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Rørth, P. 1996. A modular misexpression screen in Drosophila detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci. USA* **93**: 12418-12422. *Brand and Perrimon (1993) showed that the GAL4 transcription factor from yeast can function in flies, driving expression of reporter genes linked to GAL4 target sequences called UAS's (upstream activating sequences). Rørth takes advantage of this system to create a library of transgenic flies in which the UAS target sequences reside near random genes. By crossing these flies to lines in which GAL4 is expressed in various tissues (e.g., the eye), Rørth induces over- or mis-expression phenotypes. She calls this approach "EnhancerPiracy".*

Questions for Thought

1. What is the logic behind this screen? How does the method work? **What are the advantages of such an approach? Think of at least three advantages. Disadvantages? Think of at least three disadvantages.** Consider such factors as the choice of GAL4 line and the effort involved in making the EP collection. How does this approach compare to the gain-of-function method employed by Stevenson et al. (2001)? Four percent of the lines give a phenotype in the eye. Is that percentage reasonable?

2. What is the evidence that GAL4-driven expression of the EP-linked genes causes the observed phenotypes? Suggest at least three lines of evidence. Couldn't insertion of the EP element alone disrupt the gene? If over-expression or mis-expression really causes the phenotype, why doesn't the level of expression correlate with the mutant phenotype?

3. The author argues (p. 12420, end of first column) that the ideal enhancer piracy screen should produce mutant phenotypes by over- or mis-expression of an endogenous gene, not by producing a truncated or anti-sense transcript. Why? Isn't the goal to find all genes that might affect eye development? What is gained by limiting the screen in this way? What is lost?

4. One EP insertion targeted a gene whose product is similar to the chaperone protein DNAJ. It is thought to aid Hsp70 and other chaperone molecules in mediating protein folding, secretion and assembly of macromolecular complexes. Interestingly, Simon and colleagues identified another chaperone molecule, Hsp83, in their screen for *E(sev)*. How is it that over-expression of one chaperone (*DNAJ*) causes the same phenotype as loss-of-function mutations in another (*hsp83*)? What molecular mechanisms can account for these findings? Do these chaperones function specifically in the *sev* pathway, or are they general chaperones for all proteins? How would you distinguish these possibilities? Did these screens also identify the targets of the chaperones?

5. Biochemical studies demonstrate that Ras is normally active in a GTP-bound state and inactive when bound to GDP. GTPase activating proteins (such as GAP) catalyze exchange of GTP for GDP on Ras, thereby inactivating the protein. Geneticists describe this universally accepted relationship as

GAP —| Ras.

What, then, is the logic for building double mutants between Ras^{5703} and GAP^{EP45}?