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Review

# Stepwise Progression of Embryonic Patterning

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**It is long established that the graded distribution of Dorsal transcription factor influences spatial domains of gene expression along the dorsoventral (DV) axis of *Drosophila melanogaster* embryos. However, the more recent realization that Dorsal levels also change with time raises the question of whether these dynamics are instructive. An overview of DV axis patterning is provided, focusing on new insights identified through quantitative analysis of temporal changes in Dorsal target gene expression from one nuclear cycle to the next ('steps'). Possible roles for the stepwise progression of this patterning program are discussed including (i) tight temporal regulation of signaling pathway activation, (ii) control of gene expression cohorts, and (iii) ensuring the irreversibility of the patterning and cell fate specification process.**

## Trends

Levels of Dorsal transcription factor change over time, raising the question of how this morphogen controls patterning of the *Drosophila* embryo when it is so dynamic.

Spatiotemporal examination of a small number of Dorsal target genes had shown that gene expression is also dynamic, and this study was recently expanded using NanoString technology to provide a temporal analysis of ~70 genes in the *Drosophila* early embryo.

Quantitative, fine-scale temporal data for transcript levels in the early *Drosophila* embryo have helped to clearly define temporal roles for transcription factors and identify gene expression cohorts, providing new insight into the stepwise progression of the DV patterning gene regulatory network.

## Transcription Factor Dynamics Regulate Target Gene Expression

Subdividing the embryo into distinct domains of gene expression by combinatorial control of transcription factors is an important function of regulatory networks acting in early embryos including those of *Drosophila* [1–5]. These early patterning events influence the activation of signaling pathways to support tissue differentiation and also control cell movements required for the generation of a multilayered embryo–the developmental actions that encompass gastrulation [6,7]. To study these events at the transcriptional level in *Drosophila* embryos, previous studies of early zygotic gene expression considered one or two time-points spanning the first 4 h of early embryo development [8–11], but recent studies suggest that gene expression patterns change on the order of minutes rather than hours (e.g., [12–14]). Furthermore, only recently has it come to light that transcription factors in the early embryo exhibit changes in levels over time [15–18]. At least in part these dynamics relate to the fast nuclear divisions that encompass *Drosophila* early embryonic development and result in oscillatory inputs to target genes. Transcription factor dynamics appear to be a general mechanism of regulating gene expression [19,20] and highlight the need to study temporal regulation of developmental gene expression as a complement to previous studies of embryonic patterning in *Drosophila*, which have focused on the spatial control of gene expression [21–23].

## The Dorsal Transcription Factor Is Dynamic, As Are Its Target Genes

In the *Drosophila* embryo, the pivotal transcription factor, Dorsal (Dl), is present in a nuclear–cytoplasmic gradient along the DV axis that instructs differential gene expression, but the establishment of this morphogen gradient is atypical [24–26]. *dl* transcripts are maternally deposited and uniformly distributed [27,28]. The protein, however, is present in a nuclear gradient through differential activation of the upstream receptor, Toll [29]. Thereby, this gradient does not result from localized expression of Dorsal protein but instead involves a nuclear–cytoplasmic shift in levels of this factor along the DV axis as regulated by Toll receptor signaling [30–32]. Dorsal acts as an activator of transcription to support the expression of target genes in ventral and lateral regions of the embryo, as well as a repressor of transcription to limit the expression of a subset of target genes to dorsal regions [33–35]. In this manner, more than

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50 genes are differentially expressed along the DV axis [21,36]. High levels of nuclear-localized Dorsal in ventral regions specify the mesoderm, whereas lower levels of nuclear Dorsal in lateral regions specify the neurogenic ectoderm [37,38]. The prevailing model in the field had been that the changes in levels of Dorsal in space, along the DV axis, are important for establishing different domains of gene expression.

However, more recent studies have identified that Dorsal levels also change in time [17,39], raising the question of whether and how temporal changes of this factor impact on gene expression. How the nuclear distribution of Dorsal gives rise to precise gene expression patterns was recently investigated using live *in vivo* imaging and quantitative analysis. It was revealed that the Dorsal transcription factor gradient is highly dynamic, increasing in levels over time, and does not achieve steady-state until Dorsal levels plummet at gastrulation [13]. Until this point, during the first 3 h of development, levels of this factor build up within nuclei, from one nuclear cycle to the next, such that by cellularization a ~threefold increase is realized compared to previous nuclear cycles. In addition, Dorsal levels oscillate with each and every nuclear cycle, dropping

## Box 1. Case Studies in Transcription Factor Localization and Concentration

Two prominent transcription factors active early in *Drosophila* development are Dorsal and Bicoid. The nuclear concentrations, gradients, and embryonic localizations of both transcription factors have been characterized, and present a contrast in nuclear import strategies [13,16]. Both are imported into nuclei during syncytial nuclear cycles, but the dynamics and import rate differ between the two (Figure I). While Bicoid undergoes a rapid uptake, it also undergoes a decrease in concentration before nuclear division, indicating an overshoot and reduction in concentration to a lower steady-state. NCs 10–12 are too short to reach this overshoot and reduction, but NCs 13 and 14 show this characteristic, with the concentration of Bicoid stabilizing before mitosis, when it drops to low levels before being imported again. Dorsal, by contrast, undergoes a slower increase to maximum levels at each nuclear cycle, with no overshoot. While Dorsal never reaches a steady-state during early nuclear cycles, the concentration of Dorsal begins to level off during NC 13 and finally achieves a steady-state during NC 14, demonstrating an import mechanism different from that of Bicoid. Both Bicoid and Dorsal leave nuclei at very similar rates and times between nuclear cycles, indicating that export is likely due to rapid diffusion of the transcription factors when the nuclear envelope breaks down during mitosis.

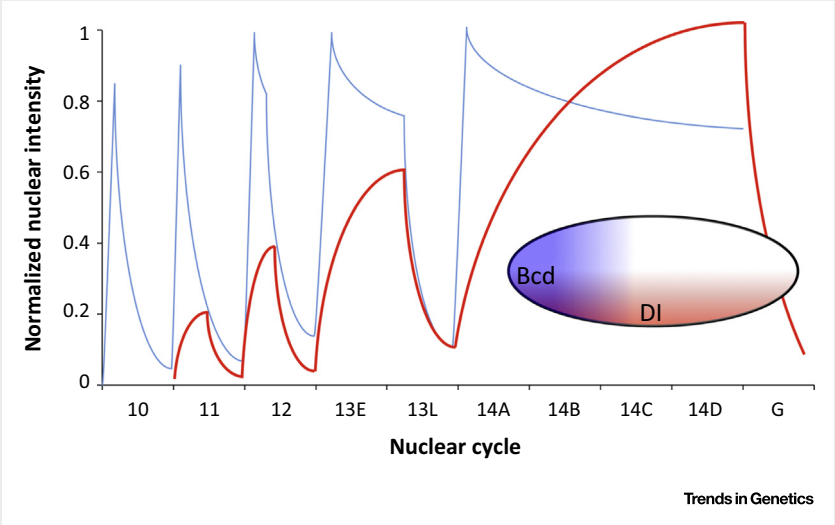


Figure I. Bicoid and Dorsal Dynamics–Comparison of Nuclear Levels. A conceptual representation of the concentration of transcription factors Bicoid (blue) and Dorsal (Dl; red) in nuclei during late nuclear cycles based on data from previous studies [13,16]. Measurements were obtained by monitoring live fluorescence intensity of Bcd–GFP or Dl–Venus fusion molecules from a single nucleus at 10% along the AP axis for Bicoid or ventral-most position for Dorsal. Nuclear intensity is normalized to the maximum for each transcription factor and overlaid. Inset is an illustration of a *Drosophila* embryo with transcription factor concentration gradients for Bicoid and Dorsal.