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Gene expression in time and space: additive vs hierarchical organization of *cis*-regulatory regions

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In higher eukaryotes, individual genes are often intermingled with other genes and spread out across tens to hundreds of kilobases, even though only small portions of their sequence are devoted to protein coding. Yet, in this seemingly extended and tangled mess, the cell is able to precisely regulate gene expression in both time and space. Over the past few decades, numerous elements, like enhancers, silencers and insulators have been found that shed some light on how the precise control of gene expression is achieved. Through these discoveries, an additive model of gene expression was envisioned, where the addition of the patterning details imparted by regulatory elements would create the final pattern of gene expression. Although many genes can be described using this model, recent work in the Drosophila bithorax complex suggests that this model may be somewhat simplistic and, in fact, regulatory elements sometimes seem to communicate with each other to form a functional hierarchy that is far from additive.

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Introduction

Throughout their lifetime, the cells of an organism must correctly respond to numerous internal and external stimuli to keep an organism alive and healthy. These responses often require the precise modification of gene expression in both time and space. This can be seen quite clearly in the process of development. During the course of development, a vast number of genes must be precisely controlled in a spatial, temporal, quantitative, and coordinated fashion. When all of these processes proceed normally, a single-celled egg is able of developing into a complete and phenotypically normal individual. Unfortunately, when things do not go normally, a malformed or unviable embryo often results. Interestingly, however, it is because the misexpression of so many genes leads to developmental phenotypes that development has proven to be such a rich source of information on the control of gene expression. Using both genetic and molecular tools, scientists have been able to dissect the process of transcriptional control during development both quickly and rigorously. Nowhere is the synergy between genetic and molecular tools more evident than in the model organism, *Drosophila* melanogaster, where the control of gene expression at numerous developmental loci is understood at an incredible level of detail. Here, we will discuss our current understanding of the control of gene expression using two classic *Drosophila* loci as models: the *even-skipped* locus, and the *bithorax* complex. In doing so, we hope to reveal some of the major lessons we have learned from the *Drosophila* system and highlight some new complexities emerging from the study of gene expression in *Drosophila*.

Enhancer additivity: the *eve* locus

One of the most thoroughly studied loci in *Drosophila* is that of the pair-rule gene *even-skipped* (*eve*). Identified in during Nobel prize winning screens of Nusslein-Volhard and Wieschaus, *eve* was first characterized as a gene required to make the even numbered abdominal segments of a *Drosophila* embryo [1]. Accordingly, hypomorphic mutations in *eve*, result in embryonic lethality with the embryos lacking even-numbered abdominal segments (and odd numbered thoracic segments). Thus, from the early genetic analysis, it was clear that *eve* played an important role in establishing the identity of alternating segments.

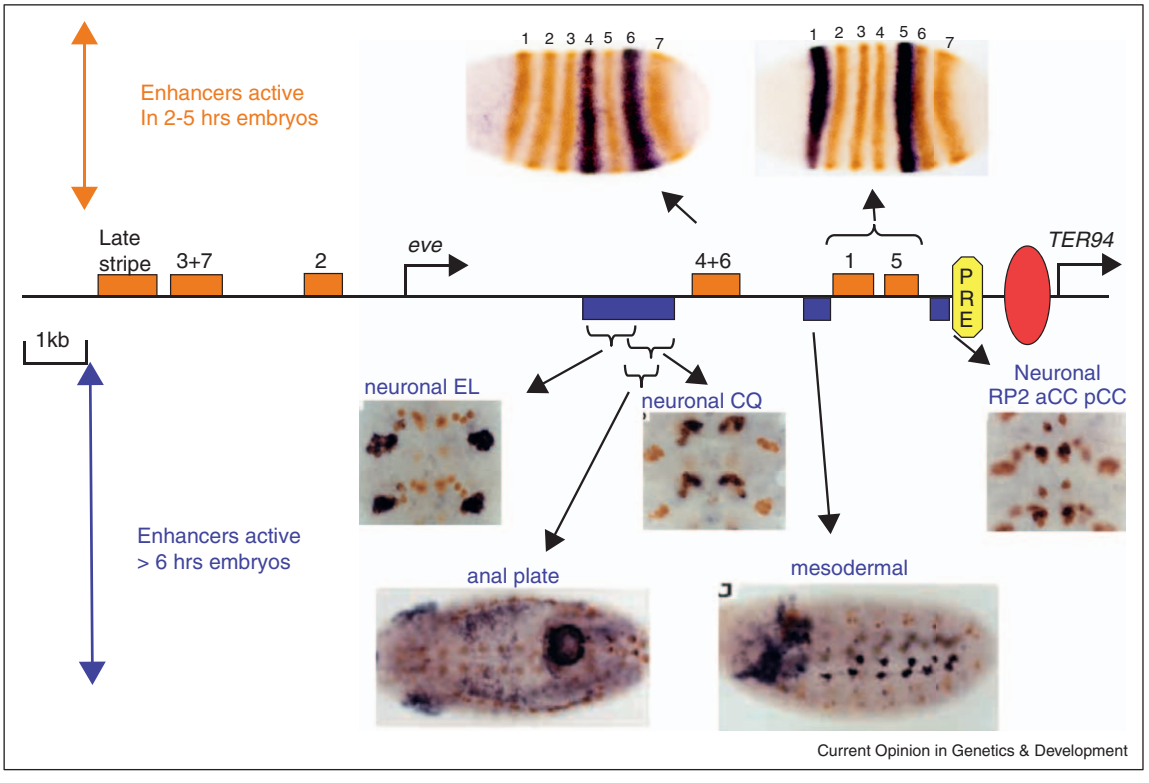
It is now known that *eve* is a primary pair-rule transcription factor and is expressed early in development in a seven-striped pattern along the antero-postero (A-P) axis [2,3] (Figure 1 top). The places where *eve* is expressed are destined to become the even numbered abdominal segments and odd numbered thoracic segments [3]. Although thus far, we have been speaking in terms of segmental units, in reality, we should be speaking in terms of embryonic parasegmentals units, since the larval segments *per se*, do not yet exist. During early development, the *Drosophila* embryo is divided into 14 metameric units, called parasegments [4]. In lieu of going into too much detail, we will say that parasegments are roughly equivalent to the future segments, but are slightly shifted relative to the segments, with parasegments one through three eventually becoming the three head segments,

parasegments four through six becoming the three thoracic segments, and parasegments seven through fourteen becoming the eight abdominal segments. *eve*, contrary to its name, is expressed in the seven odd numbered parasegments early in development.

However, the pattern of *eve* expression is not constant; it changes dramatically during the first ten hours of development [2,3]. Initially, *eve* is expressed in seven broad stripes along the A-P axis. Just before the process of germband extension, the seven stripes become thinner, with sharper anterior and posterior boundaries [5]. Later, during germband retraction, the pair-rule pattern of *eve* disappears and new patterns of *eve* expression arise (Figure 1 bottom). First, as the seven stripes start to fade an eighth more-posterior stripe begins to form in an area that will become the anal plate [2,3]. Then, *eve* starts to turn on in the developing nervous system and mesoderm [2,6–8]. Contrary to its previous pair-rule pattern, neuronal and mesodermal *eve* is expressed in specific neuroblasts, ganglion mother cells (GMCs), and dorsal mesodermal cells in each parasegment (Figure 1 bottom). Many of the neurons turning on *eve* at this stage, retain *eve* expression past embryonic development and all the way until the third instar larval stage. In all of these cell lineages, *eve* has been shown to be a crucial player in determining their eventual cell fate [7,9].

Through Herculean transgenic efforts, the ~16 kb *eve* locus has been dissected to identify more than ten separate enhancers spread both upstream and downstream of the *eve* transcriptional unit [7,10•,11•,12–14,15•,16,17,18•]. Five different enhancers have been found to control the early seven stripes of *eve* expression. These include separate enhancers that drive reporter gene expression in the first, second and fifth stripes respectively, and two other enhancers that each drive reporter gene expression in two stripes: the third and seventh stripe, and the fourth and sixth stripe [10•,13,14,15•,16]. Each of these stripe elements has been shown genetically to be under the concentration-dependent control of the gap gene products, whose expression precedes the pair-rule genes and divides the embryo into large regions along the A-P axis (Figure 1). For example, the stripe two element requires the broadly anteriorly localized Bicoid (Bcd) and Hunchback (Hb) proteins for activation, while being repressed along its anterior edge by the Giant protein and along its posterior edge by the

Figure 1



Additive enhancers of the eve locus. The central black line represents the eve DNA sequence. Shown as orange boxes are the relative positions of the stripe enhancers. Shown in blue are the tissue specific enhancers. The expression patterns of certain enhancers are shown above and below the DNA line. Stained in blue are the patterns of the specific enhancers tested (indicated by the arrows), while the wild-type expression patterns of Eve protein are stained in brown/orange. The location of the eve PRE and insulator are also indicated, as a yellow octagon and a red oval, respectively (photos taken from Fujioka *et al.*, 1999).