Individual Chromatin Fibre: Cryo-electron Microscopy and Optical Tweezers Approach

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Only few experimental techniques exist to study individual chromatin segments *in vitro*. Although they do not allow for extensive statistics, they bring the detailed information about the fibre structural organisation and its mechanical properties. We have used cryo-electron microscopy (CEM) and optical tweezers to study organisation and formation of native and reconstituted chromatin segments. High resolution CEM of linker histone containing segments suggests, that core DNA does not complete two turns on the histone octamer as suggested earlier models, but seems to preserve ~1.7 turns and the linker DNA segments become juxtaposed at ~8nm from the core centre for the distance of few nm. This "stem" organisation is likely to be responsible for the architecture of the chromatin fibre and can explain, using simple model, its compaction in higher ionic concentration.

Using optical tweezers we have investigated elastic properties and kinetics of chromatin segments formed by various reconstitution methods. Our results show that their behaviour is significantly different.

We have also evaluated the kinetics of the chromatin formation in the nuclear extract of *Xenopus laevis.* The chromatin reconstitution in nuclear extract is considered as rather slow procedure giving a good yield of chromatin structures only after several tens of minutes. Recently, however, some results has been reported suggesting much shorter times [1]. We have investigated the kinetics of the chromatin reconstitution in *Xenopus laevis* nuclear extract using optical tweezers. Typical "signature" of the nucleosomes on the stretching curve of the chromatin fiber appeared nearly immediately after exposure of the DNA template to the extract. When the fiber was completely stretched up to the limits of B-S transition forces ~65pN, all nucleosomes were destroyed and the return curve correspond to that of naked DNA. In the immediately following stretching cycle the newly formed nucleosomes were already registered. On partially extended DNA, the formation of nucleosomes was halted once the forces approached a value of 10pN. When we exposed the template DNA to the extract previously incubated with competitor DNA at concentrations typical for chromatin reconstitution (50-100ng of DNA per µl of extract) only very few nucleosomes were formed in times allowed by our experimental conditions (typically 20 minutes). Very rapid chromatin formation in single molecule experiment is therefore very likely due to the disproportional DNA/chromatin assembly complexes stoichiometric ratio.

References

[1] B. Ladoux, J.-P. Quivy, P.S. Doyle, G. Almouzni and J.L. Viovy. Sci Prog. **84**(Pt 4), 267-90. (2001)