Dintzis, Howard M. (1961) Assembly of the peptide chains of hemoglobin. *Proc. Nat'l Acad. Sci. USA* **47:**247-261.

Questões:

1. Which are the previously proposed models for protein synthesis? Why should amino acids be activated for peptide bond formation?
2. What did Dintzis mean by ribosome templates (page 248)?
3. Why did Dintzis choose reticulocytes for his studies?
4. In the experiments described in the paper what does the author consider "soluble protein" produced by reticulocytes? In the same context what would be an "insoluble protein"?
5. How radioactively labeled amino acids could help solve the problem proposed in the introduction of the paper?
6. Analyze the model of sequential chain growth proposed in figure 1.
7. Describe the fingerprinting technique used by Dintzis. Why did he use it and what were its advantages over other techniques available at the time for peptide mapping (chromatography and paper electrophoresis)?
8. What is the advantage of using ^{3}H-Leucine instead of ^{3}H-Glycine?
9. Why use precursors labeled with 2 different isotopes (^{3}H and ^{14}C)?
10. At what temperature and why were done the experiments of radiolabeled precursors incorporation?
11. Why the ribosomal proteins were not significantly labeled? How did Dintzis prove that?
12. Describe, and understand, the protocol for radioactive labeling of reticulocytes.
13. Describe the double labeling protocol with ^{3}H-Leu and ^{14}C-Leu?
14. How the fraction containing complete molecules of hemoglobin was obtained?
15. What did the fraction the author calls ribosomes contain? Why was this fraction radioactive if ribosome proteins did not incorporate the labeled precursors?
16. Why use pyridine-formic acid buffer for ion exchange chromatography? Tip: buffer pH ~ 3.5.
17. Why is the trypsin solution made in 1 mM HCl?
18. What is ninhydrin used for?
19. How was the radioactivity of the samples measured?
20. Why would the availability of radiolabeled lysine and arginine be important for the study of Dintzis?
21. What is the conclusion of the experiment shown in table 3? In this same Table, how important is column (c)?
22. Which model(s) can be discarded with the experiments shown in figure 5 and in table 3?
23. Discuss the results shown in Figures 6 and 7 against the model shown in Figure 1.

24. Describe the use of carboxypeptidase A and B for determination of the C-terminal peptide of rabbit globin. How do these enzymes work?
25. Describe the calculations made by Dintzis to obtain the velocity of globin biosynthesis.