

Microarray Analysis Of *Drosophila* Metamorphosis

Metamorphosis is thought to be an integrated set of processes controlled by a transcriptional hierarchy that coordinates the action of hundreds of genes. Instead of proceeding gene-by-gene, White and colleagues devised a microarray assay that could identify hundreds of coordinately regulated genes.

The target DNA: Patterns of gene expression during the early metamorphosis of *D. melanogaster* were examined by assaying whole animals at stages that span two pulses of ecdysone. The microarrays were constructed containing more than 4500 unique cDNA expressed sequence tag (EST) clones along with a number of ecdysone-regulated control genes having predictable expression patterns. The latter would be the positive controls. These ESTs represent approximately 30% to 40% of the total estimated number of genes in the *Drosophila* genome.

The probe: In order to quantitatively gauge expression levels, the microarrays were hybridized with fluorescent probes derived from polyA⁺ RNA isolated from the two populations of larvae. Nineteen arrays were examined representing six time points relative to puparium formation: one time point *before* the late larval ecdysone pulse; one time point *just after* the initiation of this pulse (4 hours BPF), and time points at 3, 6, 9, and 12 hours after.

More than 10% of the genes represented by the ESTs display threefold or more differential expression during early metamorphosis. Although this may seem like a high proportion of the total number of genes in *Drosophila*, the authors point out that it actually may be an underestimate, given the stringency of criteria for their selection in the database.

Differentially expressed genes fall into two major categories. The first category included those genes that are expressed at >18 hours BFP (before the late larval ecdysone pulse) but then fall to low or undetectable levels during this pulse. These represent the genes thought to be repressed by ecdysone. They make up about 44% of the developmentally regulated genes. The second category consists of genes expressed at low or undetectable levels before the late larval ecdysone pulse but which are then upregulated during this pulse. These genes are thought to be the ones induced by ecdysone, and they comprise about 31% of developmentally regulated genes. Thus, 75% of genes that changed in expression by threefold or more do so during the late larval ecdysone pulse that marks the initial transition from larva to prepupa. This result is consistent with the extreme morphological changes that are about to occur in these animals (White, 1999).

Once one knows the genes that are regulated, one can do finer tests to determine what regulates them. To test whether new targets of transcription factors could be identified in the ecdysone genetic hierarchy, the ecdysone-induced nuclear receptor DHR3 was prematurely expressed at >18 hours before puparium formation. DHR3 is responsible for the coordination of part of the transcriptional program regulating metamorphosis and can act as either a repressor or an activator of transcription, depending on the target gene. One of the most important known targets of DHR is β FTZ-F1, a nuclear receptor that is active during midprepupal development and which is responsible for the difference in the genetic response to ecdysone between the late larval and prepupal ecdysone pulses. β FTZ-F1 induction is confirmed by the microarray results. Several other genes are also induced by DHR3, when it is expressed at >18 hours BPF. One of these is represented by a novel EST (LD24139) that is induced from 3 to 9 hours APF during wild-type development (White, 1999).

The ability to perform large-scale screens such as these should greatly facilitate our analyses of complex events such as metamorphosis.

Literature Cited

White K. P., Rifkin S. A., Hurban P., Hogness, D. S. 1999. Microarray analysis of *Drosophila* development during metamorphosis. *Science* 286: 2179-2184.

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