The *Myxococcus xanthus* *dsg* Gene Product Performs Functions of Translation Initiation Factor IF3 In Vivo

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The amino acid sequence of the Dsg protein is 50% identical to that of translation initiation factor IF3 of *Escherichia coli*, the product of its *infC* gene. Anti-*E. coli* IF3 antibodies cross-react with the Dsg protein. Tn5 insertion mutations in *dsg* are lethal. When ample nutrients are available, however, certain *dsg* point mutant strains grow at the same rate as wild-type cells. Under the starvation conditions that induce fruiting body development, these *dsg* mutants begin to aggregate but fail to develop further. The level of Dsg antigen, as a fraction of total cell protein, does not change detectably during growth and development, as expected for a factor essential for protein synthesis. The amount of IF3 protein in *E. coli* is known to be autoregulated at the translational level. This autoregulation is lost in an *E. coli* *infC362* missense mutant. The *dsg*⁺ gene from *Myxococcus xanthus* restores normal autoregulation to the *infC362* mutant strain. Dsg is distinguished from IF3 of *E. coli*, other enteric bacteria, and *Bacillus stearothermophilus* by having a C-terminal tail of 66 amino acids. Partial and complete deletion of this tail showed that it is needed for certain vegetative and developmental functions but not for viability.

Myxococcus xanthus is a rod-shaped gliding bacterium found in soil. Upon nutrient limitation, 10⁵ bacteria aggregate and form a multicellular spore-filled structure called a fruiting body. Four classes of developmental mutants, Asg, Bsg, Csg, and Dsg, begin the developmental process but are arrested at characteristic points in the pathway. These mutants can be complemented extracellularly by wild-type cells or by mutants of a different class (11).

dsg mutants are able to initiate the developmental process, but their development goes awry when the cells have formed loose asymmetric aggregates, at about 6 to 10 h. These mutants are unable to transcribe a particular set of developmentally regulated genes (3). The *dsg* mutation also affects the cells during vegetative growth. Mutants form compact, heaped, smooth-edged colonies, unlike the flat, spreading wild-type colonies. These mutants are also defective in the phase variation of colony color. In the accompanying paper (5), we describe the sequence of the *dsg* gene, which contains a missense mutation in *dsg* mutants. The amino acid sequence of Dsg is 50% and 51% identical to that of translation initiation factor IF3 of both *Escherichia coli* and *Bacillus stearothermophilus*, respectively. We also show that like IF3, Dsg begins with a unique initiation codon and contains conserved residues which are critical for the function of IF3. The Dsg protein extends 66 more amino acids to its C terminus than the IF3 proteins of *E. coli*, other enteric bacteria, and *B. stearothermophilus*.

An important role of IF3 in translation initiation is to help the ribosome select the initiation codon on the mRNA. Thus, IF3 ensures the fidelity of translation by preventing initiation at an incorrect location in the message or in the wrong reading frame (12, 13, 26). Initiation begins when the 30S ribosomal subunit binds via its 16S RNA to the Shine-Dalgarno site on mRNA, tethering the 30S subunit 4 to 12 bp from the initiation

codon. The IF3 protein, which is associated with the 30S ribosomal subunit during initiation, inspects the codons near the Shine-Dalgarno site for one which will pair correctly with the anticodon on the initiator tRNA, fMet-tRNA^{fMet}, which is also associated with the 30S subunit. When the proper initiation codon is located, the 50S ribosomal subunit joins the 30S initiation complex to form the 70S ribosome, and the initiation factors are released (8).

The *infC* gene, which encodes IF3 in *E. coli*, begins with the atypical AUU initiation codon (21). Recent work shows that IF3 selects against initiation at AUU codons and for initiation at AUG, GUG, and UUG codons, the typical initiators. In fact, this atypical AUU initiation codon is involved in the translational autoregulation of *infC* expression (2, 9, 26). When IF3 levels are high, the mRNA encoding *infC* is not translated because IF3 does not recognize AUU for the initiation of translation. However, when IF3 levels are low, the *infC* message is translated because, in the absence of IF3, the ribosome shows low selectivity for the typical initiation codons. Translation of *infC* from AUU continues until the levels of IF3 are high enough to restrict translation from an AUU initiation codon, thus regulating the cellular level of the IF3 protein according to its function (1, 2, 9).

In this work, we show that Dsg reacts with anti-IF3 serum. Using anti-Dsg antibodies, we show that Dsg is present at constant levels at all times during vegetative growth and development, as would be expected of a protein involved in translation. We further show that *dsg* is able to complement *E. coli* *infC* mutants impaired in their ability to autoregulate *infC* translation. Finally, we provide evidence that the 66-amino-acid C-terminal extension of Dsg is required for function in *M. xanthus*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* and *M. xanthus* strains and plasmids used in this work are listed in Table 1. Plasmid DNA was isolated by the alkaline lysis or boiling method (15, 22). Restriction endonucleases, the Klenow frag-

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TABLE 1. Bacteria and plasmids used in this study

Strain or plasmid	Origin	Characteristics	Reference or source
Strains			
<i>E. coli</i>			
DH5 α		<i>supE44</i> Δ <i>lacU169</i> (ϕ 80 <i>lacZ</i> Δ M15) <i>hsdR17 recA1</i> <i>endA1 gyrA96 thi-1 relA1</i>	22
P90C		<i>ara</i> Δ (<i>lac-pro</i>) <i>thi</i>	19
JK382	P90C	Tn10 near <i>infC</i>	R. Simons
JK378	P90C	Tn10 near <i>infC362</i>	R. Simons
JK409	JK382	λ AUU3 (<i>ptac infC-lacZ</i>)	R. Simons
JK414	JK378	λ AUU3 (<i>ptac infC-lacZ</i>)	R. Simons
JK463	JK382	λ AUG1 (<i>ptac infC-lacZ</i>)	R. Simons
JK464	JK378	λ AUG1 (<i>ptac infC-lacZ</i>)	R. Simons
<i>M. xanthus</i>			
DK101		<i>sgL41</i>	14
DK4870	DK101	<i>dsg-429</i>	3
Plasmids			
pUC19		Ap ^r	New England Biolabs
pLK103	pUC19	Ap ^r <i>dsg</i>	This work
BGS18		Km ^r	B. Spratt
pYLC172	pGBS18	Km ^r <i>dsg-429</i>	This work
pYLC173	pBGS18	Km ^r <i>dsg</i>	This work
pYLC174X	pBGS18	Km ^r <i>dsg-Δ12</i>	This work
pYLC175X	pBGS18	Km ^r <i>dsg-Δ43</i>	This work
pYLC176	pBGS18	Km ^r <i>dsg-Δ68</i>	This work
pPLH343	pBGS18	Km ^r MX8att	P. Hartzell
pYLC130	pPLH343	Km ^r MX8att <i>dsg</i>	This work
pYLC131	pPLH343	Km ^r MX8att <i>dsg-429</i>	This work
pYLC138X	pPLH343	Km ^r MX8att <i>dsg-Δ12</i>	This work
pYLC137	pPLH343	Km ^r MX8att <i>dsg-Δ43</i>	This work
pSELECT-1		Ap ^s Tc ^r	Promega
pLK104	pSELECT-1	Ap ^s Tc ^r <i>dsg</i>	This work
pLK105	pLK104	Ap ^r Tc ^r <i>dsg-Δ68</i>	This work
pLK107	pPLH343	Km ^r <i>dsg-Δ68</i>	This work
pYLC189	pLK103	<i>dsg-429</i>	This work
pYLC187	pLK103	<i>dsg-Δ12</i>	This work
pYLC186	pLK103	<i>dsg-Δ43</i>	This work
pYLC188	pLK102	<i>dsg-Δ68</i>	This work
pSAD1		Ap ^r <i>plac infC rpmI</i>	1
pRS1346	pSAD1	Ap ^r Δ <i>plac infC rpmI</i>	R. Simon
pGEX-1		sj26	23
pLK100	pGEX-1	sj26- <i>dsg</i>	This work

ment, DNA ligase, and oligonucleotide linkers were purchased from New England Biolabs, IBI, and Boehringer Mannheim Biochemicals. Restriction fragments were separated on 0.8% agarose gels and isolated by use of Gene Clean (Bio 101, Inc., La Jolla, Calif.) or SeaPlaque low-melt-temperature agarose (FMC Bioproducts, Rockland, Maine). A restriction map of the *dsg* gene and some of the flanking restriction sites is shown in Fig. 1. Plasmid pLK103 was created by cloning of a 1.1-kb *NdeI-EcoRI* fragment which encodes the entire *dsg* gene and 86 bp of the upstream sequence into the *SmaI-EcoRI* sites of pUC19, allowing the transcription of *dsg* from the *lac* promoter. Plasmids pYLC173 and pYLC176 contain an 859-bp fragment of the *dsg* gene extending from the *XhoI* site about 40 codons into the *dsg* reading frame to an *MluI* site downstream of *dsg*. The fragment used to create pYLC176 is derived from pLK105 and contains a stop codon which truncates the C-terminal 68 codons of *dsg*. Plasmids pYLC174X and pYLC175X contain internal 579- and 491-bp fragments which extend from the *XhoI* site in *dsg* to either the *BglI* or the *SmaI* site and which truncate 12 or 43 amino acids from the C

terminus, respectively. In each of these plasmids, an oligonucleotide linker encoding a UAG stop codon (New England Biolabs) was inserted at the C-terminal restriction site. Plasmid pYLC130 contains a 2.25-kb *EcoRI-HindIII* fragment which encodes the entire *dsg* gene and upstream region. pYLC131 contains the same 2.25-kb fragment, except that it encodes *dsg-429*. pYLC138X contains a *HindIII-BglI* fragment which encodes upstream sequences and truncates 12 amino acids from the C terminus of *dsg* by the addition of the stop codon linker in the *BglI* site. pYLC137 contains a *HindIII-SmaI* fragment which truncates the C-terminal 43 amino acids from *dsg*. pLK107 contains a 2.25-kb fragment which encodes *dsg- Δ 68* and which is derived from pLK105. The *NcoI-EcoRI* fragment from pLK103 was replaced with the corresponding fragment from pYLC175X, pYLC174X, pLK105, and pYLC72m to create plasmids pYLC186, pYLC187, pYLC188, and pYLC189, respectively.

Media and reagents. *E. coli* strains were grown in Luria-Bertani broth at 37°C. *M. xanthus* was grown vegetatively in CTT broth (14) with or without 1.5% Bacto Agar (Difco) at

FIG. 1. (A) Restriction map of the *dsf* locus. The *dsf* coding region is indicated by an open box. Restriction endonuclease sites are indicated above the line. Distances in kilobases are shown below the line. The position of the Lys-180 stop codon created in pLK105, pLK107, pYLC176, and pYLC188 is indicated by an asterisk. (B) Protein encoded by the fusion of the glutathione *S*-transferase and *dsf* genes in plasmid pLK100. The boxes represent the coding regions of each gene. The molecular mass of each domain of the fusion protein is indicated in kilodaltons. *dsf* was fused to the C terminus of the gene encoding glutathione *S*-transferase at an *Xho*I site which deletes the N-terminal 40 codons of the *dsf* reading frame. Transcription of this fusion is controlled by the *ptac* promoter.

33°C. Kanamycin (40 µg/ml) and carbenicillin (100 µg/ml) were used to select for plasmid-bearing *M. xanthus* and *E. coli*, respectively, as required.

Oligonucleotide-directed mutagenesis. The lysine at codon 180 (AAG) of *dsf* in plasmid pLK104 was changed to a UAG stop codon (*dsf*-Δ68) by use of an Altered Sites in vitro mutagenesis kit from Promega. The sequence of the oligonucleotide used is GCGCCACCTACGGCGTGGGC.

Preparation of antibodies to the Dsg protein. Plasmid pLK100 encodes an in-frame gene fusion between the gene encoding glutathione *S*-transferase and all but the first 40 codons of *dsf*, under the transcriptional control of *ptac* (Fig. 1). This plasmid was created by ligating the 1.7-kb *Xho*I-*Sal*I fragment of the *dsf* gene into an *Xho*I site introduced into plasmid pGEX-1 by insertion of an *Xho*I linker (New England Biolabs) into the *Sma*I site. The fusion protein was expressed and purified as previously described (23), except that it was eluted from the glutathione-agarose beads by boiling in loading buffer (22). Several milligrams of the fusion protein were purified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The fusion protein was visualized by staining with Coomassie brilliant blue R-250, and slices of the gel containing the protein were removed. Slices of the gel containing 0.5 mg of protein were mixed with an adjuvant and used to inoculate rabbits (Josman Labs, Napa, Calif.). The antiserum was partially purified by absorption with a crude extract of *E. coli* DH5α. One volume of extract was mixed with 4 volumes of serum and incubated overnight at 4°C. Antibodies which reacted with proteins in the extract were precipitated with fixed *Staphylococcus aureus* cells (Bethesda Research Laboratories, Inc.). The cleared supernatant was used to prepare immunoblots.

Immunoblotting. The SDS-PAGE procedure described by Straus et al. (25) was used. Rabbit Anti-*E. coli* IF3 polyclonal antiserum was a gift from John Hershey (16). Anti-IF3 antiserum was used at a 1:3,000 dilution, and anti-Dsg antiserum was used at a 1:1,000 dilution.

β-Galactosidase assays. Cells were grown in Luria-Bertani broth containing 100 µg of carbenicillin per ml overnight and later diluted 1:200 into the same medium and grown to the mid-log phase ($A_{600} = 0.4$). β-Galactosidase activity was measured as previously described (17). Units of β-galactosidase are expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolyzed per minute per milligram of protein.

***M. xanthus* development.** Development of strains DK101 and DK4870 on TPM plates was performed as previously described (6). Samples were removed by scraping cell material from the plates at 2.5, 5, 7.5, and 10 h after the cells were suspended in TPM starvation medium. A sample at time zero was obtained by removing cells from the culture before the initiation of starvation. The concentration of protein in each sample was determined by the Bradford assay (17) with a Bio-Rad dye reagent.

Electroporation of *M. xanthus* with plasmid DNA. Strains DK101 and DK4870 were grown to a density of approximately 5×10^8 cells per ml in CTT broth at 33°C. The cells were pelleted at $8,000 \times g$ for 10 min at room temperature and resuspended in an equal volume of water two times. After a third wash, the cells were resuspended in a 1/100 volume of water. One to two micrograms of DNA was mixed with 40 µl of cells and electroporated at 400 Ω, 25 µF, and 0.65 kV in a 0.1-cm cuvette at room temperature. The cells were immediately diluted into CTT broth and allowed to grow for 4 to 8 h for expression of the newly acquired drug resistance. Cells in CTT broth with 0.7% Bacto Agar were plated onto CTT plates (1.5% Bacto Agar) containing 40 µg of kanamycin per ml and grown for 7 days at 33°C (28).

RESULTS

Dsg and IF3 antigens. Phylogenetically, *M. xanthus* belongs to the δ subgroup of purple eubacteria, while *E. coli* belongs to the γ subgroup (27). Nevertheless, ribosomes and their associated proteins are largely conserved across eubacterial species. To test whether Dsg, the product of the *dsf* gene in *M. xanthus*, and IF3 from *E. coli* might have similar antigenic epitopes, extracted proteins were reacted with anti-Dsg and anti-IF3 antibodies. A polyclonal anti-Dsg serum was raised against a fusion protein combining the N terminus of glutathione *S*-transferase with the C terminus of Dsg (Fig. 1). A polyclonal anti-IF3 serum that had been raised against *E. coli* IF3 (16) was a generous gift from J. Hershey. Whole-cell extracts from wild-type *M. xanthus* DK101, *E. coli* DH5α containing plasmid pUC19, and *E. coli* DH5α containing plasmid pLK103, which expresses Dsg, were separated by SDS-PAGE, transferred to nitrocellulose, and probed with the anti-Dsg and anti-IF3 sera (Fig. 2). As shown in Fig. 2, lanes 5 and 6, the anti-IF3 serum recognized a band in the *E. coli* extracts with an electrophoretic mobility of approximately 20

FIG. 2. Immunologic reaction of anti-*E. coli* IF3 serum with Dsg in Western blots. Whole-cell extracts were prepared from an *M. xanthus* strain that expresses wild-type Dsg (DK101), from an *E. coli* strain that expresses IF3 (DH5 α), and from an *E. coli* strain that additionally expresses Dsg from a plasmid [DH5 α (pLK103)]. Crude cell extracts were fractionated by SDS-PAGE, blotted onto nitrocellulose, and probed with anti-Dsg (lanes 1 to 3) or anti-IF3 (lanes 4 to 6) serum. Lanes: 1 and 4, *M. xanthus* DK101; 2 and 5, *E. coli* DH5 α (pUC19); 3 and 6, DH5 α (pLK103).

kDa; this band corresponds to IF3, as reported previously (21, 24). The anti-IF3 serum also recognized a band in the *M. xanthus* extract with an apparent molecular mass in the 30-kDa range (Fig. 2, lane 4), as well as a band with the same mobility in the *E. coli* DH5 α (pLK103) extract (lane 6). The protein band was encoded by pLK103 because it was absent from DH5 α (pUC19) (Fig. 2, lanes 2 and 5). Although the anti-Dsg serum failed to recognize a band corresponding to the IF3 protein in the *E. coli* extracts (Fig. 2, lanes 2 and 3), it did recognize one that comigrated with the ca. 30-kDa Dsg band in both the *M. xanthus* extract and the DH5 α (pLK103) extract (lanes 1 and 3). Therefore, we can conclude that the *E. coli* anti-IF3 serum can cross-react with Dsg. The failure of the anti-Dsg serum to react with the IF3 protein does not contradict an antigenic relationship because the antiserum had been raised against a fusion protein which lacks 20% of the residues from the N terminus of Dsg (Fig. 1). According to the amino acid sequence (5), the Dsg immunizing antigen would have contained an overall 36% amino acid identity to IF3 of *E. coli*. Only one protein in the *M. xanthus* extract was recognized by both the anti-Dsg and the anti-IF3 sera; we identified the band as the Dsg protein. This result is in accordance with the fact that a *dsg* DNA probe hybridized to a unique segment of *M. xanthus* DNA in Southern blotting experiments (data not shown). Thus, there appears to be only one IF3-like gene in *M. xanthus*. In addition to establishing an antigenic relationship between Dsg and IF3, the antisera are reagents for identifying the Dsg and IF3 proteins in crude extracts of cells.

Dsg in vegetative and developing cells. Protein synthesis is essential for both vegetative growth and fruiting body development. If the Dsg protein is translation initiation factor IF3 of *M. xanthus*, it would need to be present in wild-type and *dsg* cells throughout their growth and development. Accordingly, extracts of strains DK101 (*dsg*⁺) and DK4870 (*dsg*-429) were prepared from cells harvested during growth and at various times after the initiation of development on TPM agar. The amount of Dsg was then estimated visually from Western blots (immunoblots) with the anti-Dsg serum (Fig. 3). The approximately 30-kDa Dsg protein was present at 0 h (growing cells) and through 10 h of fruiting body development in the wild-type strain. No major systematic changes in Dsg levels over time were evident to the eye. The Dsg protein was also present in the missense *dsg*-429 mutant DK4870 (Gly-134 changed to Glu); again, its levels did not change over time. In fact, the levels of the Dsg antigen appeared slightly higher in the *dsg*-429 mutant than in the *dsg*⁺ strain, paralleling relative levels of *dsg* mRNA in these two strains (2a).

Dsg function in *E. coli*. *E. coli* *infC* missense mutants that are defective in the translational autoregulation of IF3 protein

FIG. 3. Western blot analysis of DK101 and DK4870 during vegetative growth and at various times after the initiation of development with anti-Dsg antiserum. Ten micrograms of crude cell extract was fractionated by SDS-PAGE, blotted onto nitrocellulose, and probed with anti-Dsg serum. The hours after the initiation of starvation are indicated below each lane. Protein size standards (Bethesda Research Laboratories) of 43 and 28 kDa are indicated (St.).

synthesis have been isolated. Regulation was monitored on the basis of the expression of β -galactosidase from an *infC-lacZ* fusion protein gene located at the phage λ attachment site, separate from the chromosomal *infC* locus (26). In a strain containing the *infC362* allele at the chromosomal locus, β -galactosidase expression was more than three times higher than in an *infC*⁺ strain. Translation of the fusion could be restored to its normal (down-regulated) state by introducing a plasmid encoding *infC*⁺, as confirmed in Fig. 4 (26). In JK414 containing plasmid pRS1346 (*infC*⁺), the levels of β -galactosidase activity were similar to those in JK409(pRS1346) (Fig. 4). These controls follow the described expression of the fusion

FIG. 4. Effect of plasmids encoding IF3 and Dsg on translational regulation of an *infC-lacZ* fusion protein in *E. coli*. Horizontal axis of the main figure: the *infC-lacZ* fusion protein in each strain has an AUU initiation codon. Strain JK409 (wild type) and strain JK414 (*infC362*) are indicated as + and 362, respectively. In the inset, the AUU initiation codon of the *infC-lacZ* fusion protein has been replaced by an AUG initiation codon. Strain JK463 (wild type) and strain JK464 (*infC362*) are indicated as + and -, respectively. In both the main figure and the inset, the genes (as indicated by the entry after "Gene") encoded by the complementing plasmids are as follows: none (pUC19), *infC*⁺ (pRS1346), *dsg*⁺ (pLK103), and *dsg*-429 (pYLC189). The vertical axes show β -galactosidase activity in nanomoles of ONPG hydrolyzed per minute per milligram of protein ($k = 1,000$). The β -galactosidase activities in the experiments shown in the inset were normalized to the cell density of the culture at the time of the assay.

(26): down-regulated in the *infC*⁺ strain but expressed at a high level in the *infC362* mutant strain. If the Dsg protein has IF3 activity in *E. coli*, it might complement the *infC362* mutant and decrease β -galactosidase activity (as observed with the *infC362* strain containing plasmid pRS1346). Strains JK409 (*infC*⁺) and JK414 (*infC362*) were transformed with plasmids encoding *dsg*⁺ (pLK103) and *dsg*-429 (pYLC189). Plasmid pLK103 (*dsg*⁺) restored translational regulation in strain JK414, indicating that Dsg is able to act like IF3. *dsg*-429, however, was unable to complement, as indicated by relatively high levels of β -galactosidase activity (Fig. 4). No Dsg antigen could be detected in immunoblots of this strain or other *E. coli* strains that contain pYLC189 by use of anti-*M. xanthus* Dsg or anti-*E. coli* IF3 serum (see lanes 3 and 5 in Fig. 7). Therefore, the conclusion that the failure to decrease β -galactosidase expression was due to the lack of Dsg expression or to instability of the mutant Dsg protein in *E. coli* cannot be excluded. Considering the likelihood that the regulatory test is more sensitive than the antibody test, one might tentatively conclude that the Dsg-429 protein lacks regulatory activity.

To confirm that the atypical (AUU) initiator of the *infC-lacZ* fusion in the experiment shown in Fig. 4 is essential for the down-regulation of *infC* expression by *dsg*⁺ from *M. xanthus*, the atypical AUU initiation codon was replaced by a typical AUG initiation codon. As shown previously (2, 26) and in Fig. 4 (inset), replacement of the AUU with an AUG for the *infC-lacZ* fusion eliminated translational autoregulation and the effect of the *infC362* mutation. The presence of *dsg*⁺ had no additional effect on the level of *infC-lacZ* expression in these strains, as expected.

Function of the carboxy-terminal segment of Dsg. The amino acid sequences of Dsg and IF3 are 50% homologous throughout the length of IF3 (5). Dsg, however, extends 66 amino acids beyond the carboxy terminus of *E. coli* or *B. stearothermophilus* IF3. To determine whether this C-terminal tail portion is necessary for vegetative or developmental functions in *M. xanthus*, *dsg* clones in which part or all of the C-terminal tail had been deleted were constructed. The ability of these truncated *dsg* genes to support viability or to complement a missense *dsg* mutant, thus restoring wild-type colony morphology or spore formation, was tested.

To determine whether the tail is essential for the viability of *M. xanthus*, a series of fragments completely internal to the *dsg* gene were each integrated into the chromosomal *dsg* locus. As diagrammed in Fig. 5, integration of a plasmid that carries an internal *dsg* fragment should produce a tandem duplication in which both *dsg* copies are mutant.

To create internal fragments of *dsg*, an in vitro deletion which removed the 5' end of the wild-type *dsg* gene, including the promoter region, the ribosome binding site, and the first 40 codons of *dsg* up to an *Xho*I site, was first made (5). In itself, this 5' deletion would be a null mutation of *dsg* that would not be expected to encode any protein. Then, internal fragments were obtained by combining the same 5' deletion with the series of *dsg* tail deletions shown in Fig. 6A. Finally, each plasmid-born internal fragment gene construct was allowed to integrate by means of single homologous recombination into the *dsg* locus in strain DK4870 (*dsg*-429). The structure of each integrant was confirmed by Southern blot analysis. When the *dsg* allele in the plasmid had no 3' deletion and only a 5' deletion, as in pYLC173, the homologous recombinant would contain one nonfunctional copy of *dsg* lacking the 5' portion of the gene described above; the second copy would be *dsg*⁺. Truncations of 12 (pYLC174X) or 43 (pYLC175X) amino acids from the 3' region also contained an in-frame UAG (stop) codon to ensure proper polypeptide chain termination.

FIG. 5. Recombination of a plasmid containing an internal fragment into a chromosome. A plasmid encoding a *Km*^r (*Kan*^r) gene and a 3'- and 5'-truncated *dsg* gene (dark shaded box) can recombine with a homologous chromosomal gene (light shaded box). This process results in two copies of the gene in the chromosome, one with a 3' truncation and one with a 5' truncation, with plasmid sequences in between.

When these plasmids were integrated into the *dsg* locus by homologous recombination, as diagrammed in Fig. 5, the two copies of the *dsg* gene, one missing the 5' sequences and one missing the 3' sequences, were separated by vector DNA. Which gene copy in the duplication also carried the *dsg*-429 mutation of the recipient depended on the site of integration relative to the location of the *dsg*-429 missense mutation.

As expected and shown in Fig. 6A, plasmid integrant colonies were obtained at a high frequency following transformation with the plasmid (pYLC173) that encodes a *dsg* gene with a 5' truncation and the normal, intact 3' region (0 amino acids deleted). Colonies were recovered at the same high frequency with a plasmid (pYLC172) that encodes a *dsg* gene with a 5' truncation and a *dsg*-429 allele, showing that this mutant allele does not decrease viability (data not shown). As expected, this recombinant strain had the *dsg* mutant phenotype. The alleles from which the last 12 and 43 codons had been deleted from the 3' end of *dsg* (pYLC174X and pYLC175X, respectively) also yielded recombinants (Fig. 6A). The frequency of such integrants was lower than that obtained from the integration of plasmids pYLC172 and pYLC173, in which the 3' region was intact, most likely reflecting the reduced extent of homologous sequences which were available for recombination. Evidently none of these deletions eliminated vital functions necessary for the growth of a colony. Constructs without the in-frame UAG at the truncated end were also prepared and integrated; they yielded results similar to those obtained with constructs containing the stop codon and are not shown.

Otherwise, wild-type colonies formed by strains containing the missense *dsg* alleles, *dsg*-429 and *dsg*-439, were more compact and more tan than wild-type colonies, which are large and yellow (3). The *dsg*-429 and *dsg*-439 mutants also failed to

FIG. 6. (A) Fragments of *dsg* integrated into the *dsg* locus to test the role of the C-terminal regions of Dsg in cell viability. The name of the plasmid containing the diagrammed *dsg* fragment is indicated at the left. Codon numbers are indicated above the line. All plasmids have the same 5' deletion through codon 40. The C terminus of Dsg, which is not homologous to that of IF3, is represented as a wavy box. The length of each fragment in base pairs is indicated below the line. Restriction sites used to construct fragments: X, *Xho*I; S, *Sma*I; B, *Bgl*I. (B) Fragments of DNA inserted into the MX8 attachment site to test the role of the C-terminal regions of Dsg in vegetative functions. The *dsg* coding region is indicated by a box (as in panel A) and the initiation and stop codons for each fragment are indicated below the line. Restriction sites are indicated above the line: S, *Sma*I; B, *Bgl*I. The location of the *dsg-429* point mutation is indicated by an asterisk. wt, wild type.

FIG. 7. Effect of C-terminal sequences on the apparent molecular weight of Dsg. Crude cell extracts were prepared from strain DH5 α containing plasmid pLK103 (*dsg*) (lanes 1 and 6) or strain JK409 containing plasmids pYLC188 (*dsg*- Δ 68) (lanes 2 and 4), pYLC189 (*dsg*- Δ 43) (lanes 3 and 5), and pUC19 (lane 7). Crude cell extracts were fractionated by SDS-PAGE, blotted onto nitrocellulose, and probed with anti-Dsg (lanes 1 to 3) or anti-IF3 (lanes 4 to 7) serum.

develop normally. These elements of the mutant phenotype could be complemented by a *dsg*⁺ allele (3). To investigate the possible role of the *dsg* tail in colony morphology and development, the ability of the 3'-truncated *dsg* alleles to complement *dsg*-429 strain DK4870 and form large yellow wild-type colonies that are sporulation proficient in development was also tested. For this purpose, DNA fragments encoding the 5' upstream region and the entire *dsg* gene or the *dsg* gene from which 12, 43, or 68 codons had been deleted from the 3' end and replaced with a UAG stop codon were cloned into plasmid pPLH343. This plasmid has sequences necessary for integration into the myxophage MX8 attachment site (Fig. 6B). The resulting plasmids were introduced into *dsg* strain DK4870 by electroporation, and their integration into the *M. xanthus* chromosome at the ectopic MX8 attachment site was confirmed with Southern blots (data not shown). *dsg*-429 strain DK4870 transformed with either wild-type *dsg* (pYLC130) or a *dsg* gene with the final 12 codons deleted (pYLC138X) was able to form large yellow colonies and spores, indicating that the *dsg* gene at the attachment site could complement the *dsg*-429 allele at the *dsg* locus and that the C-terminal 12 amino acids were not required for this complementation.

As expected, a plasmid encoding a full-length *dsg*-429 allele (pYLC131) was unable to complement colony morphology or development in *dsg*-429 strain DK4870. Plasmids with larger deletions of the 3'-coding sequences (e.g., 43 codons [pYLC137] and 68 codons [pLK107]) were unable to complement colony morphology or spore formation in *dsg*-429 strain DK4870. Together, these results show that strains containing a *dsg* allele with a deletion of the 3' 12 codons retain the capacity to form spores and wild-type colonies but that strains containing *dsg* alleles with deletions of 43 and 68 codons are not complemented.

Perhaps Δ 43 or Δ 68 fails to complement because the deletion of 3' sequences from the Dsg protein decreases the level of expression, message stability, protein stability, or proper folding. To investigate the amounts of Dsg protein in these deletion strains, extracts of *E. coli* or *M. xanthus* strains containing plasmids with 3' truncations were examined in Western blots. Neither the Dsg- Δ 12 nor the Dsg- Δ 43 or Dsg- Δ 68 protein could be detected in *M. xanthus* extracts (data not shown), although the Dsg- Δ 12 and Dsg- Δ 43 proteins must have been present, since they complemented for viability (Fig. 6A). However, proteins from all constructs could be detected at high levels in *E. coli* extracts; for example, Dsg- Δ 68 is shown in Fig. 7. Nor could IF3 be detected in *M. xanthus* when the *infC*⁺ gene from *E. coli* was coupled to the *dsg* promoter and the resulting plasmid was integrated into the MX8 attachment site. This strain was also unable to form wild-type colonies or spores when placed under developmental conditions (data not shown). It appears that the C-terminal tail is needed for certain

vegetative and developmental functions but not for viability. The complementation and Western blot data indicate that regulatory tests are more sensitive than Western blots.

Dsg tail and electrophoretic mobility. Dsg, whether produced in *M. xanthus* or *E. coli* (Fig. 3), has a mobility in SDS-PAGE corresponding to an apparent molecular mass of 32 kDa. The molecular mass predicted by its amino acid sequence is 27 kDa (5). To determine whether the discrepancy between the predicted versus the observed molecular weights of Dsg was due to the presence of the C-terminal tail, extracts of *E. coli* DH5 α containing plasmid pLK103 (*dsg*) and strain JK409 containing plasmids pUC19, pYLC188 (*dsg*- Δ 68), and pYLC189 (*dsg*-429) were probed with anti-IF3 and anti-Dsg sera (Fig. 7). The anti-IF3 serum reacted with IF3 (lanes 4 to 7), which has an apparent molecular mass of 22 kDa, similar to the molecular mass of 20.5 kDa predicted from its amino acid sequence (21, 24). Both antisera reacted with the full-length Dsg protein in the extract of strain DH5 α (pLK103) (lanes 1 and 6) and also with a band corresponding to Dsg- Δ 68 in the extract of strain JK409(pYLC188) (lanes 2 and 4). Dsg- Δ 68 runs at an apparent molecular mass of 19 kDa, similar to its predicted molecular mass of 20.5 kDa. In contrast, Dsg- Δ 12 and Dsg- Δ 43 have apparent molecular weights which are 123 and 120.5% of their predicted molecular weights, like Dsg, which also runs at 123% of its predicted molecular weight (data not shown). These data suggest that the C-terminal tail of Dsg is responsible for its anomalous electrophoretic mobility. The C-terminal tail of Dsg contains a number of proline residues which restrain the secondary and tertiary structures of the protein and possibly influence its mobility in SDS-PAGE. In Dsg- Δ 68 but not in Dsg- Δ 43 or Dsg- Δ 12, prolines 199 to 202 and proline 205 are deleted.

A band with an apparent molecular mass of 27.5 kDa was also present in Fig. 7, lanes 2 and 4. This band was the result of a fusion between β -galactosidase and Dsg, and its synthesis was due to readthrough of a stop codon 10 codons upstream of the initiation codon of *dsg*. The addition of a stop codon upstream of the *dsg* gene, which also changed the reading frame, eliminated this band (data not shown).

Effect of the Dsg tail on the translational regulation of *infC*. To investigate the influence of the Dsg tail on the translational down-regulation of the *infC-lacZ* fusion in *E. coli*, strains JK409 and JK414 were transformed with plasmids pYLC187 (*dsg*- Δ 12), pYLC186 (*dsg*- Δ 43), and pYLC188 (*dsg*- Δ 68), and the β -galactosidase activity of each strain was determined (Fig. 8). Plasmid pYLC187 (*dsg*- Δ 12) and plasmid pYLC186 (*dsg*- Δ 43) restored translational regulation to the *infC* strains, indicating that even without their C-terminal portions, the Dsg proteins could still regulate translation of the *infC-lacZ* fusion in *E. coli*. Attempts to transform strain JK414 (*infC*362) with the plasmid encoding *dsg*- Δ 68 (pYLC188) failed; no transformants could be obtained after repeated trials. Attempts to transform pYLC188 into strains with other *infC* mutant alleles also failed. Nevertheless, transformants of *infC*⁺ strain JK409 and other *infC*⁺ strains were obtained with the same DNA preparations, showing that the *dsg*- Δ 68 truncation is not a dominant lethal mutation and suggesting that it may be lethal only in combination with *infC* alleles that eliminate *infC-lacZ* down-regulation.

DISCUSSION

Dsg, the product of the *dsg* gene, resembles translation initiation factor IF3 both structurally and functionally. The Dsg amino acid sequence is 50 and 51% identical to that of the IF3 protein of both *E. coli* and *B. stearrowthermophilus*, respec-

in *M. xanthus*. Instead, it seems more likely that the failure to produce the putative D-signal is an indirect effect of the mutation of Gly-134 to Glu in Dsg. The pleiotropic effects of the *dsg* mutation, which include the formation of smaller, more tan colonies, may be due to an inability to properly translate certain genes in *M. xanthus*. It is possible that the gene which encodes the D-signal is one of the improperly translated genes and is the only one of them that is essential for development. Thus, *dsg* mutants would be complemented extracellularly by wild-type cells.

On the basis of the published work with IF3, we expect that Dsg functions during translation initiation by positioning the ribosome on the proper initiation codon as it examines the codon-anticodon interaction between the mRNA and the initiator tRNA. It is possible that the protein encoded by the *dsg-429* allele, Dsg-429, is more stringent in its selection of an initiation codon than wild-type Dsg. Since an estimated 8% of the genes in *E. coli* begin with a GUG initiation codon (8, 10), Dsg-429 may fail to recognize this or another non-AUG initiation codon as such and therefore translate certain mRNAs at a very low efficiency. According to this view, some of the vegetative and developmental functions that are defective in the *dsg-429* strain may be encoded by messages which begin with an initiation codon not recognized by Dsg-429. It is also possible that Dsg-429 is less stringent in its choice of initiation codons, again shifting the balance between messages with different initiators. The stringency issue might be resolved by comparing the translational efficiency of messages which initiate with various codons in wild-type and *dsg* strains.

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