

Meeting report

Transcription of the genome: don't read it all at once

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A report on the 22nd Annual Lorne Conference on the Organization and Expression of the Genome, Lorne, Victoria, Australia, 11-15 February, 2001

With the availability of vast amounts of genomic sequence, many new opportunities have arisen. Speakers at this Lorne Conference reported opportunities for identifying important genes, understanding global mechanisms of expression, and controlling gene output.

That looks like an interesting gene

Estimates of how many genes exist in the human genome have varied, but the announcement from Vivien Bonazzi (Celera Genomics, Rockville, USA) that there are around 35,000 genes confirmed what many of us have always known - there are a lot; so many, in fact, that it is unlikely that each can be understood in biochemical detail. The trick will be to find the most interesting genes first.

One approach is to use genome-wide mutagenesis in the mouse. Christopher Goodnow (Australian National University, Canberra, Australia) and Ruth Arkell (Medical Research Council Mammalian Genetics Unit, Harwell, UK) described results of their ethylnitrosurea (ENU) mutagenesis screens. Goodnow identified mutations associated with cancer, obesity, immune disorders, dermatitis and neurological phenotypes. Mapping and sequencing showed that one of the pedigrees of mice with an immune disorder carried a mutation in the zinc-finger transcription factor Ikaros, a protein known to play a seminal role in T- and B-cell development; Arkell also identified mice with mutations in a less well characterized zinc-finger protein, which is still under investigation. Alex Turner (Lexicon Genetics, Texas, USA) described random insertional mutagenesis using retroviral vectors and showed that the use of a variety of vectors enabled more targeted genes to be identified. Human mutations also provide

important information on function. Mitch Weiss (Children's Hospital of Philadelphia, USA) explained how the molecular basis for an inherited human anemia was revealed by sequencing a candidate gene encoding the GATA-1 transcription factor. The mutation is in the amino-terminal zinc finger of GATA-1 and reduces its interaction with its cofactor, Friend of GATA. It is likely that mutations in regulatory proteins (and particularly zinc-finger proteins, which are particularly abundant) will give rise to many viable and interesting human phenotypes.

Another method for identifying useful genes is expression profiling. Paul Meltzer (National Human Genome Research Institute, Bethesda, USA) has been working with cDNA microarrays. He has focussed on four related tumor types - Burkitt's lymphoma, Ewing's sarcoma, neuroblastoma and rhabdosarcoma - in an effort to identify genes that are differentially expressed between these tumors and are diagnostically characteristic of each. Although there is some diversity of gene expression even within individual tumors, the results are promising: it appears that a set of perhaps ten genes may be useful for diagnosis. Jennifer Taylor (Queensland Institute for Medical Research, Herston, Australia) has attempted to use cDNA profiling to uncover genes diagnostic and perhaps causative of schizophrenia. Although the results are preliminary, it is possible that this approach may provide a new avenue into understanding this complex condition.

Experiments with the nuclear factor of activated T cells (NFAT) family of transcription factors have also provided insights into brain function. Gerald Crabtree (Stanford University, USA) explained that the *NFATc4*^{-/-} knockout mice exhibited learning and memory defects. Expression profiling using cDNA microarrays confirmed that the calcium channel protein IP3RI was a target of NFATc4; this result may offer a molecular inroad towards unraveling hippocampal function.

The analysis of proteins can also provide a way of identifying key genes. Archa Fox (University of Dundee, UK) explained

how nucleoli could be rapidly purified and their proteins separated on two-dimensional gels (Figure 1). Identification required proteolysis and subsequent mass spectroscopy. Of the proteins identified so far, about a quarter are recognized nucleolar proteins, about half are known proteins not previously associated with the nucleolus, and the remainder are novel proteins. This work may provide a fuller picture of the main activities in this subnuclear organelle.

Studies on single proteins can also illuminate complex networks of activity and reveal unexpected connections. Richard Treisman (Imperial Cancer Research Fund, London, UK) has successfully teased out the mechanisms that govern activity of the serum response factor (SRF) transcription factor. The pathway involves the Rho GTPases and their effectors the Diaphanous and ROCK kinases, which regulate actin dynamics, linking transcription factor output to monomeric actin levels. The array of proteins regulating SRF function is more extensive than one might expect (almost proteomic in itself) and this work illustrates what we should

look forward to in the near future, in an age in which other challenging pathways and interconnections can be unraveled as the full cast of players in the cell become known.

Insights into how genes are read

A large part of the meeting was devoted to investigating the molecular mechanisms underlying gene regulation. Peter Cook (Oxford University, UK) explained the evidence that RNA polymerase, rather than running down DNA like a Melbourne tram on its track, appears to be fixed in the nucleus at sites termed 'transcription factories'. DNA is twisted through the fixed polymerase, and enhancers may function to deliver genes to the factories. Moreover, there is evidence that RNA polymerase is not alone but is accompanied by the translation machinery. The surprising observation that translation can occur in the nucleus may help explain phenomena such as nonsense-mediated RNA decay. The work of Rudi Grosschedl (University of Munich, Germany) on the mechanisms of chromatin opening showed that the addition

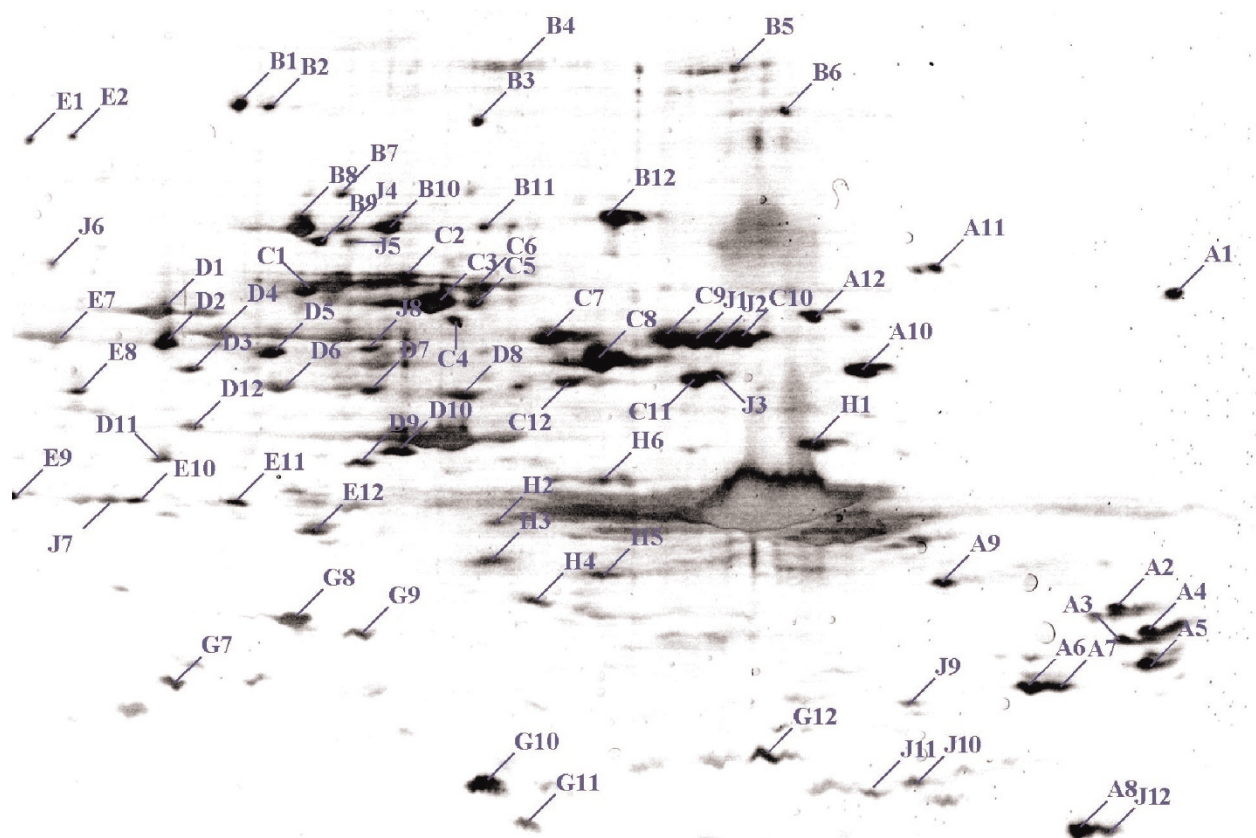


Figure 1

Proteomic analysis of nucleolar proteins. The availability of gene sequence data and advances in techniques of mass spectroscopy now allow large-scale protein identification. The sample shown is a two-dimensional gel (pH 4-7) of purified HeLa-cell nucleolar extract. Figure courtesy of A. Fox, C. Lyon, J. Anderson, M. Mann and A. Lamond.

of matrix attachment region (MAR) sequences to introduced sequences significantly increased overall levels of gene expression and histone acetylation; this is perhaps another indication that attachment is important for transcription.

Tony Kouzarides (University of Cambridge, UK) revealed that although histone acetylation is important, histone methylation also plays a key role in gene regulation. He demonstrated that a lysine methylase, Suvar 3-9, was associated with the Retinoblastoma protein. Suvar 3-9 specifically methylates lysine 9 of histone H3 but operates only if this residue is in the unacetylated state. He then went on to show that the chromodomain of the heterochromatin protein HP1 could specifically bind to methylated H3. This work suggests that the formation of silent heterochromatin may involve a step-wise process of deacetylation, methylation, and then compaction by heterochromatin-associated proteins such as HP1.

But genes that are silenced during development do not always remain silent. In some instances, reactivation can be achieved by removing histone deacetylases. The reactivation of fetal genes in the heart can lead to hypertrophy, and Eric Olson (University of Texas, Dallas, USA) has been investigating the molecular mechanisms involved. The key transcription factor MEF-2 is present in the adult heart but is inactive. It transpires that it is held in an inactive state by histone deacetylase (HDAC) enzymes, most notably HDAC-5. Phosphorylation of HDAC-5 in response to calcium signalling results in the release of HDAC-5 and its removal to the cytoplasm. This enables MEF-2 to activate gene expression.

Using technology to interfere with gene expression

In several instances, studies of natural control mechanisms have suggested means of altering gene expression for experimental or therapeutic purposes. Greg Hannon (Cold Spring Harbor Laboratory, New York, USA) spoke on RNA interference (RNAi) and the biochemical mechanisms by which double-stranded RNA can effect the degradation of homologous transcripts. Although RNAi has been used successfully to silence gene expression in many organisms, experiments in mammalian systems have in general not been promising (with the exception that RNAi seems to be effective in the early mouse embryo). Determining the key components of the RNA-induced silencing complex (RISC) and their expression patterns may help us identify systems in which silencing will be effective. Hannon has already shown that two components of RISC, the ZAP domain proteins Argonaute and Dicer, have human homologs and that RNAi can function in Chinese hamster ovary (CHO) cells. Bernie Carroll (University of Queensland, Brisbane, Australia) showed how intron-spliced RNAi was a particularly effective means of manipulating gene expression in plants. Finally, Levon Khachigian (University of New South Wales, Sydney, Australia) showed work with DNazymes, which are

analogous to ribozymes but more stable in cellular environments. These reagents were able to reduce the expression of the inflammatory-stimulated transcription factor Egr1 and may be useful in the treatment of heart conditions.

The accumulation of sequence data has far outstripped our ability to understand biological processes, but it has also facilitated the identification of important genes and enhanced our analyses of the mechanisms of expression. Hopefully, by the time of the next Lorne Genome Conference, February 2002, even more data will be available and exchanges between those working on human and kangaroo genomes will provide interesting new avenues for enquiry.