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Temporal dynamics, spatial range, and transcriptional interpretation of the Dorsal morphogen gradient

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Dorsoventral pattern of *Drosophila* embryo is specified by the nuclear localization gradient of the transcription factor Dorsal. Genetic and genomic studies of this morphogen gradient provided important insights into spatial control of gene expression in development. Recent live imaging experiments revealed hitherto unappreciated dynamics of the Dorsal gradient and posed new questions about the mechanisms of its transcriptional interpretation. Some of these questions can be answered by models in which the morphogenetic capacity of the Dorsal gradient is potentiated by spatially uniform factors, such as Zelda, a transcription factor that plays a key role in the activation of zygotic transcription. Combinatorial effects of uniform and graded factors play an important role in the transcriptional and signaling cascades initiated by Dorsal and may explain differential positioning of gene expression borders by other morphogen gradients.

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Introduction

Some of the first molecular insights into the formation and interpretation of morphogen gradients were provided by studies in *Drosophila*, at a stage when the blastoderm embryo is patterned by maternal morphogen gradients that specify the anteroposterior (AP) and the dorsoventral (DV) body axes. The formation of these gradients can be traced back to the symmetry-breaking events that occur during oogenesis. In particular, upon egg activation and fertilization, molecular nonuniformities of the extracellular matrix on the ventral side of the egg lead to activation of the Toll receptor on the ventral side of the embryo [1]. Toll signals through the highly conserved NF-κB pathway and establishes a ventral-to-dorsal gradient of nuclear localization of the transcription factor Dorsal (Dl) [2–4], which controls multiple genes gene expression domains in the embryo (reviewed in [5]).

Genes expressed at different positions along the DV axis contribute to the formation of embryonic germ layers (reviewed in [6]). For example, the expression of a growth and differentiation factor Decapentaplegic (Dpp), which is directly repressed by Dl, is confined to the dorsal part of the embryo and contributes to the formation of the dorsal ectoderm. The transcription factors Snail (Sna) and Twist (Twi) are activated by the highest levels of nuclear Dl in the ventral most regions of the embryo, and specify mesoderm. The genes encoding Short gastrulation (Sog), Brinker (Brk), and Rhomboid (Rho) are activated by lower-than-peak levels of Dl in nested domains in the lateral region of the embryo, and are essential for different aspects of the neuroectoderm (Figure 1a).

Descriptions of the Dl gradient can be found in every textbook of developmental biology and in regularly updated comprehensive reviews. Recent studies have made important steps towards making our understanding of this gradient more quantitative, by characterizing the dynamics and spatial range of the Dl gradient and elucidating new mechanisms of its transcriptional interpretation. Below we summarize the results of these studies and discuss some of the outstanding questions in the analysis of the DV patterning system.

Dynamics

Pattern formation by morphogens can be assigned to two broad classes, distinguished by the dynamics of responding tissues. In the first class, a responding tissue can be viewed as relatively static and does not significantly affect the inductive signal. Patterning of the Drosophila follicle cells by the Gurken morphogen gradient operates in this regime [7]. In the second class are systems where responding tissues are actively changing (e.g. proliferating and deforming) on time scales associated with morphogen gradient formation and interpretation. Patterning by the Dl gradient clearly belongs to the second category. Specifically, the formation and action of this gradient is strongly affected by the dynamics within the syncytial embryo, a system with multiple synchronously dividing nuclei. After the first nine divisions, which happen throughout the embryo, most of the nuclei arrange themselves in a monolayer under the plasma membrane, where they divide four more times before cellularization and gastrulation.

On the basis of the results of recent live imaging studies with GFP-tagged Dl, Dl is detected in nuclei as soon as

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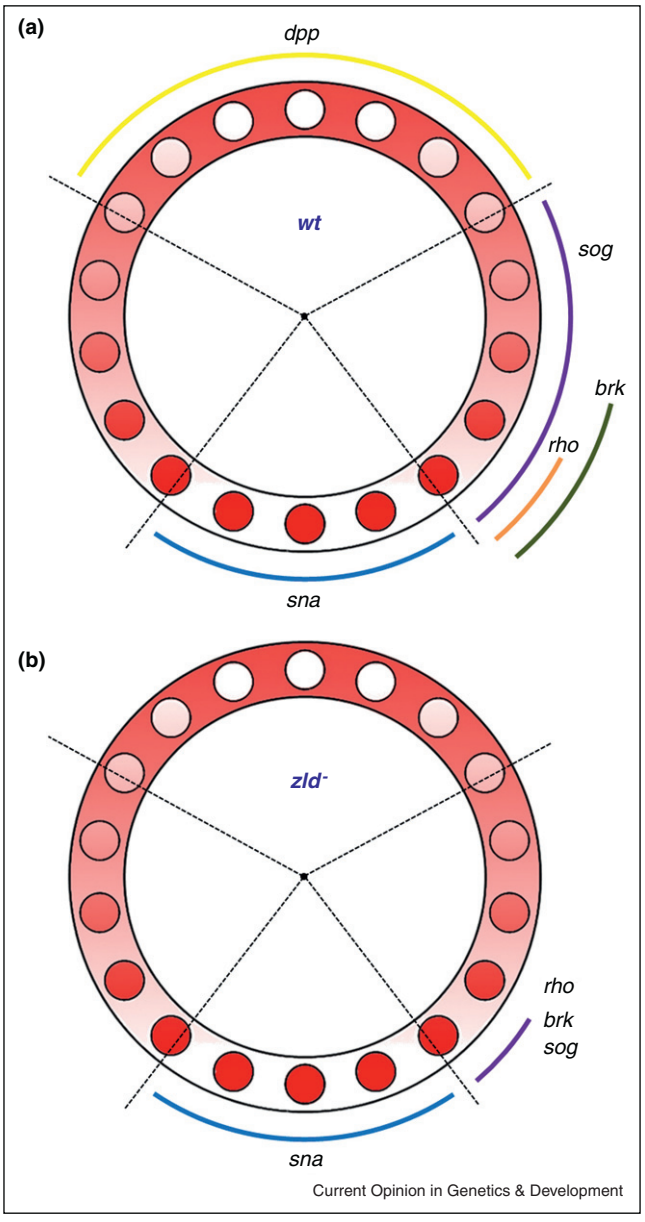
Figure 1



Schematic representation of the Dl gradient in cross sectional view, dorsal up. Dl protein (red) is localized in a nuclear concentration gradient (small circles), and subdivides the embryo into three broad domains (delineated by dotted lines). **(a)** High levels of Dl activate *sna* in the mesoderm, while progressively lower levels activate *rho*, *brk* and *sog* in nested domains in the lateral neuroectoderm. Dl also represses genes such as *dpp* to restrict their expression to the dorsal region. **(b)** In the absence of Zelda (*zld*$^{-}$), *dpp* is not expressed. *rho*, *brk*, and *sog* are expressed in the same narrow domain adjacent to the mesoderm, while the *sna* domain is largely unaffected [23•].

they reach the plasma membrane in cycle 10 [8,9,10••]. From this time on, the levels of nuclear Dl follow a sawtooth pattern, during which nuclear levels increase within the interphase and abruptly drop during mitosis. The duration of interphase progressively increases (from 10 min at cycle 10 to 25 min during cycle 13). As a consequence, the amounts of nuclear Dl reach higher levels during each progressive interphase. The spatio-temporal pattern of nuclear Dl can be summarized in a compact way as a constant shape multiplied by time-dependent amplitude, which increases during interphase and drops to baseline levels during mitosis [10••].

Time-resolved *in situ* hybridization analysis revealed that dynamics of the Dl gradient can influence the expression of its transcriptional targets. For example, transcript levels of *sna* rise during cycle 13, but then disappear for a brief period following mitosis, before starting to rise again during cycle 14 [11•]. Induced by changes in the Dl gradient, these changes in *sna* expression lead to changes in the expression of several genes in the early embryo. To summarize, the DV axis of the embryo is exposed to rapidly changing levels of Dl. These changes reflect nuclear divisions in the syncytium and play an important role in the transcriptional interpretation of the Dl gradient.

Spatial range

A key quantitative property of a morphogen gradient is its spatial range, the distance over which it can provide direct control of cellular responses. Experiments with gene fusion assays suggested that the Dl gradient is long-ranged and directly contributes to transcriptional regulation of multiple gene expression borders along the DV axis (reviewed in [5,6]). Direct tests of this conclusion required analysis of Dl gradients along the entire DV axis and in a large number of embryos. A recently developed microfluidic device provided this capability. In this device, hundreds of embryos are oriented vertically, with their DV axis normal to the optical axis of the microscope. Imaging nuclear Dl in a large number of vertically oriented fixed embryos yielded a dataset that enabled statistical analysis of the gradient range [12,13].

As a practical definition for the range, it was proposed to use the position within the tissue at which the mean value of the signal becomes indistinguishable from the basal level. For the Dl gradient, this basal value corresponds to the level of nuclear Dl measured at the dorsal-most position along the DV axis, where the activation of the Toll pathway is minimal (essentially zero). On the basis of this definition, the range of the gradient can be estimated as follows. First, by quantifying nuclear Dl gradients in a large number of embryos one constructs empirical distribution functions for the levels of nuclear Dl at multiple positions along the DV axis. Second, a pairwise statistical test is used to compare the mean values of these distributions to the mean value at the dorsal-most position. On the basis of this analysis, the range of the Dl gradient was estimated to be ~2/3 of the DV axis [13]. Clearly, this estimate is affected by the variability of the dataset, which depends, among other things on the intrinsic variability of the gradient at a specific point in time and on the length of the time window over

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which the embryos were collected. The larger is the variability, the smaller is the estimate for the spatial range. Thus, an estimate provided by this approach is the lower limit for the true range.

A similar analysis can be extended to quantify the spatial distributions of transcripts of genes controlled by Dl, in order to provide an estimate for the spatial range of transcriptional effects of the Dl gradient. This was done for *sog*; statistical analysis of the spatial distribution of nascent *sog* transcripts revealed that *sog* is expressed at ~60% of the DV axis, which agrees well with the estimate for the spatial range of Dl [13]. To summarize, based on the results of these studies Dl can be viewed as a long-range gradient that has the potential to directly contribute to gene regulation over a significant fraction of the DV axis.

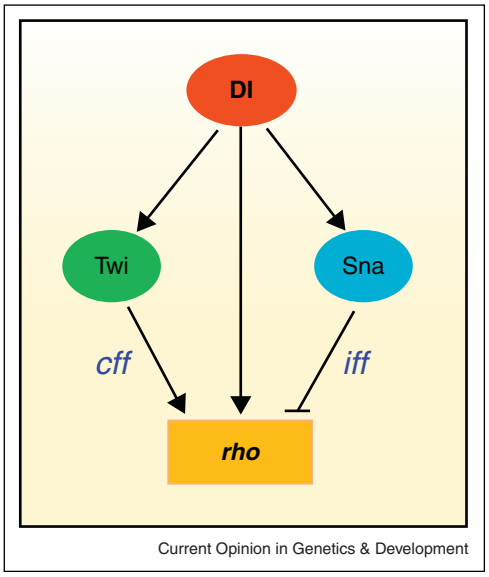
Transcriptional interpretation

The affinity-threshold mechanism has been shown to play a key role in Dl gradient interpretation [14••,15]. *twi* and *sna* contain low-affinity Dl binding sites in their enhancers and require high levels of Dl for activation, whereas *rho*, *brk*, and *sog* contain high-affinity sites and can be activated by low levels of Dl. However, without exception, transcriptional targets of Dl also depend on other regulators. For example *rho* is controlled by Dl both directly, and indirectly through Twi and Sna [15]. Twist, whose expression domain encompasses the mesoderm but also extends slightly into the neuroectoderm, functions with Dl to activate target genes such as *rho* [15] (Figure 2). Sna, whose sharp expression border delineates the mesoderm from the neuroectoderm acts as a repressor of neuroectodermal genes [15,16]. This feedforward loop architecture is common in developmental regulatory networks [17,18], and controls multiple genes expressed along the DV axis [15,19].

In feedforward loops regulators that work together with morphogens depend on them directly or through their more proximal targets. But mechanisms of gradient interpretation may rely on factors that are independent of morphogens and are distributed uniformly throughout the patterned tissue. Recent studies have identified at least two such regulators in the early embryo [20••,21]. One of them is Zelda (Zld), a Zn-finger transcription factor that plays a crucial role in the activation of the zygotic genome [20••]. Zelda was discovered based on a DNA sequence that was over-represented in the regulatory regions of early expressed genes, including Dl target genes [20••,22•,23•]. Strikingly, genetic removal of Zld affects most of early-zygotic transcription, with defects ranging from temporal delay to complete loss of expression [20••,24••]. Among the targets of Zld are genes involved in cellular blastoderm formation, cell cycle progression, sex-determination, and axis patterning.

A genome-wide analysis of Zld-DNA binding revealed that it binds patterning genes in close proximity to the

Figure 2



Feedforward loops in the Dl gene network. The feedforward loop is a regulatory motif used in transcriptional networks whereby one regulator controls a second regulator and then both regulate a common target [17,18]. In dorsoventral patterning, Dl activates Twi, a bHLH transcription factor, and then both Dl and Twi bind and activate *rho* [15], forming a coherent feedforward loop. A second incoherent feedforward loop is established when Dl activates Sna, which acts as a repressor of *rho*, introducing a negative sign into the loop. cff, coherent feed forward; iff, incoherent feedforward.

binding sites of other key transcription factors, such as Dl and Bicoid (Bcd), a morphogen that patterns the AP axis [24••,25••]. On the basis of this, it was proposed that patterning defects observed in the absence of Zld could be explained by a model based on cooperative interactions between graded regulators, such as Bcd and Dl, and Zld, which is distributed uniformly [26•]. In this model, the presence of Zld increases the probability that a graded factor will bind to the regulatory model.

While the basis of this regulated recruitment enabled by Zld is still unclear, the model makes a clear prediction about the change in the spatial domain and the robustness of expression of genes regulated by Dl: in the absence of Zld, the borders of expression domains should shrink and the expression within these domains should become noisier. This is indeed what is observed for *rho*, *brk* and *sog* in the neuroectoderm; their nested domains collapsed into the same narrow spotty pattern in the absence of Zld [24••] (Figure 3, top). Thus, Zld potentiates the morphogenetic effects of the Dl gradient, especially in progressively dorsal regions of the embryo where the levels of nuclear Dl are low and the gradient becomes flat. In addition to the narrowing of the spatial domains, the absence of Zld caused a temporal delay in transcriptional activation [24••]. This is consistent with the cooperativity model if one considers

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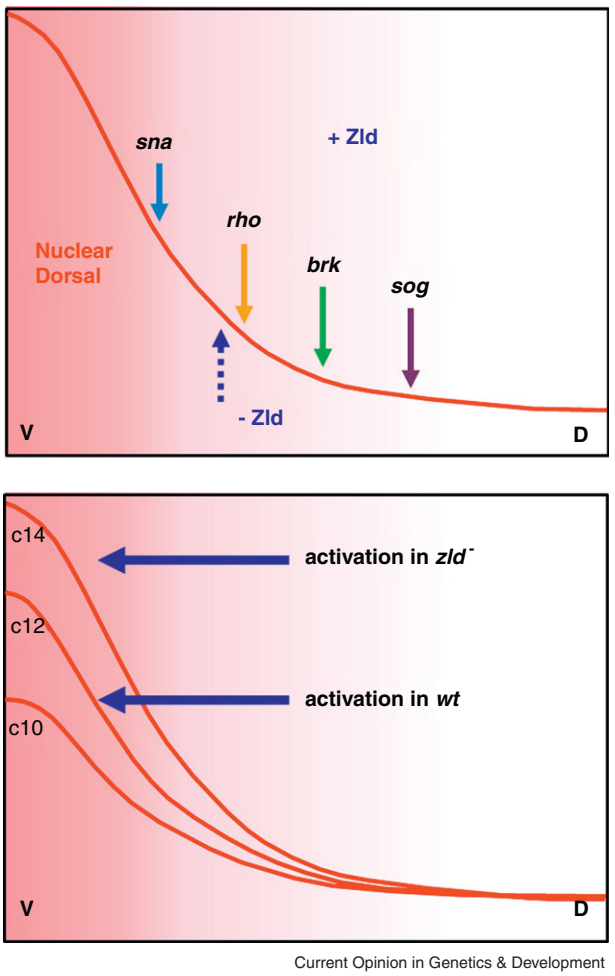
Figure 3



Zelda potentiates Dorsal morphogenetic activity spatially and temporally. Top panel: curve represents relative nuclear Dl concentrations at cycle 14 from the ventral (V) midline to dorsal (D) midline [26•]. Solid arrows delineate the dorsal border of target genes in the presence of Zld (+Zld); dashed blue arrow represents the collapse of the neuroectodermal gene borders in the absence of Zld (−Zld). Bottom panel: nuclear Dl concentration changes over time as indicated. Target genes can be activated as early as cycle 10–11 in wild-type (*wt*), but not in the absence of Zld (zld^{-}).

the fact that nuclear Dl concentrations increase over time [10••]. In the absence of Zld, lower levels of Dl present in the earlier cycles are insufficient for target gene activation (Figure 3, bottom). Thus, Zld boosts Dl activity both temporally and spatially, and provides robustness to the patterning process.

Combinatorial effects of graded and uniform factors provide an additional mechanism for the formation of nested gene expression domains, which is a hallmark of pattern formation by graded signals. In addition to the threshold affinity mechanism, which relies on a differential sensitivity to a single factor, and combinatorial mechanisms that rely on multiple graded signals, nested expression domains can arise due to differential sensitivity of target genes to uniform factors, such as Zld. Consistent with this idea, it was noticed that among the *rho*, *brk*, and *sog* enhancers [15,27,28], *rho* had the lowest Zld ChIP-binding score and *sog* the highest [24••], and it is possible that greater Zld binding leads to greater Zld–Dl cooperative interactions, which in turn leads to greater output of the Dl gradient, that is broader expression domains.

Discussion and perspectives

Since the discovery of the Dl morphogen over 25 years ago, many significant insights have been gained regarding the formation and function of the Dl gradient that can be applied to all morphogen systems. Recent imaging studies provided a quantitative description of the nuclear Dl concentration at each point along the DV axis. This taken together with the characterization of target gene expression domains along the DV axis led to knowledge about the amount of Dl protein needed to turn on (or off) each target. Further studies on the *cis*-acting enhancers of target genes confirmed that they were direct Dl targets containing Dl binding sites of varying affinity.

Historically, the affinity-threshold model explained well the differential target gene responses to the Dl gradient, however, as time went on and more target genes were studied, exceptions arose, particularly for genes expressed in regions with low levels of Dl. For example, the dorsal borders of *rho*, *brk*, and *sog* lie in the lateral region where the Dl concentration changes as little as 10% (Figure 3, top). How can an enhancer interpret such small changes? The discovery of Zld, which appears to boost Dl morphogenetic activity leading to greater 'expressivity' of target genes both temporally and spatially, provides a framework on how the combination of graded and uniform factors might control differential expression of genes along the DV axis.

The molecular mechanism behind the Zld–Dl cooperativity remains a mystery, however, an interesting feature of Zld is that it appears to bind target genes well before they are transcriptionally activated [23•,24••]. One possibility for how Zld potentiates morphogen activity is by binding target enhancers wrapped in nucleosomes, thereby displacing nucleosomes and 'opening' chromatin, which promotes accessibility of the patterning morphogens to their cognate DNA binding motifs. In this way, Zld may resemble pioneer factors, a special class of transcription factors that are the first to engage target genes and mark them for future expression (reviewed in [29,30]). In this way, the extent to which Dl binds target enhancers may be dependent not only on its concentration and the affinity of its binding-site, but also on Zld-induced changes in chromatin landscape. Thus, to further our understanding of how morphogen gradients establish robust target-gene expression domains it will be necessary to study chromatin accessibility, which may also depend on the affinity of Zld binding sites and Zld protein concentrations.

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