GENOME553 Winter 2004

Paper for Thursday 8 January 2004
Hartwell, L. H., R. K. Mortimer, J. Culotti and M. Culotti. 1973. Genetic control of the cell division cycle in yeast: V. Genetic analysis of *cdc* mutants. *Genetics* **74**: 267-286.

Questions for Thought

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 five questions, more is better. (I came up with >20 questions!). FYI: a "cistron" is a gene.

As you read, think about the questions listed below. Try to derive an answer from the paper or by thinking logically about the process. **Focus on Figure 1, Table 1, Table 3 and Figure 3**. We will discuss these questions and your questions in class.

At the beginning of class, turn in your 5 or more questions along with BRIEF answers (a few sentences) to the questions below that are typed in bold.

1) What was known at that time? What was the purpose of these experiments? **Why make temperature-sensitive mutants? What is the logic for this approach?** What predictions did they make about the phenotypes they should see?

2) How did they make temperature-sensitive mutants? Draw a flow chart of the procedure. If necessary, refer back to the previous Hartwell (1967) paper for details. How do they know their mutants are independent? Why does independence matter? Why did they use two different mutagens?

3) Once they had ts mutants, how did they determine which mutants exhibited cell-cycle defects? Why did they use several different techniques to assay cell cycle defects? How do these assays differ from each other? (What exactly are they screening for?) Why did they use a different assay than the one employed by Hartwell originally to assay cell-cycle mutants in his 1967 paper? How many mutants did they screen? Is that a lot?

4) Briefly summarize their results (Figure 1, Table 1). How many ts mutants exhibited cell-cycle defects? How did they classify their phenotypes? What is a prototype phenotype? What does the phenotype suggest about the function of the gene in regulating the cell cycle? We will come back to these questions at the end of the discussion.

5) Segregation Analysis: Are these mutations dominant or recessive? How do you know? Why does it matter? For each mutant, is the ts phenotype due to a lesion in a single gene? How do you know? Again, why does it matter?

6) Complementation Tests (Table 3, Figure 3): Describe a method for creating *MAT*□ strains from starting *MATa* strains. Why is this process necessary for carrying out complementation tests? How many genes do the authors define? How many alleles exist for each gene? Why do

some genes have multiple alleles and others only one? Are these genes the only ones required for cell-cycle regulation? How do you know? Why would one care?

7) Comparison of Alleles: The authors compare alleles by examining the number of cells generated by each mutant following shift to the restrictive temperature, and by analyzing cellular and nuclear morphology upon termination of development. **Why do the authors compare all the different alleles for each gene in such great depth? What is the rationale behind this effort to identify "exceptional" mutants?** Shouldn't the mutants all exhibit the same phenotypes, if they carry mutations in the same gene? Explain. Summarize their results. How do they explain their data?

8) What is unusual about all the alleles of *cdc7*? What does that suggest about its function?

9) "Second-division segregation" can be observed in yeast by analyzing tetrads. That is, ALL FOUR products of a single meiosis can be observed by dissecting out the haploid spores from an ascus. As a result, the orientation of homologues on the metaphase plate, the segregation of homologues and sister chromatids, and the presence of recombination events can be ascertained by analyzing the phenotypes of the four spores. Not only can one determine if two genes are linked to each other, but this tool lets one determine whether a gene is linked to a centromere! Since yeast has 16 chromosomes, linkage to a centromere or to a specific marker gene (e.g., *ade1*) greatly facilitates mapping. Why does it matter where a gene maps?

10) The authors explicitly thank Dr. Donald Hawthorne in the acknowledgements. Who was Don Hawthorne? (There is a story here...)

11) How does the genetic analysis of these *cdc* mutants contribute to our understanding of the cell cycle? What principles did the authors learn from these studies and how did these concepts help define the biological process?