

Meeting report

Genome duality

Silvia Kocanova and Kerstin Bystricky

Address: Laboratoire de Biologie Moléculaire Eucaryote (LBME), University of Toulouse, CNRS, 118 route de Narbonne, 31062 Toulouse, France.

Correspondence: Kerstin Bystricky. Email: kerstin@biotoul.fr

Published: 28 May 2009

Genome Biology 2009, **10**:308 (doi:10.1186/gb-2009-10-5-308)

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2009/10/5/308>

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A report on 'Higher Order Genome Architecture', the third meeting of the Marie Curie Conferences and Training Courses (MC-GARD), Edinburgh, UK, 1-5 April 2009.

Epigenomics is the study of epigenetics in the nuclear environment. Understanding the interplay between gene expression and positioning of specific DNA sequences motivates intense research and animates vivid discussions. New tools to investigate this relationship on a genome-wide level are continuously being developed, and in April the latest technological advances and some exciting recent results were presented at the latest Marie Curie meeting and training course held in Edinburgh. We present a few of the highlights here.

Position is everything - or is it?

The intranuclear positions of several genes have been correlated with their activity. This observation led to the hypothesis that co-regulated genes might be recruited to preassembled transcription factories, which are potentially nucleated around foci of shared transcription factors. It is unclear whether relocalization is a consequence of transcription or whether recruitment to a distinct site poises sequences for expression or silencing.

The use of genome-wide DNA adenine methyltransferase identification (Dam-ID) has enabled Maarten Fornerod (Netherlands Cancer Institute, Amsterdam, The Netherlands) and Daan Hupkes (Netherlands Cancer Institute) to analyze the nature and dynamic behavior of the genome at the nuclear periphery in a clever way. Fornerod showed that, in *Drosophila*, genes tethered to the nuclear pores are generally very large and less active than genes associated with intranuclear pore proteins not anchored to the nuclear envelope. By examining lamin-associated chromosomal

domains (LADs) during the differentiation of human embryonic stem (ES) cells to astrocytes, Hupkes was able to show that, surprisingly, reorganization of LADs - the dissociation of transcription units from the lamina - correlated with transcriptional changes occurring at single genes.

Wendy Bickmore (MRC Human Genetics Unit, Edinburgh, UK) is investigating whether recruitment of genes to the nuclear periphery affects gene expression in mammalian cells. She found that artificial tethering of a single locus near the periphery induced relocation of the entire chromosome after mitosis. In consequence, expression of a subset of genes was reduced, which may stem in part from increased levels of histone deacetylases near the nuclear periphery. Bickmore has also investigated the effects of translocations that influence nuclear localization of chromosomes. Translocation between chromosomes 11 and 22 caused the normally more peripheral chromosome 11 to be shifted toward the interior of the nucleus. This shift led to a slight overall increase in the transcription of many, but not all, of the genes on chromosome 11. Such changes in gene expression may contribute to cancer development, which is characterized by increased frequency of translocations.

Ana Pombo (MRC Clinical Sciences Centre, Imperial College, London, UK) described visualization of the intermingling of chromosome territories in resting and activated human lymphocytes using *in situ* hybridization on thin cryosections. She finds chromosome-specific differences between the two cellular states, and that these differences are in part related to the nuclear expansion that occurs during activation, but are also likely to be tied to their different transcriptional profiles. Discussing the regulation of mammalian X-chromosome inactivation, Barbara Panning (University of California, San Francisco, USA) presented evidence that contacts between the X chromosomes before X inactivation have an impact on their fates.

A hundred years ago Theodore Boveri proposed that global changes in the positioning of chromosome territories occur only in prometaphase, a hypothesis that Thomas Cremer (Ludwig-Maximilians-Universität Biozentrum, Martinsried, Germany) has confirmed by following the movement of fluorescent proteins recruited to sites of DNA damage caused by microirradiation. The absence of movement over a period of hours leaves little freedom for chromosome movement during interphase. One of us (KB) presented evidence that in cancerous, aneuploid human cell lines the relative nuclear organization of several genes and chromosome territories was largely unaffected by hormone-induced activation of transcription in cycling cells. In fact, Jill Brown (MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine) suggested that formation of intranuclear 'speckles' enriched in the splicing factor SC35 in late telophase might influence the positioning of active genes at the beginning of the cell cycle. In human hematopoietic cells, active chromatin-decondensed genes clustered near these speckles, which might explain the apparent nonrandom association between expressed genes. Work reported by Dimitris Thanos (Academy of Athens, Greece) supported this idea by proposing that preassembled activation centers - 'enhanceosomes' - responsive to interferon (IFN) were responsible for the efficient capture of the transcription factor NF κ B after interferon stimulation. In these IFN enhanceosomes the choice of the allele to be expressed was random and depended on interchromosomal interactions with specific Alu sequences. To detect and clone the NF κ B-protein-associated Alu sequences on the different chromosomes, Thanos developed a genome-wide chromatin immunoprecipitation (ChIP)-cloning technique.

3C goes global

The technique of chromosome conformation capture (3C) allows the determination of interaction frequencies between two distant chromosomal sites. Jim Hughes (MRC Molecular Haematology Unit, Weatherall Institute, Oxford, UK) presented 'microarray adapted 3C' using circularized templates to test spatial relationships between specific *cis* elements and the genes they control. The effectiveness of this '4C' (3C on chip) technique followed by high-throughput sequencing was confirmed by Hughes on the α -globin gene cluster and by Daniel Robyr (University of Geneva Medical School, Switzerland) on the β -globin locus control region. Robyr has also applied this technique to reveal intra-chromosomal and interchromosomal interactions of large intergenic noncoding regions. The role of these interactions is yet to be determined.

Whereas 4C tests genome-wide interactions with a single site, 5C (chromosome conformation capture carbon copy) goes one level further by assaying (almost) every potential interaction site against all other sites in a selected genomic region. Job Dekker (University of Massachusetts Medical

School, Worcester, USA) demonstrated the power of 5C using the human β -globin locus in an analysis involving 2.5 million pairwise interaction frequencies. Dekker reported a collaboration with Marc Marti-Renom (CIPF, Valencia, Spain) that showed that the interaction matrix obtained from 5C was suitable for three-dimensional modeling of the over 1 Mb β -globin gene cluster. In the inactive state, this 1-Mb region resembled an extended random-coil fiber, whereas in the active transcribed state it assumed a globular conformation and was involved in numerous long-range interactions. The report of the modeling sparked discussions about the potential pitfalls of this approach, due in particular to the likelihood of crosslinking and the stochastic nature of the interactions probed.

Large-scale chromatin decondensation accompanies transcription

Decondensed euchromatic regions have long been viewed as actively transcribed chromatin. The relationship between local chromatin remodeling, euchromatin and gene expression has been investigated by several groups. Using the lacO/lacI-green fluorescent protein (GFP) system to follow transgenic sequences in real time, Andrew Belmont (University of Illinois, Urbana-Champaign, USA) reported that large-scale decondensation of chromatin, which was independent of histone acetylation, accompanied heat-shock-induced transcriptional activation. He further showed that the mammalian *HSP70* gene specifically associated with interchromatin granules after heat shock. No linear transition between the compacted and decondensed states was observed. Using rapid live cell microscopy John Lis (Cornell University, Ithaca, USA) has observed that, in *Drosophila* polytene chromosomes, transcription of the two *HSP70* genes was activated within seconds of heat shock.

This activation correlated with visible local chromatin decondensation on the polytene chromosomes. Using *in situ* hybridization, decondensation of gene clusters was also examined by Ragnhild Eskeland (MRC Human Genetics Unit, Edinburgh) and Karen Leung (University of California, Davis, USA). Eskeland showed that specific decondensation of the 3' end of the Hox gene cluster during early mouse embryonic development was due to the absence of polycomb repressive complexes PRC1 and PRC2. Leung proposed that monoallelic decondensation of the imprinted snoRNA gene cluster on 15q11-13 may be linked to imprinted expression of this cluster, at least in the mouse brain.

The development of an algorithm correlating histone-modification profiles with gene expression, determined by ChIP followed by either microarray analysis (ChIP-chip) or sequencing (ChIP-seq) of the pulled-down DNA, enabled Denise Barlow (Center for Molecular Medicine, Austrian Academy of Science, Vienna) to suggest that imprinting of the insulin-like growth factor receptor locus *Igf2r* may be

achieved by preventing upregulation of one allele rather than by epigenetic silencing of the other - at least in an ES-cell imprinting model.

Epigenomics in evolution, development and disease

Cremer showed stunning images of inverted nuclear architecture in rod cells from rodent retinas. The unusual organization of heterochromatin in rod cells forms what appears as natural microlenses. Comparison of 40 species revealed that this specialized architecture is unique to nocturnal animals. Cremer also advertised developments to come using new correlation microscopy techniques that would allow the separation of two fluorescent spots to 15-nm resolution - fighting the Abbe limit.

Using transposons as tools to investigate regulatory architecture and specific expression patterns, Francois Spitz (EMBL, Heidelberg) reported that regulatory modules exist over large regions of the human genome and that changes in locus organization can lead to disease. Indeed, evolutionary reshuffling of regulatory modules tends to preserve the order of regulatory elements, creating what Spitz called "vertebrate regulatory landscapes". He showed that the range of action of enhancers is context dependent. Inspired by the dynamics of enhancer occupancy, which reflect developmental progression, Eileen Furlong (EMBL Heidelberg, Germany) has developed a prediction database for enhancer-specific temporal-spatial expression using a computational biology approach [<http://furlonglab.embl.de/methods/>]. Furlong also reported that transgene reporter assays combined with mutagenesis and genome-wide maps of transcription-factor binding for three well-characterized *Drosophila* developmental pathways revealed *cis*-regulatory epigenomic networks.

The meeting clearly showed that the data coming out of new high-throughput chromatin-interaction techniques requires computational skills, as well as biophysical modeling, and will necessitate interdisciplinary approaches in order to further advance genome research.

Acknowledgements

We thank Barbara Panning and Nick Gilbert for a helpful critique of our report.