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DOI
10.1126/science.adi8308

Publication date
2023

Document Version
Final published version

Published in
Science (New York, N.Y.)

HYPOTHESIS

How do molecular motors fold the genome?

A potential mechanism of DNA loop extrusion by molecular motors is discussed

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Many protein complexes that drive key processes in cells are "molecular motors"—assemblies that consume (electro)chemical energy to produce mechanical work. Examples include the F_oF_1 synthase rotary motor that catalyzes adenosine triphosphate (ATP) production, kinesin and myosin motors that "walk" along cytoskeleton filaments, or polymerases and helicases that move along DNA. Structural-maintenance of chromosomes protein complexes (SMCs) have only recently been identified as an entirely distinct class of DNA-translocating motors, although their key role in folding the linear DNA double helix into intricate three-dimensional structures, such as X-shaped mitotic chromosomes, was known for decades. Here, we discuss how insights from biophysical, biochemical, and structural studies are starting to yield an understanding of the mechanism by which these motors extrude loops of DNA to structure genomes.

SMCs are evolutionarily conserved from bacteria to humans. Eukaryotes feature three main classes of SMCs: condensin, which assembles mitotic chromosomes during cell division; cohesin, which regulates interphase chromosome structure and links sister chromatids (the two copies of every chromosome generated by DNA replication); and SMC5/6, which has less well understood roles in DNA damage repair and replication. All of these complexes exhibit a similar tripartite ring architecture of ~40 nm in diameter made of a dimer of coiled-coil SMC proteins and an intrinsically disordered kleisin protein, to which additional subunits attach. In the case of cohesin and condensin, these additional subunits are built from multiple repeats of "HEAT" motifs, referred to here as HEAT-A and HEAT-B subunits. At the heart of the motor are two globular ATPase head domains located at the ends of the SMC coiled coils.

SMCs generate DNA loops, which appear to be the basic motif of chromosome structure that underlie many major genomic processes, from folding mitotic chromosomes to the formation of topologically associating domains [TADs (genomic domains thought to regulate gene expression)]. Whereas earlier chromosome conformation capture mapping and polymer simulations suggested that extrusion of DNA loops by SMCs could explain many chromosomal features, direct evidence for such loop extrusion by SMCs was provided by single-molecule studies that visualized the formation of DNA loops in real time (*1*–*4*).

These studies yielded a wealth of data. Driven by ATP hydrolysis, SMCs were found to be very fast motors, reeling in DNA at a speed of ~1 kilobase pair per second in a directional and processive manner for long distances. ATP binding induces SMCs to take a step of hundreds of base pairs—strikingly different from previously characterized DNA-translocating motors that typically move a single base pair at a time. Such large steps are consistent with studies that implicated conformational changes of SMC structure that were the approximate size of the entire complex in the DNA loop extrusion process (*5*–*8*). Although fast, SMCs are also weak motors that stall if subpiconewton forces are applied to the DNA that they reel in. Another unexpected feature of SMCs is their ability to pass DNA binding proteins, such as nucleosomes, polymerases, or even other SMCs, and incorporate them into the extruded DNA loops (*9*, *10*).

The data from these single-molecule studies sparked many debates about the mechanism of DNA loop extrusion by SMCs (*11*). Based on the resemblance of the coiled-coil architecture of SMC proteins to kinesin or myosin motors that transport cargo along cytoskeletal filaments, it was initially suggested that the two globular ATPase heads might grab two distal DNA sections and bring them together, thus creating a DNA loop. This intuitive early scenario appeared, however, incompatible with the small head-to-head distances consistently observed in structural studies of SMCs.

Alternative "scrunching" models suggested that the base of a DNA loop is clamped in the vicinity of the ATPase heads, but the hinge domain at the other end of the coiled coils reaches out to grab a new DNA segment. Bending of the coils, driven by thermal fluctuations, "swings" the hinge with the new DNA section into the vicinity of the heads, where it is handed over from the hinge to the heads to merge with the DNA loop (*6*). The reverse order is also conceivable, with a DNA section bound at the hinge domain moving away from the ATPase heads while the coiled coils straighten (*12*).

Yet another hypothesis is based on the idea that opening up the lumen of the SMC-kleisin ring allows the capture of a DNA loop segment, which could be generated either by thermal fluctuations (*13*) or actively fed into the lumen by a "power-stroke" motion that is driven by the energy of ATP binding to the ATPase heads (*14*, *15*). Similar to the scrunching-type models, the newly captured loop then merges with a preexisting DNA loop held in the vicinity of the ATPase heads when the coiled coils bend and reassociate.

The different models have been tested against various experimental data: Coil bending was observed by atomic force microscopy (*5*, *6*) and cryo–electron microscopy (cryo-EM) (*7*, *8*) for multiple SMCs, predominantly at a local discontinuity in the coiled-coil superhelix that acts as an "elbow." Locking the two SMC subunits in the bent conformation prevented loop extrusion (*6*). In cryo-EM structures, DNA was bound to the inner surfaces of the SMC-kleisin ring lumen (*11*), arguing that the ring might topologically encircle DNA strands (in a manner that either protein or DNA would have to be cleaved to disengage the two) or pseudo-topologically embrace a DNA loop (in which case DNA could be pulled out of the protein ring without severing either of the two) (*15*).

Interestingly, fusing all SMC-kleisin interfaces to establish one continuous cohesin ring did not prevent loop extrusion (*2*, *9*), which argued against a classical topological model in which the SMC-kleisin ring would have to be opened to entrap DNA. Furthermore, the finding that obstacles larger than the cohesin ring could be incorporated into DNA loops (*9*) supported a nontopological model in which the extruded loop is not embraced by the SMC-kleisin lumen. The seemingly contradictory conclusions on the topology could be reconciled upon redrawing parts of the kleisin's path relative to the DNA (*11*), which implied that DNA can be temporarily cap-

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tured pseudo-topologically within the SMC-kleisin ring during the ATP hydrolysis cycle, whereas the final extruded loop is not topologically embraced by the SMC-kleisin ring.

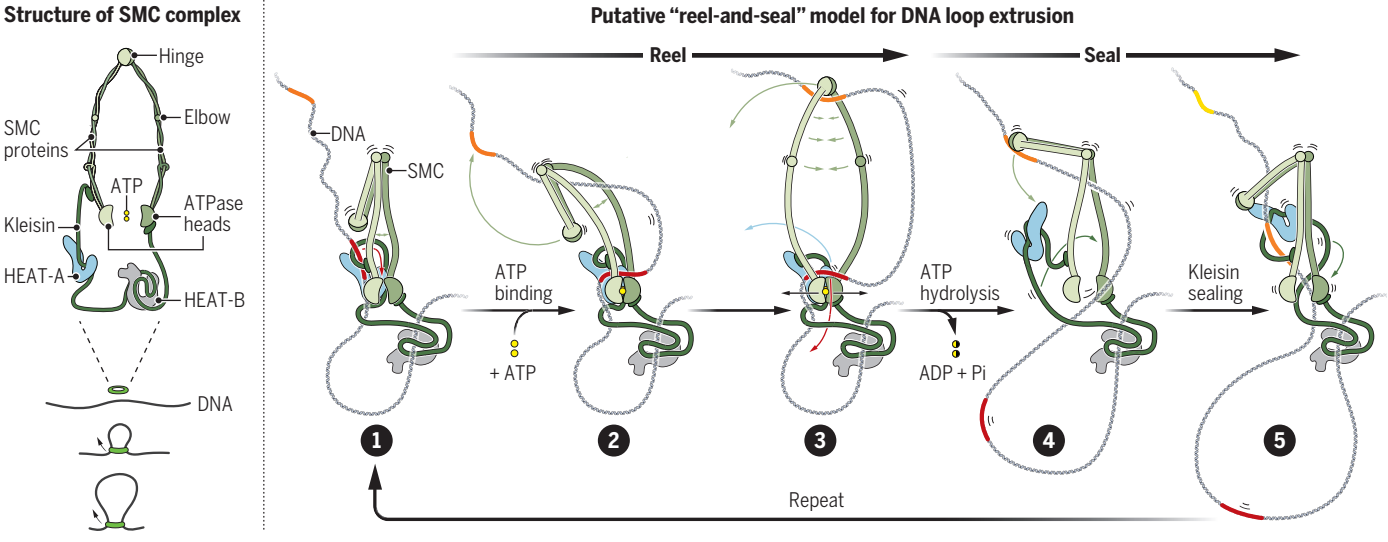
Although there are further differences between the various types of models—for example, in the way that force is generated— they all rely on large conformational rearrangements to relocate a stretch of DNA in a single reaction cycle. Below, we attempt to put together a hybrid working model that integrates central features of the different hypotheses. We base our speculations on the head engagement forms a new DNA binding site on the SMC heads and initiates a series of large-scale movements that involve a power stroke: Presumably assisted by a conformational change in HEAT-A, DNA gets clamped onto the SMC heads, which triggers a stretching and opening up of the SMC arms. These movements swing the SMC hinge away from the ATPase heads, de facto capturing a looped DNA segment inside the lumen between the disengaged arms. Clamping of the DNA onto the heads and enlarging the loop by reeling a DNA segment through the SMC lumen pro-

the SMC-kleisin lumen. Reel-and-seal extends beyond a series of previous models (*11*, *13*, *15*) by invoking the motion that unseals the HEAT-A kleisin fold, which provides the window of opportunity for the complex to pass obstacles that can be substantially larger than the diameter of the ring (*9*). Although this hypothetical scenario is compatible with most currently available data, it is intended to represent an intellectual exercise to inspire much-needed further studies.

Several key questions need to be addressed to fully elucidate the mechanism through

DNA loop extrusion by structural maintenance of chromosomes complexes

SMCs such as cohesin and condensin have a ring architecture that consists of two SMC coiled-coil subunits and a disordered kleisin subunit that is bound by HEAT-A (in humans: NIPBL for cohesin, NCAPD2 or NCAPD3 for condensin) and by HEAT-B (in humans: STAG1 or STAG2 for cohesin, NCAPG or NCAPG2 for condensin) subunits. In our proposed "reel-and-seal" model for DNA loop extrusion by SMCs, the interfaces of the SMC-kleisin ring do not need to disengage for the complex to accommodate DNA. Instead, the protein complex folds around the DNA (**1**). Upon ATP binding to the folded resting state, DNA gets clamped onto the engaged ATPase heads (**2**), which induces stretching and opening of the coiled coils and transfer of a new DNA loop between the disengaged coils (**3**). Upon ATP hydrolysis, the newly formed DNA loop is transferred into the extruded DNA loop (**4**) and the SMC relaxes into its folded state (**5**). Red, orange, and yellow indicate DNA segments that are sequentially transferred into the extruded loop.



ADP, adenosine diphosphate; ATP, adenosine triphosphate; NCAPD2, non-SMC condensin subunit D2; NCAPD3, non-SMC condensin subunit D3; NCAPG, non-SMC condensin subunit G; NCAPG2, non-SMC condensin subunit G2; NIPBL, nipped-B-like protein; Pi, inorganic phosphate; SMC, structural maintenance of chromosomes complex; STAG1, cohesin subunit SA-1; STAG2, cohesin subunit SA-2.

two best-studied SMCs: cohesin and condensin. As key features of SMC action appear to be universal across all the complexes, it will be interesting to see whether SMC5/6 or prokaryotic SMCs use a similar mechanism.

In our hybrid model (see the figure and online movie), loop-extruding SMCs do not topologically embrace DNA inside their ring-shaped structure, but rather fold their peptide chain around the DNA. The two HEAT-repeat subunits each wrap part of the long kleisin subunit around the DNA. The fold at HEAT-B provides an anchor, which allows the complex to hold onto a DNA section while enlarging the DNA loop in one direction at any given time. The second fold, at HEAT-A, positions the DNA double helix such that it can be fed onto the SMC ATPase heads upon ATP binding. ATP binding–induced SMC vides directionality to the translocation. At this point, the hinge domain contacts a new DNA section. In the next step, ATP hydrolysis and nucleotide release trigger HEAT-A to leave the ATPase heads, and the arms bend over again, which causes the segment of the captured DNA loop between the arms to move toward the heads. In this step, the original DNA section is transferred through the ATPase heads to end up in the extruded DNA loop that consequently further grows in size. The new DNA section is sealed into the HEAT-A kleisin fold and is thus positioned for clamping on top of the ATPase heads in the next cycle.

This "reel-and-seal" model explains how DNA transiently inserts a pseudotopological loop into the SMC-kleisin ring, while the final extruded DNA loop is not embraced within which SMCs extrude DNA into loops. How DNA is bound by and translocated through SMCs by a combination of topological and direct binding interactions needs to be clarified, as does the question whether translocation requires cycles of opening and closing of the SMC arms and/or cycles of stretching and bending of the arms. Whether the DNA "slides" or is handed-over from one binding site to another is also not yet clear. In addition, it will be important to understand how SMC conformational changes relate to functions other than loop extrusion, such as sister chromatid cohesion or translocation of SMCs along DNA.

Other questions concern the functions of the HEAT-repeat subunits. Whether HEAT-A might contact the SMC hinge of condensin as it does for cohesin, and

GRAPHIC: DEKKER *ET AL.*

whether HEAT-B might have a DNA-anchoring role for cohesin as it does for condensin are still unknown. An exchange between cohesin's HEAT-A variants nipped-B-like protein (NIPBL) and PDS5 may control DNA looping, but this mechanism is yet to be understood. Another outstanding question is whether SMCs extrude DNA loops unidirectionally or whether they can switch directionality by exchanging the bound DNA segments between HEAT-A and HEAT-B.

Because the looping of DNA by SMCs might change the twist of the DNA double helix, it will be important to understand the role of this induced torsion, which will supercoil the extruded DNA loop. Further insight into how the forces are generated that translocate and bend the DNA during loop extrusion will also be required. It is also not yet clear whether the energy released during ATP binding-hydrolysis cycles is used to generate these forces, or whether SMCs function as molecular ratchets that are driven by thermal motion.

Similarly, it will be of great importance to understand how SMCs can operate on chromatin fibers, where DNA is occupied by nucleosomes and polymerases. Whether SMCs step from one accessible DNA region to the next, or whether they can interact with DNA while it is wrapped around histone octamers, needs to be addressed. How cohesin's loop extrusion cycle is controlled by the architectural protein CTCF that defines the boundaries of TADs, and how post-translational modifications such as acetylation or phosphorylation of different SMC subunits affect loop extrusion, are other questions of interest. It will also be important to understand whether SMC5/6 and bacterial SMCs act differently than condensin and cohesin and whether protein complexes of similar architecture, such as the MRE11-RAD50-nibrin DNA repair protein complex, also extrude DNA. ■

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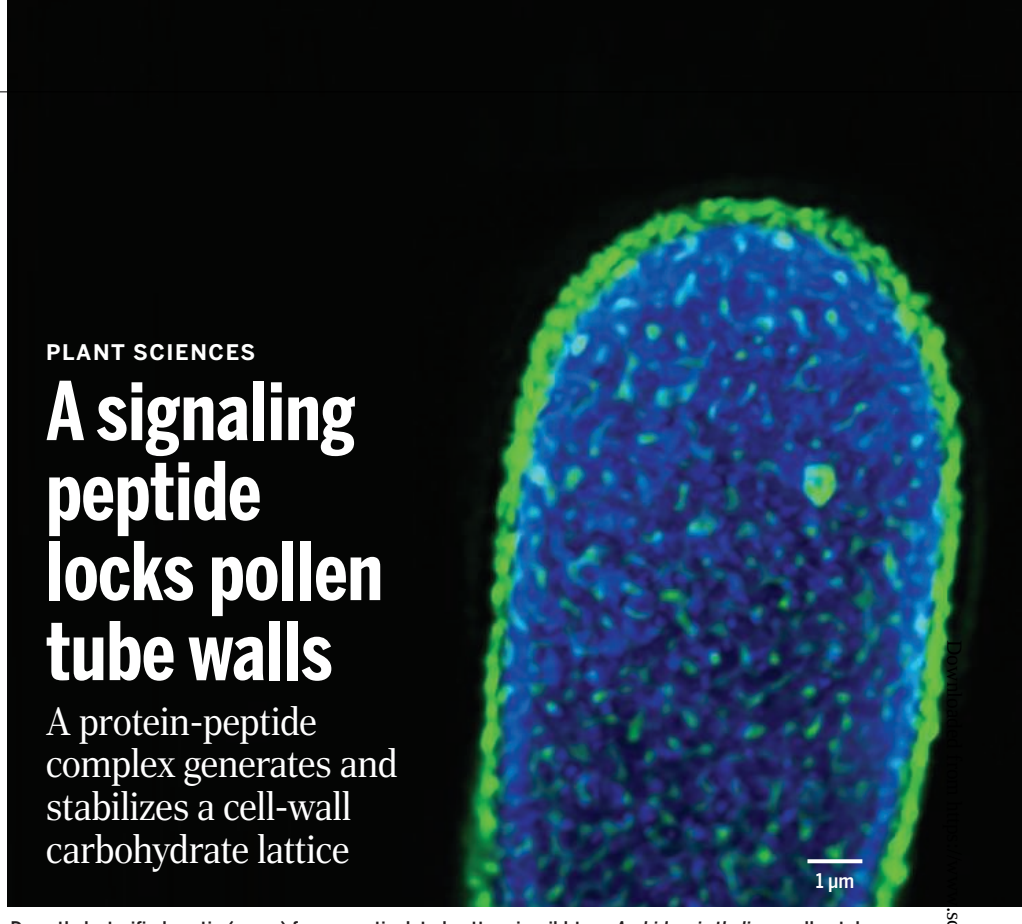
10.1126/science.adi8308

PLANT SCIENCES

A signaling peptide locks pollen tube walls

A protein-peptide complex generates and stabilizes a cell-wall carbohydrate lattice



Demethylesterified pectin (green) forms a reticulated pattern in wild-type *Arabidopsis thaliana* pollen tubes.

IMAGE: MOHNEN *ET AL.*

By **Debra Mohnen**

Pollen tube elongation in flowering plants transports sperm through the stigma and style to the egg in the ovary, enabling fertilization. Pollen tube growth entails oscillatory turgor-driven polarized cell wall expansion, which requires a malleable cell wall enriched in specific carbohydrates and proteins (*1*). However, what all of these carbohydrates and proteins are, and how they enable growing pollen tubes to elongate at rates of up to 1 cm hour^{-1} (*2*) while providing sufficient strength to prevent cell rupture, is not clear. Growing pollen tubes have a pectin-containing lattice in the wall behind the growing pollen tube tip (*3*). Many of the components of this lattice and how its formation is regulated are unknown. On page 719 in this issue, Moussu *et al.* (*4*) identify an unexpected player in this process: a peptide that forms a complex with a structural protein and binds deesterified pectin to generate and stabilize a reticulate tripartite network architecture in the pollen tube wall.

Pectin is a complex glycan found in the plant cell wall (*5*). Pollen tube growth occurs in the first 20 µm of the growing tip in a clear zone devoid of starch grains and vacuoles and enriched in vesicles containing methylesterified pectic homogalacturonan (HG) that provides a malleable wall for tip growth (*1*). HG, the most abundant pectic glycan in growing cells (*5*), is a galacturonic acid–rich homopolymer that is highly negatively charged if it is not methylesterified. The tubular lattice present behind the growing tip is enriched in Ca^{2+}–cross-linked deesterified HG that, along with cellulose and callose, strengthens the pollen tube wall (*2*, *6*). Cell wall–localized pectin methylesterases (PMEs) deesterify newly synthesized HG after delivery to the pollen tube tip (*1*), generating carboxylate groups in the HG chain that are hypothesized to sequester Ca^{2+} from preexisting HG, enabling simultaneous tip growth, wall strengthening, and avoidance of cell bursting (*7*). Transmembrane flux of additional Ca^{2+} into the wall further strengthens the HG-Ca^{2+}-HG network behind the new area of expansion.

Moussu *et al.* used elongating pollen tubes from *Arabidopsis thaliana* to investigate how cell wall polymers such as HG generate the architectural patterns that support plant cell growth. They focused on RAPID ALKANIZATION FACTOR 4 (RALF4), a member of the RALF signaling peptide family, which regulates plant growth, development, and response to

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Science **382** (6671), . DOI: 10.1126/science.adi8308