Supplemental Data: The path of DNA in the kinetochore

Kerry Bloom, Shantanu Sharma, and Nikolay Dohkolyan

Supplemental Results

Homology model of centromeric nucleosome core particle

A structural model of the centromeric nucleosome core particle was determined by substituting H3 histones in the known crystal structure of the *S. cerevisiae* nucleosome core particle [S1], with a homology model [S2] of Cse4, and replacing the palindromic 146 bp nucleosomal DNA sequence with centromeric DNA sequence [S3]. Cse4 was modeled by combining homology models of its histone-fold domain (HFD, residues 132-229) and essential N-terminal domain (END, residues 1-66). In the modeled Cse4 variant nucleosome structure, basic lysine and arginine residues in the N-terminal domain form globular structures and interact with negatively-charged DNA backbone phosphates present at the junction between linker and nucleosomal DNA (Figure S1). This is a major divergence from nucleosome core particle with histone H3, where the N-terminal histone tails are largely unstructured random coils. The highly-charged Cse4 tails are clustered at the exit and entry sites of the nucleosome, where they may restrict the mobility of Cse4 nucleosomes, as well as promote intramolecular cohesion by bending linker DNA. The Cse4 nucleosome is unique in that it is flanked by very broad (30-50 bp) hypersensitive nuclease cleavage sites [S4]. This linker DNA is susceptible to nucleolytic attack in the transition zone between the Cse4-containing nucleosome and H3-containing chromatin. Thus, Cse4 may stabilize the nucleosome core and direct the path of the DNA as it enters and exits the nucleosome. The Cse4 nucleosome represents a physiochemical interruption in chromatin organization, contributing to the unique assembly features dictated by this chromosomal locus. The model structures of Cse4 and centromeric nucleosome core particle have been deposited in the protein data bank at http://www.rcsb.org (PDB ID code 2FSB and 2FSC, respectively).

Qualitative estimates of forces on the C-loop

Cohesin rings have a large diameter (~400 Å) relative to mononucleosomes (~100 Å). We hypothesize that interactions between spatially separated interchromatid strands are dominated by strong salt-bridges formed between basic (lysine/arginine) side-chains of elongated histone tails and phosphate (DNA) of the sister chromatid strands. We have modeled the distribution of inter vs. intrastrand cohesion by the increase in these stable salt-bridge contacts formed in the C-loop (Figure 2). Fluctuations in the number of effective inter-chromatid interactions (σ_n) along the chromosome arm axis result in fluctuations in separation between the ends proximal to microtubules, σ. As intrastrand cohesins accumulate, they progressively tether flanking nucleosomes and facilitate elongation of the C-loop. In the course of this elongation, the work done by microtubule-based forces (F) is $F\sigma$ and the loss of free energy due to disruption of inter-chromatid salt bridges is $\sigma_n \Delta\Delta G_{sb}$ where $\Delta\Delta G_{sb}$ is the free energy of breaking a single inter-chromatid salt bridge. The work done by microtubule forces must compensate for the loss of favorable salt bridges and the energy associated with thermal fluctuations: $F\sigma \sim \sigma_n \Delta\Delta G_{sb} + k_B T$. However, since fluctuations in centromere separation caused by

microtubule forces $F\sigma$ far exceed thermal fluctuations $k_B T$, $\sigma \sim \left|\sigma_n \Delta\Delta G_{sb} / F\right|$. Assuming each nucleosome in the 10-kb stretch around the centromere makes a single dominant salt bridge with the sister chromatid, then σ_n is ~100 (10 kb/200 bp per nucleosome x 2 = 100). Surface salt-bridge interactions between lysine-rich histone tails, as well as $\Delta\Delta G_{sb}$, depend on salt concentration [S5], and are on the order of 10-60 kcal mol^{-1} for DNA-integration host factor complexes [S6]. If we estimate $\Delta\Delta G_{sb}$ for inter-chromatid interactions as ~20 kcal mol^{-1}, our model predicts that a F~20 pN force will lead to large positional fluctuation (σ~500 nm) of the distal end of the C-loop relative to the chromosome axis. A single microtubule has been estimated to generate at least 10 pN of force [S7,S8]. Thus, the range of force generated by the microtubule is on the order of that required to alter the position of the transition zone, and hence the distance between ends of the C-loop.

Acknowledgements
We thank Julian Haase for artistic assistance and members of the Bloom laboratory for helpful discussions. This work is supported in part by the funding from the NIH GM32238 (KB) and the Muscular Dystrophy Association MDA3720 and March of Dimes Birth Defect Foundation (5-FY03-155)(NVD).

Supplemental References

S1. White, C.L., Suto, R.K., and Luger, K. (2001). Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions. EMBO J. *20*, 5207-5218.

S2. Sali, A., Potterton, L., Yuan, F., van Vlijmen, H., and Karplus, M. (1995). Evaluation of comparative protein modeling by MODELLER. Proteins *23,* 318-326.

S3. Keith, K.C. and Fitzgerald-Hayes, M. (2000). CSE4 genetically interacts with the S. cerevisiae centromere DNA elements CDE I and CDE II but not CDE III. Implications for the path of the centromere DNA around a Cse4p variant nucleosome. Genetics *156*, 973-981.

S4. Bloom, K.S. and Carbon, J. (1982). Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. Cell *29*, 305-317.

S5. Thastrom, A., Lowary, P.T., and Widom, J. (2004). Measurement of histone-DNA interaction free energy in nucleosomes. Methods *33,* 33-44.

S6. Holbrook, J.A, Tsodikov, O.V., Saecker, R.M., and Record, M.T., Jr (2001). Specific and non-specific interactions of integration host factor with DNA: thermodynamic evidence for disruption of multiple IHF surface salt-bridges coupled to DNA binding. J. Mol. Biol. *310*, 379-401.

S7. Nicklas, R.B.(1983). Measurements of the force produced by the mitotic spindle in anaphase. J. Cell Biol. *97*, 542-548.

S8. Grishchuk, E.L., Molodtsov, M.I., Ataullakhanov, F.I., McIntosh, J.R. (2005). Force production by disassembling microtubules. Nature *438*, 384-388.

S9. Ginalski, K., Elofsson, A., Fischer, D., and Rychlewski, L. (2003). 3D-Jury: a simple approach to improve protein structure predictions. Bioinformatics *19,* 1015-1018.

S10. Eisenberg, D., Luthy, R., and Bowie, J.U. (1997). VERIFY3D: assessment of protein models with three-dimensional profiles. Methods Enzymol. *277,* 396-404.

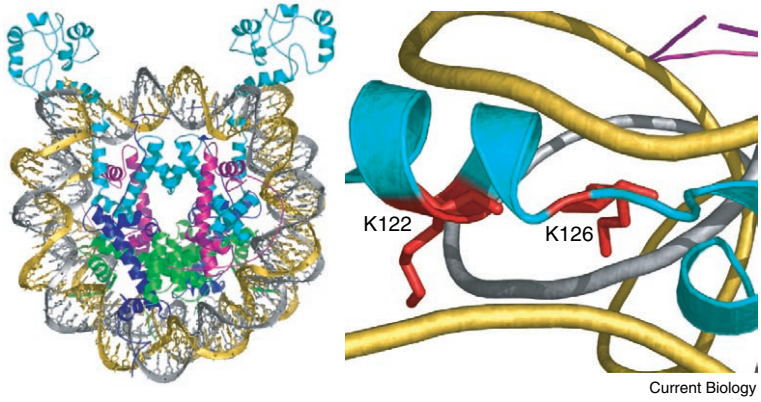

Figure S1. A structural model of the Cse4 nucleosome**.**
(Left) Cse4 (cyan), DNA (gold/silver), and histones H2A (blue), H2B (green), H4 (pink), are shown. Cse4 is modeled by combining homology models [S2] of the histone-fold domain (HFD, residues 132-229) and essential N-terminal domain (END, residues 1-66). Sequence alignments of the two domains are based on the consensus alignment predicted by the 3D-Jury Structure Prediction meta-server [S9]. 3D-Jury predicts HFD, END domains have most significant homology with histone H3 (PDB: 1ID3-A) and the N-terminus of 1,6 phosphofructokinase (PDB: 1BIF), respectively, and no significant homolog is predicted for the intermediate region (residues 67-131). The combined Cse4 model was energy-minimized using the molecular dynamics simulation procedure of the program Insight-II (Accelrys Software Inc). Evaluation of the resulting Cse4 model using Verify3d [S10] indicated that molecular geometry and stereochemistry of the Cse4 model is of good quality. The histone-fold domain of Cse4 is structurally superimposed with the known crystal structure of H3 histones present in *S. cerevisiae* nucleosome core particle (PDB: 1ID3-A, 1ID3-E) to yield the model structure of centromeric nucleosome core particle. (Right) Zoomed-in view of Cse4 interactions with centromeric DNA. Lys122 and Lys126 (red) of modeled Cse4 interact with the termini of nucleosomal DNA (gold/silver).

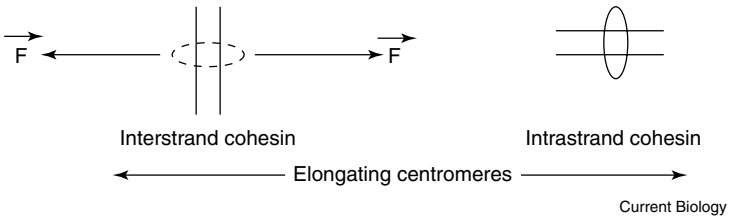

Figure S2. Comparison of interstrand and intrastrand cohesin forces.
Interstrand cohesin forces act parallel to the microtubule axis and perform work when Cse4-containing nucleosomes move away from the chromosomal axis. Intrastrand cohesin forces act perpendicular to the direction of movement of Cse4-containing nucleosomes, and do not perform work. Intrastrand cohesin progressively clamps elongating intrachromatid pairs while interstrand cohesin rings supply the restoring force opposing microtubule-based forces.