**Data Analysis Problem**

by Marianna Pap and József Szeberényi

to accompany

*The Cell: A Molecular Approach,* Eighth Edition

Geoffrey M. Cooper

**8.2 Ribosomal RNA Metabolism**

This Data Analysis Problem is also found on page 283 of the textbook.

**Source:** Warner J. R., R. Soeiro, H. C. Birnboim, Girard M., J. E. Darnell. 1966. Rapidly labeled HeLa cell nuclear RNA. I. Identification by zone sedimentation of a heterogeneous fraction separate from ribosomal precursor RNA. *Journal of Molecular Biology* 19: 349–61.

# Level of difficulty: Medium

# Corresponding chapter(s) in the textbook: Chapters 8 and 10

# Review the following terms before working on the problem: [3H]uridine labeling, nucleoli, ribosomal RNAs, velocity centrifugation, radioactivity and UV absorption measurements

# Experiment

Three cultures of HeLa cells were labeled with [3H]uridine for 25 minutes and then subjected to the following treatments:

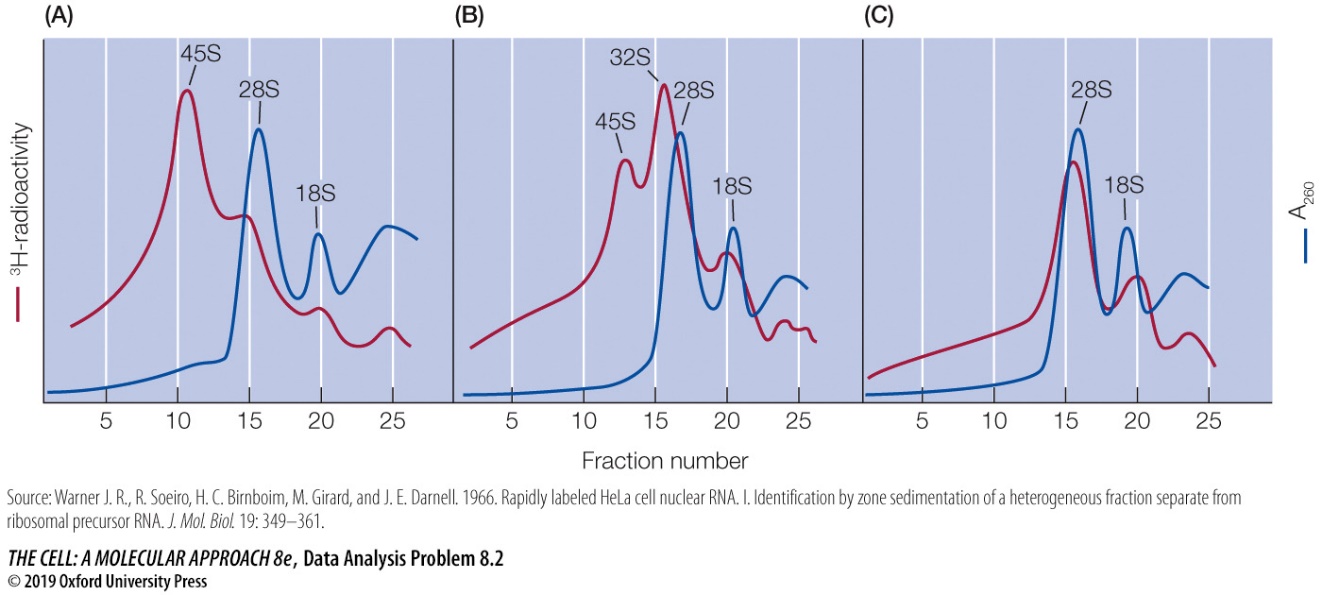
A. Control cells: no treatment

B. Incubation with actinomycin D (which inhibits RNA synthesis) for an additional 10 minutes

C. Incubation with actinomycin D for an additional 35 minutes

After incubation with actinomycin D, nucleolar RNA was isolated from the three cultures. The RNA samples were fractionated by high-speed centrifugation in a sucrose gradient, and fractions of equal volume were collected from the bottom of the tube. (The first and last fractions thus correspond to the bottom and the top of the gradients, respectively.) Radioactivity and ultraviolet absorption (A260) of the fractions were measured.

**Figures**



**Questions**

1. Why is [3H]uridine suitable for labeling newly synthesized RNA molecules?

2. What is the purpose of treating cells with actinomycin D in this experiment?

3. Why are the radioactivity and UV absorption curves so different in graph A?

4. What is the relationship between 45S, 28S, and 18S RNA species? Suggest an explanation for the presence of 32S RNA in graph B.

5. Account for the strong similarity between the UV absorption curves of the three figures.