Control of Gene Expression in Bacteriophage T7: Transcriptional Controls

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Received September 2, 1974

Summary. Two transcriptional control mechanisms of T7 can be distinguished both affecting the transcription by E. coli RNA polymerase: An early control and an "early-late" control. In wild type infections, both transcriptional control proteins appear at approximately the same time. Mutations in the early control gene have, therefore, little effect on transcription, if tested in the presence of virus RNA polymerase. Using mutants in T7 RNA polymerase, the appearance of the "early-late" control is delayed. Then, the effect of the early control gene is dramatic, its deficiency leading to an overproduction of host and early T7 RNA. The early RNA control appears to be exerted by the T7 protein kinase, the "early-late" control protein is most likely identical with the transcriptional inhibitor, which has been isolated and purified (Ponta et al., 1974). Both control proteins inhibit the initiation of RNA synthesis by E. coli RNA polymerase.

Introduction

Infection of E. coli with mutants of T7 that do not induce virus specific RNA polymerase led to a dramatic decrease of total RNA synthesis (Brunovsky and Summers, 1972). T7 mutants carrying a deletion to the left of the T7 polymerase (T7 pol) gene in addition to the defect in the RNA polymerase were blocked in this RNA shutoff (Studier, 1972; Brunovsky and Summers, 1972; Simon and Studier, 1973). Thereby the defect in RNA shutoff was correlated to a gene which was later shown to code for a virus specific protein kinase (Rahmsdorf et al., 1973, 1974). This suggested a direct involvement of the protein kinase in gene expression.

Here we describe the physiology of protein kinase negative mutants and demonstrate the existence of at least two independent RNA control mechanisms.

Materials and Methods

Bacterial and Phage Strains. T7 am193 (T7 Pol+); T7 am342,H3 (T7 Pol-, protein kinase+); T7 am342, LG26 (T7 Pol-, ligase-, termination-) were obtained from Dr. F. W. Studier, Brookhaven; T7 H280; T7 H207 and T7 H13 (all T7 Pol-) were obtained from Dr. R. Hausmann, Freiburg. Most experiments were performed with the isogenic strains: T7 am193 (T7 Pol-, protein kinase+), T7 am193 K- (T7 Pol-, protein kinase-), T7 am+K+ and T7 am*K-. T7 am193 K- was isolated by crossing T7 am342+,H3 with T7 am193. The T7 Pol+ strains are the corresponding amber+ revertants.

E. coli 011 is an amber su+ derivative of E. coli B from Dr. F. W. Studier; E. coli BB/1W is an amber su+ derivative of E. coli B from Dr. R. Hausmann. E. coli Bp-1 is amber non-permissive and defective in dark-repair (Hill, 1958). E. coli XA7007 carries the suA marker and is amber nonpermissive (obtained from Dr. J. Beckwith, Boston).

Reagents. The following biochemicals were purchased from Boehringer, Mannheim: egg white lysozyme, uridine and nucleoside triphosphates. Sodium dodecyl sulfate (SDS) was
obtained from BioRad, Richmond; histone type IIA from Sigma, St. Louis; electrophoretically purified deoxyribonuclease from Worthington, Freehold; Brij 58 from Serva, Heidelberg; β-mercaptoethanol and CsCl from Merck, Darmstadt; rifampicin from Calbiochem, San Diego; nalidixic acid from Winthrop, Newcastle upon Tyne; polyethylene glycol 6000 from Hoechst, Frankfurt. The radioactive compounds were purchased from Radiochemicals, Amersham: 14C protein hydrolysate, 14C uridine, 32P phosphate and γ32P ATP.

**Media.** M9 medium (6 g Na3HPO4, 3 g KH2PO4, 0.5 g NaCl, 1 g NH4Cl per liter) was usually supplemented with 10-4 M MgSO4 and 1% glucose. K medium is M9 minimal medium supplemented with 1.5% casamino acids, 0.05% NaCl, 0.2% glucose, 5 mM MgSO4, 0.25 μg/ml thiamine and 10 μg/ml thymine. TG medium is composed of 0.12 M Tris-HCl pH 7.5, 0.05 M NaCl, 0.02 M KCl, 0.02 M NH4Cl, 0.2 mM CaCl2, 3 mM Na2SO4, 2 μM FeCl3, 0.1 mM MgCl2, 0.25% glycerol and 0.05 mM potassium phosphate pH 7.5.

**Growth and Purification of Phage.** For the growth of phage, *E. coli* 011 or BB1W were cultured in K-medium. At late log phase (OD600 = 0.8-0.9), phage was added at a multiplicity of infection of 1/100. Lysis occurred after additional 2-21/2 hours. NaCl was then added to a concentration of 5% and the debris removed by centrifugation at 16000 g for 10 minutes. Phage were precipitated by 10% polyethylene glycol 6000. The phage were resuspended in 1 M NaCl, 0.01 M Tris-HCl pH 7.5, 0.1 mM MgCl2, 0.25 mM EDTA (Phage buffer), and purified by centrifugation through a preformed CsCl gradient (layers of 1.9, 1.7, 1.5, 1.3 g/cm3; 114 000 g for 60 minutes). Purified phage stocks had an optical density at 260 nm of above 50 and were diluted with phage buffer for further use.

**Phage Gene Nomenclature.** "0.3 RNA" and "0.3 protein" were identified according to Studier (1973). The gene numbers 2-19 correspond to those described by Studier (1969).

**Bacteriophage Crosses** were performed as described by Studier (1969).

**Infection of Cells.** *E. coli* B5-1 or XA7007 were grown at 30° in M9 or TG medium. At cell densities specified, phage was added at a multiplicity of infection of 10. Surviving cells were determined at 2 or 3 minutes. They were below 1%.

**Pulse Label in vivo.** 0.2 ml cultures were pulse-labeled with 5 μCi 14C protein hydrolysate for 2 minutes. The pulse was followed by a 2-minute chase with 1 ml tryptone broth (1% tryptone, 0.5% NaCl). Cells were then harvested on ice and pelleted. The pellets were solubilized with SDS buffer for gel electrophoresis. For RNA synthesis, 30-second pulses with 5 μCi 14C uridine per 0.2 ml aliquot were followed by a chase of 30 seconds with 1 ml M9 medium supplemented with 100 μg/ml uridine. Conditions different from these are described in figures. 32P phosphate was added in some cases at a concentration of 40 μCi/ml.

**UV Irradiation.** In order to suppress host gene expression, *E. coli* B5-1 were irradiated with UV under standard conditions (Hirsch-Kauffmann et al., 1974) for 7 minutes and allowed to shake at 30° for another 10 minutes before infection. Phage were brought to 1.0 OD600/ml by dilution with phage buffer and were placed in glass petri dishes (4 cm diameter, 1.6 ml each sample). Irradiation was allowed under standard conditions. Infection of *E. coli* B5-1 with irradiated phage was done in dim yellow light.

**SDS Polyacrylamide Slab Gel Electrophoresis.** The slab gels were prepared essentially according to Studier (1973).

**Cell-free Protein Synthesis.** The isolation of mRNA from infected cells and the protein synthesizing system have been described earlier (Herrlich and Schweiger, 1974).

**Enzyme Assays.** For the determination of enzyme activity usually aliquots of 2 × 10⁶ cells were harvested at various times after infection by mixing with the same volume of ice-cold M9 medium containing 400 μg/ml chloramphenicol. The cells were then pelleted. The preparation of crude cell extracts for the detection of enzyme activity and the enzyme assays were performed as described (Rahmsdorf et al., 1974; Herrlich and Schweiger, 1974; Herrlich et al., 1971).