Nirenberg, Marshall, Matthaei, J. Heinrich (1961) The dependence of cell-free protein synthesis in E. coli upon naturally occurring or synthetic polyribonucleotides. Proc. Natl. Acad. Sci. USA **47:**1588-1602.

Questões:

1. What technical results, obtained previously by Nirenberg´s lab, were important for this paper?
2. How were the *E. coli* cells disrupted? Make a flowchart of the protocols and try to understand each step.
3. The presence of 6 mM β-mercaptoethanol in the "standard buffer" is crucial for retaining protein synthesis activity of frozen lysate fractions. Why?
4. After reading the results of this paper explain why fractions S-30, S-100 and W-Rib were dialyzed.
5. Protein synthesis was made in the presence of phosphoenol pyruvate (PEP) kinase. Why? (consult Matthaei & Nirenberg, PNAS **47:**1580, 1961).
6. What is the difference between RNA extracted from the W-Rib fraction and the S-100 fraction?
7. Why in figures 1 and 2 the reactions attain a plateau after some amount of either soluble RNA and ribosomal RNA are added?
8. Something is consumed during the incorporation reaction (Figure 3) and leads to a plateau in ^{14}C-Val incorporation. Can you figure out what it should be?
9. Why increasing the amount of ribosomal RNA increases the incorporation of ^{14}C-Val?
10. How does chloramphenicol and puromycin affect protein synthesis? What step(s) of protein synthesis is (are) affected?
11. Table I shows three different experiments. What did the authors conclude from each one?
12. Why upon boiling ribosomal RNA "...a slight increase in activity was consistently observed."?
13. How do you explain the fact that 4% of ^{14}C-Val was incorporated at the C-terminal and 1% at the N-terminal portion of the proteins? How these measurements were done?
14. The results presented in Table 2 show that ribosomes are essential for *in vitro* protein synthesis but purified ribosomal RNA, although necessary, does not substitute intact ribosomes. Why?
15. The addition of poly-A to the reaction mixture did not stimulate protein synthesis (Table 4). Why?
16. Figure 4 shows analytical ultracentrifugation results of RNA fractionation. Where is the 4S peak?
17. Results of sucrose gradient centrifugation (Figure 5) show that the main peak of radioactive protein is not associated with soluble RNA. From what we now know about protein synthesis how you interpret this result?
18. Ribosomal RNAs from several sources (yeast, *E. coli*) as well as tobacco mosaic virus (TMV) RNA stimulate protein synthesis. Why the stimulus of homologous

preparations (*E. coli* rRNA) is not as good as TMV RNA and yeast ribosomal RNA?

19. What were the conclusions of results shown in Table 6 and Figure 6?
20. Why poly AU (A/U = 2/1 or 4/1) did not stimulate the incorporation of Phe? Can you explain this?
21. Table 7 shows a series of control experiments. Although they seem unnecessary in face of what we know today, why were they important at this time?
22. How did the authors show that the polypeptide synthesized under the control of poly-U was indeed poly-Phe?
23. What is the main conclusion of this paper and why it is so important from a historical point of view?
24. What is the conclusion of the authors about the composition of ribosomal RNA fraction when compared to soluble RNA?
25. Why boiling the ribosomal RNA fraction of *E. coli* makes the 23S and 16S peaks go to 13.1S, 8.8S and 4.4S in an ultracentrifugation analysis?
26. Why is the action of soluble RNA catalytic for precursor incorporation into protein and ribosomal RNA action stoichiometric?