Enhancer Organization: Transistor with a Twist or Something in a Different Vein?

A specific group of enhancers that act in the early *Drosophila* embryo have a highly conserved arrangement of transcription factor binding sites. Computational modeling of these enhancers suggests interesting parallels with electronic transistors and optical image processors.

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Understanding the structure and function of the *cis*-regulatory modules that control gene expression in embryos is a major goal of developmental biology [1]. *Cis*-regulatory modules — enhancers, silencers and insulators — regulate their associated promoters by integrating inputs provided by multiple transcription factors [2,3]. A typical module has on the order of 10 binding sites for a handful of transcription factors [2], and the transcriptional output of the associated promoter is a multivariable function of the levels of activators and repressors. The structure and composition of *cis*-regulatory modules can be established in studies that rely on the manipulation of the nature and arrangement of putative transcription factor binding sites. Frequently, the process of *cis*-regulatory module identification culminates in an experiment in which a synthetic regulatory module is used to drive the expression of a reporter gene in a pattern that is close to the wild-type pattern of a target gene. The output of a module is usually monitored in a small number of conditions: a wild-type background and a handful of mutants. Thus, only a small subset of the multidimensional space of inputs is explored. Furthermore, the output of a module is rarely quantified, necessitating a binary (on/off) description of the input/output function.

The relative ease of manipulating the structure of putative regulatory modules is in sharp contrast with our ability to measure the output of *cis*-regulatory modules over a wide range of multiple inputs. As a consequence, the analysis of the connection between the structure of the *cis*-regulatory modules and their function is in its early stages [4–7]. In a recent sequence of elegant papers, Levine and Papatsenko [8–10] show how such a connection might be established through a combination of computational and experimental approaches. Their work builds upon a model of transcription factor binding site occupancy for a group of type 2 neurogenic enhancers downstream of the dorsoventral patterning cascade in the early fly embryo.

The dorsoventral axis of the *Drosophila* embryo is patterned by the Dorsal morphogen gradient (Figure 1) [11]. In the ventral half of the embryo, Dorsal activates the expression of transcription factors Snail and Twist, and then synergizes with them to control other targets. A group of genes expressed in the neurogenic ectoderm, including *vein*, *vnd*, *rhomboid*, *sog* and *brinker*, are repressed by Snail and activated by Dorsal and Twist. This regulatory architecture can be viewed as a superposition of 'coherent' and 'incoherent' feedforward loops [12]: in an incoherent feedforward loop, an input activates both a target gene and its repressor; in a coherent feedforward loop, the architecture is the same, but both inputs to the target gene are activating. The incoherent feedforward loop, formed by Dorsal and Snail, represses the neurogenic genes in the ventral most part of the embryo, while the coherent feedforward loop, formed by Dorsal and Twist, maintains their expression in a lateral stripe of cells.

Earlier, the Levine lab [8] used measurements of the Dorsal, Snail and Twist profiles, along with the measurements of transcriptional outputs of experimentally validated type 2 enhancers, to fit a thermodynamic model of transcription factor binding site occupancy that predicts how local concentrations of three transcription factors control the output of the corresponding enhancer. Importantly, their model can also predict the effects of changes in binding site number and spatial arrangement within the enhancer [8]. In a follow-up analysis, Zinzen and Papatsenko [9] established that the output of type 2 enhancers in these models is very sensitive to the variation in the distance between the Dorsal and Twist binding sites, suggesting that this distance is an important structural feature of enhancer architecture. In their latest study, published recently in *Current Biology*, Papatsenko and Levine [10] demonstrate that the distance between the Dorsal and Twist site clusters in type 2 enhancers is highly conserved in twelve *Drosophila* species.

What can be the function of this highly conserved structural feature? Using the transcription factor site occupancy model, Papatsenko and Levine [10] show that the conserved spacing between binding sites between Dorsal and Twist amplifies the effect of Twist in the region of the tissue where the concentration of Twist becomes limiting. On the basis of this amplification property of their computational enhancers, the authors suggest that type 2 enhancers have a functional analogy with transistors [10]. One defining characteristic of transistors is their ability to amplify
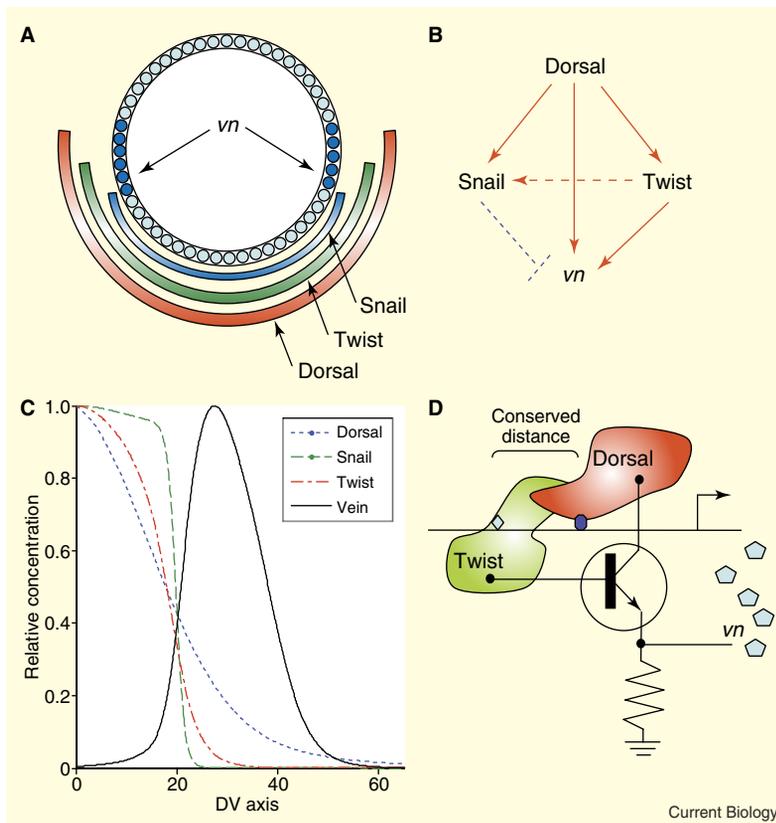
Figure 1. Transcriptional regulation downstream of Dorsal in *Drosophila*.

(A) Early patterning of the neurectoderm in the early fly embryo depends on the combinatorial action of three transcription factors: Dorsal (which acts as a morphogen), Twist and Snail [10]. (B) The gene regulatory network for *vn*, a marker of neurectoderm fate [10,11]. (C) Relative concentrations plotted along the ventral to dorsal axis of the embryo. The concentration profile of Snail drops off rapidly and for most of the expression pattern of *vn*, the role of Snail can be ignored [10]. (D) A cartoon comparison between the amplification of Dorsal signaling by Twist to stimulate *vn* transcription and a NPN transistor, which amplifies the collector current when a relatively small base current is applied [10].

signal processing capabilities features of type 2 enhancer function, which incorporates the massive parallel architecture of cellular output, leads naturally to possibilities from the fields of optical image processing, maskless lithography and holography. For instance, the objective of maskless lithography is to shape an incoming signal, ultra-violet light, into a pattern using programmable spatial light modulators that convolve a single source of light into a more complicated pattern that etches the semiconductor. Spatial light modulators can consist of arrays of tunable mirrors, micro-electro-machined devices, or single molecules with light absorption properties that are tuned with a second light source [14,15].

It is possible that new technologies based on spatially distributed and nonlinear signal processing can be inspired by a purely phenomenological analysis of biological circuits. Getting back to embryos, though, the development of quantitative experimental techniques for monitoring the output of *cis*-regulatory modules over a wide range of module architectures and external inputs appears to be the most pressing issue. The early fly embryo, which has already taught us so much about biological pattern formation, is likely to lead the way in this process.

inputs, in particular when a small input controls the output from a relatively large and constant power source. In some respects, the regulation of the type 2 enhancer can be considered as a common collector–emitter follower circuit, which provides current amplification controlled by a weak base input [13]. Granted, the analogy between electronic transistors and amplifying enhancer circuits can be only pushed so far. For example, there is no conservation of gene product like there is conservation of charge, and no clear analog to voltage in the transcriptional analogy. It is also unclear how the local input/output character of a transistor fits in the larger picture of the spatial processing that takes place across a field of cells in the embryo. Nonetheless, such

analogies are useful, because they provide an important framework for understanding and modularizing a very complicated system with the goal of subdividing the regulatory network into simpler functional components.

Perhaps the most exciting aspect to drawing parallels between electronic circuits and gene regulatory networks is the potential for flipping the analogy on its head. Can we build new devices and algorithms that operate on similar principles to enhancers? In the most general sense, the Dorsal gradient is a spatial input provided to an active medium — the cells of the early fly embryo. The early response to this input is then combined with the input to generate more complex spatial patterns. The search for an analogy that captures the tissue-scale

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Neuronal Development: Neighbors Have to Be Different

The assembly of neurons into functional circuits requires a multitude of cellular recognition events. Recent work on the hypervariable *Drosophila Dscam* gene revealed how a vast number of cell adhesion proteins contributes to neuronal patterning.

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Pattern formation during brain development occurs on different levels. After individual neurons are determined, axonal projections and dendritic arborisations form and finally the numerous interneuronal connections are specified to build the complex organization of neuronal circuits. In particular, the latter patterning processes are thought to depend on distinct cell surface molecules that mediate intra- and interneuronal recognition. Three recent papers on the hypervariable cell adhesion molecule Dscam [1–3] now beautifully illustrate that in the insect brain individual neurons possess unique surface identities necessary for axonal and dendritic patterning.

The *Drosophila* Dscam is a neuronally expressed member of the immunoglobulin (Ig) superfamily with 10 Ig-like domains and 6 type III fibronectin repeats in the extracellular portion, a single transmembrane segment and a cytoplasmic domain (Figure 1A). Three clusters of alternative exons 4, 6 and 9 encode the Ig domains Ig2, Ig3 and Ig7, respectively, and through mutually exclusive splicing potentially 19,008 distinct Dscam ectodomains can be generated [4]. In addition, two alternative exons encoding transmembrane segments target the protein either to the axonal or to the dendritic compartment [5]. Dscams' role in neuronal patterning is best understood during axon and dendrite branch segregation (Figure 1B). In larval peripheral neurons, dendritic branches of the same neuron are repelled from each other through homophilic binding of identical Dscam isoforms [6]. Similarly, in the developing CNS mushroom body neuropil, Dscam controls the segregation of bifurcated axons in sister branches [7,8]. Thus, one of Dscams' functions is to mediate intra-neuronal self avoidance of dendritic and axonal sister branches. But why are there so many isoforms?

To obtain conclusive results about the functional importance of Dscam diversity one has to deal with the extremely high number of isoforms experimentally. First approaches to Dscam complexity were based on the expression of a single isoform in *Dscam* null mutants and the analysis of reduced exon 4 variability [5,8]. Interestingly, expression of randomly chosen Dscam isoforms in single mutant mushroom body neurons restores correct sister branch segregation. Moreover, a reduction in Dscam ectodomain diversity to about 11,000 different isoforms had no effect on mushroom body patterning. Thus Dscam variability seemed dispensable for brain development.

However, recent elegant work from a number of labs has yielded exciting new insights that explain the necessity of Dscam diversity during brain development and elucidate the molecular basis of isoform-specific interactions [1–3]. Hattori and colleagues [1] used homologous recombination to create flies in which *Dscam* encodes only a single ectodomain isoform (*Dscam^single^*). Although expressed as the *Dscam^wild type^* allele and combined in equal levels to the two alternative transmembrane domains, assuring their correct subcellular localization, all *Dscam^single^* alleles are recessive lethal and cause a severe disruption of nervous system development, demonstrating for the first time that *Dscam* diversity is essential.

But is isoform diversity required for inter-neuronal recognition or intra-neuronal self-avoidance? In contrast to wild type, axonal sister branches fail to segregate in the mushroom body *Dscam^single^* animals similar to the *Dscam* loss-of-function situation (Figure 1C). Interestingly, even in heterozygous *Dscam^single^* flies, mushroom body sister branch segregation is impaired, indicating a dominant effect through the expression of a single Dscam isoform in all mushroom body neurons instead of the loss of a particular one. To further prove the model the authors established an intragenic recombination system to generate a wild-type fly with only one *Dscam^single^*