Minireview

A systems view of *Drosophila* segmentation

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Abstract

High-throughput technologies have enabled the systematic identification and characterization of most, or possibly all, of the components governing segmentation in the fruit fly *Drosophila*. What have we learned?

One of the most intensively studied processes in animal development is the division of the embryonic *Drosophila* epidermis (ectoderm) into visible segments, a process that lays the foundations for the segmented structure of the adult insect. Segmentation is governed by a program of sequential gene expression that is one of the best-defined genetic cascades in animal development [1]. It is put into motion by three maternal gene regulatory proteins - Bicoid, Hunchback and Caudal - which specify an initial 'pre-segmentation' pattern along the anterior-posterior axis, while the anterior and posterior ends of the body are specified independently by the localized activation of the maternal receptor tyrosine kinase Torso. The principal target genes of these maternal factors in the embryo's genome are known as gap genes, as their lack leads to gaps in the body pattern. The gap genes, such as *Krüppel*, *Knirps* and *Giant*, encode sequence-specific transcriptional repressors. The interplay of the maternal factors and the gap repressors constitutes one of the leading paradigms for the combinatorial control of gene expression in development. These regulatory factors bind to the enhancers of the segmentation genes to produce precisely positioned on/off repeating transverse stripes of expression for each gene, foreshadowing the subdivision of the embryo into a repeating series of body segments. The segmentation genes typically have highly complex enhancers, with multiple binding sites for each gene regulatory protein.

Detailed descriptions of individual parts of the *Drosophila* segmentation process have been available for many years, but Segal and colleagues [2] have recently taken a systems approach to segmentation by developing a comprehensive quantitative thermodynamics-based model in an attempt to describe the regulation of segmentation-gene expression in the early embryo. This analysis was made possible by two recent advances in understanding *Drosophila* segmentation. First, computational and experimental methods have identified a total of 44 different enhancers that respond to distinct combinations of maternal and gap transcription factors to direct precisely localized bands and stripes of gap and segmentation gene expression in the early *Drosophila* embryo [3-6]. Second, quantitative high-resolution imaging of the distribution profiles of each maternal and gap protein has provided precise information about the relative concentrations of these critical regulators in each nucleus of the embryo at key points during the progressive refinement of stripe formation [7,8]. Each enhancer contains a specific constellation of binding sites for maternal and gap proteins, and within each nucleus there is a particular combination of transcriptional activators and repressors that can bind to these sites.

Using this information, Segal *et al*. devised a two-component model to predict the expression, or output, mediated by each of the 44 known gap gene and segmentation gene enhancers [2]. The model incorporates some of the features used in previous site-occupancy models - such models predict the occupancy of each binding site by its corresponding transcription factor for a given gene and position in the embryo (for example, see [9-11]). The first component of the Segal model is a Boltzmann weight function (which will predict the probability that a binding site is occupied at a given concentration of transcription factor), which is computed for every sequence match to a known binding motif within a given enhancer. The second component of the model is a function that defines the transcriptional output, which depends on

the ratio of predicted activator and repressor binding sites occupied. Higher occupancy of the activator sites is predicted to produce expression at a particular location within the embryo, whereas greater occupancy of the repressor sites is predicted to produce little or no expression.

So how well did the Segal model perform? Using three parameters (concentration scaling, self-cooperativity binding strength and the expression contribution) for each input maternal and gap transcription factor, the model is in general agreement with the gene-expression patterns attributable to the enhancers in the gap genes, but produces only variable agreement with the stripes of gene expression produced by enhancers in the genes known as pair-rule genes, segmentation genes that are expressed in stripes in alternating 'segments' along the anterior-posterior axis. For many of these latter enhancers, there is only a weak correlation between the relative number of activator and repressor binding sites, the nuclear concentrations of the corresponding input transcription factors, and the experimentally observed output gene expression levels. For such enhancers, a more accurate picture means invoking 'nonlinear' mechanisms of transcriptional regulation, such as heterotypic cooperative DNA-binding interactions between different types of activators, repression by quenching (where the binding of a repressor protein appears to prevent activation of the gene by an activator bound at a nearby site) and significant contributions of low-affinity binding sites to the control of gene expression. Indeed, cooperative DNA binding and quenching have proved critical for the accurate modeling of the dorsal-ventral patterning network [10].

Cooperative binding, quenching and the importance of low-affinity binding sites are well documented in the 'pre-systems' literature. Numerous studies have established the importance of cooperative DNA binding of Bicoid in gene activation [12-14]. Repression by quenching has been shown in the prototypic stripe 2 enhancer that directs the localized expression of the pair-rule gene *eve*. Although Bicoid-binding activator sites overlap Giant- and Krüppel-binding repressor sites in this enhancer [15,16], both Giant and Krüppel are able to inhibit expression even when bound as much as 50-100 bp away from the closest activator sites [17,18]. This quenching is mediated by a co-repressor protein, CtBP, which is recruited to the DNA through interactions with a simple peptide motif, PxDSxK/R, present in the Krüppel, Giant and Knirps gap repressor proteins [19-21]. The *eve* stripe 2 enhancer contains a total of five Bicoid activator sites, three of which are low-affinity sites [22]. The analysis of natural populations of *Drosophila* led to the hypothesis that these low-affinity sites provide an intrinsic mechanism for ensuring the robustness of gene expression and enabling the modification of expression patterns during evolution [23,24].

The earlier molecular studies that established these principles were restricted to the analysis of just a handful of enhancers. Segal *et al*. [2] have now provided evidence that the mechanisms determined previously through traditional molecular studies are prevalent and generally applicable to the entirety of the segmentation process. Indeed, establishing the generality of known mechanisms is one of the great promises of systems biology.

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