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Scanning probe microscopy for imaging human chromosomes

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Various kinds of microscope using a probing tip have been introduced since the scanning tunneling microscope was invented in 1981, and form a new family of the scanning probe microscope (SPM). The atomic force microscope (AFM), one such SPM, has the advantage in creating topographic images of the sample surface at high spatial resolution in various (vacuum, air and liquid) environments. Thus, the AFM has been applied to the studies of biological samples from DNA to living cells (Ushiki 2003). In this paper, we focused on our AFM studies on the high-ordered structure of human metaphase chromosomes.

We first obtained AFM images of dried chromosomes in the ambient condition (Ushiki et al. 2002). The chromosomes - harvested from human lymphocytes by a standard procedure - were fixed with methanol-acetic acid, treated with 1% tannic acid solution and 1% OsO_4, critical point-dried in liquid CO_2, and observed using a dynamic mode (*i.e.*, intermittent contact mode) in the atmosphere. The morphology of the chromosomes prepared with this technique was well preserved; they were composed of the highly condensed chromatids of a mitotic pair, each of which was characterized by the presence of alternating ridges and grooves. At high magnification, they are composed of tightly packed chromatin fibers about 50-60 nm thick.

We then obtained AFM images of wet fixed chromosomes in a liquid environment (Hoshi et al. 2004); chromosomes prepared by the same method as above were observed using a dynamic mode in a phosphate-buffered saline (PBS) solution. For AFM imaging in liquid, the interaction force between the tip and sample was carefully adjusted to the minimum degree, otherwise chromosomes were easily deformed during scanning, because of their extreme softness in liquid environments. The surface of chromatids in these wet chromosomes was characterized by the presence of alternating ridges and grooves, as observed in the dried chromosomes.

In order to study the structure of chromosomes in the condition much closer to the physiological state, we further obtained AFM images of unfixed, or native, chromosomes in a liquid environment (Hoshi et al. 2006). The three-dimensional surface structure of the isolated chromosomes without any chemical fixation was observed at high resolution using a dynamic mode in an isolation buffer solution. The obtained features were almost the same as those found in the critical point-dried chromosomes as well as the wet fixed samples.

These features indicate that the structure of the chromosome arm is not uniform but heterogeneous because of the presence of highly condensed and less condensed regions in the chromosome.

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