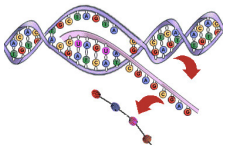

ICB 5751/ The origins of Molecular Biology

Luria SE, Delbrück M (1943) Mutations of bacteria from virus sensitivity to virus resistance

Reading guide

Introduction

1. What is the general aim of the paper?
2. Phage-resistant bacteria do not adsorb phage particles. What bacterial structure should be modified to provide bacterial resistance?
3. Phage-resistant bacteria naturally emerge in phage-infected cultures. These bacteria eventually grow and increase the turbidity of the previously clear culture (lysate) in a process that can take hours or even days. This process is known as "secondary growth". Assuming that a single mutant arises in a (liquid) bacterial culture infected by phages, at the same time that complete lysis is achieved, and that eleven hours after total lysis, the culture reaches its saturation point (5×10^9 bacteria/ml). What is the growth rate of this mutant in the medium? What is the generation time?
4. What are the TWO hypotheses that can explain the emergence of phage-resistant mutants? In what aspects these hypotheses differ?
5. How does Burnet's experiment (1929) favor the "mutation hypothesis" and why this result was not sufficient to answer the question about the origin of phage-resistant mutants?
6. Explain why one of the hypotheses about the origin of mutants requires that the number of mutants would increase with time. What did happen when Luria-Delbrück tested this hypothesis? Was the result due to an experimental artifact? Explain it.

Material and Methods

7. Describe the bacterial and phage strains used in this study.
8. The authors stated that phage-resistant mutants arise after apparent lysis of the culture and that these mutants are not lysogenic. What do they mean by that? What is the evidence for this statement?
9. How L and D proved that virus resistance is a stable phenotype?
10. What did L and D do to ensure that there were no resistant bacteria in the initial inoculum? I.e., how could they know that all resistant colonies descended from mutants that emerged during growth in the test culture (the culture that was eventually plated) and not beforehand?

11. At the end of the "Material and Methods" section, L and D hinted that mutations are pre-existent. What is this hint?

12. Two types of phage-resistant colonies have been reported: small and large. Suggest an explanation for the appearance of more than one type of colony (the answer is not in the paper).

Experimental

13. What is the aim of the experiment described in Table 1? What is the tested hypothesis and its conclusion?

14. If a group of samples is distributed according to "Poisson", the **variance** should be equal to the **mean**. Connect each of the two hypotheses about the origin of mutants with the statement above.

15. What hypothesis about the origin of mutants is corroborated by the results presented in Tables 2 and 3? Explain.

16. When *m* (mutation mean) is calculated with the formula $P_0 = e^{-m}$, the entire volume of the culture must be plated. Why is that?

17. In the paper, L and D raised the possibility that virus resistance manifests only in the offspring, but not in the bacterium that underwent the mutation. Why this idea is wrong?

18. (a) Calculate the mutation rate according to the results obtained in Expt. 23 (Table 3, p. 505). Use the method of Poisson: $P_0 = e^{-m}$ and $a = \frac{m}{N}$.

    (b) Calculate again the mutation rate using the second formula developed by L & D in the paper: $r = a \times N_t \times ln(N_t \times C \times a)$.
    r = average of mutants (in all cultures)
    a = mutation rate
    N_t = number of bacteria in each single culture
    C = number of cultures

    (c) Why do you think the calculated mutation rates not the same?

19. Poisson distribution is used to determine the likelihood of rare events. Are mutational events distributed according to Poisson? What about the number of mutants?

20. How did L and D demonstrate that mutations occur only in bacteria that are replicating?