Myxococcus xanthus Encodes an ATP-Dependent Protease Which Is Required for Developmental Gene Transcription and Intercellular Signaling

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The *bsgA* gene of *Myxococcus xanthus* plays an essential role in the regulation of early gene expression during fruiting body formation and sporulation. *bsgA* mutants behave as though unable to initiate a required cell-cell interaction and consequently fail to transcribe normal levels of many developmentally induced genes. We determined the nucleotide sequence of *bsgA*, which predicts a single gene encoding a 90.4-kDa protein. The deduced BsgA protein shares 45 and 48% amino acid identity with the *lon* genes of *Escherichia coli* and *Bacillus brevis*, respectively. The cloned *bsgA* gene was expressed in *E. coli*, and the BsgA protein was partially purified and found, like its *E. coli* homolog, to be an ATP-dependent protease. Thus, the basis for the phenotype of *bsgA* mutants is likely to be a defect in intracellular proteolysis.

Myxococcus xanthus is a bacterium that undergoes a complex multicellular response to nutrient deprivation culminating in the formation of multicellular fruiting bodies and the morphological conversion of cells into dormant spores (recently reviewed by Shimkets [20]). Genetic and biochemical evidence indicates that essential intercellular signaling occurs at multiple stages during this developmental process and that these cell interactions are required for proper transcriptional regulation of developmental genes.

One gene previously found to be required for an essential intercellular signaling event is *bsgA* (10). *bsgA* mutants appear to be blocked earlier in development than other signal mutants (18). Of 30 or so developmentally induced genes tested, including those induced immediately upon starvation, all are dependent upon *bsgA* for normal levels of transcription (10, 18). For at least some of these genes, relatively normal patterns of transcription are restored by extracellular complementation (10).

The *bsgA* gene has been cloned (11), and its product has been identified as an 89-kDa cytoplasmic protein found at relatively constant levels in both vegetative and developing cells (9). Here, we report the nucleotide sequence of the *bsgA* gene and the striking amino acid homology between BsgA and the products of the *Escherichia coli* (2, 4) and *Bacillus brevis* (16) *lon* genes. In *E. coli*, the *lon* gene encodes protease La, an ATP-dependent protease (3, 5) that uses a novel enzymatic mechanism to couple the degradation of protein substrates to the hydrolysis of ATP (12). We have partially purified cloned BsgA and demonstrated that the *M. xanthus* protein also has ATP-dependent protease activity.

MATERIALS AND METHODS

Bacterial strains and plasmids. *M. xanthus* M102 is our isolate of developmentally proficient strain DK1622 obtained from Dale Kaiser. *E. coli* BNN103 (25) has a deletion of *lon*, the gene encoding protease La. pREG1344 (9) contains the *bsgA* gene cloned on a 5.5-kbp *Sall-EcoRI* fragment and under the transcriptional control of the *trc* promoter and the

lac operator. Plasmids pREG1344Δ7-9, pREG1344Δ2-22, and pREG1344Δ4-46 are deletion variants of pREG1344 in which the cloned DNA 3' of the *bsgA* gene and various amounts of the 3' end of *bsgA* itself have been removed (Fig. 1). Deletions were formed by digestion of pREG1344 at the unique *EcoRI* site, treatment with the Klenow fragment of polymerase I to form blunt ends, and partial digestion with *HaeIII*. Appropriately sized fragments were purified from agarose, circularized with T4 DNA ligase, and transformed into BNN103. Each of the deletions extends from the unique *EcoRI* site to the *HaeIII* site at the positions indicated in Fig. 2. Genetic complementation analysis and Western blot (immunoblot) analysis of proteins encoded by the deletion fragments were performed as previously described (9, 11). pLacI^q (obtained from M. Pato) is a pACYC177 derivative that contains the *lacI*^q gene. It is compatible with pREG1344Δ7-9 and is capable of repressing the transcription of *bsgA* from pREG1344Δ7-9. pREG1344Δ7-9 and pLacI^q confer resistance to ampicillin and kanamycin, respectively.

Plasmid constructions and DNA sequencing. Restriction fragments to be sequenced were subcloned from pREG1344Δ7-9 into M13mp18 and M13mp19 or the phagemids pBS(+) and pBS(-) (Stratagene). Single strands were rescued from pBS clones by use of helper phage VCSM13 (Stratagene). The nucleotide sequence was determined for both strands with Sequenase version 2.0 (U.S. Biochemicals). Sequences were analyzed with MacVector sequence analysis software (version 3.5) and the GenBank sequence data base (versions 65 and 72).

Affinity purification of the BsgA protein from *M. xanthus*. The BsgA protein was prepared from *M. xanthus* cell lysates by immunoaffinity chromatography (15) by use of a protein G-purified anti-BsgA monoclonal antibody (9) bound to CNBr-activated beaded agarose. Cell extracts were prepared from frozen *M. xanthus* cells by lysis in phosphate-buffered saline containing 0.05% Triton X-100, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride and then brief sonication, as necessary. The extracts were clarified by centrifugation at 100,000 × *g*, and the supernatant was applied to a 1-ml anti-BsgA antibody affinity column. BsgA was eluted with 0.1 M glycine-HCl (pH 3.0) and immediately

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FIG. 1. Restriction map of the 5.5-kbp *SalI*-*EcoRI* fragment containing the *M. xanthus bsgA* gene from pREG1344. The *bsgA* coding sequence is indicated by the open box; the direction of *bsgA* transcription is from left to right. The location of the *NcoI* site used in localizing the 5' end of the gene is shown, as are the locations of the mini-Tn10 insertions Ω421-6 and Ω423-5 (from reference 11). The DNA remaining in pREG1344Δ4-46, pREG1344Δ2-22, and pREG1344Δ7-9 is indicated by the lines below the map.

neutralized. Affinity-purified protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with piperazine diacrylamide rather than bisacrylamide as a cross-linker and was transferred to a ProBlot membrane. The band containing the BsgA protein was cut out and submitted to the University of Colorado Health Sciences Center Cancer Center Micro-Sequencing Facility for N-terminal amino acid sequence determination.

Purification of cloned BsgA from *E. coli*. *E. coli* BNN103(pREG1344Δ7-9, pLacI^q) was grown at 30°C in 2 liters of Luria-Bertani (LB) medium supplemented with kanamycin and ampicillin. At a density of 4.5×10^8 cells per ml, the *bsgA* gene was induced with 1 mM isopropyl-β-D-thiogalactoside (IPTG) and incubation was continued for 4 h. Cells were harvested by centrifugation, resuspended in an equal volume of 50 mM Tris (pH 7.9) at 5°C–5 mM EDTA–250 mM KCl–20% (vol/vol) glycerol, and stored at –70°C. Frozen cells were thawed on ice, treated with lysozyme (400 μg/ml; 30 min), and sonicated as required to complete lysis. The lysate was centrifuged ($100,000 \times g$ for 1.5 h), and the supernatant was dialyzed against TMB (10 mM Tris-HCl [pH 7.9] at 5°C, 5 mM MgCl₂, 10 mM 2-mercaptoethanol) before being applied to DEAE-Sephacel (10-ml bed volume) equilibrated with the same buffer. Bound protein was then eluted stepwise with TMB containing 50 mM NaCl, 100 mM NaCl, 150 mM NaCl, and 400 mM NaCl. The 100 mM NaCl fraction, which contained all of the eluted BsgA protein, was dialyzed against TMB containing 50 mM NaCl and applied to a Mono Q column (50/5) equilibrated with the same buffer for fractionation by fast protein liquid chromatography (FPLC). Protein was eluted with a linear gradient of 0.05 to 0.2 M NaCl in TMB. Peak fractions of the BsgA protein were applied to a Sephacryl S-200 column equilibrated with TMB containing 100 mM NaCl.

Protease assay. Protease activity was assayed by measuring the degradation of [*methyl*-³H] methylated α-casein prepared as previously described (7). Samples were assayed in duplicate, with or without 2.5 mM ATP, in TMB containing [³H]casein (20 μg; 4.9×10^4 cpm) for 90 min at 30°C. Reactions were terminated by the addition of 20 μl of 3% bovine serum albumin and 25 μl of 100% (wt/vol) trichloroacetic acid (TCA), and the amount of degraded, TCA-soluble [³H]casein was determined and corrected for background TCA-soluble ³H in the absence of added protein. The ATP-dependent protease specific activity was expressed as

[(counts per minute released with ATP) – (counts per minute released without added ATP)] per milligram of protein.

Nucleotide sequence accession number. The nucleotide sequence of *bsgA* will appear in the EMBL, GenBank, and DDBJ data bases under accession number L19301.

RESULTS

The cloning of the *bsgA* gene of *M. xanthus* was previously reported (9, 11). The boundaries of the gene were previously localized to the interval between the *SalI* restriction site and the mini-Tn10 insertion Ω423-5 shown in Fig. 1 (11). In this study, the 3' end of the gene was more precisely defined by isolating a series of deletions of the *SalI*-*EcoRI* fragment as described in Materials and Methods. The smallest fragment that complemented all of the known *bsgA* mutations and produced full-length BsgA, as determined by Western blot analysis, was fragment Δ7-9. Two shorter fragments, Δ2-22 and Δ4-46, produced truncated BsgA peptides and did not complement *bsgA* mutations of *M. xanthus* (data not shown).

The nucleotide sequence of the Δ7-9 fragment and the deduced translation of the *bsgA* gene are shown in Fig. 2. Both the direction of transcription and the translational reading frame are in agreement with our previous results obtained with *bsgA-lacZ* translational fusions (9). The 827-amino-acid BsgA protein has a deduced molecular mass of 90.4 kDa, in good agreement with the 89 kDa determined by SDS-PAGE (9).

The placement of the 5' end of the coding sequence at nucleotide 312 is consistent with the genetic data placing it between the *SalI* and *NcoI* sites (11). However, within this interval there are 5 in-frame ATG codons, any of which could potentially serve as an initiator codon. The ATG codon at nucleotide 312, however, is the only one preceded by a suitable ribosome binding sequence, which is homologous to the 3' end of the *M. xanthus* 16S rRNA (19) and is correctly positioned relative to the initiator ATG codon (17). For verification of the correct translational start site, the N-terminal amino acid sequence of the BsgA protein purified from *M. xanthus* was determined. The sequence obtained, SDEKKKGS_A, corresponds to amino acids 2 through 10 of the deduced translation product (Fig. 2). This result supports the use of the ATG codon at nucleotide 312 as the initiator codon and suggests that the N-terminal f-Met residue has been removed from the mature protein. In several bacterial species, the removal of the initiator f-Met residue is a common processing event for certain classes of proteins, including those, like the BsgA protein, that have serine as the second amino acid residue (22).

Examination of the sequences 5' to the *bsgA* coding sequence failed to identify homology to an *E. coli* consensus promoter. This result was not unexpected, since *E. coli* is apparently unable to initiate transcription of the cloned *bsgA* gene by using the *M. xanthus* promoter (9). It is striking, however, that the upstream sequences are comparatively A+T rich. The 100 bases preceding the ATG start codon have an average moles percent G+C composition of 46.5%, whereas the moles percent G+C composition of the *bsgA* coding sequence and the overall moles percent G+C composition of the *M. xanthus* chromosome (20) are both 67%.

Amino acid homology between the *bsgA* gene product and protease La of *E. coli*. The predicted BsgA protein amino acid sequence shares striking homology to the deduced amino acid sequences of the *lon* genes of *E. coli* (2, 4) and *B.*

FIG. 2. Nucleotide and deduced amino acid sequences of *bsg4*. The right-hand ends of fragments $\Delta 7-9$, $\Delta 2-22$, and $\Delta 4-46$ are indicated by asterisks; the locations of the *Sal*I and *Nco*I sites shown in Fig. 1 are also indicated. Amino acid residues 2 to 10, which match the N-terminal amino acid sequence of the purified BsgA protein, are underlined. The potential ribosome binding site (RBS) at nucleotide residues 298 to 302 is also underlined.

(Fig. 3), we found that the *bsgA* gene product shares 45 and 48% amino acid identity (60 and 61% amino acid homology) with the *E. coli* and *B. brevis lon* gene products, respectively. This alignment is accommodated by use of only six single amino acid gaps or insertions and extends over virtually the entire length of each protein.

Elon	MNPERSERIEI-PVPLRDRVVVPHMVLPLFVGREKSIKRELAAMDHKKIMLYAQKEASTDEPGVNDLFTVGTASILQMLKPDGTVKVLEGLQRA	98
BsgA	MSDEKKKGAASAMPTAMAPPLINKEDIPQVLPLPLRNSVFFPGGVLPLAVGROKTIALIKDAYRDOQVIGVYTORRAEEEDPGAADLYTMGTVARIVKLLKMGEDNYSLVQGLARF	120
Blon	MGERSGKRELPLPLRGLLVYPTMVLHLDVGREKSIKRELAQAMVDNKKILLATQEEVHIEEPDAEQIYSIGTVARVQMLKPLNGTIRVLEGLQRA	97
Elon	RISALSDNGEHFSAKAEYLESPTIDER-EQEVLRVTAISQFEGYIKLNKKIPPEVLTSLSNIDDPARLADTIAAHMPLKLADQSVLEMSDVNERLEYLHAMMESEIDLLQVEKIRNRV	217
BsgA	RVVELVQEAPLYLKARYDAVEDKTSSENVEVEALGINKKLAREVIELMPELPAATLVEISITHPGHLADLIAANVDVPIEEKQAVLETVDLKARMKLVLELLNRKREILKLSNKSIDSAV	240
Blon	KIEEYLQKEDYFVVSITYLKKEKAEEN-EVEALMRSLLTHFEQYIKLSKKVSPETLTSVQDIEEPGRADVIASHLPLKMKDKQEIETVNIQERLEILLTLNNEVELELERKIGNRV	216
Elon	KKQMSQREYLLNEQMKAIQKELGEMDDAPDENEALKKRIDAAMPKEAKEAEALQKKMSPMSAEATVVRGYIDMVMQVPMNARSVKKDLRQAOEILTQHYGLERYKDRILEY	337
BsgA	KGEMSKTQREYLLRQQLKAIKEELGEMGEEEEELDELQERLKKAGLPDVEKVANKELNRLKTTIPASSEYTVARTYLDWIADLPWAKISEDNLDIENARQQLDKHFGIKKVKRILEY	360
Blon	KKQMERTQKEYLLRQMKAIQKELGDKDSRQGEVDLRAQLEKSDAPERIKAKIEKELERLEKMPSTSAEGSVIRTYIDTLFALPWTKTEDNLDIKHAEVLEDEHYGLEKPKERVILEY	336
Elon	LAVQSRVNIKIGPILCLVGGPPVGKTSLGQSIKATGRKYRMLGGVDEAEIRGHRTYIGSMPGKLIQKMAKVGKYNPLFLLEIDKMSDDMRGDPASALLEVLDPEQNVAFSDHYL	557
BsgA	LAVRKLKNDMRGPILCLVGGPPVGKTSLGQSVAKATGRKFYRLSLGGVDEAEIRGHRTYIGALPGRFIQSMKKAQTKNPVMMLEIDKLGADFRGDPASALLEVLDPEQNNVTSFSDHYL	480
Blon	LAVQKLVNSMRGPILCLVGGPPVGKTSLARSARALGREFYRLSLGGVDEAEIRGHRTYIGALPGRFIQGMKQAGTINPVFLLEIDKLSDFRGDPASALLEVLDPEQNDKFSFSDHYI	556
Elon	EVDYDLSVMFVATSSNM-IPAPLLDRMEVIRLSGYTEDEKLNIAKRHLPLKQIERNALKKGELTVDDSAIIGIIRYITREAGVRLEREISKLCRKAVKQLLLDKSKHIEINGDNLH	576
BsgA	DVPFDLSKVMFVATANQLDPIPGPLDRMEIETLTGYTTEEKQSIARHLVPLKQKELHSLPDHIDITDEALLTLTAYTREAGVRLERRIADICRAVAVEVAGGKTEQ-TINADRYK	599
Blon	EETDYLTNVMFITANSLODTPRPLDRMEVISISGYTEDEKLNIRGLVLLPKQMEDHGLKDKLQNMEDAMKLVRLYITREAGVRLNREANVCRAAKIIVGGEKKRV-VYTKATLE	575
Elon	DYLGQVRFYGR-ADNENRVQVGTGLAWTEVGGDLLTIETACVPGKGLTYTSGLGEVMOESIQAAITVVRARAELGIPNDFYEKRDHVVHVEGATPKDGPAGIAMTALVSLCTGN	695
BsgA	EILGPEMF-YSEVAERTEVPVATGLAWTAAGDGLFIEATKMGKGGMTLTGOLGDMKERPTAALSILRSKAEQLGSPNFLEKTLHLHFPAGSIPKDGPSAGVTILTALTSLLTGI	718
Blon	ALLGKPRY-RYGLAEKKDQVSVTGLAWTQAGDNLNEVSLIAGKGLTLTGOLGDMKESQAQAFSYIRSASEWIDPEFHEKNDIITHVPEGATPKDGPAGITMATVLSALTGI	694
Elon	PVRADVAMTGEITLRGQVLPILGGLKEKLLAAHGGIKTYLIPFENKRDLEEIPDNVIAADLDHPVKRIEEVLTALQNEPSGMHSLRRRCSTASTYYMAAS	797
BsgA	RVRHOTAMTGEATLRGLVPLVGGIKEKVLAAHRAKIRVILPERCKDLIDVPOARNELEFIVTHMDVYLKAALETTPYGVAGTPGGEKPEAPLKPASAEPEVA	827
Blon	PVKKEVGMTGEITLRGRLVPLGGLKEKMSAHRAGLTTIILPKDNEKDIEDIPESREALTFYPVEHLDEVLRHALTKQPVGDKK	779

FIG. 3. Comparison of the deduced amino acid sequences (in standard one-letter symbols) of *E. coli lon* (2), *M. xanthus bsgA*, and *B. brevis lon* (16), designated Elon, BsgA, and Blon, respectively. Identical amino acid residues are indicated by colons. Conservative amino acid substitutions (based on the groups I, L, V, and M; D and E; K, R, and H; Q and N; S and T; G and A; and F and Y) are indicated by dots. Gaps inserted to attain optimal alignment of the sequences are designated by hyphens. The locations of sequences matching the segment A and segment B consensus sequences of the ATP-binding domain proposed by Chin et al. (4) are indicated by horizontal lines above the Elon sequence. Ser-679 of the Elon sequence, proposed to be a catalytically active residue (1), is indicated by an asterisk.

The *lon* gene of *E. coli* has been shown to encode an ATP-dependent protease, designated protease La (3, 5). Chin et al. (4) suggested that the segment A and segment B regions of the ATP-binding domain of protease La and other proteins that bind and hydrolyze ATP conform to the consensus sequences (G/A) X_4 (G/A)(H/K/R) X_{0-1} (T/S/K/R/H) and (H/K/R) X_{5-8} Φ X Φ_2 (D/E), respectively, where X and Φ represent any amino acid and a hydrophobic amino acid, respectively. Each of the three predicted proteins contains sequences that closely match the segment A and segment B consensus sequences of the ATP-binding domain (Fig. 3). These sequences are located at identical locations within the aligned sequences and are embedded within a larger region that is particularly well conserved.

Although protease La has been designated a serine protease (24), there is no strong sequence similarity between the active sites of either La or its *M. xanthus* and *B. brevis* homologs and the active sites of other known serine proteases. However, Amerik et al. (1) determined that Ser-679 of the *E. coli* protein is essential for proteolytic activity and suggested that it is likely a catalytically active residue. Both BsgA and the *B. brevis lon* gene product contain a serine residue at the corresponding location in the aligned sequences (Fig. 3); this residue is in turn embedded within a region that is particularly well conserved among the three sequences.

An additional feature of the BsgA protein is a highly acidic region from residues 262 to 276 in which 10 of 15 residues are glutamic acid or aspartic acid, including a stretch of 5

consecutive glutamic acid residues. There is a corresponding region in protease La in which 7 of 13 residues are acidic. The acidic region of the BsgA protein, however, is not flanked on either side by basic domains as it is in protease La. We speculate that the acidic region of protease La is important for the activation of the protease by polybasic peptides (4).

The cloned BsgA protein has ATP-dependent protease activity. In light of the striking amino acid sequence homology between the predicted BsgA protein and protease La, we examined the BsgA protein for ATP-dependent protease activity. The BsgA protein is present in *M. xanthus* strains at only very low levels and is particularly unstable in *M. xanthus* cell extracts (9; data not shown). Therefore, we purified the cloned BsgA protein from an *E. coli* strain in which expression was inducible with IPTG and relatively high levels of the BsgA protein were produced (Fig. 4A). The *E. coli* host used in these experiments has a deletion of *lon*, the structural gene for protease La, so as to minimize ambiguity in our results due to copurification of the homologous *E. coli* protease.

Cell extracts of IPTG-induced *E. coli* with or without the cloned *bsgA* gene were prepared, and the dialyzed, high-speed supernatants were assayed for protease activity. There was no significant difference in the protease specific activity between the two extracts when the activity was measured in the absence of added ATP. However, when it was assayed in the presence of 2.5 mM ATP, the ATP-dependent protease activity was 15- to 44-fold higher in

FIG. 4. Expression and purification of cloned BsgA from *E. coli*. Proteins were fractionated by SDS-PAGE and visualized by staining with Coomassie blue. (A) Lanes: A and B, total cellular protein from uninduced and IPTG-induced cells containing pLacI^q but lacking the *bsgA* plasmid, respectively; C and D, total cellular protein from uninduced and IPTG-induced cells containing both pLacI^q and *bsgA* plasmid pREG1344Δ7-9, respectively. The location of the BsgA protein is indicated by the arrow. (B) Lanes: A, dialyzed, high-speed supernatant fraction; B to D, fractions obtained by successive chromatography on DEAE-Sephacel (B), Mono Q (C), and Sephacryl S-200 (D), respectively. The location of the BsgA protein is indicated by the arrow. Numbers beside gels are in kilodaltons.

extracts containing BsgA than in extracts prepared from cells lacking the *bsgA* plasmid or cells carrying a plasmid encoding a mutant, and presumably inactive, BsgA protein (data not shown). These results suggest that the BsgA-containing extracts do indeed contain increased ATP-dependent protease activity and that this activity is likely due to the BsgA protein itself.

For providing further evidence that the BsgA protein was responsible for this activity, the dialyzed, high-speed supernatant fraction was fractionated through three chromatography steps (Fig. 4B): ion-exchange chromatography on DEAE-Sephacel (lane B) and Mono Q (lane C) and gel filtration on Sephacryl S-200 (lane D). During purification, fractions were assayed for protease activity in the presence and absence of ATP and for BsgA protein by SDS-PAGE and Western blotting.

The ATP-dependent protease specific activities of the pooled peak fractions from each purification step were as follows: dialyzed, high-speed supernatant, 6,500 U/mg; DEAE-Sephacel, 40,400 U/mg; Mono Q, 52,600 U/mg; and Sephacryl S-200, 131,000 U/mg. A Coomassie blue-stained SDS gel of the corresponding fractions is shown in Fig. 4B. Through each purification step, the ATP-dependent protease was copurified with the BsgA protein. By comparison, no corresponding peak of ATP-dependent protease activity was observed following DEAE-Sephacel fractionation of extracts lacking BsgA (data not shown). In the final Sephacryl S-200 gel filtration step, the BsgA protein and the ATP-dependent protease eluted together as a sharp symmetric

FIG. 5. Elution profile for the Sephacryl S-200 chromatography step of BsgA purification. Peak material following Mono Q chromatography was fractionated on Sephacryl S-200 as described in Materials and Methods. Symbols: □, optical density at 280 nm (O.D.₂₈₀); ○ and ●, protease activity measured by the standard assay in the absence (○) or presence (●) of 2.5 mM ATP.

peak at or near the voided volume. The S-200 peak material represented an approximately 20-fold purification of the ATP-dependent protease present in the dialyzed, high-speed supernatant fraction, yielding a highly purified, although not homogeneous, BsgA protein (Fig. 4B, lane D, and Fig. 5). Protease activity in this peak fraction was completely dependent on ATP; essentially no detectable protease activity was found when the assay was done in the absence of exogenous ATP.

For obtaining an estimate of the size of the native protein, material from the S-200 peak was further fractionated by FPLC on Superose 6. The BsgA protein and the protease were coeluted at an apparent molecular mass of 380 kDa (data not shown). On the basis of a subunit molecular mass of 90 kDa, the BsgA protease, like protease La of *E. coli* (5), is likely to be a tetramer consisting of four identical subunits.

DISCUSSION

The nucleotide sequence of the *bsgA* gene of *M. xanthus* was determined. Of the several possible translational start sites, the ATG at nucleotide 312 is believed to be correct on the basis of the N-terminal protein sequence of the authentic BsgA protein affinity purified from *M. xanthus* and the presence of a suitable ribosome binding site. The size of the predicted BsgA protein, 90.4 kDa, and its predicted translational reading frame are both in agreement with our previous results.

A search of the GenBank sequence data base indicated that the deduced BsgA protein has 45 and 48% amino acid identity (60 and 61% amino acid homology) with the *lon* gene products of *E. coli* and *B. brevis*, respectively. The homology extends over most of the length of the three proteins and is particularly strong in the proposed ATP-binding domain and surrounding the proposed catalytically active serine residue (Ser-679 of the *E. coli* sequence).

The product of the *lon* gene in *E. coli* is ATP-dependent protease La. We partially purified the cloned *M. xanthus* BsgA protein and showed that it is copurified with the ATP-dependent protease. Both proteins also appear to be active as tetramers. These findings suggest that BsgA is likely to be a member of a class of proteases for which protease La is the prototype. This class of enzymes utilizes

a novel reaction mechanism in which protein degradation is coupled to the hydrolysis of ATP (12). Proteases with enzymatic properties similar to those of protease La appear to be widely distributed in both prokaryotic and eukaryotic cells (12–14). However, a regulatory role for members of this class of proteases has been demonstrated only for *E. coli* and now for *M. xanthus*.

The *bsgA* gene product of *M. xanthus* plays a critical role in the regulation of early developmental gene transcription (10, 18) and is necessary for a required cell-cell interaction at or near the onset of development (10). However, even with the identification of BsgA as an intracellular protease, its precise role in the regulation of developmental gene transcription is not obvious. However, there is ample precedent for the involvement of proteases in the regulation of transcription and other cellular processes in bacteria (12–14, 23). Among the target proteins for which proteolysis plays a role in transcriptional regulation are transcriptional repressors, such as LexA and cI (23), transcriptional activators, such as RcsA (21), and sigma factors, such as developmental stage-specific σ^K , found in sporulating *Bacillus subtilis* (6). Proteolysis also plays a role in regulating a variety of cellular processes (13), such as cell division, through an effect on SulA, and UV-induced mutagenesis, through an effect on UmcD.

Our finding that the *bsgA* gene product is an intracellular protease suggests that the role of BsgA in development is to modulate the half-life of a protein(s) involved in controlling developmental gene expression. This protease may directly affect a component of the transcriptional apparatus itself. Alternatively, it may be necessary for some aspect of *bsgA*-dependent cell signaling that is in turn required for developmental gene transcription. For example, BsgA may generate a required developmental signal, such as a small peptide, as a result of protein degradation. We isolated a particularly interesting mutant that will be useful in understanding the function of this protease and identifying its relevant substrate (unpublished data). This mutant was identified as an extragenic suppressor of a *bsgA* null mutation and has the pleiotropic properties of (i) restoring the ability of *bsgA* mutants to produce fruiting bodies and spores, (ii) restoring the ability of *bsgA* mutants to transcribe at least certain developmental genes, and (iii) allowing inappropriate, vegetative expression of at least one developmental gene, *tps*.

The properties of this mutant provide considerable support for the attractive hypothesis that the BsgA protease is required in normal development to inactivate a negatively acting regulatory factor, perhaps a transcriptional repressor, thereby allowing the transcription of *tps* and presumably other early developmental genes necessary for entry into the developmental program. Evidence for the involvement of such a repressor in the regulation of the *tps* gene has been reported by Downard et al. (8). According to this hypothesis, when the repressor is present in the cells (i.e., during vegetative growth, as well as in developing *bsgA* cells, which are unable to degrade the repressor), the transcription of developmental genes is inhibited. When the repressor is removed at the onset of development, perhaps as a result of direct proteolysis by BsgA, transcription of the regulated genes occurs. Among the genes regulated by BsgA may be those whose products mediate *bsgA*-dependent cell signaling. The pleiotropic effects of the suppressor mutation can be explained if the mutation inactivates the putative transcriptional repressor itself. In these cells, there would no longer be a requirement for the BsgA protease, and the lack

of a repressor in vegetative cells could lead to the observed constitutive expression of the regulated developmental genes. While aspects of this hypothesis require verification, it is nevertheless an attractive hypothesis to begin to explain certain aspects of early developmental gene regulation.

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