Robustness of gene expression with respect to dosage of the Dorsal morphogen

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Brief Summary

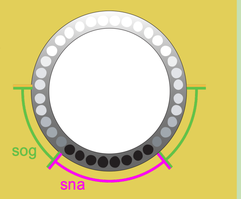
Background: In a developing tissue, proper placement of gene expression domains determines the fate of a cell. Positional information is disseminated in the form of morphogen gradients across the length of a tissue. Gene expression patterns are remarkably robust to a variety of factors, including dosage of the morphogen.

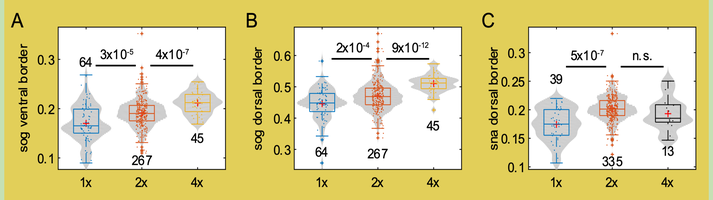
Methods: In this work, we investigate the robustness of gene expression in the dorsal ventral (DV) axis of the precellular *Drosophila* embryo, with respect to perturbations in the dosage of maternally deposited morphogen Dorsal, an NF-κB homologue. We found that the boundaries of genes regulated by Dorsal are robust to changes in dosage of the morphogen, which is paradoxical in itself, as by definition gene expression must be sensitive to morphogen concentration. In order to explain this discrepancy, we developed a mechanistic model drawing on extensive experimental and modeling work done on the Dorsal signaling system.

Results: We found that, Cactus, an inhibitor of Dorsal, which is generally believed to be only cytoplasmic, must also be present in the nucleus. Furthermore, Toll receptors, responsible for dissociating Cactus from Dorsal in the cytoplasm, must be saturated. Also, the model overwhelmingly predicts facilitated diffusion of Dorsal by Cactus complexes from dorsal to ventral regions of the embryo. In our previous work, we have shown that these mechanisms aid in proper development in wildtype embryos. In this work, we find that these three mechanisms are critical for robust gene expression when dosage of *dorsal* is compromised. Our work highlights the need for quantitative understanding of biophysical mechanisms of morphogen gradients in order to understand emergent phenotypes, such as robustness.

Experiments

- For embryos with all four dosage of Dl -
  - We imaged **Dl** and **Histone** protein and ***sna*** and ***sog*** mRNA in *Drosophila Melanogaster* embryos aged to NC 14 (approx. **2-4 hours after egg lay**), using a combination fluorescent *in situ* hybridization/fluorescent immunostaining.
  - We quantitatively analyzed these images to extract the domains of *sog* and *sna* in 1x, 2x and 4x embryos.





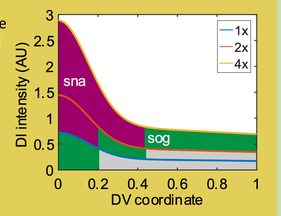
The Big Question

The shifts in gene expression boundaries is minimal across fly lines with varying dosage. How does one explain that?

Modeling

Proposition 1: Empirical scaling model of Dorsal

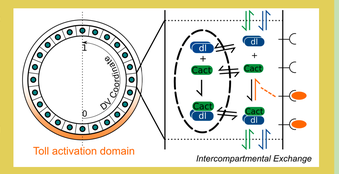
- If Dorsal scales with dosage, the domains of *sna* and *sog* extend or contract in 2x and 4x embryos.
- This may lead to catastrophic consequences for the embryo.
- This is not observed in experiments.



This model does **not** explain robustness.

Proposition 2a: Mechanistic model of Dorsal

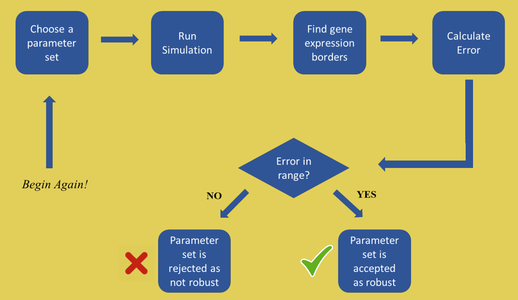
- Step 1.We used a mechanistic model of the Dorsal/Cactus/Toll system and simplified it to capture essential features of Dorsal dynamics.
- Step 2. The free parameters of the model, such as Diffusion constants "D"s and reaction constants "k"s were randomly varied, and Dl profile was simulated.
- Step 3. From the profile, sog and sna boundaries were calculated using Dl thresholds. If these boundaries were within certain error limits, the parameter set was deemed robust.



$$\frac{\partial [Dl]_{cyt}}{\partial t} = -k_b [Dl]_{cyt}[Cact]_{cyt} + k_d(x)\frac{[DC]_{cyt}}{\kappa + [DC]_{cyt}} + D\frac{\partial [Dl]_{cyt}}{\partial x^2}$$

$$\frac{\partial [DC]_{cyt}}{\partial t} = +k_b [Dl]_{cyt}[Cact]_{cyt} - k_d(x)\frac{[DC]_{cyt}}{\kappa + [DC]_{cyt}} + D\frac{\partial [DC]_{cyt}}{\partial x^2}$$

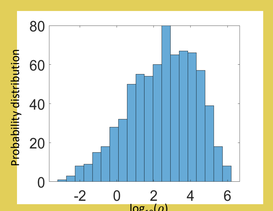
Proposition 2b: Analysis Approach



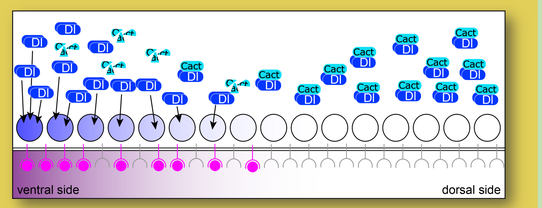
MODEL PREDICTION 1

Cactus shuttles Dl from dorsal to ventral regions.

$$\rho \sim \frac{D_{[complex]}}{D_{[free\ Dorsal]}}$$



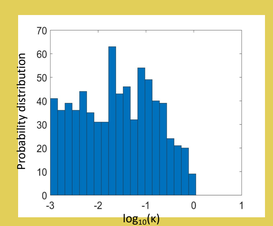
- The parameter ρ is defined as an effective ratio of the diffusion constants of Dorsal and the Dorsal/Cactus complex.
- If this ratio is greater than 1, it means that Cactus is being shuttled from the dorsal side to the ventral side.
- We see that in the majority of parameter sets, that were deemed robust, the value of ρ was greater than one.



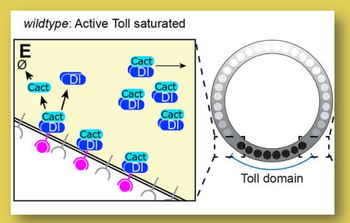
MODEL PREDICTION 2

The receptors of Toll are saturated by Dl/Cact complex

$$rate = k_d(x)\frac{[DC]_{cyt}}{\kappa + [DC]_{cyt}}$$

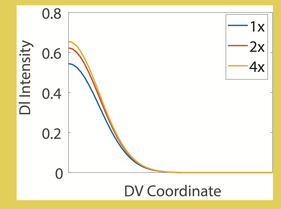


- The Michaelis Menten constant was seen to be less than one for all parameter sets that were deemed robust.
- Since all concentrations are normalized to be order 1, this means that Toll receptors are saturated by Dorsal/Cactus complexes.



MODEL PREDICTION 3

Total Dl = (Free Dl) + (Dl/Cact complex)



- In a 100% of the robust parameter sets, the concentration of Free Dl decreased to zero near the lateral regions (DV coordinate ~ 0.5).

References & Acknowledgements

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