BE 159: Signal Transduction and Mechanics in Morphogenesis

Justin Bois

Caltech

© 2021 Justin Bois and Jan Gregrowicz.
This work is licensed under a Creative Commons Attribution License CC-BY 4.0.

Questions to consider: Stapornwongkul, et al., "Patterning and growth control in vivo by an engineered GFP gradient"

Following are some questions that it will be helpful to understand when reading the paper. They are definitely not exhaustive, but useful to help you understand the motivation of the work and the experimental protocols.

1. The authors mention that in a planar geometry, transport by thermal diffusion can be problematic because of leakage. Can you think of other means of transport of morphogens among cells in a monolayer?
2. Be sure you know what the wing imaginal disc is and where it is located in the developing organism both in space and time.
3. Understanding the geometry is very important. Be sure you can sketch the geomtery of the imaginal disc. and that you understand its geometry. You should be able to sketch it on a piece of paper. Be sure you understand what is meant by the words "apical" and 'basolateral," and that you know where the anterior-posterior axis is. You should also be able to sketch how the Dpp gradient looks on the wing imaginal disc.
4. Be sure you know which plane you are looking at in all of the microscope images presented in the paper.
5. What is bone morphogenic protein (BMP)?
6. What do the authors mean by "forward engineering approach"? What could the study have looked like if they used reverse engineering?
7. Why did the authors use nanobodies and not standard antibodies?
8. The anti-GFP nanobodies, GBP1 and LaG3, have reported Kd of 0.23 nM and 25 nM respectively. The authors named their affinities high and low. How do those values compare to affinities often encountered in the cell?
9. Why couldn't a gradient form when there is leakage?

10. Why do you think that the authors modeled n_b as a function of c as they do?
11. What are S2 cells, and why did the authors transfect plasmids into them?
12. There are lots of beautiful genetics techniques at play in this paper that are easy to gloss over while thinking about how the morphogens work. In fact, the genetics techniques are what enabled the whole study. Think about/look up: What kind of techniques did the authors use to introduce exogenous genes into endogenous loci? And also: What is the flipase system and how does it work? You should be sure to understand what Fig. 4 B and C mean.
13. Why did the authors introduce glypicans?
14. Why do the authors model "hopping" as diffusion?