UNRAVELING MOLECULAR DETAILS OF CHROMATIN STRUCTURE USING ATOMIC FORCE MICROSCOPY AND OPTICAL TWEEZERS

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In contrast to prokaryotic organisms, the DNA in cells of eukyotic species is sequestered in a nucleus, which occupies about 10% of the total cell volume. Each DNA molecule is packaged into a separate chromosome, which is a large complex consisting of a single DNA molecule and many protein molecules, such as histones, RNA and DNA polymerases, gyrases, topoisomerases and many other non-histone chromosomal proteins.

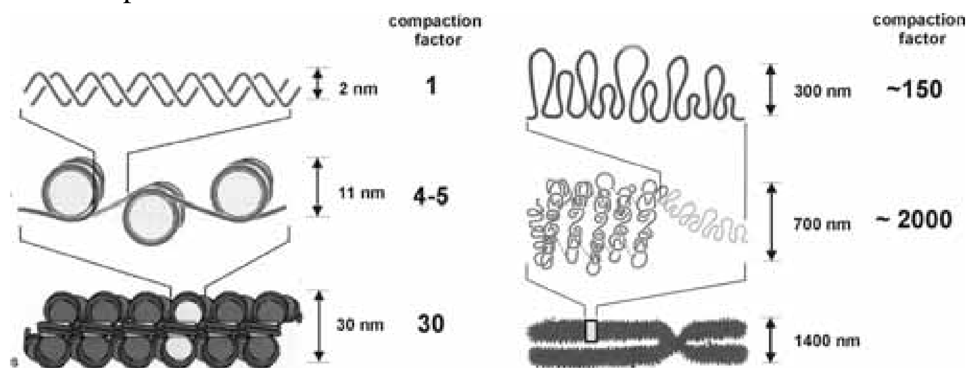

Figure 1
Different levels of DNA packaging into chromatin. (a) DNA double helix (b) “beads-on-a-string” structure (c) “30 nm fiber” (d) extended form of the chromosome (e) section of condensed chromatin (as in metaphase chromosome) (f) entire metaphase chromosome.

Starting from a protein-free DNA molecule different levels of chromatin compaction have been distinguished (Fig. 1). Eight histone proteins form a core particle around which the DNA is wrapped, which is called the nucleosome. A nucleosome is formed about every 200 bp along the DNA strand. The next level of compaction is the socalled ’30 nm’ fiber. The individual nucleosomes start to stack into a toroid-shaped structure resembling a fiber. Further compaction of the structure is achieved by folding this 30 nm fiber back and forth.

In the early days of chromatin research this structure was assumed to be static and have only one single function: packaging. Nowadays researchers are convinced that chromatin structure plays an active role in gene regulation by changing its structure on a local level. This means that chromatin is not a passive mechanism to keep the DNA packaged but is actively involved in activating and suppressing specific genes. Chromatin is not a stand-alone structure but is dynamic as a result of many external inputs, other proteins, histone-modifications, etc.

Here I will provide an overview of chromatin research ongoing in our lab. We have applied both atomic force microscopy (AFM) imaging and force spectroscopy using optical tweezers (OT) to study interactions between DNA and histone proteins on the

single molecular level. These interactions play an important role in the formation of nucleosomal arrays, which is the first packaging level of DNA into a more compact chromatin structure.

Tapping mode AFM in air was used to image nucleosomal arrays in which 208-12 DNA fragments were folded (Fig. 2). Detailed analysis of the AFM images led to the conclusion that the DNA exhibited different binding modes, suggesting a dynamic, non-rigid structure. These dynamics were studied in a more direct way using AFM imaging in a liquid environment. Tuning the buffer conditions such that the molecules are able to move allowed the observation of packaging of the DNA into a compact chromatin structure in real-time.

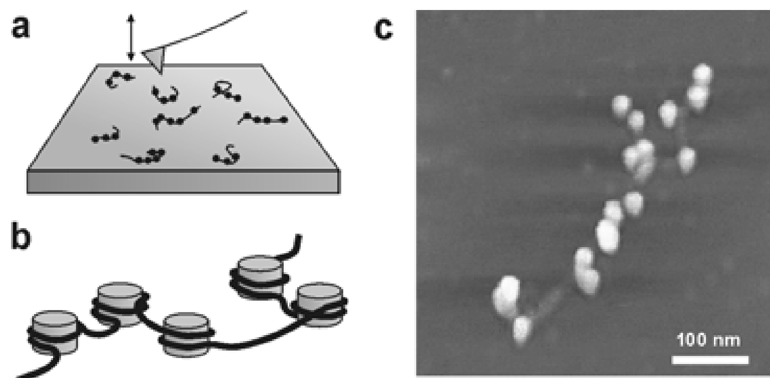

Figure 2
Atomic force microscopy of DNA-protein complexes (a) Principle of tapping mode AFM. (b) Schematic representation of the nucleosomal fiber. Cylinder represents a core histone particle, consisting of 8 histone proteins, around which the DNA is wrapped. (c) Typical AFM image of a nucleosomal fiber.

A single DNA molecule (16 um long) was attached on either end to a 2.6 um polystyrene bead. One bead was immobilized on the tip of a micropipette while the second one was held using optical tweezers. Increasing the end-to-end distance of the molecule by moving one bead away from the other, and measure simultaneously the force using the optical tweezers, allowed us to obtain a force versus length characteristic of a single DNA molecule (Fig. 3a-b). A *Xenopus laevis* cell extract was added that was known to form nucleosomal arrays on naked DNA. This caused the two beads to move towards one another, indicating compaction of DNA into a nucleosomal structure. The force versus length curve upon stretching the compacted fiber revealed forces needed to disrupt the structure and discrete opening events, which we attributed to the discrete opening of individual nucleosomes (Fig. 3c).

Figure 3
Force spectroscopy of individual DNA molecules and chromatin using optical tweezers (a) Schematic representation of a single suspended DNA molecule. (b) Typical force extension curve of a single DNA molecule. (c) Force extension curve of a chromatin fiber, revealing a stepwise opening of the structure.