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Identification of the RNA Products of the *ops* Gene of *Myxococcus xanthus* and Mapping of *ops* and *tps* RNAs

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The expression of the *ops* gene, like that of the highly homologous and closely linked *tps* gene, is induced during development of the fruiting bacterium *Myxococcus xanthus*. The RNA products of the *ops* gene have been identified and compared with *tps* RNA. The *ops* RNA was observed in developmental cells only after spore formation had commenced, and it was necessary to use a sporulation-defective mutant strain or to disrupt spores to isolate this RNA. RNA from the *ops* gene was not observed in vegetative cells but was readily detected in cells subjected to glycerol-induced sporulation. In contrast, a large amount of developmental *tps* RNA was observed in cells well before sporulation had occurred; low levels of *tps* RNA were observed in vegetative cells; and only a slight increase in *tps* RNA was found during glycerol-induced sporulation. Several *ops* and *tps* RNAs were observed in this study, and the positions of these RNAs were mapped on the *M. xanthus* genome. The 5′ ends of both the *ops* and *tps* RNAs mapped predominantly to positions about 50 bases upstream from the respective translational initiation sites. The 3′ ends of RNAs from both genes were heterogeneous. The four *ops* RNAs were 620, 775, 845, and 1,230 bases in length, while the *tps* RNAs were 612, 695, 730, and 935 bases.

Myxococcus xanthus is a gram-negative gliding bacterium with a complex life cycle (16, 25). Vegetative groups of cells prey on other microorganisms or utilize organic substrates in their environment. Starvation of these cell groups on a solid surface triggers a dramatic response by the bacteria, which is referred to as development. During development, cells move into aggregation centers to form multicellular structures called fruiting bodies. Within fruiting bodies, which may contain 10^6 cells, individual rod-shaped bacteria differentiate to form spherical myxospores. Myxospores are a resting-cell form which is relatively resistant to environmental insults such as high temperature and desiccation. Sporulation is also induced, in the absence of aggregation, by the addition of glycerol (0.5 M) to cells growing vegetatively in liquid culture (5). The germination of myxospores occurs upon provision of conditions suitable for vegetative growth and completes the life cycle.

One of the mechanisms controlling *M. xanthus* development appears to be the regulation of gene expression (2, 10, 11). Two examples of genes exhibiting developmental regulation are the *ops* and *tps* genes. These genes are about 90% homologous at the DNA sequence level and are directly repeated, with 1.4 kilobase pairs (kbp) of DNA separating them on the *M. xanthus* chromosome (13, 15). The *tps* gene encodes protein S, a very abundant protein found primarily on the outer surface of mature myxospores (3, 11, 13, 14). The expression of this gene is controlled at the transcriptional level, with *tps* RNA being found in increasing amounts from about 5 to 24 h of development (3). Three *tps* RNAs, differing in size, have been observed (3). Expression of the *tps* gene can also be detected in cells starved in liquid shaker culture in which sporulation and extensive intercellular contacts do not occur (4). Expression of the *ops* gene, observed as β-galactosidase activity in an *M. xanthus* strain containing an *ops-lacZ* fusion, is not detectable until between 24 and 48 h of development, and the induction of *ops* expression is correlated with spore formation (4). Cells in starvation shaker culture do not express the *ops* gene, but expression can be detected when sporulation is induced by the addition of glycerol to vegetative cells. Induction of *tps* expression could not be detected in glycerol-induced cells. RNA from the *ops* gene was not observed in these studies; however, the protein product of this gene has been identified and is found within myxospores (24). It has recently been shown that expression of both the *tps* and *ops* genes is under the control of the *ssbA* gene, a gene which has been implicated in cell-to-cell interactions in *M. xanthus* (7). While neither *ops* or *tps* is expressed in *ssbA* mutant strains, expression in the mutant cells can be rescued by mixing them with $ssbA^+$ cells. This result suggests that *M. xanthus* developmental gene expression is regulated by cell-to-cell interactions as well as by the nutritional environment of the cells.

Although the functions of the *ops* and *tps* gene products have yet to be elucidated, the facts that these highly homologous genes are differentially regulated and yet are subject to control by intercellular interactions make this an interesting system for comparative studies of developmentally regulated gene expression. In this communication, the identification of the *ops* RNA products is reported. To begin the more detailed analysis of the molecular mechanisms controlling *M. xanthus* gene expression, the positions of the *ops* and *tps* RNAs have been mapped and compared.

MATERIALS AND METHODS

Bacterial culture conditions. *Escherichia coli* cultures were routinely grown in LB medium (20) with ampicillin added at a concentration of 75 μg/ml to select for the maintenance of plasmids. *M. xanthus* was grown vegetatively in CYE medium (1), and glycerol-induced sporulation (5) was initiated by the addition of glycerol to 0.5 M to cells growing in CYE broth at a density of 2×10^8 to 4×10^8 cells per ml. *M. xanthus* development was achieved by plating cells on clone-fruiting (CF) agar plates as described previously (8, 22).

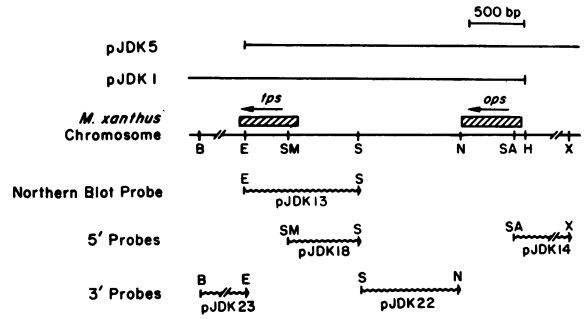

FIG. 1. cRNA probe positions for Northern blot and 5'- and 3'-end analyses of *ops* and *tps* RNA. Segments of the *M. xanthus* chromosome were inserted into the SP6 promoter plasmids pSP64 and pSP65 (19) for the synthesis of RNA hybridization probes. The source of the *M. xanthus* DNA was cloned DNA from either pJDK1 or pJDK5 (3). The positions of the *tps* and *ops* genes ▨, the direction of transcription (←), and selected restriction endonuclease cleavage sites (B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nae*I; S, *Sal*I; SA, *Sau*3A; SM, *Sma*I; X, *Xho*I) are shown. Probe RNAs (∿∿∿) are indicated with the corresponding template plasmid and the restriction sites which delineate the *M. xanthus* sequences.

Plasmids. Template plasmids for the preparation of cRNA hybridization probes were prepared by inserting appropriate *M. xanthus ops* or *tps* restriction fragments into one of the SP6 promoter plasmid vectors, pSP64 or pSP65 (19). The *ops* or *tps* DNA fragments, obtained from pJDK1 or pJDK5 (3), were purified by preparative polyacrylamide or agarose gel electrophoresis (18) prior to ligation into the SP6 promoter plasmids. Plasmid pJDK13 was constructed by inserting a 0.97-kbp *Eco*RI-*Sal*I fragment including most of the *tps* gene and its upstream region (Fig. 1) into *Eco*RI-*Sal*I-cleaved pSP65. Plasmid pJDK14 contains a 0.8-kbp *Sau*3A-*Xho*I fragment including the 5' end of *ops* inserted into pSP64. The plasmids pJDK18 and pJDK22 contain the 0.59-kbp *Sma*I-*Sal*I *tps* fragment and the 1.46-kbp *Sal*I-*Hin*dIII *ops* fragment, respectively (Fig. 1), inserted into pSP65. A final template plasmid, pJDK23, has the 0.84-kbp *Bam*HI-*Eco*RI fragment from the 3' end of the *tps* gene (Fig. 1) inserted into pSP64. All plasmids were purified by centrifugation in CsCl-ethidium bromide density gradients (17) prior to being used in in vitro transcription reactions.

***M. xanthus* strains.** The wild-type strain of *M. xanthus*, DZF1, used in this study was obtained from D. Zusman. Derivatives of DZF1 with Tn*5* insertions in either the *tps* gene (DZF3357) or the *ops* gene (DZF3353) have been described previously (3), as has the temperature-sensitive sporulation-defective strain DZF1963 (21). Tn*5* insertions in the *tps* gene (Ω106 and Ω107) or the *ops* gene (Ω200 and Ω201) (3) were transferred from the DZF1 genetic background to DZF1963 by Mx4 transduction to form strains JD26 (Ω106), JD27 (Ω107), JD28 (Ω200), and JD29 (Ω201). MX4 transduction was performed as described by Campos and Zusman (1). Selection for Tn*5*-associated kanamycin resistance in transductions was performed on CYE agar plates containing kanamycin at a concentration of 50 μg/ml.

RNA isolation. RNA was isolated from sodium dodecyl sulfate lysates by phenol extraction as described previously (3, 9). DNase (RNase free; Bethesda Research Laboratories, Inc.) was used to remove contaminating DNA. This procedure was modified to allow the purification of RNA from myxospores. Cells from two developmental plates (diameter, 100 mm) were collected by centrifugation and suspended in 0.5 ml of a solution containing 10 mM Tris (pH 7.5), 2 mM EDTA, 25 mM KCl, 200 mM sodium acetate (pH 5.5), and 1% sodium dodecyl sulfate. To this solution, 0.5 ml of buffer-equilibrated phenol and 1.0 ml of glass beads (diameter, 0.17 to 0.18 mm) were added, and the mixture was sonicated for 2 min in 30-s bursts with a Branson sonifier equipped with a microtip. Following sonication, which effectively disrupted the spores, the glass beads were removed by centrifugation, phenol extraction was repeated, and the nucleic acid in the aqueous phase was precipitated with ethanol. RNA remained intact following this procedure, as judged by the presence of sharp glyoxylated rRNA bands upon analysis by agarose gel electrophoresis.

Synthesis of cRNA probes. RNA complementary to *ops* or *tps* RNA was synthesized from template plasmids by using bacteriophage SP6 RNA polymerase (Promega Biotec, Inc.) by an established protocol (19). The template plasmids described above were cleaved with appropriate restriction endonucleases to cause runoff termination of transcription and RNA probes with precisely defined ends. [α-^{32}P]GTP (600 Ci/mmol; New England Nuclear Corp.) was used to synthesize RNA probes, and RNasin (Promega Biotec) was used to inhibit RNase which might be present in RNA synthesis reactions. Reactions were terminated by the addition of DNase for 5 min to remove the DNA template followed by phenol extraction. Unincorporated radioactive GTP was removed from the RNA probes by chromatography on a Sephadex G-75 column (Sigma Chemical Co.). The probes were collected in approximately 1-ml fractions from the column and, following the addition of 20 μg of tRNA, were precipitated with ethanol. The probe RNA was suspended in 50 to 100 μl of hybridization buffer [80% formamide, 40 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES; pH 6.7), 0.4 M NaCl, 1 mM EDTA].

Northern blot hybridization. The procedure for analysis of RNA by blot hybridization has been described previously (3). Briefly, glyoxylated RNA (15 μg) was fractionated on a 1.5% agarose gel and transferred to a nylon membrane filter (Pall Biodyne A). The detection of *ops* and *tps* RNAs was achieved by hybridization with approximately 10^7 cpm of the probe cRNA followed by autoradiography with Kodak XAR-5 film. The relative amount of *ops* RNA present during development was quantitated by scanning densitometry of the Northern hybridization autoradiogram. A Beckman DU-8B spectrophotometer equipped with a scanning program was used for this analysis.

RNase protection mapping. Test *M. xanthus* RNA was precipitated in ethanol and with carrier tRNA as necessary. The dried RNA pellets were then dissolved in 30 μl of hybridization buffer, and to these solutions were added 1 μl of the RNA probe in the same buffer. The hybridization reaction mixtures were incubated at 90°C for 5 min and then at 50°C for 16 to 24 h. Hybridization was terminated by the addition of 300 μl of RNase digestion buffer (10 mM Tris hydrochloride [pH 7.5], 5 mM EDTA, 300 mM NaCl) containing 50 μg of RNase A and 2.5 μg of RNase T_1 per ml. RNase digestion was carried out at 37°C for 1 h, 20 μl of 10% sodium dodecyl sulfate and 50 μg of proteinase K were added, and the mixture was incubated for an additional 15 min at 37°C. These samples were then extracted with phenol-chloroform and precipitated with ethanol in the presence of 20 μg of carrier tRNA. RNase-resistant hybrids were analyzed by electrophoresis on 5% or 8% denaturing polyacrylamide-8 M urea gels (17). Gels were fixed in 10% methanol–10% acetic acid and dried prior to autoradiography. RNA transcripts of known size served as standards for the determination of the sizes of RNA probe bands.

Spore counts. Spore counts were performed microscopically by determining the number of optically refractile, sonication-resistant spores in a Petroff-Hausser chamber.

RESULTS

Identification of *ops* RNA. An earlier study of the *M. xanthus ops* gene failed to identify the RNA products of this gene, even though the RNA products of the highly homologous *tps* gene were easily detected (3). However, expression of the *ops* gene could be observed when it was fused to the *lacZ* gene of *E. coli*, and developmental *M. xanthus* cells carrying the gene fusion were assayed for β-galactosidase activity. One explanation for the failure to detect *ops* RNA might be that the RNA is localized within myxospores, since *ops-lacZ* fusion activity is first detected about the time when spores are being formed. Myxospores remain intact during typical cell disruption procedures designed for the isolation of RNA.

To allow the purification of late developmental RNA by a standard procedure, use was made of a sporulation-defective mutant strain of *M. xanthus*. This strain, DZF1963, isolated by Morrison and Zusman (21), sporulates normally at 28°C but very inefficiently at 34°C. The genetic lesion responsible for the sporulation defect in DZF1963 appeared to have no detectable effect on *ops* expression, since a normal level of developmental β-galactosidase activity was detected when an *ops-lacZ* gene fusion was transduced into this strain (data not shown).

Developmental *M. xanthus* cells were tested for *ops* RNA by the Northern blot hybridization assay. In this assay, RNA isolated from strain JD26, a DZF1963 derivative with a Tn*5* insertion in the *tps* gene, was fractionated by agarose gel electrophoresis and transferred to a membrane filter for hybridization analysis with a [^{32}P]RNA probe. The *tps* insertion mutation in JD26 eliminated the synthesis of *tps* RNA which interferes with this analysis by cross-hybridizing with *ops* RNA probes. cRNA probes (19) were used to increase the sensitivity of the hybridization assay. Four *ops* RNAs were observed by Northern blot analysis of developmental JD26 RNA isolated 45 h after the initiation of development at 34°C (Fig. 2, lane 2). These RNAs (620, 775, 845, and 1,230 bases in length) were not observed in an RNA preparation from vegetative *M. xanthus* cells (lane 1). The same four RNAs were observed following analysis of developmental RNA from JD27 (lane 3). This strain, also derived from DZF1963, carries a Tn*5* insertion at another site within the *tps* gene. The *ops* RNAs were compared with the *tps* RNAs by analyzing 45-h developmental RNAs synthesized by JD28 and JD29, DZF1963 derivatives with Tn*5* insertions within the *ops* gene (lanes 4 and 5, respectively). The patterns of *ops* and *tps* RNAs were found to be distinct, with only the smallest, most abundant RNAs comigrating during agarose gel electrophoresis. The RNA probe used in the experiment shown in Fig. 2 was derived from the *tps* gene and DNA sequences upstream from this gene (Fig. 1). Identical results were observed when the probe was derived from the *ops* gene and its upstream sequences (data not shown).

***ops* RNA is located within spores.** Once *ops* RNA had been identified in the sporulation-defective strain, an attempt was made to detect RNA from this gene in a sporulation-proficient *M. xanthus* strain. The strain used in this experiment was DZF3357, a derivative of the *M. xanthus* wild-type strain DZF1 that contained a Tn*5* insertion in the *tps* gene. DZF3353, a DZF1 derivative with a Tn*5* insertion in the *ops* gene, served as a control strain containing *tps* RNA (3). Cells of the two strains were harvested from plates 47 h after the initiation of development when both rod-shaped cells and myxospores were present. RNA was isolated from the cell mixtures by using either a standard sodium dodecyl-sulfate lysis procedure or sonication in the presence of glass beads and phenol. The former treatment lyses only rod-shaped cells, while the latter disrupts both cell forms. Northern blot hybridization analysis of RNA isolated by both procedures is shown in Fig. 3. In agreement with our earlier findings, no *ops* RNA was observed when developmental RNA was isolated from DZF3357 by sodium dodecyl sulfate lysis (Fig. 3, lane 1). In contrast, *ops* RNA was readily observed in the DZF3357 RNA preparation obtained by sonication with glass beads (lane 2). *tps* RNA was observed in developmental DZF3353 RNA samples obtained by either method (lanes 3 and 4). These results indicate that intact RNA was isolated from myxospores by sonication with glass beads and that *ops* RNA resides primarily, if not exclusively, within this cell form.

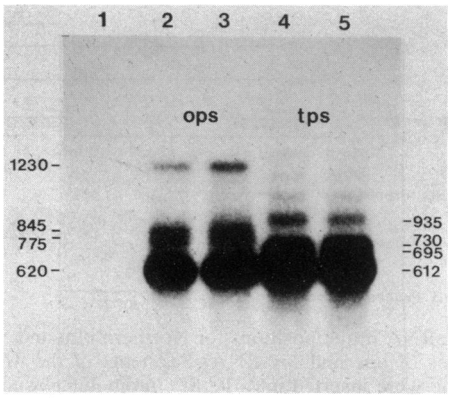

FIG. 2. Identification of *ops* and *tps* RNAs by Northern blot hybridization. RNA (15 μg) from vegetative or 45-h developmental cells was fractionated by electrophoresis on a 1.5% agarose gel. The RNA was transferred to a nylon filter and subjected to hybridization analysis with a [^{32}P]cRNA probe derived from the *M. xanthus tps* gene (Fig. 1). The vegetative RNA was from JD26 (lane 1), and developmental RNA was from JD26 (lane 2), JD27 (lane 3), JD28 (lane 4), and JD29 (lane 5). Strains JD26 and JD27 contain separate *tps*::Tn*5* insertion alleles, and JD28 and JD29 contain *ops*::Tn*5* alleles (see Materials and Methods). The sizes of the *ops* and *tps* RNAs, shown in bases on the left and right of the figure, respectively, were determined by comparison of gel mobilities with rRNA and tRNA standards and by RNA end mapping (see Fig. 5 and 6).

Time course of *ops* RNA synthesis. To examine further the correlation between *ops* RNA synthesis and spore formation, the time course of these two events during *M. xanthus* development was compared. DZF3357 (*tps*::Tn*5*) cells were collected at various times after the initiation of development, the number of spores was determined, and RNA was isolated by the glass bead-sonication technique. *ops* RNA was detected by the Northern blot hybridization assay. Under the conditions used in this experiment, *ops* RNA was first detected by 41 h and was present in larger amounts later in development (Fig. 4A). The relative amount of *ops* RNA was quantitated by scanning densitometry of the autoradiogram (Fig. 4B). Also shown in Fig. 4B is the time course of sporulation. The amount of *ops* RNA was observed to be roughly correlated with the number of spores present, indicating that *ops* RNA synthesis began at about the time that the formation of sonication-resistant, optically refractile myxospores occurred.

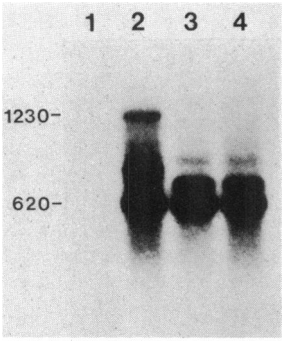

FIG. 3. Northern hybridization analysis of *ops* and *tps* RNA isolated with or without breakage of myxospores. RNA was isolated from 47-h developmental cells of strain DZF3357 (*tps*::Tn*5*) or DZF3353 (*ops*::Tn*5*) by using either a standard purification procedure or a glass-bead-sonication procedure to disrupt spores (see Materials and Methods). RNAs were detected with a ^{32}P-labeled Northern blot hybridization probe (Fig. 1). Lanes: 1, DZF3357 RNA; 2, DZF3357 glass-bead-sonication RNA; 3, DZF3353 RNA; 4, DZF3353 glass-bead-sonication RNA. All samples contained 15 μg of RNA. The sizes of the largest and smallest *ops* RNAs are indicated in bases.

Mapping of *ops* and *tps* RNAs. Northern blot hybridization experiments have indicated that multiple *ops* and *tps* RNA species exist in developmental *M. xanthus* cells (3) (Fig. 2). To investigate the relationship of the various *ops* or *tps* RNAs to one another and to compare the positions of transcripts from the two genes, the 5′ and 3′ ends of these RNAs were mapped. This was accomplished by means of a sensitive RNase protection assay in which *M. xanthus* RNA was hybridized with [^{32}P]cRNA probes synthesized in vitro. The hybrids were then treated with RNases A and T_1 to digest any probe RNA which was not complementary to cellular RNA, and the size of the protected probe RNA was determined on denaturing polyacrylamide-urea gels. From the size of the RNA probe protected and the precise position of the probe relative to the *ops* or *tps* gene, the map positions of the *ops* and *tps* RNAs were determined.

To map the 5′ ends of the *ops* and *tps* RNAs, various *M. xanthus* RNA preparations were hybridized with either the 5′ *ops* probe synthesized from *Eco*RI-cut pJDK14 or the 5′ *tps* probe synthesized from *Sal*I-cut pJDK18 (Fig. 1). When 10 μg of 45-h developmental RNA from strain JD26 (*tps*::Tn*5*) was hybridized with the 5′ *ops* probe, several probe bands (98 to 103 bases in length) were protected from RNase digestion (Fig. 5A, lane 6). The accuracy of these size determinations was estimated to be ±2 bases. The intensity of these hybridization signals (RNA bands) was dependent on the amount of *M. xanthus* RNA present in the hybridization reaction, since hybridization with 2 μg of developmental RNA resulted in much less protected probe (Fig. 5A, lane 5), and no addition of cellular RNA resulted in no protection at all (lane 1). These results indicate that the 5′ end of the RNA responsible for the largest protected RNA band is about 49 bases upstream from the *ops* translational start. *ops* RNA was not detected by this assay in vegetative RNA preparations (75 μg) from the wild-type strain, DZF1 (lane 2). Earlier studies of the expression of an *ops-lacZ* fusion indicated that *ops* is induced during glycerol-induced sporulation (4). In agreement with that observation, *ops* RNA was detected when 75 μg of 2-h glycerol-induced RNA from *M. xanthus* DZF1 or JD26 (*tps*::Tn*5*) was hybridized with the 5′ *ops* probe (lanes 3 and 4, respectively). An identical pattern of RNA-protected probe bands was detected following hybridization with either glycerol-induced or late developmental RNA, indicating that the glycerol-induced *ops* RNA did not differ significantly from *ops* developmental RNA in the positions of their 5′ ends. However, as judged by the amount of probe RNA protected, there was much less *ops* RNA present in the glycerol-induced cells.

The 5′ end of *tps* RNA was mapped in a similar manner by using the 5′ cRNA probe. When 7 μg of 24-h developmental RNA from *M. xanthus* DZF1 was hybridized with the probe RNA, two prominent bands (129 and 127 bases) were observed (Fig. 5B, lane 7). The appearance of these bands was dependent on the addition of cellular RNA to the hybridization reaction (lane 1), and less of the probe RNA was protected when only 1.4 μg of *M. xanthus* RNA was added with the probe (lane 6). The largest and most abundant of the protected probe bands indicated a 5′ end for the corresponding *tps* RNA 50 bases upstream from the translational start. This result is substantially in agreement with an earlier report of the position of the 5′ end of *tps* RNA determined by the S1 nuclease technique (12). A fainter protected probe band 100 bases in length was also observed in the hybridizations with developmental RNA. Unexpectedly, *tps* RNAs with 5′ ends identical to the most prominent developmental *tps* RNAs (represented by 129- and 127-base bands) were present in vegetative DZF1 RNA preparations (lane 2),

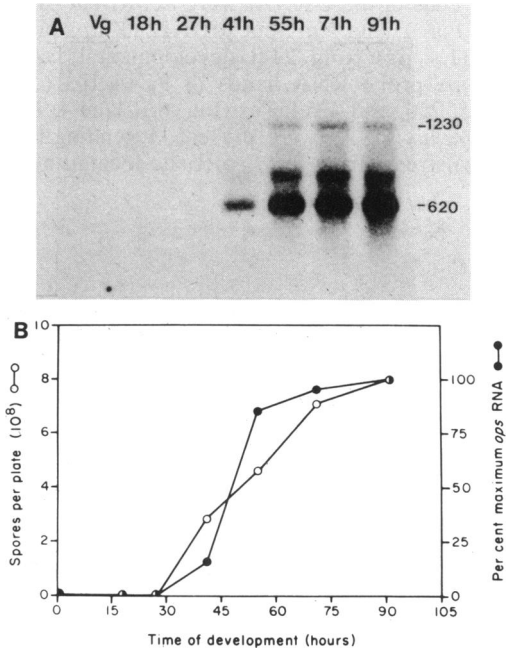

FIG. 4. Time course of *ops* RNA and sporulation during *M. xanthus* development. (A) Hybridization analysis of strain DZF3357 (*tps*::Tn*5*) RNA isolated from vegetative (Vg) cells or at the indicated time after the initiation of development. Detection of *ops* RNA, purified by the glass-bead-sonication technique (see Materials and Methods) was done as described in the legend to Fig. 2. The sizes (in bases) of the largest and smallest *ops* RNAs are indicated. (B) Sporulation and *ops* production during development. Cells were harvested from developmental agar plates at the indicated times, and the amount of *ops* RNA and the number of myxospores were determined. RNA production was quantitated by scanning densitometry of the autoradiogram shown in panel A. The number of sonication-resistant optically refractile spores was determined by direct count.

DZF1 2-h glycerol-induced RNA (lane 4), and DZF3353 (*ops*::Tn*5*) 2-h glycerol-induced cells (lane 5). Much less of this *tps* RNA was observed in vegetative RNA from DZF3357 (*tps*::Tn*5*), a mutant which has been shown by Northern blotting experiments to be unable to synthesize the mature *tps* RNA species but which might be expected to continue to initiate *tps* RNA synthesis (lane 3). A very small amount of vegetative *tps* RNA was observed in this experiment, but, again unexpectedly, a small amount of induction of *tps* RNA synthesis appeared to occur during glycerol-induced sporulation. Despite these results, it can be estimated that the concentration of *tps* RNA in maximally induced developmental cells is at least 100 times greater than in vegetative cells. A small amount of protection of a larger probe band by vegetative and glycerol-induced RNA preparations was observed (lanes 2 to 5). The *M. xanthus* RNA responsible for the protection of this probe band originates from the region upstream from the *tps*, gene since the amount of this RNA was unaffected by Tn*5* insertions known to alter the amount of the *tps* RNAs in the cell. The precise position of this vegetative *M. xanthus* RNA has not been mapped.

The results presented above indicate that the *ops* and *tps* RNAs are relatively homogeneous with regard to their 5′ ends. This implies that the various *ops* and *tps* RNAs observed in Northern hybridization experiments are generated to a large extent by heterogeneity at their 3′ ends. This was indeed found to be the case. The cRNA probes used to map the 3′ ends of these RNAs were *Eco*RI-cut pJDK23 (3′ *tps* probe; Fig. 1) and *Nae*I-cut pJDK22 (3′ *ops* probe; Fig. 1). RNA (1.4 μg) from 24-h developmental DZF1 cells protected *tps* probe RNA bands of 97 to 103 (cluster of bands), 185, 220, and 425 bases (Fig. 6A, lane 4) indicating that *M. xanthus* RNAs were present, extending those distances downstream from the *Eco*RI site located near the 3′ end of the *tps* gene. RNA from 2-h glycerol-induced DZF1 or DZF3353 (*ops*::Tn*5*) cells resulted in a pattern of protected probe bands (lanes 2 and 3, respectively), which was similar but not identical to the developmental RNA pattern (lane 4). The most noticeable difference in the two patterns was the absence of protection of a 185-base band by the glycerol-induced RNA. Vegetative RNA from DZF1 protected probe RNA bands that were indistinguishable in size from those protected by developmental RNA (lane 5); however, a longer exposure of the autoradiogram was necessary to observe this pattern of protected bands (compare lanes 1 and 5). From the locations of the 3′ ends of *tps* RNA and the location of the most abundant 5′ end, the sizes of the *tps* RNAs are estimated to be 612, 695, 730, and 935 bases.

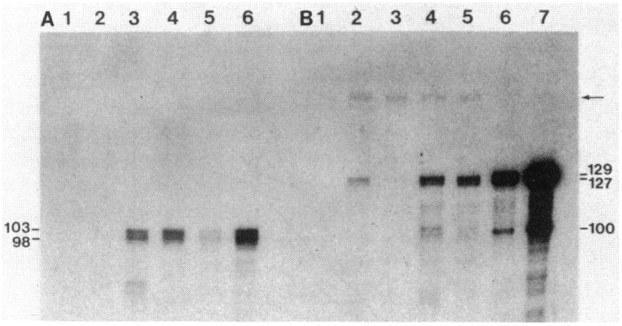

FIG. 5. Mapping of the 5′ ends of *ops* and *tps* RNA. (A) RNase protection mapping with the 5′ *ops* probe (Fig. 1) with various RNA preparations. Lanes: 1, no unlabeled RNA; 2, DZF1 (wild-type) vegetative RNA (75 μg); 3, DZF1 2-h glycerol-induced RNA (75 μg); 4, JD26 (*tps*::Tn*5*) 2-h glycerol-induced RNA (75 μg); 5, JD26 45-h developmental RNA (2 μg); 6, JD26 45-h developmental RNA (10 μg). The sizes of the protected probe RNAs (bases) are indicated at the left of the figure. (B) RNase protection mapping with the 5′ *tps* probe (Fig. 1) with various RNA preparations. Lanes: 1, no unlabeled RNA; 2, DZF1 (wild-type) vegetative RNA (75 μg); 3, DZF3357 (*tps*::Tn*5*) vegetative RNA (75 μg); 4, DZF1 2-h glycerol-induced RNA (75 μg); 5, DZF3353 (*ops*::Tn*5*) 2-h glycerol-induced RNA (75 μg); 6, DZF1 24-h developmental RNA (1.4 μg); 7, DZF1 24-h developmental RNA (7 μg). The sizes of the major protected probe RNAs (bases) are indicated at the right of the figure. The arrow points to a probe band protected by vegetative RNA. The vegetative RNA which protects the probe is not *tps* RNA and originates from transcription of the region between the *tps* and *ops* genes (see Results).

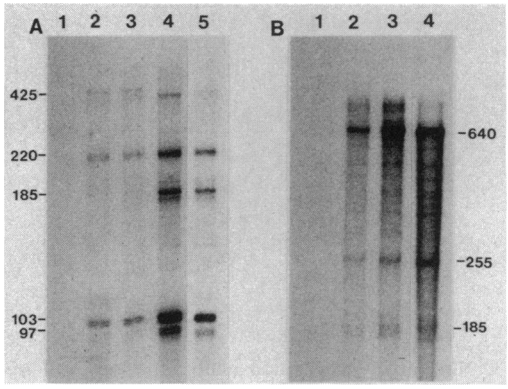

FIG. 6. Mapping of the 3′ ends of *tps* and *ops* RNA. (A) RNase protection mapping with the 3′ *tps* probe (Fig. 1) and various RNA preparations. Lanes: 1, DZF1 (wild-type) vegetative RNA (75 μg); 2, DZF1 2-h glycerol-induced RNA (75 μg); 3, DZF3353 (*ops*::Tn*5*) 2-h glycerol-induced RNA (75 μg); 4, DZF1 24-h developmental RNA (1.4 μg); 5, same as lane 1 but the autoradiogram was exposed for 30 h instead of 3 h. The sizes of the major protected probe RNAs (bases) are indicated at the left of the figure. (B) RNase protection mapping with the 3′ *ops* probe (Fig. 1) and various RNA preparations. Lanes: 1, DZF1 (wild-type) vegetative RNA (75 μg); 2, DZF1 2-h glycerol-induced RNA (75 μg); 3, DZF3357 (*tps*::Tn*5*) 2-h glycerol-induced RNA (75 μg); 4, DZF3357 55-h developmental RNA (2 μg). The sizes of the major protected probe RNAs (bases) are indicated at the right of the figure.

The 3′ *ops* probe was used to map the ends of *ops* RNA (Fig. 6B). Although these experiments resulted in the appearance of a higher level of background probe protection than 5′ *tps* mapping experiments, both 55-h developmental DZF3357 RNA (Fig. 6B, lane 4) and 2-h glycerol-induced RNA from DZF1 and DZF3357 (lanes 2 and 3, respectively) resulted in the protection of 185- (cluster of bands), 255-, and 640-base probe bands. No probe protection by vegetative DZF1 RNA was observed (lane 1). The close proximity of the 3′ end of the probe RNA with the expected 3′ end of the smallest *ops* RNA made it impossible to map precisely the position of that RNA, but the sizes of the larger *ops* RNAs are estimated to be 775, 845, and 1,230 bases. These sizes were consistent with the sizes of RNAs observed by Northern blot hybridization.

DISCUSSION

In this study, the RNA products of the *M. xanthus ops* gene have been identified. This conclusion is based on (i) hybridization of this RNA with cRNA probes to the *ops* gene

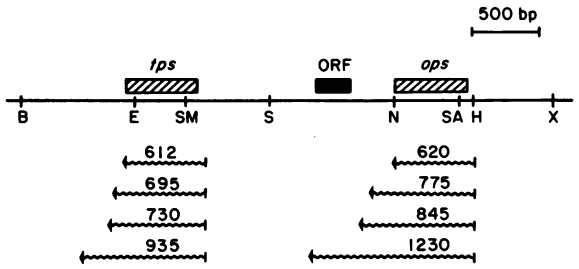

FIG. 7. Positions of the *ops* and *tps* RNAs on the *M. xanthus* chromosome. The relative positions and the sizes of the *ops* and *tps* RNAs are shown with respect to the *M. xanthus* chromosome. The direction of transcription is indicated by arrows. Selected restriction endonuclease cleavage sites are shown on the chromosome (B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; N, *Nae*I; S, *Sal*I; SA, *Sau*3A; SM, *Sma*I; X, *Xho*I). The position of a 246-base-pair open reading frame (ORF) which would be present on the 1,230-base *ops* RNA is also shown.

and the highly homologous *tps* gene, (ii) the presence of this RNA in strains with *tps*::Tn*5* insertions, and (iii) correlation of the timing of the synthesis of this RNA with the expression of an *ops-lacZ* fusion (4). During *M. xanthus* development, *ops* RNA could not be detected until sonication-resistant myxospores had formed, and the amount of *ops* RNA recovered from sporulation cultures was correlated with the number of spores present (Fig. 4). RNA from the *ops* gene was not isolated from sporulating cultures unless a procedure which disrupted spores was used (Fig. 3), indicating that *ops* RNA is localized within myxospores. This observation is in agreement with an earlier study which showed that the *ops* protein product is found within *M. xanthus* spores (24). However, synthesis of *ops* RNA does not require the formation of structurally intact spores, since this RNA was also found in late developmental cells of a sporulation-defective *M. xanthus* mutant strain (Fig. 2). RNA from the *ops* gene was observed in cells undergoing glycerol-induced sporulation, although at a relatively low level compared with that in late developmental cells. Again, this result agreed with the earlier study of *ops-lacZ* expression (4). The pattern of expression of the *ops* gene differed greatly from that of the highly related *tps* gene, a gene whose RNA and protein products are synthesized much earlier in *M. xanthus* development (3, 4, 10, 11).

Four *ops* RNAs were identified by a combination of Northern blot hybridization and RNase protection mapping of 5′ and 3′ ends. The sizes of these RNAs were estimated to be 620, 775, 845, and 1,230 bases. The 5′ ends of the developmental *ops* RNAs mapped to a small region of 44 to 49 bases upstream (−44 to −49) from the translation initiation codon, and the 5′ ends of glycerol-induced *ops* RNA also mapped to this region. Mapping of the 3′ ends of *ops* RNA showed that the various RNAs result primarily from heterogeneity at the 3′ ends of these molecules (Fig. 6B). However, some difficulties arose in precisely mapping the 3′ ends of *ops* RNA. One problem was the lack of appropriate restriction endonuclease cleavage sites near the 3′ end of the *ops* gene. The *Nae*I site used in this study was too close to the 3′ end of the smallest *ops* RNA to allow the formation of RNase-resistant hybrids, and the end of this RNA was not precisely mapped. The position of this RNA was inferred from the position of the 5′ end, and the size of the RNA was determined by Northern blot hybridization. The *ops* 3′ end mapping experiments also resulted in a relatively high background of probe protection. The reason for this is not understood, but it is probably due to a relatively high degree of heterogeneity at the 3′ ends of *ops* RNA in vivo, since *tps* RNA could be clearly mapped by the identical technique (Fig. 6A). Possibly, the half-life of the *ops* RNA is shorter than that of the *tps* RNA, which has been shown to be relatively long (3, 23). Regardless of these problems, the RNA sizes determined by end mapping of the larger *ops* RNAs agreed closely with sizes determined by Northern blot hybridization. *ops* RNAs synthesized during glycerol-induced sporulation did not differ significantly from developmental RNAs in their 3′ ends.

It was of interest to compare previously observed *tps* RNAs (3) with the *ops* RNAs identified in this study. A similar organization was found for the *tps* RNAs, with the 5′ ends of most of the RNA being located at about −48 to −50 and the 3′ ends being located at various positions downstream from the gene. From the positions of the most abundant 5′ end (−50) and the major 3′ ends, the sizes of the *tps* RNAs were determined to be 612, 695, 730, and 935 bases. These sizes are consistent with the sizes of the RNA species observed by Northern blot hybridization. In Fig. 7, the map positions of the *ops* and *tps* RNAs are shown relative to the *M. xanthus* chromosome.

Two new observations were made with regard to *tps* gene expression. First, *tps* RNA was observed during vegetative growth. This RNA, present at a low concentration, was identical to developmental *tps* RNA in map position (Fig. 5 and 6). Second, a small increase in the concentration of *tps* RNA was detected during glycerol-induced sporulation. The RNA species observed differed to some extent from developmental *tps* RNAs, with the major difference being the absence of the 695-base RNA. These observations were possible owing to the high level of sensitivity of the RNase protection assay with the cRNA probes.

The data presented in this paper are consistent with a simple model in which transcription of both the *ops* and *tps* genes is initiated at about −50, transcribes the respective protein-coding sequence, and terminates inefficiently at several downstream sites. Of course, more complex models cannot be excluded at this time. It has been noted that the homology between *ops* and *tps* extends about 100 base pairs upstream from the respective translational start (13). It will be important to determine whether the presence of this upstream region of homology has regulatory significance for the developmental and differential expression of these genes or whether nonhomologous regions farther upstream are important. The organization of the *ops* and *tps* RNAs described in this study may be significant, since segments of the *M. xanthus* genome downstream from the *ops* and *tps* genes are transcribed in a developmentally regulated fashion. For example, inspection of the DNA sequence downstream from the *ops* gene revealed an open reading frame (Fig. 7) large enough to encode an 82-amino-acid protein product which would be transcribed as part of the largest (1,230-base) *ops* RNA. It is known that deletion of this region does not result in any obvious developmental defect (6), but subtle defects may not have been detected. The similarity between *ops* developmental and glycerol-induced RNAs and among *tps* developmental, glycerol-induced, and vegetative RNAs does not suggest major differences in the promoters or terminators responsible for the synthesis of these RNAs under the various conditions. However, development of more direct methods for the study of transcription in this system (e.g., in vitro transcription systems) is necessary to confirm this point.

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