GENOME553 Fall 2011

Paper for Thursday 13 October 2011
Whiteway, M., Hougan, L., Dignard, D., Thomas, D. Y., Bell, L., Saari, G. C., Grant, F. J., O'Hara, P. and MacKay, V. L. 1989. The STE4 and STE18 genes of yeast encode potential ® and γ subunits of the mating factor receptor-coupled G protein. *Cell* **56**: 467-477.

As you read this paper, **write down questions** you have about the logic or rationale for each experiment, the method employed, and the conclusions drawn. Come up with at least three questions. Turn in your questions as homework at the beginning of class. During class we will discuss your questions and the QfT below, emphasizing the questions in bold.

Questions for Thought

1) What was known about the mating pathway at that time? How many genes were known and **what was known about their functions at the molecular level?**

Note: In a previous paper, Whiteway and colleagues describe a screen for identifying mating pathway genes. They use a genetic background that sensitizes yeast to alpha factor: a double mutant *sst1 sst2*. *SST1* encodes a peptidase that degrades "a" factor and *SST2* encodes a GTPase Activating Protein (GAP). Both down-regulate the signaling pathway to facilitate sensing the direction of hormone source and allow growth toward the cell of opposite mating type.

2) Hartwell identified *ste4* mutations but no alleles of *STE18* and *SCG1*. Why would he have missed these genes? **Specifically, what is it about the biology of *<u>these</u>* two genes that would impede the identification of mutants?** (Don't worry about the logic of Whiteway's screen and why they recovered their alleles; think in terms of Hartwell's approach and why he might have missed them.)

Note:1) *ste18*-null mutations are completely mating defective and not lethal.
Hint: 2) For *scg1*, what is the loss-of-function phenotype?

3) **How do they clone these genes? What is the logic?** How do they show they have cloned the correct genes?

4) The authors use homologous recombination to disrupt the *STE4* and *STE18* genes. Why do the null phenotypes differ from the phenotypes produced by the alleles recovered in the super-sensitive screen? **Why do they use the null alleles to analyze double mutants?** Would the results differ with the "sst" alleles?

5A) **Table 2**: Note: the authors left out the genotype at the *LEU2* locus: the diploid is *leu2/leu2*. Double mutant analyses between *scg1* and *ste4* or *ste18* show that *ste4* and *ste18* are epistatic to *scg1*. Work through the table sufficiently to convince yourself that

the markers for the double mutants (LEU^+, URA^+) show the ste18 or ste4 phenotype and not the scg1 phenotype. **Why was this result surprising? Draw a pathway to show the relationships between these genes.**

5B) **Rather than use the logic of Whiteway et al. to interpret these results, apply the logic of Hereford and Hartwell (Table 2) to analyze these interactions (i.e., what are the four ways these genes could be placed into a pathway?).** What do you conclude? How does your conclusion compare to Whiteway et al.? What is different about this pathway compared to the one analyzed by Hereford and Hartwell?

6) The authors suggest four hypotheses, with accompanying predictions, to explain their data. **What experiments would you do to distinguish among these hypotheses?** Why do the authors conclude that "biochemical analyses" should prove useful?