

WHOLE-GENOME ANALYSIS OF DORSAL GRADIENT THRESHOLDS IN THE DROSOPHILA EMBRYO

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Dorsal is a sequence-specific transcription factor related to NF- κ B. The protein is distributed in a broad nuclear gradient in the precellular *Drosophila* embryo. This gradient controls dorsal-ventral patterning by regulating at least 50 target genes in a concentration-dependent manner. Dorsal works with two additional regulatory proteins that are encoded by genes directly regulated by the gradient, Twist and Snail. To determine how the Dorsal gradient generates diverse thresholds of gene activity, we have used ChIP-chip assays with Dorsal, Twist, and Snail antibodies. This method efficiently identified 20 known enhancers and predicted another 30-50 novel enhancers associated with known or suspected dorsal-ventral patterning genes.

At least one-third of the Dorsal target genes appear to contain “shadow” enhancers. These are additional cis-regulatory sequences with activities that overlap the principal enhancer guiding the expression of the associated gene. Shadow enhancers might arise from duplications of regulatory DNAs and could provide an important source for novel patterns of gene expression during evolution.

The analysis of ~30 different Dorsal target enhancers suggest that those mediating gene expression in response to high levels of the Dorsal gradient contain a series of disordered low-affinity Dorsal and/or Twist activator binding sites. In contrast, enhancers mediating expression in response to low levels of the gradient (5% or less of the peak levels of the Dorsal protein) contain an ordered arrangement of optimal Dorsal and Twist binding sites. This organization is likely to foster cooperative occupancy of linked operator sites. We

discuss the importance of enhancer structure in mediating a sensitive threshold response to a morphogen gradient.

Although there are many examples of gene regulation via elongation of stalled polymerase (Pol) II, it is not known to what extent this mechanism is used to establish differential patterns of gene expression during *Drosophila* embryogenesis. To investigate this issue, we performed ChIP-chip assays using antibodies directed against Pol II. A specific mutant embryo was used--Toll^{10b}--that contains high, uniform levels of the Dorsal, Twist, and Snail proteins. As a result, all of the cells form mesoderm derivatives. Ectodermal derivatives, such as the CNS and extraembryonic membranes, are completely absent.

Previous whole-genome tiling arrays identified every gene that is active and inactive in Toll^{10b} mutant embryos. Neurogenic genes that are activated by intermediate levels of the Dorsal gradient are repressed due to overexpression of the Snail repressor. Although silent, most of these genes contain a peak of Pol II binding at the 5' end of the transcription unit. In contrast, genes that are uniformly expressed in these embryos display distinct Pol II binding profiles (across the length of the transcription unit). It was possible to classify 75% of all protein coding genes in the *Drosophila* genome into 3 categories based on Pol II binding profiles: uniform binding, no binding, or restricted binding near the start site. The ~3600 genes exhibiting a uniform Pol II binding profile encode housekeeping functions that are constitutively expressed throughout embryogenesis. The ~5,000 genes lacking

Pol II binding tend to be silent in the embryo, but expressed during larval and adult development. Finally, the ~1600 genes that exhibit 5' binding (i.e. stalling) tend to exhibit localized patterns of gene expression during embryogenesis and function as developmental control genes, such as Hox genes and components of the FGF, Wnt, Hedgehog, TGF β , and Notch signaling pathways.

These observations suggest that the regulation of Pol II elongation is a major mechanism of differential gene activity in the *Drosophila* embryo. We discuss the use of Pol II stalling as a mechanism of transcriptional repression, and as a means for preparing developmental control genes for rapid and dynamic induction during embryogenesis.