Nagel, James

Microarray analysis of chemokines, chemokine receptors and related pathways

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Besides chemotaxis, C-X-C and C-C chemokines function as mediators in T-cell activation and in many lymphocyte biological responses. Detailed information about downstream signalling pathways is necessary to understand the role of chemokines in normal physiology and inflammation. We have built a focused cDNA chip containing 675 known genes that are expressed and secreted by lymphoid cells and participate in their growth, signal transduction and apoptosis. In initial experiments, Jurkat T cells expressing endogenous CXCR-4 and transfected with CXCR-3 were used to examine the ability of chemokine ligands to induce unique mRNA expression. We compared transfected cells treated with the C-X-C chemokines SDF-1-, which interacts via CXCR-4, and IP-10, which interacts via CXCR-3. Further experiments examined the effects of anti-CD3, TARC, 6Ckine, IL-8, MIP-3 /LARC/Exodus, MIP-3 /ELC/ Exodus-3 and HIV gp120 glycoprotein on mRNA expression by PBMC and purified T cells. Using this approach we have observed differences in gene expression by cells treated by different ligands that bind the same chemokine receptor. This focused microarray is also useful to define candidate genes for further study, and to identify informative experimental and clinical specimens before molecular profiling on the NIA 15K chip.

Nguyen, Hong-Khanh

Minimizing the secondary structure of DNA targets with a modified deoxynucleoside and its implication for nucleic acid analysis by hybridization

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Some regions of nucleic acid targets are not accessible to heteroduplex formation with complementary oligonucleotide probes because they are involved in secondary structure through intramolecular Watson-Crick pairing. In order to form a heteroduplex, we destabilize the secondary conformation of the target to assist its interactions with oligonucleotide probes. To achieve this goal, we modified the DNA target by replacing dC with N-4-ethyldeoxycytidine (d4EtC), which hybridizes specifically with natural dG to give a G4EtC base pair with reduced stability compared with the natural GC base pair. In contrast to its natural analogue, the use of d4EtC greatly minimized the formation of the target's secondary structure in preliminary solution studies. The lower level of secondary structure allowed hybridization with a complementary probe. To characterize further the influence of d4EtC on the stability of secondary structure, hybridization of the targets is currently being studied using an array of complementary oligonucleotides scanning the sequence.

O'Hagan, Ronan

Identification of differentially regulated gene targets in the Myc and Mad family proteins

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Myc and Mad family proteins regulate diverse biological processes through their capacity to influence gene expression directly. Their biochemical profiles, as well as their behaviour in surrogate assays for transcription and transformation, support a model in which the opposing properties of Myc and Mad occur primarily through their reciprocal regulation of common gene targets. An examination of this model on several levels reveals that Myc and Mad family basic regions are not functionally equivalent in oncogenesis, that their E box-binding activity is influenced by critical interactions between flanking nucleotide sequences and non-conserved residues at position 2 of the basic region, and that there is lack of complete concordance in the genes regulated by the Myc and Mxi1 basic regions. These data support the view that the opposing biological actions of Myc and Mxi1 are likely to extend beyond reciprocal regulation of common gene targets. This complex inter-relationship is further emphasized by the finding that alterations in the basic region can influence the ability of Myc to directly repress targets, suggesting that the basic region may also have a role in Inter-mediated gene regulation. Identification of these differentially regulated gene targets provides a framework for understanding the mechanism through which Myc governs the growth and survival of normal and neoplastic cells.

Okazaki, Yasushi

Gene expression profiling using a mouse full-length 20 K cDNA microarray

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Most public-founded large-scale sequencing projects that use cDNAs are primarily using cDNA libraries which are not enriched for full-length cDNAs. Consequently, only a fraction of the resulting ESTs matches the 5′ end of the original transcript. The target of the Genome Science Laboratory of RIKEN is to clone and sequence the largest number possible of full-length mouse cDNAs in two