Meeting report

Towards the visualization of genome activity at nanoscale dimensions

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A report on the Fifth Annual Nanostructural Genomics meeting, Bar Harbor, USA, 7-10 September 2005.

It is a rare meeting where one can hear the latest developments in comparative genome analysis, relate these findings to advances in understanding both the linear and three-dimensional organization of the eukaryotic genome, and see it all beginning to fit into the context of the structure and function of the nucleus, visualized using state-of-the art labeling and microscopic techniques. These cross-disciplinary areas of research have been presented by a diverse group of scientists for the past five years at the Nanostructural Genomics meeting at the Jackson Laboratory in Bar Harbor, and the 2005 meeting again gave attendees much food for thought.

In his opening address, Timothy O'Brien (Cornell University, Ithaca, USA) outlined his view of how genomics, cell biology and optical physics all work together to create an accurate picture of nuclear structure and function, which can lead to important insights into cellular form and function. He discussed his studies of a several megabase region surrounding the mouse *piebald* locus, a genetically defined region named after a coat-color gene within it. He used comparative genomics to learn more about the nature of particular deletions in this region that cause neonatal respiratory distress and death. This information was coupled to high-resolution visualization of gene-rich and gene-poor sections of this region in the nucleus, and to the prediction of potential transcription-factor binding sites for specific genes, such as *sprouty2*, a gene involved in lung branching morphogenesis.

Chromosome sequence and structure

Considering comparative genomics at the sequence level, Ross Hardison (Pennsylvania State University, University Park, USA) discussed new algorithms designed to identify important genomic regions that may not be coding sequence but are nevertheless conserved between organisms. These algorithms, including phastCons and RP (regulatory potential), use methodology such as alphabet clustering, where different nucleotide-sequence patterns are each classified as a letter of the alphabet, to reduce complexity and identify higher-order sequence patterns that may be conserved 'in spirit', if not in exact sequence, in the genome. Some algorithms are better than others at identifying particular sequence features; for example, phastCons identifies potential microRNA genes better than RP.

Moving to the next organizational level, chromatin, Jason Lieb (University of North Carolina, Chapel Hill, USA) described a novel approach to the study of the structure of active chromatin in yeast. Using chromatin immunoprecipitation (ChIP) he has compared the pattern of sites identified by binding *in vivo* of the DNA-binding domain of the transcription factor Leu3 to the pattern obtained by 'DIP ChIP', in which naked DNA is allowed to bind the Leu3 protein *in vitro* and is then crosslinked and immunoprecipitated. By comparing the two experiments he found that promoters contain fewer nucleosomes than do other DNA sites. In addition, Lieb showed that, even at the promoter, nucleosomal organization is dynamic and influences the type of protein that binds to a particular site. Evidence of promotor-specific chromatin structure in the human genome came from Keji Zhao (National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, USA), who used ChIP in combination with serial analysis of gene expression (SAGE) to show that histone-acetylation islands in the human genome correlate with active promoter regions but not with the entire transcriptionally active gene.

The notion that the three-dimensional organization of chromatin reflects gene activity is intellectually satisfying but has

not yet been rigorously proven. Roel van Driel (University of Amsterdam, The Netherlands) described a comprehensive study that is designed to determine whether gene-rich regions, which tend to be clustered on the linear map in 'ridges' (regions of increased gene expression), occupy distinct nuclear domains. Using fluorescent *in situ* hybridization (FISH) to tag different genomic regions in a systematic way, he and his collaborators have found that, despite surprisingly large cell-to-cell variations, on average gene-rich and gene-poor regions seldom overlap spatially in the interphase nuclei of primary human fibroblasts or HeLa cells. Their initial studies also suggest that different gene-rich regions themselves might occupy non-overlapping territories within the nucleus (and gene-poor regions also appear to be within distinct territories). O'Brien presented data on this topic from the *piebald* locus, showing images (obtained by Lindsay Shopland, Jackson Laboratory, Bar Harbor, USA) of chromatin hybridized *in situ* with differently colored fluorescent bacterial artificial chromosomes complementary to either gene-rich or gene-poor regions of the *piebald* locus. In some cases, the gene-rich regions were clustered together in 'hubs', which can be loosely defined as congregations of regulatory and/or transcriptionally active genes that are not necessarily adjacent on the linear genome. In other cells, however, the gene-rich and gene-poor regions remained interspersed linearly along the *piebald* region, giving the chromosome a 'candy cane' appearance.

Job Dekker (University of Massachusetts Medical School, Worcester, USA) came at the question of the three-dimensional organization of active versus inactive chromatin from a more biochemical angle, using his previously published method of chromosome conformation capture (3C). He found that actively transcribing regions of the β-globin locus, which form decondensed 'puffs', are near one another (crosslinkable) in hubs, whereas the more compact, repressed chromatin at this site does not appear to loop out and interact at hubs. Jim McNally (National Cancer Institute, National Institutes of Health, Bethesda, USA) suggested that a puff may need to be part of a 'cloud', a region of DNA decondensed by topoisomerase II, in order for transcription to occur in his model system, a mouse mammary tumor virus tandem gene array. At a more global level, Steve Kozak (Fred Hutchinson Cancer Center, Seattle, USA) discussed his finding that differentially expressed genes in erythroid cells and neutrophils are often clustered into separate activity hubs. He likened this organization to a scale-free network such as the airline system, where there are central nodes (airports) containing multiple genes (airplanes with travelers). This is in contrast to a random network, such as a road system, where the number of links approximates the number of interactions.

Seeing is believing

There were also exciting reports on technical advances in optical microscopy. Stefan Hell (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) described a stimulated emission depletion (STED) light microscope system in which the Abbe diffraction resolution limit (the usual limit of a light microscope) has been broken. This has been achieved by inhibiting the fluorescence of molecules at the outer region of a scanning excitation spot in a saturated manner. With the use of carefully chosen dyes and focal intensity conditions, Hell has attained 10 nm optical resolution (in the lateral *x*-*y* dimension). The 4Pi microscope from Leica Microsystems, an application of Hell's earlier ideas, was also demonstrated at the meeting by Lindsay Shopland (The Jackson Laboratory) and Joerg Bewersdorff (The Jackson Laboratory). This system increases optical resolution in the axial dimension (*z*-dimension) by the use of two opposing objective lenses to propagate light from multiple directions toward the focal point, followed by a deconvolution step to give a *z*-resolution of about 80 nm (this is about five- to sevenfold greater than conventional light microscopy). This is about the size of an average gene domain. Both these systems increase optical resolution (the smallest distance detectable between two small objects) and thus also structural visualization and distinction to levels that have up to now been impossible to reach with light microscopy, and span the 100 nm region that has classically been above the practical range of electron microscopy and below that of light microscopy.

Advances in electron microscopy were not neglected. David Bazett-Jones (The Hospital for Sick Children, Toronto, Canada) discussed the use of electron spectroscopic imaging to study the structure and composition of intranuclear bodies at the nanometer level. This technique takes advantage of the fact that electrons lose differing amounts of energy depending on which elements they excite or ionize when they pass through a sample. Using this extremely informative technique, Bazett-Jones learned that subnuclear structures called promyelocytic leukemia (PML) bodies change both their structure and dynamic behavior in response to cellular stress, and showed - in collaboration with Thoru Pederson (University of Massachusetts Medical School, USA) and me - that nucleostemin, a stem-cell protein involved in cell-cycle control, is present in non-ribosome-containing compartments in the nucleolus. Michael Grunze (University of Heidelberg, Germany) updated the audience on advances in X-ray tomography in vitreous ice, and pointed out that 'quantum dot' (semiconductor nanocrystals) used as a multicolour fluorescent labels can be easily localized using simple variations of this technique.

Quantum dots can also be used as fluorescent labels for optical microscopy. Their advantage is that multiple colors can be excited at one wavelength, but one of their main disadvantages is that they are currently unsuitable for intracellular labeling in live cells. Xavier Michalet (University of California, Los Angeles, USA) reported some success towards overcoming this disadvantage with his work in

which the movement of external cell-membrane receptors was tracked on the surface of live cells using peptide-coated quantum dots as labels.

Winding up the meeting, Christoph Cremer (University of Heidelberg, Germany and The Jackson Laboratory) summarized the current state of imaging tools. He pointed out that in addition to the exciting new 4Pi and STED methodologies discussed earlier, there are also ways to dodge (rather than break) the diffraction resolution barrier in standard light microscopy. This can be done using multiply colored labels and varied types of image-acquisition techniques and careful optics calibration (that is, by spectral precision distance microscopy (SPDM) and spatially modulated illumination (SMI)). For co-localization studies in fixed cells, the distance between the centroids of two objects of different colors can now be defined in the nanometer range, even though the exact shape of each object is unresolved.

In summary, the meeting provided a delightfully unique perspective on the application of exciting experimental breakthroughs at the interface of genomics, cell biology and optical physics.

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