Epigenetics: Sowing the seeds of centromeres

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The centromere is the chromatin-based platform which accumulates microtubule binding proteins during mitosis that in turn drive chromosome segregation. Despite their size (in the order of megabases in mammals) and their conserved role, centromeres are known to occasionally leave their usual comfort zone and "jump" to a novel chromosomal site *(1, 2)*. These so-called neocentromeres are by most measures *bona fide* centromeres that produce productive sites for microtubule attachment and are segregated with high fidelity. A clue to this perplexing nomadic behavior came from the identification of a histone H3 variant, named CENP-A (or CenH3) which is incorporated into nucleosomes specifically at the centromere region *(3–5)*. Histones bind DNA in a non-sequence specific manner and as such are ideal candidates to maintain local chromatin identity largely independent of the underlying DNA sequence. This notion led to the proposal that instead of a unique DNA sequence signature it is the presence of CENP-A containing nucleosomes that identifies the position of the centromere *(6, 7)*. Consisted with this, CENP-A is a very stable component of centromeric chromatin that can survive through multiple mitotic divisions *(8)* and even appears to evade epigenetic reprogramming in the germ line *(9)*.

A strong prediction of the behavior of an epigenetic system is the ability to nucleate a structure or "mark" that in turn is propagated independent 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. Centromeres were like the rocks on the Racetrack playa in California´s Death Valley. We know they can move but no-one ever caught them in the act of moving. A recent study in this journal *(10)* now provides a plausible solution to the analogous problem in centromere mobility. They, along with another report by Foltz and colleagues *(11)* go beyond the phenomenology of human neocentromeres and experimentally induce the formation of a new centromere.

Both studies, one in Drosophila S2 cells and one in Human tissue culture cells employ chromosomally integrated arrays of bacterial Lac operator (LacO) sequences which form a recruitment platform for the ectopically expressed Lac repressor protein (LacI). The Drosophila study takes the bold approach of simply fusing Drosophila CenH3 (also known as CID) directly to the LacI protein. They tether it to the LacO site far removed for endogenous centromeres. Although highly artificial, these CenH3 molecules, literally dragged in by their tails, assemble locally into nucleosomes. Critically, they in turn

recruit more CenH3 that is not pulled in by LacI tethering but rather as a consequence of the initial CenH3 pool and expands laterally beyond the LacI binding sites. This demonstrates a positive feedback mechanism where CenH3 can be recruited to a "naïve" chromatin site in a manner dependent of CenH3 already there. Epigenetic inheritance in a nutshell.

The Foltz team takes a slightly different approach. Here, it is not CENP-A (human CenH3) that is forced onto the LacO domain but rather HJURP, a chaperone they show acts as a *bona fide* CENP-A specific chromatin assembly factor. Indeed, creating a local concentration of HJURP at a novel site is sufficient to nucleate CENP-A chromatin on the LacO array *(11)*. HJURP is the seed that sows the epigenetic centromere. It does not form a part of the stable centromere structure but its transient presence is sufficient to kick-start the process.

Is the formation of an ectopic CenH3 chromatin domain sufficient to nucleate a functional centromere? An earlier report from Cheeseman and colleagues used the same powerful LacO technique, to co-tether CENP-C 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 members of the centromere as well as proteins that are part of the kinetochore, the microtubule binding complex driving chromosome segregation during mitosis *(12)*. Indeed, at least transiently, this naive locus now becomes the site that power chromosome movement suggesting that the primary role of CENP-A nucleosomes upstream is to recruit CENP-C and –T to the centromere. These findings were further extended by recent work *in vitro* demonstrating that an array of CENP-A nucleosomes is sufficient to nucleate a functional kinetochore in Xenopus extracts *(13)*.

Previous attempts to generate neocentromeres in cells by simple CENP-A overexpression were met with mixed results, with success in Drosophila *(14)* but failure in human cells *(12, 15)*. However, similar to ectopic nucleation of CENP-C and –T *(12)*, direct tethering of CenH3 *(10)* or its chaperone HJURP *(11)* resulted in efficient recruitment of all centromere and kinetochore components tested that in turn can mediate efficient capture of microtubules emanating from the mitotic spindle and drive chromosome movement. In effect, a dicentric chromosome with two active centromeres is created. While the consequent mitotic failure and cell death highlights the detrimental consequences of such dicentric chromosomes, they preclude determining whether these new-born centromeres are in fact, heritable. The work by the Heun laboratory solved this conundrum by analyzing ectopic, plasmid-based artificial chromosomes that are not essential for cell viability *(10)*. These can replicate but have no means for active segregation during mitotic division leading to rapid loss from a dividing population. As on chromosomal sites, tethering of CenH3 to LacO containing plasmids leads to the recruitment of kinetochore proteins, microtubule binding and indeed to the stable inheritance. Importantly, seeding of such centromeres requires LacI-bound CenH3 to be present only transiently. This pool is subsequently replaced by endogenous self-replicating pools of CenH3 allowing the ectopic plasmid-based centromeres to be maintained for over a month in culture. This solves a long standing question in the

centromere field, and indeed in the broader field of epigenetics. Centromeric chromatin is not only required for centromere function but its creation is sufficient to nucleate a centromere and render it heritable.

What are the outstanding questions? Although seeding of CenH3 allows small episomal plasmids to be propagated, it is unclear whether it would be sufficient to support centromeres on chromosomal sites. The Heun team has recently reported that force expression of CenH3 in Drosophila cells triggers neocentromere function primarily adjacent to pre-existing heterochromatin suggesting a role for these domains *(14)* in centromere formation as previously demonstrated in fission yeast *(16, 17)*. Although naturally occurring human neocentromeres appear to lack heterochromatin *(18)*, this raises the question as to whether the converse can occur as well and a CenH3 triggered neocentromere can recruit heterochromatin to solidify its fate. Lastly, the seeding and subsequent inheritance of the centromere now shown by Mendiburo et al implies that CenH3 is central to a self-templating positive feedback loop. What remains, is to demonstrate what components are part of such a loop and how it works.
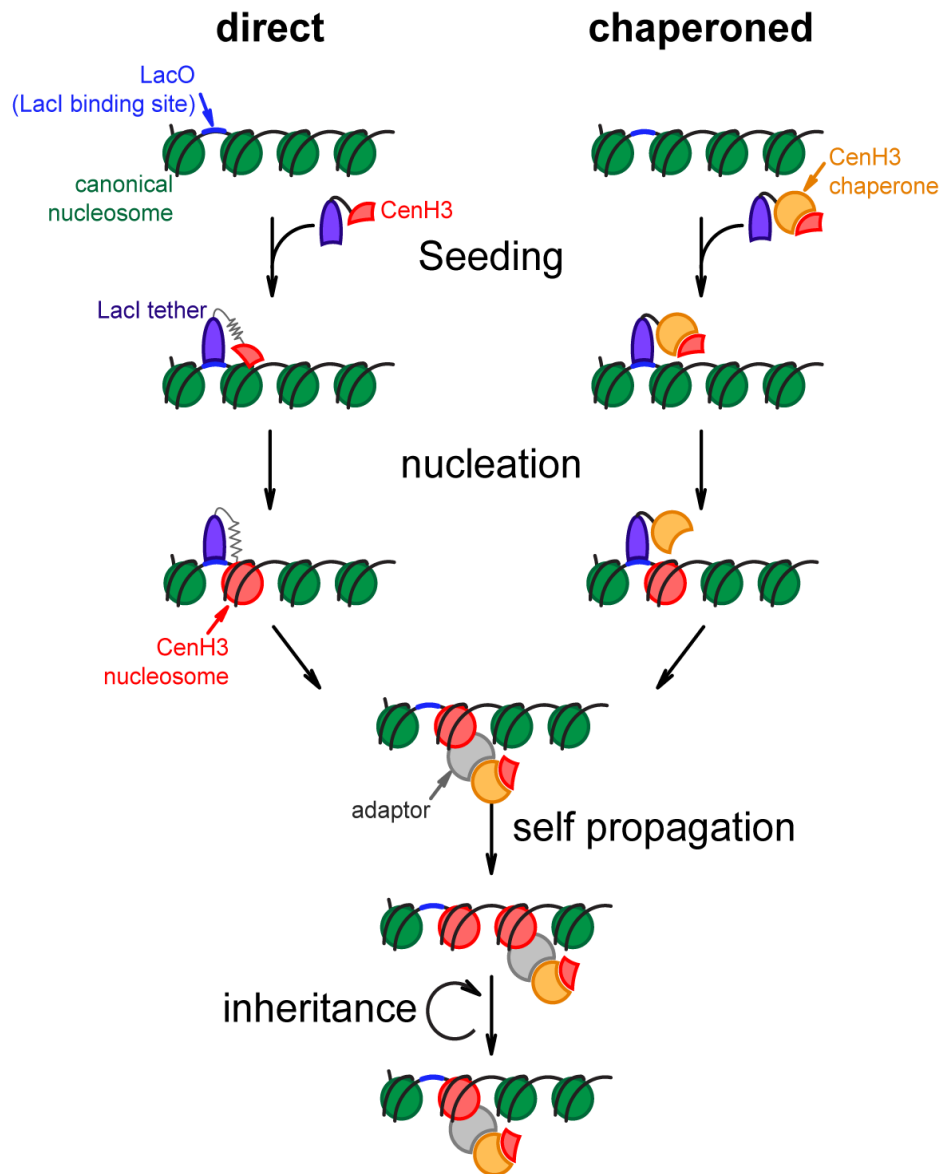
Figure:

Seeding and propagation of the epigenetic centromere. CenH3 is targeted to a naive chromatin locus either by direct fusion to locally bound LacI or through recruitment by the LacI-tethered chaperone HJURP. In either case artificially seeded CenH3 results in the nucleation of CenH3 nucleosomes. These in turn trigger the propagation of CenH3 chromatin in a self-templating manner (likely through an adaptor intermediate) without the need for the initial LacI-seed. Once formed, CenH3 chromatin propagation and turnover through cell divisions reach an equilibrium resulting in stable inheritance of the epigenetic centromere mark. Note that the nature of the adapter is outside the scope of this perspective and is depicted here as a hypothetical molecule or complex that links CenH3 nucleosomes to new CenH3 histones either directly or indirectly.

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