Meeting report

Immunological applications of genomics

Silvia Monticelli, Sonia Sharma and Anjana Rao

Address: Harvard Medical School and the CBR Institute for Biomedical Research, Boston, MA 02115, USA.

Correspondence: Silvia Monticelli. Email: monticel@cbr.med.harvard.edu

Published: 25 July 2006

Genome **Biology** 2006, **7:**321 (doi:10.1186/gb-2006-7-7-321)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2006/7/7/321

© 2006 BioMed Central Ltd

A report of the Cold Spring Harbor Laboratory meeting 'Gene Expression and Signaling in the Immune System', Cold Spring Harbor, New York, USA, 26-30 April 2006.

The 2006 meeting on Gene Expression and Signaling in the Immune System at the Cold Spring Harbor Laboratory highlighted diverse aspects of signal transduction and transcriptional regulation in lymphocytes and other hematopoietic cells. We shall discuss some of the highlights of the meeting, focusing on research related to, relying on or informed in some way by genomics, including studies of long-range interactions between *cis*- and even *trans*-regulatory regions, genome-wide transcriptional profiling and RNAi screens, epigenomic analysis, and the use of RNA interference (RNAi) and microRNAs (miRNA) to study gene expression.

Long-range gene regulation

The expression of immune-system genes is under strict control, with many of the crucial gene regulatory elements lying outside the proximal promoter regions. A number of presentations explored these distal regulatory regions and the proteins that interact with them. The specification of cell fate during the development of T lymphocytes in the thymus is associated with the differential expression of the CD4 and CD8 cell-surface co-receptor proteins, which mark two main lineages of T cells, the helper and killer (cytolytic) T cells, respectively. Expression of CD4 and CD8 is tightly controlled by *cis*-regulatory elements that function in a developmental stage-specific, subset-specific and lineage-specific manner. Wilfried Ellmeier (Medical University of Vienna, Austria) showed that activation of the murine *Cd8* locus is associated with chromatin remodeling that is mediated in part by the *Cd8* enhancers $E8_I$ and $E8_{II}$, which show a high degree of cross-species conservation. Using yeast one-hybrid screens, he and his colleagues identified the BTB/POZ domain-containing zinc-finger transcription factor MAZR as a factor that binds the $E8_{II}$ enhancer, and showed that MAZR is a transcriptional repressor of *Cd8* expression. A poster by Jamie Schoenborn and Christopher Wilson (University of Washington, Seattle, USA) described the use of two complementary approaches - chromatin immunoprecipitation (ChIP) and high-resolution, quantitative chromatin hypersensitivity to DNaseI - to identify novel regulatory elements in the murine *Ifng* locus, which encodes the cytokine interferon-γ (IFN-γ). They have applied these approaches to naive $CD4^+$ T cells (cells that have not yet encountered their antigens), to the Th1 and Th2 subsets that differentiate from naive T cells after antigen encounter, and to $CD8^+$ cytolytic T cells, identifying multiple distal regulatory elements in the *Ifng* locus. They found that these elements may contribute to properly regulated IFN-γ production *in vitro* and in response to LCMV viral infection *in vivo*.

Jeremy Boss (Emory University, Atlanta, USA) has identified novel distal regulatory regions within the class II region of the human major histocompatbility complex (MHC). One of these sites, termed *XL-9*, displayed properties of a so-called insulator site, sites that form barriers between inactive heterochromatin and active euchromatin. *XL-9* exhibited high levels of histone acetylation, blocked enhancer function in reporter assays, was enriched in the nuclear matrix fraction, and bound the insulator-binding protein CTCF *in vivo*. Boss has used the chromosome capture conformation (3C) assay, which detects long-range chromosomal interactions, to show that *XL-9* has characteristics of a genomic organizer that compartmentalizes the MHC locus into discrete transcriptional domains. He showed that *XL-9* engages with proximal regulatory regions of the flanking MHC class II genes *HLA-DRB1* and *HLA-DQA1* to form chromatin loops. Loop formation was associated with transcription of the MHC class II genes: it was induced by IFN-γ and required the activator protein RFX5 and the co-activator CIITA, which are already

known to be essential for transcription of MHC class II genes.

Several other studies used the 3C assay to evaluate long-range interactions between *cis*-regulatory elements. Amy Kenter (University of Illinois College of Medicine, Chicago, USA) studies long-range interactions in the immunoglobulin heavy-chain (IgH) locus in murine B cells, and used the 3C assay to look at interactions between the germline promoters of the constant (C)-region genes, the sites involved in immunoglobulin class switching (the recombinational replacement of one type of heavy-chain C-region gene by another in a rearranged immunoglobulin gene), and the intronic and 3′ enhancers in the IgH locus. (In this context, 'germline' denotes regulatory elements that can promote transcription at the unrearranged locus.) Interactions between the intronic enhancer Eμ and the 3′α enhancer (which are essential for expression of the immunoglobin heavy chain locus) and between Eμ and switch region γ3, were induced in B cells stimulated by treatment with bacterial lipopolysaccharide (LPS) but, importantly, not in T cells, which do not carry out class switching. These interactions were reduced in the absence of activation-induced cytidine deaminase (AID), an enzyme with a key role in both class switching and somatic hypermutation (the mutation of rearranged immunoglobulin variable-region genes) in activated B cells. These findings suggest a new function for germline regulatory elements - to form a scaffold that facilitates the interaction between switch regions upon lymphocyte stimulation, thereby promoting class switching.

Using a different approach (genomic Southern analysis) in the murine myeloma cell line F5.5, Barbara Birshtein (Albert Einstein College of Medicine, New York, USA) discovered an inversion of the IgH locus extending from the expressed rearranged heavy-chain V_H exon to a complex regulatory region to the 3′ side of the whole IgH gene cluster. The inversion is evidence that distant 3′ regulatory regions have interacted with the V_H gene being expressed. Mike Krangel (Duke University, Durham, USA) explored the role of transcription in promoting rearrangement between V and J gene segments in the T-cell receptor (TCR) α-chain locus. By introducing a transcriptional terminator downstream of gene segment $J_\alpha 56$, he confirmed a key role for transcription in targeting recombination events to J_α segments at some distance from a promoter, and also revealed a mechanism of transcriptional interference rather than promoter competition during V_α-J_α rearrangement, thus contributing to the ordered progression of recombination events characteristic of the locus.

Richard Flavell (Yale University School of Medicine, New Haven, USA) reported studies using the 3C assay showing that interactions between regulatory elements can occur not only within a chromosome, but also between chromosomes. Published work from his group has shown interchromosomal interactions in mouse T cells between the gene for IFN-γ on chromosome 10 and the clustered genes for the cytokines IL-4, IL-5 and IL-13 (the so-called Th2 cytokine locus) on chromosome 11. At the meeting Flavell described long-range interactions between the Th2 cytokine locus and the lymphotoxin (LT) locus (comprising the genes for lymphotoxin-β, tumor necrosis factor-α and lymphotoxin-α) on chromosome 17. The interactions were detectable in naive T cells but not in B cells or fibroblasts, and were strongly diminished in differentiated Th1 and Th2 cells. Flavell also described an interaction between *Ifng* and the LT locus, occurring mainly in Th1 cells, in which these genes are expressed. Deletion of the LT locus disrupted its interactions with *Ifng* and the Th2 cytokine locus as expected, but the direct Th2-*Ifng* interaction was maintained.

Taken together, these presentations underscored how long-range interactions may promote the physical separation of independent loci into discrete transcriptional domains or facilitate the coordinate regulation of coexpressed or mutually exclusively expressed genes.

Epigenetic chromatin modifications

Eukaryotic chromatin is subject to a variety of developmentally regulated covalent modifications and it is now accepted that modifications to histone proteins play an essential part in the epigenetic regulation of gene expression. Keji Zhao (National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, USA) described an epigenetic mapping strategy in which he combined ChIP and serial analysis of gene expression (SAGE) in a shotgun approach to characterize histone modifications along the entire T-cell genome. Comparing the resulting epigenetic map of histone acetylation and methylation with gene-expression levels, Zhao found, as expected, that genes repressed in T cells had high levels of methylation on lysine (K) 27 in histone H3, whereas active genes (encoding cell signaling proteins, inducible cytokines, apoptotic mediators and T-cell receptors) showed high levels of histone H3 K4 trimethylation and K9 acetylation. Most islands of histone acetylation, especially those in regions of high cross-species sequence conservation, showed enhancer function in transient reporter assays, whereas most regions of high H3 K27 trimethylation bound the Polycomb protein complexes PRC1 and PRC2 and were repressive in these assays.

The repressive effect of the Polycomb proteins appears to be due to their promotion of H3 K27 trimethylation. Matthias Merkenschlager (Imperial College, London, UK) has studied the role of the Polycomb proteins in transcriptional regulation in embryonic stem cells (ES cells). He reported that the loss of the Polycomb protein Eed, a member of the PRC2 complex that mediates trimethylation of H3 K27, in ES cells results in premature expression of several neuronal-specific genes (as assessed by replication-timing profiling) but had

modest effects on the timing of replication of the locus. This implicates PRC2 in the prevention of inappropriate expression of genes in ES cells.

Alexander Tarakhovsky (Rockefeller University, New York, USA) investigated whether the logic of histone modification might extend to non-histone proteins. He reported that G9a, a histone lysine methyltransferase responsible for H3 K9 dimethylation, contains an evolutionarily conserved sequence motif that resembles a motif in the amino-terminal tail of histone H3. This motif is lysine-methylated *in vivo* in a manner that requires, but does not affect, G9a catalytic activity. Mice with an alanine substitution at the methylated lysine residue show reduced susceptibility to viral infections, secondary to increased expression of genes controlling the antiviral response. Tarakhovsky proposed that the G9a tail is a functional histone H3 mimic that facilitates the localized assembly of transcriptionally repressive complexes.

A poster by Jian Xu and Stephen Smale (University of California, Los Angeles, USA) described a comprehensive DNA methylation analysis of lineage-specific genes in ES cells and hematopoietic stem cells. They find that control regions for typical tissue-specific genes are accessible to, and associated with, transcription factors in pluripotent stem cells, long before the genes are expressed. This contradicts the prevailing dogma that tissue-specific genes, which are fully methylated and assembled into condensed chromatin structures early in embryogenesis, are targeted for demethylation and decondensation only upon commitment to the appropriate cellular lineage.

Timing replication

A few studies focused on the connection between the timing of DNA replication and the regulation of gene expression, a complex relationship that remains largely unresolved. A beautiful example of such a connection was offered by Yehudit Bergman (Hebrew University Hadassah Medical School, Jerusalem, Israel). She showed that in differentiated pre-B cells, as opposed to stem cells, the two alleles at the κ immunoglobulin light-chain locus have an asynchronous replication pattern that is maintained in a clonal manner and propagated through many cell divisions. Rearrangement of a κ allele is associated with its early replication (the unrearranged allele replicates later), suggesting that these distinctions in replication pattern may be involved in relocating one allele to heterochromatin as well as in controlling κ rearrangement.

As part of his talk on transcriptional regulation in ES cells described earlier, Merkenschlager compared pluripotent ES cells, multipotent hematopoietic stem cells and lymphocytes by replication-timing profiling, and found that replication timing distinguishes cell types with different developmental potentials. A poster by Shoichiro Miyatake (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan) described the identification of a replication origin ($ori_{\text{IL-13}}$) downstream of exon 4 in the murine *Il13* gene; $ori_{\text{IL-13}}$ functions both in Th2 cells, which express the *Il4* and *Il13* genes, and in Th1 cells, which do not. Miyatake showed that the *Il4*/*Il13* locus is replicated very early in S phase in both Th1 and Th2 cells, despite the fact that it is only accessible to inducible transcription factors in Th2 cells. Replication initiation at $ori_{\text{IL-13}}$ required the presence of *CNS-1*, a conserved noncoding element in the intergenic region between *Il4* and *Il13*.

Dicing with gene regulation

Dicer is an RNaseIII-like enzyme that is required for generating both the short interfering RNAs (siRNAs) that mediate RNA interference (RNAi) and the microRNAs (miRNAs) that regulate gene expression. Klaus Rajewsky (Harvard Medical School, Boston, USA) described the generation of a conditional allele of the *dicer-1* (*dcr-1*) gene in the mouse. Using mice bearing this allele, his group has shown that Dicer is absolutely required for the development of the B-cell lineage. A poster from Stefan Muljo from Rajewsky's group showed that when Dicer function is ablated during the early stages of B lymphocyte development in adult bone marrow, Dicer-deficient pro-B cells do not mature to the pre-B cell stage. Rajewsky also reported that in secondary lymphoid organs such as lymph nodes, Dicer appears to be dispensable in naive B cells; however, Dicer-deficient B cells in the germinal centers (which develop in these organs during an immune response), are significantly out-competed by Dicer-sufficient B cells. To follow up on their studies of Dicer-deficient T cells, the Boston group has collaborated with the laboratories of Diane Mathis (Harvard Medical School), Christophe Benoist (Harvard Medical School), and Nikolaus Rajewsky (New York University, New York, USA) in computational studies to extract miRNA-target relationships in T cells. Klaus Rajewsky went onto describe how this was done by correlating miRNA target motifs in the 3′ untranslated regions of genes with gene-expression data from microarray analysis of Dicer-deficient T cells versus controls. Also focusing on murine B cells, Nina Papavasiliou and colleagues (Rockefeller University, New York, USA) has investigated the possibility that miRNAs regulate somatic hypermutation or class switching recombination. She reported the identification of *miR-155* as a miRNA that is dynamically expressed in response to stimulation of splenic B cells, and identified the enzyme AID as a putative target of *miR-155*.

Several groups reported microarray analyses profiling miRNA expression in various cell types. David Baltimore (California Institute of Technology, Pasadena, USA) described how his group has found that induction of *miR-146* in a human acute monocytic leukemia cell line (THP-1) stimulated with LPS is dependent on the transcription factor NFκB, and he proposed that this miRNA is part of a feedback loop that regulates NFκB activation by modulating

levels of the intracellular signaling proteins TRAF6 and IRAK4. Mark Davis in collaboration with Chang-Zheng Chen (both from Stanford University School of Medicine, Stanford, USA) has identified a microRNA that has a role in determining T-cell levels of activation: ectopic expression of this miRNA allows T cells to respond to weak agonists and antagonists with greater sensitivity.

Taking the wide view

Genome-wide transcriptional profiling is now widely used to interrogate biological processes. Harinder Singh (University of Chicago, USA) asked why mice lacking expression of the transcription factor IRF-4 show greatly diminished levels of mature immunoglobulin-secreting plasma cells. He found that *Irf4*$^{-/-}$ mature B cells are unable to induce proper temporal and stage-specific expression of the genes *Blimp-1*, *Xbp-1* and *Aid*, which together control the genetic program required for class-switch recombination and the subsequent production and secretion of immunoglobulins in plasma cells. Ken Murphy (Washington University, St. Louis, USA) reported studies on the earliest stages of mesoderm formation from murine ES cells. He has used transcriptional profiling to delineate the so-called canonical Wnt signaling pathway in ES cells, a signaling pathway that results in the entry of the transcriptional regulator β-catenin into nuclei. Combining this with inducible overexpression of constitutively-active β-catenin transgenes in ES cells, Murphy has been able to define the earliest stages of the developmental transition to mesoderm formation. He showed that the Wnt pathway is required during early mesoderm development to yield both hematopoietic and non-hematopoietic precursors, but that Wnt-dependent upregulation of the transcription factor Mesp1 induces formation of cardiac-specific mesoderm while excluding formation of hematopoietic precursors through feedback inhibition of the canonical Wnt signaling pathway.

A poster by Yousang Gwack (Harvard Medical School, Boston, USA) from our laboratory illustrated the power of genome-wide RNAi screens in *Drosophila* to identify conserved regulatory proteins in mammalian cells. These screens were designed to identify regulators of the calcium-responsive mammalian transcription factor NFAT. Although calcium-regulated NFAT proteins are not themselves present in *Drosophila*, this work took advantage of the high degree of conservation of the signaling pathway that controls NFAT subcellular localization and identified a dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) as a key NFAT kinase, and the mammalian homologs of the *Drosophila* proteins Orai and Stim as essential regulators of store-operated calcium entry. Following up on this work, one of us (S.S.) described in her poster the phosphorylation of NFAT by DYRK-family kinases, while Stefan Feske (Harvard Medical School), also from our laboratory, presented another poster describing identified defects in the human gene *ORAI1* as responsible for a defect in store-operated calcium entry through calcium-release-activated calcium channels (CRAC channels) in two patients with a severe combined immunodeficiency (SCID) syndrome. His poster then went on to describe the modified linkage analysis using genome-wide single-nucleotide polymorphism (SNP) arrays that had already linked this SCID defect to the region containing the *ORAI1* gene.

Genomics permeates every aspect of modern biology. The conference provided many examples of this, highlighting the importance of genomic approaches to our understanding of immune function. What still remains is to make the tools and technologies more generally accessible to working biologists, and more importantly, to instill an awareness of the critical importance of genomics in the day-to-day conduct of biological research.