Study reveals new mechanism for DNA folding

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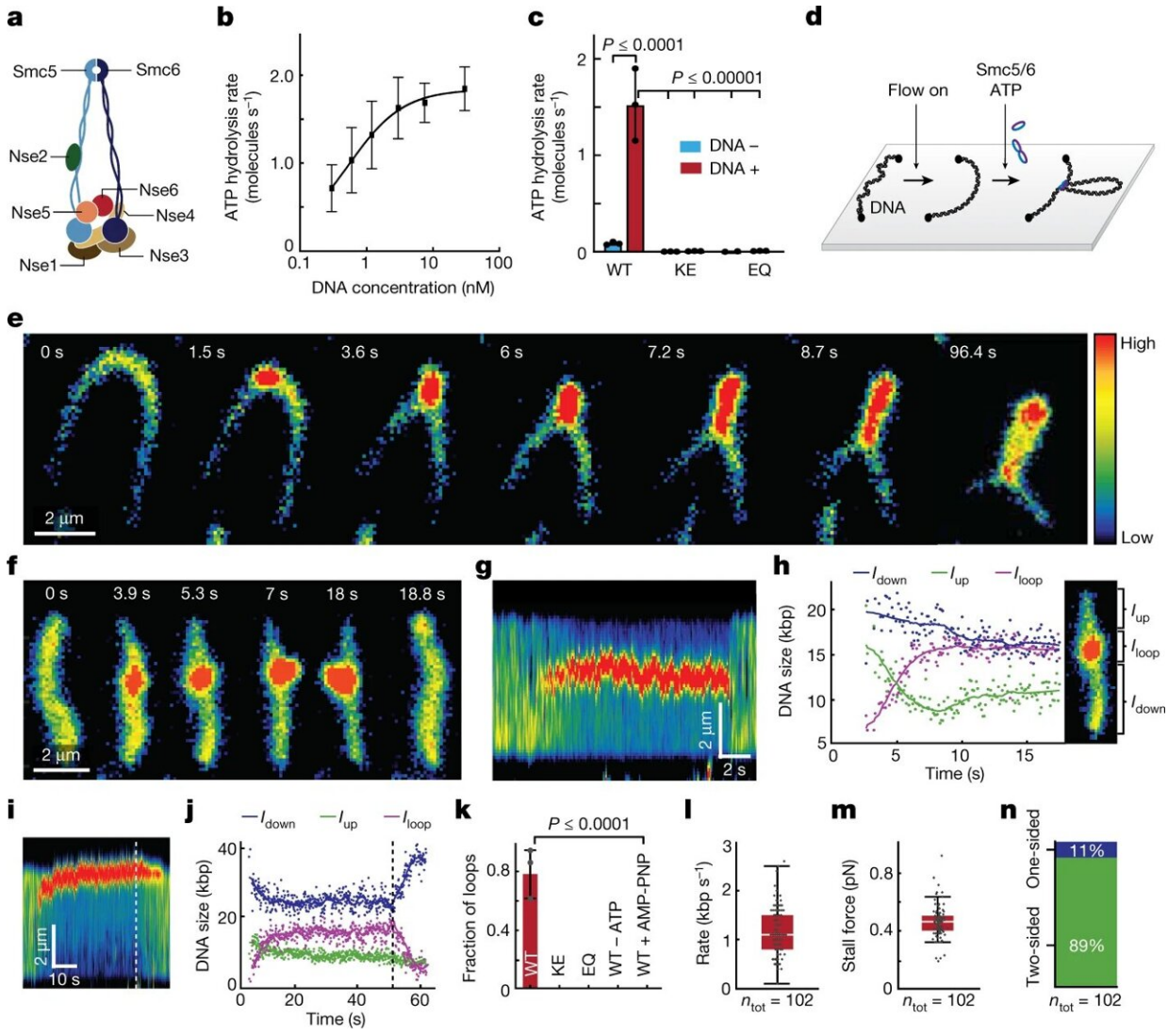

Real-time imaging of loop extrusion by Smc5/6. **a**, Cartoon of the *S. cerevisiae* Smc5/6 octameric structure. **b**, ATPase activity of the WT Smc5/6 octameric complex with different concentrations of DNA. Experimental data were fitted to

a stimulatory dose–response model by nonlinear regression; mean ± s.d. from four independent measurements. **c**, ATPase activity of WT, KE and EQ Smc5/6 complexes in the absence or presence of 30 nM plasmid DNA; mean ± s.d. from three independent measurements. **d**, Schematic of DNA loop-extrusion assay. **e**, Series of images showing DNA loop-extrusion intermediates induced by Smc5/6 complex under constant buffer flow. **f**,**g**, Images (**f**) and fluorescence intensity kymograph (**g**) of a DNA molecule showing DNA loop extrusion in the absence of buffer flow. **h**, DNA lengths calculated from the kymograph in **g** for regions outside the loop (I_{up} and I_{down}) and the loop region itself (I_{loop}). **i**,**j**, Kymograph (**i**) and calculated DNA lengths (**j**) for a loop-extrusion event followed by loop release via gradual shrinkage. Dashed lines in **i**,**j** indicate the start of loop shrinkage. Data in **e**—**j** represent typical events observed more than ten times in three independent experiments. **k**, DNA loop-forming fractions (mean ± s.d.) in the presence of ATP and 2 nM Smc5/6 WT, ATPase mutant complexes as in **c** and WT in the absence of ATP or presence of AMP-PNP. n_{tot} = 233, 121, 93, 84 and 106, respectively. **l**,**m**, Box-and-whisker plots of Smc5/6 loop extrusion showing rates (**l**) and stalling force (**m**). n_{tot} = 102 molecules, median ± 1.5× interquartile rate (IQR). **n**, Fraction of loop-extrusion events exhibiting two- or one-sided DNA reeling, as determined by observation of DNA length decrease in nonloop regions (I_{up} and I_{down} in **h**,**j**). Data in **k**–**n** are from three independent experiments. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-05963-3

A hitherto unknown mechanism for DNA folding is described in a study in *Nature* published by researchers from Karolinska Institutet and the Max Planck Institute for Biophysics. Their findings provide new insights into chromosomal processes that are vital to both normal development and to prevent disease.

The DNA in our cells is organized into chromosomes, which are highly dynamic structures that are altered when genes are transcribed, when DNA damage is repaired or when chromosomes are compacted in preparation for cell division. These processes are affected by so called SMC protein complexes (SMC, Structural Maintenance of

Chromosomes), which by mediating chromosomal interactions ensure correct spatial organization of the genome.

In humans and other eukaryotes, i.e., organisms whose cells contain a nucleus, there are three such protein complexes. Scientists have already revealed the mechanism of function for two of them. In the present study, the researchers investigated the third, the Smc5/6 complex, the function of which has remained mostly unknown.

"These results reveal the Smc5/6 complex as a new regulator of DNA folding, which can tell us more about how chromosomes are organized," says Camilla Björkegren, professor at the Department of Cell and Molecular Biology at Karolinska Institutet, who led the study together with Eugene Kim, research group leader at the Max Planck Institute for Biophysics in Frankfurt am Main.

"The discovery is also medically relevant, since DNA folding is important for normal chromosome function and for avoiding chromosomal alterations that could lead to disease."

The researchers have purified the Smc5/6 complex from yeast and, using high-resolution microscopy of individual molecules, have studied how it binds and affects individual DNA molecules. The principles of chromosomal organization are believed to be generally identical in yeast and humans, which are both eukaryotic organisms. For their experiments, the researchers both protein complex and DNA were labeled with differently colored fluorescing molecules to make them traceable through a microscope.

Their results show that the Smc5/6 complex operates by extruding an increasingly larger DNA loop, a property it shares with the other known eukaryotic SMC complexes.

The researchers have also examined how the process is regulated and found, among other things, that two Smc5/6 complexes are needed to form a loop, while single protein complexes only translocates along the DNA molecule.

Previous research indicates that Smc5/6 inhibits certain viruses and suggests that it also protects against certain types of cancer, and is important to normal fetal development. The KI researchers now want to study how these properties are related to the newly discovered mechanism.

"The next step of our research is to find out how the Smc5/6 complex's ability to make DNA loops affects their function in cells, which can increase our understanding of how Smc5/6 can function as a virus blocker, protect against cancer and contribute to fetal development," says Professor Björkegren.

More information: Eugene Kim, The Smc5/6 complex is a DNA loop extruding motor, *Nature* (2023). DOI: 10.1038/s41586-023-05963-3. www.nature.com/articles/s41586-023-05963-3

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