Journal Club

Zinc fingers and chips

Until now, the focus of most array-based studies has been the monitoring of RNA expression levels. However, the microarray format can be much more versatile. A recent paper by Bulyk *et al.*[1] explores the possibility of using DNA microarrays to investigate the sequence specificity of DNA–protein interactions. An understanding of how a transcription factor (TF) binds to DNA is crucial as it is a key step in the regulation of gene expression. Typically, insights into how DNA-binding specificity is achieved have been obtained by mutating the DNA-binding sites and/or the amino acid residues thought to be important in sequence-specific binding, and then observing the effect on binding. Phage display has enabled millions of protein variants to be selected simultaneously, but traditional methods necessitate analysing the effects of individual mutants one at a time. The main aim of this paper was to prove the principle that the rules governing TF–DNA binding specificity could be unravelled using a much more rapid and sensitive microarray method.

As a model, they chose to study DNA recognition by a mouse zinc-finger TF called Zif268. This TF is particularly valuable as a model system, as we already know from crystallographic data that the protein has three fingers in the DNA-binding domain that bind as independent modules to three tandem 3-bp subsites. To analyse protein–DNA interactions, double-stranded DNA containing the wild-type Zif268 binding sites for fingers 1, 2 and 3 was arrayed in multiple replicates onto glass slides. The array was then exposed to the wild-type Zif268 protein expressed by phage display. Bound protein was detected with a primary antibody against a phage coat protein and a fluorescently labelled secondary antibody. After various normalization controls, the fluorescence intensity of each spot was used to indicate relative protein–DNA binding affinity. A phage display library of Zif 268 variants was constructed where the amino acids in finger 2 were randomized, keeping fingers 1 and 3 wild type. These variants were tested for their ability to bind arrays that contain wild-type binding sites for fingers 1 and 3 and all possible 3-bp combinations for finger 2. Thus, the authors were rapidly able to assess the sequence-specific DNA recognition by this zinc-finger protein. To validate the use of this microarray protocol, the results were confirmed by ELISA.

Bulyk *et al.* show that microarray binding experiments can distinguish between the DNA-binding site preferences of different TFs, even if they have very similar DNA-binding specificities. The method proved highly sensitive and potentially able to determine which transcription factors are likely to bind at only a few specific sequences and which are likely to bind more uniformly throughout the genome. It could easily be used for other structural classes of DNA-binding domain and for analysing the effect of different concentrations of TFs and other cofactors. Furthermore, by arraying intergenic regions, it could be adapted to analyse binding sites throughout the genome. Clearly, this has huge potential for predicting uncharacterized TFs, for elucidating regulatory networks and ultimately for unravelling the rules that govern the specificity of DNA–protein interactions.

1 Bulyk, M.L. *et al.* (2001) Exploring the DNA-binding specificities of zinc fingers with DNA microarrays. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7158–7163

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Why do you avoid me? Migration of cortical interneurons

Two major migration processes take place in the developing brain, the guidance of axons to their targets and the migration of neuronal cell bodies from their sites of generation to functional compartments in the brain. Recent successes have greatly furthered the understanding of the genes that control axon guidance. Nature reveals its economy again as it becomes clear that these genes also underlie the guidance of migrating cells. The Netrin and Slit proteins have already been implicated in guiding cells, and now Marín *et al.*[1] show that another key system, the semaphorin–neuropilin partnership, controls the migration of cortical interneurons.

Cortical interneurons are born in the basal telencephalon and migrate long distances to the cortex, avoiding the striatum, which lies in their path. Careful examination of semaphorin and neuropilin expression revealed a complementary pattern, with striatum expressing Sema3A and Sema3F, and migrating interneuron precursors expressing neuropilin1 and neuropilin2. Using cortical slice cultures and tissue grafting from green-fluorescent protein (GFP)-labelled tissue, the authors show that interneurons avoid ectopic striatal tissue specifically. They also avoid implanted, Sema3A- and Sema3F-expressing COS cells. Direct DNA electroporation into cortical slices then showed that interneurons in neuropilin2-mutant tissue only partly avoid the striatum. Furthermore, electroporation of a dominant–negative *Neuropilin1* gene into interneurons also allowed them to migrate directly through striatal regions.

These data demonstrate convincingly that striatal semaphorins act as chemorepellants for migrating interneuron precursors and that the cells themselves use both neuropilin 1 and 2 to avoid the striatum completely. This work shows that the semaphorin–neuropilin partnership has the potential to guide a great many cell migrations in the brain. As such it could prove to be yet another powerful, underlying genetic factor in establishing compartments and boundaries in the central nervous system.

1 Marín, O. *et al.* (2001) Sorting of striatal and cortical interneurons regulated by semaphorin–neuropilin interactions. *Science* 293, 872–875

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