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Lambda Lysozyme Synthesis in the Absence of N Protein[1]

During development of phage λ, the N product stimulates synthesis of early proteins, including the Q protein (*1*). The Q protein in turn stimulates late messenger synthesis (*2*, *3*), but it is not definitely known whether N product is also directly required for stimulating late messenger or protein synthesis. In fact, different experiments have given contradictory answers to the question of whether or not the N product is directly required for late messenger synthesis. On the one hand, Konrad has presented data showing that synthesis of late messenger declines shortly after inactivating a temperature-sensitive N product (*4*). These data could imply that either N is directly required for late messenger synthesis or that the Q product is unstable and its synthesis is continuously required for late messenger synthesis. On the other hand, mutants of λ have been isolated which can grow in the absence of N product. These have bypassed their need for N by two mutations: first, a constitutive promoter for genes O, P, and possibly Q, and second, a mutation called byp, located between genes P and Q. These mutants appear to allow the synthesis of λ protein without N protein (*5*, *6*), and since they can synthesize some late protein, albeit not much, N may not be directly required for normal synthesis of late messenger.

The experiments presented here are designed to clarify the role of N in late protein synthesis. We have used the same temperature-sensitive N mutant used in Konrad's experiment, but we have examined the synthesis of phage lysozyme, a late protein, after shifting to the temperature at which N is inactivated. We found that there is no change in the rate of lysozyme synthesis for the first 10 minutes after inactivating the N product by the temperature shift (Fig. 1); thereafter its rate of synthesis slowly decreases. These kinetics are inconsistent with the notion that N is directly required for late messenger or protein synthesis, for if N were required for the step most distant from the appearance of active enzyme, i.e., the initiation of synthesis of late messenger, then the rate of lysozyme synthesis would have de-

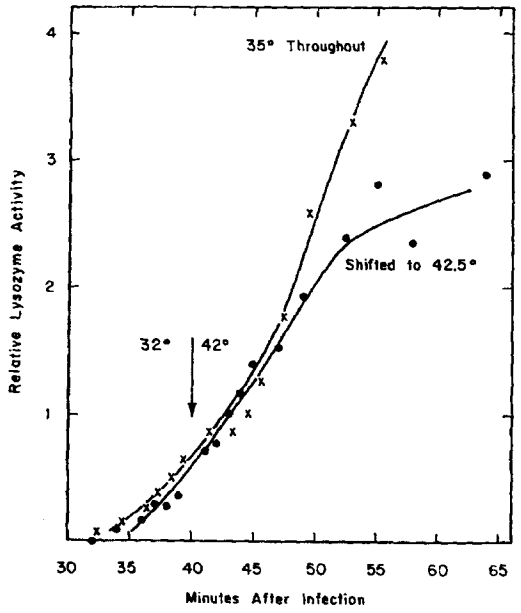

FIG. 1. Lysozyme synthesis in cells infected with N ts8 with and without a shift to 42.5°. Strain C600 was grown in broth medium to 2×10^8/ml, then resuspended in 4 ml 0.01 *M* $MgSO_4$, and λ tsN_8 (pelleted from a lysate to remove lysozyme) was added to give a moi of 5. After 15 minutes, the cells were resuspended in 100 ml broth at 2×10^8, divided into equal portions, and grown at 35°. At the indicated times, 2.5 ml were withdrawn into iced tubes. These were later sonicated and assayed for lysozyme (7). At the time of the temperature shift, an appropriate volume of broth at 62° was added to one flask to give a final temperature of 42.5° and this flask was transferred to a 42.5° water bath. The control flask received an identical volume of 35° broth at the same time. Activities have been corrected for these dilutions.

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creased within 3 minutes after inactivating N, as is seen when initiation of late messenger synthesis is blocked with rifampicin (*7*). If N protein were to be inactivated only slowly after shifting to the restrictive temperature, or if several minutes were required for the complete inactivation of all N protein, then lysozyme synthesis could continue for some time before shutting off. Such a slow inactivation does not seem likely in our case, however, for even at 37°, the wild-type N product has a half-life of 5 minutes (*8*). The temperature-sensitive N protein ought to have a very much shorter half-life at 42.5°.

A control experiment was performed to demonstrate that λNts at 35° behaves like wild-type λ. We find the synthesis of lysozyme by wild-type λ to be within 50% of the synthesis by λNts at 35°. The interpretation of the results of Fig. 1 might also be obscured if either the rate of lysozyme synthesis by wild-type λ at the higher temperature (42.5°) were much greater than its rate at 35° (since the λNts lysozyme rate was essentially the same at 35° and 42.5°, respectively) or if lysozyme decayed appreciably at the higher temperature. By extending the control experiment with wild-type λ to include a temperature shift to 42.5, we found that its apparent rate of lysozyme synthesis at 42.5° is within 25% of its rate if the infected cells had been maintained at 35° throughout. Therefore, either wild-type lysozyme synthesis at 42.5° is not significantly greater than its rate at 35°, or any increase in its rate is compensated for by an increased loss of lysozyme activity at the higher temperature.

In conclusion, lysozyme synthesis continues for 10 minutes after shifting a culture infected with N ts8 from 35° to 42.5°. This implies that N product is not directly required for late messenger or protein synthesis and that Q product may be relatively stable.

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