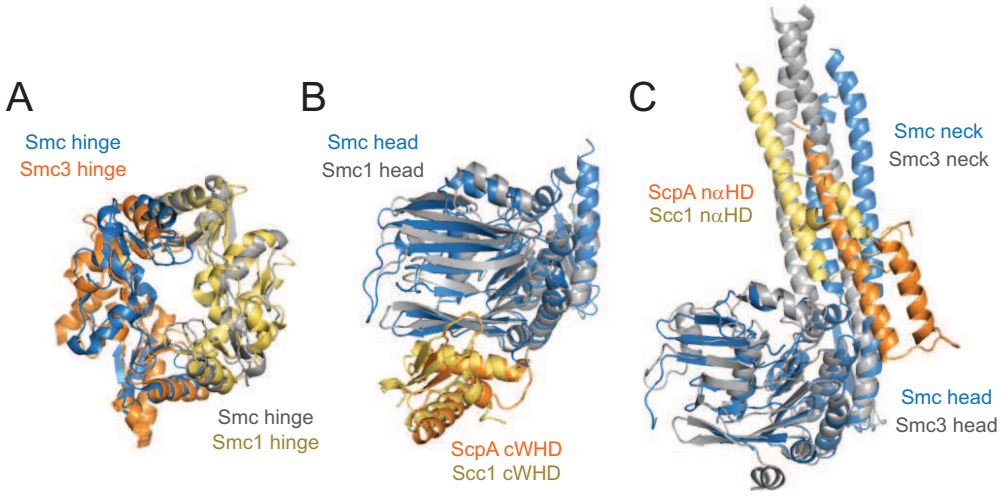

Figure 5.1: Comparison of the three core-complex interfaces in Smc–ScpAB and cohesin. Structures of the (**A**) hinge interface, (**B**) cap interface, and (**C**) neck interface are superimposed (PDB IDs: 1GXK, 2WD5, 1W1W, 4I99, 3ZGX, 4UX3).

Smc dimer (Bürmann et al., 2013). As evident from quantitative site-specific *in vivo* cross-linking experiments, this asymmetric architecture is the major form of Smc–ScpAB in *B. subtilis* cells. Moreover, genetic complementation experiments strongly support the view that it is the functionally relevant form of the complex. In fact, all three binding interfaces that mediate the assembly of the Smc–ScpA core complex are conserved in its eukaryotic counterparts (**Figure 5.1**) (Haering et al., 2002, 2004; Kurze et al., 2011; Bürmann et al., 2013; Gligoris et al., 2014). Therefore, Smc–ScpAB can be safely regarded as the structural archetype of SMC–kleisin complexes, whereby the bacterial MukBEF and MksBEF complexes probably form an outgroup.

The generalised architecture of SMC–kleisin core complexes is shown in **Figure 5.2**: At the head domains, a rod- or ring-like SMC dimer is bound by a kleisin monomer. The kleisin's N-terminal α-helical domain (nαHD) binds a surface at the head-proximal SMC coiled coil called "neck" (Bürmann et al., 2013; Gligoris et al., 2014). The cWHD of the kleisin, in contrast, binds the "cap" surface at the SMC ATPase (Haering et al., 2004; Bürmann et al., 2013). Bridging of cap and neck binding sites occurs between two different SMC proteins and generates asymmetric holocomplexes (Gruber et al., 2003; Bürmann et al., 2013). The neck-bound SMC is therefore designated ν-SMC ("nu" for neck interface) and the cap-bound SMC is designated κ-SMC ("kappa" for cap protein association) (Bürmann et al., 2013). The characteristic asymmetric architecture of Smc–ScpAB has been radicalised by eukaryotic SMC–kleisin complexes. The expression of dedicated ν- and κ-SMC proteins has allowed SMC dimers to adopt an asymmetric structure in themselves, for example at the hinge domain (Soh et al., 2015). Moreover,

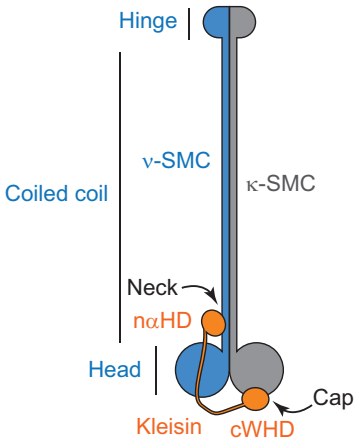

Figure 5.2: The generalised architecture of SMC–kleisin core complexes. An SMC dimer is asymmetrically bridged by a kleisin monomer.

Table 5.1: Core subunits of SMC–kleisin complexes.

Complex	**ν-SMC**	**κ-SMC**	**Kleisin**
Smc–ScpAB	Smc	Smc	ScpA
Cohesin	Smc3	Smc1, Smc1β	Scc1/Rad21, Rad21L, Rec8, Coh-3, Coh-4
Condensin	Smc2	Smc4, Dpy-27	Brn1/Cap-H, Cap-H2
Smc5–6	Smc6	Smc5	Nse4

heterodimerisation of eukaryotic SMC proteins has fostered numerous ways of specifically altering the complex by post-translational modification or subunit substitution. A list of core-subunit designations for the different SMC–kleisin complexes is shown in **Table 5.1**.

In addition to the presence of an asymmetric core complex, an emerging architectural theme is the formation of rod-shaped holocomplexes by close juxtapositioning of the SMC coiled coils (Anderson et al., 2002; Soh et al., 2015). Rod formation is conserved at least from Smc–ScpAB to condensin, but might also prevail in cohesin as suggested by structural homology and small-angle X-ray scattering experiments (Soh et al., 2015). Because the workings of a machine are dictated by its physical structure, it seems reasonable to expect that all SMC–kleisin complexes share a universally conserved mechanism.

5.2 Implications for DNA-loading and unloading mechanisms

5.2.1 DNA loading

Cohesin interacts with DNA by entrapping DNA fibres (Haering et al., 2008; Gligoris et al., 2014). Moreover, condensin probably shares this mode of DNA binding (Cuylen

et al., 2011). Quite possibly, the identity of a unifying mechanism for SMC–kleisin complexes may turn out to be DNA entrapment, which is likely to use an entry gate in the protein ring (**Figure 1.4**, p. 12). In cohesin, artificial dimerisation of the hinge blocks loading of the complex, but does not interfere with cohesion maintenance (Gruber et al., 2006). In contrast, permanent fusion of either cap or neck interface can generate functional cohesin or Smc–ScpAB complexes, respectively (Gruber et al., 2006; Bürmann et al., 2013). If an entry gate exists in SMC–kleisin complexes, then it is likely opened by transient dissociation of the hinge interface and powered by the SMC ATPase head domains. How could DNA loading at the hinge work on a structural level?

By definition, a DNA entrapment activity requires that DNA gains access to the inner volume of the protein complex. In case of Smc–ScpAB, condensin and possibly also cohesin, however, the SMC coiled coils appear to be closely juxtaposed with no space in between to accommodate DNA (**Figure 5.3**) (Soh et al., 2015). Therefore, the coiled coils would need to become separated during the loading process. Is there any evidence that this might happen? Intriguingly, site-specific cross-linking experiments indicate a DNA-dependent conformational change in the hinge-proximal coiled coils of Smc, being consistent with coiled-coil disengagement upon DNA binding (Soh et al., 2015). However, similar experiments have so far failed to detect this response in the yeast condensin complex (Soh et al., 2015). Interestingly, the coiled coils of Smc have also been observed in an open conformation by crystallography (**Figure 5.3**) (Haering et al., 2002). However, the crystallised construct contained only short stretches of coiled coil, which might have failed to pack correctly and may not reflect a relevant open conformation. Without any doubt, it will be important to determine the structure of SMC–kleisin complexes bound to DNA.

If the hinge is indeed a DNA entry gate, loading of a single DNA stretch could be performed in two ways: (I) Hinge and coiled coil interfaces could simultaneously dissociate to allow DNA entry into the complex, (II) a DNA loop could first gain access to the space in between the coiled coils and one of the loop arms could be evicted through the hinge (**Figure 5.4**). Both scenarios would be analogous to the activity of the related ABC transporters (reviewed in Locher, 2009; ter Beek et al., 2014). Scenario I would be conceptually similar to the action of ABC importers, because substrate would be transported towards the nucleotide-binding domains, whereas scenario II would correspond to the workings of ABC exporters, because substrate would be transported away from the nucleotide-binding domains. In both cases, the transport mechanism would probably require a DNA-binding activity inside the ring that changes substrate affinity during the loading process. Interestingly, the hinge dimer of many SMC proteins has been shown to contain a DNA-binding site, which presumably resides in its positively charged bottom surface (Chiu et al., 2004; Hirano, 2006; Soh et al., 2015). If the hinge dimer was to

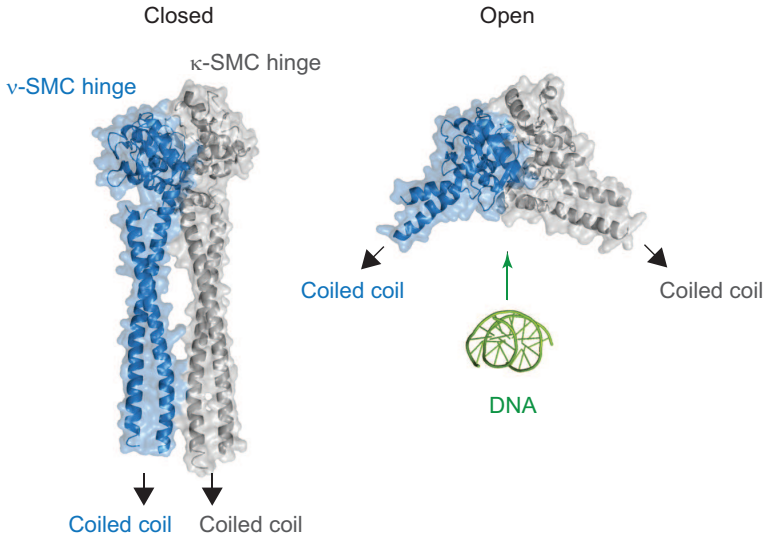

Figure 5.3: Structure of the hinge-proximal coiled coils of Smc. The coiled coils of Smc are closely juxtaposed, with no space in between to accommodate DNA (left; *Pyrococcus furiosus*, PDB: 4RSJ). For DNA entrapment inside the complex, the Smc coiled coils need to disengage, possibly adopting a V-shaped structure (right; *Thermotoga maritima*, PDB: 1GXL). A DNA dodecamer is shown in orange (PDB: 1FQ2).

dissociate during the loading reaction, this binding site would be ideally positioned for regulation by the very same dissociation event.

How is DNA loading coupled with the SMC ATPase cycle? In isolated full-length Smc protein, coiled-coil opening requires ATP-dependent engagement of the Smc heads in addition to the presence of the DNA substrate (Soh et al., 2015). Consistently, ATP promotes high-affinity DNA binding of Smc. If DNA does indeed bind the bottom surface of the hinge, then Smc's high-affinity state is probably identical with its open conformation. Possibly, ATP binding to Smc is rather used for substrate acquisition than for hinge dissociation, which might occur upon nucleotide hydrolysis. Interestingly, a recent report has proposed substrate-gate opening upon ATP hydrolysis for the asymmetric ABC exporter BmrCD (Mishra et al., 2014). For the Smc protein, early evidence for a communication between head and hinge domains came from the finding that DNA binding at the hinge allosterically activates the Smc ATPase situated 50 nm away in the head domain (Hirano and Hirano, 2006). This is also reminiscent of the related ABC transporters, where stimulation of the ATPase by the substrate probably serves as a way to tightly couple the ATPase cycle with transport (Davidson and Nikaido, 1990; Sarkadi et al., 1992; Gorbulev et al., 2001; Borths et al., 2005). Similarly, DNA binding at the Smc hinge might serve as a signal that accelerates the rate-limiting step for nucleotide turnover and, possibly, transport.

In a protein fragment of *B. subtilis* Smc comprising the hinge and one-third of the coiled

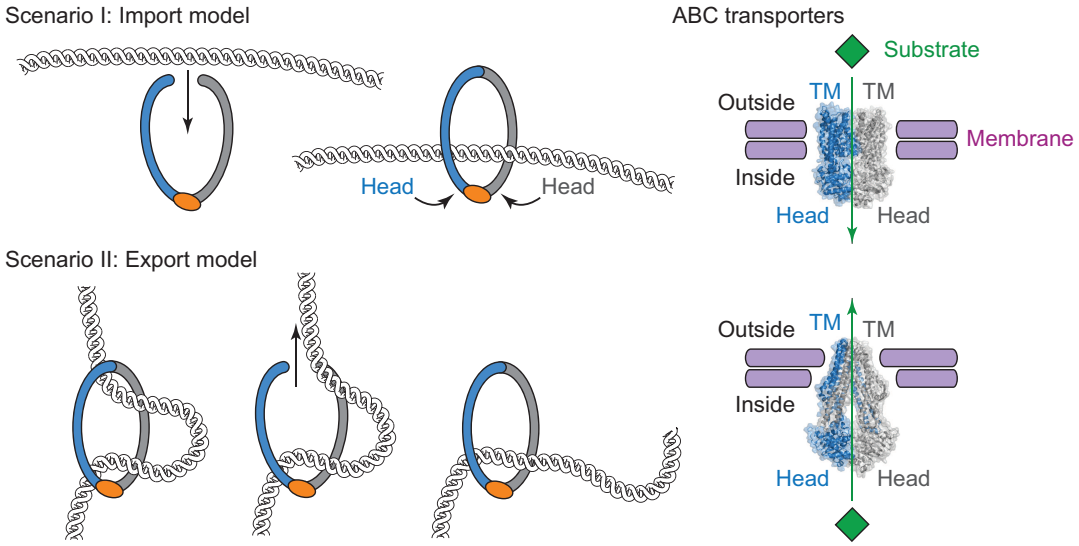

Figure 5.4: Models for DNA-fibre loading through an entry gate. In scenario I a DNA stretch is imported, whereas in scenario II a pre-formed loop is partially exported. The analogy of ABC-powered transport at membranes is shown on the right. Substrate flow is either directed towards the nucleotide-binding domains (import; BtuCD, PDB ID: 1L7V), or away from them (export; Tm0287–Tm0288, PDB ID: 4Q4H). TM, transmembrane domain.

coil, DNA binding probably results in disengagement of the coiled coils without dissociation of the hinge interfaces (Soh et al., 2015). This open DNA-bound conformation might correspond to a post-loading state of the above mentioned scenario I, or a pre-loading state of scenario II (**Figure 5.4**). Why does DNA alone promote conformational switching of truncated Smc constructs, whereas ATP is an additional requirement for full-length Smc? Compared with truncated constructs, the coiled coils of full-length Smc possibly associate more stably with each other due to formation of a more extensive interface. Therefore, the free energy of DNA binding might not be sufficient to compensate for coiled-coil dissociation in full-length Smc. For the same reason, opening might be kinetically disfavoured due to a higher activation energy of coiled-coil dissociation. Therefore, coiled-coil opening might have remained undetected in our biochemical experiments in case of very slow (incomplete) equilibration in the absence of DNA (Soh et al., 2015). Taken together, ATP dependent engagement of the Smc heads might provide free energy that could be needed to stably (and/or quickly) open the complex for DNA binding.

How could coiled-coil opening be linked to head engagement? Although the arrangement of SMC heads within the nucleotide-free complex remains to be determined, it is obvious that head engagement is not compatible with an alignment of the head-proximal coiled coils (**Figure 5.5A**). In case these regions were somewhat aligned in the nucleotide free

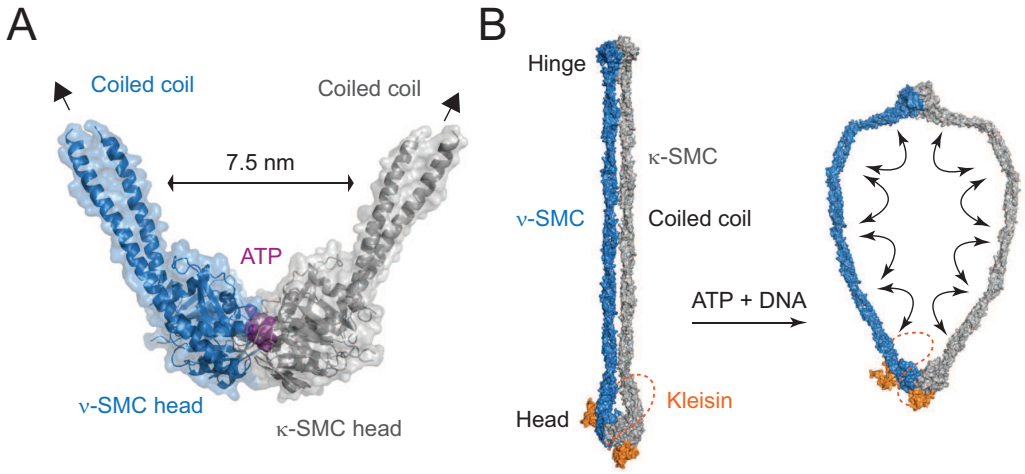

Figure 5.5: Model for SMC coiled-coil opening upon head engagement. (**A**) Model for coiled-coil conformation with engaged Smc heads. *B. subtilis* Smc heads including the neck region (PDB: 3ZGX) were superimposed onto the structure of ATP-bound *P. furiosus* Smc heads (PDB: 1XEX). The distance between the coiled coils is inconsistent with perfect rod formation in the ATP-bound state. (**B**) Tentative model for coiled-coil opening. Head engagement and DNA binding induce an "unzipping" strain in the coiled-coil interface.

complex, head engagement could simply infuse coiled-coil-disengagement strain into the SMC dimer by spreading the head-proximal coiled coils (**Figure 5.5B**). Alternatively or in conjunction, head engagement could impose strain by tilting or twisting of the coiled coils, as has been proposed for the related Rad50 protein (Lammens et al., 2011). However, the efficiency of coiled-coil disengagement in isolated Smc seems rather low in the absence of DNA (Soh et al., 2015), which might be due to poor coupling of heads and hinge, or due to low head-engagement efficiency. In any case, DNA might eventually be cleared from the hinge to enable loading of a second DNA stretch. It may be speculated that re-closing of the Smc coiled coils might push the DNA fibre towards the heads into some "storage cavity" (**Figure 5.6**). Stable association of the coiled coils could be important e.g. for keeping DNA in a fixed position after loading, or for minimising the access of non-target DNA to the inner volume of the complex. Clearly, it will be important to determine both the whereabouts of DNA inside the complex, and the conformation of the coiled coils during and after the loading reaction.

5.2.2 Complex asymmetry and the role of the kleisin

The integrity of the kleisin subunit is crucial for maintaining the association of SMC–kleisin complexes with DNA. Artificial cleavage of the kleisin removes cohesin or condensin complexes from DNA, and destroys Smc–ScpAB function presumably for the same reason (Gruber et al., 2003; Cuylen et al., 2011; Gruber et al., 2014). Possibly, cleavage of the

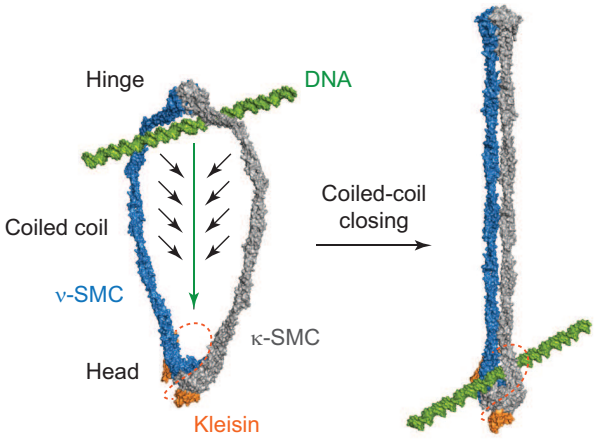

Figure 5.6: Tentative model for the fate of DNA upon SMC arm-closure. Movement of the coiled coils and DNA are indicated by black and green arrows, respectively.

kleisin promotes DNA leakage from the ring by providing an artificial opening in the complex. For cohesin, kleisin cleavage is physiologically important, as evident from the cell-cycle specific activation of separase during cohesion dissolution.

Is the kleisin merely required to keep the ring shut and recruit other accessory subunits, or does it play a role in SMC head function as well? In both Smc–ScpAB and cohesin, translational fusion of the ν-SMC and the kleisin nαHD or fusion of the κ-SMC and the kleisin cWHD, respectively, generates functional proteins. However, functionality is lost when the kleisin domain at the fusion linker is prevented from binding its SMC moiety by specific mutations in the interface (Gruber et al., 2006; Bürmann et al., 2013). Clearly, the kleisin does not simply serve as a "seal" for the complex, but has to engage both of its SMC-binding domains in order to generate functional, asymmetric complexes. What could be the reasons for this?

One possibility is that the asymmetric architecture of SMC–kleisin complexes is required for recognition (or induction) of a particular DNA geometry. In addition, asymmetry could be used for designating "front" and "back" ends of the complex, which might be relevant for loop extrusion or other directional movements along DNA. Another attractive possibility is that the DNA-loading cycle of a SMC dimer requires an intermediate or transition state in which both monomers adopt a different conformation. In this scenario, the kleisin could have evolved as a "director" that assigns specialised conformational roles to its SMC binding partners. For example, opening of the hinge or coiled-coil interfaces might involve the rotation or sliding of the coiled coils against each other, which would require movements of the SMC monomers into opposite directions. Clearly, realising an opening mechanism is probably much easier (and more likely to find an efficient solution) if an asymmetric machinery was to be used (**Figure 5.7**). Interestingly,

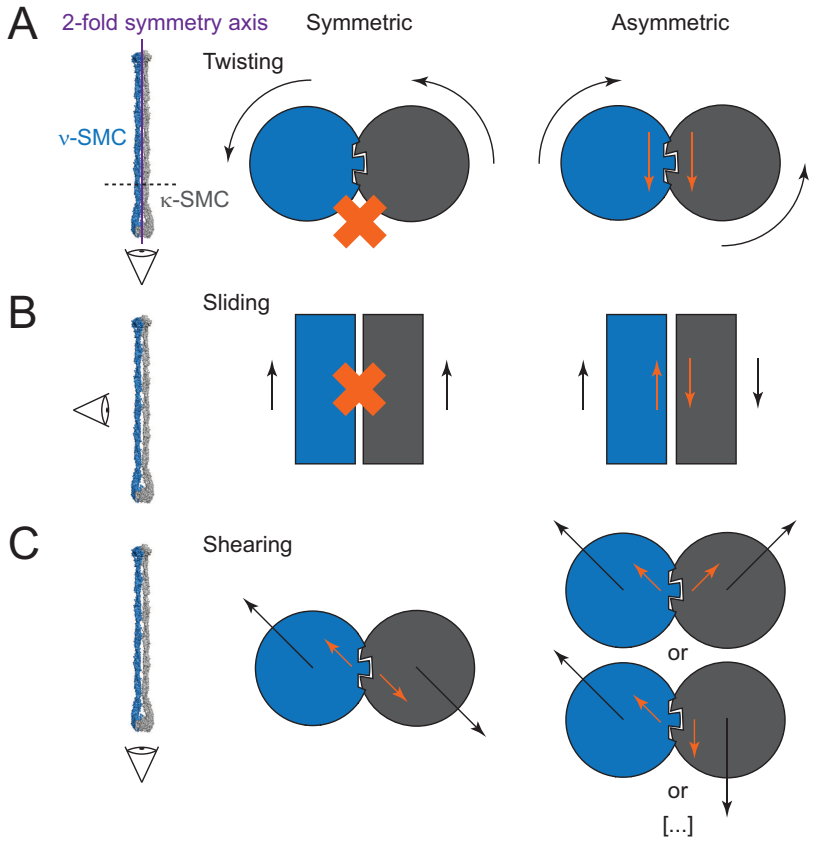

Figure 5.7: Models for conformational changes driving SMC coiled-coil or hinge disengagement. Symmetric (left) and asymmetric (right) movements with respect to the SMC twofold symmetry axis (shown in purple) are compared. Directions of coiled-coil movement are shown by black arrows, and resulting movements at the interface are shown by orange arrows. An orange X indicates no relative movement at the interface. (**A**) Coiled-coil twisting. Only an asymmetric movement results in disruption of the interface. (**B**) Coiled-coil sliding is only feasible using an asymmetric movement. (**C**) Coiled-coil shearing. Many more solutions are possible using an asymmetric mechanism.

asymmetric architectures have been observed in many of the related ABC transporters. Asymmetry is most evident for the energy-coupling factor (ECF)-type importers, which contain two completely unrelated integral membrane modules (ter Beek et al., 2011; Wang et al., 2013; Xu et al., 2013). Moreover, many heterodimeric ABC exporters have been reported to contain a degenerate nucleotide-binding site and perform highly asymmetric ATP hydrolysis cycles (reviewed in Procko et al., 2009). A general need for structural asymmetry during ABC-powered transport might come from the fact that most substrate molecules, be it sugars, vitamins, peptides, lipids, xenobiotics or DNA, might not have the proper twofold symmetry for symmetric transport through a protein complex with this particular geometry. Quite possibly, asymmetry is a more general property of ABC-powered transport than is currently acknowledged.

5.2.3 DNA exit-gate opening

Structural studies have revealed that the neck interface is a helical bundle formed by the kleisin nαHD and the ν-SMC neck region (Bürmann et al., 2013; Gligoris et al., 2014). In cohesin, this interface has been proposed to serve as a DNA exit-gate, because its covalent fusion greatly reduces cohesin's turnover on DNA and bypasses the need for Smc3 acetylation (Chan et al., 2012; Eichinger et al., 2013; Buheitel and Stemmann, 2013). Although turnover takes place for other SMC–kleisin complexes as well, the physiological role of DNA unloading has not been established (Gerlich et al., 2006a; Kleine Borgmann et al., 2013). Whatever its function, regulated DNA unloading might be a generally conserved activity of SMC–kleisin complexes and might be required for optimisation of the loading process, quality control or recycling of loaded material.

In cohesin, DNA unloading is stimulated by the Wapl protein, which does not contain any obvious cofactor binding sites. The DNA exit-gate can become locked by acetylation of the Smc3 head; however, the Smc3 acetylation site is not situated in cohesin's neck interface (Gligoris et al., 2014). This finding supports the hypothesis that Smc3 acetylation does not directly lock the exit gate. Probably, the gate is sealed by preventing Wapl from binding cohesin (Nishiyama et al., 2010).

How could DNA unloading work, and how does it energetically relate to the loading reaction? Considering the case whereby the biological function of SMC–kleisin complexes does not allow for tight DNA binding inside the complex, for example because sliding along DNA needs to be permitted (Lengronne et al., 2004), DNA transport into the complex will probably be energetically disfavoured. This is due to a high entropic cost of confining DNA inside the ring, and would agree well with the model that loading consumes ATP and does not occur spontaneously (although ATP might also be used to kinetically enhance the loading reaction). Accordingly, if DNA was not bound inside the complex, or was bound with a fast dissociation rate, and was also free to access the exit gate, DNA unloading would be thermodynamically favourable and kinetically strongly dependent on the rate of exit-gate opening. Any factor that would simply bind and destabilise the exit gate might therefore increase the unloading rate, and could catalytically promote complex turnover. Consistent with such a catalytic function, budding yeast Wpl1 associates with cohesin in substoichiometric amounts (Chan et al., 2012). Preventing the ring-opening catalyst from binding, as has been proposed for the acetylation-induced competition between Wapl and Sororin in higher eukaryotes (Nishiyama et al., 2010), could then be used to stabilise cohesin on DNA. It is conceivable that similar mechanisms operate in other SMC–kleisin complexes to regulate their biological activity.

5.3 Conclusions

SMC–kleisin complexes are fascinating cellular machines due to their (possibly) unique biochemical activity, their involvement in fundamental cellular processes and their functional importance in all domains of life. Our studies have firmly established Smc–ScpAB as the architectural archetype of SMC–kleisin complexes – a finding that was quite unexpected. Smc–ScpAB shares remarkable tertiary and quaternary structural homology with its eukaryotic cousins, whereby the most prominent architectural themes are head-module asymmetry and holocomplex rod-formation. This has a single far reaching implication: All SMC–kleisin complexes probably share a common biochemical mechanism. Consequently, our findings establish bacterial Smc–ScpAB as a very powerful and relevant model system for the analysis of one of the most widespread classes of chromosomal proteins.

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Abbreviations

ABC	ATP-binding cassette
APC	Anaphase promoting complex
ATP	Adenosine triphosphate
cWHD	C-terminal winged-helix domain
DSB	Double-strand break
ECF	Energy-coupling factor
MTOC	Microtubule-organising centre
nαHD	N-terminal α-helical domain
PDB	Protein Data Bank
rDNA	Ribosomal DNA
SCI	Sister-chromatid intertwinings
SMC	Structural maintenance of chromosomes protein
WHD	Winged-helix domain

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