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Review: SMCs in the World of Chromosome Biology—From Prokaryotes to Higher Eukaryotes

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The study of higher order chromosome structure and how it is modified through the course of the cell cycle has fascinated geneticists, biochemists, and cell biologists for decades. The results from many diverse technical avenues have converged in the discovery of a large superfamily of chromosome-associated proteins known as SMCs, for structural maintenance of chromosomes, which are predicted to have ATPase activity. Now found in all eukaryotes examined, and numerous prokaryotes as well, SMCs play crucial roles in chromatid cohesion, chromosome condensation, sex chromosome dosage compensation, and DNA recombination repair. In eukaryotes, SMCs exist in five subfamilies, which appear to associate with one another in particular pairs to perform their specific functions. In this review, we summarize current progress examining the roles these proteins, and the complexes they form, play in chromosome metabolism. We also present a twist in the SMC story, with the possibility of one SMC moonlighting in an unpredicted location. © 2000 Academic Press

INTRODUCTION

Stretched end-to-end, the DNA in any one cell of a human body would measure about 2 m. Not only does the cell manage to fit this huge length of DNA into its approximately 5-μm-diameter nucleus, it also condenses it even further prior to cell division, so that the length of a single DNA molecule is compacted nearly 10,000-fold in the metaphase chromosome. Two mechanistically distinct but interrelated processes are involved in the formation of mitotic chromosomes. In concert with replication or shortly thereafter, cohesion must be established between sister chromatids and properly maintained until the metaphase to anaphase transition. Additionally, the chromatin must be compacted to yield two condensed sister chromatids tightly paired at the centromeric regions and also along the length of the arms. It is critical for this condensation to happen in an orderly fashion so as to prevent any possible entanglement or breakage of sister chromatids during anaphase which would have dire consequences to the cell. This folding of interphase chromatin to give paired metaphase chromatids is surely one of the most visually dramatic events of the cell cycle and ultimately fundamental for ensuring the faithful segregation of genetic information during cell division (reviewed in Heck, 1997; Koshland and Strunnikov, 1996; Murray, 1998).

Data suggesting biochemical differences between interphase and mitotic chromatin came initially from studies of the synchronized nuclear cycles of *Physarum polycephalum*, a true slime mold. Histone H1 was found to be extensively hyperphosphorylated in mitosis (Bradbury *et al.*, 1974; Mueller *et al.*, 1985) and strikingly, histone phosphokinase activity added exogenously to segments of *Physarum* plasmodia was able to accelerate the initiation of mitosis (Bradbury *et al.*, 1974). Hyperphosphorylation of H1 was also observed in CHO cells, and in addition, mitotic-specific phosphorylation of serine 10 on histone H3 was noted (Gurley *et al.*, 1975). Antibodies recognizing this highly conserved epitope specifically label mitotic chromosomes in all higher eukaryotes examined to date (Van Hooser *et al.*, 1998; Wei and Allis, 1998). Mutation of this particular serine to alanine in *Tetrahymena* leads to a disruption of chromosome condensation in mitosis and meiosis (Wei *et al.*, 1999). These studies point strongly to a role for specific histone H1 and H3 phosphorylation in mitotic chromosome condensation in higher eukaryotes.

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Not unexpectedly, nonhistone chromosomal proteins also play a role in the dramatic reorganization of higher order chromosome structure during the cell cycle. Striking electron microscope images of histone-depleted mitotic chromosomes highlighted the existence of a proteinaceous substructure constraining a sea of DNA loops of 50–100 kb (Paulson and Laemmli, 1977). This "scaffold" fraction, remaining after histone extraction, was remarkably simple in composition: two major proteins (Sc1 at 170 kDa and Sc2 at 135 kDa) and a number of smaller, less abundant proteins (Lewis and Laemmli, 1982). That this fraction represented more than an artifact of biochemical extraction became clear with the identification of Sc1 as topoisomerase II (Earnshaw *et al.,* 1985; Gasser *et al.,* 1986) and Sc2 as an SMC protein (Saitoh and Laemmli, 1994). Topoisomerase II was shown to be essential for chromosome segregation in yeast (Holm *et al.,* 1985; Uemura *et al.,* 1987) and, as expected for a function in chromosome dynamics during cell division, was observed to be a marker for proliferating, and not quiescent, cells (Heck and Earnshaw, 1986).

Additional substantive breakthroughs in our understanding of chromosome behavior during the cell cycle came from the discovery of the SMC family, a novel family of chromosome-associated ATPases which appear to have essential and specific roles in the higher order dynamics of chromosome cohesion and condensation. The SMC (structural maintenance of chromosomes, formerly stability of minichromosomes) proteins were initially identified through genetic studies of chromosome segregation in *Saccharomyces cerevisiae* (Strunnikov *et al.,* 1993). The first such molecule, Smc1p, was originally characterized by frequent minichromosome nondisjunction in mutants (Larionov *et al.,* 1985) and was later shown to be essential for viability and maintaining cohesion between sister chromatids (Strunnikov *et al.,* 1993). Sequence comparisons revealed this molecule to be a member of a highly conserved and ubiquitous family. Indeed, today we know of several structurally distinct SMC subgroups playing a key role in chromosome dynamics in a host of eukaryotic organisms as well as archaebacteria and many eubacteria (Hirano, 1998, 1999; Jessberger *et al.,* 1998; Koshland and Strunnikov, 1996; Strunnikov, 1998; Strunnikov and Jessberger, 1999). Although no canonical SMC family members have been found in gram-negative bacteria, similar phenotypes are displayed by *Escherichia coli* mutants affecting *mukB* (Niki *et al.,* 1992), which encodes an SMC-like protein (despite differences at the termini) with orthologues in other bacteria. Thus, it appears that SMC proteins have an ancient origin, reflecting their fundamental role in chromosome dynamics. The current phylogeny of SMC subfamilies and their members is displayed in Fig. 1.

A typical SMC molecule ranges in mass from 115 to 165 kDa and contains five major domains, as inferred from motifs in the amino acid sequence, in which the N- and C- termini are separated by two long coiled-coils of 200–450 residues and a central, globular hinge region (Jessberger *et al.,* 1998; Peterson, 1994). The most characteristic motif is the C-terminal "DA" box which was noted to have a candidate Walker B motif (ATP hydrolysis signature) (Saitoh *et al.,* 1994; Walker *et al.,* 1982). As the N-terminal end of the molecule also contains a putative Walker A motif (ATP binding domain), it was suggested that a functional ATPase domain may form by uniting the DA box with the ATP-binding motif (Saitoh *et al.,* 1994). This could occur in either of two ways: the molecule could bend at the hinge to bring the two termini together or by dimerizing as an antiparallel coiled-coil, bringing the N-terminal domain of one subunit next to the C-terminal domain of the other. Indeed, in the case of MukB from *E. coli* and the Smc protein from *Bacillus subtilis,* it has been shown that both structures are possible (Melby *et al.,* 1998). When rotary-shadowed samples of the purified proteins were viewed by electron microscopy (EM), they both showed a striking symmetry, appearing as a flexible hinge connecting two thin, rod-like arms with terminal globular domains. A range of different conformations were also observed, in which the two arms folded tightly against each other or opened up to 180° (separating the terminal globular domains by 100 nm). To distinguish which ends of the protein corresponded to the observed globular domains, a modified MukB was created by deleting the C-terminal domain and replacing the N-terminal domain with a rod-shaped 40-kDa fragment of fibronectin. When viewed by EM, the fibronectin domain appeared at both ends, indicating that each half of the V-shaped dimer was an antiparallel coiled-coil. Although the structure of other SMC proteins in different organisms has yet to be determined, it seems likely that they share the structure of Smc in *B. subtilis* as a similar basic head–rod–tail structure is also conserved in even more distantly related molecules such as the SbcCD nuclease of *E. coli* (Connelly *et al.,* 1998). Furthermore, the frictional ratio for the one eukaryotic SMC heterodimer was found to be similar to that of MukB, suggesting that the XCAP-C/XCAP-E heterodimer (and possibly other SMC molecules) may adopt a similar conformation.

Does the antiparallel dimerization of SMCs generate a functional ATPase? Using the analogue azido-ATP, which covalently bonds proteins after light activation (Knight and McEntee, 1985), it has been demonstrated that only the N-terminal domain of

yeast and mammalian SMCs can directly bind ATP (Akhmedov *et al.*, 1998). By contrast, the C-terminal domain appears to be sufficient for DNA binding (Akhmedov *et al.*, 1998; Graumann *et al.*, 1998). However, the presence of double-stranded DNA has been shown to stimulate ATP hydrolysis in two SMC-containing complexes (Jessberger *et al.*, 1996b; Kimura and Hirano, 1997), whereas the *B. subtilis* Smc homodimer (which binds preferentially to single-stranded DNA) has a single-strand DNA-stimulated ATPase activity (Hirano and Hirano, 1998). This at least suggests that ATP hydrolysis might be enhanced by bringing together the respective ATP and DNA binding motifs of the termini. Conversely, although ATP is not required for DNA binding (Hirano and Hirano, 1998; Kimura and Hirano, 1997; Kimura *et al.*, 1999), it is clearly required for preferential binding to positively supercoiled substrates (Kimura *et al.*, 1999). Likewise, ATP binding (though not hydrolysis) is also required for the enhanced aggregation of the *B. subtilis* Smc with ssDNA (Hirano and Hirano, 1998). Of course, it remains to be seen if ATP hydrolysis itself is strictly abolished by removing the C-terminal domain. However, the ability to form antiparallel dimers is not necessarily sufficient to generate a functional ATPase. For example, the ATP-stimulated activity of a *Xenopus* SMC complex involved in chromosome condensation depends on the presence of additional non-SMC subunits (Kimura *et al.*, 1998, 1999) and the SMC heterodimer of the RC-1 recombination complex similarly requires other components for full ATPase activity (Jessberger *et al.*, 1996b). In any event, ATP hydrolysis appears to be required for the full function of SMC-containing complexes, as shown by mutagenesis of the ATP-binding domain (Chuang *et al.*, 1994; Verkade *et al.*, 1999) or the use of nonhydrolyzable ATP analogues (Kimura and Hirano, 1997).

The eukaryotic SMCs may be divided into five major groups, including the Rad18 subfamily and four other subfamilies whose members may combine as heterodimers in larger functional complexes. While the Rad18 members are only essential for DNA repair (Lehmann *et al.*, 1995; Mengiste *et al.*, 1999; Verkade *et al.*, 1999), the other SMC molecules appear to have essential nonoverlapping functions, as examples of each type are known to be required for viability (Holt and May, 1996; Michaelis *et al.*, 1997; Saka *et al.*, 1994; Strunnikov *et al.*, 1993, 1995). The ability of particular eukaryotic SMC molecules to combine as heterodimers is suggested by their co-immunoprecipitation in roughly equimolar amounts (Darwiche *et al.*, 1999; Hirano and Mitchison, 1994; Lieb *et al.*, 1998; Losada *et al.*, 1998; Schmiesing *et al.*, 1998; Sutani *et al.*, 1999) and by analogy with the observed homodimerization of bacterial SMCs (Melby *et al.*, 1998). Although the potential for eukaryotic SMCs to form homodimers has also been demonstrated, this nonpreferential association only occurred when the fusion proteins were highly overexpressed, thereby titrating out the natural SMC partner (Strunnikov *et al.*, 1995). Moreover, it seems that homodimerization is insufficient for most eukaryotic SMCs to function as their *in vitro* activity depends on the combined presence of both subunits (Kimura and Hirano, 1997; Kimura *et al.*, 1999; Schmiesing *et al.*, 1998; Sutani and Yanagida, 1997) and mutation of just one SMC partner produces defects *in vivo* (Chuang *et al.*, 1994; Lieb *et al.*, 1998; Michaelis *et al.*, 1997; Saka *et al.*, 1994; Strunnikov *et al.*, 1993, 1995). On the other hand, higher levels of oligomerization would appear to be precluded by considering the overall mass of isolated SMC-containing complexes and the known mass of the other components (Hirano *et al.*, 1997; Hirano and Mitchison, 1994; Losada *et al.*, 1998; Sutani and Yanagida, 1997; Sutani *et al.*, 1999). So far, two fundamental classes of SMC heterodimer have been described in various organisms (Heck, 1997; Hirano, 1999; Hirano *et al.*, 1995; Jessberger *et al.*, 1998). These heterodimers may associate with different sets of non-SMC subunits to yield a range of large protein complexes with diverse functions, as shown in Tables I and II and Fig. 2. The SMC2/SMC4 heterodimer seems to have a role in mediating mitotic chromosome condensation, as part of the "condensin complex" (Hirano and Mitchison, 1994; Sutani and Yanagida, 1997; Sutani *et al.*, 1999). An SMC2 homologue and another SMC4-like molecule are implicated in sex chromosome dosage compensation in *Caenorhabditis elegans* (Chuang *et al.*, 1994; Lieb *et al.*, 1996, 1998). On the other hand, the SMC1/SMC3 heterodimer forms part of a complex important for sister chromatid cohesion, dubbed "cohesins" (Guacci *et al.*, 1997; Losada *et al.*, 1998; Michaelis *et al.*, 1997; Tóth *et al.*, 1999), and is also involved in recombination as part of the RC-1 complex (Jessberger *et al.*, 1996a,b).

Unlike the SMC proteins of eukaryotes, no cofactors for the *B. subtilus* Smc have been isolated to date (Sharpe and Errington, 1999). Nonetheless, a role for the protein in chromosome structure and partitioning was clearly demonstrated by the abnormal nucleoids and accumulation of anucleate cells in *smc* mutants (Britton *et al.*, 1998; Moriya *et al.*, 1998). Similar phenotypes were also observed in *smc* null mutants of *Caulobacter crescentus* (Jensen and Shapiro, 1999). The *B. subtilus smc* mutants were also characterized by irregular subcellular localization of Spo0J [a chromosome partitioning protein which binds to sites near the origin of replication (Lin and Grossman, 1998)]. The role of the Smc

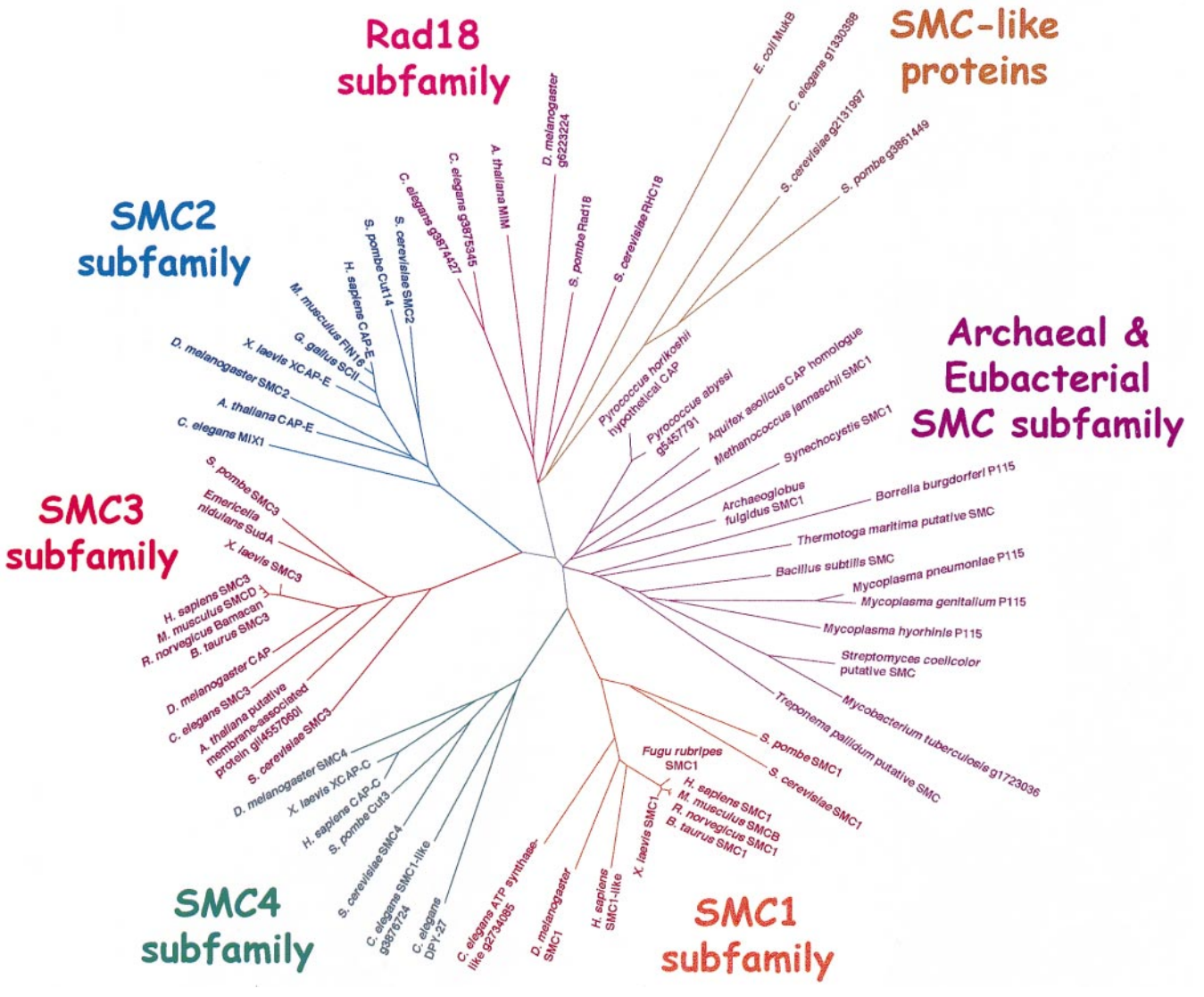

FIG. 1. Phylogenetic tree of known SMC proteins. The above tree was constructed based on alignments between SMC protein sequences generated by the ClustalW program (*Methods Enzymol.* **266,** 383–402, 1996), correcting for multiple substitutions. The various trees produced by the ClustalW program were checked by resampling with 1000 bootstrap trials and compared with neighbor-joining trees inferred using the PROTDIST and NEIGHBOR programs of Joe Felsenstein's PHYLIP package (http://evolution.genetics.washington.edu/phylip.html). The topology of the branches comprising each individual subfamily was also confirmed by using the PileUp program in the Wisconsin Package to construct separate alignments and then using a GCG interface to the tree-searching options of David Swofford's PAUP program (http://www.lms.si.edu/PAUP/about.html) to find the optimal topology by means of parsimony. Moreover, the designations of subfamilies were confirmed by analysis of distance matrix data, in which the mean distance between all members of the same SMC subfamily was compared with the mean distance of each subfamily member from all other proteins in the tree. The overall topology of the tree shown was confirmed by ClustalW alignment of partial data sets of full-length SMC molecules both with and without correction for multiple substitutions, in addition to comparison with trees based on alignment of the conserved N- and C-terminal domains alone. The correct topology of the more distantly related SMC-like proteins was confirmed by conducting all possible optimal alignments using the BestFit program in the Wisconsin Package and then constructing a neighbor-joining tree from pairwise distances calculated as distance = (length of shorter protein ÷ length of alignment) × (100% − % identity). The branch lengths in the overall tree calculated by the PHYLIP and ClustalW programs were also compared with branch lengths of trees containing only members of the same SMC subfamily. Finally, the PHYLIP and ClustalW trees were viewed using Rod Page's TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

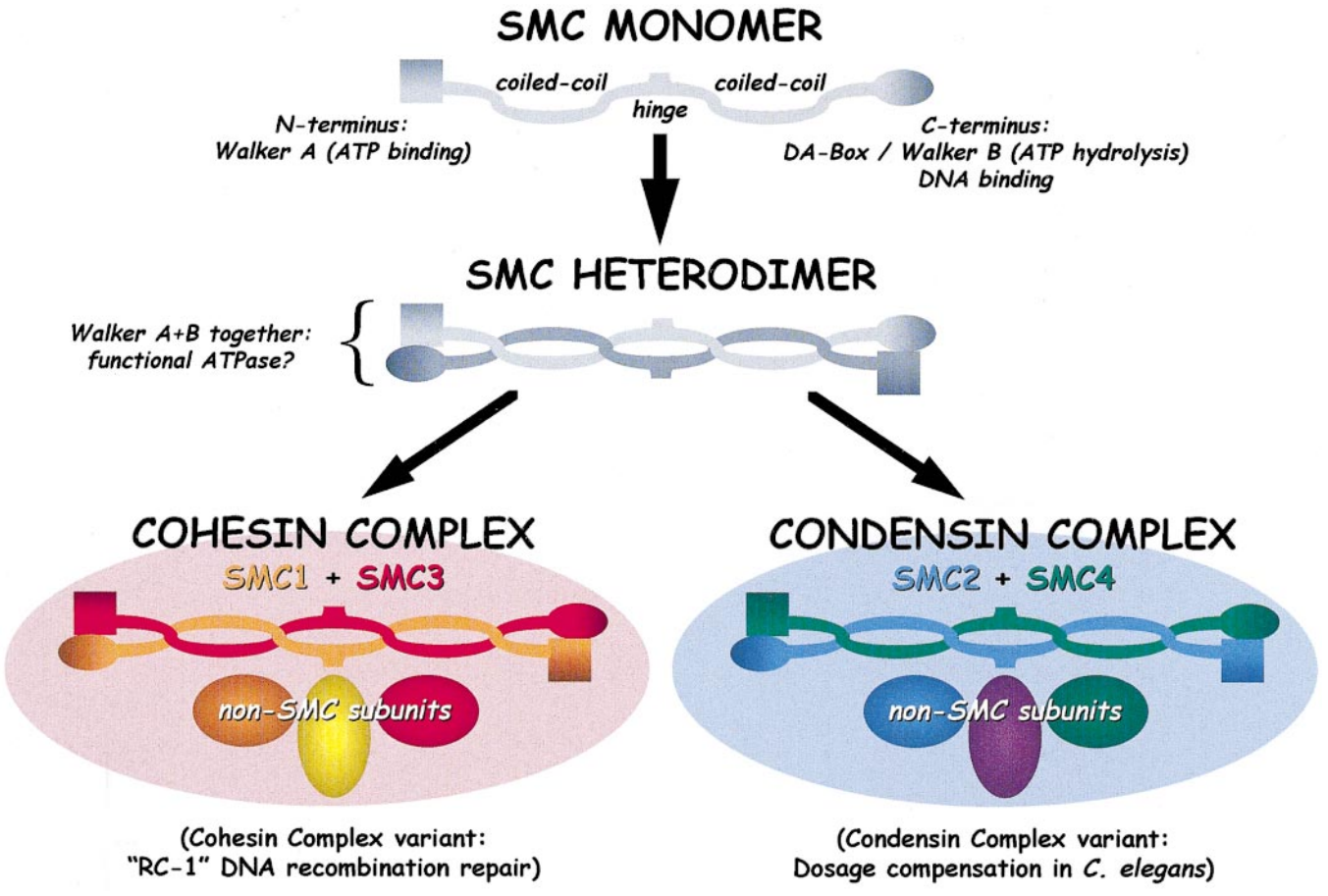

FIG. 2. Model of SMCs, from monomer to heterodimer to higher order functional complexes. The SMC monomer has an ATP binding domain near its N-terminus and an ATP hydrolysis motif near its C terminus. The regions are separated by coiled-coil domains and a flexible hinge region near the center of the molecule. When heterodimerized in an antiparallel fashion, one Walker A motif is brought into close proximity to the Walker B motif of its partner SMC. SMC heterodimers then participate in complex formation with non-SMC subunits, diagrammed as three ovals, resulting in creation of the cohesin and condensin complexes.

protein in *B. subtilus* is reflected by its chromosomal localization and its presence at the poles of the nucleoid as discrete foci (Britton *et al.*, 1998; Graumann *et al.*, 1998). As the N-terminal region of Smc is required for the formation of polar foci (Graumann *et al.*, 1998), it appears to be needed for pairing of newly replicated origins (Lin and Grossman, 1998) by mediating Spo0J localization to the pole of the nucleoid and thereby facilitating orderly segregation. However, due to the small size of bacterial cells it remains unclear whether the mutant phenotypes are caused primarily by a defect in chromosome condensation, segregation, or both (Sharpe and Errington, 1999). Nonetheless, an insight into the possible condensation activity of the *B. subtilis* Smc homodimer has come from the discovery of its ATP-dependent DNA reannealing activity (Hirano and Hirano, 1998). As bacterial nucleoids contain unconstrained negative supercoils that may be easily unpaired (Pettijohn, 1982), it has been proposed that the energy-dependent aggregation of single-stranded DNA may compact bacterial chromosomes by bringing such regions together (Hirano and Hirano, 1998). Furthermore, the increase in twist resulting from SMC-mediated restoration of base pairing may compact the DNA through the concomitant generation of compensatory positive supercoils (Sutani and Yanagida, 1997).

As DNA reannealing activities similar to that of the recombination protein recA (Weinstock *et al.*, 1979) have also been observed with the *S. pombe* cut3/cut14 heterodimer (Sutani and Yanagida, 1997), the bovine bSMC1/bSMC3 heterodimer (Jessberger *et al.*, 1996b), and even the isolated C-terminal domains of Smc1p and Smc2p from *S. cerevisiae* (Akhmedov *et al.*, 1998), it has been suggested that this may represent an activity characteristic of all SMC molecules (Yanagida, 1998). However, these eukaryotic proteins differ from the *B. subtilis* Smc homodimer as they do not require ATP for reannealing activity (Akhmedov *et al.*, 1998; Sutani and Yanagida,

TABLE I
Protein Complexes Containing SMC2 and SMC4

Species	SMC subunits		Non-SMC subunits		
	SMC2 type	SMC4 type			
Condensin complex					
S. cerevisiae	Smc2 (Strunnikov *et al.*, 1995)	Smc4	AAB67384	Brrn1	CAB41223
S. pombe	Cut14	Cut3	cnd1 (Sutani *et al.*, 1999)	cnd2 (Sutani *et al.*, 1999)	cnd3 (Sutani *et al.*, 1999)
C. elegans	MIX-1	Z69787	CAA16340	?	?
D. melanogaster	dmSMC2	dmSMC4	EST clot No. 2519	Barren (Bhat *et al.*, 1996)	EST clot No. 2199
X. laevis	XCAP-E (Hirano and Mitchison, 1994)	XCAP-C (Hirano and Mitchison, 1994)	XCAP-D2 (Kimura *et al.*, 1998)	XCAP-H (Hirano *et al.*, 1997)	XCAP-G (Hirano *et al.*, 1997)
G. gallus	ScII (Saitoh *et al.*, 1994)	?	?	?	?
H. sapiens	hCAP-E (Schmiesing *et al.*, 1998)	hCAP-C (Schmiesing *et al.*, 1998)	063880	038553	?
Dosage compensation					
C. elegans	MIX-1 (Lieb *et al.*, 1998)	DPY-27 (Chuang *et al.*, 1994, 1996)	DPY-28 (Lieb *et al.*, 1998)	DPY-26 (Lieb *et al.*, 1996)	?

1997). As SMCs do not seem to translocate directly along the DNA (Kimura and Hirano, 1997), the role of molecules such as bSMC1 and bSMC3 in recombination may be related to their role in cohesion, in which recombination is facilitated by keeping chromatids close together. However, this does not explain the renaturation activity of SMCs that are active in chromosome condensation but not cohesion. Moreover, as the isolated C-terminal domains of Smc1p and Smc2p are capable of efficient DNA reannealing

TABLE II
Protein Complexes Containing SMC1 and SMC3

Species	SMC subunits		Non-SMC subunits		
	SMC1 type	SMC3 type			
Cohesin complex					
S. cerevisiae	Smc1 (Strunnikov *et al.*, 1993)	Smc3 (Michaelis *et al.*, 1997)	Mcd1/Scc1 (Guacci *et al.*, 1997; Michaelis *et al.*, 1997)	Scc2 (Michaelis *et al.*, 1997)	Scc3 (Tóth *et al.*, 1999)
S. pombe	CAA22432	CAA15722	Rad21 (Birkenbihl and Subramani, 1992)	Mis4 (Furuya *et al.*, 1998)	?
A. nidulans	?	SudA (Holt and May, 1996)	?	?	?
C. elegans	AAB93638	CAB57898	?	?	?
D. melanogaster	dmSMC1	dCAP (Hong and Ganetsky, 1996)	AF109926	Nipped-B (Rollins *et al.*, 1999)	Stromalin (Valdeolmillos *et al.*, 1998)
X. laevis	XSMC1 (Losada *et al.*, 1998)	XSMC3 (Losada *et al.*, 1998)	XRAD21 (Losada *et al.*, 1998)	p155 (Losada *et al.*, 1998)	p95 (Losada *et al.*, 1998)
M. musculus	SMCB (Darwiche *et al.*, 1999)	SMCD (Darwiche *et al.*, 1999)	PW29 (Darwiche *et al.*, 1999)	?	Stromal antigen 1 (Carromolino *et al.*, 1997)
R. norvegicus	SMC1	Bamacan (Wu and Couchman, 1997)	?	?	?
H. sapiens	hSMC1 (Rocques *et al.*, 1995; Schmiesing *et al.*, 1998)	hCAP (Shimizu *et al.*, 1998)	hRAD21 (McKay *et al.*, 1996)	?	Stromal antigen 1 (Carromolino *et al.*, 1997)
Recombination complex					
B. taurus	bSMC1 (Jessberger *et al.*, 1996)	bSMC3 (Jessberger *et al.*, 1996)	DNA ligase III (Jessberger *et al.*, 1993)	DNA Pol ϵ (Jessberger *et al.*, 1996)	Endonuclease? (Jessberger *et al.*, 1996)

on their own,[2] it seems that the ability of SMCs to tether separate DNA molecules may simply enhance the reannealing activity by helping to bring ssDNA together. Clearly, more detailed analysis of the reactions catalyzed by the terminal domains is required to determine the mechanism of SMC-mediated recombination. Lastly, as SMC molecules have a higher affinity for AT-rich sequences such as SARs and MARs (Scaffold- or Matrix- Associated Regions) (Akhmedov *et al.*, 1998) which may be more easily melted (Bode *et al.*, 1992), it is possible that the renaturing activity of different SMC protein complexes in eukaryotes might be linked to roles in condensation and segregation, as in *B. subtilus*. We will now look more closely at the role of SMCs in these two process, beginning with their involvement in chromosome condensation.

SMCs AND CHROMOSOME CONDENSATION

The first SMC proteins exhibiting a role in chromosome condensation were found in *S. pombe*. The *cut3* and *cut14* mutants displayed a characteristic "cut" (cell untimely torn) phenotype, in which the division septum bisects the nuclear material, due to a failure in either chromosome condensation or sister chromatid segregation (Saka *et al.*, 1994). However, it was suggested that the primary defect was a failure in chromosome condensation as high rates of minichromosome loss were not observed in *cut3* mutants and centromeric DNA was reported to segregate properly to the spindle poles in both *cut3* and *cut14* mutants. The improper chromosome disjunction in these mutants therefore appeared to be a consequence of impaired chromosome condensation. Similar phenotypes were also observed in *S. cerevisiae smc2* mutants, encoding a Cut14p orthologue (Strunnikov *et al.*, 1995). Cut3p was later shown to be orthologous to Smc4p in *S. cerevisiae* (Koshland and Strunnikov, 1996). In addition, the Sc2 protein of the mitotic chromosome scaffold in chicken cells was identified as an SMC2 subfamily member, suggesting a possible structural role for these proteins in mitotic chromosome architecture (Saitoh *et al.*, 1994).

Much of our current understanding of chromosome condensation is based on the *in vitro* simulation of chromosome condensation when nuclei are added to mitotic extracts from *Xenopus* eggs. Topoisomerase II was shown to be required for mitotic chromosome condensation when either HeLa or chicken erythrocyte nuclei or demembranated sperm were added to *Xenopus* extracts (Adachi *et al.*, 1991; Hirano and Mitchison, 1993). A heterodimeric complex containing XCAP-E (SMC2-type) and XCAP-C (SMC4-type) was identified as a mitotic chromosomal component in *in vitro* assembled chromosomes (using demembranated sperm as substrate), and immunofluorescence detection of XCAP-C revealed a filamentous distribution along the chromosome axis (Hirano and Mitchison, 1994), not unlike that observed for topoisomerase II in "normal" mitotic chromosomes (Earnshaw *et al.*, 1985; Earnshaw and Heck, 1985). Two different types of condensin complex were later identified by sucrose gradient sedimentation, namely an 8S form which proved to be the XCAP-E/XCAP-C heterodimer and a larger 13S complex containing three additional subunits (referred to as XCAP-D2, XCAP-H, and XCAP-G) (Hirano *et al.*, 1997). Both the targeting of the condensin complex to chromosomes and its *in vitro* activity were shown to depend on mitosis-specific phosphorylation of these additional non-SMC subunits (Hirano *et al.*, 1997), with $p34^{cdc2}$ responsible for the hyperphosphorylation of XCAP-D2 and XCAP-H (Kimura *et al.*, 1998). XCAP-H was also found to have homology to Barren, a protein localizing to the chromosomes of mitotically active cells in *Drosophila* embryos (Bhat *et al.*, 1996). Mutants in *barren* were characterized by extensive chromatin bridges between anaphase chromosomes, in spite of centromere separation. This phenotype was reminiscent of the *cut3* and *cut14* mutants in *S. pombe*, consistent with a role for Barren in proper mitotic chromosome condensation.

A larger condensin complex has also been detected in *S. pombe* cell lysates, with a similar subunit composition to that of the *Xenopus* 13S condensin complex (Sutani *et al.*, 1999). Gene disruption has demonstrated that the additional non-SMC subunits are essential for viability and the mutants were characterized by hypocondensed chromosomes which were extended along an elongated spindle instead of clearly separating, as seen in *cut3* and *cut14* cells. Whereas the activity of the *Xenopus* condensin complex is regulated by phosphorylation of its non-SMC subunits, that of the *S. pombe* condensin appears to be controlled by mitosis-specific phosphorylation of Cut3p by $p34^{cdc2}$. This modification was also shown

[2]Considering that similar molar concentrations were used for the reactions with either full-length or partial SMC proteins, the reannealing activity of isolated SMC terminal domains appears to conflict with the inability of full-length SMCs to promote duplex formation unless they can heterodimerize (Hirano, 1999). Fortunately, this discrepancy may be explained by considering the collision rates of the different proteins with DNA. Presumably, the small, globular truncated protein has a higher collision rate than the long, rod-shaped full-length molecule because it is able to diffuse through solution more easily. Likewise, a heterodimer formed from two different SMCs has a COOH-terminal DNA binding domain at each end so it is more likely to bind to DNA. By contrast, as individual SMC proteins are unlikely to form homodimers unless they are produced in vast excess (Strunnikov *et al.*, 1995), they will only be able to bind DNA at one end and so will have a far lower collision rate.

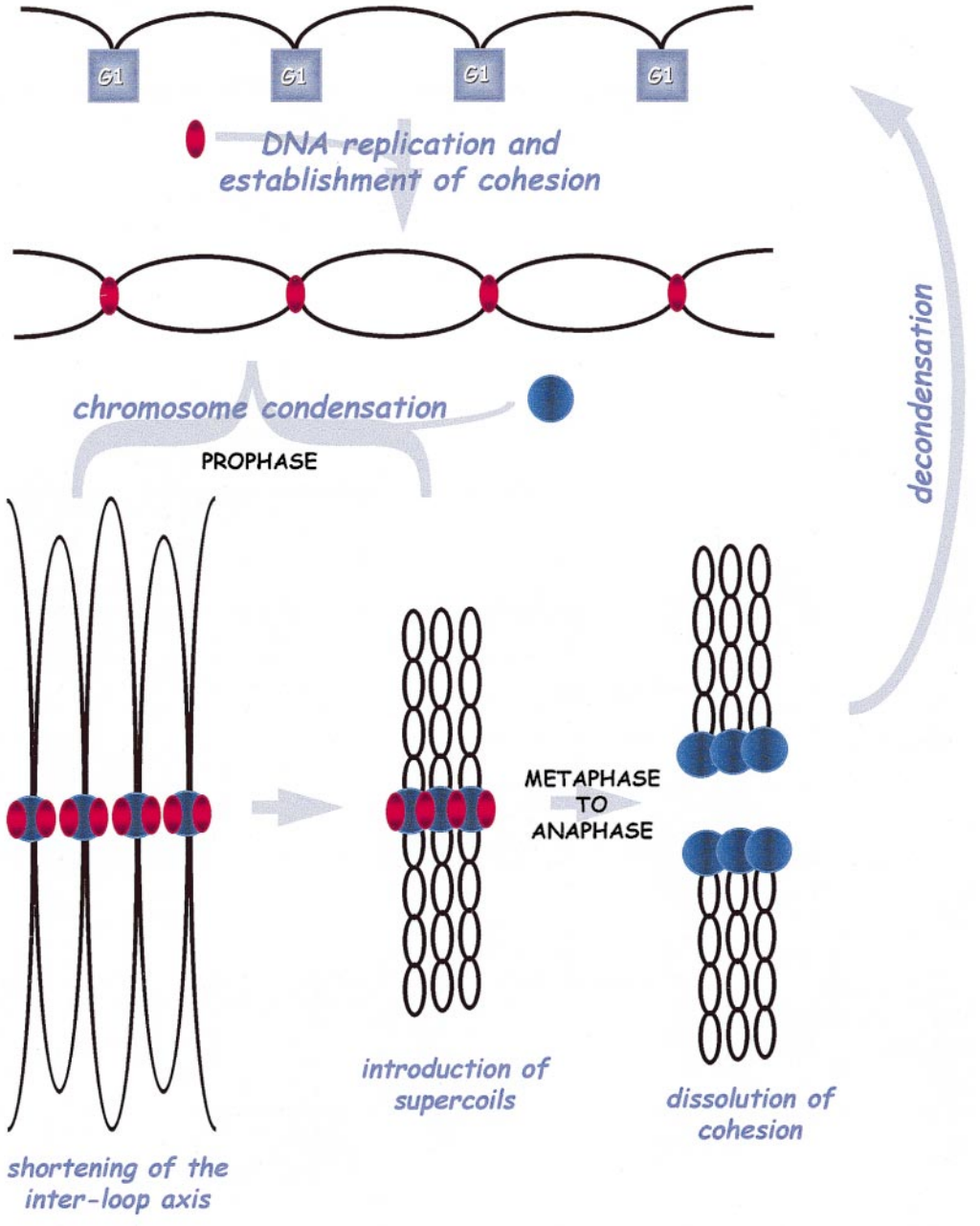

FIG. 3. Model for cohesin and condensin deposition and activity during the cell cycle. The cohesin complex (red ovals) is loaded onto chromatin either coincident with or shortly after DNA replication in S phase, thereby ensuring the attachment of sister chromatids until anaphase. The condensin complex (blue circles) then appears to be loaded during chromosome condensation in prophase. Condensation can be thought of as a two-step process resulting first in shortening of the interloop axis, followed by the introduction of supercoils to achieve the final high degree of chromosome compaction. Cohesion between the sister chromatids of the final metaphase chromosome is then dissolved at the transition to anaphase, and the sisters are segregated by the microtubule apparatus to the poles of the cell. Following nuclear envelope reformation and cytokinesis, the chromatin is decondensed in preparation for transcription and DNA synthesis.

to be essential for viability as it restricts the accessibility of a nuclear export signal (NES) in the N-terminus of the protein. As the intracellular shuttling of the other condensin subunits seems to depend on the localization of Cut3p, the phosphorylation of Cut3p during mitosis permits entry of the complex into the nucleus while the dephosphorylated form exposes the NES during interphase and therefore relegates the complex to the cytoplasm. This manner of regulating condensin activity contrasts with that of the *Xenopus* condensin complex (Hirano and Mitchison, 1994) and the chicken Sc2 protein (Saitoh

et al., 1994), which both remain in the nucleus during interphase but fail to associate with the chromosomes until mitosis. Until it can be shown that the essential activity of the *S. pombe* complex depends on the phosphorylation of any of its other subunits, one possible explanation for this shuttling appears to be that the complex is active throughout the cell cycle. Consequently, it appears that chromosome condensation during mitosis may be regulated differently in different species although the same basic protein complex appears to be involved. One should also keep in mind that *S. pombe* undergoes closed nuclear mitosis, in contrast to the observed nuclear envelope breakdown of *Xenopus* and other higher eukaryotes.

Purified Cut3p and Cut14p form a stable complex at a rather low efficiency when mixed *in vitro* (Sutani and Yanagida, 1997), so it has been proposed that the non-SMC subunits Cnd1p and Cnd3p might have a role in linking the SMCs together in the functional condensin complex. This is suggested by the report of a weak homology between Cnd1p and Cnd3p and the β subunit of the AP3 adapter protein complex, which is involved in the assembly of rod-like clathrin molecules for vesicle transport (Sutani *et al.*, 1999). Since both clathrin and SMC molecules contain two rod-like regions linked by a hinge-like region, Cnd1p and Cnd3p may enable the rod-like SMC subunits to correctly assemble. The intact *Xenopus* condensin complex was also shown to preferentially bind positively supercoiled DNA and DNA with a distorted or bent structure, such as a four-way junction (Kimura and Hirano, 1997; Kimura *et al.*, 1999). A similar affinity for cruciform DNA has also been demonstrated with Smc1p and Smc2p from *S. cerevisiae* (Akhmedov *et al.*, 1998). As bending and supercoiling of the DNA may be generated by the SMCs, this suggests that additional condensins might bind cooperatively (Kimura and Hirano, 1997), just as other proteins which strongly bind cruciform or bent DNA *in vitro* tend to bend it further (Zlatanova and van Holde, 1998).

MECHANISM OF CONDENSIN ACTION

How is the condensin complex involved in chromosome condensation? Although renaturation can contribute to supercoiling, this activity fails to fully explain the role of certain SMCs in chromosome condensation. After all, the ability to promote duplex DNA is shared by other SMCs (Jessberger *et al.*, 1996b) and indeed non-SMC proteins (Weinstock *et al.*, 1979) which have no obvious direct role in condensation. As the reannealing reaction is therefore considered to be only a part of its activity (Yanagida, 1998), how does the condensin complex interact with chromatin to induce its mitotic condensation? Based on the symmetrical structure of BsSMC (Melby *et al.*, 1998) in which each end could interact with both ATP and DNA (Akhmedov *et al.*, 1998), it has been suggested that the homodimer may function as an ATP-modulated DNA cross-linker with a "scissoring" action to induce aggregation of DNA (Hirano, 1999). The possibility of such conformational changes during SMC activity is supported by the finding that sensitivity to proteolytic cleavage of the *B. subtilus* SMC homodimer depends on the presence of ATP and ssDNA (Hirano and Hirano, 1998). By extending this concept to eukaryotic SMC heterodimers, this scissoring action was proposed to be the key mechanism underlying all SMC activities, in which the SMC2/SMC4 heterodimers involved in chromosome condensation and dosage compensation would act as intramolecular DNA cross-linkers which compact a single DNA molecule (Hirano, 1999). What impact native chromatin, in contrast to naked DNA, would have on this proposed process is anyone's guess.

An early model for condensin action was put forward by Kimura and Hirano when they found that the *Xenopus* 13S condensin complex can introduce positive supercoils into DNA, fueled by ATP hydrolysis (Kimura and Hirano, 1997). The stretches of DNA between condensin binding sites could form twisted loops by compensatory negative supercoiling, which are relaxed by treatment with either prokaryotic or eukaryotic type I topoisomerases. In agreement with previously suggested mechanisms of compaction based on the chromosome scaffold model proposed originally by Paulson and Laemmli (1977), the authors proposed that chromosome condensation might be initiated by the formation of chromatin loops by condensin-mediated supercoiling at specific sites, followed by shortening of the interloop axis and folding of the torsionally constrained loops. It was later shown that condensin reconfigures DNA in the presence of a type II topoisomerase by creating knots (Kimura *et al.*, 1999). As knotting presumably would not occur if condensin generated supercoils either by locally overwinding the DNA or by wrapping the DNA around itself, this implied that the complex operated by generating a global writhe. When the topology of the knots was determined by electron microscopy of RecA-coated DNA, it was reported that the vast majority were positive, implying that the condensin complex generated an ordered array of positive solenoidal supercoils. As the condensins were reported to bind to plasmid DNA *in vitro* at a high ratio (Kimura *et al.*, 1998, 1999), it was proposed that a high density of condensins could bind the full DNA length, touching each other to form a protein infrastructure capable of nonplanar bending of the DNA. This model neatly explains both the cooperativity of condensin binding and the observed

preference for longer DNA fragments (Kimura and Hirano, 1997) in terms of cooperative binding. Furthermore, as the two ends of the eukaryotic SMC heterodimer are similar but not identical (containing the N- and C-termini of different SMC molecules), this asymmetry might contribute to the chirality of supercoiling, provided that the complex binds DNA in a fixed orientation.

Although the high concentrations of condensins supplied *in vitro* allowed the complex to bind everywhere on the naked plasmid DNA (Hirano and Hirano, 1998; Kimura *et al.*, 1999), it is unlikely that the same is true of protein-laden chromosomes *in vivo.* Otherwise, this would conflict with the observed distribution of condensin SMCs in *Xenopus* (Hirano and Mitchison, 1994), chicken, and human cells (Saitoh *et al.*, 1994), in which they appeared restricted to the chromosome axis with concentrated staining at the centromeres (Saitoh *et al.*, 1994). The abundance of Cut3p in wild-type *S. pombe* cells predicted a density of only one condensin complex per 8 kb of DNA (Sutani and Yanagida, 1997) and a similar stoichiometry was estimated for *Xenopus* mitotic chromosomes assembled *in vitro* (Kimura *et al.*, 1999). As SMC proteins were previously observed to preferentially bind AT-rich sequences (Akhmedov *et al.*, 1998), it seems possible that nonplanar bending might be initiated at loci such as SARs. By combining these findings, one model for condensation by condensins would result from the nonplanar bending of DNA by higher order multicondensin complexes, generating positive solenoidal supercoils at defined sites along the chromosome and the simultaneous generation of negative supercoils in the intervening regions. Although these negative supercoils could be easily removed by the numerous topoisomerases in a cell, this would presumably be prevented by the binding of additional unidentified condensation factors which could stabilize these interwound loops.

Despite the insights provided by these models of condensation, the precise mode of action of SMC proteins continues to provoke discussion. In particular, the interaction between condensin complexes and topoisomerase II still remains enigmatic. Evidence of possible genetic interactions was initially provided by analysis of different *cut3* and *topoII* mutants in *S. pombe* (Saka *et al.*, 1994), although these results are equally consistent with the two proteins acting in a common pathway without direct physical interaction. A functional interaction between topoisomerase II and Barren was suggested, nonetheless, based on co-immunoprecipitation, colocalization on mitotic chromosomes, and interaction in a yeast two-hybrid assay (Bhat *et al.*, 1996). Furthermore, Barren has been reported to enhance the supercoiling activity of topoisomerase II, possibly modulating topoisomerase II-mediated decatenation of chromosomal arms. Finally, Sc2 and topoisomerase IIα have been reported to copurify in a complex found in undifferentiated mouse erythroleukemia cells (Ma *et al.*, 1993) and the two proteins cofractionate with and colocalize to the mitotic chromosome scaffold of chicken cells (Saitoh *et al.*, 1994). In contrast, the *Xenopus* condensins fail to immunoprecipitate with topoisomerase II (Hirano and Mitchison, 1994) and appear to be independently targeted to mitotic chromosomes (Hirano *et al.*, 1997). Moreover, unlike topoisomerase IIα and β (Berrios *et al.*, 1985; Meyer *et al.*, 1997; Petrov *et al.*, 1993; Zini *et al.*, 1992), Sc2 is not a component of the interphase nuclear matrix, as it readily leaked into the cytoplasm during subcellular fractionation (Saitoh *et al.*, 1994). Regardless of whether the members of the condensin complex interact directly with topoisomerase II, it is clear that their respective condensing and decatenating activities contribute synergistically to bring about chromosome condensation.

Intriguingly, there may be a functional similarity between mechanisms of chromosome condensation and the global regulation of gene expression on the *C. elegans* X chromosome, based on the involvement of an SMC2/4 heterodimer in sex chromosome dosage compensation. Transcription from each of the X chromosomes is reduced in hermaphrodites (XX) of this organism to match the level of X-linked gene expression in males (XO). The discovery that a variant SMC4 type protein (DPY-27) is an essential regulator of dosage compensation through its association with the X chromosome provided the first clue that SMC proteins might be involved in this process (Chuang *et al.*, 1994, 1996). Subsequently, MIX-1 was identified as an SMC2-type protein required for both mitosis and dosage compensation, the restricted localization of which to the X chromosome was dependent on DPY-27 (Lieb *et al.*, 1998). Like the 13S condensin complex, the dosage compensation complex consists of an SMC2/4 heterodimer and at least two non-SMC subunits, including DPY-26 and DPY-28 (Hirano, 1999). The mitotic function of MIX-1 is achieved through its association with a more conventional SMC4-type protein, suggesting that MIX-1 may have been enlisted to the dosage condensation complex through the evolution of DPY-27 as a highly specialized SMC protein, altering the higher order structure of X chromosomes by a mechanism perhaps related to that underlying chromosome condensation.

SMCs AND SISTER CHROMATID COHESION

Another aspect of mitotic chromosome dynamics in which the eukaryotic SMC proteins play a funda-

mental role is the establishment and maintenance of sister chromatid cohesion. The first SMC molecule shown to have a vital role in maintaining cohesion was Smc1p (Larionov *et al.*, 1985; Strunnikov *et al.*, 1993). Its Smc3p partner was subsequently identified in a genetic screen for *S. cerevisiae* mutants with a premature sister chromatid separation phenotype (Michaelis *et al.*, 1997). The same screen also identified Scc1p and Scc2p, two non-SMC proteins required for sister chromatid cohesion. Meanwhile, Mcd1p (identical to Scc1p) was also identified in independent screens for high-copy suppressors of an *smc1* mutant or mutants displaying enhanced inviability after mitotic arrest (Guacci *et al.*, 1997). The role of this protein in chromosome segregation was further verified by the heightened instability of circular minichromosomes in an *scc1*/*mcd1* mutant (Heo *et al.*, 1998). The functional association of either Scc1p/Mcd1p or Scc3p (another essential component of the cohesin complex) with chromosomes was then found to depend on each other and also on the presence of Smc1p, Smc3p, and Scc2p (Michaelis *et al.*, 1997; Tóth *et al.*, 1999). In particular, the Smc1p, Smc3p, Scc1p/Mcd1p, and Scc3p proteins were found to co-immunoprecipitate in roughly equal amounts, implying that they maintained cohesion as a complex (Guacci *et al.*, 1997; Tóth *et al.*, 1999), coined "cohesin." By contrast, Scc2p does not appear to be a stoichiometric component of the cohesin complex and fails to colocalize with other cohesin subunits on chromosomes but is nonetheless essential for the binding of these other subunits to chromatin (Tóth *et al.*, 1999). Its orthologue in *S. pombe*, known as Mis4, failed to coprecipitate with either Rad21p (the *S. pombe* orthologue of Scc1p/Mcd1p) or the *S. pombe* orthologue of Smc3p (Furuya *et al.*, 1998).

The need to establish cohesion during S phase was demonstrated in cells expressing Scc1p/Mcd1p exclusively from a galactose-inducible promoter (Uhlmann and Nasmyth, 1998). Ctf7p/Eco1p was later shown to be another *S. cerevisiae* protein whose activity is essential to establish cohesion along the entire length of the chromosome during S phase, although it was not required for the maintenance of cohesion (Skibbens *et al.*, 1999; Tóth *et al.*, 1999). Furthermore, Ctf7p/Eco1p does not seem to be a cohesin subunit and its presence has no effect on the association of the cohesin complex with chromosomes (Tóth *et al.*, 1999). Interestingly, a synthetic lethal interaction was observed between a temperature-sensitive mutation in *ctf7* and the yeast genes for either PCNA or an RFC-like protein (Skibbens *et al.*, 1999), suggesting that PCNA might be involved in loading the cohesin complex onto chromatin after DNA replication. It has been suggested that PCNA may play a role in the assembly of chromatin (Kelman, 1997) as its nuclear distribution in fertilized starfish eggs coincided with the chromatin distribution during the first S phase (Nomura, 1994) and mutations in the *Drosophila* gene encoding PCNA suppressed position-effect variegation (Henderson *et al.*, 1994). In addition, PCNA has been shown to bind the largest subunit of CAF-1, demonstrating a direct link between replication machinery and chromatin assembly (Shibahara and Stillman, 1999). Consequently, it was proposed that the loading of cohesin complexes onto chromatin might be coupled with PCNA-dependent DNA replication (Skibbens *et al.*, 1999). This adds weight to a previous hypothesis that cohesion might be directly coupled to passage of a replication fork (Uhlmann and Nasmyth, 1998). Although the murine orthologue of Scc1p/Mcd1p (known as PW29) and PCNA fail to exhibit similar localization patterns (Darwiche *et al.*, 1999), this does not necessarily preclude a role for PCNA in facilitating the loading of cohesins onto chromatin, as Scc1p/Mcd1p in *S. cerevisiae* also does not colocalize with Scc2p, even though the latter is essential for the efficient binding of Scc1p and other cohesin members to chromatin (Tóth *et al.*, 1999). Similarly, although the binding of cohesin subunits to chromatin occurred independently of DNA replication in *Xenopus* oocyte extracts (Losada *et al.*, 1998), this also does not prevent a possible role for PCNA as a landing pad for SMC proteins as PCNA may also bind to DNA at times other than S phase (Nomura, 1994).

The similarities between the *S. cerevisiae* and *S. pombe* proteins involved in regulating sister chromatid cohesion, combined with the conservation of factors required for chromosome condensation, suggested that the cohesion mechanism might also be evolutionarily conserved. Indeed, the vertebrate orthologues of *SMC1, SMC3,* and *SCC1* have also been shown to be essential for sister chromatid cohesion (Losada *et al.*, 1998) and proper progression of metaphase (Schmiesing *et al.*, 1998), even though these proteins appear to dissociate from chromosomes during mitosis (Darwiche *et al.*, 1999; Losada *et al.*, 1998; Schmiesing *et al.*, 1998). Nevertheless, immunoblotting showed that the murine orthologues of both *SMC1* and *SMC3* were expressed throughout the cell cycle (Darwiche *et al.*, 1999). This dissociation of the vertebrate cohesin complex from mitotic chromosomes contrasts with the pattern observed in yeast, in which Smc1p and Smc3p remain associated (Michaelis *et al.*, 1997).

Although it is possible that cohesion at this stage might be supported by other molecules (as yet unidentified), it has been shown that hSMC1 nonetheless plays a role in the maintenance of chromatid cohe-

sion as well as its establishment, even though the protein appears to be excluded from the chromosomes during mitosis. This was demonstrated by the mitotic arrest of HeLa cells microinjected during mid/late metaphase with an antibody specific for either the middle or C-terminal regions of hSMC1, whereas cells injected at early anaphase subsequently went through cytokinesis normally to yield two daughter cells (Schmiesing *et al.*, 1998). This suggests that a residual level of cohesins bound to metaphase chromosomes may be sufficient to maintain cohesion between sister chromatids until the onset of anaphase, as previously proposed (Losada *et al.*, 1998). Moreover, it has been proposed that the dissociation of most cohesins from the chromosomes at the onset of mitosis in vertebrate cells may loosen the linkage between sister chromatids, permitting reorganization of the chromatin (Losada *et al.*, 1998). This may serve to relieve a steric barrier which might otherwise prevent final condensation in such large chromosomes, as mediated by replacement of the cohesins by the condensins. This idea is supported by the prevention of interphase cells from entering mitosis by overexpression of an PW29 (SCC1)–GFP fusion protein in mouse fibroblasts (Darwiche *et al.*, 1999). Transfection with H2B–GFP or the GFP molecule itself produced no such arrest, implying that the SCC1 (PW29) protein and its complex with SMC proteins might be involved in the control of mitotic cycle progression.

So what function do the SMC proteins fulfill in the cohesin complex? Assuming that the SMC1/SMC3 heterodimers of cohesin complexes function as ATP-modulated DNA cross-linkers, it has been suggested that these molecules may form intermolecular bridges between separate DNA molecules (Hirano, 1999). Alternatively, such bridges might be produced through the association of two different Smc1/3 heterodimers (possibly mediated by Scc1p/Mcd1p or Scc3p), each of which is bound to a single chromatid (Losada *et al.*, 1998). This latter model seems plausible for *S. cerevisiae*, as dissolution of sister chromatid cohesion can be achieved by cleavage of Scc1p/Mcd1p (Ciosk *et al.*, 1998; Uhlmann *et al.*, 1999) and both Smc1p and Smc3p persist after Scc1p/Mcd1p dissociation (Tanaka *et al.*, 1999).

LOCALIZATION OF THE COHESIN COMPLEX

Having established the importance of the cohesin complex for sister chromatid cohesion, where does it bind on the chromosome? The relative distribution of cohesins along chromosomes was initially monitored by modifying existing protocols for chromatin immunoprecipitation (ChIP) in *S. cerevisiae*, in which the DNA immunoprecipitated with cohesin subunits was radiolabeled for use as a probe (Blat and Kleckner, 1999). These probes were then hybridized to a membrane containing an array of PCR-generated chromosome fragments, covering the entire length of chromosome III. A majority of cohesin binding sites identified in this way were associated with the centromere, although the complex was also shown to bind specific sites along the chromosome arms (Blat and Kleckner, 1999; Tanaka *et al.*, 1999), consistent with the discrete foci seen in chromosome spreads (Tóth *et al.*, 1999). These binding sites were found to correlate with locally AT-rich sequences, occurring roughly every ~15 kb along the chromosome (Blat and Kleckner, 1999). This preference for AT-rich sequences in centromeric regions was further corroborated by conventional ChIP analysis, using the immunoprecipitated DNA as a PCR template (Megee *et al.*, 1999). Although such regions are reminiscent of SARs, no correlation with the redundant motifs of *Drosophila* SARs was observed (Blat and Kleckner, 1999). However, this does not preclude the possibility that SARs might indeed be binding sites for cohesins, bearing in mind the differences between the short, defined centromeres of *S. cerevisiae* and the longer regional centromeres of other eukaryotes such as *S. pombe* and *Drosophila melanogaster* (Pluta *et al.*, 1995). Indeed, it was previously observed that SMC proteins bind preferentially to *Drosophila* SARs as well as sequences containing alternating poly(dA–dT) and yeast centromere regions (Akhmedov *et al.*, 1998). Interestingly, a more even distribution of cohesin binding in hydroxyurea-arrested cells suggested that cohesins bind uniformly to chromosomes at the start of S phase (Blat and Kleckner, 1999) but relocate to centromeric regions later during the cell cycle so the highest levels of centromere-bound Scc1p/Mcd1p were seen in cells arrested in M phase (Megee *et al.*, 1999).

The minimal centromere sequences required for cohesin association were then deduced by artificially inserting sequences from *CEN6* into a region which normally has low cohesin affinity (Tanaka *et al.*, 1999). Normally the insertion of additional centromeres in this manner would create unstable dicentric chromosomes, so the inserted centromeric sequences were conditionally suppressed by placing them under the control of a galactose-inducible promoter. In this way it was deduced that 130 bp containing *CDEI–II–III* was sufficient to confer cohesin binding to this sequence, whereas cohesin association with *CEN* DNA was abolished by transcription from the *GAL* promoter. The *CDEIII* sequence in particular was shown to be sufficient for weak cohesin association, but could be enhanced by adding 21 bp of *CDEII* (Tanaka *et al.*, 1999). Moreover, association was abolished by various *CDEIII* point mutations, further supporting the importance of this

element for cohesin binding (Tanaka *et al.*, 1999), in agreement with previous findings that *CDEIII* was necessary but insufficient for functional cohesion (Megee and Koshland, 1999). Despite this, cohesins were shown to differ from known centromere proteins by associating strongly with adjacent sequences as well as the centromere itself, in some cases with even higher affinity for these flanking sequences (Megee *et al.*, 1999). As ectopically placed centromeres were shown to direct Scc1p/Mcd1p binding to adjacent sequences which normally had low affinity for the protein (Megee *et al.*, 1999), it is therefore possible that the centromere primarily contributes to sister chromatid cohesion by directing the cohesin complex to AT-rich sequences in the immediate vicinity. It will be interesting to see whether similar patterns of cohesin association are found in other organisms.

THE SEPARATION OF SISTERS

How is the cohesin-mediated cohesion dissolved to allow separation of sister chromatids during mitosis? Both Scc1p/Mcd1p and Scc3p were shown to dissociate from chromosomes at the metaphase to anaphase transition (Michaelis *et al.*, 1997; Tóth *et al.*, 1999), leaving behind the bulk of Smc1p and Smc3p cohesin subunits which associate longer with the chromosomes (Tanaka *et al.*, 1999). Although the dissociation of Scc1p/Mcd1p was known to depend on the anaphase promoting complex (APC), this dissociation could be prevented by expression of a nondegradable version of Pds1p but not by other APC inhibitors (Cohen-Fix *et al.*, 1996; Michaelis *et al.*, 1997). This implied that ubiquitination by the APC was not directly responsible for Scc1p/Mcd1p destruction and suggested that Pds1p might somehow prevent Scc1p/Mcd1p dissociation. This was confirmed by deletion of the *pds1* gene, allowing sister chromatid separation and Scc1p/Mcd1p dissociation in the absence of APC function (Ciosk *et al.*, 1998; Yamamoto *et al.*, 1996). However, the premature separation of sister chromatids in *scc1*/*mcd1* mutants contrasted with the delayed separation in *pds1* mutants, inferring the involvement of other factors in the control of anaphase.

Additional insights were provided by studies of Cut2p, an *S. pombe* orthologue of the anaphase inhibitor Pds1p. This protein was shown to be degraded by the APC and copurified with Cut1p, a protein maintained throughout the cell cycle (Funabiki *et al.*, 1996a,b, 1997). Similarly, Pds1p was immunoprecipitated with Esp1p (the *S. cerevisiae* orthologue of Cut1), a protein required for sister chromatid separation (Ciosk *et al.*, 1998). As the two proteins in the complex had antagonistic effects on anaphase, the Esp1p and Cut1p were named "separins" while their inhibitors (Cut2p and Pds1p) were called "securins." Moreover, since Pds1p was not degraded by Esp1p, it appeared that securins inhibit the separins by binding to them but this inhibition was removed by APC-mediated proteolysis (Cohen-Fix *et al.*, 1996). The activity of Esp1p was then revealed when its overexpression permitted sister chromatid separation in the presence of Pds1p (Ciosk *et al.*, 1998). As sister chromatid separation in *esp1* mutants was also shown to be prevented by a failure of Scc1p/Mcd1p degradation, the separins were therefore considered to be responsible for Scc1p/Mcd1p dissociation. This role was confirmed by demonstrating that Scc1p/Mcd1p cleavage and its resultant dissociation from chromatin occurred in extracts from cells overexpressing Esp1p but not in *esp1* mutant extracts (Uhlmann *et al.*, 1999). The Scc1p/Mcd1p cleavage sites were then identified and overexpression of a cleavage-resistant protein was shown to prevent sister chromatid separation (Uhlmann *et al.*, 1999). In conclusion, the available evidence seems to suggest that dissociation of Scc1p/Mcd1p from sister chromatids in *S. cerevisiae* depends on cleavage mediated by Esp1p, which is transported to the spindle by its inhibitor Pds1p but remains inactive until the APC triggers proteolysis of Pds1p at the metaphase–anaphase transition.

As the ability of *S. cerevisiae* cell extracts to cleave Scc1p/Mcd1p correlated with the levels of Esp1p, the simplest explanation is that Esp1p is the protease directly responsible for Scc1p/Mcd1p degradation (Uhlmann *et al.*, 1999). However, until it can be demonstrated that purified Esp1p is sufficient for Scc1p/Mcd1p cleavage *in vitro*, one cannot exclude the alternative possibility that this protein might activate another protease instead. Furthermore, it remains to be seen if cleavage of the human and *Xenopus* Rad21p orthologues can be suppressed by the recently characterized functional homologue of Pds1p in *Xenopus* (Zou *et al.*, 1999). Certainly, the need to remove PW29 (the murine orthologue of Scc1p/Mcd1p) to allow separation of sister chromatids has been demonstrated by the metaphase arrest of mitotic cells when a PW29–GFP fusion protein was overexpressed (Darwiche *et al.*, 1999). However, murine PW29 protein levels appear constant throughout the cell cycle, whereas Scc1p/Mcd1p levels peak during S phase and decline thereafter (Darwiche *et al.*, 1999; Guacci *et al.*, 1997; Michaelis *et al.*, 1997). It therefore seems that anaphase occurs by a different mechanism in vertebrates, involving not just degradation of the cohesin complex but also its exclusion from the chromosomes.

CONNECTIONS BETWEEN COHESION AND CONDENSATION

Considering the involvement of complexes containing SMC proteins in both sister chromatid cohesion

and chromosome condensation, is there any structural interrelationship between these processes? To date, none of the non-SMC subunits have been found to be shared between the cohesin and condensin complexes, which would seem to indicate independent evolution of these two SMC-containing complexes. Clearly condensation is a prerequisite for proper segregation of sister chromatids, ensuring that the entire chromosome is accurately packaged to avoid such hazards as sister chromatid entanglement and cleavage of trailing chromatin at cytokinesis. A model depicting the deposition and activity of the cohesin and condensin complexes during the cell cycle is given in Fig. 3. In *S. cerevisiae* it has been demonstrated by FISH that chromosome condensation also depends on the cohesin subunit Scc1p/Mcd1 protein (Guacci *et al.*, 1997). Similarly, the dispersed staining of nuclear material and stretched chromosomes in *S. pombe rad21* mutants may reflect its involvement in chromosome condensation as well (Birkenbihl and Subramani, 1995; Tatebayashi *et al.*, 1998). It has been proposed that placement of the Scc1p/Mcd1 protein at the newly replicated chromatids provides an attachment site for recruitment of condensation proteins, suggesting a possible explanation for the mirror symmetrical, helically folded pattern often observed in the condensed sister chromatids of vertebrate cells (Baumgartner *et al.*, 1991; Boy de la Tour and Laemmli, 1988; Rattner and Lin, 1985). Nevertheless, the condensation defects resulting from mutations affecting cohesin subunits are not as severe as those affecting components of the condensin complex (Strunnikov *et al.*, 1995).

By contrast, the vertebrate cohesins do not seem to have an effect on chromosome condensation (Losada *et al.*, 1998) and neither hCAP-E nor hCAP-C are immunoprecipitated with either hSMC1 or hSMC3 (Schmiesing *et al.*, 1998). Furthermore, the binding of condensins to chromatin and chromosome condensation itself seem unaffected by cohesin immunodepletion in *Xenopus* egg extracts and cohesins similarly bind to chromatin in condensin-depleted extracts (Losada *et al.*, 1998). The differences in cohesin and condensin behavior between vertebrates and yeast may reflect the relatively small amount of condensation occurring in yeast compared to that in higher eukaryotes (Guacci *et al.*, 1994). Thus, the roles of the cohesin and condensin complexes appear to have become more specialized in higher eukaryotes so that these complexes associate and dissociate independently. Furthermore, no cell-cycle-specific phosphorylation has been observed for the cohesin subunits in *Xenopus*, unlike the mitosis-specific phosphorylation of condensin complex members (Losada *et al.*, 1998). On the other hand, the *S. pombe* Rad21 protein is initially phosphorylated in G_1/S, followed by hyperphosphorylation in G_2 which is maintained throughout mitosis (Birkenbihl and Subramani, 1995). Strikingly, the cohesin complexes from *Xenopus* egg extracts resemble condensins as they also occur as two versions with different sedimentation coefficients, in which the 9S form is a heterodimer of XSMC1 and XSMC3 while the 14S form contains at least three additional subunits (including the Scc1p/Mcd1p orthologue) (Losada *et al.*, 1998). This structural similarity between cohesins and condensins strongly suggests that they may have evolved from a common ancestor (albeit independently), particularly considering that a single SMC protein may be involved in both processes in bacteria.

A further link between cohesion and condensation in budding yeast is revealed by analysis of the Trf4 protein (topoisomerase I-related function), which binds to both Smclp and Smc2p (Castaño *et al.*, 1996; Strunnikov *et al.*, 1993) and is required for chromosome segregation (Castaño *et al.*, 1996) and rDNA chromosome condensation (Castaño *et al.*, 1996). Likewise, an additional link between the condensation and cohesion machinery has been revealed by the interaction of either Smc1p or Smc2p with different coiled-coil domains of the human HEC1 protein (highly expressed in cancer) and Tid3p, its orthologue in *S. cerevisiae* (Zheng *et al.*, 1999). This protein is required to prevent haphazard sister chromatid segregation in both organisms (Chen *et al.*, 1997; Zheng *et al.*, 1999) and to repress the ATPase activity of the 26S proteasome subunit (Chen *et al.*, 1997), suggesting possible roles in regulating the destruction of Scc1p/Mcd1p or even controlling the ATPase activity of SMC-containing complexes. Furthermore, as Tid3p was previously shown to interact with a protein required for meiotic recombination and synaptonemal complex formation (*DMC1*), this suggests a possible role for HEC1 in extending the activities of SMC proteins to recombination repair during meiosis (Dresser *et al.*, 1997). Clearly, the biochemical effects of HEC1 interaction with SMC proteins demand further study.

COHESIN PROTEINS AND MEIOSIS

The subunits of the cohesin complex also share important links with proteins required for cohesion during meiosis, as shown by the essential meiotic roles of Smc3p and Rec8p (a paralogue of Scc1p/Mcd1p) (Klein *et al.*, 1999; Parisi *et al.*, 1999; Watanabe and Nurse, 1999). The *rec8* gene was originally identified in a screen for *S. pombe* mutants with reduced meiotic recombination (Ponticelli and Smith, 1989) and the encoded protein was shown to be required for sister chromatid cohesion and pairing of homologous chromosomes during meiosis I (Krawchuk *et al.*, 1999; Krawchuk and Wahls, 1999; Mol-

nar *et al.*, 1995). Synthesis of Rec8p was specific to meiosis (unlike other cohesins) and deletion of the *rec8* gene resulted in equational rather than reductional chromosome segregation (Lin *et al.*, 1992; Watanabe and Nurse, 1999). Conversely, the replacement of Rad21p by Rec8p during mitosis produced a remarkable shift in the pattern of chromosome segregation from equational to reductional. Nonetheless, the ability of the Rad21p protein to rescue the inviability of *rec8* mutant spores showed that these proteins share common functions. Moreover, as the cohesin cleavage sites appear to be uniquely conserved between Scc1p/Mcd1p in *S. cerevisiae*, Rad21p in *S. pombe*, and the Rec8 proteins in both organisms, it seems likely that their cleavage may be similarly mediated by separins (Uhlmann *et al.*, 1999).

The Rec8 protein was originally believed to be involved in early steps of meiotic recombination, based on its early appearance and disappearance (Lin *et al.*, 1992). In agreement with this, *rec8* mutants exhibited defective linear elements (axial core-like structures formed in place of tripartite synaptonemal complexes during meiotic prophase in *S. pombe*) which were shorter and thicker than in wild-type cells (Molnar *et al.*, 1995; Parisi *et al.*, 1999). Likewise, both Rec8p and Smc3p (which are required for meiotic chromatid cohesion in *S. cerevisiae*) are essential for the formation of synaptonemal complexes and axial elements (Klein *et al.*, 1999). However, neither protein is required for the formation of double-strand breaks, implying that Rec8p and Smc3p are needed to maintain cohesion so that such lesions may be repaired (Klein *et al.*, 1999).

The *S. pombe* Rec8p protein was tightly associated with numerous chromosomal foci during prophase of meiosis I and was globally distributed around the centromeric regions, whereas Rad21p was predominantly found near the telomeres (Parisi *et al.*, 1999; Watanabe and Nurse, 1999). Just as Rad21p is phosphorylated in mitosis, Rec8p also underwent phosphorylation from prophase onward. Although the level of a Rec8–GFP fusion detected by Western blotting declined between the successive meiotic divisions, the protein persisted beyond meiosis I and remained tightly associated with centromeric heterochromatin. Similarly, Rec8p and Smc3p in *S. cerevisiae* colocalized in a continuous line along the longitudinal axis of pachytene chromosome cores while Scc1p was restricted to discrete foci (Klein *et al.*, 1999). The Rec8 protein levels were highest at the time of premeiotic DNA replication but decreased after pachytene and disappeared after anaphase II (Klein *et al.*, 1999). Correspondingly, both Rec8p and Smc3p disappeared from the chromosome arms after pachytene but persisted near the centromeres after the separation of homologous chromosomes during the first meiotic division, until anaphase of meiosis II (Klein *et al.*, 1999). Interestingly, the Rec8p localization pattern in both yeasts is similar to that of COR1 (a component of the lateral elements of synaptonemal complexes in rodent spermatocytes), suggestive of a role in synaptonemal complex formation (Dobson *et al.*, 1994; Lammers *et al.*, 1994; Yuan *et al.*, 1998).

It will be interesting to see if SMC3 and indeed SMC1 proteins are involved in maintaining meiotic cohesion in other organisms, as suggested by a high rate of expression in rodent ovaries and testes (Shimizu *et al.*, 1998; Stursberg *et al.*, 1999). The meiotic function of cohesin proteins seems to be highly conserved among eukaryotes as the *DIF1* (determinate, infertile1) gene of *Arabidopsis*, encoding an orthologue of Rec8p, was similarly shown to be essential for meiotic chromosome segregation and hence fertility (Bhatt *et al.*, 1999). In addition, the ability of the human Rec8 protein to partially complement the reduced spore viability of *S. pombe rec8* mutants suggests at least some conservation of function (Parisi *et al.*, 1999).

SMC PROTEINS AND DNA REPAIR

A number of the proteins involved in sister chromatid cohesion have also been shown to play critical roles in recombinational repair. For example, both SMC1 and SMC3 may have a role during interphase as part of the bovine recombination protein complex RC-1, in which they are complexed with DNA ligase III, DNA polymerase ϵ, and a DNA structure-specific endonuclease (Jessberger *et al.*, 1996a,b). Similarly, Rad21p of *S. pombe* was implicated in the repair of double-strand DNA breaks in irradiated cells in addition to being essential for mitotic growth (Birkenbihl and Subramani, 1992, 1995; Tatebayashi *et al.*, 1998).

However, a specific role in DNA repair is demonstrated by members of a further subgroup of the SMC family, first identified in *S. pombe* through the analysis of *rad18* mutants. A temperature-sensitive mutant was shown to be hypersensitive to both UV and γ-irradiation and also exhibited reduced rates of removal of UV photoproducts compared to wild-type cells. However, no significant difference in endonuclease activity was observed between extracts from *rad18* cells and wild-type cells, suggesting that Rad18p might be involved in repair of DNA damage by facilitating genetic recombination. This was subsequently confirmed by assaying the ability of cells to repair double-stranded DNA breaks using pulsefield gel electrophoresis (Verkade *et al.*, 1999). Correspondingly, severely reduced levels of intrachromosomal homologous recombination were demonstrated

in an *Arabidopsis mim* mutant (an orthologue of *rad18*) (Mengiste *et al.*, 1999).

Immunostaining of cells revealed that the protein is found in the chromatin compartment of the *S. pombe* nucleus, as expected for a protein directly engaged in DNA repair (Verkade *et al.*, 1999). A *rad18* mutant was completely suppressed by excess copies of *brc1* (encoding a BRCT domain protein required for proper chromosome condensation and segregation) but was synthetically lethal in combination with mutations in *brc1, fin1* (encoding a kinase which induces chromatin condensation), or topoisomerase II, suggesting that the ability of Rad18p to repair DNA lesions might be related to a role in orderly chromosome condensation. It will be interesting to see whether Rad18p shares the ability of some other SMC proteins to directly promote strand exchange (Jessberger *et al.*, 1993, 1996a,b; Sutani and Yanagida, 1997).

The deletion of *RAD18* and *RHC18* (the *S. cerevisiae* orthologue) showed that the gene is essential for proliferation in both species (Lehmann *et al.*, 1995). The mitotic defect was further characterized by the isolation of an additional temperature-sensitive *rad18* mutant (Verkade *et al.*, 1999). After irradiation, many cells underwent cytokinesis in the absence of completed chromosome segregation, resulting in cells with nuclei stretched along the division plane and bisected by septa. Such aberrant mitoses in spite of unrepaired DNA lesions also implied a role for Rad18p in maintaining the G_2 DNA damage checkpoint. This was verified by the failure of two different *rad18*/*top2* double mutants to arrest in G_2, whereas inhibition of topoisomerase II activity normally produces such an arrest (Hartwell and Weinert, 1989). However, Rad18p is not required for initiation of the checkpoint, as shown by normal phosphorylation of the Chk1 protein kinase (the final element in the signaling cascade activated by the G_2 DNA damage) in *rad18* cultures (Verkade *et al.*, 1999). It is therefore possible that Rad18p activity is induced posttranscriptionally by this G_2 checkpoint and the continued activity of this protein maintains the arrest until the damage is repaired. Thus, the Rad18 subfamily of SMC proteins appears to have multiple functions in response to DNA damage, signaling the persistence of unrepaired lesions in DNA and repairing them through a role in recombination-mediated repair.

MOONLIGHTING IN THE BASEMENT MEMBRANE?

Perhaps the most surprising result concerning SMC proteins to date is a possible additional role outside the cell. This idea is based on the identification of an extracellular, secreted proteoglycan, known as bamacan (basement membrane-associated chondroitin sulfate proteoglycan), as an SMC molecule. When rat bamacan was cloned (Wu and Couchman, 1997), the authors noted that the sequence bore no structural similarity with any chondroitin/dermatan sulfate proteoglycan reported at that time. However, they noticed that bamacan and SMC proteins from several diverse organisms shared a similar five-domain structure. Unfortunately, as none of the SMC proteins used in their comparison belonged to the SMC3 subclass, the overall sequence homology of bamacan to other SMC proteins was found to be low (even when compared with those of vertebrate origin). The human orthologue of SMC3 was cloned the following year and was initially named HCAP (human chromosome-associated polypeptide). The authors commented that this SMC protein shared 98% amino acid sequence identity with the published rat bamacan protein sequence (Shimizu *et al.*, 1998). Finally, the murine orthologue of the rat bamacan was cloned and identified as a member of the SMC3 protein subfamily, as the protein showed the same level of homology to the bovine SMC3 as it did to the rat bamacan (Ghiselli *et al.*, 1999). Meanwhile, an independent group had succeeded in cloning the murine homologue of SMC3, known as mSMCD (Darwiche *et al.*, 1999). However, at the time of writing both groups were seemingly unaware of just how close bamacan really was to SMC3. When the cDNA sequences for the murine SMC3 and murine bamacan are aligned, the corresponding predicted protein sequences are 100% identical. In other words, it appears that the mouse SMC3 is the same molecule as a component of the extracellular basement membrane.

As the murine bamacan was cloned by using the rat bamacan sequence to BLAST the *dbEST* data base, the true significance of the similarity hinges on how reliably the rat bamacan was cloned. The rat bamacan was originally isolated (Wu and Couchman, 1997) by screening a rat yolk sac carcinoma cDNA expression library with a polyclonal antiserum raised against a pool of purified proteoglycans from the murine Engelbreth–Holm–Swarm tumor matrix (Couchman *et al.*, 1996). To confirm the identity of this clone, rabbit antibodies were raised against two nonoverlapping fusion proteins encoded by subclones of the bamacan cDNA and both of these antibodies were shown to recognize the same protein by immunoblotting as the original antiserum. One of these antisera also stained extracellular matrix in tissue sections, as did the original antibody. This is strong evidence that a protein better known for its role in chromosome mechanics has a very unpredicted extracellular localization. Antibodies to the murine SMC3 were generated against a peptide in the C-terminal ATP-binding domain (Darwiche *et*

al., 1999); these antibodies demonstrated that the murine SMC3 binds to interphase chromatin and dissociates from it at the onset of mitosis, in agreement with previous studies with the *Xenopus* orthologues (Losada *et al.*, 1998). It would therefore be crucial to see whether these antibodies used on mouse tissue sections independently reproduce the pattern of staining observed with the antibodies to rat bamacan.

Naturally, one might wonder what a protein involved in chromosome dynamics might be doing in the basement membrane, outside the cell. Although it is not unheard of for the same protein molecule to have more than one distinct action (Jeffery, 1999), none of the previously recognized functions of SMC3 show any obvious connection with a role outside the cell. However, one clue to how SMC proteins could be involved in stabilizing the extracellular matrix of basement membranes may be provided by comparison with the laminins (Timpl and Brown, 1996). These molecules also have coiled-coil domains, in this case mediating heterotrimerization between the α, β, and γ chains. In addition, the globular laminin N-terminal domains mediate Ca^{2+}-dependent polymerization to yield quasihexagonal networks. These laminin networks are finally anchored in the basement membrane by integrin and dystroglycan receptors. One may conjecture that SMCs might also form more complex networks if they are secreted in sufficiently high concentration, forming chains as a result of interactions between the terminal ATPase domains of adjacent molecules. Alternatively, it is possible that secreted SMC3/bamacan may fit into a matrix through chondroitin sulfate side chains, in much the same way that perlecan (a heparan sulfate/dermatan sulfate proteoglycan) interacts with the heparan sulfate binding site in the C-terminal LG modules of laminin α chains. An additional question concerns how an SMC protein might actually reach the basement membrane. Sequence analysis of the SMC proteins using available data for nuclear localization signals (NLS) and NES reveals that each SMC may contain potential NLS and NES sequences, of which at least one candidate NES has been shown to be functional (Sutani *et al.*, 1999).

Indeed, SMC3 is not the only nuclear protein that seems to be playing an additional role outside the cell. For example, histone H1 has been shown to act as a binding protein for thyroglobulin at the cell surface of macrophages, mediating thyroglobulin endocytosis (Brix *et al.*, 1998), while titin, a constituent of muscle sarcomeres, has also been proposed to be a component of *Drosophila* chromosomes (Machado *et al.*, 1998). Another recently identified basement membrane-associated proteoglycan with candidate NES and NLS motifs, known as leprecan (Wassenhove-McCarthy and McCarthy, 1999), may possibly play a role in chromosomal dynamics as it shares more than 36% identity and 43% similarity with the synaptonemal complex protein SC56 along a stretch of 343 amino acids. In conclusion, the surprising discovery that an SMC protein may have a role outside the cell, quite apart from a fundamental role in various aspects of chromosomal dynamics, adds to a growing list of moonlighting proteins.

FUTURE PROSPECTS

The understanding of chromosome structure and behavior has been greatly enriched by the findings made over the past few years. It is already clear that the SMCs are important for chromosome cohesion, chromosome condensation, dosage compensation, and recombination repair. The original eukaryotic subfamilies of SMC1, SMC2, SMC3, and SMC4 have been joined by a fifth branch, the Rad18 subfamily. Even more exciting is the discovery and analysis of the single SMC within prokaryotes, leading to the undeniable conclusion that the SMCs are conserved not only in structure but also in function. The evolution of the single SMC to a family constituting five subfamilies is certainly a matter of intrigue, as is the possible extracellular existence of an SMC protein.

The *Xenopus in vitro* extract system, coupled with powerful genetics in *S. cerevisiae* and *S. pombe*, has predominantly contributed to the identification of these molecules and their associated proteins. Study of the SMC proteins and the complexes and processes in which they take part has not only illuminated the significant degree to which certain components and mechanisms are conserved, but also highlighted provocative questions for future study. Very likely, differences between single-celled organisms and multicellular creatures will be elucidated and with time clarified. What is clearly missing from the studies published to date is an analysis of these components in a multicellular organism amenable to developmental, genetic, and cytological approaches. The identification of the genes for SMCs and associated proteins has been greatly facilitated by the *Drosophila* genome project. This is currently leading to the identification of mutations in these genes and exploitation of the ability to examine these proteins at different times of development, in different tissues, in different types of cell cycles (e.g., rapid, synchronized early embryonic cycles lacking G1 and G2 phases versus more normal cell cycles), and in different types of chromosomes (e.g., diploid versus giant, banded polytene chromosomes). There is no doubt that the future years will be as rich for research and progress into understanding the fundamental questions of chromosome structure and behavior as the past years have been.

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