GENOME553 Winter 2004

Paper for Thursday 15 January 2004
Cho, R. J., M. J. Campbell, E. A. Winzeler, L. Steinmetz, A. Conway, L. Wodicka, T. G. Wolfsberg, A. E. Gabrielian, D. Landsman, D. J. Lockhart and R. W. Davis. 1998. A genome-wide transcriptional analysis of the mitotic cell cycle. *Molec. Cell* **2**: 65-73.

Some of these "Questions for Thought" do not have answers in the paper itself. Use your scientific/genetic common sense to derive solutions to these problems. A quick web search may provide facts to facilitate your thinking. Homework: Write brief answers to the questions in **bold**.

Questions for Thought

1) What was known about cell-cycle regulation at this time? Briefly outline the biochemical steps thought to control G1 -> S and G2 -> M transitions. (Alberts et al. wrote a whole chapter that summarizes this process. PubMed provides a slew of review articles.)

2) What were the goals of these experiments? What was the evidence for thinking transcript levels might vary during the cell cycle? Why think that these fluctuations matter? How did they approach the problem? How does their method differ from other micro-array approaches? What types of cell-cycle genes will they miss with this approach? **How do they know the genes they identify are important to the cell cycle?** How do they know that the changes in transcript levels are important for regulating the cell cycle?

3) How did they synchronize their yeast cultures? What do we know about the nature of the alleles they used? Why use two different mutants? Why use isogenic strains? What potential systematic errors were introduced using these approaches? How did they measure synchrony? Why use so many assays? How good was their synchrony? Defend your answer.

4) Previously known upstream regulatory sequences governing cell cycle-dependent transcription include the late G1 elements MCB and ECB and the early G1 element SCB.

A) Do all genes regulated in G1 have such elements? Would you expect them to? Explain.

B) Some genes that contain MCB, ECB, or SCB elements do NOT show cell-cycle dependent regulation. **Suggest THREE reasons why the presence of such a sequence might not predict cycling transcript levels.** How would you distinguish between your hypotheses?

5) The authors use their data to cluster genes with similar transcript-level patterns and then examine the upstream sequences for common motifs. How far away from the gene should one look for such sequences? What assumptions are being made about the nature of transcription? How does one know if the presence of a sequence motif is random or meaningful?

6) What do their results tell us about the biology of cell-cycle regulation? Consider genes that <u>were</u> identified and their patterns of expression, genes that were NOT identified, and transcriptional and post-transcriptional regulatory mechanisms. What are we still missing?