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The *dsg* Gene of *Myxococcus xanthus* Encodes a Protein Similar to Translation Initiation Factor IF3

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The *dsg* mutants of *Myxococcus xanthus* are defective in fruiting body development and sporulation, yet they grow normally. The deduced amino acid sequence of the *dsg* gene product is 50 and 51% identical to the amino acid sequence of translation initiation factor IF3 of both *Escherichia coli* and *Bacillus stearothermophilus*, respectively. However, the Dsg protein has a carboxy-terminal extension of 66 amino acids, which are absent from its *E. coli* and *B. stearothermophilus* homologs. The Shine-Dalgarno sequence GGAGG and 5 bases further upstream are identical in *M. xanthus* and several enteric bacteria, despite the wide phylogenetic gap between these species. The *infC* gene, which encodes IF3 in enteric bacteria, starts with the atypical translation initiation codon AUU, which is known to be important for regulating the cellular level of IF3 in *E. coli*. Translation of the Dsg protein overexpressed from the *M. xanthus dsg* gene in *E. coli* cells initiates at an AUC codon, an atypical initiation codon in the AUU class. The *dsg* mutants DK429 and DK439 carry the same missense mutation that changes Gly-134 to Glu in a region of amino acid identity.

Myxococcus xanthus, a gram-negative bacterium, undergoes multicellular development with cellular aggregation and sporulation. When development is induced by nutrient limitation, about 10^5 *M. xanthus* cells glide into a center of aggregation, in which they build a fruiting body and differentiate into spores (17). Since myxobacterial cells feed cooperatively, the fruiting body guarantees a large cell population for efficient growth when nutrients are available. The nutritional conditions that induce development include amino acid limitation, carbon and energy starvation, phosphate starvation, and the addition of competitors that inhibit the amino acid charging of a specific tRNA (16). Despite starvation, the synthesis of new and specific proteins is essential for development. The levels of more than 30 proteins, identified as bands separated by gel electrophoresis, change according to a well-defined time schedule (4, 12, 24).

The *dsg* mutants were isolated as development-deficient mutants. They grow normally in rich and minimal media, in which their growth rate is similar to that of dsg^+ cells (3). The *dsg* mutants can initiate development, form early aggregates, and express early developmentally regulated Tn*5-lac* transcriptional fusions (2). However, they fail to complete aggregation, are 10^3-fold deficient in sporulation, and are deficient in the expression of a particular set of Tn*5-lac* fusions which would normally be expressed after 4 to 6 h of development.

We previously reported the cloning and localization of the *dsg* gene to an 850-bp region of the genome. Tn*5* insertions within this segment are lethal, indicating that the *dsg* gene is essential for cell viability. However, *dsg* point mutants that are developmentally defective are viable (2, 3). This paper reports the nucleotide sequence of the *dsg* gene, the homology between the Dsg protein and translation initiation factor IF3 from both *Escherichia coli* and *Bacillus stearothermophilus*, and the utilization by the Dsg protein of the unique translation start codon AUC. IF3 serves vital cell functions in dissociating 70S ribosomal complexes when they have completed translation of an mRNA (7) and in scanning a new molecule of mRNA for the proper site to initiate translation and set the reading frame (5). Since IF3 is essential for protein synthesis, few *infC* mutants in any organism have been isolated or studied. A comparison of IF3 from *E. coli* with the wild-type and mutant forms of *M. xanthus* Dsg proteins may help to elucidate the relationships among IF3 structure, function, and regulation because of the phylogenetic distance between these proteins. The possibility that *dsg* encodes IF3 in *M. xanthus* is surprising, since a defect in the protein synthesis machinery would be expected to affect growth as well as development. Although a *dsg* null mutation is lethal, two *dsg* point mutants grow normally but are defective in development. The present work aims to understand why the *dsg* gene is vital for growth and development and how a missense mutant can grow normally but have a strong defect in development.

MATERIALS AND METHODS

Bacteria, plasmids, and culture conditions. The strains and plasmids used in this study are listed in Table 1. *M. xanthus* strains were grown in CTT broth or on CTT agar plates (10). *E. coli* strains were grown in or plated on Luria-Bertani medium at 37°C (18).

Materials. Restriction enzymes were supplied by New England Biolabs, IBI, and Boehringer Mannheim Biochemicals. ^{35}S-dATP (>1,000 Ci/mmol) was purchased from Amersham.

DNA preparation and sequencing. DNA fragments to be sequenced were cloned into pBluescript II SK^+ and pBluescript II KS^+ vectors (Stratagene, La Jolla, Calif.) and propagated in *E. coli* XL-1Blue to be used for the generation of single-stranded DNA (23). Double-stranded DNA was prepared by the modified boiling miniprep method of Holmes et al. (18). Nested ExoIII deletions of pYLC102 and pYLC107 for sequencing were obtained by use of a nested deletion kit supplied by Pharmacia LKB (Piscataway, N.J.). The dideoxynucleotide chain termination method of Sanger et al. (19) was used at 37°C with Sequenase T7 DNA polymerase as recommended by the manufacturer (United States Biochemicals, Cleveland, Ohio). To reduce electrophoretic band compression, dITP reactions were run in parallel with dGTP reactions.

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source
Strains		
M. xanthus		
DK101	*sglA1*	11
DK429	*dsg-429 sglA1*	
DK439	*dsg-439 sglA1*	
DK4870	*dsg-439 sglA1*	2
E. coli		
DH5α	*supE44* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *hsdR17 recA1 gyrA96 thi-1 relA1*	18
XL-1Blue	*supE44 recA1 hsdR17 lac*F′::Tn10 proA$^+$ lac^q *lacZ*ΔM15 *relA1 endA1 gyrA96* (Nal^r) *thi*	18
Plasmids		
pBluescript II KS$^+$	2.958-kb phagemids derived from pUC19	Stratagene
pBluescript II SK$^+$	2.958-kb phagemids derived from pUC19	Stratagene
pYLC102	0.9-kb *Sma*I-*Sma*I *dsg*$^+$ in KS^a	This work
pYLC201 to pYLC212	Deletions of pYLC102	This work
pYLC107	0.9-kb *Sma*I-*Sma*I *dsg*$^+$ fragment in KS, in an orientation opposite that in pYLC102^a	This work
pYLC701 to pYLC711	Deletions in pYLC107	This work
pYLC121	0.9-kb *Xho*I-*Mlu*I *dsg*$^+$ fragment in SK^a	This work
pYLC123	0.9-kb *Xho*I-*Mlu*I *dsg-429* fragment in KS^a	This work
pYLC124	0.9-kb *Xho*I-*Mlu*I *dsg-429* fragment in SK^a	This work
pYLC125	1.2-kb *Xho*I-*Mlu*I *dsg-439* fragment in KS^a	This work
pLK103	1.1-kb *Nde*I-*Mlu*I *dsg*$^+$	13

^{a} Each of the fragments indicated was cloned into the pBluescript II KS or pBluescript II SK vectors.

The incorporation of 7-deaza-GTP instead of dGTP in the reactions was also used to confirm sequencing results. For reliability, the sequence was determined from both strands several times and confirmed by the overlap of DNA fragments. The DNA sequence of the *dsg* initiation region in pLK103, used in the production of the Dsg protein in *E. coli*, was also determined and found to be the same as that originally determined. The DNA sequence of the *dsg* region has been assigned GenBank accession number U04438.

Purification of the Dsg protein for the N-terminal sequence determination. Fifty milliliters of *E. coli* DH5α containing plasmid pLK103, which encodes *dsg*, was grown overnight in LB medium containing carbenicillin (100 mg/liter). The cells were harvested by centrifugation at 7,000 × *g* in a Beckman JA-20 rotor at 4°C. The pellet was washed with a cold solution of 10 mM Tris (pH 7)–10 mM $MgCl_2$ and resuspended in 2 ml of the same buffer. The cells were broken by French press cell disruption, and the cell lysate was fractionated into S30 and P30 fractions by centrifugation at 30,000 × *g* (16,000 rpm in a Beckman JA-21 rotor) for 50 min at 4°C. The 30S fraction was further separated into S150 and P150 fractions by centrifugation at 150,000 × *g* (61,000 rpm in a Beckman TLA 100.3 rotor) for 2 h at 4°C. The ribosomal pellet (P150) was resuspended in 10 mM Tris (pH 7)–10 mM $MgCl_2$. Samples of the P150 fraction were separated by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis and blotted onto an Immobilon-P polyvinylidene difluoride membrane (Millipore). Proteins on the blot were visualized by staining with Coomassie brilliant blue R-250. The location of the band corresponding to Dsg was determined by comparing P150 fractions of *E. coli* DH5α(pUC19), which does not encode *dsg*, and *E. coli* DH5α(pLK103), which does. A band of approximately 30 kDa was observed only in the DH5α(pLK103) extract. This band was also found to react with anti-*M. xanthus* Dsg and anti-*E. coli* IF3 sera. The Dsg band was cut from the polyvinylidene difluoride blot and sequenced by Edman degradation at the Stanford University Protein and Nucleic Acid facility.

RNA isolation and Northern (RNA) blot analysis. *M. xanthus* RNA was isolated by use of a protocol described by Stephens et al. with a buffer containing 4 M guanidine thiocyanate (21). RNA was electrophoresed on 1.2% agarose–formaldehyde gels, blotted onto a Nytran membrane (Schleicher & Schuell, Inc., Keene, N.H.), and probed with single-stranded probes labeled by primer extension as described by Stephens et al. (21).

RESULTS

Nucleotide sequence and reading frame of the *dsg* gene. The *dsg* gene had been located in an 850-bp segment of the *M. xanthus* genome by Tn*5* insertion and deletion mutagenesis and by gene rescue (2, 3). These experiments had indicated that the *dsg* gene resided between the *Sma*I and *Mlu*I sites shown in the restriction map of Fig. 1. Two overlapping DNA fragments, an *Sma*I-*Sma*I fragment and an *Xho*I-*Mlu*I fragment, were cloned into pBluescript II SK$^+$ and pBluescript II KS$^+$ vectors (creating pYLC102 and -107 and pYLC121 to -125). Nested deletion subclones of pYLC102 and pYLC107 were generated by ExoIII-S1 nuclease digestion or by restriction enzyme digestion. With these subclones, the nucleotide sequence of the 1,306-bp *Sma*I-*Mlu*I segment of the *dsg* region was determined. The nucleotide sequence and the predicted Dsg peptide sequence are shown in Fig. 2.

To determine the transcriptional orientation of the *dsg* gene, *dsg* RNA was analyzed on Northern blots by hybridization with single-stranded DNA probes. The *dsg* RNA is transcribed in the direction from *Sma*I to *Mlu*I, left to right, as shown in Fig. 3. Two RNA transcripts, 850 and 950 bp in length, were detected, but no RNA hybridized to DNA probes of the opposite strand, as shown in Fig. 3. Single-stranded probe B (bottom strand, 5′ right and 3′ left) and the opposite single-stranded probe, probe B′ (top strand, 5′ left and 3′ right), yielded the same results as probes C (bottom strand) and C′ (top strand); namely, two transcripts were detected with probe B but not with probe B′. In addition, double-stranded probes A and E also failed to detect any transcripts, whereas probe D

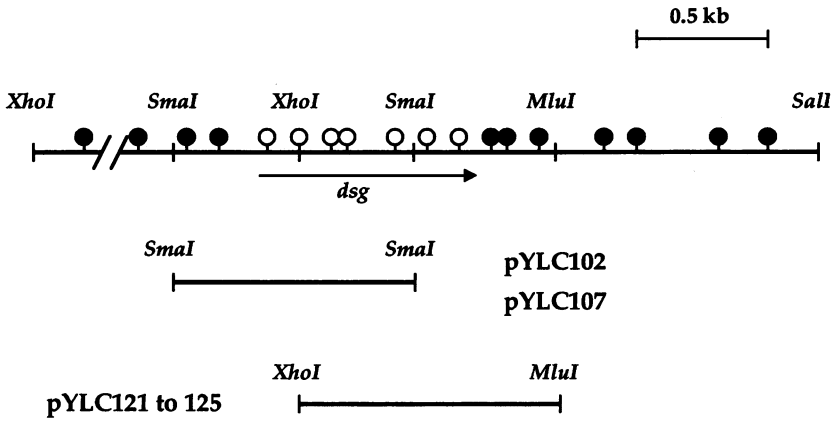

FIG. 1. Map of the *dsg* region. The positions of different Tn*5* insertions are represented by circles, closed if a single copy of the insertion in the chromosome is viable and *dsg*$^+$ and open if the insertion is lethal. On this map, the *dsg* gene resides somewhere between the leftmost *Sma*I site and the *Mlu*I site on the right. The *dsg* mRNA is depicted as an arrow pointing in the direction of transcription. pYLC102, pYLC107, and pYLC121 to pYLC125 are overlapping subclones of the *dsg* region used for DNA sequence determinations.

detected the same two transcripts as probes B and C but very faintly (data not shown). Two transcription start sites, the A at nucleotide position 127 and the T at position 231, were detected in primer extension and S1 nuclease protection experiments (data not shown).

Given the orientation of the *dsg* RNA, three forward reading frames are possible. Third-position codon G+C bias for *M. xanthus* is expected to be 85% or higher, since the overall G+C content of the *M. xanthus* genome is 67% (1, 9, 20). For the *dsg* region, only one of the three possible forward reading frames had a high third-position codon G+C bias. This frame had 86% third-position G+C; the other two frames had 70 and 50%. Moreover, only this frame was delimited by TGA stop codons separated by a distance compatible with the previous Tn*5* insertion studies and with the observed size of the *dsg* mRNA.

The open reading frame (ORF) so identified is delimited by TGA stop codons at positions 288 and 1058 in Fig. 2. Inspection of the 5′ region of the unique ORF revealed a purine-rich Shine-Dalgarno sequence, GGAGG (9, 15), at positions 299 to 303. However, no typical translation initiation codon, i.e., AUG, GUG, or UUG, was evident 12 bp or less downstream of the putative Shine-Dalgarno sequence.

Sequence comparison of the predicted Dsg protein and translation initiation factor IF3. A computer search of the Swiss-Prot data bank with the predicted amino acid sequence of the *dsg* ORF revealed a high sequence identity to translation initiation factor IF3 of both *E. coli* and *B. stearothermophilus* (Fig. 4). In the common regions, residues 1 to 180 of the *dsg* peptide sequence, the sequence of the predicted Dsg protein is 50 and 51% identical and 85 and 87% similar to the sequences of the *E. coli* and *B. stearothermophilus* IF3 proteins, respectively. The same single-codon gap following proline 164 of the Dsg protein was required in both the *M. xanthus* and the *B. stearothermophilus* sequences to align them with their cognate *E. coli* sequence. Also, the three amino acid sequences have similar hydropathy profiles (8). The pI of the Dsg protein is predicted from its composition to be 10.5; the pIs of *E. coli* IF3 and *B. stearothermophilus* IF3 are predicted to be 10.4 and 10.7, respectively. The identity and similarity of the predicted Dsg protein and the two IF3 proteins end 66 amino acids before the carboxy terminus of Dsg, and the *M. xanthus* protein is significantly longer than either of its homologs.

The nucleotide sequences for the independently isolated *dsg-429* and *dsg-439* mutants were also determined. Both mutants contain the same G-to-A mutation at nucleotide 715, in the middle of the *dsg* gene. Their sequence identity explains why the two mutant alleles were never observed to recombine with each other. This mutation would change the conserved small neutral glycine 134 residue to a larger charged glutamate residue in the part of the Dsg protein which is conserved among *M. xanthus*, *E. coli*, and *B. stearothermophilus*.

Initiation codon for the translation of *dsg*. When the region surrounding the proposed Shine-Dalgarno sequence of the *dsg* ORF is compared with the corresponding sequences upstream of the start codon of *E. coli infC* and the nucleotide sequences upstream of the IF3 initiation codons of *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Serratia marcescens* (14), striking identities appear (Fig. 5). The Shine-Dalgarno sequence and 8 bp upstream are identical. The enteric *infC* sequences all have the atypical translation start codon AUU, separated by 7 bp from the end of the Shine-Dalgarno sequence. In the *dsg* gene, there is an ATT triplet separated by 11 bp from the conserved Shine-Dalgarno sequence, a distance significantly longer than the 7-bp spacing found in the *infC* genes of *E. coli* and the four other enteric bacteria. Just preceding ATT is a related codon, ATC, at nucleotide 315, in the same frame and separated by 8 bp from the Shine-Dalgarno sequence. The use of this AUC for initiation would preserve the spacing, although AUC has not been reported as an initiation codon before.

To identify the actual protein start, the N-terminal amino acid sequence was obtained by Edman degradation of partially purified Dsg protein (see Materials and Methods). For this purpose, the *dsg* gene was overexpressed from plasmid pLK103 in *E. coli* DH5α. Although the level of IF3 is normally tightly regulated in *E. coli*, the cells apparently tolerate a higher level of the heterologous Dsg protein. Proteins from strain DH5α carrying pLK103 were isolated in two separate experiments; the N-terminal sequence of the Dsg protein band was determined twice from each preparation. In each case, the sequence of the first six amino acids at the N terminus of the Dsg protein was found to be Met Ile Arg Glu Gln Arg. This sequence matches the reading frame of the *dsg* ORF shown in Fig. 2. It shows that *dsg* is translated starting with the AUC codon at position 315, since the protein has a methionine residue

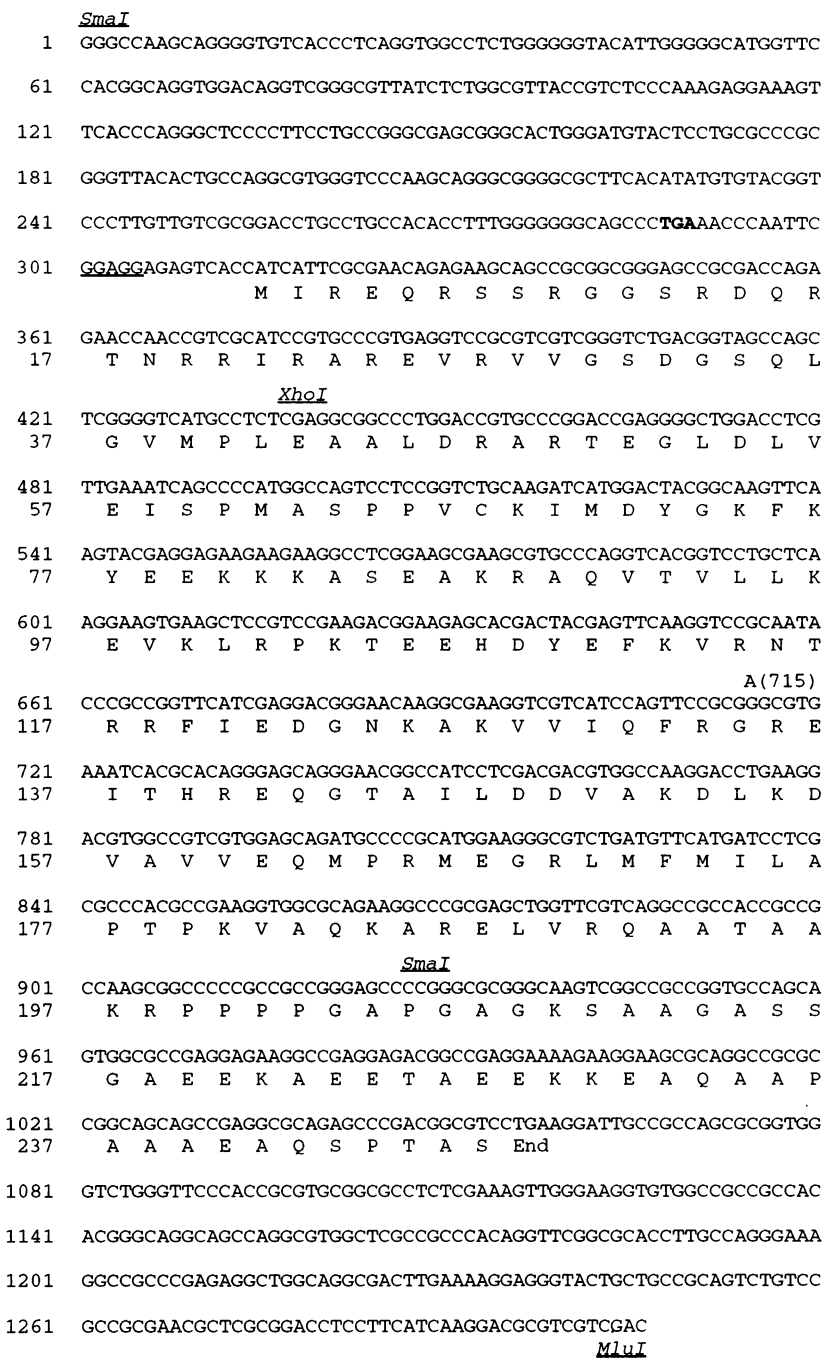

FIG. 2. Nucleotide sequence of the *dsg* region extending from the leftmost *Sma*I site in Fig. 1 to the *Mlu*I site. The sequence is shown in the transcribed direction, and the base sequence given corresponds to the mRNA. Restriction sites are labeled. The TGA stop codon of the preceding ORF is shown in boldface type. The Shine-Dalgarno sequence is underlined. The mutation in *dsg-429* and *dsg-439* of G-715 to A is shown. Translation of the sequence is shown by use of the single-letter abbreviations of the amino acids.

occupying this position rather than an isoleucine residue that would be specified by an internal AUC codon.

DISCUSSION

Originally, the *dsg* gene was defined by the *dsg-429* and *dsg-439* mutants (2). Both *dsg* mutants displayed the same phenotype: abnormal aggregation and much-delayed sporulation. At 3 days of development, sporulation was at least 10^3-fold decreased from dsg^+ levels. Both mutations were linked to Tn*5* Ω1867, with 75% cotransduction by myxophage MX8. Tn*5* Ω1867 cotransduced dsg^+ and *dsg* alleles with the same frequency, as expected for a mutation in a single locus (2).

The *dsg* gene was located to an 0.85-kb segment, within which the Tn*5* insertion blocked the ability to rescue the phenotype of both mutants. Tn*5* insertions either to the left or

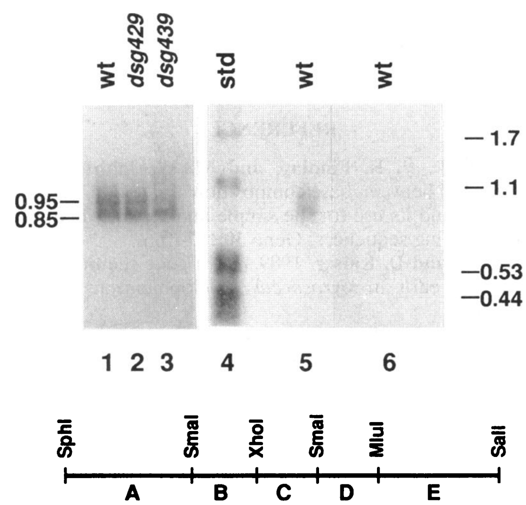

FIG. 3. Northern blots of RNAs from DK101 (wild type), DK429 (*dsg-429*), and DK439 (*dsg-439*); 10 μg of total RNA was loaded in each lane. The blots were probed with different segments of DNA from the *dsg* region defined on the map below the blots. Lanes 1, 2, 3, and 5 were probed with single-stranded fragment C (bottom strand, 5′ to 3′ runs right to left). Lane 6 was probed with probe C′ (top strand), which is the complement to probe C. The same RNA sample from DK101 was loaded in lanes 5 and 6, while a sample from a different DK101 preparation was loaded in lane 1. Lane 4 contains labeled molecular weight standards.

to the right of this segment retained the capacity to rescue (2). Although Tn*5* insertions within the 0.85-kb segment are lethal as haploids, a dominant lethal effect of the insertions can be excluded because insertions within the 0.85-kb segment are viable as partial diploids in which they are covered by a *dsg*$^{+}$, *dsg-429*, or *dsg-439* allele.

In the present report, it is shown that mRNA in the range of 850 to 950 bp hybridizes to the *dsg* region of DNA. Hybridization with single-stranded probes indicated that the mRNA was transcribed from left to right, as shown in Fig. 1. The sequence of 1,306 bp of the corresponding DNA region is presented here. One of the three possible reading frames is unique in that it has the necessary third-position codon bias toward a high G+C content and an ORF of significant length (770 bp). This ORF fits within the vital region defined by the Tn*5* insertion, within the DNA segment hybridizing to *dsg* RNA and within the segment that rescues the *dsg* mutants. We therefore conclude that this ORF encodes the *dsg* gene product.

Strikingly, the amino acid sequence encoded by the *dsg* gene is 50% identical and 85% similar to the sequences of amino acids in translation initiation factor IF3 of both *E. coli* or *B. stearothermophilus*. Since IF3 is essential for the correct initiation of protein translation, a role for Dsg as a translation factor would explain why *dsg* is a vital gene and why a Tn*5* insertion within it is lethal. It should be noted that there is only one locus related to this sequence in *M. xanthus* (13).

The independently isolated *dsg-429* and *dsg-439* mutants both have missense mutations that change Gly-134 to Glu. Since the two mutations are identical, it is now clear why the two mutants have identical phenotypes, why they show the

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P.v.            ..G.-..............ATT
S.m.           ....C.-..........A..ATT
K.p.            ...C.-.............ATT
S.t.            ...C.-..........A..ATT
E.c.        AAA--CAATT-GGAGGAATAAGGTATT
            |||  ||||| ||||||
M.x.      TGAAACCCAATTCGGAGGAGAGTCACCATCATT
            |||       || |||||
B.s.     TGGAAAAAAACCTT-GGAGGTGGCTCATTATT
```

FIG. 5. Alignment of the nucleotide sequences upstream of the translation start codons of *M. xanthus* (*M.x.*) *dsg* and *infC* from *E. coli* (*E.c.*), *B. stearothermophilus* (*B.s.*), and four other enteric bacteria: *P. vulgaris* (*P.v.*), *S. marcescens* (*S.m.*), *K. pneumoniae* (*K.p.*), and *S. typhimurium* (*S.t.*). Vertical lines or dots indicate nucleotides identical to those in the *E. coli* sequence. The initiation codons and the Shine-Dalgarno sequences are shown in boldface type.

```
Dsg           1 MIREQRSSRGGSRDQRTNRRIRAREVRVVGSDGSQLGVMPLEAALDRARTEGLDLVEISPMASPPVCKIMDYGKFKYEEKKKASEAK
E.coli IF3    1    MKGGKRVQTARPNRINGEIRAQEVRLTGLEGEQLGIVSLREALEKAEEAGVDLVEISPNAEPPVCRIMDYGKFLYEKSKSSKEQK
Bac.St.IF3    1              MSKDFIINEQIRAREVRLIDQNGDQLGIKSKQEAKEIAARRNLDLVLVAPNAKPPVCRIMDYGKFRFEQQKKEKEAR

                                                      E (dsg429 & 439 )
                                                      |
Dsg          88 RAQVTVLLKEVKLRPKTEEHDYEFKVRNTRRFIEDGNKAKVVIQFRGREITHREQGTAILDDVAKDLKDVAVVEQMP-RMEGRLM
E.coli IF3   86 KKQKVIQVKEIKFRPGTDEGDYQVKLRSLIRFLEEGDKAKITLRFRGREMAHQQIGMEVLNRVKDDLQELAVVESFPTKIEGRQM
Bac.St.IF3   77 KKQKVINVKEVRLSPTIEEHDFNTKLRNARKFLEKGDKVKATIRFKGRAITHKEIGQRVLDRLSEACADIAVVETAP-KMDGRNM

Dsg         172 FMILAPTPKVAQKARELVRQAATAAKRPPPPGAPGAGKSAAGASSGAEEKAEETAEEKKEAQAAPAAAEAQSPTAS   247
E.coli IF3  171 IMVLAPKKKQ                                                                     180
Bac.St.IF3  160 FLVLAPKNDNK                                                                    170
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FIG. 4. Amino acid sequence comparison of the Dsg protein and translation initiation factor IF3 of both *E. coli* and of *B. stearothermophilus* (*Bac. St.*). Vertical lines mark identical amino acid residues. The G-to-E mutation at amino acid 134 in the *dsg-429* and *dsg-439* mutants is shown.

same linkage to Tn*5* Ω1867, and why they have never been separated from each other by recombination. Since the change of Gly-134 to Glu is within a highly conserved part of IF3 and replaces a neutral, small amino acid side chain with a negatively charged, much larger side chain, the mutation very likely affects the function of the Dsg protein in some (unknown) way. Although this particular mutation is the only missense *dsg* mutation available, there are seven different Tn*5* insertions in *dsg* that create null alleles in the locus and three different deletions in the carboxy-terminal extension.

The DNA sequence just upstream of the *dsg* ORF contains 14 conserved bases that include the Shine-Dalgarno sequence GGAGG (Fig. 5). The conserved sequence starts with the A of a TGA codon, which may be the termination codon of a preceding ORF. Apparently, the DNA base sequence at the beginning of the *dsg* ORF has been conserved despite the contrast between the average 50% G+C content of *E. coli* and the other enteric bacteria shown in Fig. 5 and the 70% G+C content of *M. xanthus*. Evolutionary pressure for sequence divergence from *infC* is reflected in the observed divergence of the base sequence immediately downstream of the coding region of the Dsg protein, in which DNA sequence identity falls to about 50%. Conservation implies an essential role for this base sequence. Presumably, the conserved sequence of *dsg* mRNA interacts with the 30S ribosomal subunit to select the start codon and reading frame of the *dsg* gene.

Downstream of this highly conserved Shine-Dalgarno sequence, there are no AUG, GUG, or UUG translation start codons. These three are the typical start codons, which are found in 91, 8, and 1%, respectively, of a catalog of 300 ribosome binding sites (6). Instead, downstream of the conserved Shine-Dalgarno sequence in the *dsg* gene, there is an AUC immediately followed by an in-frame AUU. AUU and AUC are atypical translation start codons and are translated at 1 to 3% the level of the typical start codons in *E. coli*, as measured with a *lacZ* translational fusion (22). In *E. coli*, an AUU is the demonstrated initiation codon for IF3, and this atypical start codon is important for the regulation of IF3 expression (5). In the present work, we found that when *dsg* is expressed in *E. coli*, translation initiates at an AUC codon, also an atypical start codon. It may be similarly involved in translational regulation. AUC has not previously been reported as an initiation codon for any natural gene. A low abundance of the Dsg protein in cells as well as instability in extracts has so far precluded the isolation of sufficient protein from *M. xanthus* for an N-terminal sequence determination. *M. xanthus* may, on the one hand, use AUC to initiate *dsg* (as *E. coli* does), since AUC is the first possible start codon downstream of the Shine-Dalgarno sequence. On the other hand, *M. xanthus* may use AUU instead, because AUU starts all other known IF3 genes.

Given the sequence similarities to IF3, Kalman et al. (13) pose the following question: is the Dsg protein really translation initiation factor IF3 in *M. xanthus*? Since the Dsg protein is longer by 66 amino acid residues than other reported IF3 proteins, does this fact imply that an initiation complex in *M. xanthus*, presumably consisting of a 30S ribosomal subunit, mRNA, fMet-tRNA, and IF1, IF2, and IF3, has a structure different from that of the initiation complex in *E. coli*? Experiments done to test the necessity and function of the C-terminal tail of *dsg* are also reported by Kalman et al. (13).

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REFERENCES

1. **Bibb, M. J., P. R. Findlay, and M. W. Johnson.** 1984. The relationship between base composition and codon usage in bacterial genes and its use for the simple and reliable identification of protein-coding sequences. Gene **30:**157–166.
2. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for cell-cell interaction early in *Myxococcus* development. J. Bacteriol. **171:** 3719–3726.
3. **Cheng, Y., and D. Kaiser.** 1989. *dsg*, a gene required for *Myxococcus* development, is necessary for cell viability. J. Bacteriol. **171:**3727–3731.
4. **Downard, J., and L. Kroos.** 1993. Transcriptional regulation of developmental gene expression in *Myxococcus xanthus*, p. 183–199. *In* M. Dworkin and D. Kaiser (ed.), Myxobacteria II. American Society for Microbiology, Washington, D.C.
5. **Gold, L.** 1988. Post transcriptional regulation mechanisms in *Escherichia coli*. Annu. Rev. Biochem. **57:**199–233.
6. **Gold, L., and G. Stormo.** 1987. Translational initiation, p. 1302–1307. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
7. **Grunberg-Manago, M.** 1987. Regulation of the expression of aminoacyl-tRNA synthetases and translation factors, p. 1386–1409. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
8. **Gualerzi, C. O., C. L. Pon, R. T. Pawlik, M. A. Canonaco, M. Paci, and W. Wintermeyer.** 1986. Role of the initiation factors in *Escherichia coli* translational initiation, p. 621–641. *In* B. Hardesty and G. Kramer (ed.), Structure, function, and genetics of ribosomes. Springer-Verlag, New York.
9. **Hagen, T. J., and L. J. Shimkets.** 1990. Nucleotide sequence and transcriptional products of the *csg* locus of *Myxococcus xanthus*. J. Bacteriol. **172:**15–23.
10. **Hodgkin, J., and D. Kaiser.** 1977. Cell-to-cell stimulation of movement in nonmotile mutants of *Myxococcus*. Proc. Natl. Acad. Sci. USA **74:**2938–2942.
11. **Hodgkin, J., and D. Kaiser.** 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movement of single cells. Mol. Gen. Genet. **171:**167–176.
12. **Inouye, M., S. Inouye, and D. R. Zusman.** 1979. Gene expression during development of *Myxococcus xanthus*: pattern of protein synthesis. Dev. Biol. **68:**579–591.
13. **Kalman, L. V., Y. L. Cheng, and D. Kaiser.** 1994. The *Myxococcus xanthus dsg* gene product performs functions of translation initiation factor IF3 in vivo. J. Bacteriol. **176:**1434–1442.
14. **Liveris, D., J. J. Schwartz, R. Geertman, and I. Schwartz.** 1993. Molecular cloning and sequencing of *infC*, the gene encoding translation initiation factor IF3, from four enterobacterial species. FEMS Microbiol. Lett. **112:**211–216.
15. **Ludwig, W., K. H. Schleifer, H. Reichenbach, and E. Stackebrandt.** 1983. A phylogenetic analysis of the myxobacteria: *Myxococcus fulvus*, *Stigmatella aurantiaca*, *Cystobacter fuscus*, *Sorangium cellulosum* and *Nannocystis exedens*. Arch. Microbiol. **135:** 58–62.
16. **Manoil, C., and D. Kaiser.** 1980. Guanosine pentaphosphate and guanosine tetraphosphate accumulation and induction of *Myxococcus xanthus* fruiting body development. J. Bacteriol. **141:**305–315.
17. **Reichenbach, H.** 1966. *Myxococcus* swarming and fruiting body construction. E778/1966. Institut für den Wissenschaftlichen Film, Göttingen, Germany.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing

with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
20. **Shimkets, L. J.** 1990. Social and developmental biology of the myxobacteria. Microbiol. Rev. **54:**473–501.
21. **Stephens, K., P. Hartzell, and D. Kaiser.** 1989. Gliding motility in *Myxococcus xanthus*: *mgl* locus, RNA, and predicted protein products. J. Bacteriol. **171:**819–830.
22. **Sussman, J. K., and R. W. Simons.** Unpublished data.
23. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. Methods Enzymol. **153:**3–11.
24. **Zusman, D. R.** 1984. Developmental program of *Myxococcus xanthus*, p. 185–213. *In* R. Rosenberg (ed.), Myxobacteria. Development and cell interactions. Springer-Verlag, New York.