Meeting report

Genome packaging and expression

Merlin Crossley

Address: School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW 2006, Australia.
E-mail: M.Crossley@mmb.usyd.edu.au

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A report on the 23rd Annual Lorne Conference on the Organization and Expression of the Genome, Lorne, Victoria, Australia, 17-21 February 2002.

Now that so many genes have been catalogued, increasing attention is being paid to the behavior of chromosomal domains in the nucleus and how the molecular details of chromatin packaging fit with patterns of gene expression. Several aspects of these phenomena were discussed at this meeting.

Silent regions at the edge of the known nucleus

Significant insights have been gained by marking chromosomes and comparing the behavior of transcriptionally active and inactive regions. Susan Gasser (University of Geneva, Switzerland) has tagged yeast centromeres and telomeres with 256 copies of the *lac* operator and visualized these elements using a fusion of green fluorescent protein (GFP) with the LacI protein, which binds the *lac* operator sequence. She found that centromeres and telomeres tended to be tethered to the periphery of the nucleus. In contrast, origins of replication and other chromosomal domains moved more freely when tagged in the same way. The anchoring of heterochromatin domains - tightly packaged chromatin of the type found at centromeres and telomeres - depended on the Ku proteins, which bind to double-strand breaks in DNA. The movement of other regions seems to be independent of microtubules and the initiation of replication *per se*, but it is ATP-dependent and may be connected to ongoing transcription. David Gilbert (Upstate Medical University, Syracuse, USA) investigated the positioning of inactive late-replicating genes in synchronized mammalian nuclei and observed that the silent β-globin genes gradually moved to the periphery, where they associated with regions rich in heterochromatin protein 1 (HP1). He is now dissecting the pathway involved in this migration and in heterochromatin formation, using inhibitors and competitor peptides of histone deacetylation.

Heterochromatin: 'that other chromatin' takes center stage

The terms euchromatin and heterochromatin have been useful for many years in describing the light-staining and more darkly staining regions of chromatin that are thought to be made up of mostly active loci and silent loci, respectively. Recently, the molecular definition of these regions has been improved and, in particular, advances in our understanding of histones have been crucial. David Tremethick (Australian National University, Canberra, Australia) summarized our current knowledge of nucleosomal structure and how different histone modifications are thought to influence the packaging of nucleosomes into condensed chromatin. His work on the variant histone H2A.Z suggests that it mimics acetylated histone and promotes the formation of intermediate chromatin, which is a conformation thought to be poised for transcription. Consistent with this view, overexpression of H2A.Z facilitates activated transcription.

Tremethick also discussed his work on the promoter of human immunodeficiency virus, HIV, which indicates that histone acetylation precedes both active transcription and the recruitment of a chromatin remodeling SWI-SNF complex including the ATPase subunit Brg1. This result highlighted the role of remodeling complexes in the regulation of chromatin accessibility and provided a good introduction to the work of Craig Peterson (University of Massachusetts, Worcester, USA), who argued that many regions of DNA must be inaccessible to remodeling complexes. He has been investigating which components of chromatin might prevent access. In addition to the core histones (H2A, H2B, H3 and H4), nucleosomes also contain linker histones, such as H1 (or H5 in erythroid cells), which seal the cleft where DNA emerges from the nucleosome. The role of linker histones has long been controversial, but many

studies have argued that they inhibit gene expression and are associated with inactive chromatin. Peterson's work provides a dramatic confirmation of this view. He found that the addition of unphosphorylated linker histone H1 prevented remodeling by remodeling complexes. The unphosphorylated H1 appears to constrain the topological movement of DNA. In contrast, phosphorylation of H1 by the linker histone kinase Cdc2-cyclin B (also known as maturation promoting factor, MPF) restored remodeling. Taken together, these results provide a simple framework on which to explore the complex effects of histone H1 on gene expression.

Centromeres in focus

Work on understanding centromere structure and function is proving to have broad relevance to many biological questions. Andy Choo (Murdoch Children's Research Institute, Melbourne, Australia) described his discovery of a marker chromosome derived from human chromosome 10 that had apparently developed a 'neo-centromere' that ensures its stability through mitosis. Neo-centromeres have now been associated with many derivative chromosomes, and it seems they form readily when the normal centromere is absent. This result may, in part, explain the ability of genomes to survive large-scale translocations and rearrangements over evolutionary timescales.

The neo-centromere on the modified chromosome 10 contains typical centromeric binding proteins, such as CENP-A (a histone-H3-like protein) but, remarkably, does not contain typical centromeric sequences such as α-satellite DNA repeats. Puzzling though this result may seem, the lack of repeats has meant that Choo has been able to delimit successfully the region associated with centromeric proteins and map the neo-centromeric region. Furthermore, he has used an ingenious gene-targeting strategy to insert telomeric sections adjacent to the neo-centromere boundaries and has succeeded in creating minimal human artificial chromosomes or HACs (Figure 1). These entities have been engineered to contain *loxP* sites so that they can be used, in conjunction with vectors expressing the Cre recombinase, as vectors to carry new DNA; they should also be useful in further studies aimed at understanding the mechanisms by which centromeres operate.

Proteins, post-translational modifications and proteomes

Some people who were surprised that humans appear to have less than twice as many genes as the humble nematodes have taken comfort in the fact that our genes are more complicated and alternative splicing and elaborate post-translational modifications may generate 'respectable' levels of complexity. The study of DNA-binding proteins, such as the histones, that are basic and rich in lysine and arginine residues has highlighted the importance of lysine acetylation and methylation and arginine methylation in regulation of gene expression. George Muscat (University of Queensland, Brisbane, Australia) provided a striking example of the importance of the coactivator-associated arginine methyltransferase (CARM1/PRMT4) in muscle differentiation. This process is driven in part by the myogenic DNA-binding protein MEF-2, which recruits the steroid receptor coactivator (SRC/GRIP); it is known that SRC binds CARM1. Muscat therefore tested whether CARM1 played an essential role in muscle differentiation. Inhibition of arginine methylation by adenosine dialdehyde or using tetracycline-regulated CARM1 antisense DNA effectively prevented muscle differentiation. It is suspected that CARM1 may play a local role, methylating histone tails at key target genes, but a possible role in directly modifying its partner protein MEF2 is also being investigated.

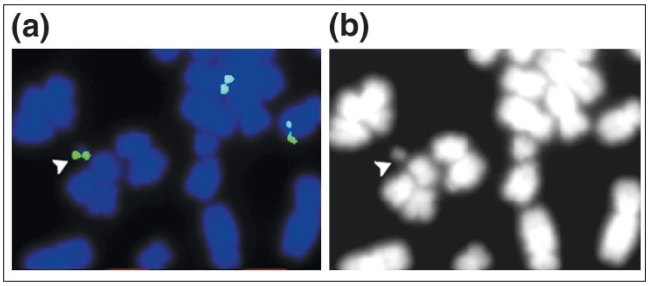

Figure 1
A human artificial minichromosome (or HAC) carrying a neocentromere. **(a)** Identification by fluorescent *in situ* hybridization (FISH) of the HAC (arrowhead) using a DNA probe for chromosome 10q25. Positive signals at the 10q25 regions of the two normal human chromosomes 10 are also visible. **(b)** Staining of the same nucleus with DAPI, which recognizes DNA, showing the relative size of the HAC compared with the other human chromosomes. Figure courtesy of Andy Choo.

Other unusual protein modifications have been identified in work on the regulation of genes in response to hypoxia. Recently, proline hydroxylation has been shown to regulate the hypoxia inducible factor (HIF), and David Lando (University of Adelaide, Australia) reported at the meeting that HIF undergoes β-hydroxylation at a conserved asparagine. Lando explained that oxygen starvation leads to the reversal of the hydroxylation allowing HIF to recruit its coactivator CBP (CREB binding protein) to activate its target genes.

In a lively finale to the meeting, Marc Vidal (Dana-Farber Cancer Institute, Boston, USA) led attendees through the strategies that can be used to combine expression data with protein-protein interaction maps in order to contribute to the annotation of genes without clear functions. Although the data remain imperfect, some impressive clustering of related genes was evident. Vidal likened his maps to the early sketches of the early explorers of America, Meriwether Lewis and William Clark, pointing out that large benefits came from simple beginnings in America. In Australia, of

course, many explorers returned empty-handed or were possibly eaten by dingos, but already the wealth of data Vidal is accumulating suggests that the genomes of model organisms are likely to be richer and more hospitable than the Australian heartland.

Molecular genetics and the history of dingos in Australia

The exact fate of certain explorers remains unknown, but details of the dingo population are now emerging. Alan Wilton (University of New South Wales, Sydney, Australia) presented a fascinating poster on analysis of mitochondrial DNA from Australian dog populations. The data are consistent with dingos having descended from domestic South East Asian pariah dogs that crossed into the continent around 5,000 years ago. The subsequent isolation of the dingo has led to it becoming a distinct population.

The conference was, as in previous years, a mixture of focused molecular biology and broader organismal biology. The genomes of humans and other animals are only beginning to be explored, and in future years it is certain that many surprises await us.