

39.314 INTRODUCTORY CYTOGENETICS

MID-TERM EXAMINATION

1 p.m. to 2:20 p.m. Tuesday, October 26, 2004

This examination is worth 15% of the course grade.

Hand in these question sheets along with your exam book.

1. (15 points) The protocol for C-banding, discussed in class, is given below.

- a. Roots are harvested, pretreated and fixed in 3:1 95% ethanol:glacial acetic acid for at least 24 h. The roots are softened in 45% acetic acid or in 0.5% aceto-carmine. Slides are prepared by the squash method and the cover glass is removed using dry-ice method. Chromosomes adhere to the slide surface.
- b. Dehydration. Typically slides are placed in 95 to 100% ethanol for 1 hour.
- c. Denaturation. Treatment with barium hydroxide for 5 to 15 min at elevated temperature 50-55° C .
- d. Renaturation. The slides are then washed with distilled water and transferred to incubation at 60° C in saline sodium-citrate solution SSC (NaCl). Incubation periods and temperature are variable.
- e. Staining. Slides are then stained with Geimsa stain and checked periodically to see how the stain is progressing. When the optimal staining has been achieved, the slides are rinsed in distilled water to remove the excess stain, air-dried, stored in xylene overnight, air dried again and the cover slip is mounted using Canada Balsam, etc.

Briefly describe the purpose of :

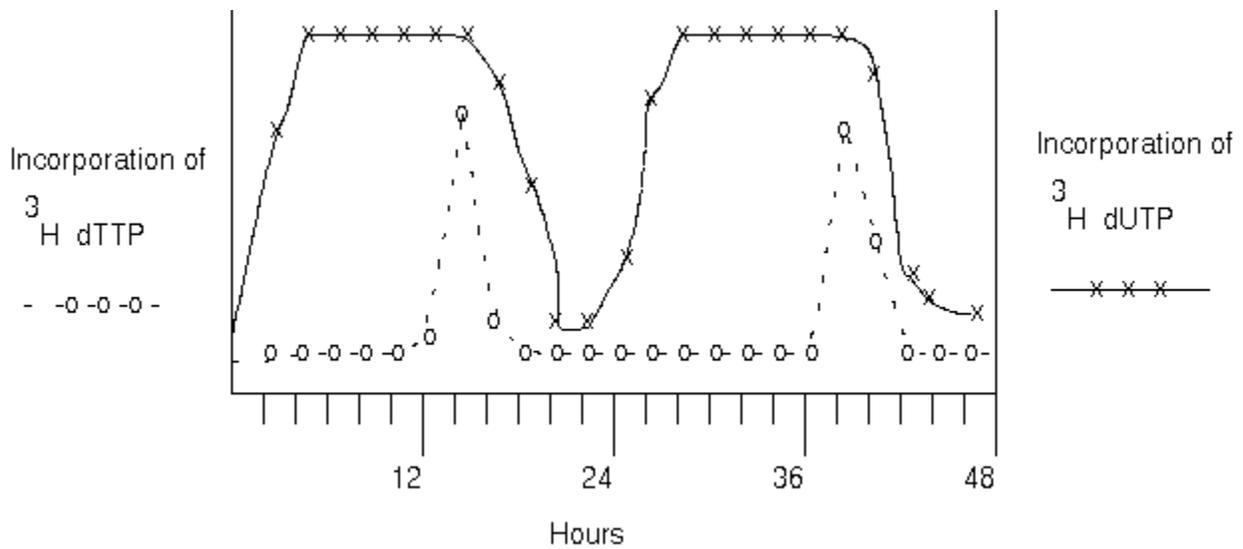
- i) The acid treatment a
- ii) The alkali treatment in c
- iii) The renaturation step in d ie. why does this step affect centromeric DNA differently and the rest of the genome?

2. Fill in the blanks. (10 points)

The chromatin domains are anchored to the _____ a _____ nuclear matrix at matrix attachment sites. Transcriptionally active domains are extended and attached to the _____ b _____ nuclear matrix. _____ c _____ regulate the flow of _____ d _____ out of the nucleus and _____ e _____ into the nucleus. The nuclear envelope is continuous with the _____ f _____ , where translation occurs. The nuclear envelope is disassembled

during _____g_____, when the membranous components of the envelope are stored as _____h_____. During _____i_____, the nuclear envelope re-forms, first around individual _____j_____, which then fuse to form a new nuclear envelope .

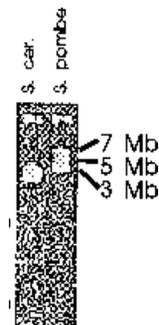
3. (10 points) Mammalian cells were grown in synchronous culture. In two parallel experiments, cells were labeled using either ^3H -dTTP or ^3H -dUTP. To measure the incorporation of isotope, a sample of cells were centrifuged every two hours, and ^3H was measured in the pellets using a scintillation counter. The results are shown below:



Explain the results.

4. (10 points) The figure below shows Pulsed Field Gel Electrophoresis (PFGE) of yeast chromosomal DNA. Gels of this type are presented as evidence that support the hypothesis that eukaryotic chromosomes are single DNA molecules.

- Describe what you would see that hypothesis were false.
- Why is it necessary to use PFGE, as opposed to a simple agarose gel?

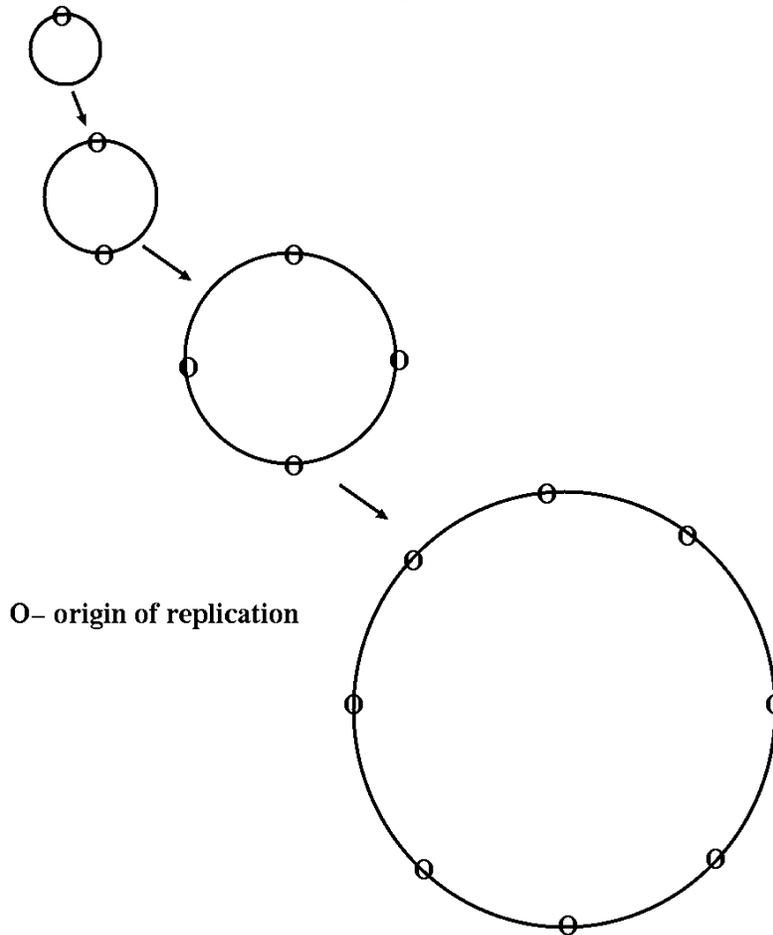


5. (15 points)

a) The figure below is a speculative model that illustrates one possible step in the evolution of the eukaryotic chromosome from a prokaryotic chromosome. As chromosomes got bigger, it took longer to replicate them. Consequently, selection favored chromosomes with multiple replication origins. What advantage would there be to having larger chromosomes? (Hint: think about the differences between prokaryotes and eukaryotes.)

b) One disadvantage of having a larger chromosome is that if a break occurs, it is harder for the two ends to find each other, such that the chromosome can recircularize. What would happen if the chromosome can't recircularize, after a break?

c) Suppose that, in a distant ancestor of the modern eukaryote, an enzyme evolved that could add short DNA sequences to the ends of broken chromosomes. What benefit would this give to the cell? To what modern enzyme would this ancestral enzyme correspond?



6. (15 points) A spool of thread represents one solution to the problem of neatly packaging a very long object in a small space. We demonstrated in class that a chromosome packages DNA roughly 10 times more efficiently than a spool packages thread. While in principle it is conceivable that a chromosome could be packaged on a "spool", there are a number of ways in which spool-like packaging would be disadvantageous, compared to the multi-level hierarchical packaging found in eukaryotic chromosomes. Considering all the things that eukaryotic chromosomes need to do, cite three reasons why hierarchical packaging is better than spool packaging.

7. (20 points)

Define the following:

a) Nucleosome

b) 30 nm fiber (solenoid)

c) What is the role of Histone H1?

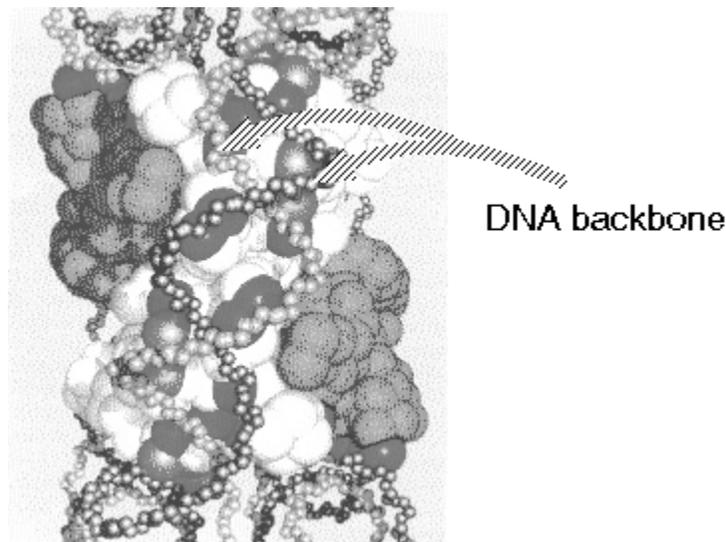
d) Which of the following is true:

Histone H1 is present in near stoichiometric quantities with respect to the number of nucleosomes

i) in transcriptionally-active chromatin

ii) in inactive chromatin

e) The accompanying figure indicates the position at which the DNA backbone interacts with the histone core particle. Describe the important features of this interaction.



8. (5 points) Data was presented indicating that the nucleus is not a sphere, but rather a more convoluted structure containing grooves and invaginations. What is the importance of this observation?

