EVIDENCE FOR TWO RNA POLYMERASES IN ARTHROBACTER, A MORPHOGENETIC BACTERIUM

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SUMMARY: Two DNA-dependent RNA polymerases have been isolated from Arthrobacter crystallopoietes, a bacterium with a distinct morphological life cycle. The two enzymes are different with respect to chromatographic and electrophoretic behavior, divalent cation requirement, and template activity with different DNA species. One appears to be similar in its properties and structure to E. coli polymerase, the other is different, but the nature of the difference is not yet clear. The possible relationship of the two enzymes in a regulatory role in the life cycle has yet to be investigated.

DNA dependent RNA polymerase has been shown to be involved in the selective transcription of DNA in several bacterial and bacteriophage systems [see (1) for a recent review], including T_4 (2) and T_7 (3) bacteriophages of E. coli and during sporulation in B. subtilis (4,5). The general picture for this enzyme indicates that there is an active "core" polymerase molecule which contains 4 protein subunits: two α, β, and β'. This "core" enzyme becomes specific and selective toward its DNA template when it combines with other protein subunits, or when one of these subunits is modified. In the case of T_4 bacteriophage [2], presence of the "sigma" subunit on the E. coli polymerase provides the transcription of certain phage genes immediately after infection. Proteins synthesized from these early messages then interact with the host polymerase to cause transcription from other portions of the phage DNA. Thus, one has a simple mechanism for control of "early" and "late" genetic information. A second example is the case of sporulating Bacillus, in which one of the β subunits of the vegetative phase polymerase is modified prior to sporulation (4,5). This modified sporulation polymerase synthesizes different RNA in an in vitro system [6]. Leighton, et. al. (7) have characterized a temperature sensitive mutant which lacks the ability

to form spores and cannot modify the vegetative polymerase, thus supporting the necessary involvement of the polymerase in sporulation.

On the basis of these two examples, it would be tempting to generalize that alteration of the original polymerase could provide for "programming" of the DNA transcription during cellular events. However, infection of E. coli by T_7 bacteriophage results in the synthesis of a separate RNA polymerase which is totally different from the host's enzyme (3). Thus, the phenomenon of modulating the activity of a single RNA polymerase molecule is not an adequate, singular explanation for the control of transcription.

In the eucaryotic organisms, the control of transcription could possibly be explained largely by the fact that distinct, different enzymes exist (8), in some cases with different nuclear locations (9). The existence of completely different enzymes in bacteria is less well documented, although the cited examples do illustrate modified forms of a single enzyme. There have been some reports of preliminary observations of more than one enzyme being found in bacteria (10,11) although the nature of the differences is not known. This present paper presents data supporting such a discovery in Arthrobacter crystallopoietes, a morphogenetic bacterium, whose life cycle has been shown to involve differential DNA transcription (12).

METHODS

Cultures of A. crystallopoietes (ATCC 15481) were grown for 16 hours in a medium containing tryptone, (0.5 % w/v), glucose (0.1% w/v), and yeast extract (0.25% w/v). RNA polymerase was isolated from 5 to 25 gram samples of these bacteria, using the techniques described by Burgess (13). The assays also followed Burgess' description, including 0.04 mM phosphate to inhibit polynucleotide phosphorylase, except when noted. DNA was isolated from the various organisms by Marmer's method (14). When isolating DNA from Arthrobacter, it is necessary to add a preliminary incubation with lysozyme (100 µg/ml, 1 hr, 37°C) and then sodium lauryl sulfate (50 µg/ml, 30 min, 37°C) to insure efficient lysis. Polyacrylamide gel electrophoresis

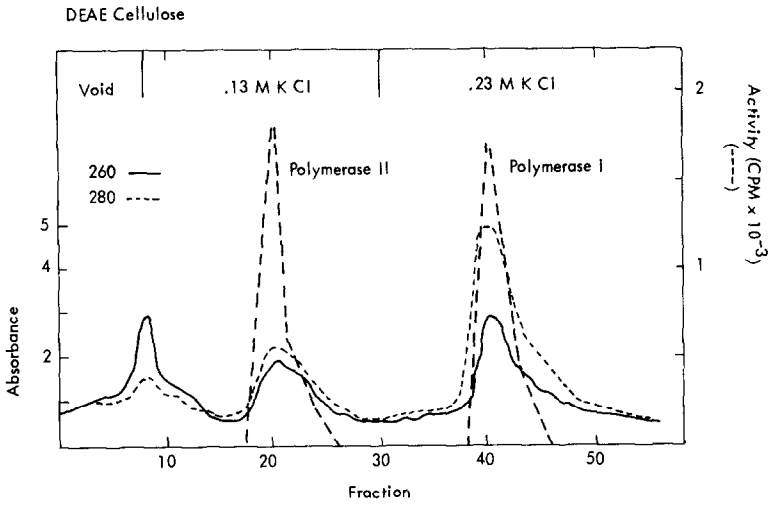

Figure 1. DEAE-CELLULOSE CHROMATOGRAPHY OF RNA POLYMERASE

RNA polymerase from Arthrobacter crystallopoietes was isolated using the general techniques described by Burgess (13). The ammonium sulfate fraction was applyed to DEAE-cellulose and eluted with a stepwise KCl gradient. All major protein peaks were assayed for activity, and indicated the presence of two activity peaks. Polymerase I is the enzyme which elutes at the same KCl concentration as the RNA polymerase of E. coli; the other activity peak is designated polymerase II. The peak at fraction 8 is uridine used to indicate the void volume.

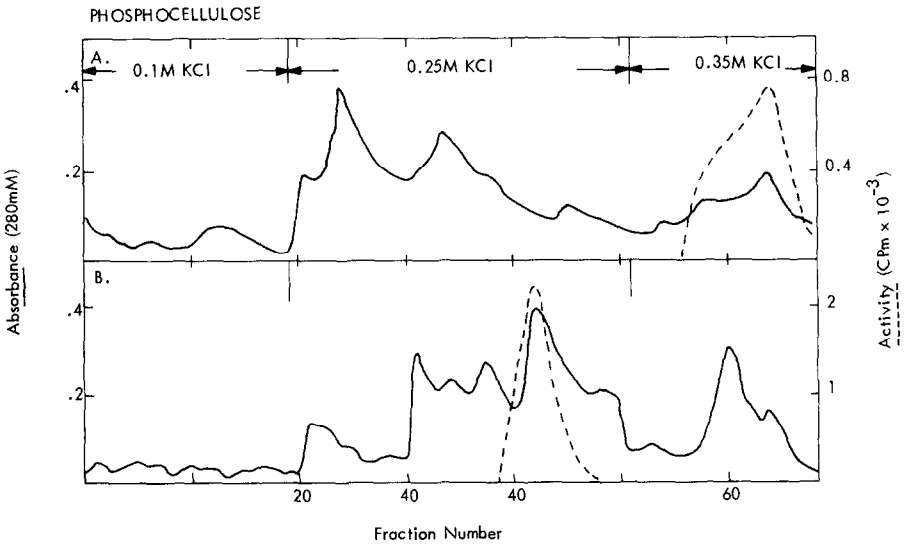

Figure 2. PHOSPHOCELLULOSE CHROMATOGRAPHY OF RNA POLYMERASE

The two polymerases isolated from DEAE-cellulose were chromatographed independendently on phosphocellulose, eluting with stepwise KCl gradients.
A. Polymerase II B. Polymerase I

was performed as described by Krakow (15).

RESULTS

Figure 1 shows that when the crude ammonium sulfate preparation (33-50% saturated fraction) was subjected to DEAE cellulose chromatography, two peaks of polymerase activity eluted. When these two enzymes were chromatographed on phosphocellulose, they retained their capacity to elute with different buffer strengths as shown in Figure 2. One of these enzymes behaves in a manner similar to the "classical" E. coli polymerase on these two ion exchange resins has been designated as polymerase I with the other designated polymerase II.

The template activity of these two enzymes with respect to various DNA's was assayed as shown in Table 1. The comparative rates of activity were different, with the differences somewhat less distinct after phosphocellulose chromatography. Both of the enzymes exhibit a change in template specificity after chromatography through phosphocellulose, as shown in Table 1. This result is similar to the case for the E. coli polymerase [8,28] and would be consistent with a loss of a sigma-like factor in that chromatographic step.

The electrophoretic behavior of the two enzymes, purified through the DEAE cellulose stage, is shown in Figure 3. While the protein stain shows that neither enzyme is very pure at this stage, the in situ assay clearly indicates that the enzyme activity is associated with different bands. This in situ assay, described by Krakow (15), requires the presence of sigma factor for the E. coli enzyme; and thus no activity could be detected by this technique for the Arthrobacter enzyme after phosphocellulose chromatography.

Both enzymes have a similar broad optimum Mg^{++} concentration at 2 millimolar, but their response to Mn^{++} is different. The optimum Mn^{++} concentration is 5 mM for I and 9 mM for II.

DISCUSSION

One initial concern in isolating a previously unreported enzyme is the

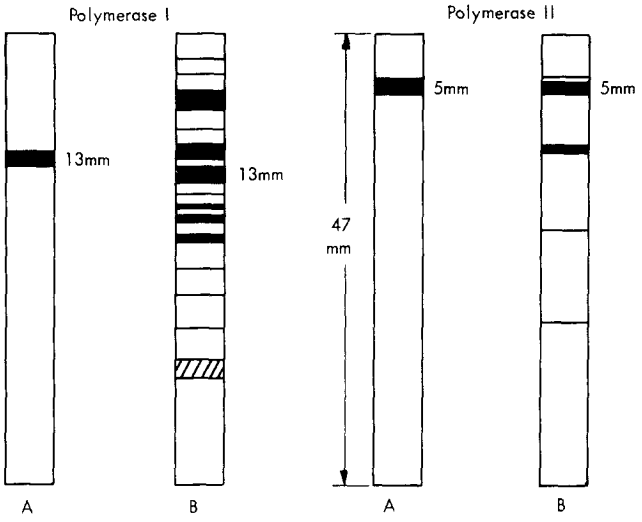

Figure 3. POLYACRYLAMIDE GEL ELECTROPHORESIS OF RNA POLYMERASE

The two polymerases isolated from DEAE-cellulose (Fig. 1) were subjected to electrophoresis on polyacrylamide gels as described by Krakow (15). Duplicate samples were run and one of these stained for protein with Coomassie Blue (Samples "A"). The other sample was subjected to an in situ assay, and the RNA product stained with Ethidium Bromide (Samples "B").

possibility that the enzyme is a contaminant or artifact. One possibility is that the new enzyme might be polynucleotide phosphorylase. However, 0.04 mM phosphate has been reported to inhibit this enzyme (13), and the presence or absence of this salt had no effect on the activity of either enzyme. In addition, one would not expect that the basic polynucleotide phosphorylase would adhere to phosphocellulose. The new enzyme might also be induced by bacteriophage infection (3). However, these bacteria are growing normally, and do not contain any virulent phage which could possibly code for this new enzyme. Finally, polymerase II is not core enzyme, because after purification through phosphocellulose, it still elutes at 0.13 M KCl if rechromatographed on DEAE cellulose. Because of the recent report (11) of a second form of polymerase found in stationary phase E. coli, it is important to point out the differences between the A. crystallopoietes enzyme and the new E. coli enzyme. The physical properties are different, since the Arthrobacter enzyme

TABLE 1 TEMPLATE ACTIVITY OF RNA POLYMERASES I AND II

	Activity* of DEAE-Cellulose Fractions		Activity* of Phospho-cellulose Fractions	
DNA source/Polymerase	I	II	I	II
A. crystallopoietes	3.0	4.0	12.0	18.0
E. coli	0.1	9.0	11.0	17.0
Calf thymus	2.0	5.0	21.0	21.0

*nM ^{14}C-AMP incorporated/mg protein

is more basic than polymerase I, while the new E. coli enzyme is more acidic. Most important, however, is the fact that the stationary phase E. coli enzyme has very little synthetic activity toward a native DNA template. Table 1 shows, however, that both Arthrobacter enzymes have considerable synthetic capability. Preliminary data indicates that A. crystallopoietes polymerase I probably has 4 subunits similar to those of E. coli. At the present time, it is not clear whether polymerase II has subunits which are entirely different from the subunits of polymerase I, or whether some of them are the same and some are modified as has been found in B. subtilis [4]. It is also possible that they might contain a similar "core" enzyme with different modulating subunits. If this latter is the case, then the whole complex of this new enzyme must be tightly bound together, since it is still a distinct enzyme after phosphocellulose chromatography. This would make it more stable than any of the complexes reported previously (1) for other bacterial RNA polymerases.

The general emerging picture for the involvement of RNA polymerase in the control of genetic transcription in bacteria shows that this enzyme has a necessary and possibly sufficient role in phage infection and sporulation. (1,6) Recent data indicates that it could be involved in stationary phase growth. (11) The current data provide sufficient cause to suspect its implication in the

control of life cycles which involve morphological changes. RNA polymerase is also suspected to be involved in the life cycle of Caulobacter, another morphogenetic bacterium (16), although the data is unclear at this time (17). For Arthrobacter, it is clear that there are either two enzymes or two different forms of the same enzyme. The intriguing possibility that they could regulate the life cycle remains to be clarified.

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