Abstract:

We present a method for the stretching of chromatin molecules that enables location-resolved optical investigation of the nucleic material with a resolution of about 6 kbp. The method relies on the equilibrium elongation that polymers experience when they are introduced into nanofluidic channels that are narrower than the Flory coil corresponding to the whole chromatin molecule. We investigate whether the elongation of reconstituted chromatin can be described by the de Gennes model, and find fair agreement.

Summary of Research:

The central question in the analysis of chromatin is telling “what” is “where.” In the post-genome era, the question of “what” is transitioning from the chemical identity of bases to the question of biological activity of known bases. We are particularly interested in the epigenetic information encoded in the local chromatin structure, especially the difference between open and closed chromatin and covalent modification of histone tails. While the majority of current studies investigate large cell population by utilizing chromatin immunoprecipitation protocols (ChIP) coupled with hybridization arrays or sequencing, our ultimate aim is the analysis of single, large chromatin molecules. We believe that such analysis is necessary for studying epigenetic variability in heterogeneous samples, such as populations undergoing embryonic or cancer development.

The starting point for our analysis is the fluorescence in-situ hybridization technique. Here, fluorescent markers are used to indicate the presence of marks along chromatin molecules. The central limitation of any such technique is the precision and reliability with which a spatial position in the sample can be attributed to a genetic location, and stretching chromatin obviously increases the genomic resolution. The idea of stretching nucleic acids prior to analysis has been widely exploited for the analysis of bare deoxyribonucleic acid (DNA).

A relatively novel technique is stretching DNA inside nanochannels [1]. Here, DNA is introduced into closed nanochannels with a cross-section comparable to a few persistence lengths or less, typically about 100 × 100 nm², and hundreds of microns long. Balancing of contractile forces, due to the maximization of entropy by random walks, and expansive self-exclusion forces establishes a stretched equilibrium state around which the molecule can fluctuate. Protein-decorated DNA can be used as well, since non-specific sticking due

Figure 1: Time-lapse movies of chromatin assembled from λ-DNA (A) and λ-DNA (B) with equal number of base pairs in nanochannels with a cross-section of about 90 nm. The scale bars are 10 µm.
to protein-wall interaction can be managed [2]. We propose here to use nanochannels to stretch single chromatin molecules.

We introduced bare lambda (λ)-DNA and chromatin formed by complexation of a whole histone mixture with λ-DNA into fused silica nanochannels with a cross-section of about $80 \times 80 \text{ nm}^2$, and 200 µm long (Figure 1). Although both molecules have the same number of base pairs, it is apparent that the chromatin molecule is considerably shortened.

Lengths of confined chromatin and DNA were analyzed by fitting the fluorescence intensity along the polymer backbone to the sum of two error functions of equal magnitude and opposite sign, and subsequent formation of histograms (Figure 2). We find that both distributions show three peaks. For bare DNA, the known physical attribution is that the central peak corresponds to full λ-DNA, the shorter peak stems from sheared DNA, while the longest peak is due to λ-DNA dimers.

In a similar fashion, we can assign peaks to the chromatin histogram, where we note that the most extended molecules also could be λ-DNA with most of the histones stripped off. We find a ratio of 0.4 for lengths of the central peak of the two histograms. Using some assumptions for the physical nature chromatin, we can theoretically predict that the ratio should be 0.3 according to the de Gennes model. We suspect that the discrepancy stems from partial stripping of histones from the DNA backbone upon insertion in the channel, as can be witnessed at the ends of some molecules.

In conclusion, we have shown that chromatin can be stretched in nanofluidic channels in a way similar to DNA, and that a common physical description based on the de Gennes model is plausible. We have also found that the stresses during channel insertion have to be carefully controlled to prevent stripping of histones from the chromatin fiber. We argue that our method has the potential to the basis of a FISH-like epigenetic analysis technique.

References: