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The *Myxococcus xanthus asgA* Gene Encodes a Novel Signal Transduction Protein Required for Multicellular Development

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The *Myxococcus xanthus asgA* gene is one of three known genes necessary for the production of extracellular A-signal, a cell density signal required early in fruiting body development. We determined the DNA sequence of *asgA*. The deduced 385-amino-acid sequence of AsgA was found to contain two domains: one homologous to the receiver domain of response regulators and the other homologous to the transmitter domain of histidine protein kinases. A kanamycin resistance (Km^r) gene was inserted at various positions within or near the *asgA* gene to determine the null phenotype. Those strains with the Km^r gene inserted upstream or downstream of *asgA* are able to form fruiting bodies, while strains containing the Km^r gene inserted within *asgA* fail to develop. The nature and location of the *asgA476* mutation were determined. This mutation causes a leucine-to-proline substitution within a conserved stretch of hydrophobic residues in the N-terminal receiver domain. Cells containing the insertion within *asgA* and cells containing the *asgA476* substitution have similar phenotypes with respect to development, colony color, and expression of an *asg*-dependent gene. An analysis of expression of a translational *asgA-lacZ* fusion confirms that *asgA* is expressed during growth and early development. Finally, we propose that AsgA functions within a signal transduction pathway that is required to sense starvation and to respond with the production of extracellular A-signal.

Myxococcus xanthus is a gram-negative, rod-shaped bacterium that inhabits the soil. Under conditions of nutrient limitation and high cell density, *M. xanthus* undergoes a developmental program that culminates in the production of spore-filled fruiting bodies. The ovoid myxospores are metabolically quiescent and are resistant to environmental extremes (for reviews, see references 10 and 44).

Tens of thousands of cells participate in the construction of a fruiting body; therefore, cell-cell communication is required for this process. Conditional sporulation mutants that are defective in cell-cell communication have been identified in *M. xanthus* (9, 14, 21). These mutants fail to release extracellular signals required for development; however, the mutants are able to develop if they are mixed with wild-type cells. Presumably, the wild-type cells supply the extracellular signal that the mutants fail to produce. This phenomenon is referred to as extracellular complementation. The conditional sporulation mutants have been placed into five classes (*asg*, *bsg*, *csg*, *dsg*, and *esg*) on the basis of extracellular complementation experiments (9, 14). Mixtures of mutants that belong to different groups are developmentally competent, whereas mixtures of mutants belonging to the same group are developmentally incompetent. The correct timing and level of expression of developmentally regulated genes require each signaling event to be carried out in sequence. As a result, each mutant class is blocked at a different stage of development (19, 21). The *asg* (A-signaling) mutants are blocked early in development, prior to the formation of aggregates of cells (25). These mutants fail to produce extracellular A-signal, which is a mixture of amino acids generated by extracellular proteolysis (27, 36). The A-signal is thought to function as an indicator of cell density during early development (26).

There are three *asg* loci: *asgA*, *asgB*, and *asgC* (24). We would like to understand how the *asg* gene products function in extracellular A-signal production. LaRossa et al. (28) found that the *asg* mutants continue vegetative growth under conditions of phenylalanine limitation, which suggests that wild-type *asg* genes are required to respond to starvation conditions. A clue to the function of *asgB* was provided by an examination of its DNA sequence (35). The deduced 163-amino-acid sequence of AsgB contains a region similar to conserved region 4 of sigma factors. Region four is located at the C terminus of sigma factors and contains a conserved helix-turn-helix motif (15, 29) that is thought to directly contact the −35 region of promoter sequences (8, 11, 46). There is no evidence for the presence within AsgB of region 2 (35), a second highly conserved region of sigma factors that is thought to interact with core polymerase and the −10 regions of promoters (15, 29); therefore, it is unlikely that AsgB is a sigma factor. Instead, AsgB may function as a transcriptional activator and/or repressor. The function of AsgB may be to alter gene expression in response to starvation, resulting in production of extracellular A-signal.

Here we report the DNA sequence of *asgA*. The deduced amino acid sequence of AsgA was found to have a remarkable similarity to those of members of the histidine protein kinase family and the response regulator family of two-component regulatory systems (34, 49). We propose that AsgA functions within a phosphorelay similar to the phosphorelay that controls initiation of sporulation in *Bacillus subtilis* (3). One of the functions of the hypothetical *M. xanthus* phosphorelay is to regulate expression of genes required for A-signal production.

MATERIALS AND METHODS

Bacteria, plasmids, and phage. *M. xanthus* DK101 carries the social motility mutation *sglA1* and is used as the wild type (16). DK476 contains *asgA476* and *sglA1* mutations (14, 24). Plasmid pLJS49 is a pBR322-derived plasmid that contains the kanamycin resistance (Km^r) gene and IS*50*L from Tn*5*, P1-specific incompatibility, and the Mx8 *attP* for insertion into the *M. xanthus* chromosome at *att* (45). Plasmid pBGS18 is a kanamycin-resistant analog of pUC18 (47). Plasmid pAK100 contains the *asgA* gene within approximately 13 kb of *M. xanthus* DNA (24). Plasmid pLP10 contains the *asgA* gene and was constructed

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by ligating the 8.6-kb *Bgl*II fragment of pAK100 into the *Bam*HI site of pBGS18. Plasmid pLP11 contains the *asgA* gene and was constructed by ligating the 1.8-kb *Kpn*I fragment of pLP10 into the *Kpn*I site of pBGS18. Plasmid pLP12 was constructed by ligating the 2.4-kb *Kpn*I fragment of pLP10 into the *Kpn*I site of pBGS18. Plasmid pLP16 was constructed by ligating the 1.8-kb *Kpn*I fragment from pLP11 into the *Kpn*I sites within the P1-derived sequences of pLJS49. Other plasmids constructed during this study are described below. Plasmids were propagated in *Escherichia coli* DH10B.

Growth and development. *M. xanthus* strains were grown at 32°C in CTT broth (1% Casitone [Difco Laboratories], 10 mM Tris hydrochloride [pH 7.6], 1 mM KH_2PO_4 [pH 7.7], 8 mM $MgSO_4$) or on CTT agar (CTT plus 1.5% Bacto agar [Difco Laboratories]) supplemented with kanamycin sulfate (Sigma) to 40 μg/ml when appropriate. CTT soft agar is CTT plus 0.7% Bacto agar. Development of *M. xanthus* was carried out on TPM agar (same as CTT agar without the Casitone).

DNA manipulations. Standard procedures for restriction endonuclease digestions, agarose gel electrophoresis, purification of DNA from agarose gels, DNA ligations, and other cloning-related techniques were used as described by Sambrook et al. (40). The primers 5′-CTCGCTCCTGGGTTC-3′ and 5′-GTTCTG CGACAGGTAGC-3′, as well as 5′-CGAAGGACACCACTTCC-3′ and 5′-CG GGAGTCGTTGTCCAC-3′, were used to amplify *asgA476* chromosomal DNA from DK476. Chromosomal DNA was prepared as described by Avery and Kaiser (2). Southern hybridizations were performed with a radioisotope-free system (Schleicher & Schuell).

DNA sequence and computer analysis. DNA sequence was determined by the dideoxy-chain termination method (42) with Sequenase version 2.0 (United States Biochemical Corp.) or a SequiTherm cycle sequencing kit (Epicentre Technologies) and custom oligonucleotide primers synthesized by the Gene Technologies Laboratory at Texas A&M University. The DNA sequence was assembled and analyzed with software from the Genetics Computer Group (1991). Codon usage was analyzed with CodonUse 3.0 (1993). GenBank searches were performed at the National Center for Biotechnology Information with the BLAST network service (1).

Construction of an *asgA-lacZ* translational gene fusion and assay for β-galactosidase. Plasmid pLP11, which contains the *asgA* gene on a 1.8-kb *Kpn*I fragment, was cut into two fragments with the enzyme *Sal*I. One *Sal*I site is located within codons 12 and 13 of the *asgA* open reading frame (ORF), and the second is located within the multiple cloning sequences at the distal end of the *asgA* insert. The large fragment containing the vector sequences and the beginning of the *asgA* ORF was isolated, and the *Sal*I ends were filled in with the large fragment of DNA polymerase I (Klenow enzyme). A 3-kb DNA fragment containing the *lacZ* gene cassette was cut out of plasmid pMC1871 (43) with *Sal*I, and the ends were filled in with Klenow enzyme. The *lacZ* cassette and the large fragment from pLP11 were ligated to form plasmid pLPJ34. The DNA sequence across the junction was determined to verify the correct orientation of the *lac* cassette and to verify that the two fragments were ligated in frame. Plasmid pLPJ34 contains little *asgA* upstream DNA (90 bp upstream of the initiator ATG). The final *asgA-lacZ* translational fusion plasmid, pDC01, was constructed by ligating the 2.4-kb *Kpn*I fragment from plasmid pLP12 to *Kpn*I-linearized pLPJ34. This final construction resulted in the addition of 2.4 kb of *asgA* upstream DNA. The DNA sequence across the junction of the *Kpn*I site immediately upstream of *asgA* was determined to verify that no sequences had been inserted or deleted during the construction or isolation of pDC01. Plasmid pDC01 was introduced into *M. xanthus* by electroporation (35). Quantitation of β-galactosidase during growth and development was performed as described by Kroos et al. (22).

Construction of plasmids for gene replacement studies. Plasmid pLP53 was constructed by ligating the approximately 7-kb *Xho*I-*Hin*dIII fragment from pLP10 into the *Sal*I and *Hin*dIII sites of pUC19. Plasmids pLPJ43, pLPJ54, and pLPJ55 were constructed as follows. Plasmid pLP53 was partially digested with *Sma*I, and linear, full-length DNA was isolated. DNA containing a Km^r gene was prepared by digesting pKC7 (38) with *Hin*dIII and *Xho*I and filling in the ends with Klenow enzyme. The 1.7-kb fragment containing the Km^r gene was purified and ligated to the linearized, full-length pLP53 DNA. The site of insertion of the Km^r cassette was verified by restriction analysis. Plasmids pLPJ43, pLPJ54, and pLPJ55 are shown in Fig. 5.

Colony hybridization. Colony hybridizations were carried out as described previously (32), except Hybond-N nylon membranes (Amersham) were used instead of filter paper, and DNA probes were labeled with random oligonucleotide primers (40).

Nucleotide sequence accession number. The 2,105-bp DNA sequence presented in Fig. 2 has been assigned GenBank accession number U20214.

RESULTS

Subcloning of *asgA*. Kuspa and Kaiser (24) isolated a strain containing a Tn*5* insertion (Ω5090) near the *asgA* locus and cloned the DNA flanking the Tn*5* by a method developed by Gill et al. (12). The resulting plasmid, pAK100, contains approximately 12.9 kb of *M. xanthus* DNA. We initially localized

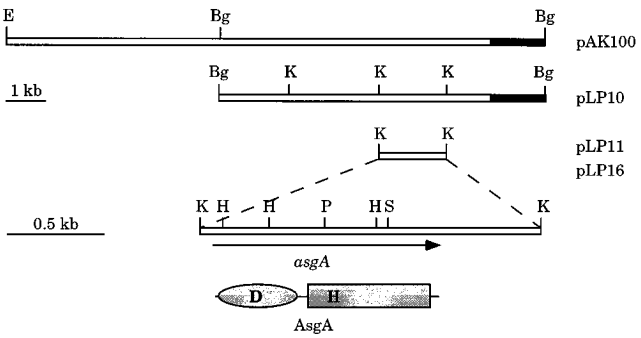

FIG. 1. Partial restriction map of *asgA* plasmids and domain organization of the deduced AsgA amino acid sequence. pAK100 is the original *asgA* clone (24). Construction of pLP10, pLP11, and pLP16 is described in Materials and Methods. An expanded version of the restriction map of the 1.8-kb *Kpn*I fragment is shown. The restriction sites are abbreviated as follows: E, *Eco*RI; Bg, *Bgl*II; K, *Kpn*I; H, *Hin*cII; P, *Pst*I; and S, *Sma*I. The arrow below the expanded restriction map represents the deduced *asgA* ORF. The diagram below the expanded map illustrates the domain organization of the deduced AsgA amino acid sequence and follows the convention used by Parkinson and Kofoid (34). The oval marked D represents the response regulator domain, and the rectangle marked H represents the histidine protein kinase domain.

asgA to the 8.6-kb *Bgl*II fragment of pAK100 and subsequently localized the gene to a 1.8-kb *Kpn*I fragment (Fig. 1). Plasmid pLP16, which contains the 1.8-kb *Kpn*I fragment and Mx8 *attP* for insertion at the *att* site of the *M. xanthus* chromosome, was found to complement strains containing any one of the 13 known *asgA* mutant alleles (data not shown). Plasmid pLP16b, which has the 1.8-kb *Kpn*I fragment in the opposite orientation relative to pLP16, was found to complement the *asgA476* mutation (data not shown).

Nucleotide sequence of *asgA* and flanking DNA. We determined the nucleotide sequence of both strands of the 1.8-kb *Kpn*I fragment as well as 336 bp upstream of the *Kpn*I end nearest the 5′ end of the deduced *asgA* ORF (Fig. 2). An ORF of 385 codons (predicted molecular weight, 42,024) is located within this sequence and begins 84 nucleotides from the upstream *Kpn*I site. The ORF has a high percentage of G + C nucleotides (93%) in the third position of the codons and exhibits a codon preference typical for *M. xanthus* (data not shown), indicating that it is likely to be a bona fide protein-encoding sequence. We detected two additional ORFs within this DNA sequence; however, it is unclear whether they encode proteins. One ORF begins upstream of the sequence presented in Fig. 2, extends to nucleotide 258, and has 85% G + C nucleotides in the third position of the codons. The second ORF begins on the bottom strand beyond the end of the sequence presented in Fig. 2, extends to nucleotide 1558, and has 87% G + C nucleotides in the third position of the codons. Neither of these ORFs shows significant similarity to sequences in the database.

Deduced amino acid sequence of AsgA and comparison to response regulators and histidine protein kinases. The deduced amino acid sequence of AsgA was found to be similar to that of members of the response regulator family and the histidine protein kinase family of the so-called two-component regulatory systems (for a recent review, see reference 34). AsgA is composed almost entirely of these two domains, with the conserved response regulator domain at the N terminus and the conserved histidine protein kinase domain at the C terminus (Fig. 1). The response regulator domain of AsgA is most similar to the response regulator domains of hypothetical protein 1 of *Xanthomonas campestris* (33), hydrogen oxidation regulatory protein HoxA of *Bradyrhizobium japonicum* (51),

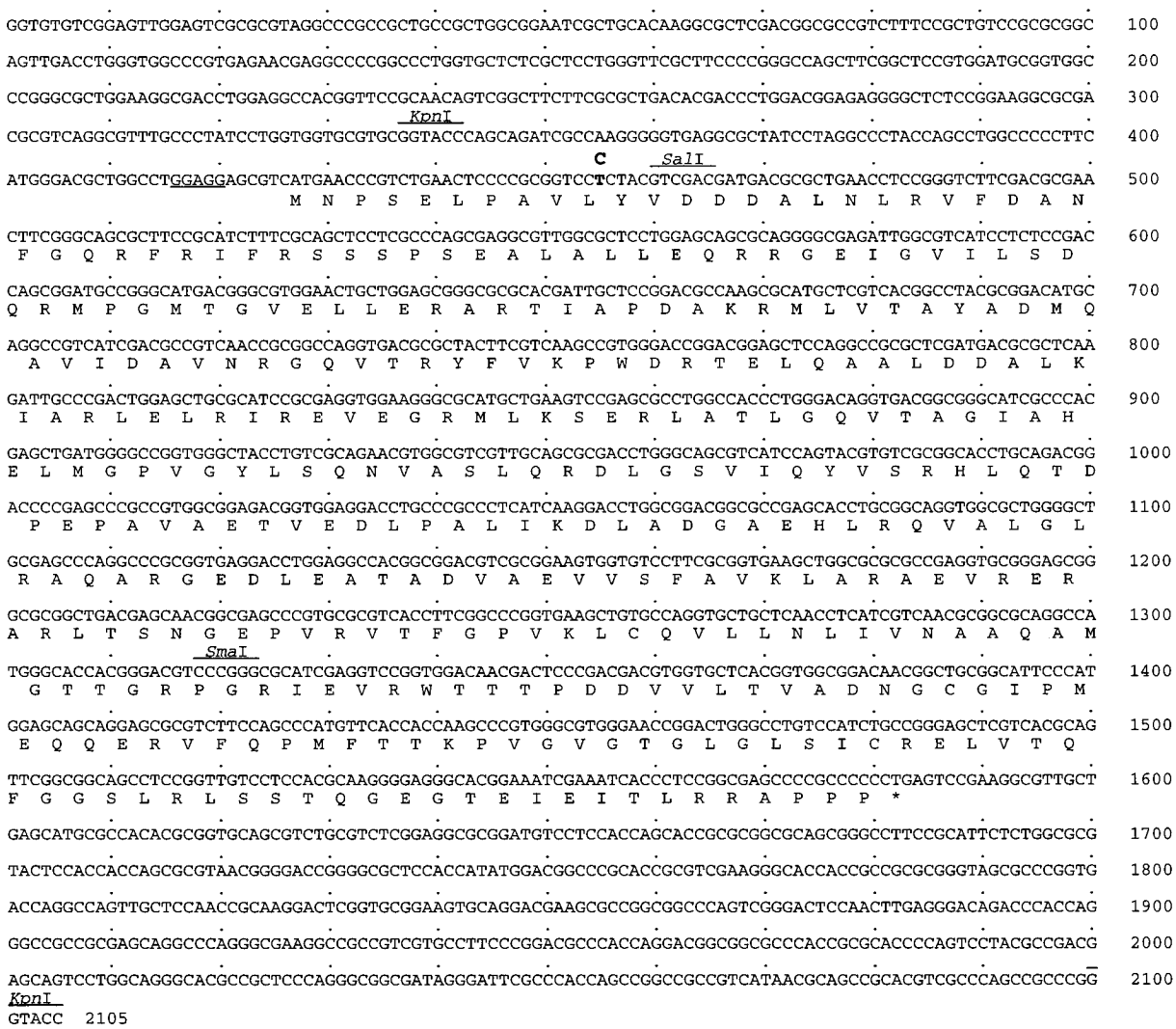

FIG. 2. Nucleotide and deduced amino acid sequences of the region of the *M. xanthus* chromosome containing the *asgA* gene. The two *Kpn*I sites that mark the ends of the *M. xanthus* DNA deduced to contain the entire *asgA* gene are indicated above the DNA sequence. The *Sal*I site that was used in the construction of the *asgA-lacZ* translational fusion is indicated above the DNA sequence. The *Sma*I site deduced to lie downstream of the *asgA476* mutation is indicated above the DNA sequence. The T-to-C transition mutation of *asgA476* is indicated by the C above the DNA sequence at nucleotide 455. A potential ribosome binding site is underlined.

Alcaligenes eutrophus and *Rhodobacter capsulatus* (6, 54), C4-dicarboxylate transport regulatory protein DctD of *Rhizobium meliloti* (53), sporulation protein Spo0F of *B. subtilis* (50), and nitrogen regulation protein NtrC of *E. coli* (37) (Fig. 3). The histidine protein kinase domain of AsgA is most similar to C4-dicarboxylate sensor protein DctB of *Rhizobium leguminosarum* (39), HupR2 protein of *R. capsulatus* (6), ornithine and arginine decarboxylase antizyme of *E. coli* (4), wide-host-range VirA protein of *Agrobacterium tumefaciens* (31), and nitrogen fixation regulatory protein FixL of *R. meliloti* (7) (Fig. 4). The ranges of percent identity and similarity of AsgA to the response regulator domains shown in Fig. 3 are 33% identical and 61% similar for hypothetical protein 1 to 29% identical and 48% similar for NtrC. For the histidine kinase domains shown in Fig. 4, the ranges are 34% identical and 61% similar for DctB to 27% identical and 54% similar for FixL.

Identification of the *asgA476* mutation. In all of the studies on A-signaling since the initial isolation and characterization of the *asg* mutants, *asgA476* has served as the representative *asgA* mutant allele (20, 23, 24, 26, 36). We conducted rescue experiments which localized the mutation in *asgA476* within the 1-kb *Kpn*I-*Sma*I fragment (Fig. 2). The *asgA476* mutation was determined by sequencing PCR products obtained with chromosomal DNA from DK476 and two sets of primers (see Materials and Methods). The two sets of primers generated two different products which together contained all of the DNA between the upstream *Kpn*I site and the *Sma*I site. A transition mutation (T to C) at position 455 was found within the 1 kb of DNA between the *Kpn*I and *Sma*I sites (Fig. 2). This mutation causes a leucine-to-proline change near the N terminus of AsgA and within a stretch of conserved hydrophobic residues. In the homologous protein CheY, these hydrophobic residues form part of its hydrophobic core (48).

Gene replacement studies. Gene replacement studies were carried out to determine the null phenotype of *asgA*. Plasmid pLP53 contains the *asgA* gene within an approximately 7-kb fragment of *M. xanthus* DNA. Derivatives of this plasmid that contain the Km^r gene inserted upstream, downstream, or within *asgA* were constructed (Fig. 5). These plasmids do not replicate autonomously within *M. xanthus* and must integrate

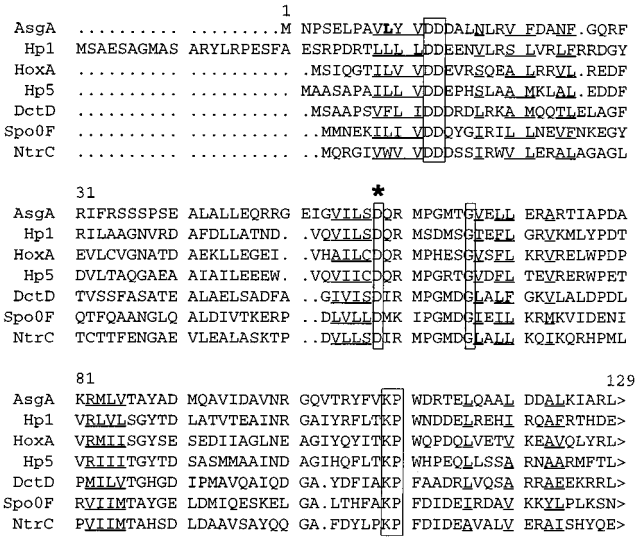

FIG. 3. Alignment of the response regulator domain of AsgA with the response regulator domains of hypothetical protein 1 of *X. campestris* (33), HoxA of *B. japonicum* (51), hypothetical protein 5′ of *R. capsulatus* (54), DctD of *R. meliloti* (53), Spo0F of *B. subtilis* (50), and NtrC of *E. coli* (37). The amino acid residue numbers are for AsgA. The boxed residues are totally conserved among the aligned sequences. The asterisk indicates the conserved aspartate that corresponds to the site of phosphorylation in CheY (41), the most extensively characterized response regulator. The underlined residues correspond to those which form the hydrophobic core of CheY (48, 49). The amino acid which is substituted in AsgA476 is in boldface (leucine at the 10th position of AsgA). The symbol > indicates that the amino acid sequence continues.

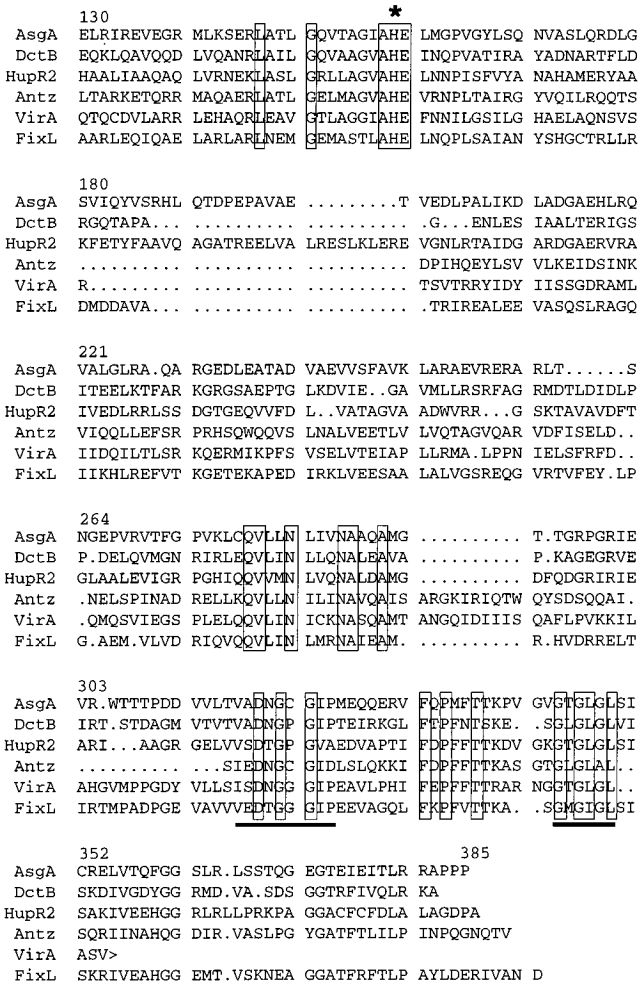

FIG. 4. Alignment of the conserved histidine protein kinase domain of AsgA with the histidine protein kinase domains of DctB of *Rhizobium leguminosarum* (39), HupR2 of *R. capsulatus* (6), ornithine and arginine decarboxylase antizyme of *E. coli* (4), VirA of *A. tumefaciens* (31), and FixL of *R. meliloti* (7). The amino acid residue numbers are for AsgA. Each aligned sequence begins with an internal residue and ends with the C-terminal residue, with the exception of the VirA sequence, which continues for a further 166 amino acids. The AsgA sequence in this figure is contiguous with the AsgA sequence in Fig. 3. The boxed residues are totally conserved among the aligned sequences. The asterisk indicates the conserved histidine that corresponds to the presumed site of autophosphorylation (34, 49). The underlined sequences indicate regions that resemble the glycine-rich sequences of nucleotide binding domains (34).

into the chromosome in order to confer kanamycin resistance. A single homologous crossover event results in the formation of a tandem duplication of *asgA* sequences with vector sequences located between the duplicated sequences. Two crossover events, one upstream of the Km^r gene and one downstream of the Km^r gene, result in a replacement of wild-type sequences with homologous DNA containing the Km^r gene. The Km^r derivatives of pLP53 were introduced into *M. xanthus* by electroporation, and the Km^r colonies were analyzed to determine whether gene replacement had occurred. Colonies containing gene replacements were distinguished from colonies containing tandem duplications by colony hybridizations with labeled vector (pUC19) sequences; the probe does not hybridize to colonies containing gene replacements because these colonies lack vector sequences. Those colonies that appeared to contain gene replacements were further analyzed by Southern hybridization (data not shown). Gene replacements were obtained at a frequency of approximately 1 to 5% after transformation with each of the Km^r derivatives. The replacement strains were analyzed for the ability to form fruiting bodies on starvation (TPM) agar. Those strains with the Km^r gene inserted upstream or downstream of *asgA* were able to form fruiting bodies, while the replacement strains containing the Km^r gene inserted within the region encoding the transmitter domain of *asgA* failed to form fruiting bodies after incubation for 3 days at 32°C (Fig. 5).

An *asgA* gene replacement was also obtained in DK6620, a strain that contains Tn*5 lac*Ω4521 (20). Tn*5 lac*Ω4521 is a developmentally regulated *lacZ* fusion gene (22) that requires functional *asg* genes or exogenously supplied A-signal for high-level expression during development (25, 27, 36). We wanted to determine whether the pattern of expression of Tn*5 lac*Ω4521 in the *asgA* gene replacement strain is similar to the pattern of expression in a strain containing *asgA476*. The results of this experiment are shown in Fig. 6. The level of expression of Tn*5 lac*Ω4521 is highest in the wild-type background and is reduced to similar levels in the *asgA476* and *asgA* gene replacement backgrounds.

Analysis of *asgA-lacZ* expression. An *asgA-lacZ* gene fusion was constructed and used to analyze expression of *asgA* during growth and development. This gene fusion contains 2.5 kb of *M. xanthus* DNA upstream of the *asgA* ATG and includes the first 12 *asgA* codons. Insertion of the *asgA-lacZ* gene fusion plasmid (pDC01) at the *asgA* locus results in the formation of a tandem duplication of *asgA* sequences that are separated by the fusion gene and vector sequences (Fig. 7B and data not shown). *M. xanthus* cells containing the tandem duplication and the *asgA-lacZ* gene fusion were spotted onto starvation (TPM) plates and harvested at various times during development for the determination of β-galactosidase specific activity

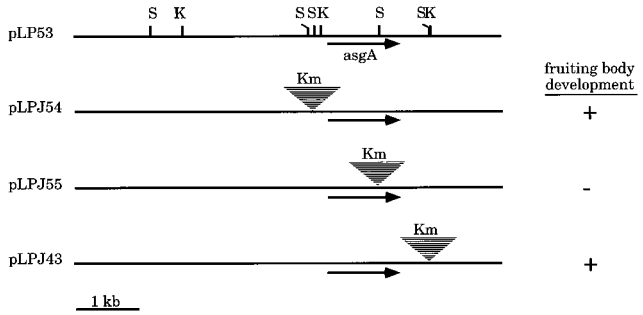

FIG. 5. Plasmids used in the gene replacement studies. A partial restriction map of the parent plasmid, pLP53, is shown at the top of the figure. The triangles represent the sites of insertion of a 1.7-kb DNA fragment containing the Km^r gene. The arrow below the map corresponds to the *asgA* ORF. The restriction sites are abbreviated as follows: S, *Sma*I; K, *Kpn*I.

(Fig. 7A). During growth, the specific activity was approximately 10 U/mg of protein. The specific activity increased roughly twofold during the first 12 h of development. *M. xanthus* cells containing the *asgA-lacZ* fusion appeared to develop normally, indicating that insertion of pDC01 does not disrupt the function of the *asgA* gene.

DISCUSSION

The deduced amino acid sequence of AsgA was found to have a remarkable similarity to those of members of the histidine protein kinase family and the response regulator family of the so-called two-component regulatory systems. In the paradigm two-component system, the conserved histidine protein kinase domain, or transmitter, is part of a transmembrane sensor protein and the conserved response regulator domain, or receiver, is part of a transcriptional regulator that mediates the cellular response to sensor signals. When the sensor is stimulated by ligand binding to its input domain, it is autophosphorylated on a histidine residue within its transmitter domain. The phosphoryl group is transferred to an aspartate within the receiver domain of the transcriptional regulator; phosphorylation modulates the activity of the DNA binding

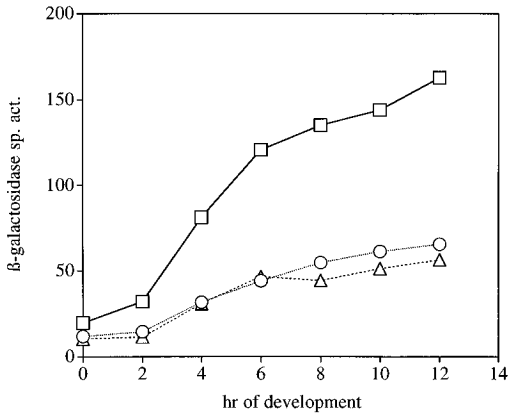

FIG. 6. Expression of the A-signal-dependent gene Ω4521 in wild-type and *asgA* backgrounds. The β-galactosidase specific activity (sp. act.) units are nanomoles of *o*-nitrophenol produced per minute per milligram of protein. The results were obtained by averaging values from two or three independent assays of single isolates. □, Ω4521 expression in DK101 (wild type); ○, Ω4521 expression in the *asgA* gene replacement strain (Km^r gene inserted within *asgA*); △, Ω4521 expression in the *asgA476* strain (DK4323).

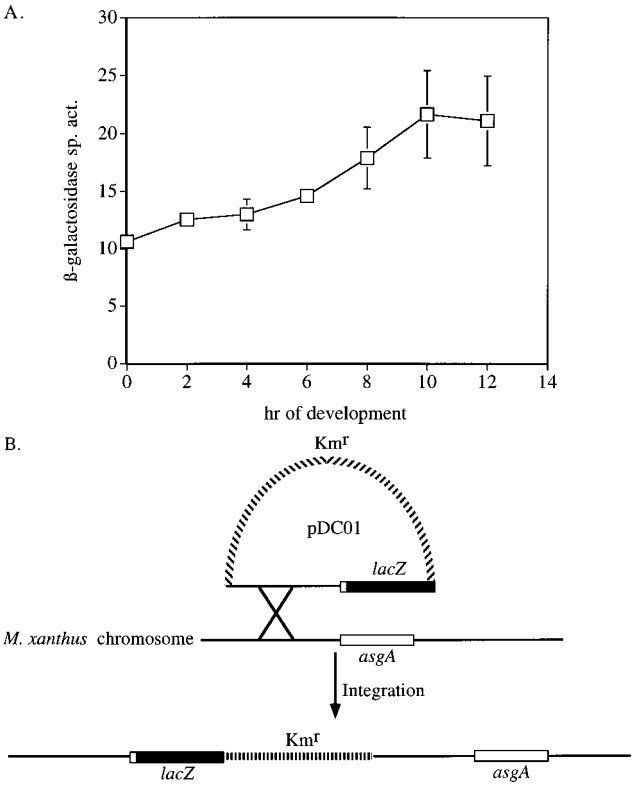

FIG. 7. (A) Expression of an *asgA-lacZ* fusion during early development. The β-galactosidase specific activity (sp. act.) units are nanomoles of *o*-nitrophenol produced per minute per milligram of protein. The results were obtained by averaging values from three assays of two independent isolates. The error bars indicate the range between plus and minus the standard deviation. (B) Insertion of the *asgA-lacZ* gene fusion plasmid (pDC01) at the *asgA* locus. Insertion of pDC01 at the *asgA* locus results in a tandem duplication of *asgA* upstream DNA, with the *asgA-lacZ* fusion and vector sequences located between the duplicated sequences. Narrow lines, *M. xanthus* DNA; open bars, *asgA* ORF; solid bars, *lacZ* sequences; striped bars, vector sequences.

(output) domain and ultimately results in a change in gene expression (34, 49).

In most of these signal transduction systems, transmitter and receiver domains are located on separate polypeptides along with their associated input and output domains, respectively. However, a number of proteins have alternative module arrangements (34). For example, *B. subtilis* Spo0F consists of a single receiver domain with no associated output domain (50), FrzZ of *M. xanthus* has two receiver domains in tandem (34, 54a), and VirA of *A. tumefaciens* contains a membrane-spanning region that is followed by a transmitter domain and a receiver domain (5). AsgA appears to consist entirely of a receiver domain followed by a transmitter domain (Fig. 1). It is the only protein we know of in which a receiver domain precedes a transmitter domain; however, a number of proteins contain both domains in the reverse order (34). In addition, AsgA appears to lack the hydrophobic, membrane-spanning regions that characterize most of the sensors, and, therefore, it is likely to function in the cytoplasm.

The presence of both a receiver and a transmitter domain within AsgA raises questions about possible interactions between the two domains. Parkinson and Kofoid (34) suggest that in those proteins that have both domains, the receiver might have a switchable autoinhibitory role in signaling. In the unphosphorylated state, the receiver could interact with the

transmitter to inhibit autophosphorylation of the transmitter or interaction with other response regulators. In this model, phosphorylation of the receiver relieves the constraints on the transmitter domain, allowing it to autophosphorylate or interact with other signaling proteins. Phosphorylation of the receiver domain could occur by the attached transmitter domain or by a transmitter associated with another protein. Mutational studies of the *arcB* and *virA* genes, each of which encodes a sensor that consists of a membrane-associated input domain followed by a transmitter followed by a receiver, suggest that the receiver plays an autoinhibitory role (5, 18).

The *asgA* gene disruption studies suggest that *asgA* is required for development but is not essential for growth. The *asgA* disruption strain, which contains the gene for Km^r inserted within the region of *asgA* encoding the transmitter domain, has a phenotype similar to that of the *asgA476* strain, which has a mutation that results in a single-amino-acid substitution near the N terminus. Both mutations result in an early developmental block, decreased expression of the *asg*-dependent gene Ω4521, and tan colony color (this work and reference 25). Although the *asgA* gene does not appear to be essential for growth, the *asg* mutants identified by Hagen et al. (14) do have an altered growth phenotype. Colonies of the *asg* mutants are tan, whereas wild-type colonies are either tan or yellow. In addition, the *asg* mutants are less cohesive than wild-type cells; in liquid culture, *asg* mutants tend to clump less than wild-type cells. The fact that the *asgA* mutants have an altered growth phenotype suggests that the *asgA* gene is normally expressed during growth. Indeed, the *asgA-lacZ* expression studies indicate that *asgA* is expressed during growth, and expression increases, at most, approximately twofold during the first 12 h of development.

What is the role of AsgA in development of *M. xanthus*? Previous work indicates that *asgA* mutants are deficient in the release of extracellular A-signal (24, 25). The A-signal, which is a mixture of amino acids and peptides (27, 36), is thought to act as a cell density signal—an extracellular signal that allows *M. xanthus* to determine whether there is a sufficiently high concentration of cells present for successful formation of fruiting bodies (26). A DNA sequence analysis of *asgB* suggests that it encodes a DNA-binding protein, and the proposed function of AsgB is to alter gene expression in response to starvation, resulting in the production of extracellular A-signal (35). Because *asgA* and *asgB* mutants are defective in A-signal production and are blocked at the same early stage of development, it is reasonable to speculate that the products of these two genes are involved in a signal transduction pathway that leads to the production of extracellular A-signal. The present study indicates that AsgA is a novel signal transduction protein that consists of a receiver domain followed by a transmitter domain. Although AsgA appears to lack input and output domains (e.g., domains that interact with a signaling ligand or bind to DNA), it is possible that the AsgA receiver domain functions as an input domain for the transmitter. Potential ligands for the putative receiver/input domain could be small phosphodonors such as acetyl phosphate and carbamoyl phosphate, which have been shown to phosphorylate receiver domains in vitro (30). In vivo, acetyl phosphate is thought to directly or indirectly control the activity of PhoB, a response regulator for the phosphate regulon of *E. coli* (52). Sensing of an intermediary metabolite such as acetyl phosphate could be the mechanism by which A-signal production is tied to the metabolic state of the cell. Alternatively, AsgA may interact with other signaling proteins that have input functions. The role of AsgA in extracellular A-signal production may be to function in the middle of a phosphorelay similar to the phosphorelay that controls the initiation of sporulation in *B. subtilis* (3). This relay is thought to allow integration of multiple developmental signals to affect activation of the transcriptional activator at the end of the relay, Spo0A (3, 13, 17). All of the components of the *B. subtilis* phosphorelay, with the exception of Spo0B, are homologous to response regulators or histidine protein kinases. At the beginning of the phosphorelay are two histidine protein kinases that are activated by unknown signals. One of the kinases, KinB, resides in the cytoplasmic membrane, while the other kinase, KinA, appears to be cytoplasmic. Phosphoryl groups are transferred from these kinases to Spo0F, which, as mentioned above, consists of a single receiver domain with no associated output domain. The phosphoryl group is transferred from Spo0F to Spo0B to Spo0A. Although Spo0A appears to be a conventional response regulator, Spo0B is not homologous to histidine protein kinases or response regulators. At the beginning of the hypothetical *M. xanthus* phosphorelay is an autokinase that senses starvation, and at the end is a transcriptional regulator of genes required for A-signal production. The transcriptional regulator at the end of the putative phosphorelay may be AsgB or a transcriptional regulator that is affected by AsgB activity. Characterization of the remaining known *asg* locus, *asgC*, as well as the identification and characterization of other genes required for extracellular A-signal production, will aid in testing the phosphorelay hypothesis.

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