nature neuroscience

Probing the brain with DNA chips

For several years now, the genetics community has been talking about DNA microarrays (DNA 'chips' and related methods) as the next quantum leap in gene technology. The enthusiasm has reached the point where reviews of the new technology are said to outnumber the published studies that use it. Nevertheless, emerging results suggest that the hype is justified, and a collection of reviews published as a supplement to the latest issue of our sister journal *Nature Genetics* affords an opportunity to examine the current state of this technology[1]. (The reviews will be freely available from 1 January at http://genetics.nature.com.) Judging from the results presented, DNA microarrays seem likely to have major impact on almost every branch of biology, including neuroscience.

In essence, microarrays consist of large numbers of DNA probes immobilized onto a solid support such as a glass slide or a filter, in a known arrangement. The probes are then incubated in a solution of labeled target sequences, which may be genomic DNA or cDNA from a tissue of interest. The presence and abundance of specific target sequences within the sample is indicated by the intensity of the hybridization signal at the corresponding probe locations. Among the most promising ways to make microarrays is the photolithographic method pioneered by Affymetrix, in which oligonucleotides are synthesized directly onto the support, using photochemical techniques with great spatial precision; the latest versions contain around a million probe sequences on a chip not much bigger than 1 cm^2. Although no other method gives such high densities, some are substantially cheaper and more accessible and can still produce arrays containing many thousands of probes.

One of the most impressive applications of chip technology is the ability to monitor the levels of thousands of transcripts simultaneously. For example, the review by Brown & Botstein in ref. 1 presents data showing how 2467 yeast genes (some 40% of the total) are affected by various environmental stimuli and mutations. The data indicate that it is possible to group genes together based on similar patterns of expression and, importantly, that functional relationships can be predicted from these patterns. It should also be possible to identify causal interactions, by asking how loss of one gene affects expression of the others. To do this systematically for all 6000 yeast genes implies a total of around 40 million datapoints, but even this ambitious goal is not beyond the realm of possibility.

It will be several years before the complete human or mouse genome sequences become available, but there are already many expressed sequence tags (ESTs) from both species in the public domain, of which a high proportion are expressed in the brain. Moreover, work will soon begin on project, sponsored by the US National Institutes of Health, to generate ESTs from different brain regions and developmental stages, first in mice and later in humans.

Many neuroscience questions will become accessible as a result. Everyone will have their own favorites, but the following might be high on the list: changes that accompany learning in the hippocampus and amygdala (where new transcription is closely correlated with context and fear conditioning respectively); changes at the end of critical periods for developmental plasticity; abnormal patterns of gene expression in animal models of brain diseases; differences between cortical areas that may determine their specific functions and connectivity patterns; and comparative studies of different primate species, to examine evolutionary relationships between monkey and human brain regions, and perhaps ultimately to identify the genetic basis of humans' unique cognitive abilities.

One problem in interpreting patterns of gene expression in the brain is its cellular complexity. What is needed is a method for examining gene expression at the single cell level, in other words for generating enough probe from a single neuron to hybridize to a chip. This is a major challenge given the small amount of RNA involved, but James Eberwine at the University of Pennsylvania believes it can be done. He has developed methods based on PCR amplification of tiny quantities of cDNA, followed by transcription of labeled RNA probes from promoter sequences incorporated into the PCR primers. A potential problem in amplifying small quantities of complex mixtures is to preserve the relative abundance of different sequences as they are amplified, but Eberwine claims that his method is accurate and robust. He can also extract RNA from samples that are fixed and stained, allowing characterization of gene expression in defined cell types. Eberwine and his collaborators are now using these techniques in a range of situations; these include abnormally stained neurons in the brains of schizophrenics, apoptotic neurons in a rat model of brain injury, individual degenerating neurons from Alzheimer's patients, and neurons in the nucleus accumbens of opiate-seeking rats. The amplification method should open up many further possibilities; among the most exciting is the potential for correlating large-scale patterns of gene expression with the physiological properties of individual neurons, using techniques in which cytoplasm is extracted through an intracellular recording electrode.

The vast datasets arising from the use of microarrays will raise new questions, but what form these will take is still unclear, and there will probably be a period of exploratory data-gathering before the questions come into sharp focus. What is clear, however, is that the ratio of manual labor to intellectual insight will be altered. To date, progress has often been limited by the sheer effort required to generate molecular data, as a generation of researchers can testify. This is about to change, and it seems likely that future progress will be limited not by the availability of data, but by the concepts and analytical tools to make sense of it all. Nowhere will the challenge be greater than in the nervous system; one likely consequence will be an urgent need for young scientists with the necessary theoretical background to analyze the behavior of complex systems, not only at the physiological but also at the molecular level.

1. The chipping forecast. *Nature Genetics* **21** (supplement), 1999.