GENOME553 Winter 2004

Paper for Tuesday 20 January 2004

Stevenson, L. F., Kennedy, B. K., and Harlow, E. 2001. A large-scale overexpression screen in *Saccharomyces cerevisiae* identifies previously uncharacterized cell cycle genes. *Proc. Natl. Acad. Sci. USA* **98**: 3946-3951.

All cells have evolved to use glucose as a carbon source, constitutively expressing the enzymes that break down this sugar. In contrast, expression of enzymes that act on other sugars, such as galactose or raffinose, is tightly regulated. Such genes are repressed when glucose is available and induced only when glucose is absent and the relevant sugar is present. These authors use this distinction to control the overexpression of yeast cDNAs, testing for cell cycle defects that result from such manipulation. Homework: Write brief answers to the questions in **bold**. **FOCUS your efforts on Tables 3 and 4.**

Questions for Thought

1) What is the goal of these experiments? **What is the logic for thinking more *cdc* genes exist?** Why think that overexpression will reveal new genes? Specifically, what molecular mechanisms might produce over-expression phenotypes?

2) How do they approach the problem? Why use a GAL promoter and a CEN vector? How do these tools work? Draw a flow chart of the screen, explaining each control. Why do the authors screen both cDNA and genomic libraries? Why do they screen 180,000 clones when only ~6,000 genes exist in yeast? Have they found all the genes that affect the cell cycle? Explain.

3) The authors anticipate that "overexpression might confer less dramatic defects than traditional loss-of-function screens". Why would one expect such a result?

4) Generally, what sorts of genes were identified and how did they characterize them? **Why did they use so many different assays?**

5) The authors analyze 36 hypothetical ORFs and found that deletion of 3 genes caused inviability while several others were viable but exhibited defects when challenged with checkpoint-inducing drugs. What is the logic for thinking these genes regulate the cell cycle? The remaining genes exhibited no phenotype when deleted (Table 3). Why did the authors recover these genes when they appear to have no role in regulating the cell cycle?

6) BLAST searches comparing the hypothetical ORFs recovered in the screen with the entire yeast gene database identified seven instances of likely genetic redundancy. Some partner genes produced cell-cycle defects when overexpressed; others did not. Some produced defects when both genes were deleted and others did not. (How were the deletions made?) Why didn't the authors recover all 14 genes in their screen? Why don't all double deletions produce a phenotype?

7) Summarize the "Rules of Thumb" that you would consider when carrying out a gain-of-function screen.