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The Path of DNA in the kinetochore

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The kinetochore is the protein-DNA complex at eukaryotic centromeres that functions as the attachment site for spindle microtubules. In budding yeast, the centromere spans 120 bp, there is a single microtubule per kinetochore, and the entire spindle is composed of 16 kinetochore microtubules plus four interpolar microtubules from each pole. There are >65 different proteins at the kinetochore, organized in at least six core multimeric complexes [1]. A spindle checkpoint network monitors the state of attachment and tension between the microtubule and chromosome. We present a model for the path of DNA in the kinetochore.

Replicated sister centromeres become maximally separated by 600-800 nm in metaphase [2]. Separation progressively decreases along chromosome arms such that sister chromatids are tightly juxtaposed at ~10 kb from the centromere [2]. The molecular glue linking sister chromatids, cohesin, is recruited to a 20-50 kb region surrounding the centromere at 3- to 5-fold higher levels than centromere-distal locations [3]. A major paradox is the accumulation of cohesin at regions of separated sister DNA strands. A second problem is the nature of the mechanical linkage coupling DNA to a dynamic microtubule plus-end. This linkage must resist detachment by mitotic forces while sliding along the polymerizing and depolymerizing microtubule lattice.

We propose that pericentric chromatin is held together via intramolecular cohesion (Figure 1), similar to a foldback structure proposed for the fission yeast centromere [4]. In contrast to fission yeast, the budding yeast core centromere (120 bp DNA wrapped around a specialized nucleosome containing two molecules of the centromere-specific histone H3 variant, Cse4) and flanking chromatin may adopt a cruciform configuration in metaphase.

Centromeric DNA is sharply bent around the Cse4 nucleosome by the CBF3 protein complex [5], forming the apex of the putative centromere-loop (C-loop). The C-loop would be approximately 22 nm in diameter (twice the diameter of a nucleosome) and held together through intramolecular cohesin bridges (Figure 1). To account for the measured distance between replicated sister centromeres, a transition zone 7-8 kb from the centromere-specific nucleosome marks the conversion from intra- to inter-molecular bridges. 7-8 kb of DNA wound 1.65X around the histone octamer is approximately 300-400nm long (~2.3 μm of B-form DNA, or 7- fold nucleosomal compaction). The proposed intramolecular linkage is therefore consistent with the appearance of separated centromeres, the apposition of DNA markers 10 kb from the centromere, and the increased concentration of cohesin at the centromere. Two alternative forms of cohesin have recently been proposed [6], perhaps reflecting the different substrates dictated by centromere-flanking chromatin vs. chromosome arms.

The budding yeast centromere is unique in having a single Cse4-containing nucleosome [7]. We derived a structural model of the centromeric nucleosome to evaluate whether the path of DNA around the nucleosome core particle is compatible with the C-loop (see Figure S1 in Supplemental Data published with this article online). The model structures of Cse4 and the centromeric nucleosome core particle are deposited in the protein data bank at http://

www.rcsb.org (PDB ID code 2FSB and 2FSC, respectively). The highly charged Cse4 tails are clustered at the exit and entry sites of the nucleosome, where they may restrict the mobility of the nucleosome as well as promote intramolecular cohesion by bending linker DNA (see the model in Supplemental Data). Thus Cse4, together with CBF3, may stabilize the nucleosome core and direct the path of the DNA as it enters and exits the nucleosome.

This model predicts that Cse4 (a CENP-A homolog) is proximal to the microtubule plus-end. The CENP-A homologs in *D. melanogaster* (CID) and *C. elegans* (HCP-3) face poleward on the mitotic chromosome 8 and 9 [8]. However, unlike a single Cse4-containing nucleosome in budding yeast, CENP-A nucleosomes are interspersed with blocks of histone-H3 nucleosomes [9]. The degree of DNA bending as DNA enters and exits the canonical CENP-A nucleosome (see Figure S1) may dictate whether single or multiple CENP-A nucleosomes comprise the kinetochore. CENP-A is highly divergent [10], indicating potential changes in its molecular architecture in different species.

Several specialized chromosome domains are organized into loop structures, including the T-loop of telomere DNA [11] and the DNA loops that characterize lampbrush chromosomes. Evidence for a centromeric DNA loop can be found in a deletion analysis of dicentric chromosomes ([12] and J.A. Brock, unpublished observations), which undergo a breakage-fusion-bridge cycle leading to chromosome rearrangements, with the predominant outcome being loss of one entire centromere and flanking DNA. Deletions arising from two DNA double-strand breaks within the C-loop are consistent with these findings. Thus, similar to the 8-kb deletion blocks of T-loops at the telomere [11], *in vivo* deletions that remove large domains of one centromere from dicentric chromosomes are indicative of loss of a structural element.

A corollary of the model is that the tip of the C-loop may be mobile relative to the chromosome axis (Figure 2). A change in the position of the transition zone relative to the centromere-specific nucleosome will alter the position of the C-loop's distal end. The C-loop tip will migrate toward the transition zone tip as interstrand cohesion is favored, and away from the transition zone as intrastrand cohesin is favored. The range of force generated by the microtubule is on the order of that required to alter the transition zone position and hence the spatial position of the C-loop (see Figure S2 and Supplemental Data). We predict that change in the position of the C-loop tip will coincide with change in the position of kinetochore microtubule plus-ends [13]. Thus, while the mechanisms are completely different, both 'ends' of the C-loop and the kinetochore microtubule are dynamic, a feature of the kinetochore that may contribute to the tension-based surveillance system.

Inducing mammalian cells to enter mitosis with unreplicated genomes [14] has allowed dissection of the kinetochore's subunit structure. Each of the 25-30 microtubule-binding sites in a mammalian kinetochore can be detached from the chromosome and still maintain an autonomous structure that includes DNA [14]. These data suggest the mammalian kinetochore is comprised of a repeating DNA-protein structural unit that is autonomous in its ability to form a C-loop and bind single or multiple microtubules. The C-loop may insert into a cylindrical kinetochore structure that encompasses both DNA and the microtubule. The C-loop predicted by our model in *S. cerevisiae* would thus represent the fundamental unit of the kinetochore across phylogeny.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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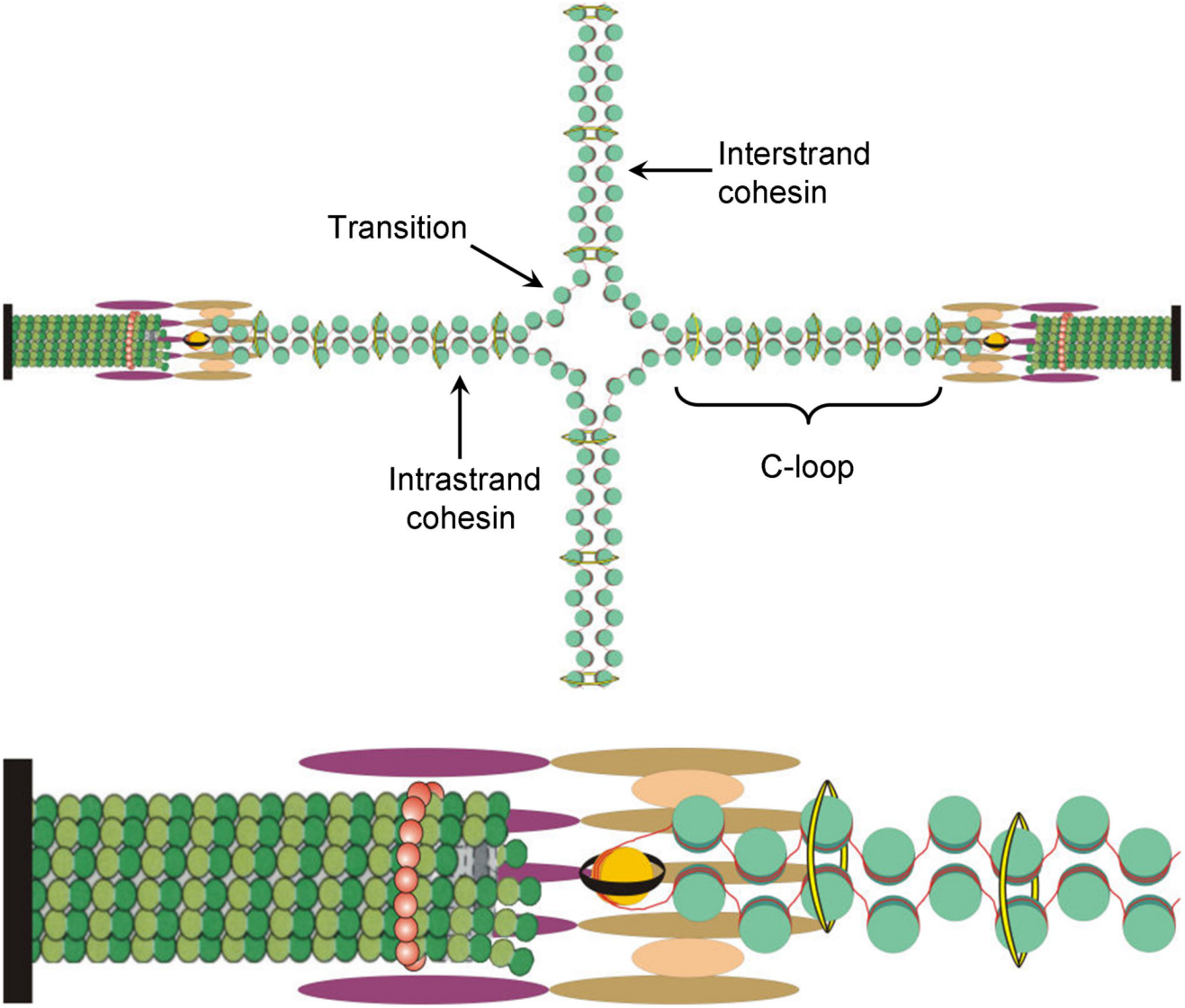

Figure 1.

Proposed structure for centromere DNA in the kinetochore. (Top) Bi-oriented sister chromatids adopt a cruciform structure. Centromere-flanking chromatin is held together by intrastrand cohesin bridges, and chromosome arms by interstrand cohesin bridges. The transition between these two regions in budding yeast is mobile and on average 7 kb from the centromere core. (Bottom) The Cse4-containing nucleosome (orange circle) and flanking nucleosomes (green circles) are proximal to the microtubule plus-end. The microtubule (left) is encompassed by the Dam1 ring (pink) [15,16] and elongated Ndc80 rods (purple)[17]. Binding of CBF3 complex (black), bends centromere DNA ~55° [5], forming a C-loop of chromatin held together by intrastrand cohesin (yellow rings). Additional kinetochore complexes (Coma and Mind in tan and blue, respectively) are proposed to link CBF3 and the C-loop to Ndc80, Dam1, and other linker complexes at the microtubule plus-end.

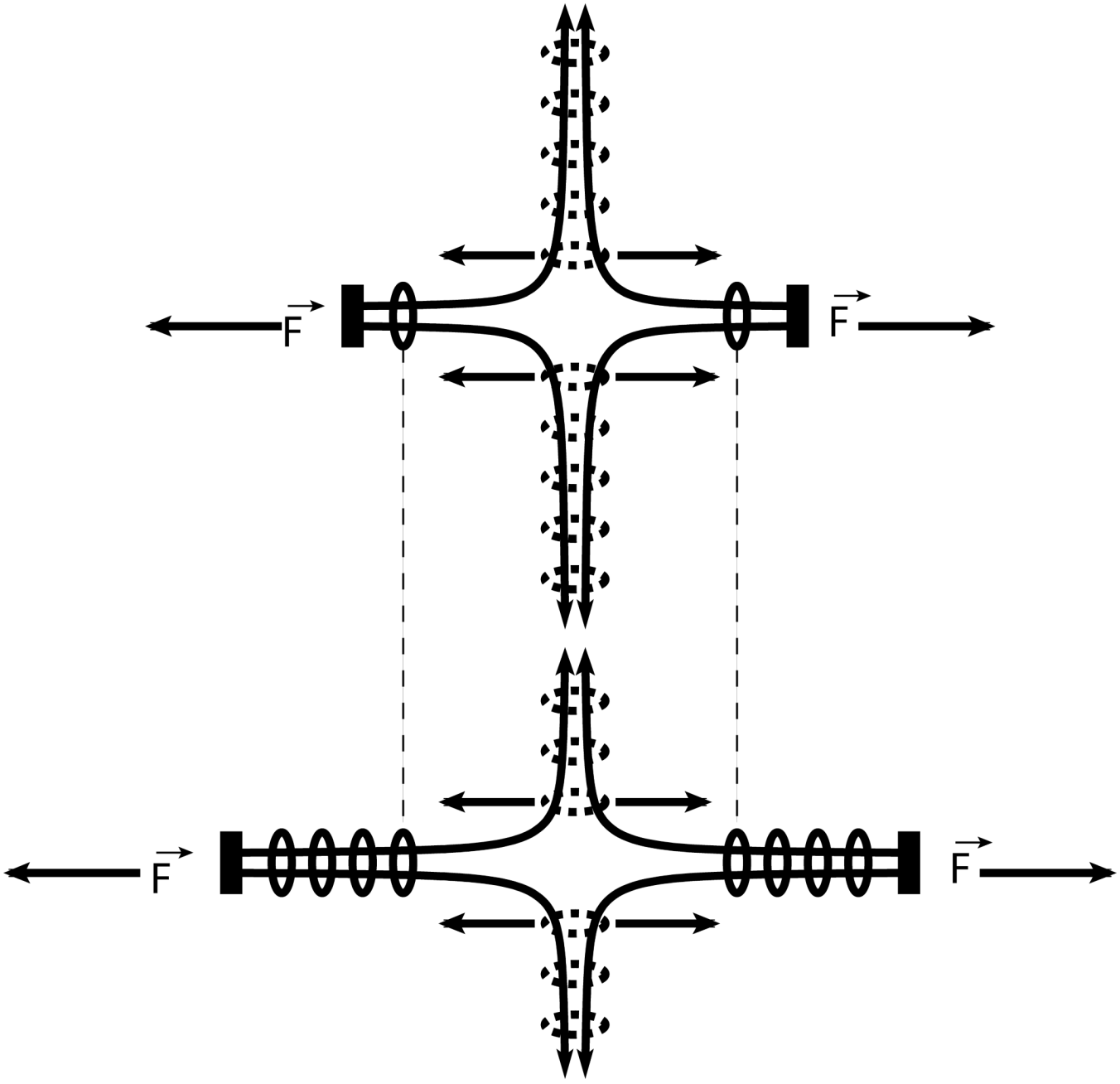

Figure 2.

Positional instability of the C-loop.We propose that cohesins (rings) form complexes on sister chromatids in both lateral (interstrand) and longitudinal (intrastrand) directions relative to the direction of microtubule-based forces (arrows). Intrastrand cohesins clamp the C-loop, facilitating its elongation and movement of centromere ends (black rectangles). Forces ($\vec{F}$ vector displacement) from attached microtubules act predominantly in the lateral direction, destabilizing interchromatid cohesins (see Figure S2). Fluorescence imaging techniques demonstrate that centromere reassociation during mitosis is infrequent ($v = 0.4\%$ of experiment time) [2] and predict elongation of the ends proximal to microtubules with a velocity of ~1 μm/min [2]. This observation suggests that the dynamic equilibrium between disruption of interchromatid cohesion and formation of intrachromatid cohesin tethers is shifted towards the

latter process (compare schematics above and below), and predicts a higher density of intrachromatid cohesin bridges along the flanking chromatin. Addition of intrastrand cohesins progressively tethers the region between transition zone and centromere ends, thereby facilitating lateral elongation of centromere ends. The cohesion-free region is fluctuating around the same mean value (denoted by dashed lines), governed by the balance between the cohesive forces and microtubule-induced forces (see discussion in Supplemental Data). From the frequency and magnitude of separation previously observed between sister kinetochores in live cells, we estimate the stabilization caused by the kinetics of conversion of inter- to intra-chromatid cohesin tethers as $\Delta\Delta G_{elong} \sim -RT \ln(l/v) \approx -3.5$ kcal mol^{-1} (see Supplemental Data). We expect the actual stabilization to be larger than $\Delta\Delta G_{elong}$ due to the limits in resolution of fluorescence microscopy. Thus, centromeric cohesin complexes may have a direct functional role in stabilizing the elongating centromere instead of producing an opposing force against pulling by microtubules. Upon loss of force the sister centromeres are predicted to return to the lowest free energy state, that of interstrand sister chromatid cohesion.