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Transcriptional specificity after mycobacteriophage I3 infection

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Abstract. Transcriptional regulation following mycobacteriophage I3 infection has been investigated. For this purpose, RNA polymerase mutants (*rif*^{r}) of host bacterium, *Mycobacterium smegmatis* have been isolated and characterised. Phage growth in *rif*^{s} and *rif*^{r} cells in presence of rifampicin revealed the involvement of host RNA polymerase in phage genome transcription. This was confirmed by studies on *in vivo* RNA synthesis as well as by direct RNA polymerase assay after phage infection. Significant stimulation in RNA polymerase activity was seen following phage infection. The maximal levels were attained in about 60 min post infection and maintained throughout the phage development period. The stimulation of polymerase activity was most pronounced when the phage DNA was used as the template. RNA polymerases from uninfected and phage-infected *Mycobacterium smegmatis* have been purified to homogeniety. The enzyme purification was accomplished by a rapid procedure utilising affinity chromatography on rifampicin-Sepharose columns. Subunit structure analysis of the purified RNA polymerase from uninfected and phage-infected cells showed the presence of α, *β, β′* and σ subunits similar to the other prokaryotic RNA polymerases. In addition, a polypeptide of 79,000 daltons was associated with the enzyme after phage infection. The enzymes differed in their properties with respect to template specificity. Phage 13 DNA was the preferred template for the modified RNA polymerase isolated from infected cells which may account for the transcriptional switch required for phage development.

Keywords. RNA polymerase; rifampicin-Sepharose; affinity chromatography; rifampicin resistance; modified RNA polymerase; transcriptional control.

Introduction

Infection with bacteriophages brings about dramatic changes in the intracellular environment of host bacteria. These major adaptations assure specific, rapid and temporally correct transcription needed for the phage growth and multiplication. Bacteriophages are known to invoke novel transcriptional controls, thereby regulating their genome transcription (Doi 1977; Travers, 1976).

We have employed *Mycobacterium smegmatis*-phage 13 system as a model to study host-virus interaction. Mycobacteriophage 13 is a temperate, transducing phage with a growth cycle in the synthetic medium lasting for 5 h comprising of a 200 min latent period and a 60–90 min rise period (Nagaraja and Gopinathan, 1980). In conformity with the slow growing nature of their hosts, the mycobacteriophages also show prolonged growth patterns. The host DNA is not degraded after phage 13 infection until the end of intracellular development period (Nagaraja and Gopinathan, 1981). Evidently, the phage must be exerting control at the level of RNA synthesis by diverting the host transcriptional machinery towards its own transcription. This

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Abbreviations used: BSA, Bovine serum albumin; DTT, dithiothreitol; DMSO, dimethylsulphoxide; SDS, sodium dodecyl sulphate; EDC, l-ethyl-3(3-dimethyl aminopropyl) carbodiimide; NTG, N-methyl-N'-nitro-N-nitrosoguanidine; PAGE, Polyacrylamide gel electrophoresis; rif^s, rifampicin sensitive; rif^r, rifampicin resistant.

transcriptional control has, therefore been investigated in detail. The results presented in this communication show that phage 13 is employing the host RNA polymerase for its genome transcription, after suitably modifying the enzyme. The RNA polymerase isolated from phage-infected cells differs markedly from the one isolated from uninfected bacteria in subunit structure and template specificity.

Materials and methods

Chemicals

Nucleoside triphosphates (ATP, GTP, CTP, UTP), calf thymus- and salmon sperm DNA, poly d(A-T), poly (G-U), rifampicin (rif), bovine serum albumin (BSA fraction V, recrystallised), dithiothreitol (DTT), dimethylsulphoxide (DMSO), sodium dodecyl sulfate (SDS) and l-ethyl-3(3-dimethyl aminopropyl) carbodiimide (EDC) were from Sigma Chemical Company, St. Louis, Missouri, USA. [^{3}H]-Uracil was from Bhabha Atomic Research Centre, Bombay. [^{3}H] -UTP was from New England Nuclear, Boston, Massachusetts, USA.

Bacteria and phage

A clear plaque forming mutant of phage 13 (Gopinathan *et al.,* 1978) was employed for all the experiments described here. The host strain used was *M. smegmatis* SN2. The bacteria and phage were grown as described elsewhere (Nagaraja and Gopinathan, 1980).

Rifampicin-resistant *M. smegmatis* cells were isolated by mutagenesis using N-methyl-N' -nitro-N-nitrosoguanidine (NTG). Bacteria growing in exponential phase were harvested, suspended in minimal medium containing 200 μg/ml of NTG and incubated for 45 min at 37°C. The cells were washed free of the mutagen, inoculated into nutrient broth and were grown overnight. The drug resistant colonies were selected on nutrient agar plates containing 100 μg/ml rifampicin.

For growth or inhibition studies, rifampicin was added to the cultures from a stock solution (4 mg/ml) in DMSO. Appropriate volume of DMSO was present in control cultures.

Preparation of cell free extracts

The cells were washed twice with extraction buffer (20 mM Tris-HCl, pH 7·4, 5 mM $MgCl_2$, 5 mM β-mercaptoethanol and 10% glycerol) and suspended in the same buffer. Extracts were prepared by passing the cell suspension through French pressure cell (18000 psi) followed by centrifugation at 2,000 *g* for 30 min. The supernatent was further centrifuged at 100,000 *g* for 90 min and this S-100 supernatent was used for all the enzyme assays.

Purification of RNA polymerase

The S-100 fraction from uninfected or 90 min post-infection cells of *M. smegmatis* was fractionated with ammonium sulphate, with pH maintained at 7·5. The protein

fraction precipitating between 30 and 50% saturation of ammonium sulphate was dissolved in a small volume of buffer A (10 mM Tris-HCl, pH 7·9, 1 mM $MgCl_2$, 0·1 mM DTT and 5% glycerol), and gel filtered on an Ultrogel column (1·2 × 70 cm), previously equilibrated with buffer A. The individual fractions (1·2 ml) were monitored for absorbance at 280 nm and enzyme activity. The active fractions were pooled and subjected to affinity chromatography on rifampicin-Sepharose column.

Preparation of rifampicin-Sepharose

AH-Sepharose (1·2 g) was washed with 0·5 M NaCl, suspended in 3·0 ml of water and 20 μmol of rifampicin dissolved in 40% dimethylformamide was added. The pH was adjusted to 4·5-6·0, and gently shaken for 2 h maintaining the pH between 4·5–6·0. EDC (200 μmol) was added, and the shaking was continued at room temperature (20°C) for 18–20 h. The reaction was continued for another 10 h in the presence of additional 200 μmol EDC, at the end of which 100 mg of glycine was added. After shaking for 1 h, the contents were washed successively with the following: (i) 100 ml of water, (ii) 100 ml of 0·1 M sodium acetate in 0·5 M NaCl (pH 4·5), (iii) 100 ml of 2 M urea in 0·5 M NaCl, (iv) 100 ml of 0·1 M sodium bicarbonate in 0·5 M NaCl, pH 9·5 and (v) excess of water.

The coupled rifampicin-Sepharose (light orange-red in colour) was stored in 0·5 M NaCl at 4°C, protected from light. Rifampicin-Sepharose was packed into a column and equilibrated with buffer A containing 50 mM KCl. The coupled rifampicin-Sepharose was relatively unstable, due to the degradation of the drug. However, if stored at 4^0C, the column could be used 2–3 times within a week. After each use, the column was washed alternately with low pH and high pH buffers containing 0·5 M NaCl.

RNA polymerase assay

The reaction was carried out essentially as described by Burgess (1969). The assay mixture contained in 125 μl, 20 mM Tris-HCl, pH 7·9, 1 mM magnesium acetate, 0·1 mM EDTA, pH 7·0, 0·1 mM DTT, 200 mM KCl, 0·4 mM potassium phosphate pH 7·5, 2·5 μg BSA, 1% glycerol, 0·4 mM each of CTP, ATP and GTP, 0·1 mM [^{3}H]-UTP (200 μCi/μmol), 5 μg calf thymus DNA and the enzyme. Potassium phosphate was added to the assay mixture to inhibit polynucleotide Phosphorylase (Burgess 1969); the presence of phosphate had no adverse effect on polymerase activity. The reaction mixtures were incubated at 37° for 5 min and 100 μ1 samples were processed for the determination of trichloracetic acid-insoluble radioactivity.

One unit of enzyme activity incorporated 1 nmol of UMP in 5 min under the conditions described. Protein estimation was carried out by the method of Lowry *et al.* (1951).

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions was carried out as described by Smith and Braun (1978) with slight modifications. Apart

from the regular constituents, the 5% acrylamide gels contained 5 mM *β*-mercapto-ethanol and 10% glycerol in Tris-glycine (pH 8·3). After electrophoresis the gels were stained and destained as described by Fairbanks *et al.* (1971). SDS-PAGE was carried out by the method of Laemmli (1970) in 7·5% gels. The gels were scanned at 280 nm using UV-190 Shimadzu double beam spectrophotometer, to determine the number of subunits.

Molecular weight determination

Molecular weight of the subunits was calculated based on the mobility of reference markers—albumin (bovinc plasma), ovalbumin, pepsin, *β*-lactoglobulin and lysozyme.

Results

Isolation and characterization of rif^r mutants of M. smegmatis

Experiments were designed to see whether phage 13 is employing the host RNA polymerase or coding for its own enzyme. For this purpose, RNA polymerase mutants *(rif^r)0* of *M. smegmatis* were isolated and characterized. All the mutants grew at 100 *μ*g/ml or more of rifampicin, while the growth of the parental strain was completely inhibited by less than 1 *μ*g/ml of rifampicin. However, the drug resistant strains showed a lag in their growth, which was even more pronounced in the presence of the drug. To rule out the possibility that the *rifr^r* mutation was at the level of permeability to the drug into the organism, the growth of the strains was examined in the presence of the antibiotic and 0·01 % SDS. At this concentration, SDS renders the cells permeable to the drug without exerting any growth inhibitory effect by myself. All the mutants isolated in the present studies were resistant to the drug even in the presence of SDS.

To confirm that the mutation was indeed in RNA polymerase, the enzyme activity was assayed in cell-free extracts prepared from wild type and *rif^r* bacteria (table 1).

Table 1. Effect of rifampicin on RNA polymerase activity.

Host strain	Infection with phage I3	Enzyme activity (cpm incorporated/5 min) Control (no drug)	In presence of rifampicin (100 *μ*g/ml)	Activity (%)
rif^s	uninfected	850	83	10
	infected	2213	163	7
rif^r	uninfected	525	550	105
	infected	1575	1560	99

The extracts were prepared from exponentially growing Dells of *M smegmatis* (uninfected or 90 min following the infection with phage I3). The S-100 fraction was used as enzyme source. The RNA polymerase reaction was carried out as described under methods. The enzyme was incubated with rifampicin for 5 min prior to the addition of the substrate.

More than 90 % inhibition of RNA polymerase activity was observed in the extracts from wild type (*rif* ^{s}) cells, before or after phage infection. In contrast, the enzyme isolated from the resistant cells was not inhibited by 100 μg/ml of rifampicin, establishing that the mutation was at the level of RNA polymerase.

Phage is utilizing host RNA polymerase for its transcription

To find out whether the phage is dependent on the host RNA polymerase or coding for its own enzyme, the effect of rifampicin on phage production was examined. Wild type and *rif*^{r} cells were infected with phage I3 and the production of phage at the end of 1 cycle of growth in the presence of rifampicin (added at different times), was monitored (figure 1).

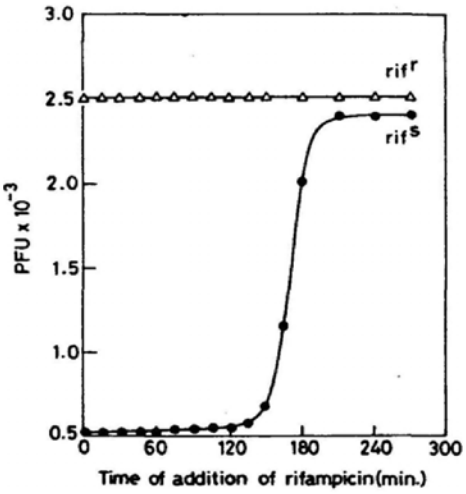

Figure 1. Effects of rifampicin on phage production.
M. smegmatis, rif ^{s} and *rif* ^{r} strains were infected with phage 13. The infected samples were exposed to 100 μg/ml of rifampicin at diferent periods during growth and the growth continued till the end of 1 cycle (300 min). The phage titers were determined at the end of one cycle of phage growth. The *rif* ^{r} mutant used in this and all the subsequent experiments was resistant upto 500 μg/ml of the antibiotic. (●) , *rif*^{s},(Δ), *rif* ^{s} .

Phage production was completely inhibited in wild type cells when rifampicin was added at any time during the phage development period (up to 160 min, in nutrient broth). On the contrary, no inhibition of phage growth was observed in *rif*^{r} cells, irrespective of the presence of drug. These results implied that in sensitive cells, inhibition of host RNA polymerase has resulted in the inhibition of transcription from phage genome. Phage production was not affected in the *rif* ^{r} cells in the presence of rifampicin due to insensitivity of the enzyme to the drug.

To establish this point firmly, the *in vivo* RNA synthesis was also examined in *rif*^{s} and *rif*^{r} cells after phage infection. [^{3}H]-Uracil incorporation into RNA was completely blocked by rifampicin in wild, type cells (figure 2). The extent of inhibition of RNA synthesis in the *rif*^{r} mutant was much less pronounced. The reduced level of

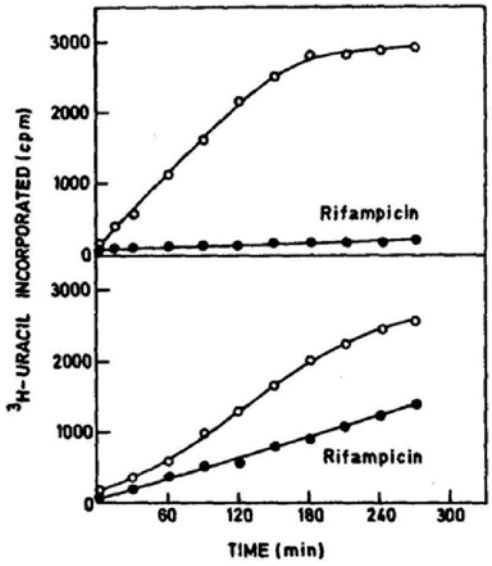

Figure 2.. *In vivo* RNA synthesis in *rif*^{s} and *rif*^{r} bacteria.

[^{3}H]-Uracil incorporation into RNA was measured during the period of phage development in the presence of rifampicin (100 μg/ml). [^{3}H]-Uracil (1 μCi/ml) was added at the time of infection. Samples were removed at different times and the acid precipitable radioactivity was determined.

Top panel: Synthesis in *rif*^{s} cells.

Bottom panel: Synthesis in *rif*^{r} cells.

(O), Control (no drug); (●), 100 μg/ml rifampicin.

RNA synthesis in *rif* ^{r} cells was consistent with the reduced growth of this strain. These results confirmed that the phage is employing host RNA polymerase for its genome transcription.

Further support for this conclusion comes from the *in vitro* RNA polymerase reaction carried out with extracts prepared from phage-infected cells (see table 1). These results also rule out that phage 13 codes for its own polymerase for the following reasons. For instance, if the phage was coding for a rifampicin-resistant enzyme, there should not be complete inhibition of RNA polymerase activity in *rif*^{s} cells infected with phage. It is evident from table 1, that there was complete inhibition. On the other hand, if phage was inducing a rifampicin sensitive enzyme, inhibition of the enzyme should be expected in the presence of the drug even in *rif*^{r} cells. There was no inhibition of activity from phage-infected *rif*^{r} cells (table 1).

RNA polymerase was stimulated after phage infection

RNA polymerase activity in the phage infected cells was higher compared to the uninfected cells (table 1). The pattern of increase in the enzyme activity after phage infection was monitored and the results are presented in figure 3. The enzyme activity increased from early times after infection, reaching the maximal values around 60 min. The extent of stimulation was 4-6 fold in different sets of experiments and was seen till the end of latent period. Significantly the stimulation was more

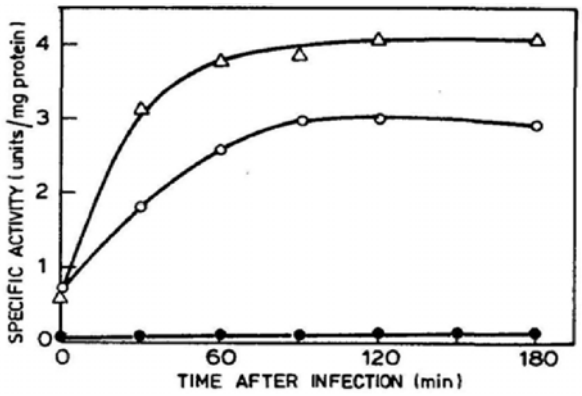

Figure 3. RNA polymerase activity after phage 13 infection.

M. smegmatis in exponential phase (1×10^8 cells/ml) was infected with phage I3 at a multiplicity of infection 15 in the presence of 1 mM $CaCl_2$ and incubated at 37°C on a shaker. The cells were removed just prior to or after phage infection at the intervals noted. The latent phase of phage 13 growth gets over at 180 min. RNA polymerase assay with templates, (Δ), phage 13 DNA and (O) calf thymus DNA;(●) in the presence 100 μg/ml refampicin with either of the templates.

pronounced when phage 13 DNA was used as a template instead of calf thymus DNA. The levels of RNA polymerase remained constant in the uninfected cells in the entire duration of the experiment.

Purification of RNA polymerases from uninfected and phage I3-infected M. smegmatis

All the above data suggested that phage I3 is employing host RNA polymerase for its transcription. The obvious question, therefore, is whether the phage is utilising the host enzyme as such or with modifications. To ascertain this point, RNA polymerase was purified from uninfected and phage I3 infected *M. smegmatis* cells.

A rapid, 3 step procedure was developed for the purification of RNA polymerase. The steps employed were ammonium sulphate fractionation, Ultrogel filtration and affinmyy chromatography on rifampicin-Sepharose. The results of purification are summarised in tables 2 and 3.

The mycobacterial enzyme, like the other prokaryotic RNA polymerases emerged immediately after the void volume from the gel filtration column as a high molecular weight protein. The enzyme was bound to the affinmyy column (rifampicin-Sepharose) very tightly and was eluted only at 1·2 M KCl (figure 4). Under similar conditions, the enzyme from *Escherichia coli* was eluted from this column at 0·6 M KCl.

The purification methods developed here resulted in electrophoretically homogeneous preparations of the enzyme from *M. smegmatis* (figure 5). The enzyme yields from phage-infected and uninfected cells were 10-16% and the increase in specific activity was 160–260 fold. The *E. coli* enzyme also appeared to be nearly homogeneous.

RNA polymerase from infected cells contains an additional component

The subunit structure of the enzyme was determined by SDS-PAGE (figure 6). The

Table 2. RNA polymerase purification from *M. smegmatis**.

Step	Total protein (mg)	Total activity (nmol UMP incorporated/ 5 min)	Specific activity (nmol UMP incorporated/5 min/ mg protein)	Fold purification
Crude (S-100) fraction	142·10	71·36	0·50	
30–50% Ammonium sulfate fraction	35·36	35·77	1·01	2
Pooled Ultrogel fraction	6·56	173·60**	26·40	53
Rifampicin-Sepharose fraction	0·085	11·08	130·35	260

M. smegmatis in exponential phase (1×10^8 cells/ml) was used for the preparation of enzyme.
*From 12 g wet weight of cells.
**The total activity at this stage was considerably more than the earlier steps, due to the removal of inhibitory materials.

Table 3. RNA polymerase purification from phage I3-infected *M. smegmatis**.

Step	Total protein (mg)	Total activity (nmol UMP incorporated/ 5 min)	Specific activity (nmol UMP incorporated/5 min/ mg protein)	Fold purification
Crude (S-100) fraction	139·20	180·80	1·30	
30–50% Ammonium sulfate fraction	33·32	56·62	1·70	1·3
Pooled Ultrogel fraction	6·32	291·18**	46·10	36·0
Rifampicin-Sepharose fraction	0·086	17·97	208·95	161·0

M. smegmatis, in exponential phase was infected with phage I3 at a multiplicity of infection of 15 in presence of 1 mM $CaCl_2$ and incubated for 90 min at 37°C on a shaker before the cells were harvested for the isolation of the enzyme.
*From 12 g wet weight of cells.
**The total activity at this stage was considerably more than the earlier steps, due to the removal of inhibitory materials.

RNA polymerase from uninfected cells showed 3 bands corresponding to molecular weight of 151,000, 95,500 and 45,500. These bands were tentatively identified as β β', σ and α subunits by analogy to the molecular weight of known prokaryotic RNA polymerases. The enzyme from infected cells had an extra band of molecular weight 79,000 in addition to other subunits. The total molecular weights of the RNA polymerases based on an α_2 $\beta\beta'\sigma$ subunit structure correspond to 488,500 and 564,500 respectively, from uninfected and phage I3-infected *M. smegmatis* (*cf.* total molecular weight of *E. coli* RNA polymerase is 493,000, Burgess, 1969).

The pure enzymes from *M. smegmatis* (uninfected or phage I3-infected), were 10 times more sensitive to inhibition by rifampicin, than the *E. coli* enzyme. At 10^{-8} M rifampicin the former was inhibited 65–75% while the *E. coli* enzyme was not affected; at 10^{-7} M drug concentration, all of them were inhibited.

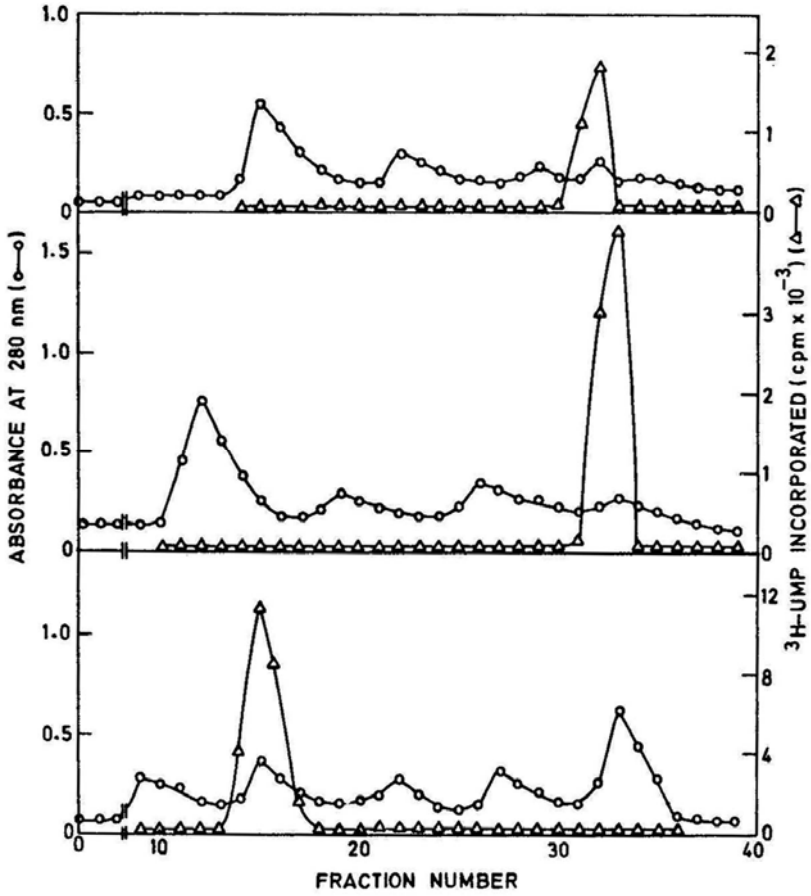

Figure 4. Affinity chromatography of RNA polymerases on rifampicin-Sepharose column
Protein fractions from Ultrogel filtration step showing enzyme activity were pooled and loaded on to the affinity column. After washing the column extensively with buffer A containing 0·1 M KCl, the enzyme was eluted using a step gradient of KCl (0·2–1·2 M) in buffer A. One ml fractions were collected and assayed for enzyme activity.
Top panel: RNA polymerase from uninfected *M. smegmatis.*
Middle panel: RNA polymerase from phage 13 infected *M. smegmatis.*
Bottom panel: RNA polymerase from *E. coli.*

Template specificity

To check whether the association of the extra polypeptide with RNA polymerase could account for changes in transcriptional specificmyies, the template recognition by the modified and the parental enzymes was compared (table 2). The enzyme from phage-infected cells transcribed phage 13 DNA to a much greater extent than all other template DNAs tested. Calf thymus- and salmon sperm DNAs also served as good templates for this enzyme, while the host DNA turned out to be poor template. On the other hand, no such specificmyy was observed for the RNA polymerase from uninfected cells. Single stranded DNA or synthetic ribopolymers were very poor templates for both the enzymes.

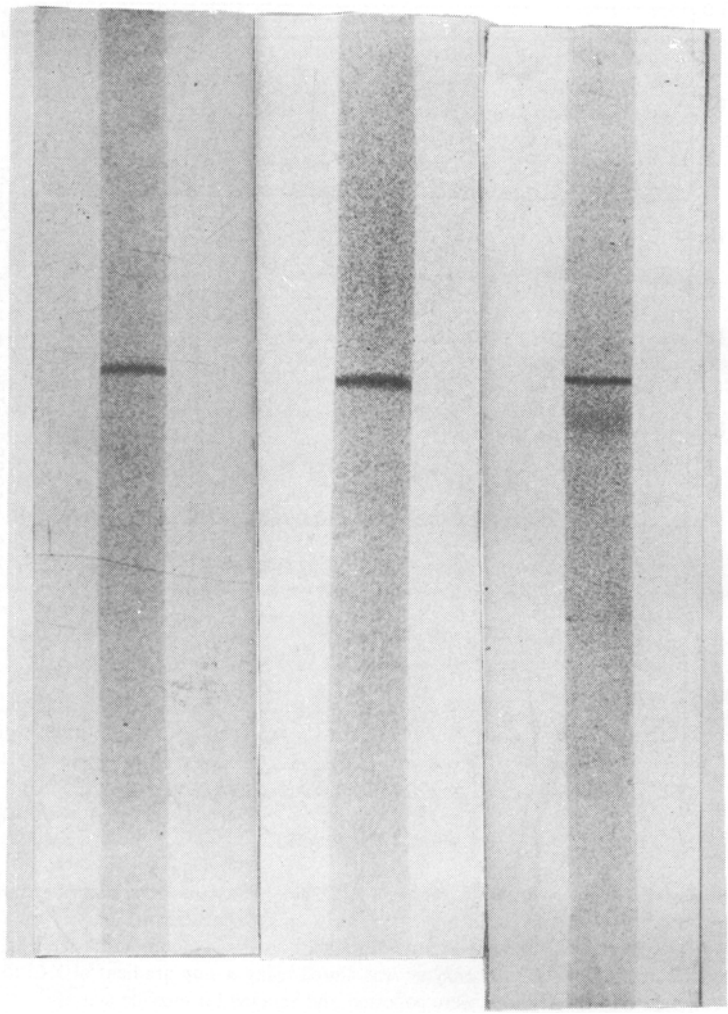

Figure 5. PAGE of RNA polymerases.
The electrophoresis was carried out in 5% gels at pH 8·8; 10 μg of enzyme after rifampicin-Sepharose affinity chromatography, was used in each gel. From left to right, RNA polymerases from uninfected- and phage I3-infected *M . smegmatis,* and *E. coli.*

Discussion

When bacteria are infected with phage, often a metabolic shift takes place channelling the macromolecular syntheses towards the phage development. Involvement of RNA polymerase in the regulation of transcriptional switch has been well documented and is primarily responsible for metabolic alterations in certain cases (Losick and Chamberlin, 1976; Doi, 1977). Different bacteriophages have evolved varying means of transcriptional control. Transcription of λ genome is regulated by antitermination phenomenon (Adhya *et al.,* 1976). Extensive changes have been observed in the subunit structure of host RNA polymerase after infection with

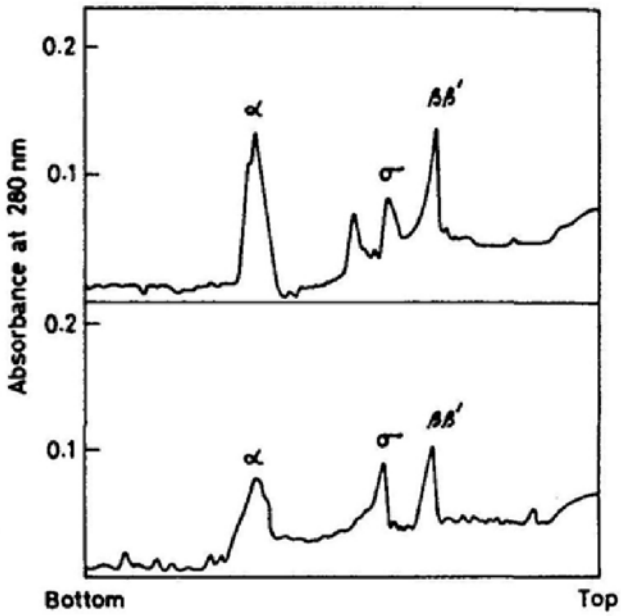

Figure 6. Subunit structure of RNA polymerase.
The purified enzymes were subjected to electrophoresis on 7·5% Polyacrylamide gels under denaturing conditions. After electrophoresis the gels were scanned at 280 nm.
Top panel: RNA polymerase from phage-infected *M. smegmatis.*
Bottom panel: RNA polymerase from uninfected *M. smegmatis.*
(*ββ′* subunits do not separate well on 7·5% gels; see Bautz, 1976 and Wiggs *et al.,* 1979)
The molecular weights of the subunits were determined from the mobility of reference markers.

Table 4. Template specificity of RNA polymerase.

	RNA polymerase activity (nmol of [^{3}H]-UMP incorporated/5 min/mg protein)	
DNA template	Uninfected	Infected
Phage I3	13·96	48·83
Mycobacterium smegmatis	16·37	14·13
Mycobacterium tuberculosis $H_{37}R_a$	13·28	12·70
Micrococcus lysodeikticus	11·66	14·21
Escherichia coli	9·64	5·47
Phage λ	18·82	20·38
φX174	7·82	6·96
Plasmid (pLM2)S_4	18·28	17·94
Calf thymus	14·96	33·60
Salmon sperm	15·68	34·24
Poly d(A-T)	14·93	17·80
Poly(G-U)	4·61	3·36

Partially purified enzyme, after Ultrogel fractionation step was used. 10 μg of protein and 5 μg of different template DNAs (native) were used in each assay. RNA polymerase reaction was initiated by the addition of substrates. Details of assay are described in text.

coliphages T4 and T5 (Stevens, 1972; Doi, 1977; Szabo *et al.,* 1975). Similarly, infection with phages SP0l and SP82 results in altered promoter recognition and template specificity of RNA polymerase from *Bacillus subtilis* (Fox, 1976; Whitelay *et al.,* 1976). A requirement for 5-hydroxymethyl uracil in DNA for the specific

transcription by the phage SP0l-modified (gene 28-product associated) RNA polymerase has been reported (Lee *et al.,* 1980). Coliphages T3 and T7 code for their own polymerases (Bautz, 1976; Doi, 1977) which carry out the transcription from late promoters. Another kind of mechanism is seen in coliphage N4 (Falco *et al.,* 1977, 1978) and phage PBS2 of *B. subtilis* (Clark *et al.,* 1974). These viruses have the RNA polymerase in the particles and is presumably injected into the host cell along with the DNA. The results presented in this paper clearly establish that the host RNA polymerase carries out phage I3 DNA transcription. Comparison of the structure of enzymes purified from uninfected and phage-infected cells show that the enzyme is in modified form after infection. A new polypeptide is found associated to the enzyme, in addition to the other subunits. The significant changes observed *in vitro* in the template specificity could be due to the binding of this factor to the host enzyme as no other changes were apparent in the enzyme structure.

The RNA polymerases from uninfected and phage I3-infected *M. smegmatis* bind to rifampicin-Sepharose column very tightly as seen from their elution profile. On the contrary, the RNA polymerase from *E. coli* is eluted at a much lower concentration of KCl indicating the lower affinity of this enzyme to rifampicin. This is consistent with the 10–100 fold higher sensitivity of mycobacterial enzymes to rifampicin, reported here and earlier for *M. tuberculosis* (Harshey and Ramakrishnan, 1976). However, mycobacterial enzyme seems to have less affinity towards heparin, because the enzyme is eluted from heparin-Sepharose column at a low salt (0·2 M KCl) concentration (data not shown). The procedure developed for purification of RNA polymerase is very rapid and the pure enzyme is obtained within 48 h. Although several procedures are available for the purification of RNA polymerases from prokaryotes, the method reported here using affinity chromatography on rifampicin-Sepharose columns could be adopted as a general method for the rapid purification of RNA polymerase from small amounts of starting material. This is the first report of the use of rifampicin-Sepharose as an affinity matrix for the purification of RNA polymerase.

Phage 13 DNA serves as the best template *in vitro* for the RNA polymerase from the phage-infected *M. smegmatis.* This immediately suggests the strong possibility that *in vivo,* the modified enzyme specifically transcribes phage 13 DNA, the bacterial DNA being less efficiently. On the other hand, RNA polymerases from various sources show differential transcription of different templates or a particular template (Iida *et al.,* 1979; Wiggs *et al.,* 1979). However, maximum specificity is seen only with the phage-induced polymerases. For instance, enzymes from phages T3 and T7 selectively transcribe their own DNA to the maximum extent (Bautz, 1976). Another extreme example is coliphage N4 (Falco *et al.,* 1977, 1978). The purified N4 polymerase transcribes single stranded N4 DNA very efficiency but not native double stranded DNA. Phage I3-modified polymerase seems to prefer native DNA templates.

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References

Adhya, S., Gottesman, M., de Crombrugghe, B. and Court, D. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin), (New York: Cold Spring Harbor Laboratory) p. 719.

Bautz, E. K. F. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin), (New York: Cold Spring Harbor Laboratory) p. 273.

Burgess, R. R. (1969) *J. Biol. Chem.,* **244,** 6160.

Clark, S., Losick, R. and Pero, J. (1974) *Nature (London),* **252,** 21.

Doi, R. H. (1977) *Bacteriol. Rev.,* **41,** 568.

Epp, C. and Pearson, M. L. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin) (New York: Cold Spring Harbor Laboratory) p. 667.

Fairbanks, G., Steck, C. L. and Wallach, D. F. H. (1971) *Biochemistry,* **10,** 2606.

Falco, S. C, Laan, K. V. and Rothman-Denes, L. B. (1977) *Proc. Natl. Acad. Sci. USA,* **74,** 520.

Falco, S. C, Zivin, R. and Rothman-Denes, L. B. (1978) *Proc. Natl. Acad. Sci. USA,* **75,** 3220.

Fox, T. D. (1976) *Nature (London),* **262,** 748.

Franklin, N. C. and Yanofsky, C. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin) (New York: Cold Spring Harbor Laboratory) p. 693.

Gopinathan, K. P., Saroja, D., Gadagkar, R. R. and Ramakrishnan T. (1978) in *Genetics of Actinomycetales* (eds E. Freerksen, I. Tarnok and J. H. Thumin), (New York: Stuttgart, G. Fisher-Verlag) p. 237.

Harshey, R. M. and Ramakrishnan, T. (1976) *Biochim. Biophys. Acta,* **432,** 49.

Iida, H., Ikehara, K. and Okada, Y. (1979) *FEBS Lett.,* **99,** 346.

Laemmli, U. K. (1970) *Nature (London),* **227,** 680.

Lee, G., Hannett, N. M., Karman, A. and Pero, J. (1980) *J. Mol. Biol.,* **139,** 407.

Losick, R. and Chamberlin, M. (eds) (1976) in *RNA polymerase* (New York: Cold Spring Harbor Laboratory).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.,* **193,** 265.

Nagaraja, V. and Gopinathan, K. P. (1980) *Arch. Microbiol.,* **124,** 249.

Nagaraja, V. and Gopinathan, K. P. (1981) *Mol. Biol. Rep.,* **8,** 11.

Smith, S. S. and Braun, R. (1978) *Eur. J. Biochem.,* **82,** 309.

Stevens, A. (1972) *Proc. Natl. Acad. Sci. USA,* **69,** 603.

Szabo, C., Dharmgrongartama, B. and Moyer, R. W. (1975) *Biochemistry,* **14,** 989.

Travers, A. (1976) *Nature* (*London*)*,* **263,** 641.

Whitelay, H. R., Spiegelman, G. B., Lawrie, J. M. and Hiatt, W. R. (1976) in *RNA polymerase* (eds R. Losick and M. Chamberlin) (New York: Cold Spring Harbor Laboratory) p. 58.

Wiggs, J. L, Bush, J. W. and Chamberlin, M. J. (1979) *Cell* **16,** 97.