Biology 7.06 Exam 1 October 4, 1994

Question 1. (20 points)

You are studying an antibiotic-resistant strain of plague bacillus (Yersinia pestis) and discover that an odd red yeast that has contaminated one of your petri plates secretes a substance that diffuses across the plate and kills the plague bacillus. Realizing that this chance discovery might lead to the identification of a new drug to treat the pathogen, you fractionate extracts of the red yeast to identify the substance that kills the plague bacillus. Eventually, you purify a protein that has the killing activity. Because the red yeast produces only small amounts of the killing protein, you decide to clone the gene for this protein and use it to make recombinant protein.

A. (8 points) Since normal yeast cells don't produce this plague killer protein, how could you clone the gene from the red yeast?

B. (4 points) You decide to produce the recombinant killer protein in E. coli. What additional DNA elements might you need for high level expression of the red yeast gene in E. coli?

C: (4 points) It turns out that expression of the killer protein kills E. coli, so you decide to produce large amounts of the protein in mammalian cells. Should you use a transient transfection system or a retroviral vector? Why?

D. (4 points) To your great disappointment, the protein you purify from mammalian cells turns out NOT to have killer activity. It does appear to be the correct size when you subject it to SDS polyacrylamide gel electrophoresis. What might be the problem?

Question 2. (20 points)

A. (3 points) There are multiple elements of a chromosome that are necessary for appropriate replication and segregation of the molecule into daughter cells. What are they and what properties do each confer on DNA molecules introduced into yeast?

ARS (or replication origin) - origin of replication

CEN (centromere) - stable inheritance of 1 copy of chromosome / cell

TEL (telomeres) - protect linear chromosome ends

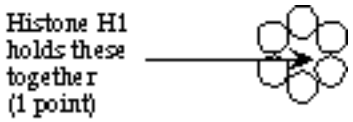
B. (17 points) Describe how the cell manages to package the human genome into a nucleus that is only 10^{-5}m. Mention the proteins involved in each aspect of packaging.

1. DNA is first split into chromosomes (2 points)

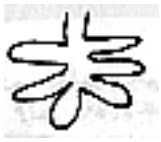
2. Then nucleosomes are formed (or 10 nm fibers) (3 points)

Nucleosomes contain 2 copies of Histones H2A, H2B, H3, and H4 (5 points)

3. These nucleosomes then form 30nm fibers (3 points)



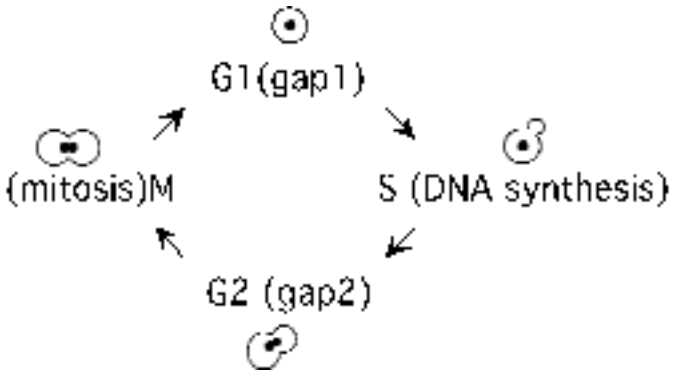
4. These then form looped domains (3 points)



Scaffold proteins or topoisomerase II hold this together. However, since this was not mentioned in class you did not have to say this.

Question 3. (20 points)

A. (4 points) Draw the four stages of the eukaryotic cell cycle and draw the corresponding yeast morphology for each stage.



2 points for cell diagram (Budding occurs around S phase)

2 points for yeast morphology

B. (6 points) How would you isolate a temperature-sensitive yeast mutant that cannot exit mitosis?

-mutagenize a population of yeast with a mutagen (ex.EMS - produces point mutations) (1 pt)

-plate out at 23 ° C (permissive temp.)

-replicate plate and grow 2nd plate at 37° C (restrictive temp.) (1 pt)

-look for colonies that grew at 23° C but not at 37° C, these are each a ts mutant gene (1 pt)

-to identify ts mutants which cannot exit mitosis, each ts mutant colony identified on 23 ° C plate is grown separately in a liquid culture at the permissive temperature.

-then shifted to the restrictive temp and an aliquot of each culture is taken and observed under the light microscope (1 pt)

-look for cultures where the cells have synchronously arrested in the final stages of mitosis (1 pt)

ie. 

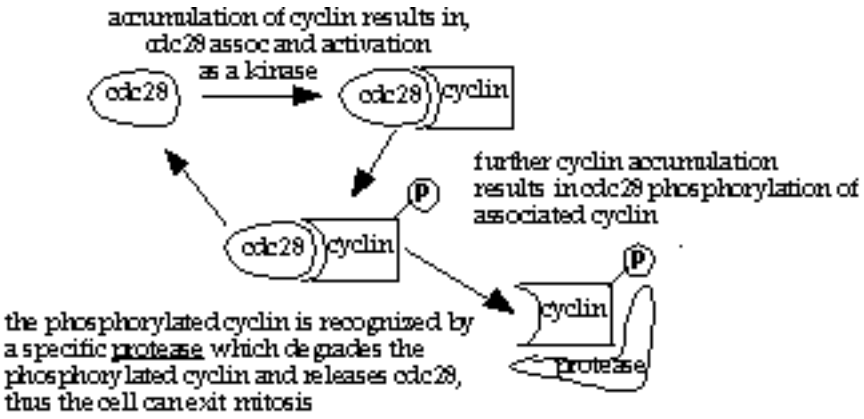
A common mistake was just trying to observe the mutant phenotype on the original 37 ° C plate which may be difficult since cells did not grow long at permissive temperature before shifting, i.e. hard to get a good population of cells to examine specific defect this way.

C. (10 points) You have cloned a wild-type copy of the gene that is defective in your ts mutant. You find that this clone encodes a protease. Explain the process that is likely to be defective in your yeast mutant.

The point of this question was to explain why a mutation in a protease might cause the phenotype observed (inability to leave mitosis) and to specifically explain the process the protease is normally involved in. Several different types of models were proposed but only one, which was discussed explicitly in detail in lecture, is an actual known biological process crucial to the completion of mitosis.

The protein kinase CDC28 is a regulator of cell cycle progression and its activity is controlled by its association with a family of proteins called cyclins (1pt).

One specific group of cyclins, mitotic cyclins, interact with CDC28 during mitosis. The presence of these mitotic cyclins is required for CDC28 to have kinase activity and for mitosis to begin. However, the absence of these mitotic cyclins is required for cells to exit mitosis. (4 pts)



- (3 pts for full description of this cdc28 / cyclin oscillator cycle)

Thus if one loses the function of the protease which recognized the CDC28-phophorylated cyclin and degrades it, cyclin levels will remain high and the cells will be unable to exit M (2 pts)

Partial credit was given for models involving other processes which could possibly give the phenotype. Some suggested that membrane protein which hold the splitting daughter cells together must be degraded for cytokinesis to finish. This is plausible, though not a known documented process. Another popular model was that the protease is needed to degrade the spindle fibers which line up the chromosomes on the equatorial plate . These fibers are made up of microtubules (microfilaments are also involved in mitotic division). However, it is depolymerization of these protein complexes which causes them to move to opposite poles, not due to protease action (see Fig. 21-46 in your text).

Question 4. (20 points)

You have a system with which you can transfect T cells with a full length HIV-1 genome and assay the expression of each HIV gene product. Explain what effects the following mutations would have on HIV gene expression and on the HIV-1 life cycle.

A. (4 points) A mutation which eliminates the catalytic activity of the reverse transcriptase.

HIV produced could not productively infect another T cell since it wouldn't be able to make a DNA copy of its RNA genome.

B. (4 points) A mutation which eliminates tat activity only.

The HIV genome could not be expressed or would not be expressed to a high level since tat is necessary for enhanced processivity and perhaps initiation of RNA polymerase.

C. (4 points) A mutation which eliminates rev activity only.

rev normally accumulates to a level where it will shut off the splicing patterns which produce it and tat and allow gag, gag-pol, and env to be produced. If rev were mutant, you would continue to produce tat & rev, but would not be able to produce gag, gag-pol, and env.

D. (4 points) A mutation which eliminates ribosomal frameshifting between the gag ans pol sequences of the mRNA.

This would eliminate translation of the gag-pol fusion protein. It would just produce gag. Without pol (reverse transcriptase) a viral particle could not productively infect a T cell (see Question #4A).

E. (4 points) A mutation which eliminates the Ψ site.

The viral RNA could not be packaged into viral particles.

(Ψ is the signal that allows RNA genome to be recognized for packaging.)

Question 5. (20 points)

You have successfully obtained ES cells heterozygous for a mutation in the flea-resistance gene using methods outlined in the handout you read on Targeted Gene Replacement. The ES cells were originally derived from mice which were homozygous for the brown coat color allele. Explain how you would use these ES cells to make a strain of mice carrying the flea-resistance mutation.

1. Inject ES cells into blastocysts from black mice. (4 points)

2. Put blastocysts into pseudopregnant female. (4 points)

3. When this female delivers look for chimeras.

(chimeras will have a mixture of black and brown in their coat) (4 points)

4. Breed chimeras to Black females. (4 points)

5. Keep brown pups - only 50% of brown pups will carry the mutation so you have to do a DNA screen (Southern) to determine which half have the mutation. (4 points)