GENOME553 Winter 2004

Paper for Tuesday 3 February 2004
Simon, M., D. Bowtell, G. S. Dodson, T. Laverty and G. M. Rubin. 1991. Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the Sevenless protein tyrosine kinase. *Cell* 67: 701-716. *By creating a background in which SEV RTK function is compromised, the authors identify seven genes that presumably function in the SEV signaling cascade. Mosaic and epistasis analyses demonstrate that at least four of these genes act in the eye, not only in the SEV pathway but with another RTK, EGFR. They clone two of the genes,* Ras1 *and* Sos. *This work, together with studies on vulval developemnt in* C. elegans, *provided the first demonstration that the Ras signaling cascade functions in a normal developmental process, compared to its aberrant role in oncogenesis.*

Questions for Thought

1. What is the logic behind this screen? Why are they looking for dominant mutations? (Aren't most mutations recessive?) What assumptions are they making?

What are the advantages of such a screen compared to other types of mutagenesis screens? Think of at least three advantages. What are the disadvantages? Again, think of at least three disadvantages.

How would you adapt this general type of approach to analyze the cell cycle in yeast? That is, what would be your design for such a screen?

2. What was the logic behind making temperature sensitive alleles of *sevenless*? If the authors did not have information about temperature-sensitive alleles of *src*, what approach could they have used to create "sensitized" conditions? Does one need a ts allele? Explain.

3. Why did the authors use the reduced pseudopupil method to score the sevenless phenotype? Why not use the behavioral assay involving the choice test? How many flies can be scored with these two approaches? What sort of false positives or negatives would result from these two different approaches?

4. Simon et al. identified 20 mutant alleles that fell into seven complementation groups. Did they identify all the genes in the *sev* pathway? Should they have, given the fact that they screened 30,000 flies? Why did they recover so many alleles of *E(sev)2A*? The authors state at the outset (top of page 703) that they expect to obtain "downstream" genes. Why not *boss*?

5. Simon now has his own lab at Stanford. Two students begin to characterize the other *E(sev)* alleles.

A) Elaine chooses to work on the III-linked line *E(sev)3D*. She maps *E(sev)3D* very carefully and finds that another scientist named Cass has already generated dozens of mutations in the region. Elaine obtains these alleles, carries out complementation tests and finds that four embryonic lethal alleles fail to complement the pupal lethality of *E(sev) 3D* ^{e0Q} and also fail to complement each other. When placed in trans to *e0Q*, the Cass alleles are semilethal, most animals dying as pupae, and the adult escapers have rough eyes. By these criteria, all five of these alleles define the same gene. Thus, Elaine was very surprised to find that NONE of Cass's alleles enhanced the sev^{ts} phenotype. Propose a hypothesis to explain these data. (Hint: why are some transheterozygotes viable?) How could you test your idea?

B) Craig analyzes the X-linked alleles of *E(sev)1A*. He finds a deficiency for the region that fails to complement the lethality associated with *E(sev)1A*. The deficiency also acts as an *E(sev)*. Surprisingly, however, the deletion is not as strong an enhancer as the two *E(sev)1A* alleles, *e3C* and *e0P*. What could explain this difference? How would you test your hypothesis?

6. How do the authors clone *Ras*? *Sos*? **How do they know they have the right genes?** How would you clone the genes, now that the genome is sequenced?

7. Why are the authors so cautious about interpreting the pathway relationships of *sev, Sos* and *Ras1*? Could these genes function in parallel, rather than as a linear pathway? Are there genetic tests that would show that *sev, Sos* and *Ras1* act in the same pathway?