

be used to define novel diagnostic subgroups.

In another use of the Lymphochip, we have discovered 33 novel lymphoid-restricted genes. Of these, 12 were not induced during *in vitro* B cell activation and were restricted in expression to germinal center B cells or memory B cells. Thus, transit of B cells through the germinal center induces a differentiation event that is marked by coordinate expression of a set of late-stage B cell differentiation genes. The expression of these genes is maintained even as the B cell leaves the germinal center microenvironment and differentiates into a non-dividing memory B cell. Some novel genes were found to be differentially regulated between centroblasts and centrocytes, showing the power of this functional genomics approach to elucidate molecular changes that occur during the process of positive and negative selection in the germinal center.

Amorese, Douglas

Characterization of cDNA microarrays fabricated using thermal ink jets

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Thermal ink jets (TIJ) have been used as a micro-delivery system for the deposition of cDNAs onto modified glass supports. We have modified TIJ heads and firing electronics, designed for the printing of ink, to form the core of a micro deposition system that efficiently, reliably and accurately deposits large numbers of cDNAs into tightly packed arrays. Data will be presented demonstrating that these arrays, generated by the TIJ process, generally perform similarly to arrays generated using conventional pen technology but offer advantages in feature quality, control of spot size and large-scale manufacturing. Quality assurance data from the drop detect and inspection systems used to qualify production runs will be presented as well as results from experiments using multiple TIJ fires to produce variable feature sizes. Lower limit of detection and two-colour competitive hybridization data collected from a new high-speed, high depth of field, autofocus scanner (under development) will also be presented.

Amundson, Sally

The 'stress chip': a specialized functional genomics application for the study of genotoxic stress response

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The cellular responses to ionizing radiation and other genotoxic stresses are complex and are regulated by a number of overlapping pathways. One such stress-signalling pathway is exemplified by the transcription factor p53, which can regulate the expression of a myriad of downstream genes in response to various stresses. We have recently demonstrated the utility of cDNA microarray hybridization to measure radiation stress-gene responses in p53 wild-type human cells. Our initial experiments also resulted in the identification of a number of new radiation-responsive genes. We have since extended these studies to a broader range of doses and timepoints, and identified a large set of genes regulated in response to

ionizing radiation, as well as other stresses. These and other newly identified stress-responsive genes are being assembled in a stress-specific array, tailored for use in future genotoxic stress experiments. By focusing our efforts on a subset of responsive genes, we hope to rapidly obtain large stress-specific databases suitable for an informatics approach to analysis. Similar analytical approaches in our laboratory have previously yielded important mechanistic insights into the function of the gene *GADD45*, forming an important precedent for the utility of informatics analysis. The wide variation in responses observed for some stress-responsive genes in different cell lines, or in response to different genotoxic stresses, highlights the importance of cellular context to stress response, as well as the need for informatics approaches to the study of stress-specific gene response pathways.

Baker, Henry

Use of global genomic expression studies to define Gcr1p as a major transcriptional activator

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In *Saccharomyces cerevisiae* the enzymes of glycolysis make up 30–60% of the soluble cellular protein. The first indication that glycolytic enzyme genes were coordinately regulated came with the isolation of *gcr1* mutants, which have severely reduced levels of most glycolytic enzymes. *GCR1* encodes a positive transcriptional activator, Gcr1p, that binds at the UAS elements of glycolytic enzyme genes with the assistance of Rap1p. An important question concerning Gcr1p is the extent of its action in the cell. Using high-density DNA microarrays, we defined a limited set of genes that are dependent on Gcr1p. For these studies we used two isogenic strains, wild type and a *gcr1* mutant grown in the permissive medium of YP supplemented with glycerol and lactic acid. RNA was collected from cells during steady-state growth and used to prepare labelled cDNA. The labelled cDNA was hybridized to DNA microarrays. ORFs that displayed threefold or higher hybridization to cDNA from wild-type cells accounted for 30% of the hybridization of the total transcriptome, whereas these same ORFs accounted for only 4% of the transcriptome of the *gcr1* mutant. We identified approximately 50 ORFs using the threefold cutoff. These 'GCR1-dependent genes' were placed in three classes: (i) glycolytic enzyme genes; (ii) ORFs encoded by Ty elements; and (iii) genes whose expression was not previously known to be dependent on Gcr1p. Of the eight glycolytic enzyme activities known to be severely affected by *gcr1* mutations, ten ORFs encoding seven of the activities were identified. Only *PGII* was not identified using the threefold cutoff; it displayed a twofold difference. In total 32 of the GCR1-dependent genes identified by microarray analysis were previously known, or were expected, to be dependent on GCR1. The expression of the remaining ORFs identified was not previously known to be dependent on GCR1. Genes encoding ribosomal proteins were not identified as GCR1 dependent, suggesting that Rap1p interacts with activators other than Gcr1p at ribosomal protein gene UAS elements. In summary, a limited set of genes is dependent on GCR1 for expression, but together they compose 30% of the yeast transcriptome. The genomic expression studies described here further establish Gcr1p as one of the most potent transcriptional activators.