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Control of Bacteriophage-induced Enzyme Synthesis in Cells Infected with a Temperature-sensitive Mutant

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The timing of "early" and "late" protein synthesis in *Escherichia coli* infected with T-even bacteriophage was studied with a temperature-sensitive phage mutant, T4 *ts*L13. This strain was completely unable to direct the synthesis of phage deoxyribonucleic acid (DNA) at 44 C because it makes a deoxycytidylate hydroxymethylase which cannot act at that temperature. However, the mutant did multiply normally at 30 C. No detectable formation of the late protein, lysozyme, occurred at 44 C, in agreement with the idea, proposed by several workers, that DNA replication is necessary for activation of late genetic functions. However, the formation of an early enzyme, thymidylate synthetase, was shut off at about 10 min, as in normal infection. This implied that separate mechanisms were responsible for cessation of early functions and activation of late ones. That the infected cell at 44 C retained the capacity for synthesis of early enzymes was shown by the fact that DNA synthesis occurred after a culture was transferred from 44 to 30 C as late as 30 min after infection. This synthesis was inhibited by chloramphenicol, indicating that de novo synthesis of an early enzyme can take place at a late period in development. It is suggested that cells infected under normal conditions maintained an appreciable rate of early enzyme synthesis throughout the course of infection.

Proteins synthesized in *Escherichia coli* infected with T-even bacteriophages fall into two categories, based upon their time of appearance in the infected cell: the so-called "early" proteins, which are mostly enzymes of deoxyribonucleic acid (DNA) metabolism, and which appear during the first 10 min after infection, and the "late" proteins, including the virus structural proteins and lysozyme, which are formed after 10 min. The mechanism by which the infected cell shuts off the synthesis of early proteins and initiates the formation of late proteins has been the object of numerous studies [for references, see Mathews (9)]. Evidence has been presented which suggests a causal relationship between the onset of phage DNA synthesis, which begins at about 10 min and the shift from early to late protein synthesis. When cells are infected under conditions which do not permit DNA synthesis, the formation of early enzymes is exaggerated and extended, and the formation of late proteins does not occur (4, 9, 11, 12, 15). Most of this work has involved ultraviolet light-inactivated phages, or amber mutants which are blocked in DNA synthesis.

To quantitatively explore the relationship between DNA synthesis and the control of phage protein synthesis, it would be helpful to have a phage-host system in which DNA synthesis can be blocked and restored at will in one culture. Temperature-sensitive mutants which are blocked in DNA synthesis present this opportunity, because the temperature of a culture can be adjusted so that DNA synthesis is either inhibited or permitted. This paper describes experiments carried out with T4 *ts*L13 (5), a mutant which grows normally at 30 C, but which cannot replicate at 44 C because it directs the synthesis of a deoxycytidylate hydroxymethylase which cannot function at the higher temperature (14).

MATERIALS AND METHODS

E. coli B was used as the host bacterium for all experiments reported here. Phage T4BO$_1$, obtained from I. Tessman, was used as the wild-type control, and is referred to herein as *ts*$^+$. T4 *ts* mutants LB-1, LB-3, and L13 were supplied by R. S. Edgar. Preliminary experiments showed that L13 was most severely blocked in DNA synthesis at 44 C, and this mutant was chosen for further study.

Growth conditions for bacteria, preparation of

extracts, and enzyme assays were as previously reported (9). In all experiments bacteria were grown at 37 C and infected at the temperature indicated in the figure legends. Stocks of *ts*L13 were prepared by growing bacteria at 37 C and infecting at 30 C. In addition, deoxycytidylate (dCMP) hydroxymethylase assays were carried out at 30 C rather than at 37 C.

DNA synthesis was measured as previously described (9). This method involves growth of bacteria in 20 μg of uracil per ml, to repress the pyrimidine biosynthetic pathway. Immediately after infection, a small amount of uracil-2-^{14}C was added to each culture, and the amount of radioactivity incorporated into DNA was followed. Infection was carried out at a multiplicity of about eight phage per bacterium, so that a negligibly small fraction of the cells (less than 0.1%) remained uninfected. Thus, any label incorporated represented synthesis of phage DNA. Since the possibility existed that unlabeled nucleotides arising from the degradation of bacterial DNA might be incorporated into phage DNA in preference to the labeled pyrimidine pool and not be detected by this method, the kinetics of uracil incorporation into bacterial DNA were followed in an uninfected culture. The rate of uracil incorporation was maximal within 6 min after addition of the isotope (Fig. 1). If one assumes that the same enzymes are present and active in the cell immediately after infection, it is most improbable that unlabeled nucleotides would be preferentially incorporated into DNA, because degradation of DNA to acid-soluble products is not observed before 10 min after infection (9).

RESULTS

The kinetics of DNA synthesis in cells infected by L13 and ts^+ at 30 and 44 C are shown in Fig. 2. DNA synthesis was undetectable in the L13-infected culture at 44 C, and was somewhat less in the ts^+-infected cells at 44 C than those at 30 C. The latter result indicated that infected cells suffered some effect of the higher temperature other than those resulting from the specific genetic lesion under study. However, ts^+ can complete a normal cycle of replication at 44 C, for the plating efficiencies of ts^+ at 30 and 44 C are equal. The plating efficiency of L13 at 44 relative to 30 C is about 6×10^{-5}

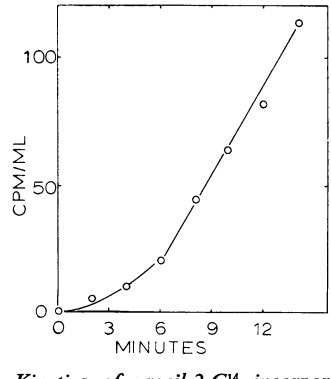

FIG. 1. *Kinetics of uracil-2-C^{14} incorporation into bacterial DNA. To a 12-ml culture of Escherichia coli B, grown in minimal medium plus 20 μg of uracil-^{12}C per ml, was added 0.04 μc of uracil-^{14}C, at time zero. Incorporation of radiactivity into DNA was followed as previously described (9).*

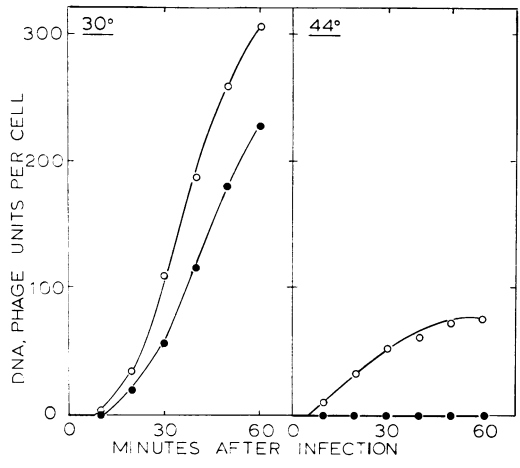

FIG. 2. *DNA synthesis in Escherichia coli B infected with T4 ts^+ (○) and L13 (●). DNA synthesis was followed by incorporation of uracil-2-^{14}C.*

Figure 3 shows the patterns of phage-induced enzyme synthesis in L13-infected cultures at 30 and 44 C and in a ts^+-infected culture at 44 C. The formation of the early enzyme, thymidylate synthetase, was shut off at about 10 to 15 min in all cases, as in a normal infection at 37 C. However, the late enzyme, lysozyme, was not formed in detectable quantities in L13-infected cells at 44 C, although a significant amount of the enzyme was made at that temperature by cells infected with the wild-type strain.

That the failure to observe lysozyme synthesis with L13 at 44 C was not due simply to a generalized cessation of protein synthesis is shown by the experiment of Fig. 4. In this experiment, protein synthesis was determined by following the incorporation of ^{14}C-leucine into acid-insoluble material. Although the rate diminished at late periods in L13-infected cells at 44 C, significant incorporation was observed as late as 60 min after infection.

Another point of interest (Fig. 3) was that L13 was capable of making dCMP hydroxymethylase at both 30 and 44 C. It was apparent, however, that the enzyme was completely nonfunctional at 44 C, since DNA synthesis was undetectable. Moreover, the enzyme was unstable at 44 C, since activity determined in extracts of L13-infected cultures at 44 C decreased to zero late in

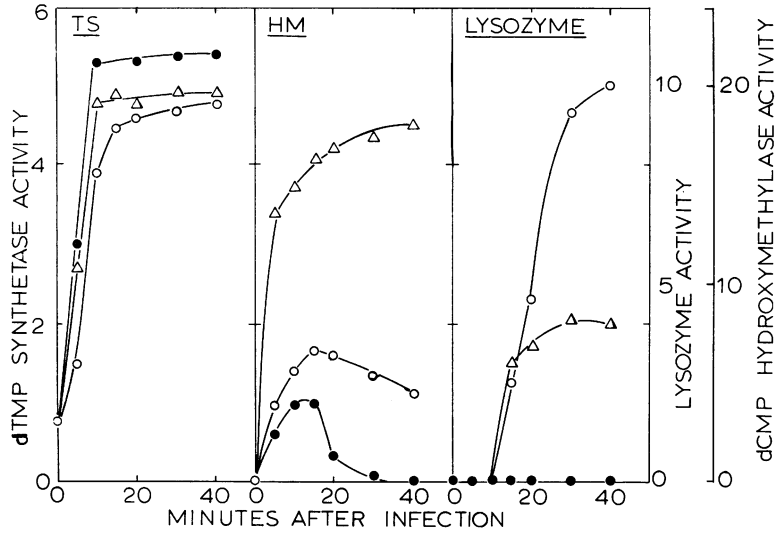

FIG. 3. *Phage-specific enzyme formation in Escherichia coli B infected with L13 at 30 C* (○), *L13 at 44 C* (●), *and ts⁺ at 44 C* (△). *TS, thymidylate synthetase; HM, deoxycytidylate hydroxymethylase.*

infection. These results are in accord with the studies of Wiberg and Buchanan (14). These authors found that dCMP hydroxymethylase extracted from cells infected with L13 at 30 C was inactive at 44 C. However, they did not test L13-infected cultures a 44 C to determine whether an enzyme was formed which could be detected at 30 C

The low activity of the hydroxymethylase extracted from L13-infected cells at 30 C, compared with that obtained from *ts*⁺-infected cells at 30 C, suggested that, in the L13 cultures at 30 C, dCMP hydroxymethylase activity was the rate-determining step for DNA synthesis. This could, in turn, account for the fact, seen in Fig. 2, that DNA synthesis was slower at 30 C with L13 than with *ts*⁺

An interesting feature of kinetic curves for the time course of synthesis of early enzymes is that, once maximal activity is achieved, at about 10 to 15 min after infection, the specific activity does not decline appreciably later in infection. If early enzyme formation were completely shut off at 10 to 15 min, then one would expect the specific activity to decrease at later times, for the rate of overall protein synthesis does not decrease notably, under our conditions, for at least 60 min [see Mathews (9)]. This suggests that early enzyme formation does proceed, albeit at a greatly decreased rate, throughout the entire course of infection. The L13 system presented an opportunity to test this idea. As shown in Fig. 2, L13 was incapable of initiating DNA synthesis at 44 C. However, we found that, when such a culture was transferred from 44 to 30 C as late as 40 min after infection, some DNA synthesis occurred following the transfer. Figure 5 shows the results of experiments in which cultures were switched from 44 to 30 C at 20 and at 30 min. In both cases, significant restoration of DNA synthesis was observed. This restoration was partially, but not completely, inhibited by chloramphenicol. This suggested that there are two aspects to this initiation of DNA synthesis: (i) the reactivation, or prevention of inactivation, of previously formed dCMP hydroxymethylase; and (ii) the synthesis of new enzyme molecules de novo. It should be observed that the concentration of chloramphenicol was well in excess of a concentration

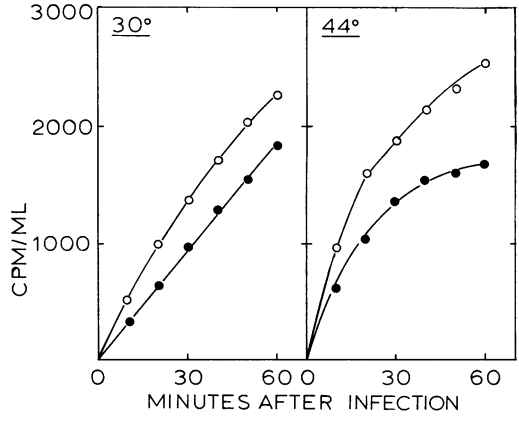

FIG. 4. *Incorporation of leucine-¹⁴C into protein in Escherichia coli B infected with ts⁺* (○) *and L13* (●).

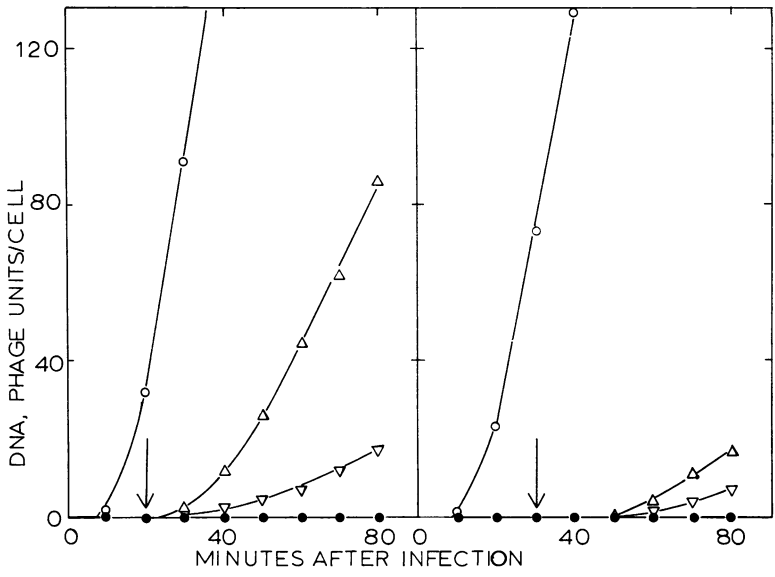

FIG. 5. *DNA synthesis in L13-infected cultures after transfer from 44 to 30 C. In the left-hand portion of the figure, transfer was carried out at 20 min after infection; in the right-hand part, 30 min. Symbols for both graphs:* ○, *control culture kept at 30 C;* ●, *control culture kept at 44 C;* △, *culture transferred from 44 to 30 C at the indicated time;* ▽, *cultures transferred at the indicated times, but 50 μg of chloramphenicol per ml added 2 min before transfer.*

previously shown (10) to inhibit early enzyme synthesis completely. Moreover, similar results were obtained when puromycin was used to block protein synthesis.

DISCUSSION

The present experiments indicate two general features of the regulation of phage-specific protein synthesis which have not previously been reported in detail. (i) Whereas the activation of late protein synthesis is dependent upon the replication of DNA, the cessation of early protein synthesis is not replication-dependent. (ii) Although the control of synthesis of late proteins early in infection is absolute, in that no late proteins are formed before about 10 min, the reverse is not true; i.e., early proteins can be formed, although at a reduced rate, late in infection. Both of these findings are in accord with previously published work, both from this laboratory and from others.

Regarding the first statement above, Mathews (9) showed that when DNA synthesis is limited, by deprivation of thymine in a culture of thymine-requiring bacteria and phage, normal shutoff of early enzyme formation is observed, although there is a delay in the activation of lysozyme synthesis. This suggests that one can observe a dissociation of the functions controlling the shutoff of early protein synthesis and the activation of late protein synthesis. The present studies confirm this. It would be of interest to study late protein synthesis in the L13 system by use of the sensitive fluorescent-antibody technique developed by Sercarz (12), who showed that, in cells infected with amber mutants blocked in DNA synthesis, the inhibition of formation of viral antigens was complete.

The results described here and in our previous paper (9) suggest that the genetic information for synthesis of late proteins comes only from newly synthesized DNA. It was estimated (9) that between 5 and 10 phage equivalents of DNA per cell must be formed before the lysozyme gene can be fully expressed. If input DNA alone could direct the synthesis of lysozyme late in infection, then one might expect detectable levels of the enzyme in the absence of DNA synthesis if infection were carried out at high multiplicity. However, in experiments with the L13 system at 44 C and with amber mutants completely blocked in DNA synthesis (9), we have been unable to detect any lysozyme formation in cultures infected at multiplicities as high as 20.

In interpreting these results, it must be stressed that not all phage-specific proteins fall into the "early" and "late" categories described here. Internal protein, for example, is synthesized throughout the course of infection (2), and the synthesis of dihydrofolate reductase by T4 has been shown to be controlled by a different control mechanism in comparison with the other

early enzymes (9, 13). In addition, the formation of ribonucleotide reductase has been reported to occur later than the early proteins but earlier than the late ones (3). However, the majority of phage proteins thus far identified do seem to fit the overall pattern of early or late formation, and it appears reasonable, therefore, to seek a general mechanism which could account for this pattern.

The present picture of the regulation of protein synthesis in T-even, phage-infected cells suggests that there are mechanisms controlling both transcription and translation. Hall, Nygaard, and Green (8) have shown that both early and late phage messenger ribonucleic acid (mRNA) molecules are present in T2-infected cells late in infection. Edlin (6) has proposed a model which assumes that late mRNA is more stable, and more securely bound to ribosomes, than early mRNA. This model predicts that there will be a low level of early enzyme synthesis late in infection, a prediction which is confirmed in the present study.

Some translational control is expressed early in infection as well, as demonstrated by Bautz et al. (1), who showed that the lysozyme gene is transcribed once very early in infection. Translation of the resultant mRNA, however, does not occur, and transcription of the gene does not resume until several minutes later. The activation of late-function genes may be causally related to a physical change involved in DNA replication. Frankel (7) has described a very large molecular species of DNA which is found in the replicating pool of T4. This species does not occur in cells infected with amber mutants which are blocked in DNA synthesis. It is possible that this species must be formed for continuous transcription of late genes to occur. In addition, the formation of the large molecule may be a multistep process, which can begin but not be completed in the absence of DNA synthesis. This could, in turn, account for the observed dissociation between the shutoff of early enzyme synthesis and the activation of late protein formation. In this context, it would be valuable to know whether late mRNA can be formed in the absence of DNA synthesis. Experiments designed to answer this question are now in progress.

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