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A Segment of *Myxococcus xanthus ops* DNA Functions as an Upstream Activation Site for *tps* Gene Transcription

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A segment of DNA located between 131 and 311 base pairs (bp) upstream from the transcriptional start of the *Myxococcus xanthus ops* gene (−131 to −311) was shown to function as an upstream activation site (UAS) for developmentally regulated transcription from the *tps* gene promoter region. The activation of early developmental transcription by the *ops* UAS was independent of orientation and could be increased by the addition of a second copy of the UAS. The *ops* UAS segment continued to function when placed 1.5 kbp upstream from the transcription initiation site. DNA from the *tps* promoter region was required for transcriptional activation by the *ops* UAS, and a specific requirement for the sequence of *tps* DNA between −34 and −66 was demonstrated. Several specific *ops* UAS DNA-protein complexes were observed after incubation of this DNA segment with an extract of early developmental *M. xanthus* cells. Extracts of vegetative cells contained much less *ops* UAS-specific DNA-binding activity. When the distance between the *tps* and *ops* genes was increased from 2 to 15 kbp by insertion of a transduced segment of DNA, the amount of developmentally induced *tps* RNA was found to be about one-third that found in wild-type *M. xanthus*. Our observations suggest that the regulatory region of the *ops* gene functions not only to control *ops* gene expression but also to increase early developmental expression of the *tps* gene located about 2 kbp downstream on the *M. xanthus* chromosome.

Development (fruiting body formation) in the gram-negative, gliding bacterium *Myxococcus xanthus* involves the aggregation of large numbers of cells into mounds and the differentiation of rod-shaped vegetative cells into ovoid myxospores (20, 33, 37). After nutrient depletion, cells plated on an agar surface aggregate during the first 24 h, and sporulation occurs during the next 24 h within the multicellular mounds. Sporulation can also be induced in vegetatively growing cultures by the addition of glycerol (0.5 M) (9). Glycerol-induced myxospores form rapidly (3 to 5 h), but they differ in ultrastructure from fruiting body spores (18).

The *M. xanthus tps* and *ops* genes have been the focus of studies on the regulation of developmental gene expression in our laboratory. These genes are separated by 1.4 kilobase pairs (kbp) on the *M. xanthus* chromosome (Fig. 1), and they are about 90% identical at the DNA sequence level (16, 17). The expression of both *tps* and *ops* is activated during development, but the temporal patterns of expression differ (4, 7, 8). The *tps* gene, which is expressed at a low level vegetatively, begins to be highly expressed about 5 h after the initiation of development. A very similar temporal pattern of expression is observed in low-nutrient or starvation shaker culture in the absence of the extensive cell-cell contact that occurs during development on a solid surface. The abundant protein product of the *tps* gene (protein S) is found as a layer on the outer surface of mature myxospores (18). The *ops* gene is not expressed until much later in development, after sonication-resistant myxospores have formed within multicellular mounds (5, 8). This gene is expressed during glycerol-induced sporulation, whereas the *tps* gene is not. However, the *ops* gene is not activated during starvation shaker culture when the *tps* gene is highly expressed. The protein product of the *ops* gene is found inside myxospores (36). It is interesting that, like other developmentally regulated genes, the *tps* and *ops* genes are controlled by intercellular signaling (19). Normal expression of the *ops* gene depends on signaling controlled by the *asgA*, *asgB*, *bsgA*, and *csg* genes, whereas *tps* gene expression depends only on the *asgA*, *asgB*, and *bsgA* genes (12, 21, 22). These genes are believed to be involved in the production of signals that are part of the cell-cell communication which is required for *M. xanthus* to progress through development.

The *tps* and *ops* genes are regulated at the transcriptional level (4, 7). The high degree of relatedness between *tps* and *ops* continues upstream about 100 bp from the translational start sites, and transcription is initiated at corresponding positions within the related sequences, about 50 bp upstream from the translational start sites (4). The regulatory regions of the *tps* and *ops* genes have been localized by deletion analysis of the regulatory DNA sequences (6). These studies have shown that a segment of *tps* DNA extending 95 bp upstream from the transcriptional start site (−95) and containing the transcriptional and translational initiation sites is capable of directing developmentally regulated *tps-lacZ* gene fusion expression. Similarly, an *ops* DNA segment extending upstream to −131 is sufficient for regulated expression of that gene. However, the regulated expression of both genes is increased by the inclusion of additional upstream DNA. This is particularly true in the case of the *ops* gene, for which additional upstream DNA to −208 results in a substantial increase in expression.

The apparent ability of distant *cis*-acting DNA sequences to regulate transcriptional initiation of the *ops* gene suggested that they may be functioning as an upstream activation site (UAS) or enhancer. These regulatory DNA sites are involved in transcriptional activation, and they have been identified frequently in eucaryotic genes and less often in procaryotic genes (1, 23, 35). Enhancers and UAS elements function in either orientation and at long and variable distance from RNA initiation sites. Enhancers, but not UAS elements, also function downstream from the transcriptional

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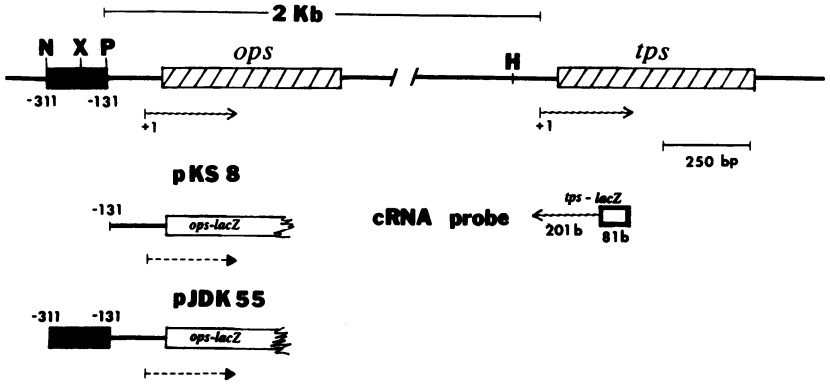

FIG. 1. Maps of the *tps* and *ops* genes on the *M. xanthus* chromosome, the *tps-lacZ* cRNA probe, and *ops-lacZ* fusions. The *tps* and *ops* genes are shown on the *M. xanthus* chromosome with selected restriction endonuclease sites. The protein-coding regions of the two genes (▨) and the direction of RNA synthesis from the transcription initiation sites at +1 (⟿) are indicated. The 180-bp *ops* UAS located between −131 and −311 (■) is shown to be about 2 kbp upstream from the *tps* transcriptional start. The *tps-lacZ* cRNA probe was synthesized as described in Materials and Methods and consisted of 81 bases from *lacZ* sequences and 201 bases from the *tps* gene. The 3′ end of this cRNA corresponds to 95 bases upstream from the *tps* transcriptional initiation site. The *ops-lacZ* fusion genes contained on plasmids pKS8 and pJDK55 are shown (6). They differ only in that the pKS8 fusion gene lacks the 180-bp *ops* upstream activation site, whereas the pJDK55 fusion gene contains this DNA segment. The GenBank accession numbers for the DNA sequence of the *tps* and *ops* region of the *M. xanthus* chromosome are JO1745 and JO1746. Restriction sites: N, *Nci*I; X, *Xmn*I; P, *Pvu*II; H, *Hin*fI.

initiation site. It is not clear whether this distinction between enhancers and UAS elements is mechanistically significant. These regulatory elements are bound by proteins that are required to interact with other regulatory proteins at the transcriptional start. For example, in *Escherichia coli*, glutamine synthetase production is controlled by an activated form of the *ntrC* protein that binds to a UAS element and is involved in the formation of an open *glnA* promoter complex with the σ^{54} form of RNA polymerase (30, 32).

In this study, we examined the function of a segment of *ops* DNA, located at −131 to −311, in developmental gene expression. This segment of *M. xanthus* DNA, which is involved in *ops* gene regulation, is shown to function as a UAS for transcription at the *tps* gene promoter. The ability of this DNA segment to activate early developmental gene expression is shown to be correlated with the presence of a specific DNA-binding activity(ies) in crude extracts of early developmental cells. Our results suggest a role for the *ops* UAS in developmental regulation of both the *ops* and *tps* genes despite the fact that this DNA segment is located about 2 kbp from the *tps* gene.

MATERIALS AND METHODS

Bacterial culture conditions. *M. xanthus* was grown in Casitone (Difco Laboratories)-yeast extract medium (3). Early development in liquid shaker culture was initiated by suspending *M. xanthus* cells in CF medium (14) at a cell density of about 4×10^8/ml (8). The conditions for development on CF agar plates have been described elsewhere (29). *E. coli* cultures were grown by standard procedures (25).

Plasmids. The gene fusion plasmids described in this report are all originally derived from the *lacZ* translational fusion vector pMLB1034 (34). Plasmids pJDK59, pJDK60, and pJDK61 are derived from the *tps-lacZ* fusion plasmid pSK1-11 (6). This fusion gene has 172 bp of *tps* DNA from the site of fusion with *lacZ* to a position 66 bp upstream from the *tps* transcriptional start site (Fig. 2) and contains a Tn*5* insertion in the ampicillin resistance gene of pMLB1034. The *tps-lacZ* plasmid was cleaved at an *Xba*I site located immediately upstream from the *tps* DNA. The *Xba*I site was filled in by using the Klenow fragment of DNA polymerase and ligated with a gel-purified (26) 180-bp *ops* DNA fragment (−131 to −311 fragment) (Fig. 1). This *ops* DNA fragment was obtained from pJDK55 (6) by *Eco*RI and *Pvu*II cleavage, and the *Eco*RI end was filled in to create doubly blunt ended molecules. After ligation, kanamycin-resistant *E. coli*

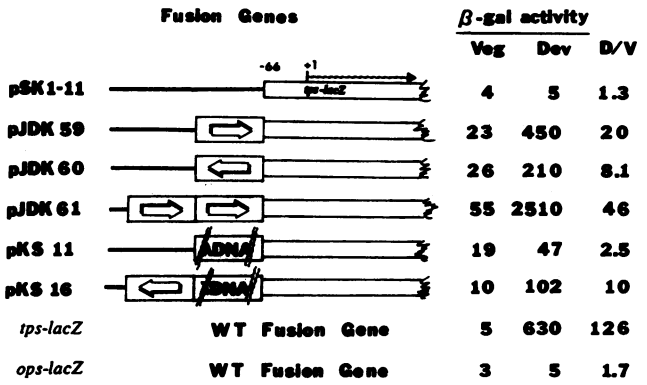

FIG. 2. Effect of the *ops* UAS on production of *tps-lacZ* fusion β-galactosidase activity. Fusion genes are designated according to the *E. coli* plasmid on which they reside. The pSK1-11 *tps-lacZ* fusion, which was used to measure gene expression, contains *tps* DNA in the transcriptional initiation region with an upstream border at −66. The 180-bp *ops* UAS (rectangle with arrow) is indicated. The arrow pointing to the right indicates the orientation of the DNA segment found on the *M. xanthus* chromosome with respect to the *ops* gene transcriptional start. The bacteriophage lambda DNA indicated is a 1.5-kbp *Kpn*I fragment of phage DNA. The wild-type (WT) *tps-lacZ* and *ops-lacZ* fusion genes have the complete complement of chromosomal sequences normally found upstream from the respective genes. These fusion genes are found in *M. xanthus* DZF3427 (*tps-lacZ*) and DZF 3361 (*ops-lacZ*) (8). The β-galactosidase specific activity (nanomoles per minute per milligram of protein) was determined for representative fusion gene transductants growing vegetatively (Veg) or incubated for 24 h in CF shaker culture (Dev). The ratio of the specific activities from early developmental and vegetative cells (D/V) is shown for each fusion construction.

MC1000 (34) transformants were screened by colony hybridization (25) for the presence of the 180-bp *ops* DNA fragment. The *Eco*RI-*Pvu*II *ops* DNA fragment was ^{32}P labeled by nick translation to serve as a hybridization probe (25). Plasmids that contained the *ops* DNA segment were screened by restriction enzyme digestion to determine the orientation and the number of copies of the *ops* DNA.

Plasmid pKS16 (Fig. 2) is derived from pJDK60. A unique *Kpn*I site located between the *tps* and *ops* DNA sequences in pJDK60 was used to insert a 1.5-kbp *Kpn*I fragment of bacteriophage lambda DNA (25). Plasmid pKS11 was constructed by cleaving pKS16 with *Sma*I and *Cla*I to remove a 2.5-kbp fragment of DNA containing bacteriophage lambda, *tps*, and *lacZ* sequences. This DNA fragment was inserted into the plasmid vector pKS7 (6), cleaved with *Sma*I and *Cla*I, to regenerate the *tps-lacZ* fusion sequences of pKS16 with the 1.5-kbp lambda DNA fragment positioned upstream (Fig. 2).

Plasmids pKS17, pKS18, and pKS19 (see Fig. 4) are derived from the *tps-lacZ* fusion plasmids pSK1-10, pSK1-12, and pSK1-13 (6), respectively. These plasmids were constructed by ligation of the purified 180-bp *ops* DNA fragment into the filled-in *Xba*I sites of the *tps-lacZ* plasmids as described above for the construction of pJDK59.

Bacteriophage P1 transduction. Plasmid DNA sequences containing the gene fusions were transferred from *E. coli* to *M. xanthus* by P1 transduction as described previously (31). The fusion genes integrated into the *M. xanthus* chromosome at random sites via Tn*5*-mediated transposition (5). Transductants were selected by plating on Casitone-yeast extract medium containing kanamycin (50 μg/ml).

RNA isolation. RNA was isolated from sodium dodecyl sulfate lysates of *M. xanthus* cells as described previously (4, 7).

RNase protection assay. RNA produced from *tps-lacZ* genes or the chromosomal *tps* gene was detected by hybridization to a ^{32}P-labeled cRNA probe, followed by RNase digestion as described previously (4, 27). The cRNA probe used contains *tps* and *lacZ* sequences (Fig. 1) and was synthesized by using the bacteriophage T7 RNA polymerase as described elsewhere (6). This cRNA probe allows the simultaneous analysis of RNA synthesis from the intact *tps* gene and *tps-lacZ* gene fusions and contains *tps* sequences extending upstream to −95 (*Hin*fI site; Fig. 1). RNA initiated at the normal *tps* transcription initiation site results in protected probe bands of 187 and 106 bases for the *tps-lacZ* and *tps* genes, respectively.

β-Galactosidase assays. The assay of β-galactosidase has been described elsewhere (28). Vegetative and early developmental cells were disrupted by sonication; late developmental cells, including myxospores, were broken with vigorous agitation by using zirconium beads (diameter, 0.15 mm) (6).

Crude cell lysates for gel mobility retardation analysis. Vegetative or early developmental *M. xanthus* cells in liquid culture (125 ml at 4×10^8 cells per ml) were harvested by centrifugation and stored at −70°C. Extracts were prepared essentially by the method of Gross et al. (13). Cell pellets were suspended in 0.25 ml of solution A (0.01 M Tris hydrochloride [pH 7.9], 25% sucrose, 0.1 M NaCl) and incubated on ice for 15 min. This procedure was followed by the addition of 0.06 ml of solution B (0.3 M Tris hydrochloride [pH 7.9], 0.1 M EDTA, 4 mg of lysozyme per ml) and incubation on ice for an additional 10 min. Finally, 0.31 ml of solution C (1.0 M NaCl, 0.02 M EDTA [pH 7.0], 0.08% deoxycholate) was added, and incubation was continued for 10 min. The resulting lysates were centrifuged for 40 min at 30,000 × *g*. The supernatants, with a protein concentration of 5 to 6 mg/ml, were immediately used for gel mobility retardation assays. Protein was assayed with a Pierce BCA protein assay kit.

TABLE 1. β-Galactosidase activity produced from *ops-lacZ* fusions

Fusion gene[a]	Sp act[b] (nmol/min per mg)		Dev/Veg[c]
	Vegetative	Developmental	
pKS8	5	27	5
pJDK55	4	235	60
DZF3361	3	670	220

[a] The fusion genes are designated according to the *E. coli* plasmids on which they reside (Fig. 1) (6). Strain DZF3361 has an *ops-lacZ* fusion with the complete complement of *ops* upstream DNA (8).

[b] Measured in crude lysates of vegetative or 72-h fruiting body cells from developmental plates. Data for the pKS8 and pJDK55 fusions are averages from analysis of five independent transductants for each fusion gene.

[c] Ratio of the developmental specific activity to the vegetative specific activity.

Gel mobility retardation assay. Various amounts of crude cell extract were incubated with a ^{32}P-labeled DNA probe and 2 μg of poly(dI-dC) · poly(dI-dC) (Pharmacia) at 30°C for 10 min to allow DNA binding. In addition, the 20-μl binding reactions contained 12% glycerol, 12 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-NaOH (pH 7.9), 5 mM $MgCl_2$, 4 mM Tris hydrochloride (pH 7.9), and 0.6 mM EDTA. The samples were then subjected to electrophoresis on a 5% native polyacrylamide gel in low-ionic-strength buffer. The conditions for DNA binding (2) and gel electrophoresis (10, 11) have been described. After electrophoresis, the gels were dried for autoradiography. The probe DNA fragments used were the 180-bp *ops* DNA fragment (Fig. 1, −131 to −311) labeled with [α-^{32}P]dATP at the *Eco*RI site by the fill-in reaction (25) and a 98-bp *tps Hae*III-*Ava*I fragment labeled by the fill-in reaction with [α-^{32}P]dCTP. The latter fragment, which served to test nonspecific DNA-binding activity, is located at +28 to +125 with respect to the transcriptional start of the gene.

RESULTS

***ops* DNA as a UAS.** Fusions of the *M. xanthus ops* gene with the *E. coli lacZ* gene were tested for developmental gene expression under control of the *ops* gene regulatory region. The gene fusions differed in the amount of upstream *ops* gene DNA that they contained (Fig. 1). When the segment of *ops* gene DNA located between −131 and −311 was removed, there was a dramatic drop in the level of developmentally regulated fusion gene expression, from 235 to 27 U (Table 1). Despite the low level of expression, the pKS8 (−131) fusion gene was expressed in a normal *ops* gene temporal pattern beginning late in *M. xanthus* development, and transcription appeared to initiate at the same site utilized in the intact *ops* gene (6). These observations indicated that the *ops* DNA between −131 and −311 was functioning at a considerable distance from the *ops* transcription initiation site to regulate transcription and suggested that this DNA segment might contain a UAS.

To test this possibility, the 180-bp *ops* DNA segment located from −131 to −311 was placed upstream from the promoter region of a *tps-lacZ* gene fusion, pSK1-11 (6). This *tps-lacZ* fusion lacks *tps* gene DNA upstream from −66, but it retains *tps* DNA in the region of transcriptional initiation

and is not developmentally regulated. When placed upstream from the *tps-lacZ* fusion, the 180-bp *ops* DNA segment caused a large increase in developmentally regulated expression (23 U for the pSK1-11 fusion versus 430 U for the pJDK59 fusion; Fig. 2). However, it was surprising that this segment of a gene expressed late in development activated gene expression early in development; that is, the *ops* DNA segment restored the normal temporal *tps* pattern of expression on the deleted *tps-lacZ* fusion gene. The 180-bp *ops* DNA fragment activated early developmental gene expression in either orientation, although less activity was observed in one orientation than in the other (Fig. 2, the pJDK59 fusion gene versus the pJDK60 fusion gene). A further increase in expression was observed when two tandem copies of the *ops* DNA fragment were located upstream of the *tps-lacZ* sequences (Fig. 2, pJDK61). Since these fusion genes were expressed early in development, it was not possible to determine whether the 180-bp *ops* DNA segment was having any effect on late developmental expression, but this *ops* DNA did not appear to have any effect on gene expression in response to glycerol-induced sporulation (data not shown). The addition of glycerol to vegetatively growing *M. xanthus* cells results in activation of *ops* gene expression (8).

RNA from the fusion genes was analyzed by an RNase protection assay to determine whether specific initiation of transcription was occurring from the *tps* gene initiation site. In this experiment, RNA was isolated from cells growing vegetatively or after a 20-h incubation in starvation shaker culture (early development). The production of specifically initiated, developmentally induced *tps-lacZ* RNA was observed when the 180-bp *ops* DNA fragment was positioned upstream from the transcription initiation site. The *tps-lacZ* bands in lanes 3, 5, and 7 of Fig. 3A correspond to RNA initiated at the same position as that observed for initiation of *tps* gene RNA. In this experiment, no vegetative *tps-lacZ* RNA was detected (Fig. 3A, lanes 2, 4, and 6). No developmental *tps-lacZ* RNA was observed from the pSK1-11 (−66) fusion gene (Fig. 3A, lane 8). In agreement with the amount of developmental β-galactosidase activity observed, the pJDK61 fusion gene with two copies of the 180-bp *ops* DNA produced the most fusion gene RNA (Fig. 3A, lane 7) and the pJDK60 fusion gene produced the least (Fig. 3A, lane 5).

Despite the results presented in Fig. 3A, the 180-bp *ops* DNA segment also appeared to be having an effect on vegetative expression of the fusion genes (Fig. 2). For example, vegetative expression of the pJDK61 fusion was observed to be reproducibly higher than that of the pJDK59 fusion. To investigate vegetative transcription in more detail, larger amounts (50 μg) of total RNA from the various fusion gene strains were analyzed by the RNase protection assay. In each of the fusion genes containing the *ops* DNA segment, three protected probe bands were observed (Fig. 3B, lanes 2 to 4) corresponding to RNAs initiating at the normal *tps* gene initiation site (+1), a site about 33 bases upstream (−33), and a site(s) upstream from the *tps* DNA sequences present in these fusion genes (upstream from −66). The amounts of the different *tps-lacZ* RNAs varied, but again more of the RNA was observed from the pJDK61 fusion gene. No RNA was observed from the deleted *tps-lacZ* fusion pSK1-11 (Fig. 3B, lane 1).

The ability of the 180-bp *ops* DNA segment to function at greater distance from the *tps* transcriptional start was tested by inserting a 1.5-kbp segment of bacteriophage lambda DNA between the *tps* and *ops* DNA segments. Despite the increased distance, this *ops* DNA segment continued to activate developmentally regulated transcription (Fig. 2, pKS16 fusion gene). Insertion of the lambda DNA segment alone had little if any effect on expression (Fig. 2, pKS11 fusion gene), since a small (two- to threefold) increase in β-galactosidase activity has been observed routinely for fusion genes in the absence of detectable transcriptional activation (e.g., Fig. 2, pSK1-11 fusion gene).

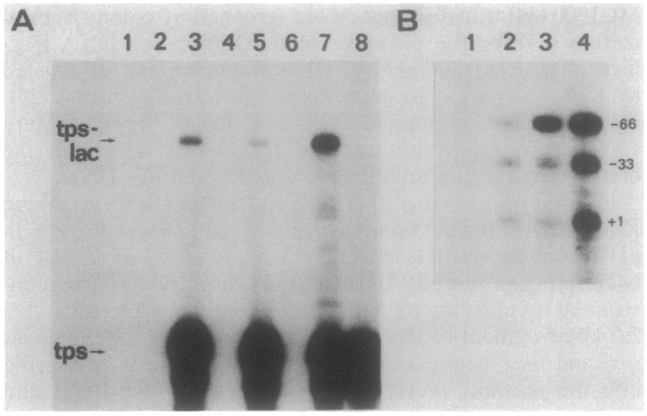

FIG. 3. RNase protection analysis of *tps-lacZ* fusion gene RNA production in vegetative and developmental cells. (A) RNA (10 μg) from vegetative or 24-h developmental cells was analyzed for *tps-lacZ* expression by the RNase protection assay with a ^{32}P-labeled cRNA probe derived from the 5′ end of the fusion gene (Fig. 1). The positions of protected probe bands corresponding to correctly initiated *tps-lacZ* (187 bases) and *tps* (106 bases) RNA are indicated. Lanes: 1, no added cellular RNA; 2, vegetative pJDK59 fusion RNA; 3, developmental pJDK59 RNA; 4, vegetative pJDK60 RNA; 5, developmental pJDK60 RNA; 6, vegetative pJDK61 RNA; 7, developmental pJDK61 RNA; 8, developmental pSK1-11 RNA. (B) Vegetative RNA (50 μg) from the fusion gene strains was analyzed by the RNase protection assay for expression of *tps-lacZ* RNA as for panel A. The positions of protected probe bands corresponding to correctly initiated *tps-lacZ* RNA (+1) and RNA initiating 33 bases (−33) and more than 66 bases (−66) upstream from the correct site are indicated. Position −66 corresponds to the end of *tps* DNA sequences present in the pSK1-11 fusion gene. The autoradiographic exposure time for this analysis was about four times longer than was used for panel A. Lanes: 1, pSK1-11 RNA; 2, pJDK59 RNA; 3, pJDK60 RNA; 4, pJDK61 RNA.

The ability of the 180-bp *ops* DNA fragment to activate *tps* gene transcription in an orientation-independent manner and at variable distance from the transcriptional initiation site indicates that this DNA segment is functioning as a UAS analogous to those found in other systems (2, 23, 35). It will be referred to as the *ops* UAS.

Functional localization of the *tps* gene promoter. Our results suggested that the *ops* DNA was functioning as a positive regulatory element which was required in conjunction with the *tps* promoter region for developmentally regulated transcription initiation. It has been noted that there is some similarity between the presumptive *tps* gene −35 and −10 regions and those of the *E. coli* consensus promoter sequence (15). The functional requirement for the *tps* DNA sequences was tested by placing the *ops* UAS upstream from fusion genes with varying amounts of *tps* upstream DNA. The deletion derivatives of the *tps-lacZ* fusion gene are not expressed developmentally in the absence of the *ops* UAS (6). A fusion gene with *tps* DNA upstream to −81 (pKS17) was expressed in a developmentally regulated fashion like the pJDK59 fusion gene which contains *tps* DNA to −66 (Fig. 4). However, it was apparent that the pKS17 fusion

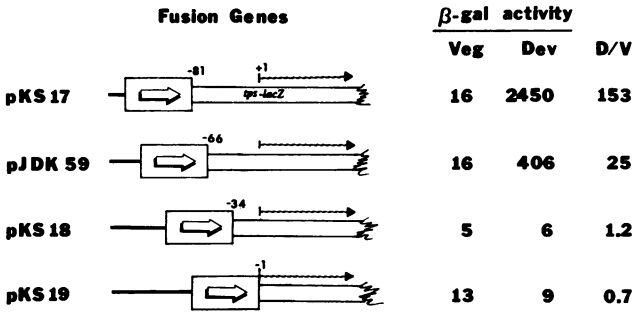

FIG. 4. Effect of deletion of *tps* DNA sequences on the production of *ops* UAS-directed *tps-lacZ* β-galactosidase activity. The amount of vegetative (Veg) or 24-h developmental (Dev) β-galactosidase activity produced by various fusion genes is shown. The fusion genes all have a single copy of the *ops* UAS element in its normal orientation with respect to the transcription initiation site (+1) plus various amounts of upstream *tps* gene DNA as indicated. The ratio of developmental to vegetative activity (D/V) is also shown.

gene was more highly expressed than the pJDK59 gene (2,450 versus 406 U). In contrast, the expression of fusion genes with upstream *tps* DNA to −34 (pKS18) or −1 (pKS19) was not activated by the *ops* UAS. These results are consistent with the notion that the *tps* gene promoter is located within the *tps* DNA downstream from −66 and that it is involved in the activation of developmentally regulated gene expression by the *ops* UAS.

Development-specific protein binding to the *ops* UAS. The ability of the *ops* UAS to activate early developmental gene expression suggested that there might be a corresponding change in the binding of this DNA by a regulatory protein(s). DNA-binding activities specific for the *ops* UAS were identified by using the gel mobility retardation assay (10, 11). When extracts of early developmental cells were tested for the *ops* DNA-binding activities, several complexes were observed (Fig. 5A, lanes 6 to 9). These were apparently DNA-protein complexes, since they were sensitive to proteinase K (data not shown). The complexes appeared to be specific for the *ops* UAS element, since no protein-DNA complexes were observed when a segment of downstream *tps* DNA (+28 to +125) was incubated with the developmental cell extract (Fig. 5A, lane 10). A particularly prominent complex, observed at low developmental extract protein concentrations, is indicated in Fig. 5A. A complex with the same mobility as this developmental complex was observed when vegetative cell extracts were tested for binding to the *ops* UAS (Fig. 5B, lanes 2 to 5). However, much less of this complex was formed by the vegetative extract, and it was not detected at the same autoradiographic exposure as that shown for the developmental complexes (Fig. 5A, lanes 2 to 5). Other specific protein-DNA complexes were not observed after incubation of the *ops* UAS segment with the vegetative extract, although some of the probe DNA was retained at the origin at high vegetative cell extract protein concentrations (Fig. 5A, lanes 4 and 5).

Increasing the chromosomal distance between the *tps* and *ops* genes lowers *tps* gene expression. The *ops* UAS segment is

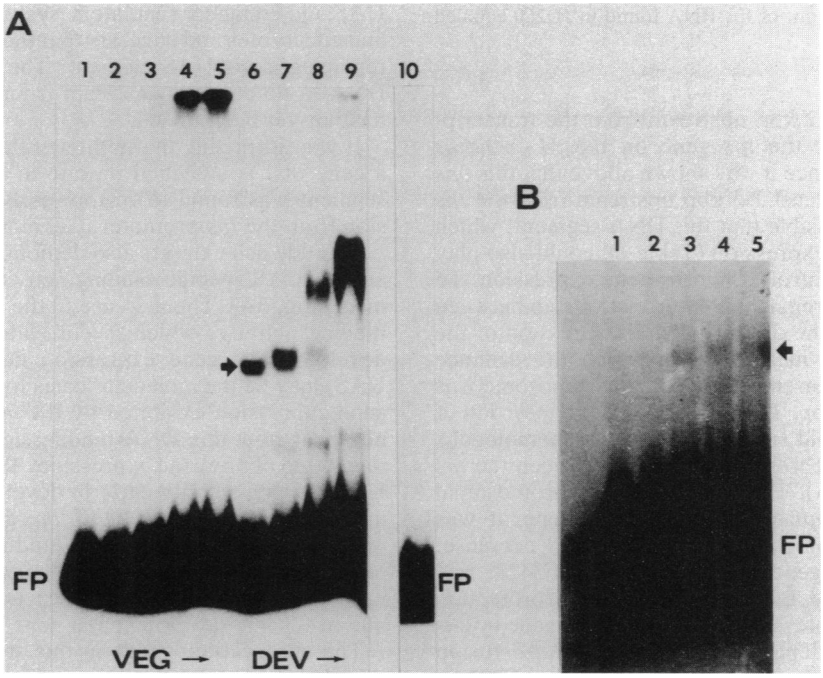

FIG. 5. Gel mobility retardation analysis of vegetative and developmental protein binding to the *ops* UAS element. (A) Crude lysates of vegetative or 16-h developmental cells were incubated with ^{32}P-labeled DNA probes and then subjected to gel mobility retardation analysis. The mobility of the free 180-bp *ops* UAS probe (FP) is shown in lane 1. Lanes 2 to 5 demonstrate the effect of incubation of the *ops* UAS probe with increasing amounts of a vegetative extract (3, 6, 9, and 12 μg of protein, respectively); lanes 6 to 9 show the effect of incubation of the same probe with increasing amounts of a developmental extract (3, 6, 9, and 12 μg of protein, respectively). The effect of incubation of a *tps* downstream probe (see Materials and Methods) with 12 μg of developmental crude extract protein is shown in lane 10, with the position of the free probe (FP) indicated. (B) A fivefold-higher autoradiographic exposure of lanes 1 to 5 from panel A. The position of the free probe (FP) is marked, and an arrow indicates the presence of a protein-*ops* DNA complex with the same mobility as the developmental complex marked with an arrow in lane 6 of panel A.

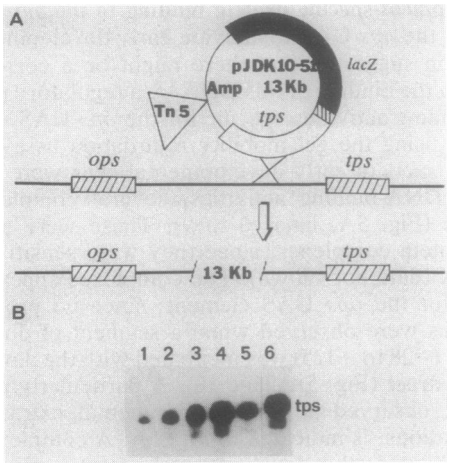

FIG. 6. Production of *tps* RNA in *M. xanthus* with an increased chromosomal distance between the *tps* and *ops* genes. (A) Integration of the *tps-lacZ* fusion plasmid pJDK10-51 into the *M. xanthus* chromosome by homologous recombination (8) results in a 13-kbp increase in the distance between the *tps* and *ops* genes (from 2 to 15 kbp). *M. xanthus* DZF3427 contains one copy of this plasmid integrated into the chromosome. (B) The production of 20-h developmental *tps* RNA was measured by using the RNase protection assay with a ^{32}P-labeled *tps-lacZ* probe as for Fig. 3. In lanes 1 to 4, 1.5-, 3.75-, 7.5-, and 15-μg amounts of total RNA from the wild-type strain DZF1 were tested for the relative amount of *tps* RNA production. RNA (15 μg) from the pJDK10-51 insertion strain DZF3427 (lane 5) and a strain (JD260) with pJDK10-51 inserted at another chromosomal location (lane 6) was analyzed in the same way. Scanning densitomitry was used to determine that the level of *tps* RNA observed in DZF3427 was about one-third that found in the wild type and that the amount of *tps* RNA found in JD260 equaled that in the wild type.

normally located about 2 kbp upstream from the transcriptional initiation site for the *tps* gene on the *M. xanthus* chromosome (Fig. 1). Since it was shown above that this *ops* UAS could function at least 1.5 kbp upstream from the *tps* promoter, it seemed possible that this DNA segment, which is involved in *ops* gene expression (Table 1), could also play a role in the positive control of the *tps* gene expression. To begin to investigate the regulatory interrelationship between the *tps* and *ops* genes, the developmental expression of the *tps* gene was analyzed in a strain in which the distance between the two genes on the *M. xanthus* chromosome was increased by about 13 kbp. This occurred by the insertion of a *tps-lacZ* fusion plasmid, pJDK10-51 (8), by homologous recombination into the chromosomal DNA between the *tps* and *ops* genes (Fig. 6A). By increasing the chromosomal distance between the *ops* UAS and the *tps* gene, it was expected that expression of the *tps* gene would decrease. Expression of the *tps* gene in this strain, DZF3427, was measured quantitatively by using the RNase protection assay. In this assay, expression of the intact *tps* gene in this strain was analyzed independently of the *tps-lacZ* fusion gene also present. As expected, the amount of *tps* RNA in DZF3427 (Fig. 6B, lane 5) was found to be about one-third the amount found in the wild-type strain, DZF1 (Fig. 6B, lane 4). However, the insertion of pJDK10-51 into the chromosome not only increased the chromosomal distance between the *tps* and *ops* genes but also introduced a second copy of the *tps* gene regulatory region. To determine whether the additional copy of *tps* gene regulatory DNA might be responsible for lowering *tps* gene expression (e.g., by the titration of a positive regulatory factor), *tps* gene expression was measured in a strain in which pJDK10-51 was integrated into the chromosome at a site distant from the *tps* region by Tn*5*-mediated transposition (5). In this strain, JD260, *tps* gene RNA was present at about the same level as was found in the wild-type strain (Fig. 6B, lanes 6 and 4). Thus, the low level of *tps* gene expression in DZF3427 was apparently not due to an additional cellular copy of *tps* regulatory DNA and may have been caused by the increase in chromosomal distance between the *tps* and *ops* genes.

DISCUSSION

The 180-bp *ops* DNA fragment, located between 131 and 311 bp upstream from the transcriptional start of the gene (−131 to −311), has been shown to function as a UAS for transcription from the *tps* gene promoter. The *ops* UAS was shown to restore developmentally regulated transcription to a deleted *tps-lacZ* gene fusion that was missing important upstream positive regulatory DNA (Fig. 3A). This deleted *tps* regulatory region, which contains the *tps* transcription initiation site and DNA upstream to −66, almost certainly contains the *tps* promoter, and the requirement for *tps* DNA between −66 and −34 for developmental expression was shown by using this heterologous system (Fig. 4). The *ops* UAS was shown to be capable of activating developmental transcription in either orientation (Fig. 3A) and at considerable distance (>1.5 kbp) from the regulated promoter (Fig. 4). Two of these elements apparently had a synergistic effect on the amount of developmental expression (Fig. 2). This study demonstrates that the −131 to −311 portion of the *ops* gene regulatory region shares characteristics with other UAS and enhancer regulatory systems found in procaryotes and eucaryotes and suggests that the *ops* UAS is functionally replacing a *tps* UAS element. The ability of the *ops* UAS DNA to function downstream from the regulated promoter has not yet been tested.

It was surprising that although the *ops* gene UAS is from a gene that is switched on late in development, this DNA segment was found to restore early developmental expression from the *tps* promoter. Gel mobility retardation studies with crude cell extracts also demonstrated the appearance of an *ops* UAS-specific binding activity(ies) early in development (Fig. 5A). The increase in the *ops* UAS-specific DNA-binding activity, which is correlated with increased early developmental gene expression under control of the *ops* UAS, may be the molecular basis for the temporal control of gene expression exhibited by the *ops* UAS. However, it is not clear how this DNA-binding activity is involved in the regulation of *ops* gene expression. The appearance of the *ops* UAS-binding activity early in development and the requirement of that DNA segment for *ops* gene expression (Table 1) may indicate that the DNA-binding activity observed is necessary but not sufficient for *ops* gene activation. Alternatively, there may be another DNA-binding activity required for late developmental gene activation.

The observations made in this study raise the possibility that the *ops* UAS DNA segment functions in *M. xanthus* to modulate expression of the *tps* gene from a chromosomal distance of about 2 kbp. As mentioned above, the *ops* UAS continued to activate early developmental transcription when placed more than 1.5 kbp upstream from the *tps* promoter. Also, the separation of the chromosomal *tps* gene from the *ops* gene by 13 kbp resulted in a drop in *tps* gene expression to about one-third that found in the wild-type strain (Fig. 6B). In an earlier study, the *tps* DNA segment

from −95 to +106 was shown to be sufficient to direct early developmental activation of transcription, and this occurred even when the fusion gene was inserted at random sites distant from the *tps-ops* locus in *M. xanthus* (6). However, these fusion genes were always expressed at a lower level than a fusion gene with the normal complement of chromosomal DNA upstream from the *tps* gene. All of these observations are consistent with the notion that this DNA site(s) upstream from the *ops* gene plays a role in regulating the level of *tps* gene expression.

Vegetative expression of *tps-lacZ* expression was also influenced by the *ops* UAS. RNase protection analysis of vegetative *tps-lacZ* RNA indicated that correctly initiated RNA was produced in the presence of the *ops* UAS but not in its absence (Fig. 3B). RNA was also initiated at −33 and at an undetermined site(s) upstream from the *tps* DNA sequences present in the pSK1-11 (−66) fusion gene. The amounts of these RNA species varied with the *ops* UAS orientation, and all three species were produced in increased amounts when two tandem segments were present (pJDK61). The activation of vegetative expression may be explained in part by the presence of a vegetative binding activity specific for the *ops* UAS (Fig. 5B). The relatively small amount of this activity that was observed resulted in an *ops* UAS complex with the same mobility as a complex observed with early developmental cell extracts. In any event, the *ops* UAS element apparently influences in some way the choice of sites for transcription initiation.

Our results are consistent with a model in which a positive regulatory protein binds to the *ops* UAS and interacts with RNA polymerase at the *tps* promoter to activate developmental transcription. The localization of the activities involved to specific segments of the regulatory DNA and the ability to construct heterologous combinations or regulatory elements will facilitate further analysis of this regulatory system. It appears that there is an interesting interaction between the regulatory regions of the *tps* and *ops* genes, and this interaction is currently being explored in more detail. An understanding of the interrelationship will require the comparative analysis of the precise DNA sequences and the specific proteins that are involved in *tps* and *ops* gene expression.

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