Polyadenylic Acid Synthesis Activity of Purified DNA-dependent RNA Polymerase from Caulobacter*

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Characterization of purified DNA-dependent RNA polymerase (EC 2.7.7.6) of Caulobacter crescentus, strain CB15 has led to the conclusion that this enzyme catalyzes poly(A) synthesis in the absence of template. Poly(A) synthetase activity co-purifies with both holoenzyme and core polymerase on DNA-cellulose columns, and core polymerase purified to 98% homogeneity by glycerol gradient centrifugation is still capable of catalyzing poly(A) polymerization. Both RNA synthesis and poly(A) polymerization activities are sensitive to rifampicin. In addition, RNA polymerase purified from partially rifampicin-sensitive mutants exhibits the same partial sensitivity in vitro to the drug in the synthesis of RNA and poly(A). The enzyme used in these studies was prepared by a simple method which allows a high yield of pure RNA polymerase from large batches of exponential cells. The procedure includes high speed centrifugation of cell extracts, DEAE-cellulose column, DNA-affinity chromatography, and low salt glycerol gradient centrifugation. Holoenzyme can be resolved into core and $\sigma$ subunit by either DNA-cellulose chromatography or glycerol gradient centrifugation, and the latter step allows recovery of pure $\sigma$ factor.

The Gram-negative-stalked bacterium Caulobacter crescentus undergoes a series of well-defined morphological and functional changes during its life cycle, making it a simple and attractive model for study of developmental regulation (Poindexter, 1964; Newton et al., 1975; Shapiro, 1976). Previous studies have shown that transcription (Newton, 1972) and translation are needed for normal development throughout most of the cell cycle. Furthermore, the pattern of protein synthesis changes sequentially during the development of these cells (Cheung and Newton, 1977). These results support the view that differential gene expression, presumably at the level of transcription, plays an important role in the control of development in C. crescentus.

A direct approach to the study of transcriptional control is to examine the properties of the DNA-dependent RNA polymerase. We have devised a simple purification procedure which allows for efficient recovery of RNA polymerase with high yields of enzyme activity from large quantities of exponential cells. $\sigma$ subunit can be resolved from core polymerase and recovered by glycerol gradient centrifugation. In the characterization of purified enzyme, we have found that both holoenzyme and core polymerase are capable of catalyzing polyadenylyic acid synthesis in the absence of template. This is of particular interest because of the report that a significant fraction of the messenger RNA of C. crescentus cells is polyadenylated (Ohta et al., 1975). Both biochemical and genetic evidence for the identity of poly(A) synthetase activity with a component of the DNA-dependent RNA polymerase is presented below.

MATERIALS AND METHODS

Cells and Growth Conditions

Caulobacter crescentus, strain CB15, was obtained from the American Type Culture Collection (ATCC 19089). Cells were grown in complex medium (Pye; Poindexter, 1964) made of 0.2% Difco Bactopeptone, 0.1% Difco yeast extract, and 0.02% MgSO$_4$. $\cdot$7H$_2$O in local well water adjusted to pH 6 before autoclaving. Cells were grown at room temperature and harvested in log phase using the Sharples continuous flow ultracentrifuge and stored frozen.

Rifampicin-resistant mutants of C. crescentus isolated previously in our laboratory can be grouped into two general phenotypic classes. The first class, which includes strains Cl-Rf1, Cl-Rf2, and Cl-Rf3, are derived from cys strain Cl (Newton and Allebach, 1975) and they are resistant to at least 50 $\mu$g/ml of rifampicin in Pye medium; growth of the wild type strain CB15 is completely inhibited by 1 $\mu$g/ml of the drug. The second class of rifampicin-resistant mutants, which includes strains C12-Rf6, C24-Rf6, and C26-Rf6, grow and divide normally in 10 $\mu$g/ml of rifampicin, but cell morphology is distorted and the growth rate is reduced at 50 $\mu$g/ml. These latter strains are referred to as partial rifampicin-resistant mutants.

Materials

Most chemicals used were commercially available, analytical grade reagents. Whatman microgranular (preswollen) DEAE-cellulose DE52 was obtained from Whatman. Protein standards for SDS-polyacrylamide electrophoresis, electrophoretically pure pepsin, calf thymus DNA (New England Biochemicals). Bovine serum albumin, dithiothreitol (Cleland's Reagent), rifampicin, highly polymerized calf thymus DNA for use as template in assays, imidazole, unlabeled nuclease triphosphates, Coomassie brilliant blue, and PMSF were obtained from Sigma Chemical Co. Radiolabeled nucleotide triphosphates were purchased from New England Nuclear.

1 M. Osley and A. Newton, unpublished observations.

2 A. Newton and E. Allebach, unpublished result.
Reagents for SDS-gel electrophoresis were purchased from Bio-Rad.

**Buffers**

All buffers were prepared from analytical grade chemicals without further purification in deionized distilled water. Stock solutions of 1 M imidazole, pH 6.5, at 25°, 0.1 M EDTA (pH 7.9), 0.1 M dithiothreitol (stored in a brown bottle, and 1 M MgCl₂·7H₂O were kept at 4° and diluted to make the various buffers. Buffer I contained 0.01 M dithiothreitol, 0.1 mM EDTA, and 10% (v/v) glycerol. Buffer II contained 0.05 M imidazole (pH 6.5), 10 mM MgCl₂·7H₂O, 0.2 mM KC1, 1 mM dithiothreitol, 0.1 mM EDTA, and 20% (v/v) glycerol. Dithiothreitol was added to the buffers just prior to use from a 0.1 M stock solution.

**RNA Synthesis Activity**

The procedure for the assay was modified slightly from Burguess (1969). The standard assay mixture contained, in a final volume of 0.25 ml: enzyme, 0.04 M Tris (pH 7.9 at 25°), 0.01 M MgCl₂, 0.1 mM EDTA, 0.1 phenylmethylsulfonfyl fluoride, 0.16 mM KC1, 0.6 mg/ml of bovine serum albumin, 0.15 mM concentrations of UTP, CTP, and GTP, 0.15 mM ['³²P]ATP (1 mCi/mmol), 0.4 mM potassium phosphate (pH 7.5), and 0.15 µg/ml of calf thymus DNA. Potassium phosphate was used in the assays to inhibit polyribonucleotidase phosphorylase activity. The reaction was started by the addition of labeled ATP after preincubation of the assay mixture plus enzyme for 10 min at 30°. Assays were incubated at 30° for 10 min, chilled in ice, and precipitated with 3 ml of 5% trichloroacetic acid. After 30 min at 4°, the precipitate was collected on a Whatman GF/A glass fiber filter paper, washed four times with 3 ml of 2% ice-cold trichloroacetic acid, and then three times with 2 ml of chilled absolute ethanol. All trichloroacetic acid solutions contained 0.01 M sodium pyrophosphate. The filters were dried in an oven at 70° for 30 min and then counted in 4 ml of toluene scintillation fluid (16 g of 2,5-diphenyloxazole and 0.4 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene) in 4 liters of toluene. One unit of RNA synthetic activity is defined as the amount of protein that catalyzes the incorporation of 1 n mole of AMP into acid-precipitable material in 10 min of incubation under the conditions described above. The specific activity is expressed as units of enzyme per mg of protein. Protein was measured by the method of Lowry et al. (1951), using crystalline bovine serum albumin as standard.

**Poly(A) Synthetic Activity**

The assay mixture was adapted from Iwakuara et al. (1974) and contained in a final volume of 0.25 ml: enzyme, 0.12 M Tris (pH 7.9 at 25°), 0.12 mM MnSO₄, 0.2 mM dithiothreitol, and 0.05 mM ['³²P]ATP. The enzyme was preincubated in the assay mixture at 30° for 10 min without ['³²P]ATP, which was then added to start the reaction. Assays were incubated for 2 h at 30°, chilled in ice, and then precipitated with 5 ml of 5% trichloroacetic acid. The precipitate was processed for scintillation counting as described above. One unit of poly(A) synthetic activity is defined as the amount of protein that catalyzes the incorporation of 1 n mole of AMP into acid-precipitable material in 2 h of incubation under these conditions.

**Purification**

Purification procedure for the DNA-dependent RNA polymerase used in these studies is described in the miniprint supplement.*

**Assays for Enzymatic Impurities**

**Ribonuclease (RNase)** — Degradation of RNA was measured by monitoring the size distribution on sucrose gradients. ['³²P]labeled ribosomal RNA from C. crescentus (kindly supplied by N. Ohta, Department of Biology, Princeton University) in 0.1 M sodium acetate at pH 5.2 (30 µg, 260,000 cpm) and 25 µg of purified C. crescentus RNA polymerase were incubated for 1 h at 30° in a total volume of 0.25 ml of the buffer used in the RNA polymerase assay but without DNA or nucleoside triphosphates (nuclease buffer). The reaction was then chilled in ice and an aliquot of 0.2 ml was centrifuged for 9 h at 39,000 rpm on a 5 to 30% sucrose gradient. This is a sensitive assay for endonuclease activity because the gradient resolves the 18 S and 23 S species of RNA and any nicks or breaks alter the profile of the RNA. A second assay designed to detect the utilization of labeled RNA by nuclease was performed by incubation of ['³H]RNA from C. crescentus with enzyme as above for 12 h at 30°. Then the reaction mixture was chilled in ice and 500 µg of yeast tRNA in 0.5 ml of 0.3 M sodium acetate was added. RNA was precipitated by addition of 300 µl of absolute ethanol and centrifuged and the supernatant was counted in 3 ml of Bray’s scintillation fluid (Bray, 1960).

For quantitation of RNase activity, 4 µg of ['³H]poly(U) in 50 µl (1 mM UTP) was substituted for C. crescentus RNA and incubated with enzyme at 30° for 10 min, all in 4 mM phosphate buffer, pH 7.4. RNase activity was expressed as the radioactivity solubilized in 10 min under these conditions.

**Deoxyribonuclease** — Endonuclease activity was assayed according to Greene et al. (1974) with some modifications. Reactions were carried out at 37° for 20 min in 30 µl of 0.1 M Tris (pH 7.5), 0.05 M NaCl, and 0.5 mM MgCl₂, containing 1 or 2 µg of enzyme and 0.8 µg of supercoiled PM2 DNA; the incubation was stopped by adding 5 µl of 5% SDS. Electrophoresis was then performed using 0.7% agarose in a Tris/borate buffer (Greene et al., 1974) at 40 V overnight. Gels were soaked in the Tris/borate electrophoresis buffer containing 0.5 µg/ml of ethidium bromide for 15 min and DNA bands were visualized with ultraviolet light (from Ultra-Violet Products, Inc.). Supercopied, open circular, and linear forms of DNA were distinguished by their differential mobilities on these gels and the amounts of nicked and intact PM2 DNA were determined by comparison to standards. A similar endonuclease assay was performed with ['³²P]labeled SV40 DNA (kindly supplied by A. Sen, Department of Biochemistry, Princeton University), but the products were analyzed on alkaline sucrose gradients which could separate close circular DNA (supercoiled) from open circular or linear DNA (Form I). In 100 µl of nuclease buffer (see below), 10 µg of ['³²P]-labeled SV40 supercoiled DNA (about 10,000 cpm) were incubated at 30° for 1 h and the resultant DNA profile was analyzed on alkaline sucrose gradients as described by Meyer (1972).

An assay for exonuclease and large amounts of endonuclease activities is based on solubilization of DNA, ['³²P]-labeled SV40 DNA (3'161 cpm in 0.5 µg) and 10 µg of enzyme were incubated at 30° for 12 h in 100 µl of nuclease buffer. The reaction was stopped by addition of 100 µl of 10% trichloroacetic acid and then centrifuged in a Beckman 152 microfuge for 5 min. The acid-soluble counts released were determined by precipitating the supernatant (100 µl) in 3 ml of Bray's scintillation fluid.

**DNA**

The temperate Caulobacter bacteriophage, LC72, was purified by repeated centrifugation followed by equilibrium centrifugation through a CsCl density gradient. DNA from LC72 and C. crescentus strain CB15, was purified by phenol extraction (Marmur, 1963; Melli and Bishop, 1970). C. crescentus was grown in Pye medium; a typical yield from 5 g of cells was 12 mg of DNA with an absorbance ratio (A₂₆₀/A₂₈₀) of 0.504.

**RESULTS**

**Stability and Purification of C. crescentus RNA Polymerase**

In purifying C. crescentus RNA polymerase, it was found that the enzyme was easily inactivated by the presence of high concentrations of salt, e.g. 1 M KCl at 4°, despite the presence of 1 mM dithiothreitol and 10% (v/v) glycerol. The enzyme assay was also inhibited under these conditions. Furthermore, a partial inactivation of the enzyme occurred even when salts were rapidly removed after ammonium sulfate precipitation. For this reason, the purification procedure was designed to avoid using high concentrations of salt (Cheung, 1976).

To minimize selective enzyme degradation or inactivation...
during purification, the stability of enzyme activity in cell extracts was examined over the pH range 2 to 12 using citrate, phosphate, imidazole, and borate buffers. These results showed that the enzyme was most stable at pH 6.5 in imidazole buffer in the presence of 1 mM dithiothreitol and 20% (v/v) glycerol. All purification steps were carried out under these conditions.

The purification procedure used in this study was developed independently of a method described recently by Amemiya et al. (1977). Both of the procedures use DEAE-cellulose and DNA-cellulose chromatography, but they differ in other respects. Among these are the avoidance of high salt concentrations at all stages and the use of glycerol density gradient centrifugation as a final purification step in the procedure described (see next section).

Resolution of α Subunit

The core enzyme and α subunit can be resolved in two different ways. As reported previously (Amemiya et al., 1977) holoenzyme and core polymerase can be recovered by DNA-cellulose chromatography (Fig. 3). In addition, we have found that core enzyme and the putative α subunit can be isolated by glycerol gradient centrifugation. When holoenzyme purified on a DNA-cellulose column was layered on a 15 to 30% (v/v) glycerol gradient in Buffer II containing 0.05 M KCl and centrifuged, the peak fractions of activity contained core polymerase (see next section) and purified α was recovered from Fractions 15 to 20 (Fig. 4). SDS-gel electrophoresis of these fractions (Fig. 5) and proteins of known molecular weight (not shown) shows characteristic core subunits of 165,000 (β'), 155,000 (β), 48,000 (α), and 96,000 (α'). The 96,000-dalton protein (α'), as well as the other proteins, have been designated by analogy to the subunits of the E. coli RNA polymerase (Burgess, 1969).

The holoenzyme and core RNA polymerase purified as described above showed no difference in catalytic activity under the standard assay conditions when T4 or calf thymus DNA were used as templates (Table II). However, with Caulobacter phage LC72 DNA, the holoenzyme was 2-fold more active than the core polymerase (Table II). We have also been able to distinguish between holoenzyme and the core RNA polymerase of C. crescentus by differences in metal ion preferences (Table II). Although optimal RNA polymerase activity requires Mg++, holoenzyme is 6- to 7-fold more active than core polymerase when Mn2+ was substituted for Mg++ with bacteriophage T4 DNA as template. The holoenzyme was also more active with native CB15 DNA as template when Mn2+ was substituted for Mg++ in the assay.

Attempts to reconstitute holoenzyme from purified core and α subunit have not been successful. This may reflect the lability of isolated α or suboptimal conditions for reconstitutive...
Purified Caulobacter RNA Polymerase with Poly(A) Synthesis

TABLE II
Metal ion preference in RNA synthesis

| DNA template | Mg²⁺ | Mn⁺⁺ |
|--------------|------|------|
| Holoenzyme   | Core | H/C² | Holoenzyme |
| CB15         | 2.7  | 2.4  | 1.2        | 0.6  | 0.3  | 2.1 |
| T4           | 1.3  | 1.2  | 2.8        | 0.2  | 0.03 | 7.2 |
| LC72         | 0.67 | 0.35 | 1.0        | 0.2  | 0.03 | 7.2 |
| Calf thymus  | 2.19 | 2.39 | 1.0        | 0.2  | 0.03 | 7.2 |

*H/C is the ratio of specific activity (nanomoles/mg) of holoenzyme versus specific activity of core polymerase.

*Not assayed.

SDS-Gel Electrophoresis of Purified RNA Polymerase

To assess the purity of the enzyme, samples from each step of the purification were analyzed by electrophoresis on 10% SDS-polyacrylamide slab gels (Fig. 5). The resolution of holoenzyme and core polymerase on a DNA-cellulose column is also shown.

The purity of the RNA polymerase preparation was determined from tracings of photographic negatives of stained SDS-polyacrylamide gels. The major contaminants in the holoenzyme after the DNA-cellulase column step are in two bands (cf. Slot 2, Fig. 5) that account for less than 5% of the total protein. Core polymerase at the same stage of purification has about 15% of the total protein. However, after glycerol gradient centrifugation the core polymerase contained less than 2% contaminating proteins.

Enzymatic Purity

Purified RNA polymerase was assayed for contamination by nuclease and polynucleotide phosphorylase as described below.

Ribonuclease - The sedimentation profile of ribosomal RNA from C. crescentus was not altered after incubation for 1 h at 30°C with 25 μg of purified RNA polymerase holoenzyme; however, 0.01 μg of RNase A gave complete digestion of the RNA under similar conditions (Fig. 6). Thus, no endonuclease activity was detected in the purified enzyme under these conditions. The purified core polymerase (10 μg from glycerol gradient) also failed to solubilize radioactive CB15 in 10% trichloroacetic acid after incubation for 24 h at 30°C, whereas 0.01 μg of RNase A completely degraded the RNA after incubation for only 1 h (data not shown).

Deoxyribonuclease - Since it was necessary to remove DNA by treatment with DNase during the purification (see miniprint supplement), the purified RNA polymerase was examined for contaminating DNase activity. PM2 DNA, incubated for 20 min at 37°C with 2 μg of purified enzyme (Fraction 4, Table I), exhibited the same agarase gel pattern as the control (no enzyme). DNAse I (0.001 μg) under the same conditions nicked 90% of the DNA. Similarly, SV40 DNA retained the same sedimentation profile on alkaline sucrose gradient after incubation with 10 μg of purified C. crescentus RNA polymerase (see "Materials and Methods"; data not shown). Thus, no contaminating endonuclease activity could be detected in the purified RNA polymerase preparation.

We also observed no solubilization of SV40 DNA even after incubation with 10 μg of polymerase for 12 h at 30°C, whereas 0.01 μg of DNAse I rendered 1 μg of SV40 DNA completely soluble in trichloroacetic acid after 10 min of incubation at 30°C.

Polynucleotide Phosphorylase – Polynucleotide phosphorylase activity was assayed by the exchange of [³²P]inorganic phosphate (New England Nuclear) into ADP (Reiner, 1969). E. coli polynucleotide phosphorylase (0.1 μg from P-L Biochemicals) exchanged about 10,000 cpm in 30 min at 30°C, while 10 μg of purified polymerase gave less than 1% of the above exchange.

Poly(A) Synthesis

Since a significant fraction of the unstable messenger RNA in C. crescentus is polyadenylated at the 3'-OH terminus (Ohta et al., 1975), we examined purified fractions of RNA polymerase for poly(A) synthetase. Traces of activity that catalyzed the synthesis of poly(A) from ATP in the absence of template were observed in RNA polymerase purified on DEAE-cellulose. The total poly(A) synthetase activity in the RNA polymerase preparation increased significantly after DNA-cellulase chromatography, presumably because of removal of contaminating proteins.
moval of nucleases or inhibitors. Poly(A) synthetase fractionated on DNA-cellulose with the holoenzyme (Peak I) and core polymerase (Peak II, Fig. 3). Under these assay conditions the incorporation of ATP into poly(A) is linear with time for at least 2 h, but it was linear with enzyme only above 5 μg of protein/ml (data not shown). Consequently, poly(A) synthesis was routinely assayed with 5 μg/ml or more of polymerase preparations.

Several results indicate that the poly(A) synthetic activity is catalyzed by the RNA polymerase and not by an active, minor contaminant, like polynucleotide phosphorylase, in the purified preparation. These results include: (a) both holoenzyme and core polymerase have poly(A) synthesis activity as shown above, and it is unlikely that a contaminant could copurify with both enzymes on DNA-cellulose; (b) the rate of poly(A) synthesis is approximately 5% that of template-primed RNA synthesis (Fig. 3 and Table III) and SDS-gel electrophoresis showed that each of the several contaminating proteins amounted to no more than 1% of the total protein in the purified RNA polymerase (Fig. 5); (c) RNA polymerase preparations used in these experiments contained less than 0.1% polynucleotide phosphorylase activity (see "SDS Gel Electrophoresis of Purified RNA Polymerase"); and (d) the poly(A) synthetase in C. crescentus is more active with Mn⁺⁺ than with Mg⁺⁺ (Table III). This metal ion preference contrasted with the polynucleotide phosphorylase from E. coli, which is more active with Mn⁺⁺ than with Mg⁺⁺ (Table III). This metal ion preference contrasts with the polynucleotide phosphorylase from C. crescentus as well as with the holoenzyme purified from C. crescentus (Amemiya et al., 1977). We determined the sensitivity of the same purified enzyme preparations to rifampicin in the synthesis of poly(A). The results (Table V, Fig. 7) showed that this activity is also inhibited by rifampicin, although somewhat less so than the DNA-dependent RNA synthesis reaction. Since rifampicin inhibits E. coli RNA polymerase by binding to the β subunit of the enzyme (Chamberlin, 1974) this result suggests that at least the β subunit of C. crescentus RNA polymerase is involved in polyadenylate acid synthesis.

### Table IV

| Amount of rifampicin (μg/ml) | Percentage residual enzyme activity in RNA synthesis (%) |
|-------------------------------|---------------------------------------------------------|
|                               | CB10 | Cl-Rf3 | C12-Rf6 |
| 0                             | 100% | 100%   | 100%    |
| 0.2                           | 100% | 100%   | 100%    |
| 1.6                           | 0%   | 100%   | 75.5%   |
| 3.2                           | 0%   | 0%     | 62.2%   |
| 8.4                           | 0%   | 0%     | 43.3%   |
| 16                            | 0%   | 0%     | 42.5%   |
| 40                            | 0%   | 0%     | 34.0%   |

* Condition not assayed.

### Table V

| Amount of rifampicin (μg/ml) | Percentage residual enzyme activity in poly(A) polymerization (%) |
|-------------------------------|---------------------------------------------------------------|
|                               | CB10 | Wild type | Cl-Rf3 | C12-Rf6 |
| 0                             | 100% | 100%      | 100%   | 100%    |
| 0.4                           | 10.5%| 100%      | 90.6%  | 88.8%   |
| 0.8                           | 5.7% | 100%      | 92%    | 70.5%   |
| 2                             | 3.9% | 2.6%      | 95.2%  | 81.8%   |
| 16                            | 0%   | 0%        | 90.6%  | 71.3%   |
| 10                            | 3.6% | 2.1%      | 88%    | 65.5%   |

* Condition not assayed.

* One hundred per cent of original activity also at 40 μg of rifampicin/ml of reaction mixture.
rifampicin-resistant mutants. The in vitro sensitivity of RNA polymerase in the mutants (see "Materials and Methods") was examined in cell extracts purified by high speed centrifugation (cf. Table I, Fraction 2) and sedimentation through low salt glycerol density gradients described in Fig. 1. RNA polymerase from the peak fractions in each gradient was pooled and samples of 100 μl each were incubated with rifampicin as indicated for 5 min at 30° before standard polymerase assay mixture was added. Residual RNA polymerase activity at 30° was determined for CB15 (○), Cl-Rf3 (rifampicin-resistant mutant, ▲), and C12-Rf6 (the partially rifampicin-resistant mutant, ◆) as described in text.

Poly(A) Synthesis in Rifampicin-resistant Mutants

To eliminate the possibility that poly(A) synthesis is catalyzed by a contaminating activity that is also sensitive to rifampicin, we have examined poly(A) and RNA synthesis in rifampicin-resistant mutants. The in vitro sensitivity of RNA polymerase in the mutants (see "Materials and Methods") was examined in cell extracts purified by high speed centrifugation (cf. Table I, Fraction 2) and sedimentation through low salt glycerol gradients; the profiles of RNA polymerase activity were similar to that of the wild type (cf. Fig. 1). Three rifampicin-resistant strains studied (C1-Rf1, C1-Rf2, and C1-Rf3) exhibited complete resistance to 10 and 20 μg/ml of rifampicin in vitro, and the RNA polymerase activity from a fourth mutant, C12-Rf6, was only 50% inhibited by 10 μg/ml of rifampicin (Fig. 8). The wild type CB15 enzyme was completely inhibited at 0.2 μg/ml in the partially purified preparation.

Strains C1-Rf3 and C12-Rf6 were examined in more detail. RNA polymerase from these strains was purified through the DNA-cellulose chromatography step (Table I, Fraction 4) to allow for detection of poly(A) synthetase activity, and the response of holoenzyme and core polymerase to rifampicin in RNA synthesis (Table IV) and poly(A) polymerization was determined (Table V). The purified enzyme from strain C1-Rf3, which grew well in the presence of 50 μg/ml of rifampicin, was completely resistant to the drug, as assayed by both RNA synthesis and poly(A) polymerization activities. Purified RNA polymerase from the partially resistant mutant, C12-Rf6, was only partially inhibited by rifampicin in the two assays. These parallel sensitivities of the mutant enzymes to rifampicin in both assays strongly support the conclusion that core RNA polymerase of C. crescentus is responsible for both RNA synthesis and poly(A) synthesis.

DISCUSSION

Purified RNA polymerase from C. crescentus, strain CB15, catalyzes both polyadenylic acid synthesis in the absence of template and DNA-dependent RNA synthesis. This conclusion is supported by several lines of evidence: i) enzyme purified to 98% homogeneity on glycerol gradient catalyzes both of these activities; ii) poly(A) synthetic activity co-purifies with holoenzyme and core polymerase on DNA-cellulose column; iii) both poly(A) synthesis and RNA polymerase activity exhibit in vitro sensitivity to rifampicin; and iv) purified RNA polymerases from partially rifampicin-sensitive strains show reduced sensitivity in both RNA synthesis and poly(A) synthesis. These results indicate that poly(A) synthesis is catalyzed by the core polymerase and that at least the β subunit is required for synthesis.

Although we do not know the physiological significance of the poly(A) synthesis activity, the result is interesting because a fraction of the polyosomal RNA in C. crescentus is polyadenylated at the 3'-OH terminus (Ohta et al., 1975). A fraction of the unstable RNA in E. coli also contains poly(A) tracts at the 3'-OH end (Srinivasan et al., 1975) and Ramanarayanan and Srinivasan (1976) have characterized an enzyme which catalyzes the polymerization of ATP and ADP, as well as ATP exchanges into ADP and ATP. This latter enzyme is distinct, however, from the DNA-dependent RNA polymerase of E. coli.

The poly(A) synthetase activity of C. crescentus is described above also differs in its properties from other poly(A) polymerases present in E. coli. One of these is a ribosome-associated enzyme that catalyzes RNA-dependent poly(A) polymerization (August et al., 1962). This enzyme is more active with Mg²⁺ than with Mn²⁺, and it is apparently not associated with the DNA-dependent RNA polymerase in these cells. Chamberlin and Berg (1964) reported a template-dependent poly(A) synthesis activity in RNA polymerase; they attributed the activity to a slippage by the RNA polymerase on the DNA template when only adenosine triphosphate is present.

The purification procedure for DNA-dependent RNA polymerase used in this study was developed for C. crescentus, strain CB15. The scheme described in the miniprint supplement results in a very high recovery of purified holoenzyme and core polymerase (Table I). Although DEAE-cellulose and DNA-affinity chromatography steps described in this procedure are also used in a procedure developed independently by Amemiya et al. (1977) for the purification of RNA polymerase...
from C. crescentus, the two methods differ in a number of ways. We have noted that RNA polymerase in C. crescentus is very easily inactivated by high concentrations of salt and therefore we have avoided steps that require the use of high ionic strengths. In addition, the final step of our purification is centrifugation through a glycerol gradient. This procedure removes some trace contaminants and it allows the recovery of a purified σ subunit.

The holoenzyme and core polymerase from C. crescentus can be separated by DNA-cellulose chromatography, and they can be distinguished in two different assays. Although optimal RNA polymerase activity requires Mg2+, the holoenzyme catalyzes RNA synthesis 10-fold more actively than the core enzyme when Mn2+ is substituted for Mg2+ (Table II). A second way to distinguish between the two polymerases is their relative sensitivity to rifampicin, as reported previously for the enzyme from strain CB13 (Amemiya et al., 1977): the holoenzyme is consistently more resistant to the drug in both RNA and poly(A) synthesis. This can be observed for the enzymes from both wild type cells and rifampicin-resistant mutant cells (Tables IV and V). The greater drug resistance of the holoenzyme activity may reflect a tighter initiation complex between this form of the enzyme and the template, as shown for E. coli RNA polymerase (Chamberlin, 1974). Unfortunately, neither of the assays allowed us to detect the reconstitution of holoenzyme from purified core polymerase and σ factor of C. crescentus. This failure may be due to any number of causes, including inadequate conditions for assay, or reconstitution, and the lability of resolved σ factor. Although Amemiya et al. (1977) did not recover σ factor in their resolution of C. crescentus holoenzyme, they were able to demonstrate the stimulation of core polymerase by E. coli σ subunit, using T7 DNA and Caulobacter bacteriophage ϕChK DNA as templates.

We have previously shown that transcription is required throughout the cell cycle for normal development in C. crescentus (Newton, 1972), and changes in the patterns of protein synthesis in the various cell types (Cheung and Newton, 1977) also suggest that differential gene expression may play an important role in cell differentiation. Despite the high yield of purified RNA polymerase achieved in this procedure (Table I), we have not detected multiple forms of σ or core as determined by SDS-polyacrylamide gel electrophoresis of the constituent proteins. This result is consistent with an earlier finding that swarmer cells and stalked cells contain the same RNA polymerase (Bendis and Shapiro, 1973). We have also been unable to detect any modified subunits by electrophoresis in 8 M urea. None of the results is conclusive, however, since the enzyme recovery from cell extracts has been less than 100% using any of the published procedures. Differential transcriptional specificity in vivo could be mediated by a minor polymerase species that is lost during purification or by loosely bound modulating proteins that do not co-purify with the core or holoenzyme. We are investigating these two possibilities.

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Additional references are found on p. 2261.
**Purified Caulobacter RNA Polymerase with Poly(A) Synthesis**

**Introduction**

A simple purification of the 32P-ribonucleoside 5'-triphosphate from a chloramphenicol-induced extract of Caulobacter crescentus was used to obtain RNA polymerase and nucleic acid polymerase activity from the top fraction of retained nucleic acids.

**Methods**

**Chemical characterization**

The RNA polymerase was purified by gel filtration on a Sephadex G-50 column, followed by ultracentrifugal analysis of the nucleic acid polymerase activity. The purified enzyme was then analyzed for its nucleic acid polymerase activity, which was found to be associated with the top fraction of the column.

**Results**

A summary of the nucleic acid polymerase activity is provided in Table 1. The enzyme showed high activity in the top fraction of the column, with a specific activity of 2261 units/mg protein.

**Discussion**

The results obtained suggest that the 32P-ribonucleoside 5'-triphosphate is associated with the top fraction of the column, indicating that the nucleic acid polymerase activity is associated with this fraction.

**References**

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**TABLE 1**

| Fraction | Nucleic Acid Polymerase Activity (units/mg protein) |
|----------|--------------------------------------------------|
| 1        | 2261                                             |
| 2        | 543                                              |
| 3        | 250                                              |

**Figures**

![Figure 1](http://www.jbc.org/)

![Figure 2](http://www.jbc.org/)

![Figure 3](http://www.jbc.org/)

![Figure 4](http://www.jbc.org/)
Polyadenylic acid synthesis activity of purified DNA-dependent RNA polymerase from Caulobacter.
K K Cheung and A Newton

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