DISSECTING CHROMOSOMES, ONE AT A TIME!

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The atomic force microscope (AFM) is proving to be as much a tool as an imaging device. Recently, Stefan Thalhammer, Robert Stark, Stefan Müller, Johannes Wienberg and Wolfgang M. Heckl have added another useful technique to this repertoire.[1] They demonstrated that the AFM can be used to cut out an identified section of DNA and then extract that section for amplification.

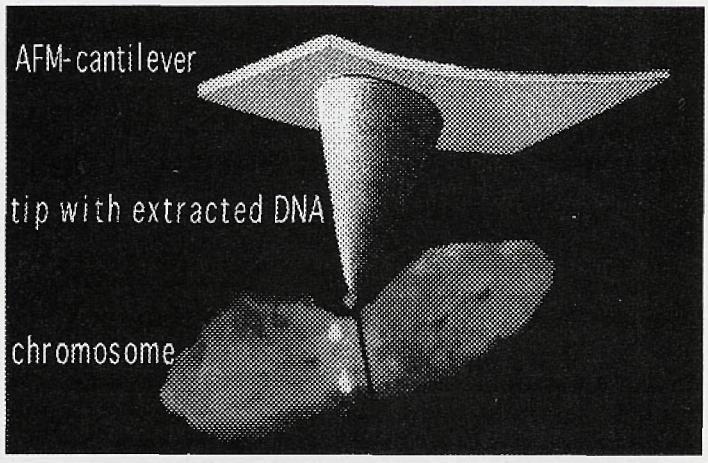
They began by flow sorting chromosomes, and then increased the specific amount of the target DNA with a "pre-set" *in situ* hybridization with chromosome-specific DNA to provide a specimen rich in chromosome 2. To identify the target area, selected chromosomes were examined with the AFM in the non-contact mode using stiff cantilevers. It was reasoned that this mode of examination would avoid contamination. However, their studies presented evidence that the tip made minimal contact during this phase, resulting in a slight contamination. After selecting the site for transection of chromosome 2, the loading force of the tip was increased to 45 mN by adjusting the piezo z voltage. One line scan was then performed across the chromosome, resulting in a cut later measured at 100 nm in width. That portion of the chromosome stuck to the tip by hydrophilic interactions, so the cantilever (with the tip and extracted portion of the chromosome) could be transferred to a tube with an appropriate buffer, and then the DNA was amplified. A topoisomerase I digestion was first performed to increase the effectivity of the amplification. To generate the probe, the DNA that had been relaxed by the digestion procedure was amplified using modified degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR) cycling conditions for 33 cycles. Secondary DOP-PCR was carried out in the presence of biotin-16-dUTP to label some of the amplified probes. Standard fluorescent *in situ* hybridization (FISH) was performed to visualize the chromosome band-specific probes. The biotinylated probes were detected with avidin coupled to fluorescein isothiocyanate (FITC) and observed with an epifluorescence microscope.

The FISH analysis showed a distinct signal corresponding to the site of the cut. A fluorescence intensity profile done to confirm the FISH experiment showed that the maximum signal was around the 2p12 band. Thalhammer *et al.* demonstrated that very discrete genetic probes could be generated by this technique. They suggested that microfabricated tips for the AFM could be designed for even more specific dissection of chromosomes. They also suggested a tip design that would snap off the contaminated end used in the imaging process, leaving a clean tip for the dissecting and probe extraction.

It is quite remarkable that a small piece can be dissected from a selected site on a chromosome and then can be amplified to create a specific genetic probe. This technique, that Thalhammer *et al.* termed "one cut—one probe," could be used for physical fine mapping of chromosomes, especially useful for evolutionary studies. It will be very interesting to see nanodissection of chromosomes with the AFM join the repertoire of methods that are merging molecular biology with morphologic techniques.

1. The author gratefully acknowledges Dr. Wolfgang Heckl for reviewing this article.

2. Thalhammer, S., R.W. Stark, S. Müller, J. Wienberg, and W.M. Heckl, The atomic force microscope as a new microdissection tool for the generation of genetic probes, J. Structural Biol. 119.232-237, 1997



Front Page Image

Nerve Fibers Growing from an Explanted Chick Dorsal Root Ganglion (DRG) in Culture

DRG's are neuronal cell clusters situated along the spinal cord, where they relay sensory information to the spinal cord and higher brain centers. Because they are easily isolated and cultured they are popular whenever primary neuronal cells are needed in an assay system. They can be taken from embryonic or adult animals and cultured either as a whole or dissociated into single neurons. Huber and Brösamie, for example, are using DRG cultures to assess the fiber growth-promoting properties of various substrates (see following article on page 18 of this issue). The DRG shown on the cover was taken from embryonic day 13 chick (8 days before hatching), explanted in a culture dish, and a crystal of the fluorescent lipophilic neuronal tracer DiI placed on top of it. The DiI distributes in the cell membranes and thus labels the outgrowing nerve fibers. The preparation was observed on an inverted Olympus microscope at a magnification of 100 x.

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