GENETICS

Sowing the Seeds of Centromeres

How can a centromere relocate to a new chromosomal position?

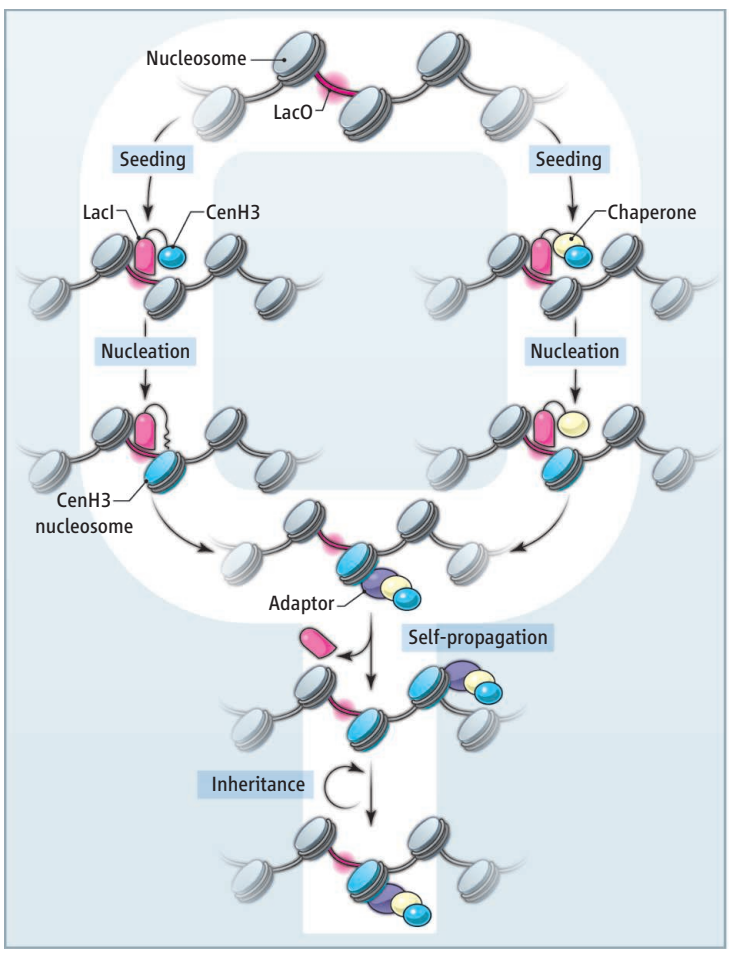
Lars E. T. Jansen

The centromere is a chromatin-based platform that accumulates microtubule-binding proteins that drive chromosome segregation during cell division. Despite their size (on the order of megabases of DNA in mammals) and conserved role, centromeres have the remarkable capacity to leave their usual comfort zone and to reform at a new chromosomal site (*1*). Although found rarely, these so-called neocentromeres are by most measures bona fide and segregate chromosomes with high fidelity. What accounts for this nomadic behavior?

A clue came from the identification of a variant of the histone protein H3 called centromere protein A (CENP-A), which is incorporated into nucleosomes specifically at the centromere region (*2*, *3*). Histones (the protein constituent of nucleosomes) bind DNA in a non–sequence specific manner, yet can heritably differentiate genomic regions. This is achieved either by the local decoration of canonical histones with chemical modifications or by replacement with variant histones. As such, histones are ideal candidates to epigenetically maintain chromatin identity. This notion led to the proposal that instead of a unique DNA sequence signature, it is the presence of CENP-A nucleosomes that identifies the position of the centromere (*4*). Consistent with this, CENP-A nucleosomes are not turned over except by dilution on newly replicated centromeres during cell division (*5*) and appear to evade epigenetic reprogramming in the germ line (*6*).

A strong predictor of the behavior of an epigenetic system is the ability to nucleate a structure or "mark" that in turn is propagated independently of the initial trigger, much like a seed that triggers subsequent autonomous growth of a crystal. Until very recently, such direct evidence for the epigenetic nature of the centromere was lacking. Although we've known that centromeres can move, they have yet to be caught in the act. Two recent studies provide plausible solutions for how neocentromeres form, going beyond phenomenology by experimentally inducing the formation of a new centromere (*7*, *8*).

Both studies, one in cultured fruit fly cells (*Drosophila melanogaster* S2 cells) (*7*) and one in human tissue culture cells (*8*), employed arrays of bacterial Lac operator (LacO) DNA sequences integrated into a defined chromosomal site to form a recruitment platform for the ectopically expressed Lac repressor protein (LacI). The *Drosophila* study by Mendiburo *et al*. (*7*) simply fused *Drosophila* centromeric histone H3 (CenH3, the fly homolog of CENP-A; also called CID) directly to LacI and tethered the fusion protein to a LacO DNA site, far removed from endogenous centromeres (see the figure). Although highly artificial, these hybrid CenH3 molecules, literally dragged in by their tails, assembled locally into nucleosomes. Critically, they in turn recruited endogenous CenH3 that was not pulled in by LacI but rather as a consequence of the initial pool of CenH3-LacI fusion protein; recruitment expanded laterally beyond the LacO DNA sites to which the initial pool bound. Analogous to the template-directed duplication of genetic information in DNA, a protein structure (CenH3 nucleosome) is "replicated" based on the initial template, a cycle that is a central feature of epigenetic mechanisms.



Seeding and propagation. CenH3 is targeted to a naïve chromatin locus either by direct fusion to locally bound LacI (*7*) or through recruitment by the LacI-tethered chaperone HJURP (*8*). This results in the nucleation of CenH3 nucleosomes, which in turn triggers the propagation of CenH3 chromatin in a self-templating manner (likely through an adaptor intermediate) without the need for the initial LacI seed. CenH3 chromatin propagation and turnover through cell divisions reach an equilibrium, which results in stable inheritance of the epigenetic centromere mark.

Barnhart *et al*. (*8*) took a slightly different approach—it was not CENP-A that was forced onto the LacO domain but rather Holliday junction–recognizing protein (HJURP), a chaperone for CENP-A. The authors show that HJURP acts as an assembly factor for CENP-A nucleosomes in vitro. Indeed, creating a local concentration of HJURP at the LacO array was sufficient to nucleate CENP-A chromatin. Thus, HJURP is the seed that sows the epigenetic centromere; it is not a part of the stable centromere structure but its transient presence kick-starts the process.

An earlier report (*9*) used the same powerful LacO technique to tether both CENP-C

Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal. E-mail: ljansen@igc.gulbenkian.pt

CREDIT: Y. HAMMOND/SCIENCE

Downloaded from http://science.sciencemag.org/ on May 12, 2020

Published by AAAS

and CENP-T, two centromere components acting downstream of CENP-A, onto an ectopic chromosomal site. Remarkably, this was sufficient to trigger the recruitment of other centromere constituents (but not CENP-A), as well as the kinetochore, the microtubule-binding complex that drives chromosome segregation during cell division. Indeed, at least transiently, this naïve locus became a site that powered chromosome movement, which suggests that a key role of CENP-A–containing nucleosomes is to recruit CENP-C and CENP-T to the centromere. These findings were further extended by the demonstration that a defined array of CENP-A nucleosomes is sufficient to nucleate a functional kinetochore in extracts from *Xenopus laevis* oocytes (*10*).

Previous attempts to generate neocentromeres in cells by simple CenH3/CENP-A overexpression were successful in *Drosophila* (*11*) but failed in human cells (*9*, *12*). However, as observed in the earlier CENP-C/T tethering experiments, artificial nucleation of CENP-A or its chaperone HJURP results in efficient recruitment of kinetochore components that capture spindle microtubules and mediate chromosome movement (*7*, *8*). In these experiments, a new centromere was created in addition to the original one, and this led to mitotic failure and cell death. Although this highlights the detrimental consequences of such dicentric chromosomes, it precludes determining whether these "new-born" centromeres are heritable. Mendiburo *et al*. addressed this by analyzing ectopic, plasmid-based artificial chromosomes that are not essential for cell viability. These can replicate but have no means for active segregation, which leads to their rapid loss from a dividing population of cells. As on chromosomal sites, tethering of CenH3 to LacO-containing plasmids led to the recruitment of kinetochore proteins and microtubule binding. Seeding of such centromeres required LacI-bound CenH3 to be present only transiently. This pool was subsequently replaced by endogenous self-replicating pools of CenH3 nucleosomes, which allowed the ectopic plasmid-based centromeres to be maintained for over a month in cultured cells. Thus, centromeric chromatin is not only required for centromere function, but its creation is sufficient to nucleate a centromere and render it heritable.

Although seeding of CenH3/CENP-A allows small episomal plasmids to be propagated for some time, it is unclear whether it can support heritable centromeres on chromosomal sites and how natural neocentromeres form. Forced expression of CENP-A can trigger neocentromere function primarily adjacent to preexisting heterochromatin (regions of the genome where gene expression is generally suppressed) (*11*, *13*, *14*). Although naturally occurring human neocentromeres appear to lack heterochromatin (*15*), this suggests that a particular local chromatin environment permissive for CENP-A assembly is required for neocentromere formation. The experimental seeding and subsequent inheritance of the centromere implies that CENP-A is central to a self-templating positive-feedback loop. What remains is to demonstrate the components that are part of such a loop and how it works.

References

1. P. E. Warburton, *Chromosome Res.* **12**, 617 (2004).
2. W. C. Earnshaw, B. R. Migeon, *Chromosoma* **92**, 290 (1985).
3. K. F. Sullivan, M. Hechenberger, K. Masri, *J. Cell Biol.* **127**, 581 (1994).
4. P. E. Warburton *et al*., *Curr. Biol.* **7**, 901 (1997).
5. L. E. T. Jansen, B. E. Black, D. R. Foltz, D. W. Cleveland, *J. Cell Biol.* **176**, 795 (2007).
6. D. K. Palmer, K. O'Day, R. L. Margolis, *Chromosoma* **100**, 32 (1990).
7. M. J. Mendiburo, J. Padeken, S. Fülöp, A. Schepers, P. Heun, *Science* **334**, 686 (2011).
8. M. C. Barnhart *et al*., *J. Cell Biol.* **194**, 229 (2011).
9. K. E. Gascoigne *et al*., *Cell* **145**, 410 (2011).
10. A. Guse, C. W. Carroll, B. Moree, C. J. Fuller, A. F. Straight, *Nature* **477**, 354 (2011).
11. A. M. Olszak *et al*., *Nat. Cell Biol.* **13**, 799 (2011).
12. A. A. Van Hooser *et al*., *J. Cell Sci.* **114**, 3529 (2001).
13. H. D. Folco, A. L. Pidoux, T. Urano, R. C. Allshire, *Science* **319**, 94 (2008).
14. K. Ishii *et al*., *Science* **321**, 1088 (2008).
15. A. Alonso, D. Hasson, F. Cheung, P. E. Warburton, *Epigenetics Chromatin* **3**, 6 (2010).

10.1126/science.1218162

BIOCHEMISTRY

Enzymes in Coherent Motion

A single-molecule approach reveals coherence in long trajectories of enzyme conformational dynamics.

H. Peter Lu

Subtle conformational changes often play a crucial role in enzyme functions, and enzyme conformations are highly dynamic. The use of only a static structural characterization from an ensemble-averaged measurement at equilibrium is therefore often inadequate for predicting dynamic conformations and understanding correlated enzyme functions. Single-molecule approaches (*1*), which investigate individual molecules under specific physiological conditions, are a powerful tool for characterizing and analyzing complex enzymatic reaction dynamics and correlated conformational dynamics. On page 319 of this issue, Choi *et al*. (*2*) report a novel approach to probing single-molecule conformational dynamics that reveals the conformational motions of the enzyme's active site during enzymatic reaction turnovers.

The authors studied T4 lysozyme, an enzyme that catalyzes the hydrolysis of the glycosidic bonds in the polysaccharide found in bacterial cell walls (*3*). During the enzymatic reaction, an active substrate-enzyme complex forms, followed by chemical transformation and product release (see the figure, panel A). Choi *et al*. attached the T4 lysozyme molecule to a single-walled carbon nanotube (SWNT) device (see the figure, panel B). In their single-molecule enzymatic reaction assay, enzyme molecular motions create changes in electrostatic potentials that can be converted and amplified into dynamically changing electron fluxes. Using this approach, the authors were able to record hinge-bending motions of the T4 lysozyme active site under enzymatic reactions with high time resolution and signal clarity.

The authors detected two different electronic signals caused by the lysozyme hinge-bending motions: Slow signal oscillations result from the transduction of catalytic turnover events at ~15 Hz, and fast signal oscillations correspond to nonproductive binding events at ~330 Hz. These results are in good agreement with earlier results from single-molecule fluorescence resonance energy transfer (smFRET) spectroscopy under enzymatic reactions (see the figure, panel C) (*4*–*6*). This is encouraging, as the conclusions are reached from very different

Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, OH 43403, USA. E-mail: hplu@bgsu.edu

Downloaded from http://science.sciencemag.org/ on May 12, 2020

Published by AAAS

Science

Sowing the Seeds of Centromeres

Lars E. T. Jansen

Science **335** (6066), 299-300.
DOI: 10.1126/science.1218162

ARTICLE TOOLS http://science.sciencemag.org/content/335/6066/299

REFERENCES This article cites 15 articles, 7 of which you can access for free
http://science.sciencemag.org/content/335/6066/299#BIBL

PERMISSIONS http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.

Copyright © 2012, American Association for the Advancement of Science

Downloaded from http://science.sciencemag.org/ on May 12, 2020