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RNA Polymerase of *Myxococcus xanthus*: Purification and Selective Transcription In Vitro with Bacteriophage Templates

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DNA-dependent RNA polymerase from vegetative cells of the gram-negative, fruiting bacterium *Myxococcus xanthus* was purified more than 300-fold by a modified Burgess procedure (Lowe et al., Biochemistry **18**:1344–1352, 1979), using Polymin P precipitation, 40 to 65% saturated ammonium sulfate fractional precipitation, double-stranded DNA cellulose chromatography, A5m gel filtration chromatography, and single-stranded DNA agarose chromatography. The last step separated the RNA polymerase into a core fraction and an enriched holoenzyme fraction. The core enzyme showed a subunit structure similar to that of the *Escherichia coli* polymerase, as follows: β′ and β (145,000 and 140,000 daltons, respectively) and α (38,000 daltons). A comparison of the core enzyme and the holoenzyme implicated two polypeptides as possible σ subunits. These polypeptides were closely related, as indicated by peptide analysis. *M. xanthus* RNA polymerase was capable of transcribing DNAs from *E. coli* phages T7, T4, and λ, *Bacillus subtilis* phage φ29, and *M. xanthus* phages Mx1, Mx4, and Mx8. Transcription of T7 and φ29 DNAs was stimulated by KCl, whereas transcription of Mx1, Mx4, and Mx8 DNAs was inhibited by KCl. Magnesium ion dependence, rifampin and heparin sensitivities, and spermidine stimulation of *M. xanthus* RNA polymerase activity were similar to those found with *E. coli* RNA polymerase. The pH optimum of *M. xanthus* RNA polymerase activity was more basic than that of *E. coli* polymerase. *M. xanthus* RNA polymerase was capable of selective transcription in vitro when DNAs from phages T7 Δ111, φ29, and Mx1 were used. The molecular weights of the resulting phage RNA transcripts made by *M. xanthus* RNA polymerase (as determined by agarose-acrylamide slab gel electrophoresis) were the same as the molecular weights of the transcripts synthesized by *E. coli* RNA polymerase. No discrete transcripts were detected as the in vitro RNA products of *M. xanthus* phage Mx4 and Mx8 DNA transcription. Southern blot hybridization analysis was used to confirm the identity of the T7 ΔD111 A_1 transcript synthesized by *M. xanthus* RNA polymerase. Three transcripts (transcripts A, B, and C; molecular weights, 2.55×10^6, 1.95×10^6, and 1.56×10^6, respectively) were identified as in vitro RNA products of *M. xanthus* phage Mx1 DNA transcription when either *E. coli* or *M. xanthus* RNA polymerase was used. A Southern blot hybridization analysis indicated that the *E. coli* RNA polymerase and the *M. xanthus* RNA polymerase transcribe common *Sal*I restriction fragments of Mx1 DNA.

Myxococcus xanthus is a gram-negative, gliding bacterium which grows in soil or on the bark of living trees (24, 42). *M. xanthus* cells feed on decaying organic matter or on other microorganisms by excreting extracellular enzymes that are capable of hydrolyzing a variety of macromolecules. The myxobacteria generally move over solid surfaces in large groups called swarms. Presumably, a swarm provides the critical cell mass needed by the myxobacteria to attack and digest other microorganisms or to degrade complex organic matter (15). Under conditions of starvation on a solid surface, a developmental program is triggered. As indicated by studies of mutants (31, 38), the developmental program of *M. xanthus* appears to involve two pathways that are parallel and to some extent independent, namely, aggregation and sporulation. The aggregation pathway directs the movement of cells inward toward aggregation centers, where 10^5 to 10^6 cells form raised mounds. The sporulation pathway controls the conversion of individual rod-shaped cells (length, 4 to 8 μm; diameter,

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0.5 μm) to ovoid, environmentally resistant resting cells called myxospores. In *M. xanthus*, the raised mounds of myxospores are termed fruiting bodies.

The developmental program of *M. xanthus* is associated with major changes in the pattern of gene expression. At least 25% of the major soluble proteins observed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis show significant changes in their rates of production; several significant changes also occur in the membrane proteins (20). Two major development-specific proteins have been purified and studied in more detail. Protein S, the most abundant developmental protein, first appears in the soluble fraction of cells, accounting for as much as 15% of the total protein synthesis (20, 22). At later times of development, this protein self-assembles to form a spore surface coat (21). The second protein, myxobacterial hemagglutinin, is a lectin which accounts for about 5% of the total developmental protein synthesis (10, 11); this protein accumulates in the periplasmic space and on the cell surface (at the cell poles) at the time that the cells aggregate (32, 33). In vitro transcription of the genes of these proteins should provide some clues concerning how gene expression is developmentally regulated in *M. xanthus*.

To facilitate in vitro studies of transcriptional control with DNA from *M. xanthus*, RNA polymerase was purified from vegetative cells of *M. xanthus* by using a modified Burgess procedure (4, 26). This procedure has been used to purify RNA polymerases from a variety of bacterial species (40). The optimal assay conditions and sensitivities to various inhibitors and stimulators were determined by using the DNA from a deletion mutant (ΔD111) of coliphage T7. This well-studied template (36, 40) was chosen because it contains a strong major promoter (A_1) and terminator which are recognized in vivo by *Escherichia coli* RNA polymerase and in vitro by a wide variety of bacterial RNA polymerases (40).

The ability of purified *M. xanthus* RNA polymerase to transcribe phage templates selectively in vitro was investigated. The selectivity of transcription is the ability of RNA polymerase to recognize specific promoters and terminators to form discrete transcripts (7). It is necessary to demonstrate selectivity if meaningful conclusions are to be drawn from in vitro transcription experiments. We analyzed the transcription products of the *M. xanthus* and *E. coli* RNA polymerases on various phage templates by agarose-acrylamide electrophoresis and by hybridizing the RNA to DNA restriction fragments, using the method of Southern (35). These methods have provided good evidence for the selective transcription of DNA templates in vitro by the *M. xanthus* enzyme.

MATERIALS AND METHODS

Materials. Materials were obtained from the following sources: unlabeled ribonucleoside triphosphates, salmon testis DNA, polydeoxyadenylic acid-polydeoxythymidylic acid [poly(dA-dT)]-containing DNA, lysozyme, rifampin, deoxycholate, heparin, and spermidine, from Sigma Chemical Co.; Bio-Gel A5m and polyacrylamide gel reagents, from Bio-Rad Laboratories; agarose (Seakem ME), from Marine Colloids; SDS, from BDH Chemicals, Ltd.; Tris and ammonium sulfate (ultrapure), from Schwarz/Mann; dithiothreitol and phenylmethylsulfonyl fluoride (PMSF), from Calbiochem; [^{3}H]UTP and [^{3}H]CTP, from Amersham Corp.; [α-^{32}P]CTP, from ICN Pharmaceuticals; Polymin P and 2-mercaptoethanol, from Eastman Organic Chemicals; Trasylol (aprotinin), from FBA Pharmaceuticals; and *Staphylococcus aureus* V8 protease, from Miles Laboratories, Ltd. T7 ΔD111, Mx1, Mx4, and Mx8 DNAs were prepared by phenol extraction of cesium chloride-purified phage particles. T4$^+$ DNA was a gift from Michael Chamberlin. Proteinase K-treated φ29 DNA was a gift from Barry L. Davison. Lambda DNA was provided by Daryl Faulds of our laboratory. *Sal*I and *Hpa*I endonucleases were obtained from New England Bio-Labs.

Bacterial and bacteriophage strains. RNA polymerases were purified from *M. xanthus* strain DZF1(FB) (34) and *E. coli* BG156 (obtained from Michael Chamberlin). The bacteriophage T7 deletion mutant ΔD111, which lacks promoters A_2 and A_3 (14), was obtained from Michael Chamberlin. The following three *Myxococcus* phages were used: Mx8 (29) and Mx1 (1, 2), which were obtained from Dale Kaiser, and Mx4 (6, 16).

Bacteriophage DNAs. DNAs from coliphage T7 ΔD111 and *M. xanthus* phages Mx1, Mx4, and Mx8 were prepared by phenol extraction of CsCl-purified phage particles. Redistilled phenol was saturated with 1.0 M Tris (pH 8.0) before use. T7 ΔD111 was grown on *E. coli* B. The *M. xanthus* bacteriophages were grown on strain DZ1 (43). The phage particles remaining in the supernatant from a low-speed centrifugation of chloroform-treated phage lysates were pelleted by centrifugation at 27,000 rpm for 2 h. The resulting phage pellets were suspended and subjected to CsCl density gradient centrifugation (35,000 rpm, 20 h). Table 1 summarizes the physical properties of these templates.

Cells. Vegetative cells of *M. xanthus* DZF1(FB) were grown to a density of 2×10^9 cells per ml (two-thirds of stationary density) in CYE broth (6) at 30°C in a New Brunswick fermentor with vigorous agitation and aeration. The cells were quickly chilled with ice, harvested with a Sharples continuous flow centrifuge, and frozen at −70°C until they were used.

Buffers. Grinding buffer, TGED buffer (which contained Tris, glycerol, EDTA, and dithiothreitol), and storage buffer were prepared as described by Burgess and Jendrisak (4), with the following modifications: TGED and grinding buffers contained 10% (vol/vol) glycerol, 1.0 mM EDTA, and 0.2 mM dithiothreitol; grinding buffer contained 1.0 mM PMSF and Trasylol (10^6 K.I.V. per liter); TGED used prior to the double-

TABLE 1. Summary of the bacteriophage templates

Bacteriophage	Host	DNA mol wt (10^6)	Guanine-plus-cytosine content (mol%)	Reference(s)
T7 Δ111	*E. coli*	25	50	14
φ29	*B. subtilis*	12	34	12
Mx1	*M. xanthus*	150	50	1, 2
Mx4	*M. xanthus*	39	70	6, 16
Mx8	*M. xanthus*	37	70	29

stranded DNA cellulose step contained 10 mM $MgCl_2$ (TGEDM buffer) and 1.0 mM PMSF; and storage buffer contained 0.3 mM dithiothreitol and 10 mM $MgCl_2$.

Protein determinations. Protein concentrations were determined by the method of Lowry et al. (27). Alternatively, concentrations of purified RNA polymerase were determined spectrophotometrically by using extinction coefficients [$E_{280}(1\%)$] of 6.2 for the holoenzyme and 5.5 for the core enzyme (26); the values determined by this method correlated well with the values determined by the method of Lowry et al. Corrections for light scattering were not made. If the two methods differed significantly, the spectrophotometric determination was used.

RNA polymerase assay. For quantitation of polymerase activity, the standard assay mixture contained (in a volume of 100 μl) 40 mM Tris (pH 8.3), 10 mM $MgCl_2$, 0.2 mM EDTA (pH 8.0), 0.2 mM dithiothreitol, 0.4 mM unlabeled ribonucleoside triphosphates, 150 mM KCl, 2 μCi of [^{3}H]UTP or [^{3}H]CTP, and 4.0 μg of DNA. If necessary, RNA polymerase was diluted into storage buffer immediately before use. All reactions were carried out at 30°C for 5 min unless stated otherwise; 3 ml of ice-cold 10% (wt/vol) trichloroacetic acid (TCA) containing 0.01 M sodium pyrophosphate was added to stop the reaction. The RNA was precipitated for 20 min on ice and then collected on glass fiber filters that had been presoaked in 5% (wt/vol) TCA containing 0.01 M sodium pyrophosphate. The filters were washed four times with 3 ml of 5% TCA–0.01 M sodium pyrophosphate and twice with 95% ethanol and then dried. The radioactivity was determined by liquid scintillation counting (counting efficiency, 35%); 1 U of RNA polymerase activity corresponded to incorporation of 1 nmol of CMP in 10 min at 30°C. Incorporation of CMP was linear with respect to time for 15 min under the conditions described above. The specific activity was calculated from the activity observed in 5 min. The proportion of enzyme activity that was resistant to rifampin (as determined by a prebinding protocol [28]) was determined under standard conditions, except that the KCl concentration was 50 mM and DNA (T7 ΔD111) was used at a concentration of 100 μg/ml. The enzyme (enzyme/DNA ratio, 2) was prebound for 10 min, and the reaction was started with ribonucleoside triphosphates with and without 10 μg of rifampin per ml and continued for 5 min. *M. xanthus* RNA polymerase preparations were always purified through the single-stranded DNA agarose step of the purification procedure. The *E. coli* RNA polymerase preparation utilized was purified from *E. coli* BG156 by the method of Burgess and Jendrisak (4), as modified by Lowe et al. (26). The *E. coli* enzyme used for rifampin-resistant (rapid-start) complex formation was provided by Michael Chamberlin. The standard assay with 40 mM Tris (pH 7.9), T7 ΔD111 DNA, and [^{3}H]UTP was used to monitor activity during purification.

Cell lysis and Polymin P precipitation. *M. xanthus* vegetative cells (60-g batches) were lysed by the EDTA-lysozyme-deoxycholate procedure (26). This treatment was used on frozen cells since freezing and thawing helped disrupt the cell wall structure. Since *Myxococcus* contains proteases, 1.0 mM EDTA, 1.0 mM PMSF, and Trasylol were added to the buffer to minimize proteolysis during cell lysis. In addition, lysis was performed at 0°C in an ice water slurry to minimize proteolysis further. Typically, spheroplast formation and lysis took approximately 40 min.

After cell lysis, RNA polymerase and 10% of the soluble protein were precipitated with 0.25% Polymin P. The resulting Polymin P pellet was then washed with 0.35 M NaCl, which removed 67% of the protein but left more than 95% of the polymerase activity in the pellet. The RNA polymerase was solubilized with 1.0 M NaCl. The solubilized polymerase was then subjected to fractional 40 to 65% ammonium sulfate precipitation. This step was found to be very important; RNA polymerase yields were very low in its absence. The 65% saturated ammonium sulfate slurry was diluted twofold with 40 mM Tris (pH 7.9)–1.0 mM EDTA–0.2 mM dithiothreitol–65% saturated ammonium sulfate before centrifugation to lower the glycerol concentration and allow tight pellet formation. Based on specific activity, an apparent 58-fold purification was achieved at this stage.

Double-stranded DNA cellulose chromatography and Bio-Gel A5m gel filtration. RNA polymerase purified through the Polymin P and ammonium sulfate steps was chromatographed on double-stranded DNA cellulose. Double-stranded DNA cellulose chromatography was performed as described by Lowe et al. (26), with the following modifications: salmon testis DNA (6 mg/ml) was used instead of calf thymus DNA; a 30-ml column was loaded at a rate of 50 ml/h and eluted at the same flow rate with a 100-ml linear 0.15 to 0.75 M NaCl gradient. The enzyme was eluted with a shallow 0.27 to 0.40 M NaCl gradient. Peak activity fractions (fractions 68 to 85) and side fractions (fractions 60 to 67 and 86 to 95) were pooled separately; together, these fractions represented 30% of the activity loaded onto the column. Chromatography on DNA cellulose removed many contaminating proteins, but little nucleid acid (ratio of absorbance at 280 nm [A_{280}] to A_{260}, 1.15). The specific activity of the RNA polymerase was increased from 127 to 224 U/mg by this step of the purification.

The pooled peak fractions eluted from double-stranded DNA cellulose were concentrated by ammonium sulfate precipitation (65% saturated) and applied to a Bio-Gel A5m column, as described by Burgess and Jendrisak (4), using a 40-ml column (ratio of height to depth, 40) and a flow rate of 5 ml/h. The first peak contained almost no protein and had a ratio of A_{280} to A_{260} of 1.0. The main peak contained the RNA polymerase activity. The specific activity of the RNA polymerase was doubled by Bio-Gel A5m gel filtration, but significant losses of protein and activity were incurred. The nucleic acid content was reduced, as

indicated by an increase in the ratio of A_{280} to A_{260} from 1.15 to 1.70.

Purification of core enzyme by using single-stranded DNA agarose. Peak fractions (fractions 42 to 48) from the Bio-Gel A5m column were diluted twofold to 0.25 M NaCl and then applied to a single-stranded DNA agarose column. Single-stranded DNA agarose chromatography was performed as described by Lowe et al. (26), using salmon testis DNA. A 2.5-ml column was eluted with proportionately lower buffer volumes and flow rates. Core enzyme and holoenzyme-enriched activities were eluted with 0.4 and 1.0 M NaCl, respectively, as shown in Fig. 1. The core enzyme fractions had some activity on poly(dA-dT) but very little activity on T7 ΔD111 DNA. In contrast, the holoenzyme-enriched fractions were quite active on both T7 ΔD111 DNA and poly(dA-dT). The remaining nucleic acids were removed, as shown by an increase in the ratio of A_{280} to A_{260} to 1.86. Recovery was quite high, and the specific activity of the RNA polymerase was increased from 435 to 720 U/mg.

Polyacrylamide gel electrophoresis. [^{32}P]RNA was analyzed by electrophoresis in 1.75% acrylamide–0.7% agarose slab gels (17), followed by autoradiography. *E. coli* polymerase transcripts of *Bacillus subtilis* phage φ29 made in vitro were used as molecular weight standards (12). For protein analysis, SDS-polyacrylamide electrophoresis was performed in slab gels (37) by using a discontinuous buffer system (25) and an acrylamide-bisacrylamide ratio of 30:0.8. Samples were precipitated on ice in 10% TCA for 1 h, followed by a single wash in 1.0 ml of 5% TCA and two washes in 1.0 ml of 95% ethanol. Air-dried samples were then suspended in solubilization-reduction buffer by using a sonicating water bath and boiled for 30 s. Samples were electrophoresed at a constant current (25 mA) through 14 cm of a 9% polyacrylamide separating gel, followed by staining with Coomassie blue, destaining, and drying.

Peptide analysis. To determine biochemical relatedness, proteins were cut from dried gels and mixed with *S. aureus* V8 protease, and peptides were analyzed on a second polyacrylamide gel (15%), as described by Cleveland et al. (9).

Effects of KCl concentration. Standard assays were performed by using [^{3}H]UTP with or without 150 mM KCl added on a variety of DNA templates. DNA preparations were dialyzed against 10 mM Tris (pH 8.3)–1 mM EDTA–10 mM KCl to minimize salt contributions from the DNA preparations. Assays without added KCl contained 5 mM NaCl due to the 0.1 M NaCl present in storage buffer.

Optimal concentrations of $MgCl_2$ and ribonucleoside triphosphates. Standard RNA polymerase assays with T7 ΔD111 DNA and [^{3}H]CTP were used to determine $MgCl_2$ and ribonucleoside triphosphate requirements. For the $MgCl_2$ requirement determinations, EDTA was omitted from the assays. For determinations of ribonucleoside triphosphate requirements, the concentrations of three of the ribonucleoside triphosphates were held constant at 0.4 mM, and the concentration of the ribonucleoside triphosphate being tested was varied from 1 to 400 μM. Each of the values in the CTP experiment was corrected for the changing specific activity of [^{3}H]CMP incorporated.

Determination of pH optima. The pH of 100 mM Tris buffer that allowed maximal [^{3}H]CMP incorporation into T7 ΔD111 RNA was determined. Standard assays were performed at pH 7.0 to 9.4.

Sensitivity of RNA polymerase to heparin or rifampin. Standard assays with T7 ΔD111 DNA were used to determine the sensitivities of *E. coli* and *M. xanthus* polymerases to heparin. The sensitivity of *M. xanthus* RNA polymerase to rifampin was determined by using a modified assay procedure. Crude extracts of *M. xanthus* prepared by sonication and low-speed centrifugation were assayed by using reaction mixtures containing ribonucleoside triphosphates at concentrations

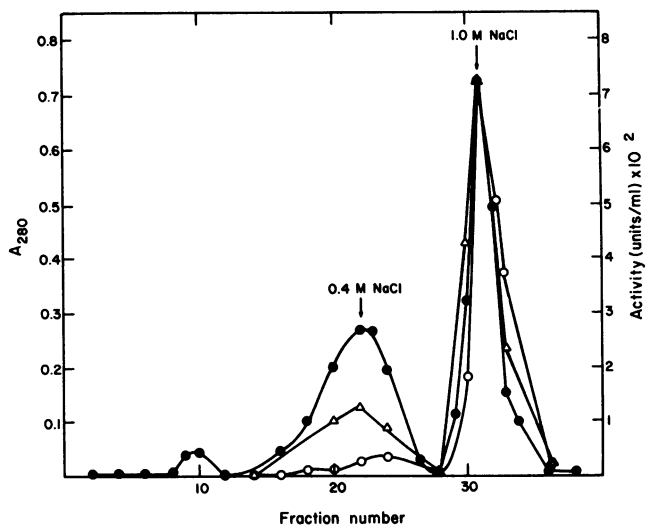

FIG. 1. Chromatography of RNA polymerase on single-stranded DNA agarose. A 3.5-mg amount of *M. xanthus* RNA polymerase from a Bio-Gel A5m column was chromatographed on a 2.5-ml column as described in the text; 0.5-ml fractions were collected and assayed for A_{280} (●) and for activity on T7 ΔD111 DNA (○) and poly (dA-dT) (△).

of 0.15 mM, 40 mM Tris (pH 7.9), and 40 μg of salmon testis DNA per ml; 10 μl of crude extract was used in each 100-μl assay mixture.

Agarose-acrylamide electrophoresis. [^{32}P]RNA was analyzed by electrophoresis in 1.75% acrylamide–0.7% agarose slab gels (17), followed by autoradiography. *B. subtilis* phage φ29 RNA transcripts made in vitro by *E. coli* RNA polymerase were used as molecular weight standards (12). [^{32}P]RNA was removed from an agarose-acrylamide gel by extraction with 10 mM Tris–1.0 mM EDTA. Gel slices were macerated and centrifuged, and the resulting supernatant was withdrawn and used for Southern blot hybridizations. The extraction efficiency was approximately 50%.

DNA restriction and agarose gel electrophoresis. After overnight incubations under appropriate conditions, DNA samples (2 μg/lane) cleaved by *Sal*I and *Hpa*I restriction endonucleases were analyzed by horizontal 0.8% agarose slab gel electrophoresis (30), stained in ethidium bromide (1 μg/ml), and photographed under UV light.

RNA synthesis. RNA for gel analysis and Southern blot hybridizations was synthesized in an assay mixture containing (in a volume of 100 μl) 100 mM Tris (pH 7.9), 10 mM $MgCl_2$, 0.2 mM EDTA (pH 8.0), 0.2 mM dithiothreitol, 0.4 mM unlabeled ribonucleoside triphosphates, 150 mM KCl, [α-^{32}P]CTP (250 to 500 cpm/pmol), 100 μg of DNA per ml, and 1.6 mM spermidine; the reaction was performed at 30°C. Heparin (50 μg/ml) was added at 1.5 min to prevent reinitiation. The reaction was quenched with 25 μl of stop buffer (36). Samples (5 μl) were removed for determinations of the amount of RNA synthesized before gel analysis.

Southern blot hybridizations. Hybridizations of [^{32}P]RNA to restriction fragments of DNA bound to nitrocellulose were performed as described by Southern (35), with the following modifications: nitrocellulose filters with bound DNA fragments were prehybridized in a solution containing 50% formamide, 50 mM sodium phosphate (pH 6.5), 1% (wt/vol) glycine, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 0.5 mg of sonicated denatured salmon testis DNA per ml, and 0.5% SDS for 1 h at 42°C; hybridizations were performed in a solution containing 50% formamide, 5× SSC, 20 mM sodium phosphate (pH 6.5), 0.1 mg of sonicated, denatured salmon testis DNA per ml, and 0.5% SDS for 24 h at 42°C; after hybridization, filters were washed once in 2× SSC–0.5% SDS (25°C), three times in 5× SSC–0.5% SDS–50% formamide (42°C), and once in 2× SSC–0.5% SDS–50% formamide (42°C); and filters were then air dried and subjected to autoradiography. RNA to be hybridized was synthesized as described above for the RNA gel analysis experiments, except that the reaction was stopped by raising the concentration of EDTA to 25 mM; the unincorporated ribonucleoside triphosphates were removed by gel filtration, using a sterile 10-ml Bio-Gel P30 column.

Elongation rate determinations. [^{32}P]RNA was synthesized by using either T7 ΔD111 or Mx4 DNA. At different times samples were withdrawn, mixed with stop buffer (36), and subjected to agarose-acrylamide electrophoresis. The mobilities of the centers of mass of the RNA spots were measured and average molecular weights were determined by using φ29 transcripts as standards (12). The RNA sizes were plotted against time, and mean chain elongation rates were calculated.

RESULTS

RNA polymerase was purified from vegetative cells of *M. xanthus* as described above. Figure 2 shows an SDS-polyacrylamide gel electrophoresis analysis of protein fractions from various stages of the purification. RNA polymerase containing subunit σ is shown in Fig. 2, lane F. This polymerase has a subunit structure typical of RNA polymerases purified from other gram-negative organisms (3). The subunit molecular weights were determined by using *E. coli* RNA polymerase as the standard. Assuming molecular weights of 150,000 and 145,000 for the β′ and β subunits of the *E. coli* enzyme, respectively, and 70,000 and 36,500 for subunits σ and

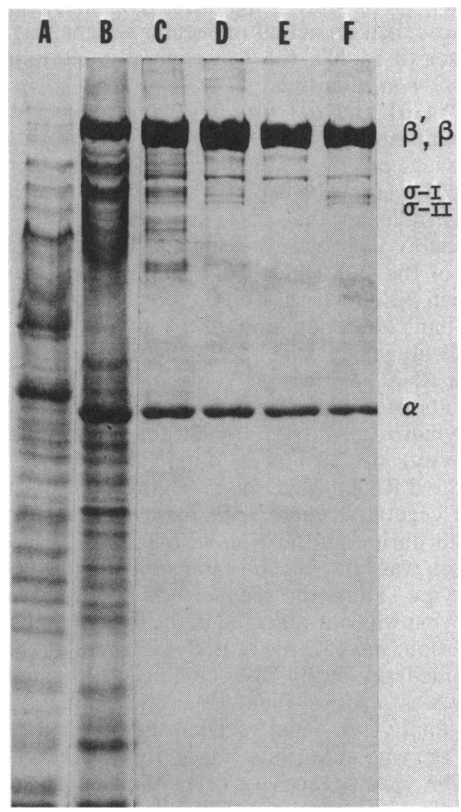

FIG. 2. SDS-polyacrylamide gel electrophoresis of samples at different stages of purification. The following samples were TCA precipitated and electrophoresed as described in the text: lane A, low-speed supernatant (50 μg); lane B, 40 to 65% saturated ammonium sulfate fraction (50 μg); lane C, DNA cellulose pooled peak fractions (30 μg); lane D, Bio-Gel A5m pooled peak fractions (25 μg); lane E, single-stranded DNA agarose 0.4 M NaCl eluate (20 μg); lane F, single-stranded DNA agarose 1.0 M NaCl eluate (15 μg).

α, respectively (3, 4), the molecular weights of the *M. xanthus* β′ and β subunits were estimated to be 145,000 and 140,000, respectively, and the molecular weight of the α subunit was estimated to be 38,000. Two proteins, designated σI and σII, copurified with the holoenzyme activity. These two proteins were very similar in apparent molecular weight to the σ subunit of the *E. coli* enzyme (3) and were the only two proteins that consistently copurified with the holoenzyme activity as opposed to the core enzyme activity. This result was corroborated in four subsequent purifications of holoenzyme. The molecular weights of σI and σII were estimated to be 73,000 and 70,000, respectively, based on the molecular weight of the *E. coli* σ factor (70,000, as determined by sequence analysis [5]). On SDS gels, *E. coli* σ factor has an apparent molecular weight of 86,000. If *M. xanthus* σ factor differs significantly from *E. coli* σ factor, then its actual molecular weight may be closer to 86,000, the value initially determined for *E. coli* σ factor.

The σI and σII proteins were subjected to peptide analysis (Fig. 3). The same two major cleavage products were observed when either σI or σII was cleaved by *S. aureus* V8 protease. This indicates that these two polypeptides are probably very closely related. A peptide analysis of the 110,000-dalton protein migrating between ββ′ and σ showed no relatedness to the σ proteins (data not shown).

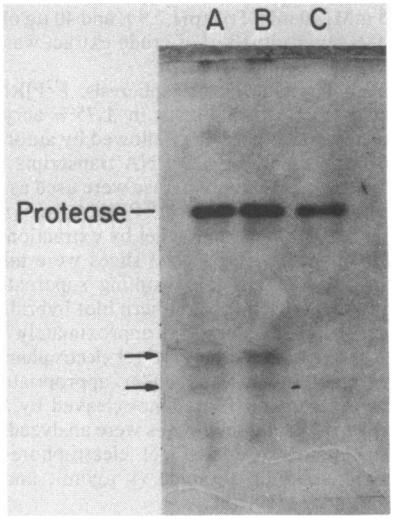

FIG. 3. Peptide analysis of σI and σII. The method of Cleveland et al. (9) was used to determine the relatedness of the putative *M. xanthus* RNA polymerase σ subunits, as described in the text. The lanes contained the σII protein band and protease (lane A), the σI protein band and protease (lane B), and protease alone (lane C).

Yield, purity, and specific activity of *M. xanthus* RNA polymerase. Table 2 summarizes the results of our purification of *M. xanthus* RNA polymerase. An overall yield (based on specific activity) of 7.9% was obtained; 1.65 mg of purified RNA polymerase was obtained from 60 g of vegetative cells. Specific activity was monitored during purification with T7 ΔD111 DNA, which was not efficiently transcribed by the core enzyme. Thus, the recovery of 1.5 mg of core enzyme was not reflected in the overall yield. In addition, the 15.3 mg of active enzyme obtained as side fractions by DNA cellulose chromatography was also not included in the estimation of the final yield. Figure 2 illustrates the purity of the enzyme at various stages of purification.

The specific activity of purified *M. xanthus* RNA polymerase was 720 U/mg when it was assayed on T7 ΔD111 DNA before dialysis against storage buffer. The specific activity declined during storage at −20°C and stabilized at about one-half of its original value after 4 months of storage. Subsequent purifications yielded RNA polymerase of approximately the same specific activity as the enzyme reported here. The *M. xanthus* RNA polymerase activity was completely dependent on exogenous DNA. The incorporation of UMP into RNA was proportional to the amount of enzyme added over the range from 0.135 to 13.5 μg of added enzyme. This shows that the enzyme was stable to dilution in storage buffer. If the enzyme was diluted in TGEDM containing 0.1 M NaCl, activity was lost at dilutions lower than 1:20 (0.68 μg of added enzyme). Spermidine in α-^{32}P-containing reaction mixtures stimulated activity 1.5-fold when T7 ΔD111 or φ29 DNA was transcribed with either *M. xanthus* or *E. coli* RNA polymerase.

M. xanthus RNA polymerase (specific activity, 550 U/mg; 15% active molecules) was capable of forming rapid-start complexes on both Mx1 DNA and T7 ΔD111 DNA. The proportion of activity that formed rapid-start complexes was 40% when T7 ΔD111 DNA was used and 30% when Mx1 DNA was used. The *E. coli* RNA polymerase (specific activity, 2,000 U/mg; 37% active molecules) formed 90% rapid-start complexes when it was assayed on either template.

Specific activity on various DNA templates and salt effects. *M. xanthus* RNA polymerase was assayed by using a variety of DNA templates, with and without added KCl (Table 3). The resulting activities were compared with the activity of *E. coli* polymerase assayed under the same conditions. The specific activity of *M. xanthus* polymerase on T7 ΔD111 DNA was lower than the value reported above (Table 2) because some activity was lost during storage at

TABLE 2. Purification of RNA polymerase from *M. xanthus*[a]

Fraction(s)	Vol (ml)	Protein		Activity		Yield (%)	Purification (-fold)	A_{280}/A_{260} ratio
		Concn (mg/ml)	Total amt (mg)	Sp act (U/mg)	Total activity (U)			
Low-speed supernatant	390	16.7	6,513	2.2	14,300	100		0.56
40 to 65% saturated ammonium sulfate precipitate	65	2.4	256	127	19,800	132	58	1.0
Double-stranded DNA cellulose								
Peak fractions (fractions 68–85)	17.5	0.8	14	224	3,240	21.6	102	1.15
Side fractions (fractions 60–67 and 86–95)	18.0	0.85	15.3	180	2,760	18.4	81	1.13
Bio-Gel A5m peak fractions	3.5	1.0	3.5	435	1,560	10.4	197	1.70
Single-stranded DNA agarose								
0.4 M NaCl eluate	2.5	0.6	1.5	34	52	0.35		1.84
1.0 M NaCl eluate	1.8	0.9	1.65	720	1,188	7.9	327	1.86

[a] The starting material was 60 g (wet weight) of vegetative cells. RNA polymerase activity was assayed with T7 ΔD111 DNA, as described in the text; 1 U of activity represented 1 nmol of [^{3}H]UMP incorporated in 10 min at 30°C. Protein concentrations were determined by the method of Lowry et al. (27). The 0.4 and 1.0 M eluates from single-stranded DNA agarose chromatography were assayed before dialysis in storage buffer.

−20°C. The T7 ΔD111 template was the most efficient template surveyed for both enzymes. The addition of 150 mM KCl stimulated the *E. coli* enzyme about 50% of T7 ΔD111, T4, and *B. subtilis* phage φ29 DNAs. KCl also stimulated *M. xanthus* RNA polymerase on these templates, but to a lesser degree. KCl inhibited *E. coli* and *M. xanthus* RNA polymerase activities on the DNAs from all three *M. xanthus* phages (Mx1, Mx4, and Mx8). This inhibition was more dramatic for *E. coli* polymerase, especially when Mx1 and Mx4 DNAs were used. The specific activities obtained when coliphage λ and the synthetic copolymer poly(dA-dT) were used as templates were relatively insensitive to the concentration of KCl for both enzymes. *M. xanthus* polymerase synthesized φ29 RNA much less efficiently than *E. coli* polymerase did. The only templates that *E. coli* polymerase transcribed less efficiently than *M. xanthus* polymerase were Mx4 and Mx8 DNAs.

TABLE 3. Specific activities of *E. coli* and *M. xanthus* RNA polymerases on a variety of DNA templates[a]

Template	*E. coli* RNA polymerase sp act (U/mg) with:		*M. xanthus* RNA polymerase sp act (U/mg) with:	
	No KCl	150 mM KCl	No KCl	150 mM KCl
T7 ΔD111	850	1,400	390	445
φ29	550	850	110	150
Mx1	295	95	170	105
Mx4	120	50	180	125
Mx8	110	70	185	120
λ	165	140	120	120
T4	725	1,275	230	270
poly(dA-dT)	860	875	385	395

[a] Standard conditions were used to assay various templates as described in the text. Each assay mixture contained 5 μg of *M. xanthus* RNA polymerase or 3 μg of *E. coli* RNA polymerase. In some assays 150 mM KCl was added in addition to the 5 mM NaCl present in the enzyme samples; 1 U of activity represented 1 nmol of [^{3}H]UMP incorporated in 10 min at 30°C.

$MgCl_2$ and ribonucleoside triphosphate requirements. The requirements for Mg^{2+} ions and all four ribonucleoside triphosphates were determined by using T7 ΔD111 DNA and the standard assay. $MgCl_2$ had to be present at a concentration of at least 10 mM to obtain maximal activity for both *E. coli* and *M. xanthus* RNA polymerases. Both enzymes displayed 50% activity at an $MgCl_2$ concentration of 0.8 mM.

The concentration of each of the four ribonucleoside triphosphates was varied independently; efficient transcription was dependent on each of the ribonucleoside triphosphates. The concentrations which allowed 50% maximal activity for each ribonucleoside triphosphate in the presence of the other three at concentrations of 0.4 mM were as follows: ATP, 0.027 mM; GTP, 0.016 mM; CTP, 0.027 mM; UTP 0.021 mM. A small amount of synthesis in the absence of a particular ribonucleoside triphosphate was observed for each ribonucleoside triphosphate except UTP. In each case a minimum concentration of approximately 0.1 mM was required to obtain maximal activity.

Determination of optimal pH. Optimal pHs were determined for *E. coli* and *M. xanthus* RNA polymerases. The optimal pH for *E. coli* RNA polymerase was 8.0, compared with 8.3 for *M. xanthus*. Tris buffer was required at a mini-

mum concentration of 25 mM for the standard assay when T7 ΔD111 DNA was used. Tris was present at pH 7.9 in these reaction mixtures in order to conform with the conditions used by other workers in experiments with *E. coli* polymerase; the same conditions were used for *M. xanthus* RNA polymerase. *M. xanthus* RNA polymerase activity was only 80% of the maximum value at pH 7.9. The standard assays used to monitor activity during the RNA polymerase purification procedure were performed at pH 7.9 because the true optimum was not known at that time. *E. coli* RNA polymerase activity at pH 8.3 is only 80% of the maximum activity at pH 7.9.

Transcriptional inhibitors. The in vitro sensitivities of *M. xanthus* and *E. coli* RNA polymerases to the transcriptional inhibitors rifampin and heparin were determined. The 50% level of inhibition by rifampin was 17 ng/ml (20 μM) for *M. xanthus* RNA polymerase. This was in good agreement with the value obtained for *E. coli* RNA polymerase when purified enzyme was used (M. Temple, M.S. thesis, University of California, Berkeley, 1976). Although the concentrations of heparin that inhibited the reaction 50% (*E. coli*, 3.4 μg/ml; *M. xanthus*, 1.7 μg/ml) were similar, the *E. coli* enzyme responded to a lower concentration than the *M. xanthus* RNA polymerase and was not inhibited as effectively at higher concentrations. Streptolydigin at a concentration of 100 μg/ml completely inhibited *M. xanthus* RNA polymerase in vitro.

Chain elongation rates. To study the ability of *M. xanthus* RNA polymerase to transcribe various phage DNA templates, it was necessary to compare the chain elongation rates of the *M. xanthus* and *E. coli* enzymes so that comparable amounts of RNA product could be analyzed. *E. coli* and *M. xanthus* RNA polymerases were incubated with DNA extracted from coliphage T7 ΔD111 or *Myxococcus* phage Mx4. At different times, samples of the RNA synthesis reaction mixtures were withdrawn, and the ^{32}P-labeled RNA products were analyzed by agarose-acrylamide gel electrophoresis, as described above. Heparin clearly blocked re-initiation under these conditions, producing fairly synchronous transcription. Omitting heparin significantly increased the proportion of radioactivity that ran as low-molecular-weight RNA (data not shown). The mobilities of the RNA bands were measured for each time interval, and the average molecular weights were determined by using standards consisting of the in vitro transcripts of *B. subtilis* phage φ29. These in vitro transcripts cover a size range from 300 to 5,000 nucleotides (12) and are linear with respect to the logarithm of molecular weight. The sizes of the RNA products as a function of time of incubation are shown in Fig. 4. After a brief lag,

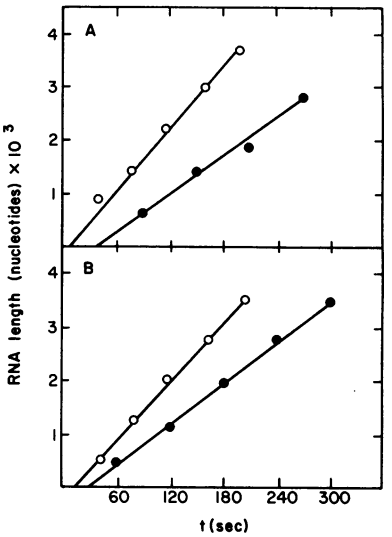

FIG. 4. Elongation rates of RNA polymerases from *E. coli* and *M. xanthus* on phage T7 ΔD111 and Mx4 DNAs. *E. coli* RNA polymerase or *M. xanthus* RNA polymerase was incubated with phage T7 ΔD111 DNA or phage Mx4 DNA as described in the text. At different times, 100-μl samples were withdrawn from reaction mixtures and subjected to gel electrophoresis and autoradiography. The amount of RNA product was determined from the total amount of [α-^{32}P]CMP (250 cpm/pmol) incorporated into 100-μl samples. The mobilities (center of mass) of the RNA bands were measured for each time interval, and the average molecular weights were determined by using φ29 standards. The sizes of the RNA products are plotted as a function of the time of incubation. (A) T7 ΔD111 DNA used with *E. coli* RNA polymerase (○) or *M. xanthus* RNA polymerase (●). (B) Mx4 DNA used with *E. coli* RNA polymerase (○) or *M. xanthus* RNA polymerase (●).

E. coli RNA polymerase synthesized RNA at a rate of 19 nucleotides per s at 30°C with either T7 ΔD111 DNA (guanine-plus-cytosine content, 50 mol%) or Mx4 DNA (guanine-plus-cytosine content, 70 mol%). *M. xanthus* RNA polymerase showed a lag of about 30 s and then synthesized RNA at a rate of 13 nucleotides per s at 30°C, again using either template. It should be noted that these reactions were carried out under the standard conditions used for the *E. coli* polymerase. Under the conditions used, the *M. xanthus* enzyme, which has a higher optimum pH, shows only 80% of its maximal activity.

Transcription of T7 ΔD111 DNA as analyzed by RNA gels. The DNA of coliphage T7 is a well-defined template for in vitro transcription studies. The in vitro transcription map of phage T7 when *E. coli* RNA polymerase was used is shown in Fig. 5. The mutant T7 ΔD111 contains an 1,100-base pair (bp) deletion that removes

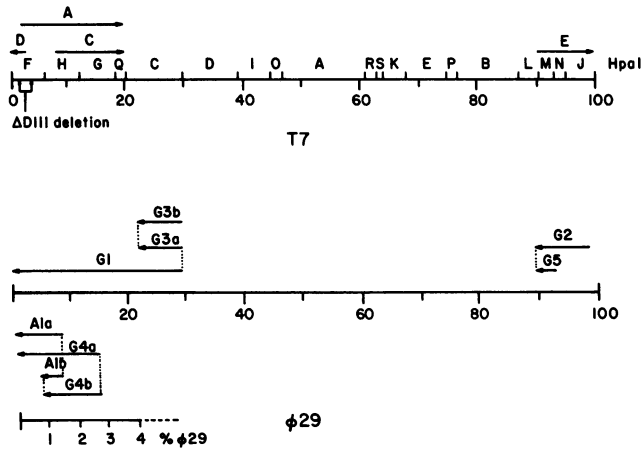

FIG. 5. In vitro transcription maps of *E. coli* phage T7 ΔD111 and *B. subtilis* phage ϕ29 DNAs. The positions of the T7 ΔD111 transcripts were taken from Stahl and Chamberlin (36). The positions of the *Hpa*I restriction fragments were taken from McDonnell et al. (30). The positions of the ϕ29 transcripts were taken from Davison et al. (13). The lower left corner of the figure shows an enlargement of the first 3% of the ϕ29 genome.

promoters A_2 and A_3, leaving a template with a single major promoter (A_1) and three minor promoters (designated C, E, and D) (14). Transcripts initiated in vitro at promoters A_1 and C terminate at the 20% terminator, forming the following two easily resolved transcripts: A (2.25 megadaltons) and C (1.7 megadaltons). Transcripts initiated at minor promoters E and D terminate at the ends of the DNA, giving the following two additional transcripts: E (1.2 megadaltons) and D (0.075 megadaltons). Although these promoters are generally recognized by a wide variety of bacterial RNA polymerases (40), differences in promoter utilization do occur.

Figure 6 shows the in vitro transcripts synthesized from T7 ΔD111 DNA by *E. coli* (20% active molecules [Fig. 6, lane 1]) and *M. xanthus* (10% active molecules [lane 2]) RNA polymerases. The transcripts were synthesized under free-initiation conditions at enzyme/DNA molar ratios of >10. *E. coli* RNA polymerase synthesized transcripts A_1, C, E, and D. *M. xanthus* RNA polymerase also synthesized RNAs with mobilities similar to those of transcripts A_1, C, and E. Transcript D could be detected with *E. coli* RNA polymerase but not with *M. xanthus* RNA polymerase after long exposures of the gel. However, we do not know whether small but undetected amounts of transcript D may have been synthesized since this transcript was difficult to detect even with the *E. coli* polymerase. *M. xanthus* RNA polymerase appeared to recognize promoter A_1 more efficiently than promoter C, which was read more efficiently than promoter E, establishing a gradient of promoter strength (A_1 > C > E). This was also observed in Fig. 6, lane 3.

Transcription of T7 ΔD111 DNA and Southern blot hybridization. Figure 6 also shows that a large amount of ^{32}P-labeled RNA migrated as disperse high-molecular-weight material. Initially, it was thought that this material was "readthrough" RNA that failed to be terminated. To characterize this RNA and the presumed T7 transcripts more carefully, hybridization of the RNA to restriction fragments of the template was undertaken.

Figure 7, lane 1, shows the *Hpa*I restriction digest of T7 ΔD111 DNA. The deletion in T7 ΔD111 DNA reduced the size of *Hpa*I restriction fragment F (Fig. 5) from 2,500 to 1,400 bp (30) and the size of the A_1 transcript from 7,133 to 6,030 nucleotides. Fragment F of T7 ΔD111 migrated just above fragment L. RNA polymerase initiating transcription at the A_1 promoter has been shown to transcribe fragments F (1,400 bp), H (2,312 bp), and G (2,464 bp), in that order (30). Therefore, we allowed the *E. coli* and *M. xanthus* RNA polymerases to transcribe approximately 3,000 bp of T7 ΔD111 DNA. The RNA was then hybridized to Southern blots of the *Hpa*I fragments shown in Fig. 7, lane 1. Figure 7, lane 2, shows that fragments H and F were the primary restriction fragments that hybridized with labeled RNA. However, *M. xanthus* RNA polymerase (Fig. 7, lane 3) also produced minor amounts of RNA which hybridized with restriction fragments A, B, C, D, I, and J. At higher enzyme/DNA ratios, many restriction fragments were labeled, and less than 50% of the labeled RNA was bound to restriction fragments H and F. This suggests that the disperse high-molecular-weight RNA in Fig. 6, lanes 2 and 3, might not be readthrough RNA but, alternatively, might represent nonselective transcription (i.e.,

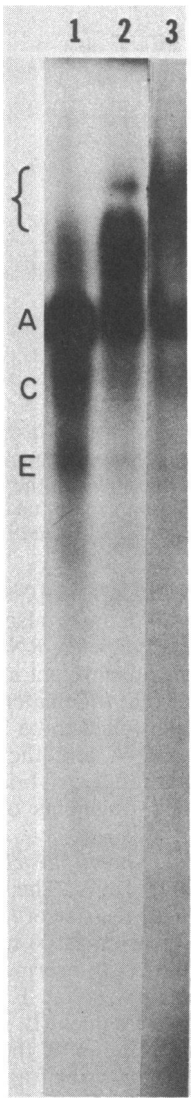

FIG. 6. Electrophoretic analysis of RNA transcripts from T7 ΔD111 DNA synthesized by *M. xanthus* and *E. coli* RNA polymerases. The reaction conditions, slab gel electrophoresis, and autoradiography were as described in the text, except that KCl was present at a concentration of 100 mM. Lane 1, Transcripts made in 8 min by 2.3 μg of *E. coli* RNA polymerase (enzyme/DNA ratio, 11); lane 2, transcripts made in 12 min by 2.7 μg of *M. xanthus* RNA polymerase (enzyme/DNA ratio, 13); lane 3, transcripts made in 15 min by 0.8 μg of *M. xanthus* RNA polymerase (enzyme/DNA ratio, 4). The amount of TCA-precipitable radioactivity applied to each track and the total amount of α-^{32}P-labeled RNA were as follows: lane 1, 30,000 cpm and 1.2 nmol, respectively; lane 2, 25,000 cpm and 0.3 nmol, respectively; lane 3, 80,000 cpm and 0.28 nmol, respectively. The bracket indicates the position of the disperse high-molecular-weight RNA (see text).

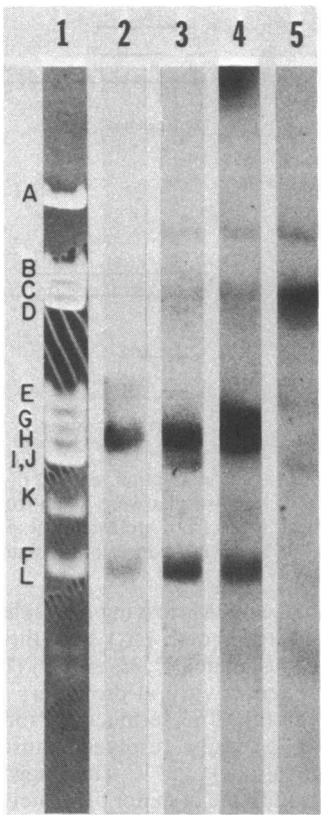

FIG. 7. Hybridization of RNA synthesized in vitro by *E. coli* and *M. xanthus* RNA polymerases to *Hpa*I restriction fragments of T7 ΔD111 DNA. *Hpa*I restriction fragments of T7 ΔD111 DNA were separated on agarose gels (lane 1) and then transferred to nitrocellulose for hybridization analysis of the RNA, as follows: lane 2, RNA synthesized for 3 min (~3,000 nucleotides) with *E. coli* RNA polymerase (enzyme/DNA ratio, 4); lane 3, RNA synthesized for 4.3 min (~3,000 nucleotides) with *M. xanthus* RNA polymerase (enzyme/DNA ratio, 4); lane 4, RNA eluted from the A_1 transcript region of the gel shown in Fig. 4, lane 3; lane 5, RNA eluted from the disperse high-molecular-weight region of Fig. 4, lane 3.

RNA which results from transcription of DNA regions that are not included in the defined program of selective transcription established by using *E. coli* RNA polymerase). To investigate this possibility further, the labeled RNAs from Fig. 6, lane 3, corresponding to the A_1 transcript and the disperse high-molecular-weight RNA (Fig. 6, lane 3, bracket) were extracted from the gel and hybridized to Southern blots of the *Hpa*I restriction fragments shown in Fig. 7, lane 1. Figure 7, lane 4, shows that the labeled RNA corresponding to the A_1 transcript hybridized to fragments G, H, and F, as predicted. In addition, a small amount of labeled RNA hybridized

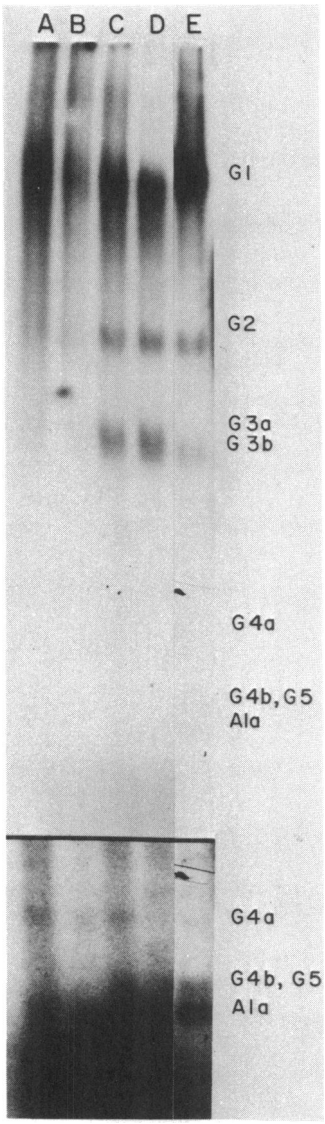

FIG. 8. Electrophoretic analysis of RNA transcripts synthesized from ϕ29 DNA by *M. xanthus* and *E. coli* RNA polymerases at varying concentrations of KCl. The reaction mixtures contained 2 μg of *E. coli* RNA polymerase or 5 μg of *M. xanthus* RNA polymerase. The reaction conditions, gel electrophoresis, and autoradiography were as described in the text, except that the reaction times were 14 min and 50 μg of heparin per ml was added at 10 min. Lanes A through D contained RNA synthesized by using *M. xanthus* RNA polymerase at the following concentrations of KCl: lane A, no added KCl; lane B, 50 mM; lane C, 100 mM; lane D, 150 mM. Lane E contained RNA synthesized by using *E. coli* RNA polymerase and 40 mM KCl. Equal amounts of TCA-precipitable radioactivity were applied to each lane. The total amounts of ^{32}P-labeled RNA (250 cpm/pmol) synthesized in the reactions were as follows: lane A, 0.68 nmol; lane B, 0.68 nmol; lane C, 0.68 nmol; lane D, 0.84 nmol; lane E, 1.6 nmol. The lower-molecular-weight transcripts could be resolved only with longer exposures of the photographs and are shown at the bottom.

to several higher-molecular-weight fragments. As Fig. 6 shows, this could result from trailing of the disperse RNA into the A_1 region. Figure 7, lane 5, shows that the labeled RNA corresponding to the disperse high-molecular-weight material did not appear to be A_1 readthrough RNA. Figure 5 predicts that RNA of this size (15,000 nucleotides) which includes the A_1 transcript should hybridize primarily with fragments G, H, F, C, and, possibly, D. Instead, the RNA hybridized primarily with fragment B, with significant amounts also bound to fragments A, C, D, I, and J. Thus, despite the appearance of inefficient termination displayed in Fig. 6, lane 3, the termination efficiency at the 20% terminator of phage T7 may actually be quite high with *M. xanthus* RNA polymerase. However, since fragment B appears to be complementary to most of this RNA, the data suggest that the *M. xanthus* enzyme actually recognizes a promoter in this region which is not recognized by the *E. coli* enzyme.

Transcription of ϕ29 DNA. An in vitro transcription map of *B. subtilis* phage ϕ29 DNA is shown in Fig. 5. Eight transcripts can be resolved on agarose-acrylamide gels and have been given the following designations: G1 (1.74 megadaltons), G2 (0.66 megadaltons), G3a (0.43 megadaltons), G3b (0.38 megadaltons), G4a (0.16 megadaltons), G5 and G4b (0.11 megadaltons), A1a (0.093 megadaltons), and A1b (0.43 megadaltons). A1b is too small to detect with the gel system used in this study, and G5 and G4b comigrate and are not resolved. Whereas *E. coli* RNA polymerase synthesizes all of these transcripts in vitro, *B. subtilis* polymerase fails to produce transcripts G3a, G4a, and G5 (G4b) (12, 13). Figure 8 shows that *M. xanthus* RNA polymerase synthesized all seven of the detectable transcripts under the appropriate conditions (ionic strength, 100 mM KCl). At low salt concentrations transcripts G2, G3a, G3b, and G4b (G5) were not detected. This also occurs with *E. coli* and *B. subtilis* RNA polymerases (12, 13), suggesting that salt is required by RNA polymerases to terminate these transcripts in vitro. These are the only ϕ29 transcripts that have internal terminators; the transcripts that terminate at the ends of the genome (G1, G4a, and A1a) could be visualized under low-salt conditions.

Transcription of *M. xanthus* phage DNA templates. To examine in vitro transcription in a homologous system, DNAs were purified from *M. xanthus* bacteriophages Mx1, Mx4, and Mx8. Several attempts were made to identify

discrete RNA transcripts synthesized from Mx4 and Mx8 DNA templates with both *M. xanthus* and *E. coli* RNA polymerases. Although the elongating RNA could be visualized easily (data not shown), no discrete transcripts were detected.

However, three discrete transcripts were found when the in vitro RNA products synthesized from phage Mx1 DNA were separated electrophoretically on agarose-acrylamide gels. These transcripts were observed with both *E. coli* RNA polymerase (Fig. 9, lane 1) and *M. xanthus* RNA polymerase (lane 2). The molecular weights of the three transcripts were determined by using the A_1, C, and E transcripts of T7 ΔD111 as standards. The molecular weights were as follows: A, 2.55×10^6 (6,890 nucleotides); B, 1.95×10^6 (5,270 nucleotides); C, 1.56×10^6 (4,215 nucleotides). The transcripts formed by *E. coli* and *M. xanthus* RNA polymerases were identical in molecular weight.

To characterize these transcripts further, the RNA products of the *E. coli* and *M. xanthus* polymerases were hybridized to *Sal*I restriction fragments of phage Mx1 DNA. *Sal*I endonuclease was chosen because it cleaves Mx1 DNA into a reasonable number of discrete restriction fragments (Fig. 10, lane 1). RNA polymerase from *E. coli* or *M. xanthus* was then incubated with phage Mx1 DNA under conditions which permitted about 2,000 nucleotides of synthesis and then hybridized to a Southern blot prepared from the *Sal*I digest of Mx1 DNA. The results are presented in Fig. 10, lanes 2 and 3. Most of the RNA hybridized to only a limited number of restriction fragments; the RNA synthesized with *E. coli* polymerase hybridized to fragments I, J, L, M, and Q (Fig. 10, lane 2), and the RNA synthesized with *M. xanthus* polymerase hybridized with fragments D, I, J, L, and M (Fig. 10, lane 3). Although *E. coli* RNA polymerase did not appear to synthesize RNA complementary to fragment D and *M. xanthus* polymerase failed to synthesize RNA complementary to fragment Q, both enzymes transcribed RNA which was complementary to fragments I, J, L, and M.

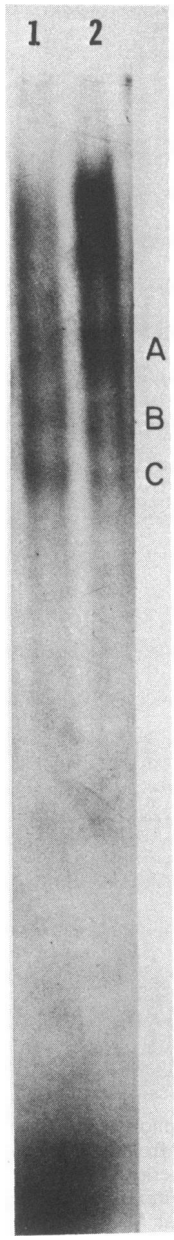

FIG. 9. Electrophoretic analysis of RNA transcripts from phage Mx1 DNA. The RNA polymerase reactions, gel electrophoresis, and autoradiography were performed as described in the text. Lane 1, Transcripts made in 22.5 min by *E. coli* RNA polymerase (1.3 μg; enzyme/DNA ratio, 40); lane 2, transcripts made in 22.5 min by *M. xanthus* RNA polymerase (1.3 μg; enzyme/DNA ratio, 40). The total amount of [α-^{32}P]CMP (500 cpm/pmol) incorporated into RNA (reaction volume, 50 μl) and the TCA-precipitable radioactivity applied were as follows: lane 1, 82 pmol and 40,000 cpm, respectively; lane 2, 110 pmol and 50,000 cpm, respectively.

DISCUSSION

In this paper, we describe the purification of DNA-dependent RNA polymerase from *M. xanthus*. The Burgess Polymin P purification procedure was chosen to purify *M. xanthus* RNA polymerase for a variety of reasons. First, the procedure had been used successfully to purify RNA polymerases from several bacterial species (40). Also, the procedure can be scaled down easily, depending upon the requirements of the particular research goal (19). And finally, the

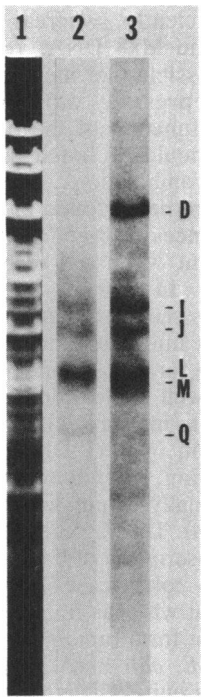

FIG. 10. Hybridization of RNA synthesized by *E. coli* and *M. xanthus* RNA polymerases to *Sal*I restriction fragments of Mx1 DNA. RNA polymerase reactions and Southern blot hybridization were performed as described in the text and in the legend to Fig. 9. Lane 1 contained Mx1 DNA fragmented with *Sal*I restriction enzyme and then subjected to agarose gel electrophoresis. The transcriptionally active fragments are indicated. The molecular weights were as follows: fragment D, 4.5×10^6; fragment I, 2.8×10^6; fragment J, 2.4×10^6; fragment L, 1.8×10^6; fragment M, 1.7×10^6; and fragment Q, 1.45×10^6. T7 ΔD111 DNA restriction fragments were used as standards for molecular weight determinations. Lane 2 contained DNA fragments which hybridized with RNA synthesized for 2.16 min (~2,000 nucleotides) by using *E. coli* RNA polymerase. Lane 3 contained DNA fragments which hybridized with RNA synthesized for 3.0 min (~2,000 nucleotides) by using *M. xanthus* RNA polymerase. The amounts of ^{32}P-labeled RNA added to the hybridization reactions were as follows: lane 1, 11 pmol (5,500 cpm); lane 2, 18 pmol (9,000 cpm).

procedure allows rapid purification with satisfactory yield, purity, and specific activity. Although significant modification was required, the Burgess procedure was used successfully to purify *M. xanthus* RNA polymerase more than 300-fold.

Protease activity presented a substantial problem in recovering active RNA polymerase from *M. xanthus*. The addition of 1.0 mM EDTA, 1.0 mM PMSF, and Trasylol helped minimize proteolytic degradation during the initial steps of the purification, but this treatment was not sufficient to allow significant recovery of active RNA polymerase. Working at 0°C during the lysis step also helped minimize proteolysis. The entire purification procedure was performed quickly and without interruption to ensure adequate recovery of activity. Despite all of these efforts to minimize proteolysis, several early attempts to purify *M. xanthus* RNA polymerase produced low yields of active enzyme. Only by incorporating fractional ammonium sulfate precipitation before DNA cellulose chromatography was it possible to obtain significant amounts of sigma-containing holoenzyme.

Single-stranded DNA agarose was effective for separating a core fraction and an enriched holoenzyme of *M. xanthus* RNA polymerase. A nearly quantitative yield was observed, and additional nucleic acid was removed. The purity of the enriched holoenzyme fraction (Fig. 2, lane F) appeared to be very high. The enriched holoenzyme fraction contained one major protein contaminant with a molecular weight of 110,000. This contaminant resembled protein X (a contaminant discussed by Burgess [3]) in molecular weight and in its ability to bind to core enzyme. This protein has been observed in *E. coli*, *B. subtilis*, and *Lactobacillus curvatus* RNA polymerase preparations, and it has been suggested that it might be a degradation product of the β′ subunit (3). A protein of the same apparent molecular weight was the sole major contaminant in the preparation of *E. coli* RNA polymerase purified by the Polymin P method used in our studies. Additional minor contaminants of *M. xanthus* RNA polymerase were revealed when larger amounts of enzyme were subjected to gel electrophoresis.

The percentage of active molecules of *M. xanthus* holoenzyme can be estimated roughly from specific activity (8). The specific activity of *M. xanthus* RNA polymerase was 720 U/mg. Based on a holoenzyme molecular weight of 0.5×10^6 and a chain elongation rate of 13 nucleotides per s, it is possible to calculate a maximal specific activity of 3,900 U/mg. Thus, the *M. xanthus* holoenzyme preparation probably contained about 20% active molecules. A similar calculation for the *E. coli* RNA polymerase purified for this work indicated that the *E. coli* preparation contained about 25% active molecules when it was transcribing T7 ΔD111 DNA.

If *E. coli* RNA polymerase is preincubated with DNA in the absence of ribonucleoside triphosphates, stable complexes are formed that are capable of initiating transcription when they are challenged with a mixture of ribonucleoside triphosphates and rifampin. This has been used as a rough estimate of σ factor content (28, 40). Both *M. xanthus* and *E. coli* RNA polymerases formed rapid-start complexes on both T7 ΔD111

DNA and Mx1 DNA at 30°C with 10 min of prebinding. This indicated that *M. xanthus* RNA polymerase contained a reasonable amount of σ factor and that Mx1 DNA contained promoter-like sites.

M. xanthus RNA polymerase preparations contained two proteins that behaved like σ factor (σI and σII). These two proteins appeared to be closely related on the basis of the peptides generated by *S. aureus* V8 protease digestion. Both forms of σ could have been present in vivo (either resulting from divergent evolution or in vivo modification), but in view of the protease problems encountered, a more likely explanation is that the σII protein was an in vitro cleavage product of the σI protein that retained the ability to bind to holoenzyme. It seems likely that σI or σII must function as the σ subunit of RNA polymerase because these proteins were the only proteins found in substantial quantities in enriched holoenzyme preparations and not in core enzyme preparations. The holoenzyme preparation contained 20% active molecules, and 40% of these molecules were capable of forming rapid-start complexes. This implies that the σ content should have been sufficiently high to detect on polyacrylamide gels. The 110,000-molecular-weight contaminant was present in enriched holoenzyme preparations, but was also present in core enzyme preparations. It is unlikely that this contaminant was σ factor because the activity of *M. xanthus* core enzyme on T7 ΔD111 DNA was low. This implied that *M. xanthus* core polymerase was deficient in σ content, which is required for efficient utilization of T7 ΔD111 DNA. It is possible that additional σ-like factors (18, 41) were present as minor contaminants or were lost during the purification process.

When the specific activities of *E. coli* and *M. xanthus* RNA polymerases on various templates are compared, it should be kept in mind that the slower elongation rate of the *M. xanthus* RNA polymerase at pH 7.9 was reflected in these activities. Also, the enzyme reaction conditions were optimized for T7 ΔD111 DNA and may not have been optimal for all of the other templates. T7 ΔD111 DNA was chosen to monitor purification and optimize reaction conditions because this DNA is very well characterized and has a single, strong promoter that is known to be recognized in vivo by a variety of bacterial RNA polymerases (40). This DNA was also the most efficiently utilized DNA template tested. Differences between the specific activities of the *M. xanthus* and *E. coli* RNA polymerases on the φ29, Mx4, and Mx8 DNA templates cannot be explained solely on the basis of differences in percentages of active molecules and chain elongation rates. *M. xanthus* transcribed φ29 DNA relatively inefficiently, whereas *E. coli* transcribed Mx4 and Mx8 DNAs relatively inefficiently. It is possible that these relative incompatibilities represent different promoter strengths as a function of the polymerase utilized (40). It should be noted that these were heterologous combinations; these inefficient combinations are never found in vivo.

Other differences between the two enzymes became apparent when the responses to KCl were examined. In general, *M. xanthus* polymerase was less sensitive than *E. coli* polymerase to both salt inhibition and salt stimulation. The mechanism for salt stimulation or salt inhibition is not well understood, making these results difficult to interpret. Salt inhibition of *E. coli* transcription of Mx1 DNA was the most dramatic inhibition. Activity was reduced sharply and was virtually eliminated at 200 mM KCl (data not shown). This is similar to the effect of KCl on the transcription of T4 and T7 DNAs by *B. subtilis* RNA polymerase (12). An interesting difference is that whereas *B. subtilis* RNA polymerase does not form rapid-start complexes on T7 DNA (40), *E. coli* RNA polymerase forms such complexes quite efficiently on Mx1 DNA.

The magnesium ion and ribonucleoside triphosphate experiments were performed primarily to determine whether these components were limiting or inhibitory at the concentrations used in the standard assay. The significance of the shift in the pH optimum for the *M. xanthus* enzyme relative to the *E. coli* enzyme is not known. The difference in the results of the heparin sensitivity determinations for *E. coli* and *M. xanthus* RNA polymerases may have been partially due to the fact that the *E. coli* RNA polymerase was used at a higher concentration than the *M. xanthus* RNA polymerase.

The data presented above show that *M. xanthus* RNA polymerase is capable of selective transcription on a variety of phage DNA templates. This selectivity is, to a first approximation, the same selectivity demonstrated by *E. coli* RNA polymerase in vitro. The use of T7 and φ29 DNAs as templates allows the identification of specific transcripts by using two well-defined systems with published transcription maps.

The T7 phage genome is the most well characterized of these templates. The T7 ΔD111 deletion mutant has only one major promoter, A_1, and one terminator. In addition, this mutant has minor promoters C, E, and D. The relative efficiencies with which these promoters are utilized at low enzyme/DNA ratios reflect the relative strengths of these promoters as a function of the RNA polymerase used. The *M. xanthus* RNA polymerase utilized these promoters with relative efficiencies in the following order: A > C > E. Transcript D either was not

produced or was produced in small quantities. This gradient of promoter strength is similar to that observed when *Caulobacter cresentus* or *L. curvatus* RNA polymerase was used in a similar experiment (40); it differs from *E. coli* RNA polymerase in that transcript C formation is not drastically reduced at low enzyme/DNA ratios.

The Southern blot hybridization analysis of T7 ΔD111 transcription clearly demonstrated that the large, major transcript made by *M. xanthus* RNA polymerase not only has the same molecular weight as the A_1 transcript, but also has A_1 sequence homology. The nature of the disperse high-molecular-weight RNA was less clear. This RNA was not homologous to the A_1 transcript and therefore is likely to be caused by transcription from a promoter(s) in the late region rather than transcription through the A_1 terminator. Since this RNA was primarily homologous to only one restriction fragment, fragment B, the data suggest that the *M. xanthus* enzyme has a slightly different specificity in vitro than the *E. coli* enzyme on the T7 ΔD111 DNA template. Several cloned *Hpa*I restriction fragments from the late region of T7 (including fragment B) have been shown to contain weak promoters utilized in vivo by *E. coli* RNA polymerase (39). *E. coli* RNA polymerase recognizes tight-binding sites in the late region of T7 in vitro and is capable of initiating transcription at a low frequency at these sites (23). It is possible that the RNA synthesized by *M. xanthus* RNA polymerase in vitro which was homologous to the restriction fragments containing late T7 DNA (notably fragment B) was initiated at these tight-binding sites. Since the *M. xanthus* RNA polymerase preparations used contained some core enzyme, it is not known whether *M. xanthus* σ factor is required to produce these late region transcripts in vitro.

The φ29 phage genome is also a useful template for the study of in vitro transcription. The RNA transcripts made by *B. subtilis* and *E. coli* RNA polymerases in vitro when φ29 DNA is used are consistent with the in vivo transcription pattern observed in *B. subtilis* during early phage infection (13). At a KCl concentration of 100 mM *M. xanthus* RNA polymerase was able to recognize at least five and possibly six promoters that are also binding sites for *B. subtilis* RNA polymerase and *E. coli* RNA polymerase. Considering the wide taxonomic diversity of these organisms, this certainly provides additional evidence for the universality of promoter recognition in bacteria (40). In addition, at least two of the three φ29 terminators utilized in vitro by *E. coli* RNA polymerase were recognized by *M. xanthus* RNA polymerase.

When the φ29 DNA template is used, *E. coli* RNA polymerase makes three transcripts in vitro (G3a, G4a, and G5 [G4b]) that are not made efficiently by the *B. subtilis* RNA polymerase. However, at high enzyme/DNA ratios, two of these three transcripts can be detected, indicating that the failure of *B. subtilis* to utilize these promoters efficiently in vitro is a result of lowered promoter strength and not a result of a total lack of recognition ability. *M. xanthus* RNA polymerase synthesizes the same transcripts in vitro as *E. coli* RNA polymerase. Thus, *M. xanthus* polymerase more closely resembles *E. coli* polymerase in the recognition of promoter strengths on φ29 DNA than *B. subtilis* polymerase.

We were unable to demonstrate defined transcripts synthesized by RNA polymerases from *M. xanthus* or *E. coli* in vitro when phage Mx8 or Mx4 DNA was used as a template. RNA was synthesized in vitro, but it did not show any termination. If the elongating Mx4 and Mx8 RNAs observed on gels were initiated at promoter sites, there are several possible explanations for the failure to terminate. For example, the transcripts may be very long in vivo, making their analysis in vitro difficult by conventional techniques. Alternatively, the terminators may not be recognized in vitro under the assay conditions used; rho factor may be required for the recognition of these terminators in vitro.

The formation of discrete transcripts was observed when phage Mx1 DNA was transcribed with *E. coli* or *M. xanthus* RNA polymerase. Both RNA polymerases seemed to recognize the same promoters since they both made transcripts A, B, and C. Active promoter recognition by our *E. coli* RNA polymerase preparation was strong since more than 90% of the active molecules were capable of forming rapid-start complexes on this template. *M. xanthus* RNA polymerase was also capable of forming rapid-start complexes on the Mx1 template. However, termination of these transcripts may not have occurred at internal terminators, since all three of these discrete transcripts (transcripts A, B, and C) could have been formed by termination at the ends of the DNA.

Further evidence of specific promoter recognition on the phage Mx1 template was obtained by Southern blot hybridization analysis. Only 6 of the more than 25 discrete *Sal*I restriction fragments of Mx1 DNA appeared to be transcribed efficiently in vitro when either *E. coli* or *M. xanthus* RNA polymerase was used. Four of these fragments were made by both enzymes and may represent the transcripts containing the promoters for transcripts A, B, and C. The transcription of fragment D by only the *M. xanthus* polymerase and the transcription of fragment Q by only the *E. coli* polymerase may represent a difference in strength or specificity of promoter recognition in vitro. In any case, it

is clear that specific initiation occurred in vitro on Mx1 DNA when either *E. coli* or *M. xanthus* RNA polymerase was used.

Purification and preliminary characterization of *M. xanthus* RNA polymerase has shown that this enzyme is similar to *E. coli* RNA polymerase in many respects. Chromatographic behavior, general salt effects, subunit molecular weights, responses to inhibitors and stimulators of transcription, and general reaction conditions were all similar for the two enzymes. Some differences in elongation rate, template utilization efficiency, degree of salt effects, and pH optimum were found. These may reflect evolutionary or ecological differences between the two organisms. The analysis of transcription with several bacteriophage templates showed that despite the wide taxonomic gap between *E. coli* and *M. xanthus*, the *M. xanthus* enzyme exhibits selectivity in vitro similar to that of the *E. coli* RNA polymerase. Since the RNA polymerase of *M. xanthus* appears to show selective transcription on several phage DNA templates, it would be of interest to investigate transcription by this enzyme on cloned developmental genes.

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