GENOME553 Winter 2004

Paper for Thursday 29 January 2004
Reinke, R. and S. L. Zipursky. 1988. Cell-cell interaction in the Drosophila retina: the *bride of sevenless* gene is required in photoreceptor cell R8 for R7 cell development. *Cell* **55**: 321-330.

The authors identify a new gene, boss*, required for R7 cell development. Mosaic analysis demonstrates the function of this gene in the neighboring R8 cell, revealing a signaling pathway central for ommitidial assembly.*

Questions for Thought

1. What was known at this time?

2. Briefly describe the mutagenesis screen used to identify *boss*. Why was *boss* the only gene identified in this screen? Is 10,000 a lot of flies? Why didn't the authors obtain mutations in *sevenless*?

3. Why did the authors make so many additional alleles of *boss*? (What did they hope to learn from this effort?)

4. Why did they make double mutants with *sev*? That is, what is the logic behind making double mutants with mutations that cause the EXACT same phenotype?

5. We will go through Figure 4 in detail. How are mosaic patches generated in the eye? When are clones induced? Why irradiate at that time? Why do the authors use two markers, *chaoptic* and *white*? What are essential features of these markers?

Figure 4C and Table 1: What is the evidence that *boss* is NOT required in R7? What is the evidence that it IS required in R8? How far can BOSS diffuse? Which ommitidia demonstrate this principle? How would the data in Table 1 appear if *boss* could be expressed in R8 or R1 or R6?

If suitable markers existed, could this method be used in *C. elegans*? Why or why not?

6. A new student in Larry Zipursky's lab decided to repeat Rosemary Reinke's mosaic analysis. Kowing it is important to "Go big or stay home!", he used the exact same protocol that Reinke used but looked at 30,000 flies. Using the w^+ marker, he identified 300 flies that exhibited mosaic patches. Most of these flies gave the same result that Reinke saw, that is, if R8 was mutant for *chaoptic*, R7 failed to develop. Six flies, however, did not show the same phenotype. Instead, ommitidia with chaoptic R8 cells still had clear R7 cells. Explain this paradoxical result.

7. **You have cloned the *boss* gene and have both an RNA probe and an antibody to BOSS protein. Would you still do mosaic analysis, or would you simply look at the RNA and protein expression patterns?** (Think about the work involved to do transmission EMs, as done here. *In situ* hybridization and immunohistochemistry can be performed on whole tissues, not sections, and would take about a week total. Does that information affect your decision?) **Justify your decision.**

8. Here is a gene, *boss*, that must be expressed in R8 for R7 to develop properly. R8 touches all of the photoreceptor cells and *sev* is expressed not only in R7 but in all other cells except R2, R5 and R8. Why don't all the cells that express *sev* become R7 cells? Suggest experiments to test your hypotheses.