New Technologies Measure Genome Domains

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High-resolution microscopy and novel labeling procedures enable 3-D studies of the functional architecture of gene domains in cell nuclei.

Although fluorescence light microscopy has made considerable progress in improving resolution over the past decade, measuring the size of specific domains of the human genome with high precision in 3-D conserved cell nuclei remains a challenge.

The chromatin in the cell nucleus is not randomly organized. Specific fluorescence DNA probes and 3-D fluorescence microscopy have shown that the cell nucleus comprises distinct chromosome territories and subchromosomal domains (e.g., telomeres, centromeres, replication units, band domains, transcription units) organized in a hierarchy. The architecture of these elements depends on their size and their functional activity.

Computer simulations of chromatin and nuclear elements suggest that chromatin compaction of genes depends on their activity and accessibility for macromolecules. Further investigations require novel nanotechnology techniques. Recent experimental data indicate that gene activity is correlated to changes in the chromatin compaction and structure of individual genes in dimensions considerably beyond light microscopy’s Abbe resolution limit.

Improving resolution

Recent improvements in microscope resolution provide first evidence for the elucidation of gene compaction and chromatin folding in 3-D conserved cell nuclei. For example, spatially modulated illumination microscopy demonstrates superior distance resolution for fluorescent nano-objects. Furthermore, the technology can measure the diameter of fluorescently labeled gene targets with a precision of a few nucleosomes, as has been demonstrated for several gene loci in lymphocyte cell nuclei.

The spatially modulated illumination microscope (Figure 1) uses structured laser illumination to obtain information about the fluorescent objects. Two counter-propagating laser beams couple into an interferometric setup and interfere, yielding a standing wave field. A piezoelectric element moves the specimen in the direction of the optical axis in increments of 20 to 40 nm. At each of 200 to 400 axial positions, a CCD camera records a fluorescence image of the object, similar to the process that occurs in a wide-field fluorescence microscope. The excitation wave field strongly modulates the detected fluorescence emission intensity through the complete image stack. The stack contains precise information about the axial position and the spatial extension of the fluorescence intensity.

![Figure 1. In a spatially modulated illumination microscope, two gas lasers produce interferometric illumination. Focusing the illumination beams into the back focal planes of the objectives produces a standing wave in the object space. The specimen slide moves through the object space in 20-nm steps; a CCD camera captures a wide-field fluorescence image at each position.](image-url)
distribution of the analyzed objects in a size range of 40 to 200 nm, based on the nonmodulating part of the curve and additional assumptions of the fluorochrome distribution (Figure 2).

After appropriate calibration of the modulation contrast as a size measure, we can determine gene diameters with a precision of a few nucleosomes; i.e., some 10 nm.\textsuperscript{11,12}

Recently we have shown the feasibility of such measurements in fixed cell nuclei for FISH-labeled domains of p53 (45 kb), p58 (85 kb) and c-myc (120 kb) using specific DNA probes obtained from bacterial artificial chromosome (BAC) clones. From the average size measurements of 103 ± 12 nm (c-myc), 119 ± 14 nm (p53) and 123 ± 10 nm (p58) we have estimated the average volumes (and gene compaction) in lymphocytes for c-myc to 5.7 × 10^{-4} \text{µm}^3 (1:396), for p53 to 8.9 × 10^{-4} \text{µm}^3 (1:129) and for p58 to 9.7 × 10^{-4} \text{µm}^3 (1:235).

The standard deviations indicate precisions in the gene diameter of one to two nucleosomes. The values are about two orders of magnitude smaller in the gene volume than a laser spot volume in a confocal laser scanning microscope.

Better labels

Labeling techniques now require some upgrades to avoid fixation and the destructive influence of denaturation that may occur during standard FISH. We developed a procedure that omits the thermal denaturation of the chromatin\textsuperscript{13} for use in a technique that we call combinatorial oligo FISH.\textsuperscript{14}

In standard FISH, the DNA bases of a single-stranded DNA probe bind to their complementary bases of a single-stranded (i.e., thermally denatured) DNA target via Watson-Crick pairs. In contrast, combinatorial oligo FISH uses the ability of purine bases to bind another purine or a complementary pyrimidine via (reverse) Hoogsteen pairing, so that the single-stranded DNA probe labels the double-stranded chromatin target.

Because of binding energy requirements, the optimum length of the thirdstrand probes is only 10 to 35 nucleotides, compared with typical BAG probe lengths of about 100,000 nucleotides. Obtaining a specific label of a given chromatin target requires complete bioinformatic analysis of the human genome database.

This technique selects only purine target sites that specifically colocalize at a given gene locus. For these, DNA or PNA oligo probes can be synthesized as thirdstrand probes and used for specific labeling of a genome target (Figure 3).

Combinatorial oligo FISH has several advantages over standard FISH:

- The probe design enables labeling of any target site in the human genome, in contrast to BAC probes.
- Because it uses Hoogsteen pairing, the technique can omit denaturation of the chromatin target, also allowing specific FISH labeling of vital cells.
- The probes are much shorter than a gene target. For example, for abl (Figure 4) 606 nucleotides label a domain of 186,000 target nucleotides. This should considerably reduce any effects of modifications of the target structures by the probe incorporation.

Combining techniques

In a first proof, we have combined the microscopy with the labeling technique for size measurements of the abl gene in 3-D conserved, fixed lymphocyte nuclei of one healthy donor. Because of the size of the probes, only 62 fluorochrome molecules...
attach to one target site, which has required a considerable improvement of the sensitivity of the detection system.

In this example, 42 target loci revealed an average diameter of $77 \pm 22$ nm using the size calibration curve described in reference 11. Assuming that the loci have a spherical shape, we have estimated the volume at $2.3 \times 10^{-4}$ µm$^3$ and the compaction ratio at 1:821 on the basis of a linear DNA length of 340 nm for 1000 nucleotides.

Such data can be used for investigations of the abl region in blood cells of leukemia patients to determine the correlation of breakpoint region compaction and the risk for translocation induction.

These data show that, with spatially modulated illumination microscopy and combinatorial oligo FISH, novel techniques are on hand that offer new ways to provide further information on epigenetic mechanisms for gene activity and chromosome aberration induction; e.g., leukemia correlated translocations. Such investigations may be needed to understand regulatory functions of chromatin and nanoscale nuclear organization.

Meet the authors

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References


Figure 4. Spatially modulated illumination microscopy acquired 200 images (optical sections) of a labeled abl genome domain with an axial image distance of 20 nm (abscissa unit). The curve shows the axial intensity distribution of the labeled domain from each optical section along the Z-axis (abscissa). The AID is a direct measure of the spot size, which in this case has a diameter of 96.8 nm after background subtraction and application of a size calibration curve.