more miRNAs expressed at lower levels. Many of the miRNAs were isolated from mixed-stage RNA preparations (*2*, *3*). Biochemical collection of miRNAs from selected cell types or finely staged preparations may reveal rare miRNAs that act in particular cells or at particular times. But the comprehensive detection of miRNAs expressed in few cell types or under particular conditions may demand informatic approaches based on the now-extensive training set of miRNAs revealed in these papers.

In fact, the number of genes in the tiny RNA world may turn out to be very large, numbering in the hundreds or even thousands in each genome. Tiny RNA genes may be the biological equivalent of dark matter—all around us but almost escaping detection, until first revealed by *C. elegans* genetics and then more comprehensively charted by these papers. The next step is to figure out whether these regulatory RNAs use principles of amplification and systemic spread that have selected for their conservation as well as their ramification into so many apparently new sequences.

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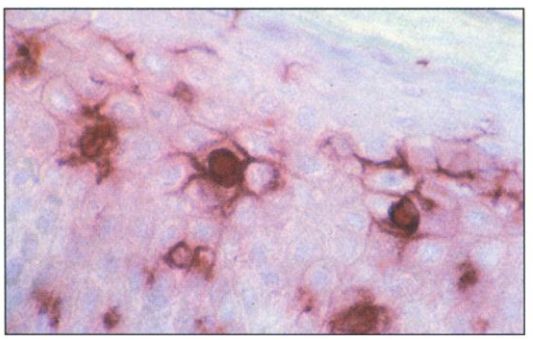
PERSPECTIVES: IMMUNOLOGY

Chip Shots—Will Functional Genomics Get Functional?

Robert L. Modlin and Barry R. Bloom

Of every 10 people infected with the tubercle bacillus, only one will develop tuberculosis; of a hundred individuals infected with the malaria parasite, only 0.5% will die. When people are exposed to the same point source of infection, some become ill, for example from enteritis caused by *Escherichia coli* in contaminated food, whereas others do not; some may die from influenza whereas others suffer only mild symptoms. How can we understand the basis for these variations in susceptibility to infection? In the genomic age, the easy answer could be that pathogens switch on different genes or differentially alter expression of the same genes in susceptible versus resistant individuals. But surely variations in disease susceptibility cannot be explained so readily. Indeed, given the presumed strong selective pressure of infection on the human genome, it is striking how few genes exclusively expressed in the immune system are strongly associated with disease susceptibility, and how small the effects of known polymorphisms (sequence differences) in individual genes appear to be (*1*). On page 870 of this issue, Huang *et al.* (*2*) compare the gene expression profiles of human dendritic cells challenged with three different pathogens (a bacterium, a yeast, and a virus). In particular, these investigators wanted to know whether antigen-presenting dendritic cells could discriminate the three microbial challenges and whether discrimination reflected the program of genes activated uniquely by the interaction of a specific pathogen (or its products) with the dendritic cells. As they report, gene chip array analysis revealed that the patterns of dendritic cell gene expression varied according to the pathogen encountered, with some genes being uniquely activated.



Dendritic cells on the frontline. Photomicrograph of skin dendritic cells (Langerhans cells) from a patient with leprosy. Dendritic cells, components of the innate immune response, recognize and respond to microbial pathogens by activating T cells (adaptive immunity) and by releasing inflammatory mediators that mobilize neutrophils and macrophages (innate immunity). Patterns of dendritic cell gene expression differ according to the microbial pathogen encountered (*2*).

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Dendritic cells, members of the body's innate immune system (see the figure), are a good choice of immune cell for this type of study. They are important for initiating both adaptive immunity (activating T and B cells expressing receptors that recognize microbial antigens) and innate immunity (activating phagocytic cells such as neutrophils and macrophages). With their gene chip arrays, Huang *et al.* (*2*) discovered a hierarchy of gene expression in human dendritic cells. The outright winner, the *E. coli* bacterium, activated 685 genes; next came the influenza virus with 531 genes activated; and last came yeast (*Candida albicans*), which activated the fewest genes (289). Of the 6800 genes on the chip array, about 2000 were expressed in resting dendritic cells, and 1330 genes showed alterations in expression after infection. Based on database annotations, the expressed genes were categorized into the following families: innate or adaptive immunity, immune receptors, immune transcription, glycolysis and energy, apoptosis, growth factors, tissue remodeling, cell stress, and immune inhibitors. Despite the fact that the three microbes are completely different organisms, a large number (166) of core genes were activated by all three pathogens, and a smaller number of overlapping genes were activated by two of the three pathogens. Some genes were activated early, some 8 hours after infection, and others 12 hours after infection. The innate immune response appears to be programmed to respond to widely different pathogen challenges with a common core pattern of gene activation. More interesting still, pathogen-specific responses were also observed—118 and 58 genes were activated only by *E. coli* or influenza virus, respectively. Curiously, *Candida* did not induce any pathogen-specific genes. This could imply that the core genes induced by all three pathogens would be sufficient to combat yeast infection. Alternatively, other genes not represented on the chip array, or immune cells in addition to dendritic cells, may be required to deal effectively with this pathogen.

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To explore how these three pathogens activate unique patterns of gene transcription, Huang *et al.* studied the responses of cultured dendritic cells to molecules produced by these microbes. For example, lipopolysaccharide from the cell wall of *E. coli*, yeast cell wall–derived mannan, and viral double-stranded RNA activate the innate immune system through distinct receptors that recognize specific biochemical patterns. These microbial ligands triggered only a subset of genes induced by the whole live pathogen. Generally, the live pathogen was a more potent gene activator than its ligand. For example, 5 to 10 times more mRNA and protein of inflammatory cytokines (tumor necrosis factor–α, p40, and interleukins IL-12 and IL-10) were elicited by *E. coli* than by lipopolysaccharide. Curiously, in some cases the pattern of genes triggered by the microbial ligand failed to correlate with that induced by the intact pathogen; for example, the set of genes activated by yeast-derived mannan was more similar to that elicited by *E. coli* than by *C. albicans*. But what does all of this information really tell us?

In the case of cancer, microarray analysis of different tumors at different stages of malignancy has yielded information that could not be discerned by pathological examination. For example, gene arrays have been used to classify leukemias (*3*), breast cancers (*4*), and melanomas (*5*), and in some cases to predict the survival of cancer patients. An elegant gene chip study has shown that the signaling protein RhoC is essential for metastasis of melanomas (*6*). Such research is paralleled by studies of in vivo gene expression in microbial pathogens using several sophisticated approaches including in vivo expression technology (IVET) (*7*), molecular beacons (*8*), and terrain mapping (*9*). These techniques, combined with gene chip studies of immunologic responses to microbial pathogens and their ligands, are already yielding important functional information (*10, 11*).

The interaction between a pathogen and its host is clearly complex. It is one thing for the expression of 1000 genes to be altered by infection and neatly classified into families, but quite another to know which of these genes are crucial for host defense, and which promote microbial invasion, survival, and pathogenesis. Different pathogens (and their products), which presumably interact with different patterns of cell receptors, stimulate both common and unique signaling pathways and activate the expression of a number of different genes. The study of gene chip arrays is often referred to as "functional genomics," and classifying genes whose expression is altered on the array allows us to speculate about what these genes do.

But, which of the activated genes are crucial for protective immunity, which have little to do with the immune response, and which are involved in tissue injury? Array data narrow down the possibilities, and as such are enormously valuable, but annotations alone cannot answer these questions. Huang *et al.* did confirm in vitro that some of the cytokines produced by dendritic cells in response to *E. coli* were able to increase chemotaxis of neutrophils, key inflammatory cells. It remains to be determined whether these cytokines do the same in vivo.

How will functional genomics become functional? In a world of multiple genes and quantitative traits controlling complex phenomena, such as immunity to infection, how do we learn which genes count and which do not? And how will your automated health care provider of the future, analyzing a drop of your blood before a trip to an exotic destination, discern worrisome susceptibilities to nasty pathogens (and provide you with the appropriate therapeutic drug or vaccine)? Perhaps answering these questions will require reverting to the quaint hypothesis-driven, low-throughput approach once known as experimental biology.

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PERSPECTIVES: ASTRONOMY

Brown Dwarfs

John E. Gizis

Below about 75 times the mass of Jupiter (75 M_J), astronomical objects cannot achieve core temperatures hot enough for sustained nuclear fusion of hydrogen. These failed stars are called brown dwarfs. The hydrogen burning limit thus defines the cutoff between stars and brown dwarfs, but the term "brown dwarf" is usually also taken to refer to objects that form through collapse and fragmentation of an interstellar cloud (like stars) rather than originating in a circumstellar disk (like planets).

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It has long been expected that star formation will produce brown dwarfs, particularly because the time scales of brown dwarf formation are too short for hydrogen burning to be relevant. However, detection of brown dwarfs has proved difficult because they are very faint. Star and planet formation models have to account for the properties of these objects at the extreme low-mass end of the stellar mass distribution. Thanks to improved telescopes and detectors, the observational characterization of brown dwarfs is now well under way. But although models of the evolution of brown dwarfs are well developed, models of their formation are still in a preliminary state.

The first unambiguous brown dwarfs were discovered in 1995. Since then, hundreds have been identified in star-forming regions, young open clusters, and the field. Nearby, old brown dwarfs in the field have been identified with the Deep Near-Infrared Survey (DENIS), Two Micron All-Sky Survey (2MASS), and the Sloan Digital Sky Survey (SDSS). New spectral classification systems have been developed to characterize their properties (*1–4*). Over the course of 100 million to 10 billion years, an individual brown dwarf will evolve through spectral classes M (with an effective temperature $T_{\rm eff} > 2200$ K), L (2200 K > $T_{\rm eff} > 1400$ K), and T (1400 K > $T_{\rm eff} > 700$ K) to as yet unobserved cooler states.

There is now wide agreement that brown dwarfs are numerous but not a substantial source of dark matter. Counts of brown dwarfs in the field, open clusters, and star-forming regions indicate that brown dwarfs represent a small (less than 15%) fraction of the stellar mass but a substantial fraction by number (*5*).

If the brown dwarf population is a result of the star formation process, it should share properties with stars. Many—if not most—stars are found in multiple systems, and the formation of circumstellar disks is also common. Some 20% of L dwarfs in the 2MASS survey turn out to be doubles when imaged with the Hubble Space Telescope. The brown dwarfs in these pairs are between 1 and 10 astronomical units (AU; 1 AU equals the Sun-Earth distance) apart, indicating that there are few wide brown dwarf–brown

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