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Requirement of a Phage-Induced 5′-Exonuclease for the Expression of Late Genes of Bacteriophage T5

(electrophoresis/transcription/"late protein" synthesis)

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ABSTRACT **Amber mutants of bacteriophage T5 defective in gene *D15*, which codes for a 5′-exonuclease, do not express late genes. Electrophoretic separation in sodium dodecyl sulfate–polyacrylamide gels of the proteins induced by this mutant in nonpermissive *Escherichia coli* show a virtual absence of late proteins. Synthesis of lysozyme and serum-blocking power is very low whereas the extent of synthesis of an early enzyme, deoxyribonucleoside monophosphokinase, is similar to that in wild-type infections. It is proposed that one requirement for the expression of late T5 genes is the introduction of gaps or nicks in the T5 DNA so that late transcription can occur.**

After infection of permissive cells of *Escherichia coli* by bacteriophage T5, the expression of the phage genome is controlled in a well-defined temporal sequence. Three classes of phage-specific proteins are synthesized in three distinct time periods (1). "Pre-early" proteins are synthesized from about 1 to 8 min after infection at 37°, and are coded for by the 8% segment of T5 DNA that is initially transferred to host cells. The mechanism for shutting off the synthesis of pre-early proteins is encoded in this initial 8% DNA segment (1) and is carried out by a protein coded for by gene *A1* (2, 3). "Early" proteins are synthesized from about 5 min until 20 min after infection, at which time their rate of synthesis starts to decrease considerably. The delay in the turn-on of synthesis of early proteins is due to the unusual mode of transfer of T5 DNA. As stated above, an initial 8% DNA segment is transferred to host cells first, and only after certain pre-early proteins have been synthesized (4, 5) can the remaining 92% of the phage DNA, which carries early as well as "late" genes, be transferred. Hence there is an obligatory delay between the onset of synthesis of pre-early and of early proteins. "Late" proteins are synthesized from about 13 min after infection until lysis at about 45 min. Moyer and Buchanan (6) showed that the delay in onset of synthesis of late proteins is due to a requirement for early proteins.

In this paper, we identify one of the early genes whose integrity is required for the turn-on of synthesis of late proteins. The product of this gene has been identified as a 5′-exonuclease, which is required for generating genome-sized molecules of T5 DNA from replicating DNA structures of much higher molecular weight (7–9). A possible role that such a nuclease may play in the turn-on of synthesis of late proteins is presented.

MATERIALS AND METHODS

Bacteria and Phage. *E. coli* F, a nonpermissive host (su^-) for amber mutants of T5 (10) was used in this study. All the amber mutants in gene *D15* were isolated and characterized in this laboratory (10, 11).

Growth and Infection of Cells. Bacteria were grown in maleate glucose salts medium (12) and harvested by centrifugation. The cells were washed by resuspension in maleate salts buffer containing 1 mM $CaCl_2$ followed by sedimentation and resuspension at 5×10^9 cells per ml in the same buffer. The concentrated cell suspension was incubated at 37° for 30 min with slight aeration to exhaust residual nutrients. At the end of this starvation period the cells were infected with an average of 5–6 phages per bacterium. After 15 min at 37° for adsorption of the phage, the suspension was diluted 1:10 into maleate glucose salts medium containing 1 mM $CaCl_2$ and aerated vigorously at 37°. Phage growth is initiated at the time of this dilution, which is designated "zero time" for each infection.

Pulse-Labeling of Proteins for Electrophoresis. Proteins were pulse-labeled essentially as described by McCorquodale and Buchanan (1), except that in the present studies the proteins were labeled with ^{14}C-labeled reconstituted protein hydrolysate (0.2 μCi/ml). Pulse-labeled proteins were prepared for electrophoresis as described by Studier (14).

Electrophoresis and Autoradiography. Electrophoresis of radioactive proteins was performed in Na dodecyl sulfate–polyacrylamide gel slabs (13–15). The composition of the separating gel was similar to that of Laemmli (16). Electrophoresis of proteins was carried out in 10% gels for 8–10 hr at 10 mA. The slabs were fixed with 12.5% trichloroacetic acid for 30 min and stained overnight with RDS-L Coomassie blue protein stain concentrate. Destaining was carried out by diffusion into 7.5% acetic acid in 5% methanol. Drying of gels and autoradiography were performed as described (17).

Enzyme Assays. Lysozyme activity was determined as described by Sekiguchi and Cohen (19). 1 Unit of lysozyme is the amount of enzyme that gives $\Delta A_{450\ nm}/min = 0.01$. Deoxyribonucleoside monophosphokinase was assayed according to the method of Fessler *et al.* (20), as modified by McCorquodale and Buchanan (1). 1 Unit of deoxyribonucleoside monophosphokinase is defined as the amount of enzyme catalyzing the conversion of 1 μmol of deoxyribonucleoside 5′-monophosphate per hour.

Estimation of Serum-Blocking Power. Serum-blocking power was measured using antiserum prepared against $T5^+$ (k = 100–500 min^{-1}) and using T5 as the tester phage according to the method of DeMars (18). The results are expressed as phage equivalents per ml of phage-infected culture by comparing with a standard curve prepared against T5 *A1 · amH27* in su^- cells.

Measurement of Incorporation of [^{14}C]Leucine into Acid-Insoluble Protein. 2-ml Samples of phage–bacterium complexes in maleate salts buffer containing 1 mM $CaCl_2$ were removed at various intervals and rapidly mixed with 0.2 ml of warmed (37°) maleate glucose salts medium containing 1 mM $CaCl_2$ and 0.25 μCi (10 μg) of [^{14}C]leucine. After incubation for 2 min at 37°, 2 ml of cold 15% trichloroacetic acid, and 0.05 ml of 0.5% serum albumin were added. The acid-insoluble proteins were collected on a glass-fiber filter (Reeve Angel, Grade 984 H) and washed with 10 ml of 5% trichloroacetic

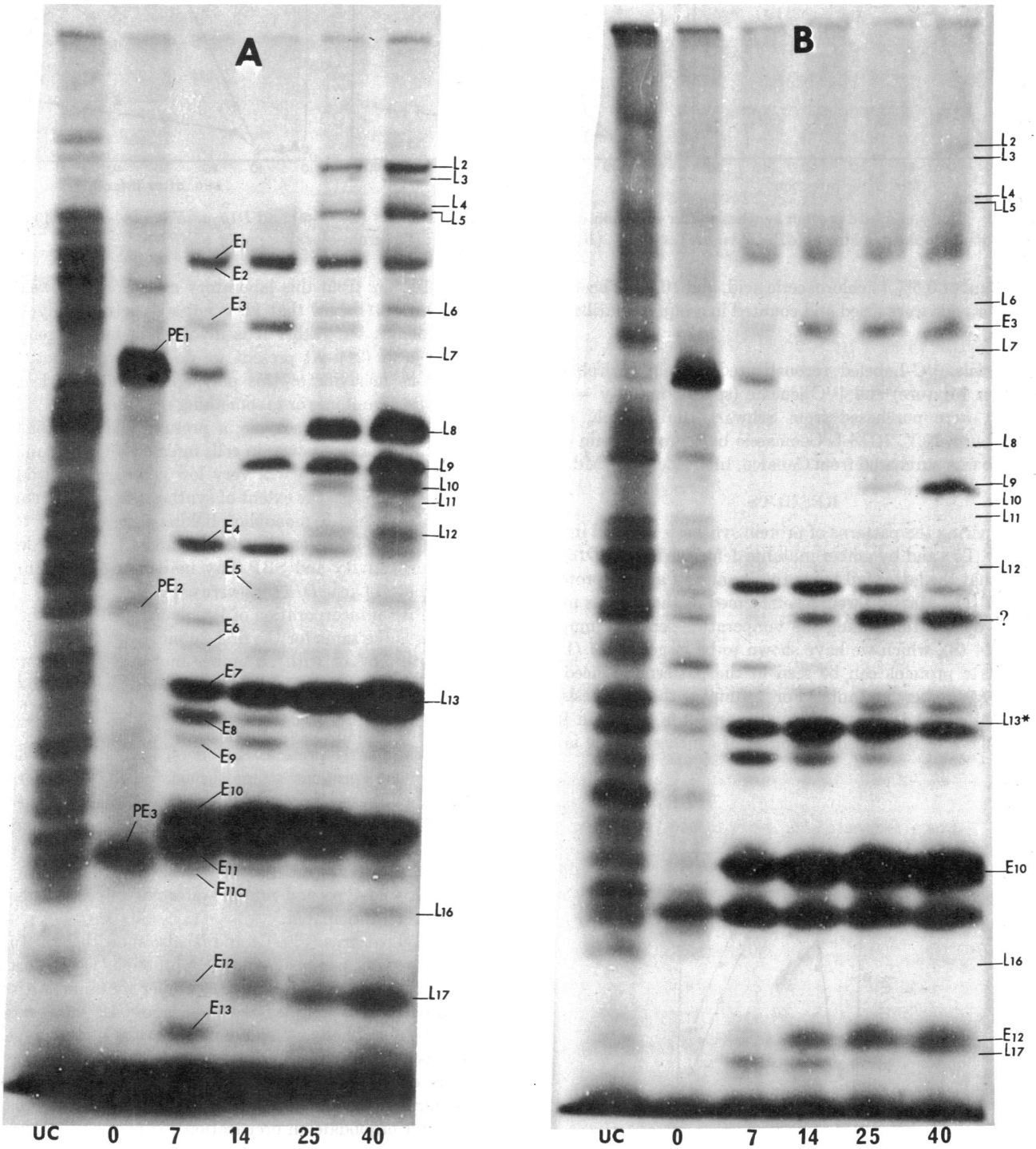

FIG. 1. Autoradiograms of pulse-labeled polypeptides from *E. coli* F (su$^-$) infected with T5 after separation in 10% Na dodecyl sulfate–polyacrylamide gels: (*A*) T5 wild type; (B) *D15·amH50. Numbers* below the autoradiograms indicate the times when 4-min pulses were begun. The designations *UC*, *PE*, *E*, and *L* represent uninfected cells, pre-early polypeptides, early polypeptides, and late polypeptides, respectively. The complete designations of the various polypeptides will be made elsewhere (Chinnadurai and McCorquodale, in preparation). Similar results have been obtained with *D15·amH8* and *D15·amH33a*. [* Polypeptide L13 is completely absent in cells infected with the amber mutant in gene *D15*. The band seen during late times corresponding to that position is E7. These two bands, which migrate together, can be resolved in 15% gel (results not shown). Similarly, the absence of L17 was also confirmed in 15% gels.]

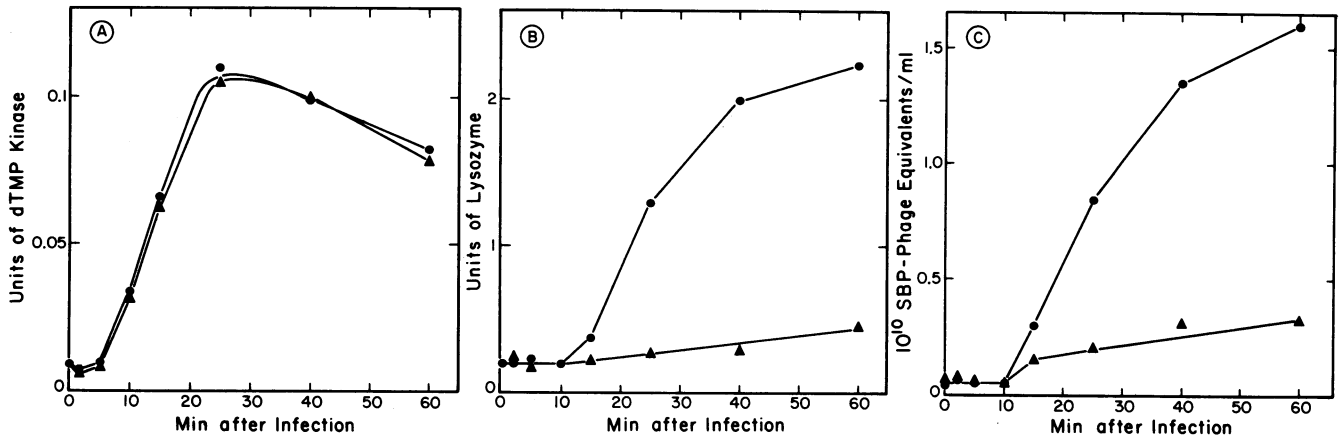

FIG. 2. Early and late protein synthesis after infection of *E. coli* F (su^-) with $T5^+$ (●——●) and *D15·amH50* (▲——▲). (*A*) deoxynucleoside monophosphokinase (*dTMP kinase*) activity; (*B*) lysozyme activity; (*C*) serum-blocking power (*SBP*).

acid, 10 ml of 0.5% trichloroacetic acid, and 10 ml of acetone. The filter pads were dried and counted in a liquid scintillation counter.

Materials. ^{14}C-Labeled reconstituted protein hydrolysate (Schwarz Mixture) and [^{14}C]leucine (specific activity = 312 Ci/mol) were purchased from Schwarz BioResearch, Inc., Orangeburg, N.Y. RDS-L Coomassie blue protein stain concentrate was purchased from Canalco, Inc., Rockville, Md.

RESULTS

In comparing the patterns of protein synthesis induced in su^- cells by $T5^+$ and by amber mutants defective in gene *D15*, we noted that the latter fail to synthesize almost all late proteins (Fig. 1) [Dr. Martin Zweig has informed us that he has made similar observations using the temperature-sensitive mutant HA70R4 (8), which we have shown to be in gene *D15* (11)]. Some late proteins can be seen in the patterns induced by gene-*D15* amber mutants. For example, the synthesis of polypeptide L9 is observed, but in amounts less than in a wild-type infection. Also, the onset of synthesis of L9 is delayed. Results from this laboratory and those of Zweig and Cummings (21) show that L9 is not a structural polypeptide of the phage, but rather it is involved in morphogenesis of the T5 head (21). At present we do not know whether the anomalous behavior of this polypeptide is due to leakiness of our amber mutants or to something else.

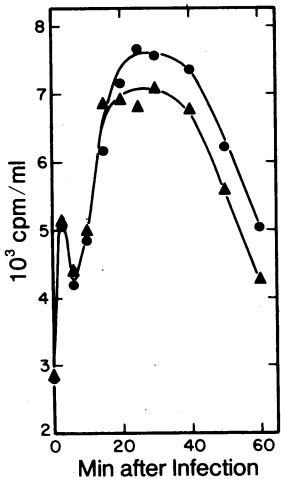

FIG. 3. Rate of incorporation of [^{14}C]leucine into acid-insoluble proteins in su^- cells infected with $T5^+$ (●——●) and with *D15·amH50* (▲——▲).

The synthesis of lysozyme, a presumed late protein (22), and serum-blocking power in cells infected by amber mutants defective in gene *D15* was very low compared to wild-type infections, whereas the extent of synthesis of an early enzyme, deoxyribonucleoside monophosphokinase, was similar to that of a wild-type infection (Fig. 2*A*, *B*, and *C*). The low level of lysozyme activity and SBP may be due to the leakiness of the mutants of gene *D15* that were used.

The rate of incorporation of [^{14}C]leucine into acid-insoluble proteins is the same for the first 20 min after infection of su^- cells with $T5^+$ or with an amber mutant defective in gene *D15*. After 20 min, the rate in cells infected with the *D15* mutant is somewhat less than in cells infected with $T5^+$ (Fig. 3). This difference in rates is not great but is reproducible. If the synthesis of early proteins is shut off normally and the synthesis of late proteins is not turned on, the overall rate of protein synthesis during the late period should decrease considerably. However, since the synthesis of three early proteins (E3, E10, and E12) continues through the late period at undiminished rates (see below), the observed difference in rates between wild-type and mutant-infected cells is not as great as might be anticipated.

The gel patterns (Fig. 1*A* and *B*) also show that the turn-on and shut-off of pre-early and early proteins (1) are normal in su^- cells infected with gene-*D15* amber mutants, with three exceptions. Polypeptides E3, E10, and E12 show extended periods of synthesis. We have recently identified polypeptide E10 as the product of gene *D5*, and it plays a dual role in phage development. It is required for the synthesis of DNA and for the shut-off of synthesis of early proteins (Chinnadurai and McCorquodale, in preparation). It may be that the shut-off of synthesis of this early polypeptide, as well as E3 and E12, is effected by the synthesis of late proteins.

One polypeptide, indicated by the question mark in Fig. 1, is synthesized in su^- cells infected with amber mutant *D15·amH50*, but is not synthesized in cells infected with $T5^+$. We do not know if it is an NH_2-terminal fragment of the polypeptide coded by gene *D15*, since we have been unable to de-

termine the location in our gels of the intact polypeptide coded by this gene.

DISCUSSION

Our results demonstrate that the product of gene *D15*, a 5′-exonuclease (7, 8), is necessary for the proper turn-on of synthesis of late proteins. We believe that this turn-on is regulated at the level of transcription, since the synthesis of the three classes of proteins specified by T5 (1) follows the synthesis of three corresponding classes of T5-specific RNA (6, 23).

We know that transcription of late genes can occur without replication of T5 DNA that carries a functional gene *D15* (24). However, the transcription of late genes from unreplicated T5 DNA may require a modification of the input parental DNA by the 5′-exonuclease specified by gene *D15*. A clue to the nature of this modification is provided by the work of Carrington and Lunt (9), who show that high-molecular-weight replicating T5 DNA and mature-sized T5 DNA, excised from the replicating DNA, differ from DNA isolated from T5 particles in that they both bind strongly to nitrocellulose powder whereas mature T5 DNA does not. Nitrocellulose powder binds single-stranded DNA but not double-stranded DNA. It may be that one function of the 5′-exonuclease is to produce single-stranded regions in T5 DNA which, in turn, are necessary for transcription of late T5 genes. The 5′-exonuclease is also necessary for the excision of mature-sized T5 DNA from replicating T5 DNA, which is probably in the form of concatemers (9). The mature-sized T5 DNA excised from such concatemers still contains regions of single-stranded DNA, but these regions must disappear as the DNA is matured and packaged into heads. These observations imply that replicating concatemers of T5 DNA, newly excised genome lengths of T5 DNA, and unreplicated parental T5 DNA can all be utilized for the transcription of late genes as long as a functional gene *D15* is present and is expressed.

In addition to our speculation that single-stranded regions in T5 DNA are required for late T5 transcription, it may be that the transcription of some late T5 genes requires only a nick rather than a gap. It is possible that the T5-induced exonuclease could introduce such nicks even though Frenkel and Richardson (8) reported that it possesses no detectable endonuclease activity *in vitro*. However, they do suggest that, *in vivo*, it may possess a highly specific endonuclease activity not yet detected.

A suggestion that combines these two hypotheses is that the exonuclease may only be necessary to prevent repair by a ligase of specific single-strand nicks that are introduced by this nuclease or by another enzyme (8). The nicks, by this suggestion, are sufficient to permit late transcription.

The requirements suggested here for late T5 transcription are similar, in principle, to those for late T4 transcription. Late T4 transcription requires a "state" of the DNA different from the state of parental DNA (25). The two systems may differ, however, in the detailed state required for late transcription. For example, late T4 transcription appears to require only single-strand nicks (26) whereas late T5 transcription may require single-strand gaps as well. Also, late T4 transcription normally requires continuous T4 DNA replication (26–29) whereas late T5 transcription does not (24).

A defect in gene *D15* appears to have no effect on the shut-off of synthesis of early proteins. They continue to be synthesized during late times, but at a decreased rate, just as occurs in cells infected with wild-type phage. Three exceptions are polypeptides E3, E10, and E12, which show extended periods of synthesis (see *Results*). These findings suggest an active, albeit sluggish, mechanism for the shut-off of synthesis of early T5 proteins.

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