X-ray study of nucleosome organization in single mitotic chromosomes

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Background: The large-scale scale (> 10 nm) organization of DNA inside cells is not well understood, and is biologically important. In eukaryote cells, DNA is organized into repeated units called nucleosomes, each of which is a roughly 10 nm diameter particle containing 146 base-pairs (50 nm) of DNA, wrapped around an octamer of 'histone' proteins. Despite their large size and molecular weight, nucleosomes can be purified and crystallized, with the result that we know their structure with almost atomic detail. However, at the next level up – scales beyond 10 nm – we know relatively little about how nucleosomes are organized into chromosomes.

A chromosome can be thought of as a long string of nucleosomes, separated by roughly 30 base pair (10 nm) 'linker' DNA segments. Short stretches of these strings, called 'chromatin fibers' can be visualized in the electron microscope. However, electron microscopy is not able to study the dense three-dimensional organization of chromosomes under physiological (hydrated) conditions.

Optical microscopy tells us about large-scale motions and reorganizations of chromosomes, but there is a 'window of ignorance' about chromosome structure for scales between 10 and 250 nm. This is precisely the range of length scales where it is believed that non-histone proteins fold chromatin fiber into chromosomes.

Our group at UIC uses micromanipulation and force microscopy methods to study chromosome structure, focusing mainly on the 'mitotic' or folded form of the chromosome occurring during cell division. The structure of this organized form of the chromosome is again poorly understood at 10 to 250 nm length scales: we have demonstrated that rather straightforward physical experiments can give new (and surprising) information about that structure. Our methods also suggest a strategy for carrying out challenging, but exciting, X-ray studies of mitotic chromosome structure.

Chromosome Micromanipulation: Our lab's approach to mitotic chromosome structure is to isolate single chromosomes from dividing cells, using small glass 'micropipettes' as tools. We pull and cut these pipettes to have tip inside dimension of roughly 2 microns, about the same diameter as the mitotic chromosomes in the cells that we are studying. Our main body of experiments involves use of the mitotic chromosome stretching modulus as a reporter on structural changes that we generate biochemically, via spraying of enzymes or other molecules from an additional pipette.

The mitotic chromosome is very soft condensed matter: the Young modulus of a mitotic chromosome is roughly 100 Pa: compare with the GPa moduli of globular proteins. A mitotic chromosome is also ~1/3 liquid water by volume. Native conditions must be studied: e.g. preparations for studies in vacuum will have disastrous effects on structure (note the relevant surface tension is 10^2 Pa $\cdot$ 10^{-6} m = 10^{-4} N/m).

An example of how this type of experiment can be useful is given by the surprising results of experiments carried out with enzymes that cut DNA at specific sequences. We have found that the native elastic response is first eliminated, and then

the chromosome is completely destroyed, by enzymes which have a 4-base-pair target; however, enzymes with a 5 and 6 base-pair target have no effect. This indicates that the interior structure of the mitotic chromosome is supported by chromatin itself, and that proteins serve to 'cross-link' the chromatin together. This eliminates one popular model for mitotic chromosome structure based on an axial protein 'scaffold' to which 'loops' of chromatin are tethered. The protein component 'mitotic scaffold' is therefore a disconnected structure.

X-ray Study of Single Mitotic Chromosomes: It is intriguing to think about carrying out X-ray study of individual mitotic chromosomes. An attractive objective of such studies is determination of positional correlations of nucleosomes. The interior of the mitotic chromosome can be thought of as a high-density (roughly half by volume) colloidal gel, with the nucleosomes playing the role of 10 nm colloidal particles. The wavelengths relevant to this project are in the range 5 to 100 nm, and might be probed in a scattering experiment with soft X-rays.

We have developed a technique to put single (or a few) mitotic chromosomes into the end of a micropipette, which then acts as a very small sample cell. The micropipettes are 3 micron OD, 2 micron ID, so the mass of sample cell is similar to the mass of chromosome. Note that in this setup, chromosomes are oriented, so that orientational ordering of nucleosomes (suggested by optical studies using polarized light) might be observable.

A major problem is small sample size: one chromosome is 2 by 2 by 20 microns. A requirement for a successful experiment is therefore a highly focused, micron-scale beam plus an experiment where precise optical alignment of a sample with the beam can be carried out. At present we are planning preliminary experiments with 10 nm and 50 nm silica particles in micropipettes at ChemMatCARS, to more precisely quantify the sample size problem.

If it can be developed, the ability to use small-angle scattering to study hydrated samples at the micron scale may have a range of applications in studies of micron-scale samples with nanoscale positional order.

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Further Reading:

Micromechanics of chromatin and chromosomes, J.F. Marko, M.G. Poirier (2003) *Biochem. Cell Biol.* **81**, 209-20 (short review article on chromosome structure).

Mitotic chromosomes are chromatin networks without a contiguous protein scaffold, M.G. Poirier and J.F. Marko (2003) *Proc. Natl. Acad. Sci USA* **99**, 15393-15397 (research report on the micromanipulation/nuclease digestion experiment discussed above).