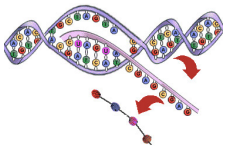

ICB 5751/The origins of Molecular Biology

THOMAS J. KELLY AND HAMILTON SMITH (1970). A restriction enzyme from *Haemophilus influenzae*. II. Base sequence of the recognition site.

Roteiro de leitura

Abstract & Introduction

1. What is the aim of the work described in the paper?

2. What is the size of the T7 genome (based on information given in the paper)?

3. Why only 0.1% of T7 genome is digested by the endonuclease? What is the significance of this finding?

Materials and Methods

4. How is it possible to distinguish between ^{33}P uniformly labelled DNA with ^{32}P end-labelled DNA? Could the labelling be reversed, i.e., uniformly labelled with ^{32}P and end-labelled with ^{33}P?

5. What are the substrate and product of pancreatic DNase?

6. What are the substrate and product of the snake venom phosphodiesterase?

7. Explain how the size of the oligonucleotide chains was determined. What was the purpose of removing the 5'-phosphoryl groups with alkaline phosphatase?

Results

8. What is the meaning of the concept "even duplex break" in Fig. 1?

9. Still in Fig. 1, describe the function of each enzyme. What are the "several nucleases" mentioned in the text?

10. In Fig. 2 what is the difference between (a) and (b)? Explain the result obtained.

11. What did the authors conclude regarding the fact that different proportions of dAMP and dGMP were obtained (see Table I)? How did they reach this conclusion?

12. What feature of exonuclease I was used to obtain a dinucleotide at the end of the digested fragment?

13. How was the second nucleotide in the recognition site of enzyme R identified?

14. How was the 5'-trinucleotide determined? Draw a flowchart.

15. Describe how did the authors conclude that the restriction site contains complementary ends as shown in Fig. 8a?

16. What is the expected frequency for the site GTPyPuAC in *E. coli* chromosome (50% GC)?

17. What is the relation between the average T7 DNA fragment size digested with Endo R and the fact that the restriction site is GTPyPuAC?

18. Describe the experiment represented in Fig. 11. What is the conclusion? What is the maximum number of fragments that would be expected if there would be two more residues in the restriction site?

Discussion

19. Can *H. influenzae* DNA be digested by Endo R? Explain. What evidences did the authors have?

20. What is the actual name of endonuclease R?

21. Finding out the EcoRI (G'AATTC) restriction site would be easier, harder or equally challenging compared to EndoR? Explain your answer.

22. Bonus question: The purification of Endo R was not complete, later it became clear that there were two different REs. What was the second RE? Why Smith and Kelly did not realise that there was more than one enzyme?