TRANSCRIPTION SPECIFICITY OF AN RNA POLYMERASE FRACTION FROM BACTERIOPHAGE SP01-INFECTED *B. SUBTILIS*

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1. Introduction

We have isolated and purified a DNA-dependent RNA polymerase from phage SP01-infected *B. subtilis*. SP01 is a large, virulent virus of *B. subtilis* [1] whose transcription program is shown in fig. 1. Early RNA (*e*, *em*) which appears within the first minute after infection, independently of viral protein synthesis, can be synthesized *in vitro* on native SP01 DNA template by purified *E. coli* or *B. subtilis* RNA polymerase. Middle RNA (m, m_1ℓ, m_2ℓ) also first appears before SP01 DNA replication but the capacity to make this RNA derives from viral gene expression. Late viral RNA (ℓ) appears after the onset of SP01 DNA replication [2–5]. We show here that a RNA polymerase purified from cells 10 min after infection at 37°C directs the synthesis of middle RNA transcriptions *in vitro*.

2. Methods

B. subtilis 168 M (indole⁻) growth, infection with phage SP01, preparation of unlabeled *in vivo* RNA and of viral DNA were done as described previously [2]. RNA polymerase was prepared from disrupted cells (grown in CHT medium [2] with 0.4% nutrient broth and 0.25% yeast extract) 10 min after high multiplicity infection with wild type virus. The method is similar to those previously published by others [6, 7] involving the following steps, in sequence: high speed centrifugation at high ionic strength, $(NH_4)_2SO_4$ fractionation of the supernatant, chromatography on agarose, DEAE cellulose, calf thymus DNA cellulose and phosphocellulose. Details will be published elsewhere. Other methods are indicated in the figure captions.

3. Results and discussion

Three major fractions of RNA polymerase activity were resolved by DNA cellulose chromatography of enzyme from infected cells. One of these fractions was passed over phosphocellulose to remove host initiation factor [6, 7] and loosely associated proteins to yield "phage infected core" which has a relatively high specific activity on phage SP01 DNA. ^{3}H-labeled RNA was synthesized with native SP01 DNA (in excess) by infected "core" polymerase for only 2 min with initiation limited to 30 sec by the addition of rifampicin and was subjected to hybridization competition with *in vivo* RNA (fig. 2). Unlabeled chloramphenicol (CM) RNA (○; *e*, *em*) and RNA isolated 28 min after infection

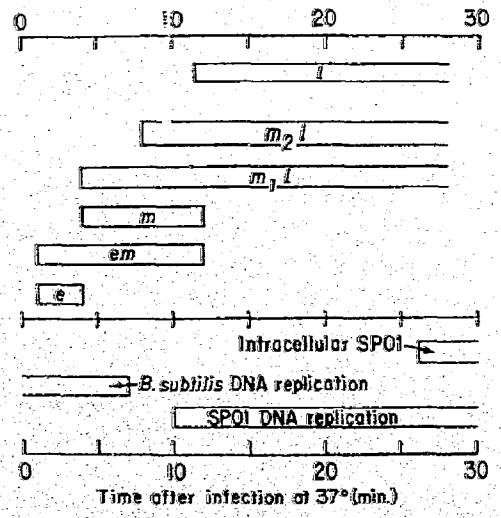

Fig. 1. SP01 transcription program. The time of first appearance and synthesis of viral transcripts is shown above; three cellular events are shown below.

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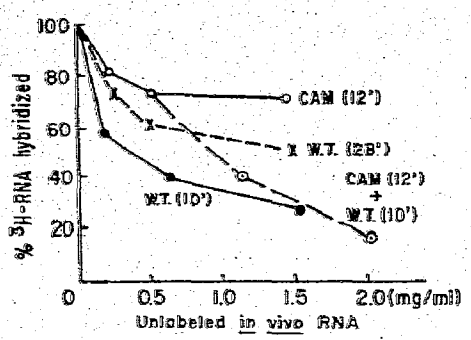

Fig. 2. Hybridization competition of phage core RNA polymerase-directed SP01 RNA. Hybridization of *in vitro* synthesized [^{3}H]RNA to SP01 DNA was competed by unlabeled RNA from SP01 infected *B. subtilis*: (o—o—o) CM (12 min) early RNA; (•—•—•) 10 min middle RNA; (×—×—×) 28 min late RNA (⊙—⊙—⊙) mixed competition, 0.51 mg/ml CM (12 min) RNA with increasing concentrations of 10 min RNA. [^{3}H]RNA was synthesized at 30°C with 180 μg/ml SP01 DNA, 22 μg/ml RNA polymerase, 1 mM each of ATP, GTP and CTP, 0.1 mM [^{3}H]UTP (290 cpm/pmole), 0.1 mM EDTA, 0.1 mM DTT, 0.8 mM spermidine and 100 μg/ml bovine serum albumin which were preincubated 10 min. Synthesis was started by making the sample 10 mM $MgCl_2$; rifampicin (5 μg/ml) was added at 30 sec. After 2 min, the reaction was stopped by adding EDTA and SDS, and the RNA was extracted with phenol. Hybridization competition followed [4], with 0.02 μg/ml [^{3}H]RNA and 10 μg/ml SP01 DNA. Input radioactivity, 798 cpm. Hybridization in absence of competing RNA: 535 cpm. Radioactivity bound to membrane in absence of DNA: 4 cpm.

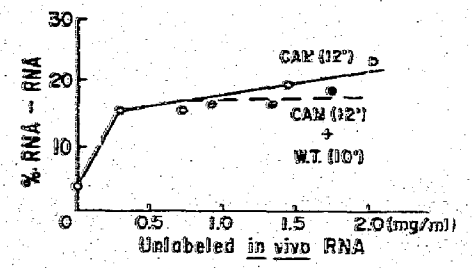

Fig. 3. Anti-messenger content of *in vitro* RNA. RNAase resistance of RNA–RNA duplexes was assayed according to [9]. (o—o—o) CM (12 min) early RNA; (•—•—•) 0.73 mg/ml CM (12 min) and increasing amounts of 10 min RNA; 0.04 μg/ml [^{3}H]RNA synthesized as described in fig. 2 (2.0×10^5 cpm/μg).

Table 1
RNA–RNA duplexes between *in vivo* and *in vitro* RNA.

In vivo RNA	RNAase resistance after hybridization (%)
CM (12 min)	22
10 min	20
28 min	18
none	6

The assay was carried out according to [9] with 1.4–1.5 mg/ml *in vivo* RNA and 0.04 μg/ml *in vitro* [^{3}H]RNA.

(×; $m_2\ell$, ℓ, some $m_1\ell$) compete significantly less well than middle *in vivo* RNA (•; *em*, *m*, $m_1\ell$, some $m_2\ell$). A mixed competitor assay shows middle RNA transcripts predominating in the [^{3}H]RNA: a fixed amount of unlabed CM RNA competes against early species in the ^{3}H-labeled *in vitro* RNA; the substantial competition by added cold 10 min RNA (⊙) now involves middle RNA only. It appears that neither *e* early RNA nor its complement is made since there is little additional competition by CM RNA (*e*, *em*) in a mixed competitor assay with 10 min RNA; the latter should compete out middle RNA and *em* RNA (data not shown). In contrast, [^{3}H]RNA synthesized *in vitro* with holoenzyme from uninfected *B. subtilis* is known to be early (*e* and *em*) RNA: chloramphenicol RNA is a very effective competitor, but 28 min late RNA and 10 min middle RNA are not [5, 4].

The asymmetry of the RNA synthesized *in vitro* by phage-infected "core" polymerase was measured by RNA–RNA duplex formation with unlabeled *in vivo* RNA [9]. SP01 RNA synthesized in infected cells does not contain a substantial fraction of anti-messenger. (No combination of labeled *in vivo* RNA with excess unlabeled *in vivo* RNA converts more than 6% of radioactivity to RNAase resistance.) However, several preparations of *in vitro* RNA contained varying proportions of material complementary to *in vivo* RNA. The 2 min *in vitro* RNA with the highest anti-messenger content is described in table 1. Further analysis of the anti-*in vivo* messenger (fig. 3) shows that a substantial fraction of it is anti-early RNA and anti-late RNA but that *the middle RNA is asymmetric.* This contrasts with RNA synthesized under the same conditions with RNA polymerase core from uninfected *B. subtilis* which is highly symmetrical and contains anti-middle RNA.

SDS acrylamide gel electrophoresis of phage-infected "core" shows the α, β and β' subunits of host RNA polymerase, one additional polypeptide and a trace of another. The latter two polypeptides appear not to be present in comparably purified core RNA polymerase from uninfected cells. The phosphocellulose chromatography [6, 7] which generates the phage infected "core" separates a fraction which stimulates RNA synthesis on SP01 DNA with RNA polymerase core and behaves, in that respect, like host σ factor. The specific ability of infected cell core enzyme to synthesize middle RNA transcripts is not list on a second passage over phosphocellulose or on sedimentation through a high salt glycerol gradient.

Three novel properties of this enzyme should be emphasized: i) It is a virus-modified, purified, relatively stable RNA polymerase which retains transcription specificity for viral transcripts that are not transcribed by unmodified bacterial RNA polymerase. By way of contrast, the exhaustive and detailed analysis of *E. coli* RNA polymerase modification after phage T4 infection has yielded no comparable result (reviewed in [10]). In fact, only one comparable prior report deals with a very unstable enzyme that transcribes the left half of phage λ DNA [11] but, unaccountably, not from the *in vivo* promotor which is located near the opposite end of linear λ DNA. ii) The enzyme appears to execute two steps of the control sequence of viral gene expression (fig. 1): it directs the synthesis of middle RNA; apparently it does not direct the synthesis of (*e*) early RNA which is shut off after the first few minutes *in vivo*. How these control functions are exerted, whether they involve a single modification of the RNA polymerase core, or whether they can be uncoupled [5], remains to be determined. iii) The enzyme has some transcriptional specificity but is a "core" in the commonly accepted meaning of the term. Its properties might be taken as contradicting the σ model of transcriptional control, at least in its simplest form. But, does a tightly bound peptide function here, like σ factor, in directing middle RNA synthesis? The isolation and reassociation of RNA polymerase subunits [12] might help to resolve these questions.

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