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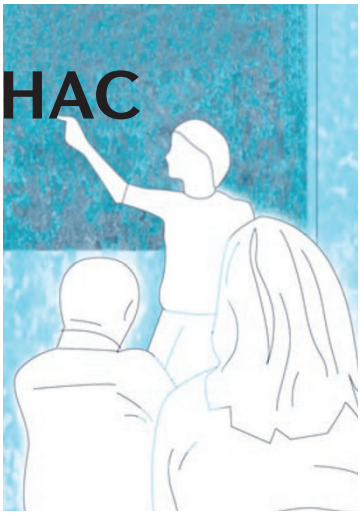
CHROMOSOMES

Introducing HAC



Kinetochores are essential for proper chromosome segregation. The site of kinetochore assembly has been proposed to be controlled by epigenetic mechanisms; however, the chromatin 'signature' of human kinetochores has remained elusive. Nakano *et al.* now describe a novel human artificial chromosome (HAC) that allows for the first time the manipulation of the epigenetic state of chromatin within an active kinetochore. They show that heterochromatin induction at the centromeres abolishes kinetochore function.

Previously, artificial chromosomes that were introduced into human cells contained authentic α-satellite sequences — repetitive DNA that is found at the centromeres of human cells — that contained a DNA-binding site for the centromere protein CENP-B (the CENP-B box). The newly engineered HAC is based on a synthetic repeated DNA sequence called the alphoid^{tetO} dimer. One part of the dimer consists of the natural α-satellite containing the CENP-B box sequences; the other part contains a synthetic α-satellite sequence with the CENP-B box replaced by a tetracycline repressor (tetR) binding site (tetO), which can be used to target desired tetR fusion proteins into the kinetochore.

First, the authors confirmed that the alphoid^{tetO} HAC assembled with kinetochore proteins and segregated correctly as an independent chromosome during mitosis. Fluorescent proteins fused to tetR were efficiently targeted to alphoid^{tetO} and did not affect kinetochore function. Furthermore, chromatin immunoprecipitation (ChIP) analysis showed that key markers of centromeric chromatin, such as the centromere-specific histone variant CENP-A and dimethylated Lys4 on histone H3 (H3K4me2), were present at the alphoid^{tetO} HAC.

Next, Nakano *et al.* studied the effect of altering the centromeric chromatin state on kinetochore function by targeting a transcriptional activator (tTA), which leads to an open chromatin conformation, or a repressor (tTS), which induces a closed chromatin structure, to the alphoid^{tetO}. In both cases, the chromatin alterations caused mis-segregation and loss of HAC, although tTA had a much milder effect on kinetochore function than tTS. Interestingly, these results support recent cytological observations that suggest that heterochromatin is not compatible with kinetochore function. To further confirm these findings, Nakano and colleagues showed that binding of tTS to alphoid^{tetO} increased the level of repressive chromatin marks at the centromeres and abolished the binding of CENP-A. Targeting of the heterochromatin protein-1α (HP1α) — a non-histone protein that is involved in heterochromatin formation and gene silencing in many organisms — to the alphoid^{tetO} was by itself sufficient to disrupt kinetochore structure and function.

The use of the alphoid^{tetO} HAC will enable the systematic alteration of the 'histone code' within the kinetochore, which has long remained inaccessible to direct targeted manipulation, and the definition of how the epigenetic signature of centromere chromatin affects kinetochore function.

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ORIGINAL RESEARCH PAPER Nakano, M. *et al.* Inactivation of a human kinetochore by specific targeting of chromatin modifiers. *Dev. Cell* **14**, 507–522 (2008)
FURTHER READING Cheeseman, I. M. & Desai, A. Molecular architecture of the kinetochore–microtubule interface. *Nature Rev. Mol. Cell Biol.* **9**, 33–46 (2008)