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Looping the Genome with SMC Complexes

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Abstract

SMC (structural maintenance of chromosomes) protein complexes are an evolutionarily conserved family of motor proteins that hold sister chromatids together and fold genomes throughout the cell cycle by DNA loop extrusion. These complexes play a key role in a variety of functions in the packaging and regulation of chromosomes, and they have been intensely studied in recent years. Despite their importance, the detailed molecular mechanism for DNA loop extrusion by SMC complexes remains unresolved. Here, we describe the roles of SMCs in chromosome biology and particularly review in vitro single-molecule studies that have recently advanced our understanding of SMC proteins. We describe the mechanistic biophysical aspects of loop extrusion that govern genome organization and its consequences.

Contents

1. INTRODUCTION

The length of genomes in various species varies considerably, from the largest known genome of ~150 billion base pairs (bp) of *Paeonia japonica* (1) to 3.4 billion bp for *Homo sapiens*, down to 0.11 million bp for the bacterium *Nasuia deltocephalinicola* (2). All cells require their DNA to be compacted at least 1,000 fold in order to fit within the finite space of a micrometer-sized nucleus (or cell volume for bacteria). Additionally, information encoded in the genome continuously needs to be accessed and processed. Accordingly, the genome is constantly and actively reshaped to sustain all cellular functions. Structural maintenance of chromosomes (SMC) complexes play vital roles at all stages of chromosome biology throughout the cell cycle, from forming the characteristic textbook X-shaped mitotic chromosomes to actively regulating gene expression, controlling replication timing, and conferring genome stability. To do so, SMCs have evolved to be regulators of DNA looping, as large-scale (0.1–1 Mbp) loops constitute the basic motif of the spatial structure of the genome in all domains of life.

SMC complexes are multi-subunit motor proteins that use the energy of ATP hydrolysis to fold genomes into loops throughout the cellular life cycle. Eukaryotic cells have three classes of SMC complexes—cohesin, condensin, and SMC5/6—that exhibit distinct roles. For example, condensin plays a central role in chromosome compaction and segregation, whereas cohesin is essential for sister chromatid cohesion and interphase genome organization, and SMC5/6 is implicated in DNA repair and checkpoint responses. Most archaea and bacteria, on the other hand, feature one major type of SMC proteins, for example, MukBEF in *Escherichia coli* and SMC–ScpAB in *Bacillus subtilis*, whereas other SMC-like proteins (e.g., MksBEF) appear to coexist in certain bacteria (3). These complexes facilitate the resolution of sister chromosomes before cell division, thus exhibiting functional similarity to eukaryotic condensin. DNA loop extrusion activity has been demonstrated on a single-molecule level for all eukaryotic SMCs, whereas some of the best in vivo evidence that SMC complexes extrude DNA loops comes from studies in bacteria.

Going back in history, the first documented observation of structural aspects of chromosomes was made in 1882 by Walther Flemming (**Figure 1*a***) (4). He observed emanating loops in axolotl oocyte lampbrush chromosomes, just a decade after DNA was identified (5). The notion of a proteinaceous scaffold to which loops of 30–90 kb are anchored prevailed for more than a century (6) after its initial description by Flemming. However, later research showed that lampbrush chromosomes actually result from high levels of transcription, which causes the transcript-decorated

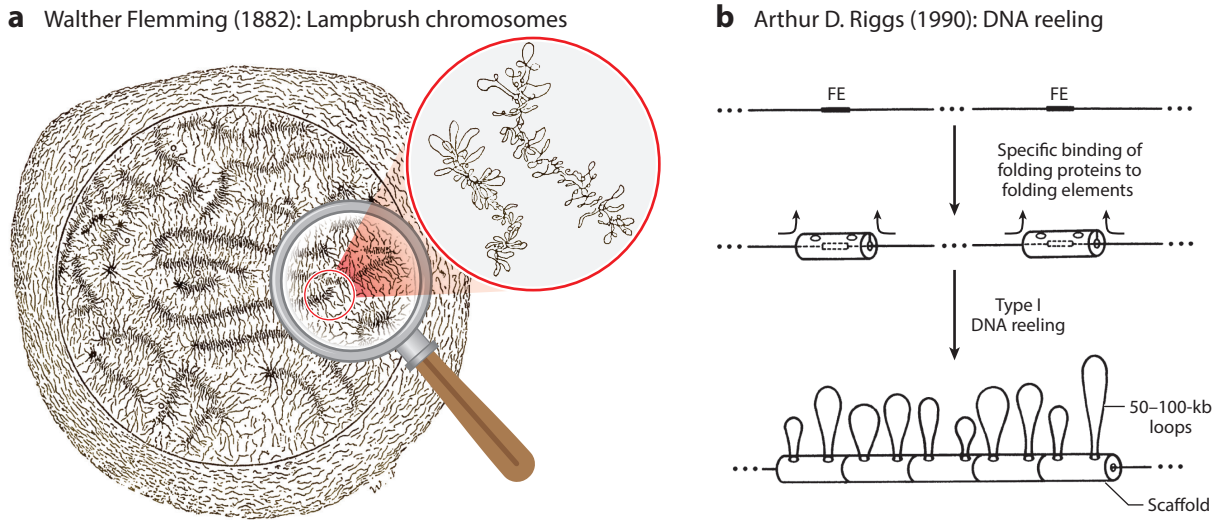
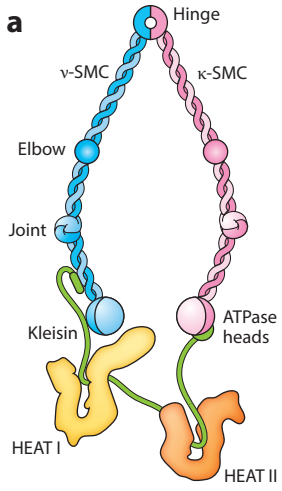


Figure 1

Historic precursors to the DNA loop extrusion hypothesis. (*a*) In 1882, Walther Flemming observed DNA loops in axolotl oocytes using light microscopy. These loops were due to the repulsion of highly expressed genes. (*b*) Arthur D. Riggs first proposed a DNA reeling mechanism in cis to explain the formation of mitotic chromosomes, inspired by the bidirectional translocation of bacterial type I restriction enzymes. Even though details have changed, the principle of Riggs' mechanism remains valid until today. Panel *a* adapted from Reference 183 with permission from The Royal Society; panel *b* adapted from Reference 15. Abbreviation: FE, folding element.

DNA to repel itself into a loop-like structure (7, 8). DNA looping proved to be an attractive mechanism throughout history to explain a range of phenomena such as transcription initiation of prokaryotic operons (9), the action of enhancers on promoters in eukaryotes (10), and recombination (11). Looping between genomic loci can occur over distances of hundreds of base pairs via dimerization of DNA-bound proteins or through supercoiling (12). Such relatively small loops have to overcome an energy cost associated with the bending of DNA on the length scale of the 50 nm persistence length of DNA, for which DNA elasticity is a main determinant. As loops become longer, the DNA twisting and bending energies diminish, but this comes at the expense of an entropic cost, which is reflected in the decrease in the contact probability of any two genomic loci as their genomic separation increases (13). To achieve DNA looping over long genomic distances, an active energy-consuming "DNA reeling" mechanism was proposed. Early evidence came from bacterial type I restriction enzymes, specifically EcoK, which were proposed to bind to recognition sites and translocate bidirectionally away until another EcoK is encountered (14). In 1990, Arthur D. Riggs postulated that such a mechanism can be used to ensure specific looping in cis, i.e., along the same chromosome (15), an idea that was indeed confirmed with single-molecule experiments two decades later (16). Quantitative estimations of the compaction of mitotic chromosomes by Riggs were sound: DNA reeling and encounters of 1,000 loops of 50–100 kb in length would result in an axial protein backbone of ~5 μm long, roughly the size of a mammalian mitotic chromosome (**Figure 1*b***).

Even before Riggs' postulation of DNA reeling, a gene named stability of minichromosomes 1 (*SMC1*) was discovered in *Saccharomyces cerevisiae* (17, 18), while the discovery of more SMC genes in various organisms followed in the 1990s. The annotation of the acronym SMC for this ring-shaped protein complex (**Figure 2*a*,*b***) was later changed to structural maintenance of chromosomes. Although initially discovered for their deterministic impact on chromosome segregation, the relation of SMC proteins to Riggs' postulated DNA reeling activity was not



b

		Bacteria		Eukaryotes		
		Bacillus subtilis	*Escherichia coli*	SMC5/6	Cohesin	Condensin
Core subunits	ν-SMC	SMC	MukB	Smc6	Smc3	Smc2
	κ-SMC	SMC	MukB	Smc5	Smc1	Smc4
	Kleisin	ScpA	MukF	Nse4	Scc1	Brn1
Accessory subunits	KITE	ScpB	MukE	Nse1 Nse3		
	HEAT I				Scc2 Pds5	Ycs4
	HEAT II				Scc3	Ycg1
				Nse2 Nse5 Nse6		

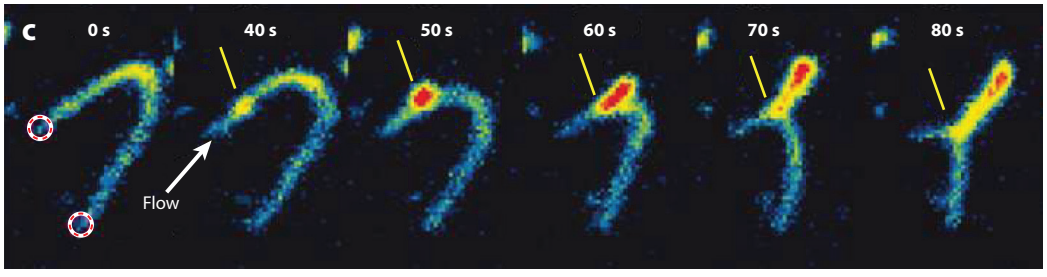


Figure 2

SMC complexes extrude loops of DNA. (*a*) General architecture of SMC complexes. The core of an SMC complex is characterized by a pair of ∼50-nm long antiparallel coiled-coil protein subunits named SMCs. These SMCs dimerize on one end (the hinge), and at their other end (the ATPase head domains), they mutually connect through a kleisin protein, resulting in a ring-like structure. The kleisin recruits additional regulatory subunits. One family of these regulators, the Kite dimers, interacts with SMC–kleisin rings from bacteria, archaea, and the eukaryotic SMC5/6 complex. Condensin and cohesin further evolved by replacing Kite domains with Hawk domains (184). Condensin has two Hawks (Ycg1 and Ycs4 in yeast) and cohesin has three (Scc3, Scc2, and Pds5 in yeast). While Ycg1 and Ycs4 are stably associated with condensin, as is Scc3 with cohesin, Scc2 and Pds5 transiently associate with cohesin and compete for cohesin binding (185). Both Kite and Hawk proteins play a crucial role in DNA binding and ATPase activity of the SMC complexes (128, 186, 187). In the case of SMC5/6, three additional subunits exist, namely Nse2, Nse5, and Nse6. (*b*) Subunits comprising SMC complexes in certain bacteria and Eurkaryotes. (*c*) Single-molecule imaging demonstrating DNA loop extrusion. The flow is directed toward the top right; yellow lines indicate the position of the extruded loop. Image reproduced from Ganji et al. (21). Abbreviations: Hawk, HEAT-repeat proteins associated with kleisins; Kite, kleisin interacting winged-helix tandem elements; SMC, structural maintenance of chromosomes.

straightforward. This model, one of many at the time, was largely ignored until the development of chromosome conformation capture technologies over a decade later. Two seminal papers, one focused on mammalian cells and the other on the bacterium *Bacillus subtilis*, that were published within months of each other provided the first in vivo data that hinted toward the fact that SMC complexes could function as loop extruders (19, 20). These studies led to further in vivo and in vitro experiments that culminated in single-molecule visualizations of SMC-driven DNA loop extrusion (**Figure 2*c***) (21).

In this review, we discuss recent advances in the field of SMC proteins, focusing on the role of in vitro single-molecule studies, which continue to be pioneering in advancing our understanding

of SMC proteins. After briefly reviewing the various roles of SMC proteins during mitosis and interphase, we dive into the various structures of SMC proteins found throughout the ATPase cycle. We discuss the currently unresolved but heavily debated mechanism of DNA loop extrusion and the effects of abundant roadblock DNA-binding proteins on chromatin that might interfere with loop extrusion in vivo.

2. THE VERSATILE ROLES OF SMCS IN CHROMOSOME BIOLOGY

While initially discovered and studied for their role in chromosome segregation, it became clear that SMCs are also involved in mitotic chromosome formation, interphase chromosome organization, transcriptional regulation, and DNA repair. Here we highlight the most prominent functions of SMC proteins through the stages of the cell cycle.

2.1. Activity of SMCs During Cell Division

As cells replicate and divide, two of the most well-known SMCs work in symbiosis for faithful chromosome segregation: DNA compaction is driven by loop extrusion via condensin, while sister chromatid cohesion is established by cohesin. While the formation of DNA loops was suggested to underlie mitotic chromosome condensation early on (6, 22, 23), a novel spur of interest in the loop-extrusion hypothesis arose when it appeared that the elongated mitotic chromosome structure and segregation of the newly replicated chromosomes could be elegantly recapitulated with simulations (24, 25). Merely assuming a loop-extrusion mechanism, for which condensin was a strong candidate to be the driving protein, was sufficient to self-organize compacted chromosomes. These simulations, combined with Hi-C chromosome-capture analyses, further explained the separate roles of two types of condensin present in vertebrates: Condensin II is nuclear and binds chromatin in prophase, whereas condensin I is cytoplasmic and thus can act on chromatin only after nuclear envelope breakdown (26, 27). It was deduced that condensin II first extrudes large DNA loops ~400 kb long, whereas condensin I extrudes ~80-kb long nested loops within these 400-kb loops (28, 29) (**Figure 3*a***). Notably, the estimates from the Hi-C and quantitative microscopy methods aligned remarkably well with very early estimates of loop sizes made by Riggs (15) and Nasmyth (30).

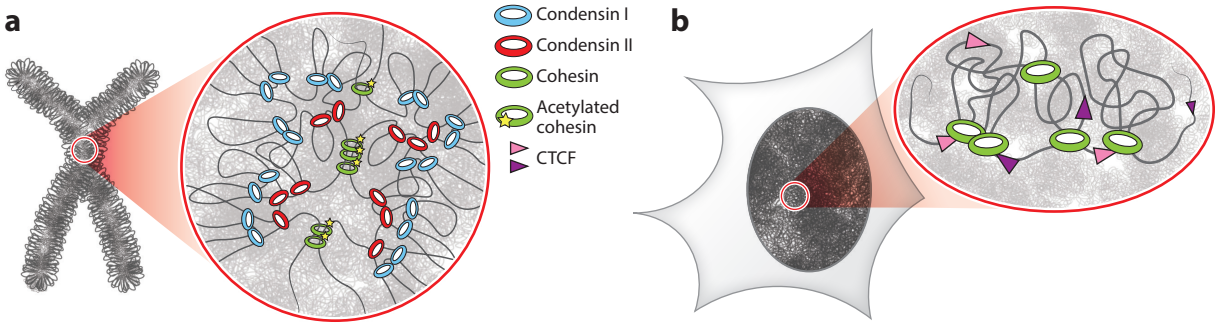


Figure 3

SMC complexes are active throughout the cell cycle. (*a*) Mitotic chromosomes in vertebrates are formed through looping of the genome by condensin I and II, whereas cohesin is responsible for sister chromatid cohesion by topologically encircling the two sisters. Acetylation of cohesin (denoted by a star) prevents loss of cohesin at this stage by preventing the unloader, WAPL, from acting on cohesin. (*b*) In interphase, cohesin is the dominant SMC, as it extrudes DNA loops along the genome. Blocking of cohesin by convergently oriented CTCF sites (differently colored *triangles* denote CTCF in different orientations bound to DNA) results in topologically associated domain formation. Abbreviations: CTCF, CCCTC-binding factor; SMC, structural maintenance of chromosomes; WAPL, wings apart-like protein homolog.

In contrast to eukaryotes, sister chromatid cohesion is absent in prokaryotes. In prokaryotes, the resolution and segregation of sister chromatids are coupled to replication. Bacterial SMCs load onto chromosomes at parS centromeric sequences adjacent to the replication origin, with the help of the chromosome-partitioning protein ParB (31). Hi-C analysis of *B. subtilis* suggested that SMCs translocate and perform loop extrusion of the entire megabase-sized chromosome until the terminus of replication is reached (32, 33). This causes the two arms of the circular chromosome to become juxtaposed. SMC proteins are required for segregation of replication origins, and the chromosomes are segregated by the ParB–ParA system and entropic forces (34, 35). In bacteria without the ParABS system, such as *E. coli*, the SMC-like MukBEF complex loads nonspecifically on the genome (36, 37). By increasing chromosome occupancy of MukBEF and imaging the genome, it was observed that MukBEF complexes form a chromosomal axial core from which DNA loops emanate, reminiscent of mitotic chromosome condensation in eukaryotes (38). Although it is difficult to come up with an alternative model for how SMC complexes organize bacterial chromosomes based on the in vivo evidence, a direct proof of loop extrusion by the bacterial complexes at the single-molecule level has been lacking to date, although multiple groups have tried to demonstrate this.

The second important aspect of chromosome segregation in division, sister chromatid cohesion, depends critically on cohesin. Sister chromatid cohesion is established during S phase, concomitant with DNA replication (30). Cohesin can be loaded onto the DNA via two independent pathways: Cohesin complexes can load de novo during or right after replication or existing chromatin-associated cohesins can be converted into cohesive ones (39–42). Cohesin associates with both sister chromatids. The prevailing model is that a single ring-shaped cohesin complex (43) encircles one double-stranded (ds) DNA molecule from each sister chromatid (44) (**Figure 3*a***) (for more focused reviews of cohesin's role in sister chromatid cohesion, see 45, 46). While mechanistic details of the process are still lacking, in vitro studies showed that cohesin, when topologically bound to a dsDNA molecule, is capable of capturing and topologically embracing another ssDNA, but not dsDNA, molecule in the presence of Scc2 and ATP (47). Such a scenario is reminiscent of replication, during which cohesin could topologically bind to the replicated leading strand and then capture the lagging strand before its conversion to dsDNA. Cohesin can also stably bridge two dsDNA molecules in an ATP-dependent manner (48). However, it is unclear how cohesin can distinguish between sister chromatids and other chromatin and whether the bridging of dsDNA molecules is efficient, since bulk in vitro assays have failed to detect such an association (47). Recently, a cohesin mutant was identified that is defective in sister chromatid cohesion but can still perform DNA loop extrusion (49). This suggests that these functions can be genetically separated. The authors raise the intriguing possibility that CTCF may convert loop-extruding cohesin into cohesive cohesin, even though the mechanism remains as yet unknown (50). Since CTCF and human minichromosome maintenance 3 (MCM3) (a part of the replisome) contain the same peptide sequence that is predicted to interact with a conserved binding site of cohesin, formed by its kleisin and STAG1 subunit (51), it is conceivable that such a proposed conversion can also occur when extruding cohesins encounter the replisome. The final conversion from a topologically bound cohesin to a cohesive cohesin can then occur by catching the newly replicated lagging strand as proposed by Murayama et al. (47), by interacting with other replisome components (39), or at converging replication sites (52). Since cohesion between sister chromatids is very stable, in extreme cases lasting for decades, as in human oocytes, such cohesive cohesin must be protected from DNA release. Acetylation of SMC3 by the acetyltransferase Eco1 in yeast [ESCO1/ESCO2 in humans (53–55)] protects cohesin from release by WAPL and PDS5 (56–58) (**Figure 3*a***). SMC3 acetylation is likely coupled to cohesion establishment during replication, since Eco1 associates with the replication machinery (59). It is thus conceivable that

the topological encircling of one or two dsDNA molecules occurs in vivo but is readily resolved by WAPL when cohesin is not acetylated. A bridging activity of two DNA molecules has also been suggested by experiments demonstrating that a loop extrusion-independent mechanism also contributes to the shaping of chromosomes (60). dsDNA–dsDNA capture events that have been observed previously in vitro (48) are produced by multimers of SMCs that do not necessarily have to topologically entrap DNA. Such weak protein–protein interactions are reminiscent of the clustering or phase separation of SMCs by bridging-induced phase separation, arising from the fact that SMC proteins contain at least two DNA binding sites; a DNA molecule longer than 3 kb can form a loop between these sites and then serve as a nucleation site for additional SMCs (61). The interaction of several SMC molecules was observed by multiple approaches (62–65). In particular, the coexpression of two allelic mutations in SMCs, which each individually fail to condense and cohere chromatids, rescued the phenotype (63), suggesting a functional interaction between SMC molecules.

2.2. Activity of SMCs During Interphase

While condensin and cohesin join forces in forming mitotic chromosomes during cell division, their roles change during interphase; in this phase, chromosome organization is mainly governed by DNA loop extrusion by cohesin, while condensin plays only a modest role.

First, we address the formation of topologically associated domains (TADs) and their implications for transcriptional regulation. Studies of the interphase organization of chromosomes were significantly accelerated when chromosome conformation capture (3C) methods advanced to a resolution of <100 kb. Self-interaction maps of chromosomes revealed regions, called TADs, of higher contact probabilities compared to their surroundings (66, 67). These TADs span several hundred kilobase pairs and are often accompanied by strong chromosomal interactions between the loci demarcating TAD boundaries (68). A hallmark of most TADs is that their boundaries are bound by CTCF and cohesin (66, 69–71), and the large majority of TADs disappear when either of these two components is deleted (72–76). The loop-extrusion hypothesis, originally formulated to explain the condensation of mitotic chromosomes, proved to be successful in explaining TAD formation as well (20, 77–79): Cohesin was suggested to bind and extrude DNA loops until it encounters a CTCF molecule, which acts as a boundary element (**Figure 3*b***). This hypothesis could also explain two major observations in cells that are depleted of WAPL, a factor that releases cohesin from chromatin. Effectively increasing the residency time of cohesin on chromatin upon WAPL depletion led to longer loops in Hi-C experiments (74, 76) and a dramatic compaction of chromatin and redistribution of cohesin into axial chromosome regions, termed vermicelli (80).

DNA loop extrusion by cohesin during interphase, and the associated formation of TADs, likely plays a fundamental role since roughly 2/3 of all TAD boundaries are invariant among cell types and between species (66, 81). This high degree of evolutionary conservation is poorly understood, and recent single-cell analyses reveal the highly dynamic and cell-to-cell variant nature of loops and TADs (82–84). Yet TAD boundaries may constitute a structural framework that enables or disfavors interactions between cis-regulatory sequences, which is corroborated by the fact that most enhancer–promoter interactions occur within a TAD (85, 86). Even though the contact probability within TADs is only two- to fivefold higher than between TADs (87), computational studies found an up to twofold insulation and three- to fivefold facilitation of enhancer–promoter contacts, depending on the relative positioning of regulatory elements (**Figure 4*a***) (87–89).

Rearrangements of TADs and the accompanying deregulation of genes may thus exert an evolutionary pressure on keeping TAD boundaries intact (90), providing an intriguing hypothesis to explain the strong evolutionary conservation of TADs. This hypothesis predicts that the structural integrity of the genome should have a broad effect on the expression levels of genes. However, this

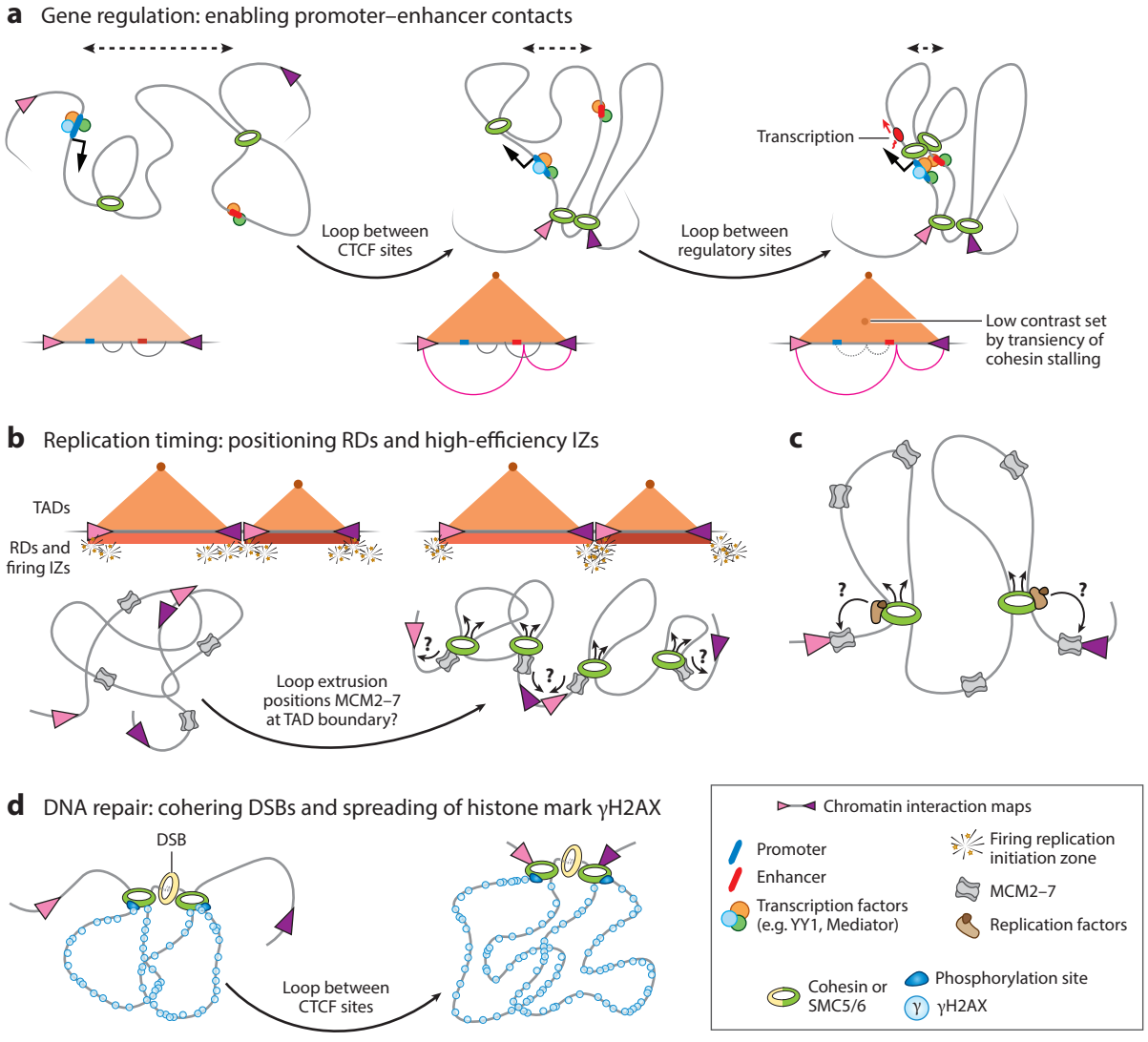


Figure 4

DNA loop extrusion in gene regulation, replication, and DNA repair. (*a*) An enhancer and promoter on an unconstrained genome segment may have a low contact probability due to the large intervening distance between their sites. DNA loop extrusion between CTCF sites located at the encompassing TAD can constrain the polymer and enhance contacts between regulatory sites. When additional loops are enriched between regulatory sites, even for a short time by, e.g., transiently stalling loop extrusion (cf. **Figure 6**), their contact probability is boosted. Due to the transiency and low frequency of their occurrence, these interactions may remain rather hidden in chromatin interaction maps (*orange triangles*). (*b*) RDs overlap with TADs. Without loop extrusion, IZs appear diffuse, while ongoing loop extrusion localizes IZs to TAD boundaries. (*c*) Enrichment of cohesin at CTCF sites due to loop extrusion may bring other replication factors to MCM2–7 complexes residing at CTCF sites. (*d*) DNA DSBs may be sensed and cohered by cohesin and/or SMC5/6 to enable homologous recombination. Loop extrusion by cohesin or SMC5/6 may enable spreading of the DSB response marker γH2AX by reeling DNA through phosphorylation sites located at the DSB. Abbreviations: γH2AX, phosphorylated histone H2AX; CTCF, CCCTC-binding factor; DSB, double-strand break; IZ, initiation zone; MCM2–7, minichromosome maintenance protein complex; RD, replication domain; SMC, structural maintenance of chromosomes; TAD, topologically associated domain.

is not ubiquitously the case, since the effect of cohesin and CTCF loss on transcription genome wide is modest (72, 75) and occurs mainly after a few days of depletion (73), with some genes not being affected at all by structural alterations (for examples, see 91–94). These contradictory views may be reconciled by the notion that TAD formation by DNA loop extrusion is solely a means to provide a rough structural framework in which specificity and maintenance is governed by transcription factors and the transcription machinery, which, in turn, may interact with DNA loop extrusion (95) (see Section 5). Active loop extrusion and blocking thereof constitutes a mechanism that can be regulated by the cell, e.g., by CTCF (un)binding or the establishment or dissolution of loops with the help of NIPBL (the human analog of the cohesin Scc2 subunit) or WAPL. The specificity and selection of interaction partners from a pool of regulatory elements within a TAD may be tuned by the binding of transcription factors and the formation of transcriptional condensates, which may also ensure maintenance of interactions after establishment by loop extrusion (96–98) (**Figure 4*a***). However, it is important to note that DNA loop extrusion is likely not a strict requisite for the regulation of a variety of elements such as stable housekeeping genes, promoters without partnering enhancers, promoter–enhancer pairs with exceptionally high affinity [such as Sox2 and its distal enhancers (99)], and promoters that can be readily looped over short distances without the additional help of the active looping machinery (100). Instead, promoter–enhancer pairs separated by long genomic distances and genes with quick expression changes, e.g., in response to external stimuli, might be more affected by the presence of cohesin and CTCF (101).

How does TAD formation relate to replication timing? Mammalian genomes replicate from multiple origins that fire in a distinct temporal order, called the replication-timing program. Domains spanning 400–800 kb can change their replication timing during development and are called replication domains (RDs) (102). Remarkably, the boundaries of RDs have a near one-to-one correspondence to TAD boundaries (**Figure 4*b***), and replication timing changes happen in genomic regions spanning one or multiple TADs (103). Do RDs then depend on both cohesin and CTCF, just like TADs? Early-firing replication origins colocalize with loop anchors at TAD boundaries, demarcated by cohesin and CTCF binding (104) but not by cohesin alone (105). Ablation of cohesin causes replication origins to become more diffuse, while ablation of WAPL, and thus a gain of longer loops compared to the wild type, localizes replication origins to TAD boundaries (**Figure 4*b***). A molecular mechanism linking TADs and RDs is not yet resolved. Nevertheless, a prominent candidate for being involved in both processes is the minichromosome maintenance protein complex MCM2–7, a key enzyme of the replication machinery. Two recent studies have shown that the replication complex, in particular MCM2–7, is a semipermeable barrier to cohesin loop extrusion (106, 107). This suggests that cohesin can be positioned by replication factors such as MCM2–7 and/or can be recruited de novo in order to perform both DNA loop extrusion and sister chromatid cohesion (40). If responsible for localization of replication origins, MCM2–7 should be preferentially loaded at or relocated to TAD boundaries in a fashion that depends on cohesin and CTCF (105) (**Figure 4*b***). However, no correlation between MCM localization and TAD boundaries is known to date, and a molecular mechanism for this remains to be shown. Alternatively, other factors important for replication initiation might be localized by active loop extrusion to specifically activate MCM complexes at TAD boundaries (**Figure 4*c***). In conclusion, while there is a striking correlation between replication timing and TAD boundaries, as well as documented interactions between cohesin and the prereplication complex, the molecular mechanism that couples DNA loop extrusion and replication timing awaits further investigation.

Loop extrusion has also been implicated in DNA repair. DNA double strand breaks (DSBs) threaten the integrity of the genome; they are highly deleterious and, if unrepaired, can cause genome instability. SMC proteins are essential components of the DNA repair pathways, yet

details on their contribution remain largely unresolved. The high-fidelity homologous recombination pathway depends on cohesin's ability to mediate cohesion between sister chromatids (108). Both SMC5/6 (109) and cohesin are recruited to DSBs, possibly even together (110). In the case of cohesin, the recruitment depends on the presence of Scc2, indicating de novo loading of cohesin rather than conversion of previously bound complexes (111–113). Both cohesin and SMC5/6 have been shown to bind ssDNA (114, 115), which may be essential for sensing ssDNA overhangs of DSBs in the repair through homologous recombination (**Figure 4*d***).

In a loose analogy to the concerted action of DNA loop extrusion and sister chromatid cohesion during mitosis, these two mechanisms may also be concomitantly at work during DNA repair. One of the first signaling events following DSB is the phosphorylation of H2AX (in mammals) to form γH2AX (116). Arnould et al. (112) observed that both cohesin and the ATM kinase remain bound at the DSB, yet the γ-H2AX mark was enriched up to 1 Mbp away from the DSB within a CTCF-bound TAD boundary. Given the observation that components of the DNA repair machinery block cohesin progression in vivo (117), the authors proposed that cohesin is stalled at DSBs and extrudes loops only on one side, which also generates the stripe pattern that has been observed around CTCF sites (**Figure 4*d***). Based on these observations, the authors argued that cohesin's one-sided loop-extruding activity is necessary to reel DNA close to the DSB, while the ATM kinase deposits the γ-H2AX mark on the extruded DNA. Mechanistic details and whether the ATM kinase can directly interact with cohesin (rather than spreading from nucleosome to nucleosome) remain unknown. It is, however, also conceivable that, at least in part, SMC5/6 takes over the loop-extruding function at DSBs. The finding that SMC5/6 is a symmetrical loop extruder with two independent motors (118) indicates that it may potentially bridge the DSB, consistent with an equal spreading of γH2AX on both sides of the DSB. However, for now, the roles of DNA loop extrusion and cohesion by cohesin and SMC5/6 in DNA repair remain barely understood.

Another important biological process where loop extrusion plays a crucial role is the assembly of antigen receptor genes by V(D)J recombination in B and T cells of the vertebrate immune system (for a detailed review, see 119).

3. BIOPHYSICS OF DNA LOOP EXTRUSION BY SMCS

3.1. Single-Molecule Visualization of Loop Extrusion by SMC Complexes

Despite extensive evidence for DNA loop extrusion by condensin and cohesin, the high complexity of the cellular environment, the existence of a plethora of interacting partners, and their dissimilarity to any other characterized DNA translocases posed considerable barriers to identifying the molecular functions of these complexes. Biochemical and biophysical in vitro analyses of the isolated complexes were therefore performed to elucidate the protein activities. In early years, inspired by the discovery that cohesin holds sister chromatids in mitotic chromosomes, as well as by the fact that the ATPase hydrolysis rate is very low, SMC complexes were often thought of as static chromatid linker molecules rather than active loop extruders. The first ATP-dependent activity of purified condensin to be observed in in vitro biochemistry analysis was that 13S condensin in *Xenopus laevis* egg extracts introduced positive supercoils into a circular DNA in the presence of TOP I (120, 121), a finding recently supported by single-molecule experiments (122). The possibility that condensin could actively compact DNA in an ATP-dependent fashion first emerged from single-molecule magnetic tweezer experiments with condensin I from *Xenopus laevis* eggs (123) and from budding yeast (124).

Evidence that condensin is a motor protein was obtained using single-molecule imaging, which showed that individual fluorescently labeled budding yeast condensins translocated unidirectionally along stretched and surface-anchored fluorescently labeled DNA in an ATP-dependent

manner (125). Soon thereafter, the first direct evidence that condensin is a DNA-loop-extruding motor was provided using another single-molecule visualization assay (21). In this breakthrough experiment, a DNA molecule was tethered at its two ends in a loose configuration such that the tension within the molecule was low. Upon stretching the DNA by applying a sideways liquid flow, the DNA was visualized, and a fluorescently labeled condensin could be observed to rapidly reel DNA into a loop upon ATP hydrolysis (21). These real-time visualization experiments unambiguously proved that a single condensin complex can extrude a large (tens of kilobase pairs) loop of DNA. Loop extrusion was found to occur in an asymmetric manner in which DNA was reeled into the loop from only one side, while the other side of the DNA was anchored at the safety belt of the HEAT II Ycg1–kleisin subunits of condensin. Condensin was found to be a very fast motor, with DNA loop extrusion occurring at speeds up to 1.5 kbp/s, but also a weak motor, as a tension of less than 1 pN on the DNA that was reeled in was sufficient to stall the motor action.

Unlike condensin, early in vitro single-molecule studies of cohesin focused on the ATP-independent diffusion of human (126) and *Saccharomyces pombe* cohesin (127) along DNA. These experiments showed that cohesin can topologically load onto DNA and diffuse along the strand, even passing over some DNA-bound proteins (EcoRI, dCas9, TetR), but not across transcribing RNA polymerase (RNAp) or CTCF. Direct evidence that cohesin is able to drive DNA loop extrusion was provided using human cohesin with a similar assay previously employed for visualizing condensin-mediated loop extrusion (95, 128). These experiments revealed that human cohesin requires NIPBL–MAU2, the HEAT I subunit of cohesin that acts as a cohesin loader onto chromatin, not only for initiating but also for maintaining ongoing loop extrusion (95). Based on the structural similarities among SMC complexes, it seems plausible that loop extrusion activity might not be a function limited to condensin and cohesin but might be a universal feature shared by all SMC complexes. Indeed, in vitro magnetic tweezer experiments showed that SMC5/6 compacts DNA in an ATP-dependent manner under low tension (129), resembling ATP-dependent compaction by condensin (123, 124). Recently, DNA loop extrusion induced by the budding yeast SMC5/6 complex was observed (118). The study showed that Nse5/6, the subunits specific only to the SMC5/6 complex, negatively regulates DNA loop extrusion by reducing loop initiation.

Real-time imaging of the DNA loop extrusion process by purified SMC complexes has proven to be a powerful approach as it not only allows the direct visualization of the extrusion process but also provides mechanistic insight and enables quantitative estimations of the properties of the loop extrusion at the single-molecule level. In Sections 3.1.1.–3.1.5., we discuss several important characteristics of loop extrusion that were revealed by in vitro single-molecule experiments.

3.1.1. Loop extrusion rate. Ganji et al. (21) observed that yeast condensin reels DNA into a loop at speeds of up to 1.5 kbp/s, with an average of 0.6 kbp/s. Notably, this is a very fast rate that exceeds the speed of other DNA translocases (such as helicase or polymerases) by orders of magnitude, indicating that these SMC complexes constitute a unique new class of motor proteins. Similar values were also found for different SMC complexes, e.g., for human cohesin [0.5–1 kbp/s (95, 128)], for the cohesin from interphase *Xenopus* egg extract [2 kbp/s (130)], for human condensin I and II [~1 kbp/s and 0.5 kbp/s, respectively (131)], and for yeast SMC5/6 [1 kbp/s (118)]. Given the fact that all of these purified SMC complexes exhibit similarly low ATP hydrolysis rates, i.e., 1 or a few ATP molecules per second, the length of DNA that the SMC complexes reel into loop per ATP hydrolysis cycle (i.e., the step size) is likely to be conserved across SMC complexes. In the case of condensin, single step sizes were recently measured to be ~20–45 nm, that is, up to 200 bp per step (132), roughly consistent with the estimated step sizes from the imaging experiments. Furthermore, it has not escaped our notice that the step size equals the size of the SMC complex, which immediately suggests that a complex-wide conformational change is involved in

the stepping. The rate of extrusion was also experimentally determined in vivo using Hi-C analyses. The rate of chromosome arm juxtaposition by bacterial SMC complexes in *B. subtilis* (32) and *Caulobacter crescentus* (133) was found to be 0.3–0.8 kbp/s, corroborating the range observed in single-molecule experiments. In eukaryotes, however, from matching Hi-C experiments to simulations, the DNA extrusion rates of cohesin and condensin were estimated to be lower, on the order of 0.1 kb/s (134), which may suggest that other factors, such as cellular crowding and roadblocks (e.g., DNA-bound CTCF and other proteins, transcription machinery, etc.), play a role in slowing down these motor proteins.

3.1.2. Stalling force. Another important property shared by all loop-extruding SMCs is that they stall if the tension on the DNA that is reeled in becomes too large. The growth of a loop thus saturates and reaches a plateau when extrusion is performed on double-tethered DNA substrates as more and more DNA is transferred to the loop and tension within the non-extruded DNA inevitably increases (21). The stalling force of SMC complexes is low, subpiconewton, which is much lower than that of most other DNA-processing enzymes (135). The estimated stalling forces for different SMC complexes exhibit similar values: 0.4–1 pN for condensin (21, 132), 0.1–0.8 pN for cohesin (128, 130), and 0.5 pN for SMC5/6 (118).

3.1.3. Symmetry of loop extrusion. SMC complexes can reel DNA into the loop from one side or from both sides. Ganji et al. (21) first made the surprising observation that DNA loop extrusion by yeast condensin proceeds in a one-sided manner, i.e., asymmetrically. This posed a major theoretical challenge: Could one-sided extrusion perform the large variety of tasks that were thus far credited to two-sided extrusion (25, 28)? Experiments with a condensin mutant showed that robust one-sided extrusion depends on Ycg1–Brn1, which firmly anchors the condensin onto DNA (21, 136). Recent work showed that deletion of Ycg1 in *Chaetomium thermophilum* turned condensin from a one-sided to a two-sided motor (137). The physiological reason behind the strong asymmetry of condensin-driven loop extrusion in yeast cells is so far not well understood. Since human condensin I and II seem to be able to extrude loops in both a symmetric and asymmetric manner (131), asymmetric extrusion appears to be a weakly conserved feature among eukaryotic condensin complexes. Unlike condensin, cohesin and SMC5/6 were observed to extrude loops symmetrically in a two-sided manner. The differences in (a)symmetry may relate to the number of SMCs required for loop extrusion. Condensin was convincingly shown to act as a loop extruder as a single SMC complex. Cohesin likely also extrudes loops as a single complex (95). We hypothesize that two-sided extrusion by cohesin, at the microscopic level, may not occur simultaneously but rather in an alternative fashion in which a motor switches the sides of extrusion (R. Barth, I. Davidson, J.-M. Peters, C. Dekker, unpublished data). In contrast, SMC5/6 extrude loops as dimers (118), and these dimers may extrude DNA symmetrically by the two motor complexes independently translocating along DNA, thus reeling in DNA simultaneously. It remains to be seen whether dimeric loop extrusion is a shared characteristic for all kleisin interacting winged-helix tandem element (Kite)-based SMC proteins (i.e., the SMC5/6 and bacterial SMC complexes). Importantly, analysis of DNA loop formation in bacteria suggested that bacterial SMC complexes are also dimeric extruders (32).

3.1.4. Topology of SMCs and DNA. Since the geometry of SMC proteins is a ring, the specific topology of how SMC proteins interact with DNA has long been actively investigated. In vivo chemical cross-linking (44) and in vitro biochemical studies (44, 129, 138) suggested that SMC complexes can topologically load onto DNA, or in other words, a DNA molecule can be encircled by the trimeric SMC–kleisin ring. It was suggested that the entry of the DNA would be achieved by opening of the hinge, while the exit gate would be the SMC–kleisin interface

(139, 140). A recent study from Shaltiel et al. (137), however, showed that DNA remained bound when incubated with a fusion complex made by internal covalent linkage of the trimeric ring of condensin, and the DNA would reside within the two chambers created by the interface of the kleisin and the two HEAT-repeat proteins associated with kleisins (Hawk) domains, thus remaining as a pseudotopological configuration within the trimeric ring. It thus appears possible that the traditional immunoprecipitation method, in combination with a high salt wash, does not discriminate between pseudotopological and topological loading of SMCs onto DNA, casting some doubt on early claims of a strictly topological entrapment of DNA by SMC rings.

The link between topological loading of SMCs and their loop extrusion activity has long remained unclear. Recent work from Davidson et al. (95) and Shaltiel et al. (137) provided clues in this direction. They used cohesin and condensin in which the trimeric core ring complexes were covalently linked and showed that these complexes were, remarkably, still able to extrude DNA loops, thereby excluding the possibility that topological loading is required for loop extrusion. Recent experiments from Pradhan et al. (141) furthermore showed that the covalently linked cohesin can also accommodate DNA-bound obstacles that are much larger than the ring size into the extruded loop, thus challenging topological and pseudotopological mechanisms of loop extrusion and instead indicating a nontopological mechanism.

3.1.5. Conformational states of SMCs. Since the loop extrusion function of SMC complexes was discovered, a major open question has been to understand how these proteins extrude DNA loops at the mechanistic level. To this end, it is critical to identify the conformational states of SMC complexes and how these conformations link to one another during loop extrusion. Recently, many high-resolution cryo–electron microscopy (cryo-EM) structures of SMCs became available in different states of the ATP-hydrolysis cycle, and the dynamics of the conformational transitions of SMCs were captured with high-speed atomic force microscopy (AFM) and fluorescence resonance energy transfer (FRET), all providing clues for resolving the mechanism of loop extrusion by SMCs (for a recent review on SMC structures and conformations, see 142).

Recent structural analyses of SMCs captured in the ATP-free (or apo) and ATP/DNA-bound state reveal striking structural similarities across different SMC complexes. In the apo state, SMCs are in a folded conformation in which the juxtaposed SMC coiled-coil arms are folded toward an ATPase head (111–115), with which the HEAT I (112, 113) and Kite (114, 116) domains are predominantly associated. In the case of condensin, another apo conformation, a so-called apo-bridged state, was reported in which two SMC ATPase heads are separated by a large distance and bridged by the HEAT I domain (112). Upon ATP binding, the two SMC ATPase heads do engage by sandwiching two ATP molecules, which appears to induce a conformational change that allows stable clamping of DNA within the complex (28, 83, 105, 119–122). The SMC head engagement leads to a partial opening of the SMC arms, giving it a V-shape in which a DNA strand is bound above the engaged heads and below the HEAT I or Kite domains. In this clamped state, the DNA is sandwiched by the kleisin from above and the engaged heads from below.

Although a juxtaposed folded conformation of SMCs in the apo state was commonly reported in cryo-EM data, conformational changes of condensin (143) and cohesin (118) observed by AFM and FRET reveal that SMC arms can be well separated and exhibit dynamic transitions between open and closed states. For condensin, dynamic and flexible SMC arms were reported (143, 144), and the SMC complex appeared to toggle between an open state and a state in which the hinge collapsed onto the globular domain. Similarly, for cohesin, an alignment of coiled-coil arms and their bending toward the Smc3 head was reported, similar to the cryo-EM data (118). Upon ATP binding, condensin and cohesin seem to undergo different conformational transitions. While binding of ATP led to a folded or collapsed conformation of condensin (117), the

ATP-binding-induced head engagement was reported to change cohesin from a folded conformation to an open conformation (118).

4. MODELS OF LOOP EXTRUSION

While the field has not converged on a final understanding of the molecular gymnastics that underlie loop extrusion by SMC proteins, progress has been made toward establishing a model for DNA loop extrusion at the molecular level. A variety of different models have been proposed that all aim to account for the available data, including the experimental finding that steps in the DNA extrusion process involve very large conformational changes—of the order of the size of the SMC ring itself (132). Here we discuss recent models, which we categorize into three classes according to their shared characteristics (**Figure 5**).

4.1. The Scrunching/Swing-and-Clamp Model

Based on AFM observations of the large conformational transitions of condensin complexes between an extended open O shape to a collapsed butterfly B shape, triggered by the binding of ATP (132), a scrunching model was suggested for condensin-mediated loop extrusion (**Figure 5*a***). In this model, DNA binding occurs at the safety belt of HEAT II Ycg1–kleisin (136), as well as at a second binding site at the hinge. Upon ATP binding, the hinge-bound DNA is transferred to the ATPase head domains by the conformational change from the O to the B shape and bound there at a third DNA-binding site. Indeed, recent magnetic tweezer data confirmed that it is the ATP binding, rather than hydrolysis, that is associated with the actual step of drawing DNA into the extruded loop (132). Upon ATP hydrolysis, condensin returns to the O shape and stochastically captures a new DNA segment for the next step in the cycle.

Recent work from Bauer et al. (145) proposed a more detailed model of loop extrusion driven by human cohesin, the swing-and-clamp model, which shares similarities with the scrunching model. In this model, the DNA transfer is controlled by the DNA-loader HEAT I (NIPBL), which in the apo state binds at the DNA-bound hinge and upon ATP binding dissociates from the hinge and associates with the heads, while carrying DNA. The transfer of DNA-bound NIPBL from hinge to heads is achieved by the hinge swinging toward the SMC3 head in the apo state, whereupon the ATPase heads dimerize and DNA is clamped in between NIPBL and the engaged heads. The subsequent ATP hydrolysis leads to disengagement of the heads, disassembly of the clamp, and unfolding of the SMC elbows, thus bringing the hinge back to the original position and getting the motor ready for the next cycle. While the swing-and-clamp model may explain DNA translocation, it is not fully clear thus far how this would lead to loop extrusion, which may involve a coordinated action with STAG1/2. Notably, the swing-and-clamp mechanism does not agree with data from yeast condensin, in which ATP binding was found to trigger the conformational change from the open to the collapsed shape (132). For both the scrunching model and the swing-and-clamp model, it remains unclear how a mechanism that involves random grabbing of nearby DNA by the hinge would provide a sustained directionality to the loop extrusion.

4.2. The Brownian Ratchet Model

This model is based on the recently resolved DNA-gripping state from fission yeast cohesin (146, 147). This model suggests that a Brownian ratchet is built from the Scc3–hinge and Scc2–head modules, which are juxtaposed during the ATP-bound state but allow unidirectional DNA diffusion upon ATP hydrolysis (**Figure 5*b***). According to this Brownian ratchet model, DNA arrives in the gripping state without passing the kleisin N-terminal gate, which prevents DNA passage upon the subsequent ATP-hydrolysis-induced ATPase head disengagement. The stochastic Brownian

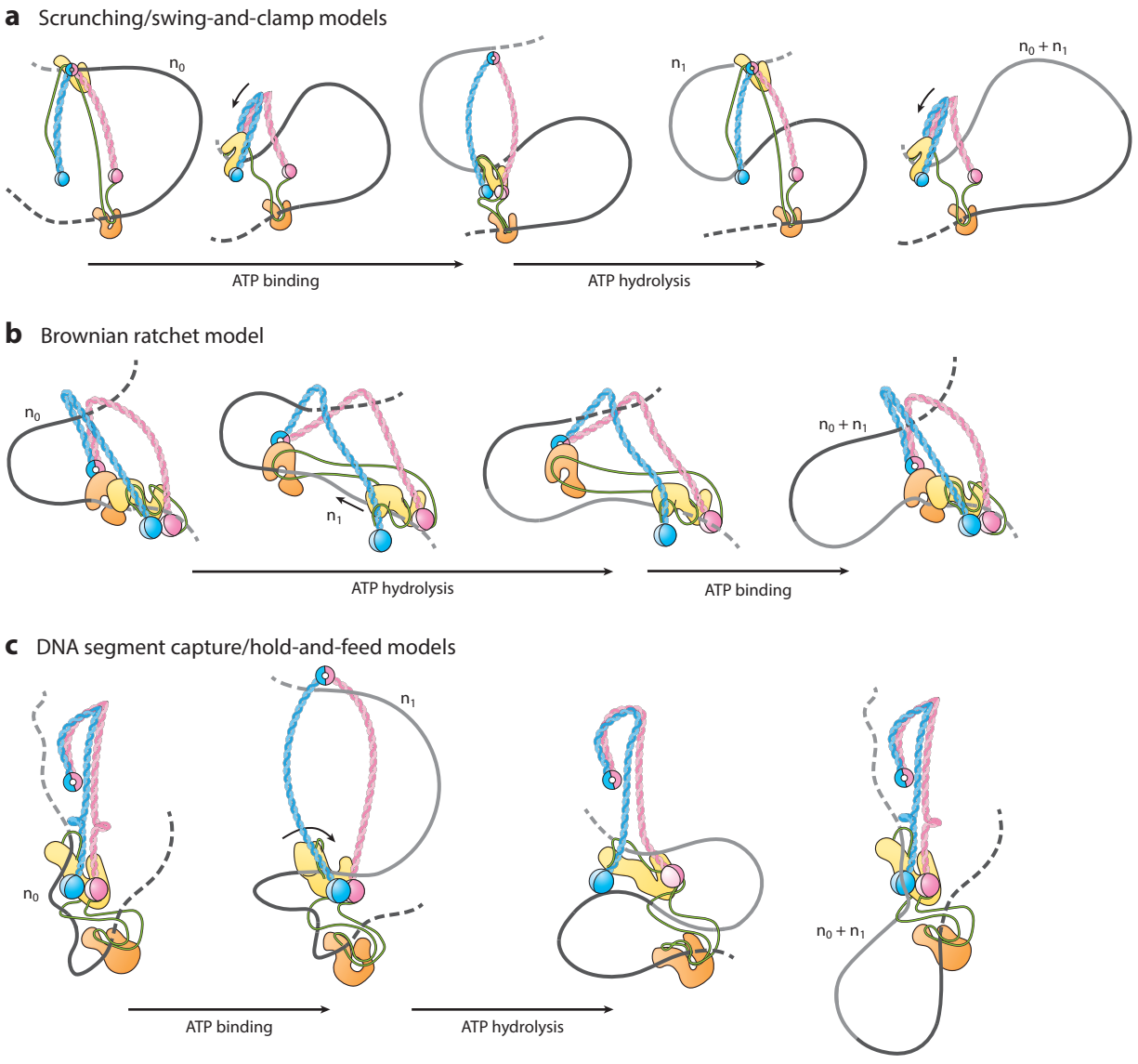


Figure 5

Loop extrusion models. (*a*) The scrunching/swing-and-clamp model. The SMC complex binds to DNA at the HEAT I (located near the hinge) and HEAT II subunits. Folding of the SMCs and a subsequent ATP head–engaged open conformation allow the transfer of a DNA segment between the hinge and the heads, thereby enlarging a loop. (*b*) The Brownian ratchet model. Upon ATP binding, SMC complexes adopt a gripping state in which DNA is pseudotopologically loaded. A swinging motion of the HEAT I domain, driven by Brownian motion, turns the DNA bend into a loop. (*c*) The DNA segment capture/hold-and-feed model. A DNA loop is initially captured by the interfaces of the kleisin and HEAT domains. ATP binding results in a power-stroke tilting motion of the HEAT I–kleisin chamber, allowing the complex to capture new DNA into the SMC ring. Upon ATP hydrolysis, the opening of the ATPase heads and the folding or zipping-up action of the SMC arms allow the transfer of the new DNA into the previously established loop. The DNA trajectory is adopted from Oldenkamp & Rowland (142), which is compatible with all cross-links tested by Shaltiel et al. (137).

swinging motion of the Scc3-hinge module turns the DNA into a loop and is responsible for the subsequent loop growth. The low DNA affinity of the Scc3-hinge module results in DNA release, and the Scc3-hinge module subsequently returns to form a new DNA-gripping state upon ATP binding. This model, however, seems to be incompatible with some experimental observations, e.g., Scc2's association with the hinge in the apo state (145) and the deletion of Ycg1 (analogous to Scc2) in *C. thermophilum* condensin, which did not impair the loop extrusion activity of condensin (137). Furthermore, the Brownian ratchet model can explain only the passage of roadblocks that are smaller than the SMC lumen, while the passing of large roadblocks can be realized only by the opening of the SMC ring (146). However, it was recently shown that a covalently closed SMC ring is able to bypass roadblocks 200 nm in diameter (141).

4.3. The DNA Segment Capture/Hold-and-Feed Model

The DNA segment capture model (148, 149) was inspired by features of prokaryotic SMC complexes in which the conformation of SMC–ScpAB complexes transitions between the ATP head–disengaged juxtaposed and head-engaged opened states (150). The model assumes that, in the apo state, a small loop of DNA is captured in the lower compartment created in between the juxtaposed SMC arms and the kleisin (**Figure 5*c***). Upon ATP binding, the opening of the SMC coiled-coil arms enables another loop to be captured in the upper compartment created within the head-dimerized SMC arms. Subsequent ATP hydrolysis and the associated opening of the ATPase heads merges the two compartments, followed by the zipping-up action of the SMC arms, which allows the transfer of the DNA loop within the upper compartment to the lower compartment. When iterated, this reaction extrudes a loop. A recent modification of this model (149) combines the loop capture in the upper compartment with an ATP-induced power stroke by the folding kleisin that feeds a DNA loop into the SMC ring. This is inspired by recent work by Shaltiel et al. (137), who proposed a hold-and-feed mechanism, which exhibits large similarities to the DNA segment capture model (137). This model specifies that a small loop is captured in the apo state by the kleisin–Ycs4 and kleisin–Ycg1 chambers. ATP binding–induced head engagement enables the power-stroke tilting motion of the Ycs4–kleisin chamber and swings the DNA within the chambers to allow the capture of new DNA into the SMC ring. Subsequent ATP hydrolysis and head disengagement changes the complex to the apo-bridged conformation (151), allowing the merging of the new loop into the lower compartment. At the start of the next cycle, the Ycs4–kleisin chamber is tilted back to the apo conformation together with a new segment of DNA to enlarge the loop. Although many aspects of this model are consistent with experimental observations and simulations (149), some predictions of this model regarding obstacle bypass remain inconsistent with experimental observations (152), and the mechanism of capturing a DNA loop into the upper compartment in each cycle and feeding it to the existing loop remains somewhat speculative. It appears that each of the models so far can explain a subset of the experimental data but not all of them, and a fully satisfying model has not yet been achieved. Oldenkamp & Rowland (142) recently provided a nontopological variant of the hold-and-feed model that is consistent with all cross-linking data by Shaltiel et al. (137), as well as obstacle bypass experiments (141) (**Figure 5*c***).

5. FROM NAKED DNA TO CHROMATIN

The in vitro studies demonstrating DNA loop extrusion by condensin, cohesin, and SMC5/6 were all performed on bare DNA (21, 95, 118, 128, 131). Of crucial importance, a direct translation of the DNA loop extrusion mechanism to in vivo systems is not straightforward, as chromatin is decorated by a plethora of DNA-interacting proteins. While Hi-C data provide strong evidence for loop extrusion in vivo, it is not immediately clear how the various loop extruders interact with the DNA-binding proteins that putatively act as roadblocks for loop extrusion. In the remainder of

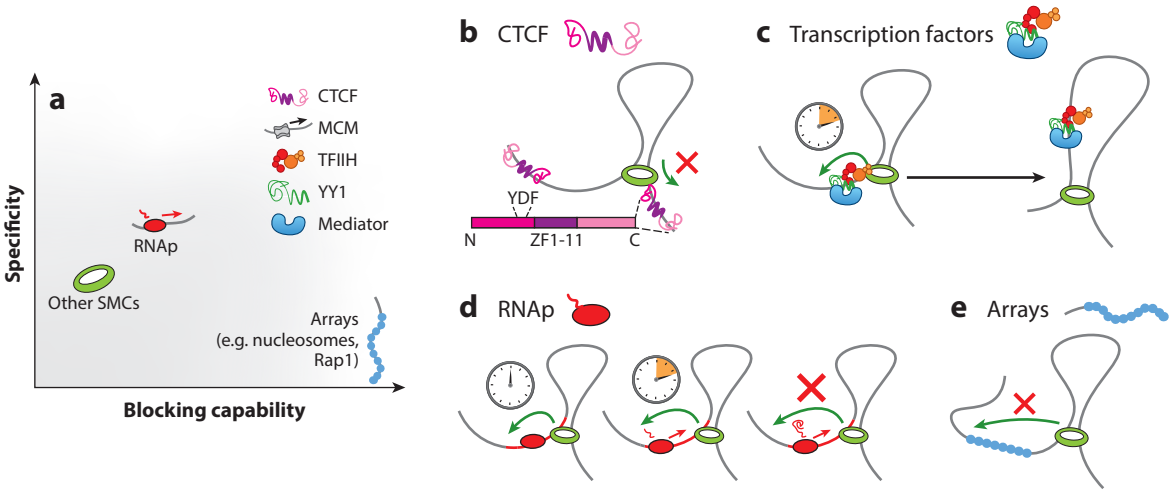


Figure 6

Loop extrusion on chromatin. (*a*) DNA-binding roadblocks characterized according to the specificity of their interaction with SMCs and their expected ability to block loop extrusion. (*b*) CTCF interacts with cohesin primarily (though not exclusively) with its YDF motif, located on the N terminus. (*c*) Transcription factors such as TFIIH, YY1, and Mediator may temporarily stall loop extrusion, biasing interactions between regulatory sites. (*d*) While promoter-bound RNAp does not constitute a strong roadblock to DNA loop extrusion, it may become progressively more effective in blocking loop extrusion upon transcription, as SMCs may interact with the RNA transcript. (*e*) A dense array of closely packed DNA-binding proteins may constitute a roadblock to loop extrusion if this protein binding induces a stiffening of the DNA. Abbreviations: RNAp, RNA polymerase; SMC, structural maintenance of chromosomes, YY1, Yin Yang 1; ZF, zinc finger domain.

this section, we discuss some frequently occurring DNA-binding proteins and sort them into two categories (**Figure 6*a***): (*a*) roadblocks that specifically interact with the SMC through biochemical interactions [e.g., CTCF, MCM3, TFIIH, Yin Yang 1 (YY1), Mediator] and (*b*) physical roadblocks (e.g., RNA and RNAp, nucleosomes, arrays of DNA binding proteins such as RAP1, other SMCs).

The best-studied and most prominent interaction partner of cohesin is CTCF (71, 153). Loop extrusion by cohesin that is stalled by convergently oriented CTCF molecules (67, 77, 81, 154) gives rise to the appearance of TADs (66, 67). An evolutionarily conserved surface formed by STAG1/2 and kleisin specifically interacts with three N-terminally located amino acids, YxF, on CTCF (51) (**Figure 6*b***). In vivo deletion experiments suggest, however, that more than these three amino acids are responsible for the interaction of cohesin and CTCF (155) and the genomic insulation at CTCF sites (156). Recent estimates from live-cell tracking of loci adjacent to TAD boundaries suggest that wildtype CTCF is an imperfect roadblock to loop extrusion (83, 157). These results are supported by recent in vitro experiments showing that CTCF's ability to block cohesin's loop extrusion depends strongly on DNA tension and that cohesin–CTCF encounters can result in loop extrusion direction reversal and stably bound loops, as well as loop slippage, all of which are dependent on DNA tension (158). For now, it remains incompletely understood how binding of CTCF's YxF motif to cohesin is modulated and how that binding interferes with its loop-extruding activity. Some other proteins also contain the YxF motif, e.g., human MCM3 (which is part of the replication machinery) and TFIIH (part of the transcription machinery). While to date no known functional association between cohesin and TFIIH has been reported, MCM3 was indirectly shown to block DNA loop extrusion by noting that CTCF-anchored loops gained intensity upon depletion of MCM3, similar but independent from the WAPL depletion phenotype (106). Single-molecule assays showed that diffusion of DNA-bound cohesin is indeed

severely impeded by MCM3 and the YxF motif. However, loop extrusion stalling by MCM could not be measured in vitro.

Beyond CTCF, other factors also modulate SMCs. Intriguingly, work in *B. subtilis* has identified the site-specific recombinase XerD as an SMC unloader (159), while MatP may have this role in *E. coli* (36). Like CTCF, XerD bound to specific DNA elements limits the extent of loop extrusion in vivo, although in this case, it appears SMC is actively unloaded from the chromosome. Biophysical characterization of this novel unloader requires the development of an in vitro assay for the bacterial SMC complex. Furthermore, Mediator has been reported to interact with both cohesin (160, 161) and NIPBL–Mau2 (160, 162), potentially as a means of forming specific DNA loops between Mediator-bound promoters and enhancers. Similarly, Yin Yang 1 (YY1) preferentially occupies enhancers and promoters, and enhancer–promoter interactions are lost upon YY1 depletion (163, 164). However, simple DNA binding of factors such as Mediator or YY1 is not likely to be sufficient to establish contacts between elements located up to several hundred kilobase pairs apart. Cohesin could loop the intervening space and, if stalled at regulatory elements, facilitate the contact, which could thereafter be maintained by the dimerization of transcription factors (**Figure 4*a***). An interaction between YY1 and cohesin, and also condensin, has been reported (165), supporting this view. If enhancer–promoter contacts need to be established, this might occur only infrequently, e.g., only upon external stimuli, and once established, they could be maintained by various factors without cohesin (**Figure 6*c***). These factors might cause such interactions to be nearly invisible in population-averaging mapping methods, whereas single-molecule in vitro experiments have the ability to show whether and to what extent such an association exists.

Next, we describe some proteins that do not feature a biochemical interaction with SMCs but may act as physical roadblocks for loop extrusion. For example, elongating RNAps have been suggested to interact with cohesin. In particular, the notion that cohesin is found in islands between two convergently oriented genes led to the idea that active transcription can push intragenic cohesin toward transcription termination sites (**Figure 6*d***) (126, 166).

This pushing seems to impact not only passively bound cohesin but also loop-extruding cohesins, as evidenced by the fact that relocation of cohesin by transcription is accompanied by formation of new cis contacts (167, 168).

Computational modeling of Hi-C maps from bacteria (169) and humans (168) suggests that active transcription affects the DNA-looping pattern around genes, as loop extruders are temporarily stalled at elongating RNAp, where the stalling time depends on the direction of loop extrusion with respect to the direction of transcription. This may present a mechanism by which contacts between regulatory elements and RNAp can be maintained during elongation and termination (170, 171).

The first single-molecule experiments showed that transcribing RNAp can block diffusing cohesin (126). However, loop-extruding yeast condensin overcomes promoter-bound RNAp with ease (141). This result calls into question the efficiency of RNAp as an interacting partner to SMC proteins. But in vitro studies have so far investigated only loop extrusion encounters with inactive promoter-bound RNAp, and actively transcribing RNAp might resemble the in vivo scenario closer. In fact, it might be the RNA transcript rather than the RNAp that interacts with cohesin and thereby stalls loop extrusion, and significant blocking may, in fact, be observed only once the RNA transcripts grow to a sizeable length (**Figure 6*d***). This hypothesis is supported by the fact that the cohesin subunits STAG1/2 are RNA-binding proteins (172). Furthermore, SMC proteins may encounter each other on the same DNA during loop extrusion. For example, the bacterium *B. subtilis* carries eight parS sites within ~800 kbp around its origin of replication, onto which BsSMC loads with the help of ParB (173, 174), and SMC–SMC encounters are likely

frequent. Single-molecule experiments involving two loop-extruding condensin complexes showed that condensins can pass each other and form a double loop, a new loop motif that was called a Z-loop (175). Loop-extruding SMCs loaded at these clustered parS sites are thus able to traverse one another upon encounter (173, 174). Furthermore, simulations have shown that, even though yeast condensin is a one-sided loop extruder (21), the traversal of condensins can rescue the formation of mitotic chromosomes by allowing further compaction than one-sided extruders without traversal would allow (176).

The bypassing of nucleosomes by DNA loop extruders is also crucial for support of the loop-extrusion hypothesis in vivo. As expected, condensin from humans and yeast indeed incorporates nucleosomes into compacted extruded DNA loops (131, 141). While DNA loop extrusion is not considerably affected by single nucleosomes, it is conceivable that dense arrays of nucleosomes may prohibit cohesin binding (177), and stiffening of nucleosome arrays of nucleosomes by histone tail acetylation (178, 179) may stall loop extrusion (**Figure 6*e***). An analogous phenomenon may be exploited in *S. cerevisiae* to resolve accidental fusions of telomeres. Stalling of loop extrusion due to an array of closely packed Rap1-binding sites at the fusion may localize it to the abscission site upon cell division (180). Even though condensin can take steps of up to several hundred base pairs, it is conceivable that a stiff segment of DNA may halt loop extrusion if the Rap1-bound array is longer than the step size in physical space (i.e., ∼40 nm) (132) or when Rap1 binding causes stiffening (181) such that the bending energy of the Rap1-bound DNA segment cannot be overcome (137, 148) (B. Analikwu, R. Barth, A. Deshayes, A. Katan, J. van der Torre, S. Marcand, C. Dekker, unpublished data).

Unexpectedly, it appears quite difficult to rigorously stop SMCs since there is, to date, no known impermeable roadblock to DNA loop extrusion. Even the biochemical interactions between cohesin and CTCF's YxF motif have a dissociation constant in the micromolar range (51), implying that CTCF is also an imperfect roadblock. We hypothesize that closely spaced arrays of DNA-bound proteins may be the sole roadblock with the capability to completely block loop extrusion.

6. CONCLUSION AND OUTLOOK

The looping of the genome by SMC complexes through DNA loop extrusion is an old idea that was rejuvenated by in vivo Hi-C experiments. Single-molecule demonstrations of DNA loop extrusion (21, 95, 128) provided the key in vitro evidence and have enabled molecular insights into the underlying mechanism. These studies have demonstrated the ability to quantify the loop extrusion, as well as the interaction kinetics between SMC complexes and potential roadblocks. This also informs simulations that use the observed kinetics of in vitro experiments to resemble in vivo data more closely. While initial single-molecule experiments used model substrates of naked DNA, recent in vitro work has been designed to approach more complicated molecular constructs, e.g., by incorporation of potential roadblocks (106, 131, 141) or deletion experiments (137). This will allow some of the many open questions in the field to be addressed. One example concerns the heavily debated regulation of genes: the persistent question of whether enhancer–promoter contacts are necessary to induce transcription, and whether such contacts may be made by modulating loop extrusion around regulatory sites. Another outstanding challenge is to reconstitute loop extrusion by bacterial and archaeal SMC complexes to investigate the similarities and differences in the underlying mechanism of loop formation. Questions regarding the roles of SMC proteins in replication and DNA repair are starting to emerge only now. Unfolding the molecular mechanisms governing DNA loop extrusion will not only contribute to our understanding of its role throughout the cell cycle but eventually also shed light on the causes of associated diseases such as cohesinopathies (182) and offer potential diagnosis and treatment targets.

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AUTHOR CONTRIBUTIONS

All authors developed the ideas, compiled the information, and wrote the review together. All authors have read and agreed to the published version of the manuscript.

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LITERATURE CITED

1. Pellicer J, Fay MF, Leitch IJ. 2010. The largest eukaryotic genome of them all? *Botanical J. Linnean Soc.* 164(1):10–15
2. Bennett GM, Moran NA. 2013. Small, smaller, smallest: the origins and evolution of ancient dual symbioses in a phloem-feeding insect. *Genome Biol. Evol.* 5(9):1675–88
3. Petrushenko ZM, She W, Rybenkov V. 2011. A new family of bacterial condensins. *Mol. Microbiol.* 81(4):881–96
4. Flemming Z. 1882. *Zellsubstanz, Kern und Zellteilung*. Leipzig, Ger.: F.C.W. Vogel
5. Kossel A. 1879. Ueber die chemische Zusammensetzung der Peptone. *Biol. Chem.* 3(1–2):58–62
6. Paulson JR, Laemmli UK. 1977. The structure of histone-depleted metaphase chromosomes. *Cell* 12(3):817–28
7. Leidescher S, Ribisel J, Ullrich S, Feodorova Y, Hildebrand E, et al. 2022. Spatial organization of transcribed eukaryotic genes. *Nat. Cell Biol.* 24(3):327–39
8. MacGregor H. 2013. Lampbrush chromosomes. In *Brenner's Encyclopedia of Genetics*, ed. S Maloy, K Hughes, pp. 190–94. Berlin, Heidelberg: Springer Berlin Heidelberg. 2nd ed.
9. Englesberg E, Squires C, Meronk F. 1969. The L-arabinose operon in *Escherichia coli* B/r: a genetic demonstration of two functional states of the product of a regulator gene. *PNAS* 62(4):1100–7
10. Müller HP, Sogo JM, Schaffner W. 1989. An enhancer stimulates transcription in trans when attached to the promoter via a protein bridge. *Cell* 58(4):767–77
11. Wood C, Tonegawa S. 1983. Diversity and joining segments of mouse immunoglobulin heavy chain genes are closely linked and in the same orientation: implications for the joining mechanism. *PNAS* 80(10):3030–34
12. Matthews KS. 1992. DNA looping. *Microbiol. Rev.* 56(1):123–36
13. Mirny LA. 2011. The fractal globule as a model of chromatin architecture in the cell. *Chromosome Res.* 19(1):37–51
14. Studier FW, Bandyopadhyay PK. 1988. Model for how type I restriction enzymes select cleavage sites in DNA. *PNAS* 85(13):4677–81
15. Riggs AD. 1990. DNA methylation and late replication probably aid cell memory, and type I DNA reeling could aid chromosome folding and enhancer function. *Philos. Trans. R. Soc. B* 326(1235):285–97
16. Seidel R, van Noort J, van der Scheer C, Bloom JGP, Dekker NH, et al. 2004. Real-time observation of DNA translocation by the type I restriction modification enzyme EcoR124I. *Nat. Struct. Mol. Biol.* 11(9):838–43
17. Larionov VL, Karpova TS, Kouprina NY, Jouravleva GA. 1985. A mutant of *Saccharomyces cerevisiae* with impaired maintenance of centromeric plasmids. *Curr. Genet.* 10(1):15–20

18. Strunnikov AV, Larionov VL, Koshland D. 1993. SMC1: An essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. *J. Cell Biol.* 123(6):1635–48
19. Wang X, Le TBK, Lajoie BR, Dekker J, Laub MT, Rudner DZ. 2015. Condensin promotes the juxtaposition of DNA flanking its loading site in *Bacillus subtilis*. *Genes Dev*. 29(15):1661–75
20. Sanborn AL, Rao SSP, Huang SC, Durand NC, Huntley MH, et al. 2015. Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *PNAS* 112(47):E6456–65
21. Ganji M, Shaltiel IA, Bisht S, Kim E, Kalichava A, et al. 2018. Real-time imaging of DNA loop extrusion by condensin. *Science* 360(6384):102–5
22. Rattner JB, Lin CC. 1985. Radial loops and helical coils coexist in metaphase chromosomes. *Cell* 42(1):291–96
23. Saitoh N, Goldberg IG, Wood ER, Earnshaw WC. 1994. ScII: An abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J. Cell Biol.* 127(2):303–18
24. Alipour E, Marko JF. 2012. Self-organization of domain structures by DNA-loop-extruding enzymes. *Nucleic Acids Res*. 40(22):11202–12
25. Goloborodko A, Imakaev MV, Marko JF, Mirny L. 2016. Compaction and segregation of sister chromatids via active loop extrusion. *eLife* 5:e14864
26. Hirota T, Gerlich D, Koch B, Ellenberg J, Peters JM. 2004. Distinct functions of condensin I and II in mitotic chromosome assembly. *J. Cell Sci.* 117(26):6435–45
27. Ono T, Losada A, Hirano M, Myers MP, Neuwald AF, Hirano T. 2003. Differential contributions of condensin I and condensin II to mitotic chromosome architecture in vertebrate cells. *Cell* 115(1):109–21
28. Gibcus JH, Samejima K, Goloborodko A, Samejima I, Naumova N, et al. 2018. A pathway for mitotic chromosome formation. *Science* 359(6376):eaao6135
29. Walther N, Hossain MJ, Politi AZ, Koch B, Kueblbeck M, et al. 2018. A quantitative map of human condensins provides new insights into mitotic chromosome architecture. *J. Cell Biol.* 217(7):2309–28
30. Nasmyth K. 2001. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* 35:673–745
31. Gruber S, Errington J. 2009. Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in *B. subtilis*. *Cell* 137(4):685–96
32. Wang X, Brandão HB, Le TBK, Laub MT, Rudner DZ. 2017. *Bacillus subtilis* SMC complexes juxtapose chromosome arms as they travel from origin to terminus. *Science* 355(6324):524–27
33. Wang X, Hughes AC, Brandão HB, Walker B, Lierz C, et al. 2018. In vivo evidence for ATPase-dependent DNA translocation by the *Bacillus subtilis* SMC condensin complex. *Mol. Cell.* 71(5):841–47.e5
34. Jun S, Mulder B. 2006. Entropy-driven spatial organization of highly confined polymers: lessons for the bacterial chromosome. *PNAS* 103(33):12388–93
35. Gogou C, Japaridze A, Dekker C. 2021. Mechanisms for chromosome segregation in bacteria. *Front. Microbiol.* 12:685687
36. Nolivos S, Upton AL, Badrinarayanan A, Müller J, Zawadzka K, et al. 2016. MatP regulates the coordinated action of topoisomerase IV and MukBEF in chromosome segregation. *Nat. Commun.* 7(1):10466
37. Japaridze A, van Wee R, Gogou C, Kerssemakers JWJ, Dekker C. 2022. MukBEF-dependent chromosomal organization in widened *Escherichia coli*. bioRxiv 2022.07.13.499882. **https://doi.org/10.1101/2022.07.13.499882**
38. Mäkelä J, Sherratt DJ. 2020. Organization of the *Escherichia coli* chromosome by a MukBEF axial core. *Mol. Cell.* 78(2):250–60.e5
39. Srinivasan M, Fumasoni M, Petela NJ, Murray A, Nasmyth KA. 2020. Cohesion is established during DNA replication utilising chromosome associated cohesin rings as well as those loaded de novo onto nascent DNAs. *eLife* 9:e56611
40. Zheng G, Kanchwala M, Xing C, Yu H. 2018. MCM2–7-dependent cohesin loading during S phase promotes sister-chromatid cohesion. *eLife* 7:e33920

41. Takahashi TS, Yiu P, Chou MF, Gygi S, Walter JC. 2004. Recruitment of *Xenopus* Scc2 and cohesin to chromatin requires the pre-replication complex. *Nat. Cell Biol.* 6(10):991–96
42. Takahashi TS, Basu A, Bermudez V, Hurwitz J, Walter JC. 2008. Cdc7–Drf1 kinase links chromosome cohesion to the initiation of DNA replication in *Xenopus* egg extracts. *Genes Dev.* 22(14):1894–905
43. Gruber S, Haering CH, Nasmyth K. 2003. Chromosomal cohesin forms a ring. *Cell* 112(6):765–77
44. Haering CH, Farcas AM, Arumugam P, Metson J, Nasmyth K. 2008. The cohesin ring concatenates sister DNA molecules. *Nature* 454(7202):297–301
45. Peters J-M, Nishiyama T. 2012. Sister chromatid cohesion. *Cold Spring Harbor Perspect. Biol.* 4(11):a011130
46. Onn I, Heidinger-Pauli JM, Guacci V, Ünal E, Koshland DE. 2008. Sister chromatid cohesion: a simple concept with a complex reality. *Annu. Rev. Cell Dev. Biol.* 24:105–29
47. Murayama Y, Samora CP, Kurokawa Y, Iwasaki H, Uhlmann F. 2018. Establishment of DNA-DNA interactions by the cohesin ring. *Cell* 172(3):465–77.e15
48. Gutierrez-Escribano P, Newton MD, Llauró A, Huber J, Tanasie L, et al. 2019. A conserved ATP- and Scc2/4-dependent activity for cohesin in tethering DNA molecules. *Sci. Adv.* 5(11):eaay6804
49. Nagasaka K, Davidson IF, Stocsits RR, Tang W, Wutz G, et al. 2022. Cohesin mediates DNA loop extrusion and sister chromatid cohesion by distinct mechanisms. bioRxiv 2022.09.23.509019. **https://doi.org/10.1101/2022.09.23.509019**
50. Liu Y, Dekker J. 2022. CTCF–CTCF loops and intra-TAD interactions show differential dependence on cohesin ring integrity. *Nat. Cell Biol.* 24:1516–27
51. Li Y, Haarhuis JHI, Sedeño Cacciatore Á, Oldenkamp R, van Ruiten MS, et al. 2020. The structural basis for cohesin–CTCF-anchored loops. *Nature* 578(7795):472–76
52. Cameron G, Gruszka D, Xie S, Nasmyth KA, Srinivasan M, Yardimci H. 2022. Sister chromatid cohesion establishment during DNA replication termination. bioRxiv 2022.09.15.508094. **https://doi.org/10.1101/2022.09.15.508094**
53. Ladurner R, Kreidl E, Ivanov MP, Ekker H, Idarraga-Amado MH, et al. 2016. Sororin actively maintains sister chromatid cohesion. *EMBO J.* 35(6):635–53
54. Nishiyama T, Ladurner R, Schmitz J, Kreidl E, Schleiffer A, et al. 2010. Sororin mediates sister chromatid cohesion by antagonizing Wapl. *Cell* 143(5):737–49
55. Higashi TL, Ikeda M, Tanaka H, Nakagawa T, Bando M, et al. 2012. The prereplication complex recruits XEco2 to chromatin to promote cohesin acetylation in *Xenopus* egg extracts. *Curr. Biol.* 22(11):977–88
56. Zhang J, Shi X, Li Y, Kim BJ, Jia J, et al. 2008. Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast. *Mol. Cell.* 31(1):143–51
57. Ben-Shahar TR, Heeger S, Lehane C, East P, Flynn H, et al. 2008. Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. *Science* 321(5888):563–66
58. Ünal E, Heidinger-Pauli JM, Kim W, Guacci V, Onn I, et al. 2008. A molecular determinant for the establishment of sister chromatid cohesion. *Science* 321(5888):566–69
59. Song J, Lafont A, Chen J, Wu FM, Shirahige K, Rankin S. 2012. Cohesin acetylation promotes sister chromatid cohesion only in association with the replication machinery. *J. Biol. Chem.* 287(41):34325–36
60. Kinoshita K, Tsubota Y, Tane S, Aizawa Y, Sakata R, et al. 2022. A loop extrusion-independent mechanism contributes to condensin I-mediated chromosome shaping. *J. Cell Biol.* 221(3):e202109016
61. Ryu JK, Bouchoux C, Liu HW, Kim E, Minamino M, et al. 2021. Bridging-induced phase separation induced by cohesin SMC protein complexes. *Sci. Adv.* 7(7):eabe5905
62. Cattoglio C, Pustova I, Walther N, Ho JJ, Hantsche-Grininger M, et al. 2019. Determining cellular CTCF and cohesin abundances to constrain 3D genome models. *eLife* 8:e40164
63. Eng T, Guacci V, Koshland D. 2015. Interallelic complementation provides functional evidence for cohesin–cohesin interactions on DNA. *Mol. Biol. Cell.* 26(23):4224–35
64. Srinivasan M, Scheinost JC, Petela NJ, Gligoris TG, Wissler M, et al. 2018. The cohesin ring uses its hinge to organize DNA using non-topological as well as topological mechanisms. *Cell* 173(6):1508–19.e18
65. Xiang S, Koshland D. 2021. Cohesin architecture and clustering in vivo. *eLife* 10:e62243
66. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, et al. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485(7398):376–80

67. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159(7):1665–80
68. Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, et al. 2009. Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus. *Nature* 460(7253):410–13
69. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, et al. 2012. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485(7398):381–85
70. Parelho V, Hadjur S, Spivakov M, Leleu M, Sauer S, et al. 2008. Cohesins functionally associate with CTCF on mammalian chromosome arms. *Cell* 132(3):422–33
71. Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, et al. 2008. Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature* 451(7180):796–801
72. Rao SSP, Huang SC, Glenn St Hilaire B, Engreitz JM, Perez EM, et al. 2017. Cohesin loss eliminates all loop domains. *Cell* 171(2):305–20.e24
73. Nora EP, Goloborodko A, Valton AL, Gibcus JH, Uebersohn A, et al. 2017. Targeted degradation of CTCF decouples local insulation of chromosome domains from genomic compartmentalization. *Cell* 169(5):930–44.e22
74. Gassler J, Brandão HB, Imakaev M, Flyamer IM, Ladstätter S, et al. 2017. A mechanism of cohesin-dependent loop extrusion organizes zygotic genome architecture. *EMBO J.* 36(24):3600–18
75. Schwarzer W, Abdennur N, Goloborodko A, Pekowska A, Fudenberg G, et al. 2017. Two independent modes of chromatin organization revealed by cohesin removal. *Nature* 551(7678):51–56
76. Wutz G, Várnai C, Nagasaka K, Cisneros DA, Stocsits RR, et al. 2017. Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins. *EMBO J.* 36(24):3573–99
77. de Wit E, Vos ESM, Holwerda SJB, Valdes-Quezada C, Verstegen MJAM, et al. 2015. CTCF binding polarity determines chromatin looping. *Mol. Cell.* 60(4):676–84
78. Fudenberg G, Imakaev M, Lu C, Goloborodko A, Abdennur N, Mirny LA. 2016. Formation of chromosomal domains by loop extrusion. *Cell Rep.* 15(9):2038–49
79. Nichols MH, Corces VG. 2015. A CTCF code for 3D genome architecture. *Cell.* 162(4):703–5
80. Tedeschi A, Wutz G, Huet S, Jaritz M, Wuensche A, et al. 2013. Wapl is an essential regulator of chromatin structure and chromosome segregation. *Nature* 501(7468):564–68
81. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, et al. 2015. Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. *Cell Rep.* 10(8):1297–309
82. Flyamer IM, Gassler J, Imakaev M, Brandão HB, Ulianov SV, et al. 2017. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. *Nature* 544(7648):110–14
83. Gabriele M, Brandão HB, Grosse-Holz S, Jha A, Dailey GM, et al. 2022. Dynamics of CTCF- and cohesin-mediated chromatin looping revealed by live-cell imaging. *Science* 376(6592):476–501
84. Beckwith K, Ødegård-Fougner Ø, Morero N, Barton C, Schueder F, et al. 2021. Visualization of loop extrusion by DNA nanoscale tracing in single human cells. bioRxiv 2021.04.12.439407. **https://www.biorxiv.org/content/10.1101/2021.04.12.439407v2**
85. Smith EM, Lajoie BR, Jain G, Dekker J. 2016. Invariant TAD boundaries constrain cell-type-specific looping interactions between promoters and distal elements around the CFTR locus. *Am. J. Hum. Genet.* 98(1):185–201
86. Spielmann M, Lupiáñez DG, Mundlos S. 2018. Structural variation in the 3D genome. *Nat. Rev. Genet.* 19(7):453–67
87. Zuin J, Roth G, Zhan Y, Cramard J, Redolfi J, et al. 2022. Nonlinear control of transcription through enhancer–promoter interactions. *Nature* 604(7906):571–77
88. Doyle B, Fudenberg G, Imakaev M, Mirny LA. 2014. Chromatin loops as allosteric modulators of enhancer-promoter interactions. *PLOS Comput. Biol.* 10(10):e1003867
89. Symmons O, Pan L, Remeseiro S, Aktas T, Klein F, et al. 2016. The Shh topological domain facilitates the action of remote enhancers by reducing the effects of genomic distances. *Dev. Cell.* 39(5):529–43
90. Krefting J, Andrade-Navarro MA, Ibn-Salem J. 2018. Evolutionary stability of topologically associating domains is associated with conserved gene regulation. *BMC Biol.* 16(1):87

91. Ghavi-Helm Y, Jankowski A, Meiers S, Viales RR, Korbel JO, Furlong EEM. 2019. Highly rearranged chromosomes reveal uncoupling between genome topology and gene expression. *Nat. Genet.* 51(8):1272–82
92. Williamson I, Kane L, Devenney PS, Flyamer IM, Anderson E, et al. 2019. Developmentally regulated Shh expression is robust to TAD perturbations. *Development* 146(19):dev179523
93. Alexander JM, Guan J, Li B, Maliskova L, Song M, et al. 2019. Live-cell imaging reveals enhancer-dependent *Sox2* transcription in the absence of enhancer proximity. *eLife* 8:e41769
94. Benabdallah NS, Williamson I, Illingworth RS, Kane L, Boyle S, et al. 2019. Decreased enhancer-promoter proximity accompanying enhancer activation. *Mol. Cell.* 76(3):473–84.e7
95. Davidson IF, Bauer B, Goetz D, Tang W, Wutz G, Peters JM. 2019. DNA loop extrusion by human cohesin. *Science* 366(6471):1338–45
96. Lee R, Kang M-K, Kim Y-J, Yang B, Shim H, et al. 2022. CTCF-mediated chromatin looping provides a topological framework for the formation of phase-separated transcriptional condensates. *Nucleic Acids Res.* 50(1):207–26
97. Conte M, Irani E, Chiariello AM, Abraham A, Bianco S, et al. 2021. Loop-extrusion and polymer phase-separation can co-exist at the single-molecule level to shape chromatin folding. *Nat. Commun.* 13:4070
98. Goel VY, Huseyin MK, Hansen AS. 2022. Region Capture Micro-C reveals coalescence of enhancers and promoters into nested microcompartments. bioRxiv 2022.07.12.499637. **https://doi.org/10.1101/2022.07.12.499637**
99. Chakraborty S, Kopitchinski N, Eraso A, Awasthi P, Chari R, et al. 2022. High affinity enhancer-promoter interactions can bypass CTCF/cohesin-mediated insulation and contribute to phenotypic robustness. bioRxiv 2021.12.30.474562. **https://doi.org/10.1101/2021.12.30.474562**
100. Rinzema NJ, Sofiadis K, Tjalsma SJD, Verstegen MJAM, Oz Y, et al. 2021. Building regulatory landscapes: enhancer recruits cohesin to create contact domains, engage CTCF sites and activate distant genes. bioRxiv 2021.10.05.463209. **https://doi.org/10.1101/2021.10.05.463209**
101. Horsfield JA. 2022. Full circle: a brief history of cohesin and the regulation of gene expression. *FEBS J.* **https://doi.org/10.1111/febs.16362**
102. Ryba T, Hiratani I, Lu J, Itoh M, Kulik M, et al. 2010. Evolutionarily conserved replication timing profiles predict long-range chromatin interactions and distinguish closely related cell types. *Genome Res.* 20(6):761–70
103. Pope BD, Ryba T, Dileep V, Yue F, Wu W, et al. 2014. Topologically associating domains are stable units of replication-timing regulation. *Nature* 515(7527):402–5
104. Li Y, Xue B, Zhang M, Zhang L, Hou Y, et al. 2021. Transcription-coupled structural dynamics of topologically associating domains regulate replication origin efficiency. *Genome Biol.* 22(1):206
105. Emerson DJ, Zhao PA, Cook AL, Barnett RJ, Klein KN, et al. 2022. Cohesin-mediated loop anchors confine the locations of human replication origins. *Nature* 606:812–19
106. Dequeker BJH, Scherr MJ, Brandão HB, Gassler J, Powell S, et al. 2022. MCM complexes are barriers that restrict cohesin-mediated loop extrusion. *Nature* 606(7912):197–203
107. Jeppsson K, Sakata T, Nakato R, Milanova S, Shirahige K, Björkegren C. 2022. Cohesin-dependent chromosome loop extrusion is limited by transcription and stalled replication forks. *Sci. Adv.* 8(23):eabn7063
108. Sjögren C, Nasmyth K. 2001. Sister chromatid cohesion is required for postreplicative double-strand break repair in *Saccharomyces cerevisiae*. *Curr. Biol.* 11(12):991–95
109. De Piccoli G, Cortes-Ledesma F, Ira G, Torres-Rosell J, Uhle S, et al. 2006. Smc5–Smc6 mediate DNA double-strand-break repair by promoting sister-chromatid recombination. *Nat. Cell Biol.* 8(9):1032–34
110. Potts PR, Porteus MH, Yu H. 2006. Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. *EMBO J.* 25(14):3377–88
111. Ström L, Karlsson C, Lindroos HB, Wedahl S, Katou Y, et al. 2007. Postreplicative formation of cohesion is required for repair and induced by a single DNA break. *Science* 317(5835):242–45
112. Arnould C, Rocher V, Finoux AL, Clouaire T, Li K, et al. 2021. Loop extrusion as a mechanism for formation of DNA damage repair foci. *Nature* 590(7847):660–65

113. Ünal E, Heidinger-Pauli JM, Koshland D. 2007. DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). *Science* 317(5835):245–48
114. Shi Z, Gao H, Bai XC, Yu H. 2020. Cryo-EM structure of the human cohesin-NIPBL-DNA complex. *Science* 368(6498):1454–59
115. Alt A, Dang HQ, Wells OS, Polo LM, Smith MA, et al. 2017. Specialized interfaces of Smc5/6 control hinge stability and DNA association. *Nat. Commun.* 8(1):14011
116. Shroff R, Arbel-Eden A, Pilch D, Ira G, Bonner WM, et al. 2004. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Curr. Biol.* 14(19):1703–11
117. Piazza A, Bordelet H, Dumont A, Thierry A, Savocco J, et al. 2021. Cohesin regulates homology search during recombinational DNA repair. *Nat. Cell Biol.* 23(11):1176–86
118. Pradhan B, Kanno T, Igarashi MU, Loke MS, Baaske MD, et al. 2023. The Smc5/6 complex is a DNA loop-extruding motor. *Nature* 616:843–48
119. Peters J-M. 2021. How DNA loop extrusion mediated by cohesin enables V(D)J recombination. *Curr. Opin. Cell Biol.* 70:75–83
120. Kimura K, Hirano T. 1997. ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. *Cell* 90(4):625–34
121. Kimura K, Rybenkov VV, Crisona NJ, Hirano T, Cozzarelli NR. 1999. 13S condensin actively reconfigures DNA by introducing global positive writhe: implications for chromosome condensation. *Cell* 98(2):239–48
122. Kim E, Gonzalez AM, Pradhan B, van der Torre J, Dekker C. 2022. Condensin-driven loop extrusion on supercoiled DNA. *Nat. Struct. Mol. Biol.* 29:719–27
123. Strick TR, Kawaguchi T, Hirano T. 2004. Real-time detection of single-molecule DNA compaction by condensin I. *Curr. Biol.* 14(10):874–80
124. Eeftens JM, Bisht S, Kerssemakers J, Kschonsak M, Haering CH, Dekker C. 2017. Real-time detection of condensin-driven DNA compaction reveals a multistep binding mechanism. *EMBO J.* 36(23):3448–57
125. Terakawa T, Bisht S, Eeftens JM, Dekker C, Haering CH, Greene EC. 2017. The condensin complex is a mechanochemical motor that translocates along DNA. *Science* 358(6363):672–76
126. Davidson IF, Goetz D, Zaczek MP, Molodtsov MI, Huis in 't Veld PJ, et al. 2016. Rapid movement and transcriptional re-localization of human cohesin on DNA. *EMBO J.* 35(24):2671–85
127. Stigler J, Çamdere G, Koshland DE, Greene EC. 2016. Single-molecule imaging reveals a collapsed conformational state for DNA-bound cohesin. *Cell Rep.* 15(5):988–98
128. Kim Y, Shi Z, Zhang H, Finkelstein IJ, Yu H. 2019. Human cohesin compacts DNA by loop extrusion. *Science* 366(6471):1345–49
129. Gutierrez-Escribano P, Hormeño S, Madariaga-Marcos J, Solé-Soler R, O'Reilly FJ, et al. 2020. Purified Smc5/6 complex exhibits DNA substrate recognition and compaction. *Mol. Cell.* 80(6):1039–54.e6
130. Golfier S, Quail T, Kimura H, Brugués J. 2020. Cohesin and condensin extrude DNA loops in a cell-cycle dependent manner. *eLife* 9:e53885
131. Kong M, Cutts EE, Pan D, Beuron F, Kaliyappan T, et al. 2020. Human condensin I and II drive extensive ATP-dependent compaction of nucleosome-bound DNA. *Mol. Cell.* 79(1):99–114.e9
132. Ryu J-K, Rah S-H, Janissen R, Kerssemakers JWJ, Bonato A, et al. 2022. Condensin extrudes DNA loops in steps up to hundreds of base pairs that are generated by ATP binding events. *Nucleic Acids Res.* 50(2):820–32
133. Tran NT, Laub MT, Le TBK. 2017. SMC progressively aligns chromosomal arms in caulobacter crescentus but is antagonized by convergent transcription. *Cell Rep.* 20(9):2057–71
134. Banigan EJ, Mirny LA. 2020. Loop extrusion: theory meets single-molecule experiments. *Curr. Opin. Cell Biol.* 64:124–38
135. Kato Y, Miyakawa T, Tanokura M. 2018. Overview of the mechanism of cytoskeletal motors based on structure. *Biophys. Rev.* 10:571–81
136. Kschonsak M, Merkel F, Bisht S, Metz J, Rybin V, et al. 2017. Structural basis for a safety-belt mechanism that anchors condensin to chromosomes. *Cell* 171:588–600
137. Shaltiel IA, Datta S, Lecomte L, Hassler M, Kschonsak M, et al. 2022. A hold-and-feed mechanism drives directional DNA loop extrusion by condensin. *Science* 376(6597):1087–94

138. Murayama Y, Uhlmann F. 2014. Biochemical reconstitution of topological DNA binding by the cohesin ring. *Nature* 505(7483):367–71
139. Collier JE, Nasmyth KA. 2022. DNA passes through cohesin's hinge as well as its Smc3-kleisin interface. bioRxiv 2022.05.30.494034. **https://doi.org/10.1101/2022.05.30.494034**
140. Nasmyth K. 2011. Cohesin: a catenase with separate entry and exit gates? *Nat. Cell Biol.* 13(10):1170–77
141. Pradhan B, Barth R, Kim E, Davidson IF, Bauer B, et al. 2021. SMC complexes can traverse physical roadblocks bigger than their ring size. *Cell Rep*. 41(3):111491
142. Oldenkamp R, Rowland BD. 2022. A walk through the SMC cycle: from catching DNAs to shaping the genome. *Mol. Cell.* 82(9):1616–30
143. Ryu J, Katan AJ, van der Sluis EO, Wisse T, de Groot R, et al. 2020. The condensin holocomplex cycles dynamically between open and collapsed states. *Nat. Struct. Mol. Biol.* 27:1134–41
144. Eeftens JM, Katan AJ, Kschonsak M, Hassler M, de Wilde L, et al. 2016. Condensin Smc2-Smc4 dimers are flexible and dynamic. *Cell Rep*. 14(8):1813–18
145. Bauer BW, Davidson IF, Canena D, Wutz G, Tang W, et al. 2021. Cohesin mediates DNA loop extrusion by a "swing and clamp" mechanism. *Cell* 184(21):5448–64
146. Higashi TL, Pobegalov G, Tang M, Molodtsov MI, Uhlmann F. 2021. A Brownian ratchet model for DNA loop extrusion by the cohesin complex. *eLife* 10:e67530
147. Higashi TL, Eickhoff P, Sousa JS, Locke J, Nans A, et al. 2020. A structure-based mechanism for DNA entry into the cohesin ring. *Mol. Cell.* 79(6):917–33.e9
148. Marko JF, De Los Rios P, Barducci A, Gruber S. 2019. DNA-segment-capture model for loop extrusion by structural maintenance of chromosome (SMC) protein complexes. *Nucleic Acids Res*. 47(13):6956–72
149. Nomidis SK, Carlon E, Gruber S, Marko JF. 2022. DNA tension-modulated translocation and loop extrusion by SMC complexes revealed by molecular dynamics simulations. *Nucleic Acids Res*. 50(9):4974–87
150. Lee H, Avila LBR, Gruber S, Lee H, Avila LBR, et al. 2017. Structure of full-length SMC and rearrangements required for chromosome organization. *Mol. Cell* 67(2):334–47
151. Lee BG, Merkel F, Allegretti M, Hassler M, Cawood C, et al. 2020. Cryo-EM structures of holo condensin reveal a subunit flip-flop mechanism. *Nat. Struct. Mol. Biol.* 27(8):743–51
152. Pradhan B, Barth R, Kim E, Davidson IF, van der Torre J, et al. 2022. Can pseudotopological models for SMC-driven DNA loop extrusion explain the traversal of physical roadblocks bigger than the SMC ring size? bioRxiv 2022.08.02.502451. **https://doi.org/10.1101/2022.08.02.502451**
153. Rubio ED, Reiss DJ, Welcsh PL, Disteche CM, Filippova GN, et al. 2008. CTCF physically links cohesin to chromatin. *PNAS* 105(24):8309–14
154. Tang Z, Luo OJ, Li X, Zheng M, Zhu JJ, et al. 2015. CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. *Cell* 163(7):1611–27
155. Pugacheva EM, Kubo N, Loukinov D, Tajmul M, Kang S, et al. 2020. CTCF mediates chromatin looping via N-terminal domain-dependent cohesin retention. *PNAS* 117(4):2020–31
156. Nora EP, Caccianini L, Fudenberg G, So K, Kameswaran V, et al. 2020. Molecular basis of CTCF binding polarity in genome folding. *Nat. Commun.* 11(1):5612
157. Luppino JM, Park DS, Nguyen SC, Lan Y, Xu Z, et al. 2020. Cohesin promotes stochastic domain intermingling to ensure proper regulation of boundary-proximal genes. *Nat. Genet.* 52(8):840–48
158. Davidson IF, Barth R, Zaczek M, van der Torre J, Tang W, et al. 2023. CTCF is a DNA-tension-dependent barrier to cohesin-mediated loop extrusion. *Nature* 616:822–27
159. Karaboja X, Ren Z, Brandão HB, Paul P, Rudner DZ, Wang X. 2021. XerD unloads bacterial SMC complexes at the replication terminus. *Mol. Cell.* 81(4):756–66.e8
160. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, et al. 2010. Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467(7314):430–35
161. Ramasamy S, Aljahani A, Karpinska MA, Cao TBN, Cruz JN, Oudelaar AM. 2022. The Mediator complex regulates enhancer-promoter interactions. bioRxiv 2022.06.15.496245. **https://doi.org/10.1101/2022.06.15.496245**
162. Mattingly M, Seidel C, Muñoz S, Hao Y, Zhang Y, et al. 2022. Mediator recruits the cohesin loader Scc2 to RNA Pol II-transcribed genes and promotes sister chromatid cohesion. *Curr. Biol.* 32(13):2884–96.e6

163. Weintraub AS, Li CH, Zamudio AV, Sigova AA, Hannett NM, et al. 2017. YY1 is a structural regulator of enhancer–promoter loops. *Cell* 171(7):1573–88.e28
164. Beagan JA, Duong MT, Titus KR, Zhou L, Cao Z, et al. 2017. YY1 and CTCF orchestrate a 3D chromatin looping switch during early neural lineage commitment. *Genome Res*. 27(7):1139–52
165. Pan X, Papasani M, Hao Y, Calamito M, Wei F, et al. 2013. YY1 controls Igκ repertoire and B-cell development, and localizes with condensin on the Igκ locus. *EMBO J*. 32(8):1168–82
166. Busslinger GA, Stocsits RR, van der Lelij P, Axelsson E, Tedeschi A, et al. 2017. Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl. *Nature* 544(7651):503–7
167. Heinz S, Texari L, Hayes MGB, Urbanowski M, Chang MW, et al. 2018. Transcription elongation can affect genome 3D structure. *Cell* 174(6):1522–36.e22
168. Banigan EJ, Tang W, van den Berg AA, Stocsits RR, Wutz G, et al. 2022. Transcription shapes 3D chromatin organization by interacting with loop-extruding cohesin complexes. bioRxiv 2022.01.07.475367. **https://www.biorxiv.org/content/10.1101/2022.01.07.475367v1**
169. Brandão HB, Paul P, van den Berg AA, Rudner DZ, Wang X, Mirny LA. 2019. RNA polymerases as moving barriers to condensin loop extrusion. *PNAS* 116(41):20489–99
170. Lee K, Hsiung CCS, Huang P, Raj A, Blobel GA. 2015. Dynamic enhancer–gene body contacts during transcription elongation. *Genes Dev*. 29(19):1992–97
171. Schaaf CA, Kwak H, Koenig A, Misulovin Z, Gohara DW, et al. 2013. Genome-wide control of RNA polymerase II activity by cohesin. *PLOS Genetics* 9(3):e1003382
172. Pan H, Jin M, Ghadiyaram A, Kaur P, Miller HE, et al. 2021. Cohesin SA1 and SA2 are RNA binding proteins that localize to RNA containing regions on DNA. *Nucleic Acids Res*. 48(10):5639–55
173. Brandão HB, Ren Z, Karaboja X, Mirny LA, Wang X. 2021. DNA-loop-extruding SMC complexes can traverse one another in vivo. *Nat. Struct. Mol. Biol.* 28(8):642–51
174. Anchimiuk A, Lioy VS, Bock FP, Minnen A, Boccard F, Gruber S. 2021. A low Smc flux avoids collisions and facilitates chromosome organization in *Bacillus subtilis*. *eLife* 10:e65467
175. Kim E, Kerssemakers J, Shaltiel IA, Haering CH, Dekker C. 2020. DNA-loop extruding condensin complexes can traverse one another. *Nature* 579(7799):438–42
176. Banigan EJ, van den Berg AA, Brandão HB, Marko JF, Mirny LA. 2020. Chromosome organization by one-sided and two-sided loop extrusion. *eLife* 9:e53558
177. Muñoz S, Minamino M, Casas-Delucchi CS, Patel H, Uhlmann F. 2019. A role for chromatin remodeling in cohesin loading onto chromosomes. *Mol. Cell.* 74(4):664–73.e5
178. Otterstrom J, Castells-Garcia A, Vicario C, Gomez-Garcia PA, Cosma MP, Lakadamyali M. 2019. Super-resolution microscopy reveals how histone tail acetylation affects DNA compaction within nucleosomes in vivo. *Nucleic Acids Res*. 47(16):8470–84
179. Portillo-Ledesma S, Tsao LH, Wagley M, Lakadamyali M, Cosma MP, Schlick T. 2021. Nucleosome clutches are regulated by chromatin internal parameters. *J. Mol. Biol.* 433(6):166701
180. Guérin TM, Béneut C, Barinova N, López V, Lazar-Stefanita L, et al. 2019. Condensin-mediated chromosome folding and internal telomeres drive dicentric severing by cytokinesis. *Mol. Cell.* 75(1):131–44.e3
181. Le Bihan Y-V, Matot B, Pietrement O, Giraud-Panis M-J, Gasparini S, et al. 2013. Effect of Rap1 binding on DNA distortion and potassium permanganate hypersensitivity. *Acta Crystallogr*. D69(3):409–19
182. Cummings CT, Rowley MJ. 2022. Implications of dosage deficiencies in CTCF and cohesin on genome organization, gene expression, and human neurodevelopment. *Genes* 13(4):583
183. Oscar H. 1890. *Lehrbuch der Entwicklungsgeschichte des Menschen und der Wirbeltiere*. Jena, Ger.: Gustav Fischer
184. Wells JN, Gligoris TG, Nasmyth KA, Marsh JA. 2017. Evolution of condensin and cohesin complexes driven by replacement of Kite by Hawk proteins. *Curr. Biol.* 27(1):R17–18
185. Kikuchi S, Borek DM, Otwinowski Z, Tomchick DR, Yu H. 2016. Crystal structure of the cohesin loader Scc2 and insight into cohesinopathy. *PNAS* 113(44):12444–49
186. Haarhuis JHI, van der Weide RH, Blomen VA, Yáñez-Cuna JO, Amendola M, et al. 2017. The cohesin release factor WAPL restricts chromatin loop extension. *Cell* 169(4):693–707.e14
187. Yu Y, Li S, Ser Z, Kuang H, Than T, et al. 2022. Cryo-EM structure of DNA-bound Smc5/6 reveals DNA clamping enabled by multi-subunit conformational changes. *PNAS* 119(23):e2202799119

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Errata

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