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REVIEW ARTICLE

The SMC complexes, DNA and chromosome topology: right or knot?

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Abstract

Topology is the study of geometric properties that are preserved during bending, twisting and stretching of objects. In the context of the genome, topology is discussed at two interconnected and overlapping levels. The first focuses the DNA double helix itself, and includes alterations such as those triggered by DNA interacting proteins, processes which require the separation of the two DNA strands and DNA knotting. The second level is centered on the higher order organization of DNA into chromosomes, as well as dynamic conformational changes that occur on a chromosomal scale. Here, we refer to the first level as "DNA topology", the second as "chromosome topology". Since their identification, evidences suggesting that the so called structural maintenance of chromosomes (SMC) protein complexes are central to the interplay between DNA and chromosome topology have accumulated. The SMC complexes regulate replication, segregation, repair and transcription, all processes which influence, and are influenced by, DNA and chromosome topology. This review focuses on the details of the relationship between the SMC complexes and topology. It also discusses the possibility that the SMC complexes are united by a capability to sense the geometrical chirality of DNA crossings.

Keywords: condensin, cohesin, Smc5/6 complex, MukB, topoisomerases, chromosome structure and dynamics

Introduction

DNA topology

The successful execution of DNA metabolic processes must overcome inherent physical constraints presented by the DNA molecule. These constraints arise primarily from the intertwined double-stranded nature of DNA. DNA replication, transcription and strand invasion during homologous recombination all entail the separation and unwinding of DNA strands. If the DNA molecule is closed, that is, has fixed ends that cannot rotate, this unwinding will lead to over-winding in another part of the molecule. The over-wound portion is said to be positively supercoiled, while under-wound DNA is negatively supercoiled. (for a more precise explanation of DNA topology, see box 1 and Figure 1) (Mirkin, 2001; Witz and Stasiak, 2010). The term "supercoil" comes from the fact that at a certain level of increased or decreased twist, the DNA will fold on itself and create a coil of the DNA helix, i.e. a supercoil. Twist-induced supercoils can easily be observed by pulling apart two intertwined ropes with fixed ends (Figure 2). The coiling of the DNA helix is called writhe, and the topological status of DNA is determined by the sum of the twist and writhe. The terms "supercoiling" and "supercoils" are generally used to describe the topological status of a DNA molecule regardless of the distribution between twist and writhe. Moreover, writhe can be accommodated in two different conformations: plectonemic or toroidal (also called solenoidal) supercoils (Figure 1). Within cells, positive supercoils can be found ahead of an advancing replication fork or RNA polymerase, while negative are detected behind the transcription machinery. DNA topology can also be altered by DNA-binding proteins. The wrapping of DNA around the histone octamer is a good example, which constrains negative toroidal supercoiling in eukaryotic DNA.

For better understanding of the reasoning around topology and SMC complexes, a short introduction to the handedness, or geometrical chirality, of helices and coils is also needed. Furthermore, it is important to appreciate that the handedness of negative and positive supercoils

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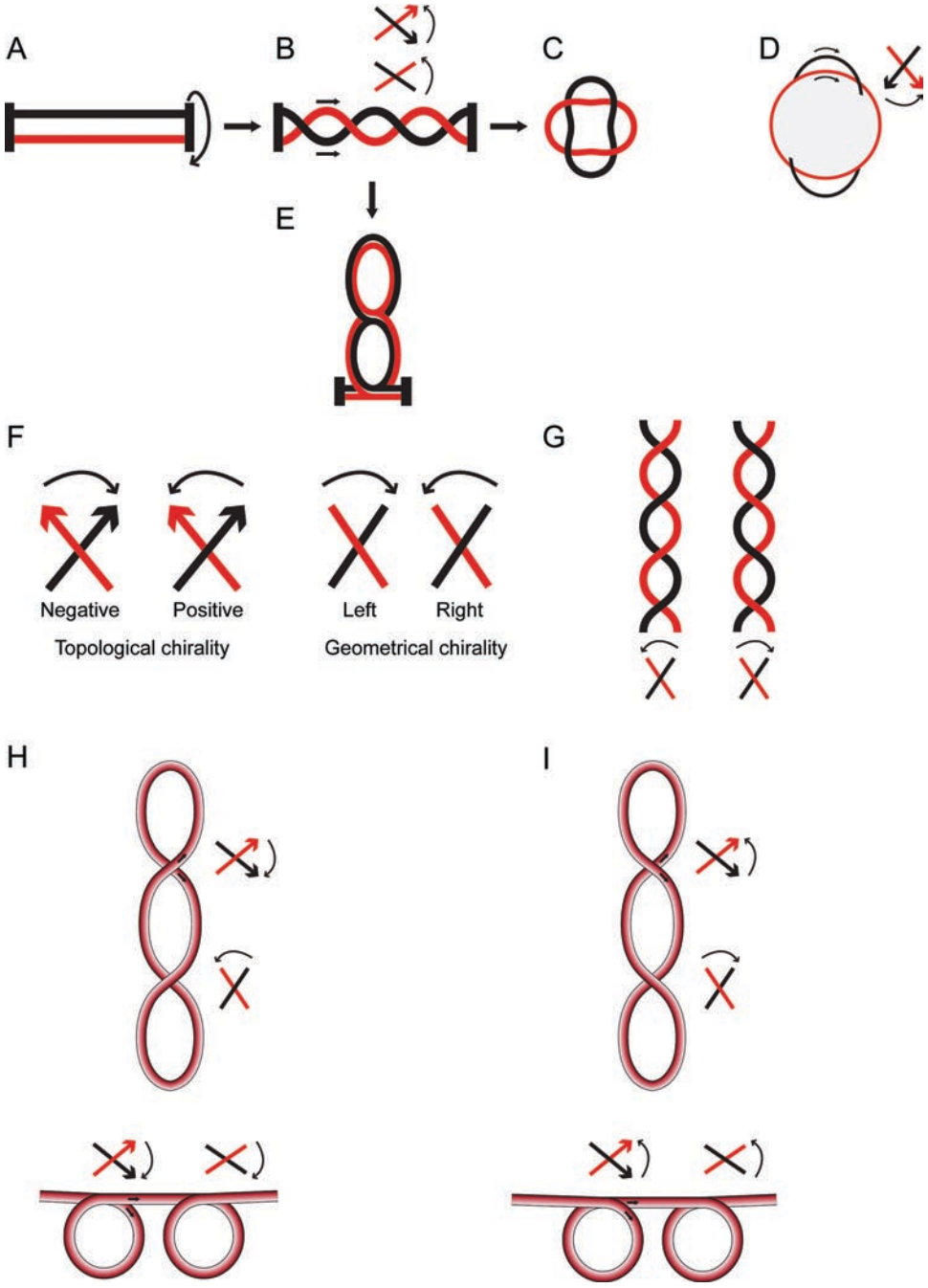

Figure 1. Basic topological concepts: linking number, twist, writhe and chirality. (A) Schematic depiction of two DNA strands (horizontal read and black lines) with fixed ends. (B) Two full rotations of one end introduce twists into the molecule. (C) Covalent closure of a DNA results in the topological linkage of the two strands of the helix. (D) To determine linking number (Lk) one strand is set to represent the edge of an imaginary surface (red line). The linking number is equal to the algebraic (sign-dependent) sum of intersections between this line and the other strand (black line). Here Lk = +2. (E) Lk can be present as twist (Tw) as in B, or writhe, which is when the DNA helix twists around itself in space as in E. As shown, the twist in (B) (Tw = +2, Wr = 0) is converted to writhe (Tw = 0, Wr = +2). (F) The chirality of DNA crossings is determined by the illustrated sign conventions. Topological chirality takes the path of the DNA into account. To determine the topological sign of a node, the direction in which the overlying vector must be rotated to be aligned with the underlying vector is determined. The rotation cannot exceed 180°. If the overlying vector is rotated clockwise, the node is negative. If the overlying vector is rotated counter-clockwise, the node is positive. Geometrical chirality is independent of the directionality of the DNA crossing. Instead, the overlying segment is rotated through the smallest angle required to align it with the underlying segment. If the overlying segment is rotated clockwise, the crossing is left-handed. If the overlying segment is rotated counter-clockwise, the crossing is right-handed. The sign conventions show that the DNA helix illustrated in (B) is positive and right-handed. (G) Right- and left-handed DNA helices. (H) DNA supercoils can be maintained in plectonemic (top) or toroidal (bottom) conformations. Here, both conformations contain negative supercoils, though the handedness of the plectonemic structure is right and the toroidal structure left. (I) Positive supercoils maintained in plectonemic, left handed (top) and toroidal, right handed (bottom) conformations.

Box 1.

The topology of a closed circular DNA, or a DNA region with fixed ends, is described by three parameters. The first paramenter is linking number (Lk). Lk is the total sum of complete turns one DNA strand makes around the other strand. Lk itself equals the sum of twist (Tw) and writhe. Tw represents the total number of helical turns in a circular DNA, that is, the total number of times the two strands twist around each other. Writhe describes the total number of times the DNA helix crosses itself in space, and can also be thought of as a net measurement of the left- or right-handedness of a curve (See Figure 1).

$$Lk = Tw + Wr$$

In a closed DNA molecule, Lk is fixed. A change in Lk can only be accomplished by breakage of one or both DNA strands, twisting of the strands relative to each other and religation of the break. Considering the invariability of Lk, it becomes apparent from the above equation that changes in Tw are compensated for by changes in Wr, and vice versa. The most predominant DNA conformation in nature is the B-form, a right-handed double helix with a pitch (γ) of 10.5 bp per helical turn. For a DNA molecule N bp long, Tw is thus given by:

$$Tw = N/\gamma$$

In a completely relaxed DNA molecule Lk corresponds to Tw:

$$Lk_0 = Tw_0 = N/\gamma$$

A DNA molecule with an Lk value that differs from Lk_0 is said to be supercoiled, and results from the introduction of Tw or Wr. The difference between Lk and Lk_0 is the linking difference (τ).

$$\tau = Lk - Lk_0$$

τ can be positive or negative in sign, and indicates the degree of positive or negative DNA supercoiling, respectively. Negative values for τ denotes a DNA molecule that is unwound relative to a relaxed DNA and positive values denote a DNA molecule that is overwound relative to a relaxed DNA molecule.

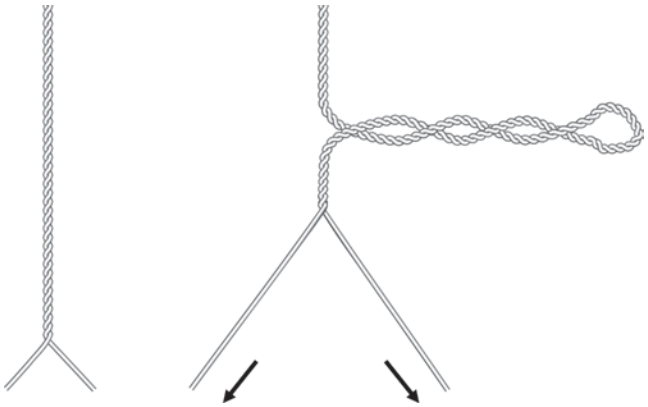

Figure 2. Illustration of twist-induced supercoiling. If the ends of a twisted rope with fixed ends are pulled apart, the increase in twist eventually results in the coiling of the rope upon itself. Note that the pulling introduces positive supercoils, which fold into left-handed plectonemic structures.

changes if folded in either plectonemic or toroidal conformations. An easy way to determine the handedness of a helix (or coil) is to align a thumb with its path, holding the rest of the fingers slightly curved in a 90^0 angle to the thumb. If the helix is right-handed, the fingers of the right hand will curl in the same direction as the turn of the helix. If the helix is left-handed, it will be necessary to use the left hand for the fingers to curl in same direction as the turn of the helix. A more technical method for determining handedness is detailed in Figure 1. Using either method one can tell the handedness of DNA, as well as of different types of supercoils. This will show that negative supercoils are folded in a right-handed way when present in a plectonemic structure, but are left-handed in toroidal supercoils. Conversely, positive supercoils will fold into left-handed plectonemes and right-handed toroidals (Figure 1). The primary regulators of DNA supercoiling in cells are topoisomerases, specialized enzymes that catalyze transient breaks in DNA. Topoisomerases have been reviewed elsewhere, and we will only briefly introduce their function (Wang, 2002). The topoisomerases are classified into two main categories, type I and type II enzymes. Type I topoisomerases introduce single-strand DNA breaks, allowing for the rotation of one part of the DNA molecule, and then religate the break. Type II topoisomerases produce double-strand DNA breaks, and facilitate the passage of a DNA helix through the break prior to religation. Through these reactions type I and II topoisomerases alter the linkage between DNA strands, thereby changing its twist and/or writhe. This is crucial for a large number of DNA metabolic reactions, and telling examples are those of transcription and replication. When prying apart the DNA double helix, a transcription unit generates positive supercoils in front of the polymerase and negative supercoils behind (Figure 3). Similarly, the advancement of replication forks generates positive supercoils. Without removal of the supercoils by topoisomerases, the topological tension will increase to a level that blocks both transcription and replication. An alternative way to prevent accumulation of supercoils is to allow the transcription or replication unit to follow the turn of the DNA helix. In the case of replication, such rotation will lead to lead to intertwining of the sister chromatids behind the fork (Figure 3) (Postow et al., 2001; Wang, 2002). To facilitate further reading on sister chromatid intertwinings here and elsewhere, a clarification of the nomenclature for sister chromatid intertwining can help. When replication takes place on a closed DNA molecule (plasmid or circular genome) the intertwining are called pre-catenanes until replication is completed, when they become catenanes. If replication of a linear chromosome is considered, the intertwinings are simply called sister chromatid intertwinings (SCI). Regardless of the nomenclature, all catenations/SCIs have to be removed at anaphase to allow full segregation of chromosomes. This removal is called decatenation, and it is executed by type II topoisomerases.

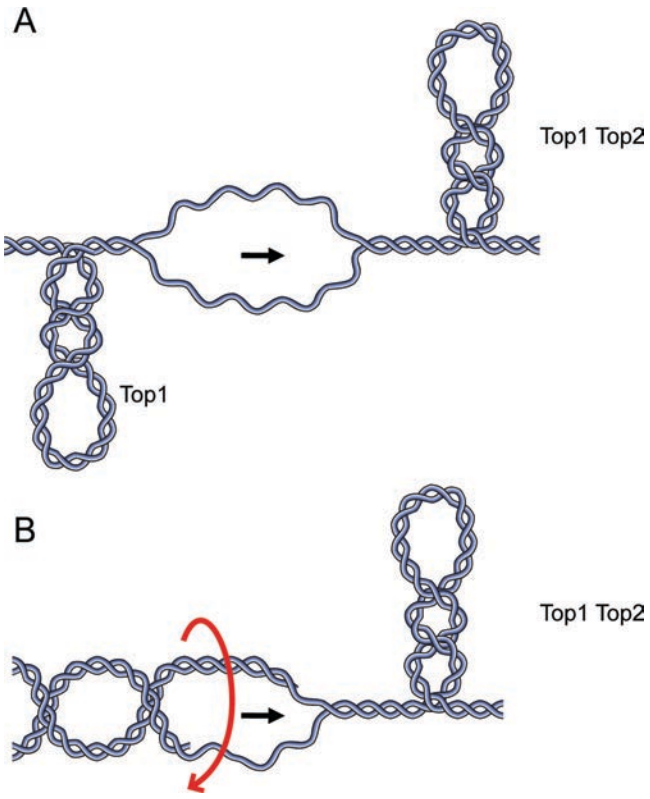

Figure 3. Topological changes as a result of DNA transcription and replication. (A) The separation of DNA strand and the movement of the transcriptional machinery (black arrow) induce positive supercoils in front of the polymerase and negative supercoils behind it. Successful execution of transcription depends on supercoil removal by topoisomerases. (B) Positive supercoils are also generated in front of advancing forks (black arrow) during DNA replication. In addition to removal by topoisomerases, these supercoils can be resolved through rotation of the fork with the turn of the DNA helix (red arrow), resulting in right-handed sister chromatid intertwinings behind the fork.

Chromosome topology

An important clarification concerning the terminology used in this review should be made. "Chromosome topology", as applied here, is used as a collective term to describe particular aspects of higher order organization of DNA into chromosomes, the dynamics of long-range intra- or interchromosomal interactions, the entrapment of DNA loops, as well as DNA catenation between chromosomes. This terminology has primarily been used in order to clearly contrast local changes in DNA topology to the potential consequences of those changes on a chromosomal scale.

Eukaryotic DNA is first organized into nucleosomes in which units of 147bp of DNA are wrapped in a double turn around histone octamers, which contain one pair each of histones H2A, H2B, H3 and H4 (Finch et al., 1977; Kornberg, 1974). As stated, this wrapping constrains negative writhe in the DNA. The resulting nucleosome array forms a 10 nm chromatin fiber commonly described as beads on a string. In the next level of organization, in which the linker histone H1 plays a critical role, interactions between nucleosomes form a 30 nm wide fiber (Davies and Small, 1968; Ris and Kubai, 1970; Sivolob and Prunell, 2003). Depending on experimental conditions, DNA and origin of cells, this fiber has been shown to fold into different structures and to be more or less abundant *in vivo*. Its impact on DNA and chromosome topology therefore remains to be elucidated. The existence of higher levels of chromatin organization beyond the 30 nm fiber is undisputed, but the exact nature of these levels is unresolved (Woodcock and Ghosh, 2010). One recurring concept in chromosome topology is the formation of chromatin loops. Chromatin looping is involved in the regulation of gene enhancer and promoter interactions and the looping out of co-regulated gene groups (Kadauke and Blobel, 2009). Moreover, through a variety of scenarios, large-scale chromatin loops have been envisioned to contribute to higher order chromatin structure. For example, loops of supercoiled DNA can be observed as a "halo" following histone removal, suggesting some level of uniform, loop-mediated organization, as well as the existence of an insoluble nuclear matrix (Vogelstein et al., 1980). The nuclear matrix itself is proposed to be composed of a dynamic network of as many as 400 proteins (Elcock and Bridger, 2010; Mika and Rost, 2005; Tsutsui et al., 2005). To date, the exact influence of the nuclear matrix on chromatin remains unclear, and must be clarified before higher levels of chromatin organization can be fully elucidated.

Chromosome structure beyond the 30 nm fiber has been studied most extensively in the highly compacted mitotic chromosome. One of the first models of mitotic chromosome organization is the radial loop model, and was built upon the concept of a metaphase protein scaffold (Marsden and Laemmli, 1979). The protein scaffold was proposed following initial studies of histone-depleted mitotic chromosomes, which revealed DNA loops attached to a core that included subunits of the SMC complex condensin (see below) and the type II topoisomerase Topo II (Top2) (Gasser et al., 1986; Laemmli et al., 1978; Saitoh et al., 1994). The protein scaffolding concept was further strengthened by the observation that both condensin and Top2 display an axial distribution along metaphase chromosomes (Earnshaw and Heck, 1985; Maeshima and Laemmli, 2003; Ono et al., 2003; Tavormina et al., 2002). Subsequent observations, however, placed the idea of a mitotic protein scaffold in doubt. For instance, Top2 depletion leaves *in vitro* assembled mitotic chromosome structure unperturbed, and nuclease treatment is sufficient to disintegrate micromanipulated chromosomes (Hirano and Mitchison, 1993; Poirier and Marko, 2002). Alternative concepts for mitotic chromosome organization include a chromatin network stabilized by protein crosslinks and hierarchical chromatin folding into a metaphase chromosome stabilized by condensin acting as axial "glue" (Kireeva et al., 2004; Poirier and Marko, 2002). Though the exact nature of the protein components of mitotic chromosomes is still debated, Top2 and condensin make essential

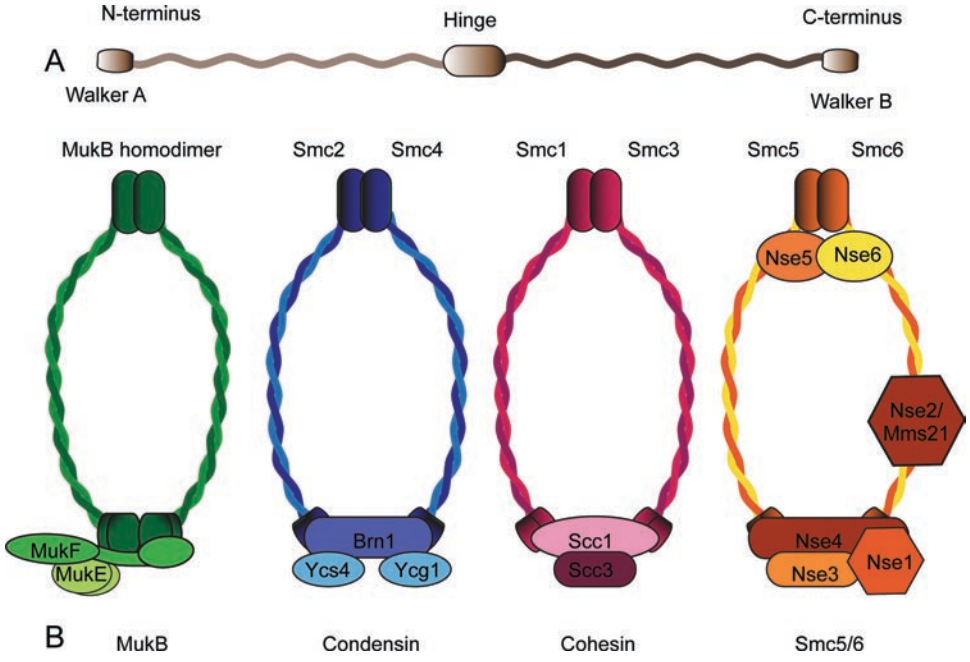

Figure 4. Basic SMC complex composition and architecture. (A) SMC proteins have a conserved domain structure consisting of a central hinge region flanked by two coiled-coil arms, and globular N- and C-termini containing Walker A and Walker B nucleotide binding motifs, respectively. (B) The *E. coli* MukB, and *S. cerevisiae* condensin, cohesion and Smc5/6 complexes are shown as examples of the basic structures of SMC complexes.

contributions to the structure of mitotic chromosomes, including mediating chromosome compaction and resolution at anaphase (Adachi et al., 1991; Hirano et al., 1997; Hirano and Mitchison, 1994; Holm et al., 1989; Uemura et al., 1987). In addition to structural roles, budding yeast Top2 has also been suggested to coordinate replication and transcription by topologically insulating transcription units within DNA loops (Bermejo et al., 2009). Moreover, while condensin appears to drive SCI removal by Top2, another SMC family member, the Smc5/6 complex, seems to facilitate their formation (see below).

Basic structure and composition of SMC protein complexes

The core of functional SMC complexes is formed by a dimer of SMC proteins (Figure 4) (Fousteri and Lehmann, 2000; Haering et al., 2002; Hirano and Hirano, 2002; Melby et al., 1998). Individual SMC proteins display a characteristic primary structure consisting of five conserved domains. Each SMC protein contains a flexible hinge region flanked by two long coiled-coil domains. The N- and C-termini form globular domains that contain Walker A and Walker B nucleotide binding motifs, respectively. The central hinge region of SMC proteins facilitates folding of the molecule that allows an antiparallel association of the protein arms and brings the Walker A and Walker B motifs into close proximity, constituting a complete ATP-binding pocket (Haering et al., 2002; Hirano and Hirano, 2002; Melby et al., 1998). All SMCs bind and hydrolyse ATP, and the ATPase activity is critical for the function of the complexes (Arumugam et al., 2003; Fousteri and Lehmann, 2000; Hirano et al., 2001; Hirano and Hirano, 1998; Kimura and Hirano, 1997; Verkade et al., 1999; Weitzer et al., 2003). The hinge domain also mediates dimerization between two SMC proteins.

The most well-studied bacterial SMC proteins are *Escherichia coli* (*E. coli*) MukB and *Bacillus subtilis* (*B. subtilis*) Smc, both of which form homodimeric complexes (Britton et al., 1998; Hirano and Hirano, 2002; Moriya et al., 1998; Niki et al., 1992; Niki et al., 1991). Based upon the identification of either MukB or Smc across different species, it was widely believed that bacteria in general contained a single SMC complex. However, a third family of bacterial SMC proteins was recently identified, MksBEF (MukBEF-like SMC), and shown to coexist with MukB or Smc in particular species (Petrushenko et al., 2011). In eukaryotes, six SMC genes have been identified that produce three distinct heterodimeric complexes. Cohesin, responsible for maintaining sister chromatid cohesion from S-phase until chromosome separation at anaphase, contains a heterodimer of Smc1 and Smc3 (Guacci et al., 1997; Losada et al., 1998; Michaelis et al., 1997; Toth et al., 1999). Condensin, composed of Smc2 and Smc4, is necessary for the proper compaction and segregation of mitotic chromosomes, and exists in two forms in metazoans, condensin I and II (Freeman et al., 2000; Hirano et al., 1997; Schmiesing et al., 2000; Sutani et al., 1999). Smc5 and Smc6 form the Smc5/6 complex, which has been shown to be necessary for DNA repair and was recently implicated in managing replication-induced topological stress in budding yeast (Fousteri and Lehmann, 2000; Kegel et al., 2011; Taylor et al., 2001). Associated with the central SMC core of each complex are specific non-SMC proteins, and all

Table 1. SMC complex orthologs from representative eukaryotic organisms.

	S. cerevisiae	*S. pombe*	*D. melanogaster*	*X. laevis*	*H. sapiens*
Cohesin					
	Smc1	**Psm1**	**SMC1**	**SMC1**	**SMC1a**
	Smc3	**Psm3**	**SMC3**	**SMC3**	**SMC3**
	Scc1/Mcd1*	Rad21*	RAD21*	RAD21*	RAD21*
	Scc3	Psc3	SA	SA1, SA2	SA1, SA2
Condensin					
	Smc2	**Cut14**	**SMC2**	**CAP-E**	**CAP-E**
	Smc4	**Cut3**	**SMC4**	**CAP-C**	**CAP-C**
	Ycs4	Cnd1	CAP-D2/CG31989	CAP-D2/ CAP-D3†	CAP-D2/ CAP-D3†
	Ycs5	Cnd3	CAP-G/?	CAP-G/ CAP-G2†	CAP-G/ CAP-G2†
	Brn1*	Cnd2*	Barren*/CG14684†	CAP-H*/ CAP-H2†	CAP-H*/ CAP-H2†
SMC5/6					
	Smc5	**Spr18**	**SMC5**	**SMC5**	**SMC5**
	Smc6	**Rad18**	**CG5524**	**SMC6**	**SMC6**
	Nse1	Nse1	NSE1	NSE1	NSE1
	Nse2/Mms21	Nse2	–	NSE2	NSE2
	Nse3	Nse3	–	–	MAGEG1
	Nse4/Qri2*	Rad62*	–	–	NSE4a/NSE4b*
	Nse5	Nse5	–	–	–
	Nse6/Kre29	Nse6	–	–	–

SMC proteins are in bold. *Kleisin subunit. †Condensin II. Dash indicates the ortholog remains unidentified.

complexes contain a representative of the kleisin protein family (Haering et al., 2002; Schleiffer et al., 2003). The kleisin subunit interacts with the head domains of both SMC proteins and imparts an overall ring-shaped architecture to the complexes (Fennell-Fezzie et al., 2005; Haering et al., 2002; Onn et al., 2007; Palecek et al., 2006; Schleiffer et al., 2003) (Figure 4). An exception is MukF, which exists as an elongated $(E_2F)_2$ complex with MukE (Fennell-Fezzie et al., 2005; Petrushenko et al., 2006a; Woo et al., 2009). As opposed to the kleisin subunits of other SMC complexes, a recent structural study revealed that the elongated shape of MukEF prevents it from simultaneously binding the engaged head domains of a single MukB dimer, indicating that MukBEF functions as a multimolecular assembly as opposed to individually (Woo et al., 2009). Orthologs of SMC protein complexes from representative organisms are listed in Table 1.

SMC protein complexes – background

E. coli MukB was originally identified in a screen designed to detect mutations that produced anucleate cells following cell division (Niki et al., 1991). Additional phenotypes resulting from MukB inactivation include temperature sensitivity, nucleoid unfolding and chromosome decondensation (Niki et al., 1991; Weitao et al., 2000a; Weitao et al., 2000b). As previously stated, MukB interacts with two non-SMC proteins, MukE and MukF (Yamanaka et al., 1996; Yamazoe et al., 1999). Deletion of either MukE or MukF confers phenotypes similar to MukB inactivation (Fennell-Fezzie et al., 2005; Yamanaka et al., 1996). Null mutations of the *B. subtilis* MukB homolog, Smc, results in the same defects in chromosome structure and segregation as in *E. coli* cells lacking MukB function (Britton et al., 1998; Moriya et al., 1998). Two non-Smc proteins, the kleisin ScpA and ScpB, were identified by virtue of their interaction with Smc (Fennell-Fezzie et al., 2005; Mascarenhas et al., 2002). Further genetic analysis revealed an epistatic relationship between Smc, ScpA and ScpB (Mascarenhas et al., 2002; Soppa et al., 2002).

The first component of a eukaryotic condensin complex was discovered through investigations of proteins responsible for the organization of mitotic chromosomes in HeLa cells. This led to the identification of the DNA scaffolding proteins SC1 and SC2 (Lewis and Laemmli, 1982). SC1 was subsequently shown to be Top2 and SC2 a condensin subunit (Earnshaw et al., 1985; Saitoh et al., 1994). In *Schizosaccharomyces pombe* (*S. pombe*), the condensin subunits Cut3 (Smc4) and Cut14 (Smc2) were identified through library complementation of mutants with a "Cut", i.e. chromosome missegregation phenotype (Saka et al., 1994). By virtue of sequence similarity to *SMC1*, budding yeast *SMC2* was identified and shown to confer chromosome missegration and decondensation phenotypes when mutated. Sequence analysis further led to the suggestion that Smc2 was part of an SMC subgroup that included *Xenopus laevis* (*X. laevis*) XCAP-E (Smc2) and Cut14 proteins (Strunnikov et al., 1995). In testing the function of proteins involved in building mitotic chromosome structure, extracts from unfertilized *X. laevis* eggs were used to assemble mitotic chromosomes from sperm chromatin. Protein analysis of the generated chromatids identified XCAP-B (Top2), XCAP-C (Smc4) and XCAP-E (Smc2) (Hirano and Mitchison, 1994). XCAP-C was found to be necessary for the formation of the mitotic chromosomes, as chromosome assembly was prevented by an XCAP-C specific antibody (Hirano and Mitchison, 1994). Further, similarity between XCAP-C and XCAP-E to previously identified yeast Smc1 and

the chicken scaffolding condensin component SC2 was established (Saitoh et al., 1994). Immunoprecipitation with antibodies against XCAP-E or XCAP-C resulted in the recovery of a complex that contained three non-SMC subunits of condensin, XCAP-D2, XCAP-G and XCAP-H. This 13S complex was found to contain the condensation activity and named the 13S condensin complex. Another 8S condensin complex, containing only XCAP-E and XCAP-C, was also recovered, but lacked condensation activity (Hirano et al., 1997).

The cohesin *SMC1* gene was originally identified in a *Saccharomyces cervisiae* (*S. cerevisiae*) mutant that displayed increased loss rates of a centromeric plasmid, a defect that resulted from nondisjunction during plasmid segregation (Larionov et al., 1985). Subsequent sequence analysis revealed that Smc1 shared structural similarities to MukB (Strunnikov et al., 1993). Later, screens designed to isolate yeast mutants with increased chromosome loss rates and sister chromatid separation prior to anaphase led to the identification of SMC1, SMC3, SCC1/MCD1 and SCC3 (Guacci et al., 1997; Michaelis et al., 1997; Toth et al., 1999). In *X. laevis*, immunoprecipation experiments against SMC1 and SMC3 identified a 14S complex composed of SMC1, SMC3, XRad21 (Scc1) and one of two Scc3 orthologs, SA1 or SA2 (Losada et al., 1998).

Characterization of the Smc5/6 complex has mostly arisen from work around DNA repair. A screen for DNA damage sensitive mutants in *S. pombe* identified Rad18, later found to be Smc6 (Lehmann et al., 1995; Nasim and Smith, 1975; Verkade et al., 1999). Spr18 (Smc5) was subsequently identified as an Rad18 interaction partner (Fousteri and Lehmann, 2000). The non-SMC elements Nse1-6 were identified by virtue of two-hybrid interaction or copurification with Smc5 (McDonald et al., 2003; Pebernard et al., 2004; Pebernard et al., 2006). Nse4 was also independently identified as Rad62 in a DNA damage sensitivity screen (Morikawa et al., 2004). A combination of sequence analysis, DNA damage sensitivity screens and analysis of uncharacterized essential genes identified the *S. cerevisiae* Smc5/6 complex (Fujioka et al., 2002; Hazbun et al., 2003; Hu et al., 2005; Prakash and Prakash, 1977a; Prakash and Prakash, 1977b). Human orthologs have been identified for Smc5, Smc6 and Nse1-4 (Taylor et al., 2008; Taylor et al., 2001). Thus far, no human orthologs of Nse5 or Nse6 have been found.

SMC complexes and DNA topology

Studies to determine how SMC protein complexes exert their effect on DNA and chromosomes have proceeded along several lines of investigation. It was found that the chromosome segregation and condensation defects of *mukb*/*smc* mutants were partially suppressed by lowering the expression of type I topoisomerase (Topo 1) or overexpression of type II topoisomerase IV (Topo IV). Both conditions are thought to increase net negative supercoiling within the genome (Lindow et al., 2002; Sawitzke and Austin, 2000; Tadesse et al., 2005). This indicates that the phenotypes of *mukb/smc* mutants are due to a general increase of positive supercoiling, which is counteracted by altering the activities of topoisomerases. In line with that idea, *smc* mutants are hypersensitive to inhibition of DNA gyrase, which decreases levels of negative supercoiling (Lindow et al., 2002). However, *smc* mutants produce plasmids with increased levels of negative supercoiling (Lindow et al., 2002). Thus, the findings only allow the general conclusion that the topological balance is perturbed in *mukb/smc* mutants, and that Mukb/Smc function is likely to be linked to a change in topology. The contradicting results also urge us to underline the following. Perturbation of the topological status by inhibition of a protein needed for maintaining it will influence all processes which require or lead to a change in DNA topology. If the perturbation allows cell survival, this pleitropic effect can end in a new topological balance that might not directly reflect the function of the protein that was initially inhibited. It is therefore possible that the combination of *mukb/smc* mutants with other "topology-mutants" results in a balance that would not be expected simply by adding together the effects of both single mutants. The balance might also be different in chromosomes as opposed to plasmids. Similar caveats are of course true for studies of all type of cellular events, but might be especially relevant when investigating DNA and chromosome topology as they influence, and are influenced by, most DNA-based processes. Moreover, *smc* and topoisomerase mutations have been shown to change protein expression patterns, likely via effects on transcription (Tadesse et al., 2005). This complicates the analysis of topology mutants even further.

Another commonly used method to investigate the topological functions of SMC complexes is to investigate the topology of DNA plasmids after incubation with purified SMC proteins or complexes. *X. laevis* condensin is capable of introducing positive supercoils into plasmid in an ATP-dependent manner (Kimura and Hirano, 1997) (Figure 5). This activity is dependent upon the presence of a type I topoisomerase, the non-SMC components of the condensin complex, as well as mitosis specific post-translational modifications of the non-SMC subunit XCAP-D2 (Kimura et al., 1998). It was proposed that positive superhelicity is introduced into the plasmid through ATP-dependent toroidal wrapping of the DNA by the condensin complex. This action produces the formation of compensatory negative supercoils that are subsequently removed by the topoisomerase, imparting an overall positively supercoiled character to the DNA. The supercoiling activity of *X. laevis* condensin was correlated with the generation of positive three-node knots, called trefoils, in a similar assay using nicked circular dsDNA and a type II topoisomerase (Kimura et al., 1999). The type and chirality of knots produced in this assay are indicative of the relative conformation of DNA segments prior to topoisomerase II action. The production of positive trefoils therefore confirmed that the complex is indeed capable of organizing at least two torodial DNA loops

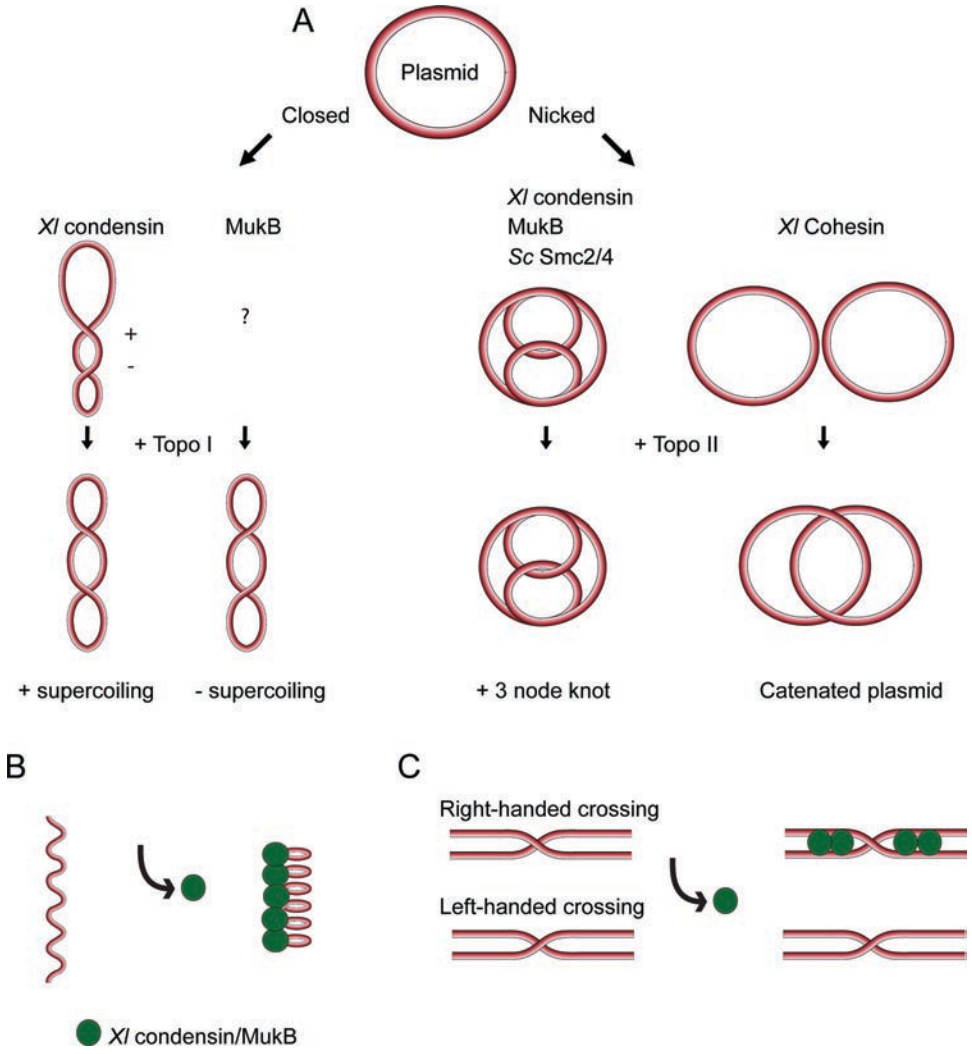

Figure 5. A summary of the effects of SMC proteins and complexes on DNA topology *in vitro*. (A) The plasmid substrates (top) was used in either closed circular (left) or nicked (right) form. (Left) *X. laevis (Xl)* condensin induces positive supercoiling in the closed plasmid substrate, resulting in compensatory negative supercoiling in another portion of the molecule. Topo I treatment removes negative supercoils, leaving the plasmid positively supercoiled. In a similar assay, MukB produces a negatively supercoiled plasmid after passing through an undefined intermediate. (Right) When incubated with a nicked plasmid, *X. laevis* condensin, MukB and *S. cerevisiae (Sc)* Smc2/4 organize two right-handed loops in a nicked plasmid. Treatment by Topo II treatment generates +3 node knots. The same assay with *X. laevis* cohesion stimulates intermolecular plasmid interactions that produce catenated dimers following Topo II treatment. (B) *X. laevis* condensin and MukB contract DNA in a single molecule assay. (C) MukB preferably bridges right-handed DNA crossings.

in a right-handed configuration (Figure 5). *S. cerevisiae* Smc2/4 and *E. coli* MukB both produce positive trefoils in knotting assays as well, but differ from the *X. laevis* condensin in several aspects. Positive chiral knotting of DNA by yeast SMC2/4 occurs without an accompanying change in net supercoiling, while MukB introduces negative supercoiling (Petrushenko et al., 2006a; Stray et al., 2005; Stray and Lindsley, 2003). Further, neither Smc2/4 nor MukB requires non-SMC subunits for knotting or supercoiling activity (Petrushenko et al., 2006a; Stray and Lindsley, 2003). To the contrary, the addition of MukEF to MukB results in a MukBEF complex incapable of binding or reshaping DNA (Petrushenko et al., 2006b). These differences remain unexplained, and it is uncertain how condensins change DNA topology. Electron microscopic studies suggest that *X. laevis* condensin is able to wrap DNA about the head domains of the complex, thereby introducing positive gyres into DNA (Bazett-Jones et al., 2002). Condensin has also been proposed to actively overwind DNA or to trap DNA in a positive writhe (Kimura et al., 1999). Despite the above described discrepancies and the still unclear mechanistic details, a common feature of the *E. coli*, *S. cerevisiae* and *X. laevis* condensins is that all create right-handed knots in nicked plasmids when incubated in the presence of Top2. This indicates that they all organize DNA in right-handed toroidal supercoils. Interestingly, this type of geometrical organization, two DNA molecules which cross in a right-handed fashion, is a reoccurring theme in investigations of SMC complexes and topology (see below).

Cohesin has been less characterized biochemically than the condensin complexes. In a knotting assay

using nicked circular dsDNA and type II topoisomerase, cohesin purified from HeLa cell extracts generated primarily intermolecular catenated DNA molecules, as opposed to intramolecular knots as condensin (Losada and Hirano, 2001). Additionally, in a ligation assay with linear DNA fragments, incubation with cohesin prior to the addition of *E. coli* DNA ligase produced linear multimers. Both activities occurred independently of SMC ATP activity. The tendency of cohesin to facilitate intermolecular interactions between DNA molecules is in line with its known role in sister chromatid cohesion.

If the effect of cohesin on DNA topology has been less studied compared to condensin, this is even truer for the Smc5/6 complex. So far it is known that its ATPase activity is stimulated by DNA (Fousteri and Lehmann, 2000). Our unpublished data indicate that the budding yeast Smc5/6 complex introduces positive supercoils into plasmid DNA, but more definitive investigations are required (Kanno and Sjögren, unpublished).

Even though the above bulk experiments investigating changes in DNA topology are revealing, important additional details have been provided by single molecule studies on MukB and condensins. It has been shown that *X. laevis* condensin is capable of contracting a single DNA molecule in an ATP-dependent mechanism (Strick et al., 2004). In agreement with plasmid reshaping assays, only mitotic condensin displayed DNA contracting activity. MukB is also able to contract DNA, as shown in experiments using magnetic tweezers to measure decreases in DNA extension in the presence of the protein (Cui et al., 2008). Consistent with prior observations of MukB activity, ATP was not required for DNA contraction. Instead, ATP was found to regulate the initiation of DNA contraction, while having no appreciable effect on condensation once begun. It was recently reported that MukB supports bridging between two DNA fragments (Petrushenko et al., 2010). This activity was found to occur in two separate steps, the initial binding of MukB to DNA, followed by subsequent binding to a second, protein-free DNA fragment. This bridging was modulated by ATP, unlike the role of MukB in plasmid reshaping. Moreover, MukB bridging of a single pair of parallel DNAs was more efficient when the DNAs were crossed in a right-handed manner, while left-handed crossings were poor substrates for bridging. This is in line with the findings that MukB organizes plasmid DNA into right-handed toroidal supercoils, and is yet another sign that at least one part of the (topological) function of SMC complexes might be determined by the chirality of DNA crossings.

Condensin and chromosome topology

Early observations indicating that SMC complexes influence chromosome topology and function together with topoisomerases came from studies of mitotic chromosome condensation and decatenation. In bacteria, mutations of either MukB or Topo IV perturb chromosome segregation (Kato et al., 1990; Niki et al., 1991). In flies, condensin and Topo II interact with each other, and mutations of either protein trigger similar segregation defects (Bhat et al., 1996). Topo IV overexpression in *B. subtilis* results in chromosome hypercompaction and partially rescues *smc* mutants, while underexpression leads to chromosome decondensation (Tadesse et al., 2005). Budding yeast strains carrying *smc2* or *smc4* temperature sensitive alleles display chromosome segregation defects that are specifically exacerbated at the rDNA locus (Freeman et al., 2000; Strunnikov et al., 1995). The rDNA segregation defect was shown to be rescued by the ectopic expression of a viral Topo II, strongly suggesting that catenations underlie the segregation defect and that endogenous Topo II requires condensation to resolve it (D'Ambrosio et al., 2008a). In *Drosophila melanogaster* (*D. melanogaster*) S2 cells depleted of SMC4, the chromosomal distribution of Topo II is disrupted, and produce extracts with reduced Topo II decatenation activity (Coelho et al., 2003). A number of recent reports have shed some light on the interplay between condensins and type II topoisomerases in bacteria and yeast. In *E. coli*, MukB was found to stimulate Topo IV activity through a direct physical interaction (Hayama and Marians, 2010; Li et al., 2010). Further, a MukB isolate containing a point mutation that failed to rescue the temperature sensitive phenotype of *mukb* strains was shown to interact with Topo II with reduced efficiency (Li et al., 2010). Another study examined the relationship between decatenation and DNA supercoiling of mitotic plasmid DNA in yeast (Baxter et al., 2011). In a conditional *top2* background, centromeric plasmids undergoing replication transitioned between two different forms of supercoiled catenated dimer, a premitotic form, and a mitotic form whose appearance coincided with chromosome segregation. The transition from premitotic to mitotic catenated dimer was shown to be accompanied by a topological conversion from negative to positive supercoiling. Generation of the positively supercoiled form of catenated dimer was found to be dependent upon the presence of mitotic spindles and condensin. In *in vitro* titration experiments, notable differences in the kinetics of Top2 activity on the different forms of catenated dimers were observed. Relatively high levels of Top2 were necessary to decatenate the negatively supercoiled premitotic dimers, and this activity occurred simultaneously with supercoil relaxation. In contrast, decatenation of positively supercoiled mitotic dimers preceded supercoil relaxation and required much lower levels of enzyme. These results led to the proposal that positive supercoiling generated by condensin and mitotic spindles results in the preferential exposure of intermolecular catenanes to Top2 action.

The genome organizing effects of condensin during interphase have been studied at the tRNA genes in yeast. The *S. cervisiae* 274 tRNA genes are dispersed among all 16 chromosomes, but form clusters that are associated with the nucleolus (Haeusler et al., 2008; Thompson et al., 2003). In a process termed tRNA gene-mediated (tgm) silencing, tRNA clustering induces silencing of RNA

polymerase II transcription near tRNA genes (Wang et al., 2005). Genome-wide analysis showed that condensin binds all tRNA genes, an association thought to be mediated through an interaction with the polymerase III transcription factors TFIIIB and TFIIIC (D'Ambrosio et al., 2008b; Haeusler et al., 2008). In a plasmid-based reporter assay, disruption of any condensin subunit relieved tgm silencing. Further, *in situ* hybridization showed that tRNA gene clustering was either defective, or wholly absent in condensin subunit mutants (Haeusler et al., 2008).

Condensin may also function to limit the interaction between distant DNA regions. In certain *D. melanogaster* cell types, numerous rounds of DNA replication without cell division gives rise to polytene chromosomes, which are composed of arrays of closely associated sister chromatids (Edgar and Orr-Weaver, 2001). Polytene chromosomes of nurse cells are disassembled during development. In condensin II mutants, this disassembly of polytene chromosomes is compromised, suggesting that condensin antagonizes chromatid pairing (Hartl et al., 2008). Conversely, overexpression of the Cap-H2 condensin II subunit was sufficient to induce the disassembly of normally persistent polytenic chromosomes of salivary gland nuclei. In somatic cells, Cap-H2 mutants increased transvection, a process in which a gene is transcriptionally activated or repressed in *trans* by regulatory elements located on the homologous chromosome (Duncan, 2002). It was therefore proposed that condensin-generated supercoiling could physically disrupt chromosome alignment or establish a higher-order structure that restricts the accessibility of regions prone to *trans* association.

Cohesin and chromosome topology

In addition to its well-established canonical function in sister chromatid cohesion, numerous lines of evidence implicate cohesin in mediating intrachromosomal interactions as well. Cohesin has been shown to cooperate with the mammalian zinc-finger transcription factor CCCTC-binding factor (CTCF). CTCF performs a wide variety of cellular tasks, including regulating the interaction between gene enhancers and promoters by mediating DNA looping (Phillips and Corces, 2009). Using chromatin immunoprecipitation (ChIP)- based assays, a number of studies have detected an extensive overlap between genomic CTCF and cohesin binding sites (Parelho et al., 2008; Wendt et al., 2008). While the full significance of this co-localization on chromosomes is not currently understood, recent studies have provided insight into the functional relationship between CTCF and cohesin at particular loci. In human CD4 T cells, CTCF and cohesin co-localize to the developmentally regulated IFNG gene, encoding the cytokine interferon-γ (Hadjur et al., 2009). CD4 T cells are the progenitors of T helper (T_H) 1 and 2 cells. During T_H1 differentiation, IFNG becomes inducible, while differentiation of T_H2 cells results in silencing of IFNG. Chromosome conformation capture (3C) detected T_H1 specific chromosomal interactions at the IFNG locus that were disrupted by RNA interference (RNAi) against the cohesin subunit RAD21. The disruption of 3C interactions was associated with a reduction of basal IFNG transcription levels, as well as inducibility. Together, these data indicate that cohesin plays a critical role in developmentally regulated topological restructuring at the IFNG locus, as well as provide a possible mechanism for its contribution to transcriptional regulation.

Cohesin is also required for maintaining CTCF-mediated loops at the imprinted insulin-like growth factor 2 (*IGF2*)-*H19* locus (Nativio et al., 2009). IGF2 and H19 are normally expressed solely from the paternal and maternal alleles, respectively (Wendt and Peters, 2009). 3C experiments in mouse cells have verified that the allele-specific expression pattern of *IGF2-H19* indeed reflects specific DNA looping that allows or impedes communication between the respective gene and distant enhancer elements. In human breast epithelial cells, depletion of RAD21 by RNAi resulted in the disruption of CTCF-mediated 3C interactions, as well as induced a switch in IGF2 expression from monoallelic to bi-allelic.

ChIP sequencing of human breast and liver cell lines revealed that cohesin binds a significant number of genomic binding apart from CTCF (Schmidt et al., 2010). Instead, cohesin binding sites were found to be enriched for co-occupancy with cell-type specific transcription factors. In breast cancer cells, cohesin binding sites were co-occupied by the transcriptional regulator estrogen response (ER)- α. ER-α was recently shown to promote long-range DNA interactions and proposed to coordinate gene transcription through the formation of loop anchors that bring together co-regulated genes (Fullwood et al., 2009). Among all ER-α binding sites, cohesin enrichment was shown to occur preferentially in a subset participating in loop anchoring. Further, genes co-occupied by both ER-α and cohesin, as opposed to ER alone, were found more likely to be transcriptionally responsive to estrogen treatment. These data suggest that cohesin might also contribute to gene regulation through chromosomal looping independently of CTCF.

DNA looping by cohesin has also been suggested to play a role in the organization of chromosomes undergoing replication. During S-phase, DNA replication origins cluster together in discrete foci termed replication factories (Jackson and Pombo, 1998; Kitamura et al., 2006). Intervening DNA segments emanate from replication factories in loops, the size of which is known to be correlated with the spacing between origins (Buongiorno-Nardelli et al., 1982; Lemaitre et al., 2005). Origin spacing itself has been shown to be determined by replication fork velocity in the previous S-phase (Courbet et al., 2008). A recent study reported that down-regulation of cohesin in HeLa cells resulted in a delay of S-phase progression and longer DNA loops associated with replication factories (Guillou et al., 2010). The S-phase delay appeared to result from a reduction of activated origins within replication factories suggesting that cohesin could contribute

to the topological structure of interphase chromosomes by stabilizing replication factory associated DNA loops.

Smc5/6 complex and chromosome topology

The Smc5/6 complex has been most strongly associated with DNA repair, and several lines of evidence indicate that it like other SMCs could influence DNA and chromosome topology. In budding yeast the Smc5/6 complex is enriched at repetitive rDNA and telomeric sequences, and in strains carrying *smc5* or *smc6* temperature sensitive alleles both loci display segregation defects (Torres-Rosell et al., 2005). This is also true for any chromosomal region after treatment with replication inhibiting drugs such as MMS (methyl methanesulfonate) (Ampatzidou et al., 2006; Miyabe et al., 2006; Pebernard et al., 2006). The segregation error was found to be due to a defect in resolving recombination intermediates between sister chromatids (Branzei et al., 2006; Mankouri et al., 2009; Sollier et al., 2009). It was subsequently shown that recombinational repair of rDNA normally takes place by the exclusion of the DNA double strand break outside the nucleolus (Torres-Rosell et al., 2007b). In a *smc6* mutant relocalization of an induced DNA double strand break is compromised and homologous recombination occurs within the nucleolar compartment. It has also been observed in *D. melanogaster* that double strand breaks in repetitive heterochromatin must first be excluded from the heterochromatic domain before the strand-invasion protein, Rad51, can associate (Chiolo et al., 2011). Exclusion of Rad51 was dependent upon a functional Smc5/6 complex, as RNAi depletion of Smc5 or Smc6 resulted in the formation of Rad51 repair foci within heterochromatin. Together this argues that Smc5/6 either prevents the repair to occur within and/or facilitates the exclusion of the damage DNA from repetitive and heterchromatic regions. In both cases it could be a sign of a role in setting chromosome topology.

Recent work in budding yeast has also implicated Smc5/6 in the resolution of replication-induced supercoiling. Inhibition of Smc5/6 was shown not only to inhibit recombination, but also to facilitate replication termination in the rDNA repeats (Torres-Rosell et al., 2007a)). Moreover, ChIP on chip and ChIP sequencing analysis shows that the Smc5/6 complex associates to all chromosomes as a consequence of replication (Kegel et al., 2011; Lindroos et al., 2006). The frequency of Smc5/6 chromosome arm-binding sites increases with chromosome length, and artificial chromosome shortening or circularization decreases and increases Smc5/6 binding frequency, respectively. In addition, *smc5/6* mutants display a late replication delay that specifically affects longer chromosomes. This phenotype was also detected in cells lacking functional Top1 or Top3. As Top1 is considered to be the main player in the resolution of replication-induced positive supercoils and Top3 might remove topological tension at replication termination sites (Mankouri and Hickson, 2007; Wang, 2002), this argues that Smc5/6 also removes topological tension. Two findings led us to propose that it executes this function by stabilizing sister chromatid intertwinings behind the replication fork, thereby facilitating its rotation and preventing the accumulation of positive supercoils ahead the replication machinery (Figure 6). First, the amount of chromosomal-associated Smc5/6 increases in *top2-4* mutants in which the number of SCIs increases. Second, the number of catenations between replicated plasmids was lower in a *smc6 top2* double mutant as compared to *top2* single. Notably, the SCIs which form by fork rotation in the direction which prevents the accumulation of supercoils are right-handed. Possibly, the Smc5/6 complex can perform its function in replication because it displays the same chiral preference for DNA crossings as eukaryotic condensin and the bacterial SMC complexes.

Conclusion

Ever since the discovery of SMC proteins and complexes, their tight relation to DNA and chromosome topology has been evident. This is especially true for the bacterial complexes and eukaryotic condensins, which topological functions have been extensively studied *in vitro* and *in vivo*. This has shown that condensin acts together with Top2, and has large impacts on both DNA and chromosome topology. The findings also suggest that their ability to change DNA topology *in vitro* is related to their roles in arranging higher order chromosome structure *in vivo*. However, the details of this relationship remain obscure, partly because it is difficult to predict how a function that leads to a change in topology of naked DNA will be executed in the context of chromatin and chromosome dynamics. How do histones, histone modifications, chromatin folding, and the possible changes in topological status they all introduce, influence the function of a specific SMC complex? Other important, but difficult questions to answer are: How long-lived is a change in topological tension within the DNA of a cell, and how large an impact does it have on the function of SMC complexes and chromosome topology? When a SMC complex arranges loops, does DNA topology determine loop size? For example, is the reported crosstalk between replication speed and chromosome loop size transmitted via DNA topology and SMC complexes (Courbet et al., 2008)? To address these and related question new tools are needed that allow detailed dissection of DNA topological phenotypes and their effect on chromosome topology. Genome-wide analyses of topological tension using psoralen crosslinking or transcriptional efficiency as read outs have been conducted by the team of J. Roca (Bermudez et al., 2010; Joshi et al., 2010). These approaches and techniques that allow rapid and detailed resolution of topological status in wild type cells, or after short periods of perturbation, promise to provide valuable new insights. Combined with a set of SMC complex mutants that lack certain aspect of their ability to alter DNA topology *in vitro*, these tools could shed more light on the relationship between

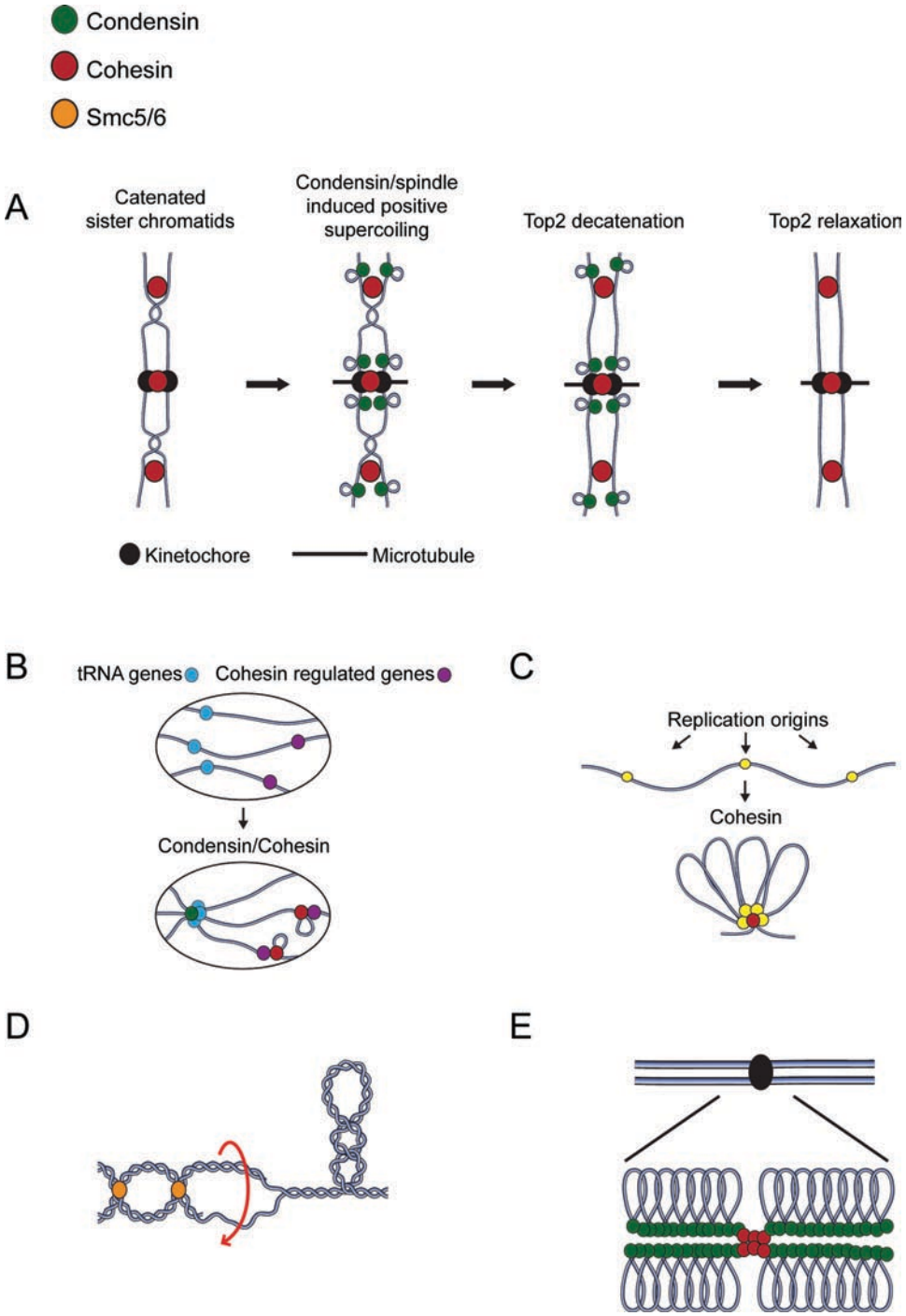

Figure 6. Schematic illustrations of some of the potential roles of SMC proteins in chromosome topology. (A) Condensin facilitates decatenation of sister chromatids. Shown is a model proposing that following chromosome replication, positive supercoiling induced by condensin and mitotic spindles direct Top2 to remove catenations prior to DNA supercoil relaxation. (B) Condensin organizes the clustering of tRNA genes and cohesin regulates gene transcription by promoting chromatin looping. (C) Cohesin organizes replication origins within replication factories. (D) The Smc5/6 complex helps remove replication-induced topological stress by facilitating fork rotation through stabilization of sister chromatid intertwinings. (E) Cohesin and condensin organize mitotic chromosomes. The region surrounding the centromere of a mitotic chromosome is highlighted. Condensin promotes chromosome compaction of chromsome arms, and cohesin holds sister chromatids together at the centromere.

the functions of SMC complexes in DNA and chromosome topology.

Finally, while writing this review it became clear that a reoccurring theme in the studies of topology and SMC complexes is a preference for right-handed DNA crossings. Condensin and MukB organize plasmids into a right-handed writhe (Kimura et al., 1999; Petrushenko et al., 2006a; Stray et al., 2005; Stray and Lindsley, 2003).

MukB preferentially bridges DNA molecules, which are crossed in a right-handed way (Petrushenko et al., 2010). The Smc5/6 complex appears to have affinity for the right-handed SCIs (Kegel et al., 2011). Moreover, both Smc5/6 and cohesin are recruited to sites of DNA double strand breaks, which are repaired by homologous recombination (Lindroos et al., 2006; Strom et al., 2004; Unal et al., 2004). Structural determination of a synthetic double Holliday junction, the most common recombination intermediate, shows that the DNA helices are joined in a right-handed structure (Ortiz-Lombardia et al., 1999). It is interesting to speculate that SMC complexes are able to recognize, and/or organize two DNA molecules into this geometric chirality. Even though more detailed studies are required to verify this ability and its relevance to the *in vivo* functions of SMC complexes, it could be a key to a better understanding of how they perform their multiple roles within a cell.

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