Bacteriophage SPO1 DNA- and RNA-Directed Protein Synthesis in vitro: The Effect of TF1, a Template-Selective Transcription Inhibitor

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Summary. Transcription factor one (TF1), a protein synthesized after infection of B. subtilis by phage SPO1, is a specific inhibitor of SPO1 in vitro transcription. In this paper, we investigate the effect of TF1 on SPO1-specific in vitro protein synthesis, using SPO1 DNA or messenger RNA as templates. Protein synthesis is measured by incorporation of radioactive amino acids into acid insoluble form, synthesis of a phage-specific enzymatic activity, and analysis of radioactive polypeptides by acrylamide gel electrophoresis. TF1 is shown to inhibit the DNA-dependent synthesis of all SPO1 proteins which can be made in vitro, but to have no specific effect on RNA-dependent protein synthesis in vitro.

Introduction

Protein and RNA synthesis in cells of B. subtilis, infected by bacteriophage SPO1, proceeds in an ordered manner: any particular molecular species being synthesized for a characteristic portion of the latent period (Gage and Geiduschek, 1971; Levinthal et al., 1967). A template specific inhibitor of SPO1 DNA-dependent RNA synthesis, transcription factor one (TF1), is produced during SPO1 infection (Wilson and Geiduschek, 1969; Johnson and Geiduschek, 1972). In this paper, we investigate the possibility that TF1 may also have a role in the regulation of translation.

E. coli extracts support the synthesis of complete, enzymatically active proteins when supplemented with bacteriophage DNA or m-RNA templates (Gold and Schweiger, 1971). When SPO1 DNA is added to such an extract, a small set of proteins is synthesized. The electrophoretic mobilities of the proteins made in vitro are similar to those of the proteins which are made earliest in a normal SPO1 infection (Shub, in preparation). Transcription of SPO1 DNA by purified E. coli RNA polymerase is similarly restricted, primarily to those RNA species which are made earliest in a normal infection, and subsequently repressed (Geiduschek et al., 1968). Deoxycytidylate deaminase, an enzyme specified by SPO1 but absent from E. coli extracts, is also synthesized in response to several B. subtilis phage DNAs (Schweiger and Gold, 1970). On the other hand, RNA extracted from SPO1-infected bacteria stimulates the production of those

Abbreviations: TF1, transcription factor one; SDS, sodium dodecyl sulfate; CM, chloramphenicol.
proteins that were being made in the cell at the time of extraction of the RNA (Shub, in preparation).

TF1 was purified as an inhibitor of SPO1 DNA-directed RNA synthesis. TF1 binds to SPO1 DNA and titrates sites on DNA rather than RNA polymerase, and its effect is specific for SPO1 and several very closely related phage DNAs (Wilson and Geiduschek, 1969). However, it is possible that the sites on DNA which are recognized by TF1 might also be transcribed into RNA molecules; such sites might be contiguous with, or contained in, coding sequences for protein synthesis. It is, therefore, possible that TF1 might inhibit both the synthesis and the subsequent function of the appropriate m-RNA. We are able to test this possibility directly by comparing the effect of TF1 on cell free protein synthesis stimulated by SPO1 DNA and by RNA extracted from cells infected with SPO1.

Methods

Procedures for in vitro protein synthesis, gel electrophoresis, assay of dCMP deaminase enzyme activity and extraction of bacteriophage-specific nucleic acids are described in the accompanying article (Shub, 1975).

Assay of β-glucosyl Transferase. Assay conditions and units of activity are those of Gold and Schweiger, 1971.

Amino Acid Incorporation. 14C-leucine (50 μCi/μmole) was included in reaction mixtures. After standard incubations, aliquots were diluted into H2O and precipitated with 5% CH3COOH. After heating at 90°C for 30 min, samples were filtered on Whatman glass fiber filter paper (GF/C) and counted in a scintillation counter. Incorporation is expressed as pmols of leucine per 50 μl reaction.

TF1. TF1 was prepared as described elsewhere (Johnson and Geiduschek, 1974) from B. subtilis 168 M infected with wild type SPO1. Cells were collected 20 minutes after infection. The TF1 used for these experiments had been purified to the final phosphocellulose step. It had a specific activity, in the standard in vitro RNA synthesis inhibition assay (Wilson and Geiduschek, 1969) of 175 units of TF1 activity per unit of absorbance at 235 nm.

Results

When SPO1 DNA was used to stimulate the cell free protein synthesizing system, TF1 produced a dramatic inhibition of both amino acid incorporation and dCMP deaminase synthesis (Table 1). T4 DNA-directed protein synthesis, on the other hand, was insensitive to TF1. The highest levels tested produced a 10% stimulation of amino acid incorporation and a 25% inhibition of T4 specific enzyme synthesis. It should be pointed out that at these high levels of TF1, protein from the TF1 preparation is in excess of phage DNA on a weight basis. It can be seen, furthermore, that when SPO1 DNA is the template, inhibition of dCMP deaminase and incorporation of leucine both exhibit the same dependence on TF1 concentration (Fig. 1).

The inhibition of SPO1 DNA-directed protein synthesis is several fold more sensitive to TF1 concentration than is the transcription reaction with purified E. coli RNA polymerase (compare Fig. 1 of this paper with Fig. 2 in Wilson and Geiduschek, 1969) but this is due to the higher concentrations of the interacting components in our study (Johnson, G. G. and Geiduschek, E. P., to be published). The salt concentration in the cell free protein synthesizing system is slightly above that which is optimal (Wilson and Geiduschek, 1969) in the standard assay for TF1 (with SPO1 DNA and purified RNA polymerase). The fact that