

Patterning Lessons from a Dorsalized Embryo

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A paper by Nunes da Fonseca and colleagues in this issue of *Developmental Cell* shows that, to pattern its dorsoventral axis, the beetle *Tribolium* utilizes many of the same genes used in flies, but in very different ways: rather than relying on maternal information, it uses Dorsal and Dpp as part of two coordinated ancestral self-organized systems.

Establishment of primary axes—anterio-posterior (AP) and dorsoventral (DV)—during development creates the framework for patterning the embryo. This ensures that appropriate segments and organs form at specific positions. In *Drosophila*, localized maternal morphogens play critical roles: Bicoid specifies the AP axis while Dorsal specifies mesoderm, ectoderm, and extraembryonic fates along the DV axis (for review: Stathopoulos and Levine, 2002). These studies in *Drosophila* have largely driven our understanding of axis specification, but flies clearly exhibit a derived mode of early development. Therefore, the study of other insects that have retained ancestral modes is necessary to understand the basic principles of axis determination. A paper by Nunes da Fonseca et al. (2008) in this issue of *Developmental Cell* describes an ancient self-organizing system governing DV patterning in the flour beetle *Tribolium*.

The *Drosophila* embryo patterns all of its segments simultaneously prior to gastrulation (“long germ” development). Specification of DV identity by maternal factors happens all at once. In contrast, most insects, including *Tribolium*, utilize “short germ” development, where extraembryonic membranes occupy most of the egg while the embryo occupies only a small portion, leaving room to pattern only the most anterior segments. Posterior segments form after gastrulation from a growth zone (for review: Liu and Kaufman, 2005). These very different modes of development necessitate important differences in DV patterning, for which Nunes da Fonseca and colleagues provide insightful explanations.

Drosophila DV determinants are provided maternally. Dorsal, a rel/NF- κ B

transcription factor, is found uniformly in the embryo. A ventral signal established during oogenesis leads to activation of Spätzle, which binds to its receptor Toll on the embryo's surface (Morisato and Anderson, 1994; Stein and Nüsslein-Volhard, 1992). The resulting signaling cascade leads to a gradient of nuclear Dorsal: high Dorsal concentrations in ventral nuclei activate zygotic genes (e.g., *twist*) that specify mesoderm, while lower nuclear concentration specifies neuroectoderm (for review: Stathopoulos and Levine, 2002). Dorsal also contributes to the formation of an opposing gradient of Decapentaplegic (Dpp), which specifies dorsal ectoderm and extraembryonic tissue (Ferguson and Anderson, 1992). These two gradients allow the designation of the full range of DV cell types.

The authors analyzed the function of DV genes in *Tribolium* (*Tc-genes*). Their disruption by RNAi leads to dorsalized embryos. In *Drosophila*, extraembryonic structures are the most dorsal structure controlled by the DV system: amnioserosa and dorsal ectoderm are merged and expanded in *Toll* mutants (Anderson et al., 1985). Extraembryonic tissues are controlled by both AP and DV systems in *Tribolium*: they are still present dorso-anteriorly in *Tc-Toll* RNAi embryos. However, the embryo lacks DV polarity, with loss of ventral genes *short gastrulation* (*Tc-sog*) and *Tc-twist* (*Tc-twi*).

Data presented in this issue suggest that DV patterning in *Tribolium* results from a self-organized system that does not rely extensively on maternal information, in sharp contrast to the highly deterministic control by localized maternal factors in *Drosophila*. Self-patterning along the DV axis in the absence of maternal information was in fact suggested over

30 years ago by Sander, who observed regeneration along the DV axis of longitudinal embryonic fragments of the leafhopper *Euscelis* (Sander, 1975). Meinhardt and Gierer (2000) have proposed a model for how such a self-organized system could minimally be achieved. Their model requires an initial activation event that is self-reinforcing and is also coupled to autoinhibition. If self-reinforcement has a more restricted range than inhibition, this can generate pattern. This is exactly what seems to happen for patterning along *Tribolium* DV axis.

While the *Drosophila* Dorsal gradient remains stable until gastrulation, Tc-Dorsal is highly dynamic: initially very broad, it progressively retracts toward the ventral side before disappearing at gastrulation (Chen et al., 2000). The relevant *Tc-Toll* is exclusively zygotic. Its weak uniform activation leads to broad nuclear Tc-Dorsal, but a ventral bias, likely from a maternal cue, leads to enhanced ventral uptake of Dorsal. Because *Tc-Toll* is also a Tc-Dorsal target, this represents the self-activation aspect of the model: ventral nuclear uptake of Dorsal leads to increased *Tc-Toll*, and thus to more ventral signaling that reinforces Tc-Dorsal activation (Figure 1).

How, then, is autoinhibition achieved? Cactus, the negative regulator of *Drosophila* Dorsal nuclear import, fits with the model: *Tc-cactus* (*Tc-cact*) is a zygotic target of Tc-Dorsal expressed early in a narrow ventral domain. There is, however, a further twist: while *Tc-dorsal* expression is lost after gastrulation, *Tc-cact* is upregulated in a domain that coincides with *Tc-twi* (another Tc-Dorsal target). *Tc-cact* is both an early target of Tc-Dorsal and a late target of Tc-Twist, which explains why *Tc-dorsal* disappears at gastrulation.

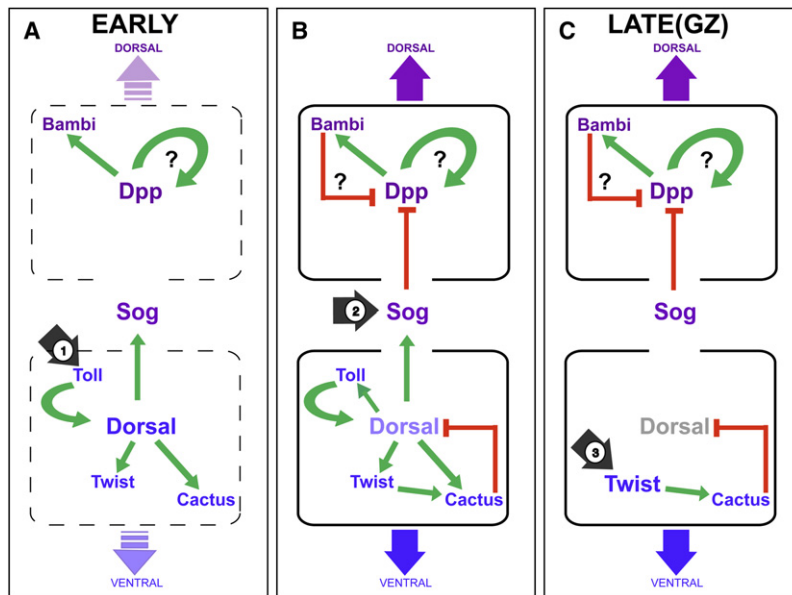


Figure 1. Model of DV Patterning in *Tribolium*

(A) A self-organized system biased ventrally (perhaps by a maternal system similar to *Drosophila*) leads to robust ventral activation of Tc-Dorsal (arrow 1).

(B) A second self-organized loop involves Dpp. It is likely biased dorsally by Tc-Sog, which links the two systems (arrow 2). This permits the specification of a range of DV cell fates in emergent posterior segments.

(C) After gastrulation, Twist expression in the growth zone leads to ventral patterning of posterior segments (arrow 3).

In *Tc-twi* RNAi embryos, *Tc-cact* expression is lost and the Dorsal gradient is stabilized and resembles *Drosophila*'s (Figure 1). Therefore, DV patterning in *Tribolium* has all the features of the self-organizing system of Meinhardt and Gierer: Dorsal is self-reinforcing through activation of *Tc-Toll* while a broader (presumably spatial and temporal) autoinhibition results from activation of *Tc-cact*, first directly, then by a feedforward loop through *Tc-twi*.

Could this self-organized system serve to pattern posterior segments that sequentially emerge in short germ insects? This could be the case, since all segments (including posterior segments) are dorsalized in *Tc-Toll* RNAi embryos, with loss of mesodermal *Tc-twi*. Their active growth zone lacks the most ventral values—the mesenchymal precursors of the mesoderm—while embryonic ectoderm is preserved. However, the *Tc-dorsal* system does not appear to function in the growth zone because no nuclear Dorsal is found there.

The model that emerges from these observations in *Tribolium* is that DV pat-

ternung in anterior segments results from a self-organized system. The initial ventral bias (arrow 1, Figure 1) might result from a weak signal similar to the strong maternal system found in *Drosophila*. Patterning of ectodermal and mesodermal fates in posterior segments must occur via forward induction from the differentiated anterior region to the unpatterned growth zone, through activation of *Tc-twi* (arrow 3, Figure 1).

Loss of DV polarity in *Tc-twi* RNAi embryos was expected to lead to loss of ventral and dorsolateral ectoderm and gain of dorsal ectoderm around the circumference of the embryo. However, while the most ventral positions are indeed lost, DV patterning is still obvious in these embryos, but occurs along the wrong axis: alternate rings of ectoderm expressing various DV markers are found along the AP axis, i.e., orthogonal to their normal position. This is due to a second self-organized system: The dorsal specifier Dpp appears to be self-reinforced through an unknown mechanism. Although *Tc-sog* was suspected to be the negative element, it is another negative

factor regulating Dpp, *Tc-bambi*, whose expression depends on *Tc-dpp*, which might play this role. Instead, *Tc-sog* is a target of Tc-Dorsal that coordinates the two self-organized systems. *Tc-Toll* RNAi embryos lack the cue (Tc-Sog?) that biases the Dpp self-organized system dorsally (arrow 2, Figure 1). Instead, the self-organized system uses a stripe of *Tc-dpp* along the AP axis as a template: artificial stripes of DV positions now form along the AP axis!

Therefore, Dpp signaling plays a more significant and autonomous role in DV specification in *Tribolium* than in *Drosophila*. This ancestral role is supported by the extensive use of Dpp in DV systems of other animals. The Dorsal network was likely later co-opted and became superimposed on Dpp to establish DV polarity. Interestingly, the interactions that support the self-organized nature of the Tc-Dorsal system likely represent fundamental features of the NF- κ B pathway that are also found in other organisms and contexts, including innate immunity. In flies, the self-organized nature of the Dorsal system was lost and has been replaced with a highly deterministic system that relies, as does the AP system, on strong prelocalized maternal clues.

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