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Suppression of TGA Mutations in the *Bacillus subtilis spoIIR* Gene by *prfB* Mutations

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An unexpectedly high proportion of TGA nonsense mutations was obtained in a collection of chemically induced mutations in the *spoIIR* locus of *Bacillus subtilis*. Of 11 different mutations obtained, TGA mutations were found in four codons, whereas only three codons yielded missense mutations. Six suppressors of the TGA mutations were isolated, and five of the suppressing mutations were mapped to the *prfB* gene encoding protein release factor 2. These are the first mutations shown to map to the *B. subtilis prfB* locus. The sequence of the *prfB* gene was completed, and two revisions of the published sequence were made. The five *prfB* mutations also resulted in suppression of the *catA86*-TGA mutation to between 19 and 54% of the expression of *catA86*$^+$, compared to the readthrough level of 6% in the *prfB*$^+$ strain. N-terminal sequencing of suppressed *catA86*-TGA-specified protein demonstrated that the amino acid inserted at UGA because of the *prfB1* mutations was tryptophan.

The genetic code shows small but fundamental differences in various organisms, suggesting that the code has evolved to meet special requirements of the host (reference 12 and references therein). Some of the variability of the code is strikingly evident in the codon UGA (29). UGA is one of the three translation termination codons. In *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis*, UGA is encountered less frequently than UAA and more frequently than UAG (4, 13). Although UGA is a termination codon, in *E. coli* and *S. typhimurium* UGA can be decoded at very low frequency; when this occurs, the amino acid inserted is tryptophan (29). Thus, UGA is viewed as a "leaky" termination codon (34). The extent of readthrough of UGA in wild-type *E. coli* appears to be on the order of 10^{-5} to 10^{-2} per termination at UGA, and the readthrough efficiency of UGA seems to depend on the context in which UGA is found (13, 29). Readthrough as Trp is distinct from the special case of UGA coding for selenocysteine, where a codon context of 40 nucleotides is involved (14).

Although UGA functions as a stop codon in *B. subtilis*, the efficiency of UGA readthrough is quite high. With a *catA86* reporter gene with UGA inserted into two different sites, the efficiency of UGA readthrough was approximately 6% (26). Similar UGA readthrough values were obtained with *Staphylococcus aureus* as host (25, 26). Thus, in these very different gram-positive species, UGA is substantially leakier than is observed in the *Enterobacteriaceae*. N-terminal sequencing of the protein specified by *catA86* containing UGA at codon 7 demonstrated that tryptophan was the inserted amino acid. *Mycoplasma* are "wall-less" gram-positive bacteria, and in many *Mycoplasma* species UGA is not a termination codon but rather directly encodes tryptophan (4, 7, 18). The very high level of readthrough of UGA in *B. subtilis* and *S. aureus* suggested that it might be difficult to obtain TGA nonsense mutations in most coding sequences within these organisms.

In the present study, we demonstrate the isolation of several mutations in *spoIIR* which cause a defect in sporulation and result from a change of a sense codon to UGA. The sporulation phenotype associated with the TGA mutations allowed the isolation of second-site mutations that suppress TGA nonsense mutations. We demonstrate here that five of six such UGA suppressors result from mutations in the structural gene (*prfB*) for release factor 2 (RF2).

MATERIALS AND METHODS

Media. *B. subtilis* was grown in modified Schaeffer's sporulation medium (2 × SG) without glucose and on Schaeffer's sporulation agar (33). Addition of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (100 μg/ml), chloramphenicol (4 μg/ml), neomycin (3 μg/ml), erythromycin (10 μg/ml), and lincomycin (1 μg/ml) (resistances to the latter two are encoded by the *erm* gene and the combination is referred to as Erm^r) or of spectinomycin (50 μg/ml) was done as required.

Strains and plasmids. *B. subtilis* 168 strain BR151, *trpC2 metB10 lys-3*, was used as the parent strain in all experiments. *E. coli* DH5α (GIBCO/BRL) was used to maintain plasmids. pPL708C2 contains a constitutively expressed version of *catA86*, a gene that codes for chloramphenicol acetyltransferase (CAT). pPL708C2 UGA-7 contains TGA as replacement for *cat* codon 7, which is Glu (GAA) in the wild-type gene (26). Both plasmids were transformed into strain BR151 and derivatives of BR151 containing *prfB1* mutations to generate strains containing either the wild-type *cat* gene (pPL708C2) or the mutant *cat* gene (pPL708C2 UGA-7).

Isolation of *prfB* mutants. *prfB1* was isolated from an ethyl methanesulfonate (EMS)-mutagenized (9) culture of MLK940. MLK940 contained *spoIIR152* with a TGA mutation and a *cotE-lacZ* fusion (to indicate σ^E activity) linked to a Cam^r determinant (38). The *prfB1* mutant was isolated and exhibited a LacZ$^+$ phenotype on sporulation agar plates containing X-Gal. The *prfB2* suppressor strain and the *prfB3*, *-4*, and *-5* suppressor strains were isolated as Spo$^+$ colonies from UV-mutagenized derivatives of BR151 containing *spoIIR151* and *spoIIR74*, respectively.

Cloning of the gene encoding *prfB1*. A library of Cam^r Tn*10* insertions (31) made in BR151 was transduced into MLK984 (a BR151 derivative containing *prfB1*) by using transduction phage PBS-1 (17), and colonies that were Cam^r and Spo$^+$ at 42°C were isolated. DNA from each Cam^r and Spo$^+$ clone was isolated and transformed back into MLK984, and linkage of the Tn*10*s was determined. One Tn*10* that was 65% cotransformed to *prfB1* was cloned, along with its flanking chromosomal DNA, to make pMLK252. This plasmid did not contain *prfB*. It was used as a probe for a lambda DNA library. A lambda clone that hybridized to pMLK252 and corrected the *prfB1* mutation was subcloned to yield pMLK262, carrying the 800-bp *Sac*I-*Hin*dIII fragment of the insert-phage junction ligated with *Sac*I-*Hin*dIII-digested pBluescript KS (Stratagene). pMLK262 corrected the *prfB1* mutation. The adjacent 900-bp *Eco*RI-*Hin*dIII fragment was cloned in pBluescript KS to make pMLK266.

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TABLE 1. Description of *spoIIR* mutants

Allele	Amino acid change	Base change	Sporulation (% of parental *spo*$^+$ strain BR151)	Mutagen[c]
spoIIRΔneo		Insertion at codon 111	$<2.9 \times 10^{-6}$	
spoIIR11		Deletion of TA	1.4×10^{-1}	NA
spoIIR29	Gln→ochre	CAA→TAA	4.0×10^{-3}	MHA
spoIIR40	Gln→amber	CAG→TAG	7.7×10^{-3}	MHA
spoIIR44	Arg→Cys	CGT→TGT	4.8×10^{-4}	MHA
spoIIR74	Trp→opal	TGG→TGA	2.9×10^{-1}	MHA
spoIIR144	Gly→opal	GGA→TGA	6.1×10^{-3}	NA
spoIIR151	Trp→opal	TGG→TGA	2.3×10^{-4}	MHA
spoIIR152 (*spoIIR4*[a])	Trp→opal	TGG→TGA	6.7×10^{-3}	EMS; MHA[b]
spoIIR153 (*spoIIR3*[a])	Cys→Tyr	TGT→TAT	6.1×10^{-3}	EMS; MHA[b]
spoIIR157L (*spoIIR1*[a])	Pro→Leu	CCG→CTG	3.5×10^{-1}	EMS
spoIIR157S (*spoIIR2*[a])	Pro→Ser	CCG→TCG	7.0×10^{-1}	EMS; MHA[b]

[a] Original allele name (20).
[b] Same mutant allele was obtained by two different mutagenesis protocols (see text).
[c] NA, nitrous acid; MHA, methoxyamine.

PCR amplification and sequencing. Both the *spoIIR* and *prfB* mutant DNAs were PCR amplified with a GeneAmp kit (Perkin-Elmer Cetus). The *spoIIR* gene primers were 5′CACCCTGCACGTTTATCCCAGGCTCTCC3′ and 5′GCAG TTGATAAAACATCCGTTCACCCCG3′, and the *prfB* primers were 5′GTGG TTGATATCGGACGAAATGCCC3′ and 5′GCAGCAGTGAAATCAAGGAT ATAAG3′. One primer in each reaction was phosphorylated with dATP and T4 polynucleotide kinase, and after amplification, the phosphorylated strand was degraded with lambda exonuclease (5). The remaining strand was sequenced with Sequenase 2.0 (Amersham) according to the manufacturer's instructions.

Other methods. Sporulation frequency was determined as heat-resistant spores per milliliter of culture 16 h after the initiation of sporulation (27). CAT assays were performed by the colorimetric procedure of Shaw (37). Protein was assayed according to the method of Bradford (8).

CAT UGA-7 protein was affinity purified with chloramphenicol caproate agarose (Sigma) and eluted with 10 mM chloramphenicol. The purified protein was subjected to automated N-terminal sequencing by Edman degradation. Typically, sequencing was allowed to proceed through 15 cycles (15 amino acid residues).

Nucleotide sequence accession number. The complete nucleotide sequence of the *prfB* gene is available from GenBank under accession no. AF013188.

RESULTS AND DISCUSSION

TGA nonsense mutations in *spoIIR*. The *spoIIR* gene is required for the coordination of transcriptional events during sporulation. Sporulation is a developmental process requiring the concerted efforts of two cells, the mother cell and the forespore (also called the prespore). These two cells express separate genetic programs that are sequentially controlled by a set of sigma factors that are coordinated by cell-cell signaling (15). The SpoIIR protein is produced by the forespore with sigma factor F and initiates transcriptional events in the mother cell by activating σ^E (20, 24).

Previously, we obtained the four mutations that defined the *spoIIR* locus by EMS mutagenesis of an appropriately marked strain (20). In order to obtain further mutations in *spoIIR*, we utilized directed mutagenesis of transforming DNA (3). We obtained two *spoIIR* mutants by nitrous acid mutagenesis and nine by methoxyamine mutagenesis (11). DNA sequence analysis indicated that we had obtained, in total, 10 distinct point mutations in *spoIIR*, of which 6 were nonsense mutations, including TGA mutations in four separate codons (Table 1). Three of the mutations obtained by the directed mutagenesis were identical to three of the mutations obtained by mutagenesis of bacteria with EMS. The SpoIIR protein is predicted to contain 224 residues, including a 23-residue leader sequence that is thought to be cleaved during protein secretion (20). In only 3 of 224 residues were missense mutations obtained, and in 2 of these residues mutations were obtained more than once (Table 1). In contrast, of the four codons that could yield TGA by a single-base transition, three did so; the fourth codon, W218, encoded the residue that is only seven residues from the C terminus, and a nonsense mutation there may have no phenotype. An additional TGA mutation resulted from a transversion of GGA which encoded G144. Of the nine codons that could yield TAA or TAG by a single-base transition, two did so. There were thus two surprises from this analysis: the disproportionate number of nonsense mutations and the occurrence of TGA mutations. The disproportionate number of isolated nonsense mutations may indicate that most amino acid substitutions in *spoIIR* cause no phenotypic change. We are aware of only one other report of a TGA (opal) nonsense mutation in *B. subtilis* (22).

Previously, we had supposed that high readthrough levels of UGA nonsense codons in wild-type *B. subtilis* curtailed the ability to isolate such mutants (26). For example, there is sufficient readthrough of the *catA86* UGA-7 mutation that the mutant *catA86* gene confers Cam^r to the bacterium. However, the wild-type level of UGA readthrough in the *spoIIR* mutants must not be sufficient to produce enough *SpoIIR* to support sporulation, and thus we were able to identify several mutants with TGA nonsense mutations of this gene.

Isolation and characterization of UGA suppressor mutants. In order to determine how *SpoIIR* acts, we attempted to identify second-site suppressor mutants of *spoIIR*. To do so, we screened EMS-mutagenized (9) colonies of our original four *spoIIR* mutants (20) for the activation of σ^E by using a *lacZ* fusion to a σ^E-directed gene, *cotE*, that was present in each of the strains. After mutagenesis, the bacteria were plated on Schaeffer's sporulation agar containing X-Gal, and colonies exhibiting β-galactosidase activity were isolated. Only one of the *spoIIR* mutants, *spoIIR4* (20), produced a β-galactosidase-positive colony that was neither a revertant nor an up mutant of the endogenous β-galactosidase gene. The *spoIIR4* mutant had a TGA mutation at codon 152 (Table 1) (this mutation is hereafter referred to as *spoIIR152*). As shown below, the suppressor mutation was found to be a UGA suppressor and to map in *prfB*. For clarity, the name *prfB* is used here and throughout, even though the evidence placing the mutation in *prfB* is presented later. This suppressor mutation has been named *prfB1*. σ^E transcriptional activity during sporulation was increased in the presence of *prfB1* over that of the *spoIIR152* strain, although it remained approximately one-third that of the *spoIIR*$^+$ strain (Fig. 1). The presence of the suppressor mutation also increased the sporulation frequency of the *spoIIR152* mutant approximately 450-fold (Table 2). We ob-

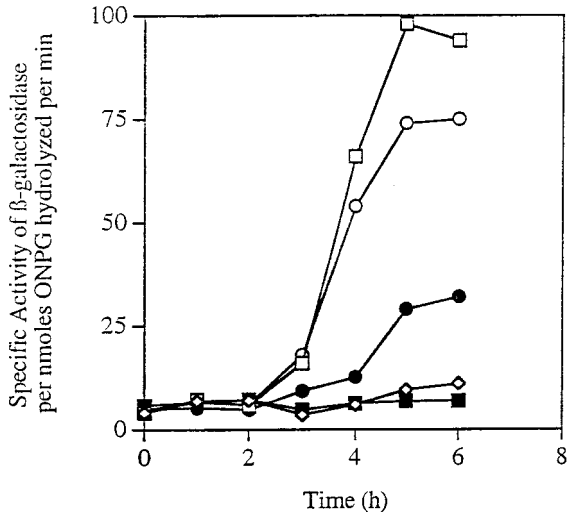

FIG. 1. Restoration of σ^E activity to *spoIIR* bacteria by *prfB1*. β-Galactosidase activity from the σ^E-controlled p1 promoter of *cotE* fused to *lacZ* in the following BR151 derivatives: □, *spoIIR*$^+$; ■, *spoIIR152*; ●, *spoIIR152 prfB1*; ○, *spoIIR*$^+$ *prfB1*; and ◇, endogenous β-galactosidase activity of the parent strain BR151 that does not contain the *lacZ* fusion. ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

tained no suppressors of the *spoIIR152* mutation that did not need *spoIIR* for σ^E activation.

The *prfB1* mutation on its own impaired sporulation, and interestingly, this effect was much greater at higher temperatures. *prfB1* bacteria produced 8.2×10^6 heat-resistant spores per ml of culture at 37°C and only 180 spores per ml at 42°C, whereas the wild-type strain produced about 2×10^8 spores per ml at both temperatures (Table 3). The sporulation of *prfB1* bacteria appears to be blocked at an early stage of sporulation. There was no detectable formation of the asymmetric septum, one of the earliest sporulation-specific morphological markers (32). In contrast to the sporulation phenotypes, there was only a slight effect on the growth rate of *prfB1* at 42°C, with a growth rate (mass doublings/hour) of 1.34 for the *prfB1* strain compared to 1.52 for the *prfB*$^+$ parent. At 37°C, the growth rates were similar for the two strains, 1.32 for the *prfB1* strain and 1.31 for the *prfB*$^+$ parent.

TABLE 2. Sporulation of suppressed *spoIIR* mutants at different temperatures

Relevant genotype[a]	Spores per ml[b]		Relative increase in spores per ml	
	37°C	42°C	37°C	42°C
spoIIR152	6.2×10^3	1.5×10^3		
spoIIR152 prfB1	2.7×10^6	1.4×10^2	428	−11
spoIIR152 prfB2	2.8×10^6	1.2×10^6	444	800
spoIIR152 prfB3	9.8×10^5	1.1×10^5	156	73
spoIIR152 prfB4	1.8×10^6	4.0×10^5	290	267
spoIIR152 prfB5	4.3×10^5	1.8×10^5	69	120
spoIIR151	4.2×10^4	1.2×10^4		
spoIIR151 prfB2	1.3×10^7	2.5×10^6	309	208
spoIIR74	4.0×10^5	1.3×10^5		
spoIIR74 prfB3	5.9×10^7	1.3×10^7	148	100
spoIIR74 prfB4	1.4×10^8	2.6×10^7	350	200
spoIIR74 prfB5	3.0×10^7	1.7×10^7	75	131

[a] Strains are isogenic sets derived from *B. subtilis* 168 strain BR151.
[b] Spores per milliliter were determined as heat-resistant spores per milliliter of culture. A strain with an insertion mutation in *spoIIR* produced less than 10 spores per ml.

TABLE 3. Sporulation of *prfB* mutants at different temperatures

Relevant genotype[a]	Spores per ml[b]		% of parental strain BR151	
	37°C	42°C	37°C	42°C
prfB$^+$	2.0×10^8	2.4×10^8		
prfB1	8.2×10^6	1.8×10^2	5.5	0.00014
prfB2	5.6×10^7	5.7×10^7	24	33
prfB3	1.2×10^8	8.0×10^7	60	33
prfB4	1.9×10^8	5.7×10^7	95	24
prfB5	1.6×10^8	1.2×10^8	80	50

[a] Strains are an isogenic set derived from *B. subtilis* 168 strain BR151.
[b] Spores per milliliter were determined as heat-resistant spores per milliliter of culture.

The *spoIIR152* mutant and strains containing TGA mutations affecting codons 74 and 151 were used in a second type of screen in which suppressors that exhibited a Spo$^+$ phenotype after UV mutagenesis were isolated. In this screen, the *spoIIR152* mutant did not yield any Spo$^+$ colonies that contained extragenic suppressors, although five intragenic revertants were isolated from the 5×10^7 colonies that were screened. With *spoIIR151*, we isolated one Spo$^+$ extragenic suppressor, and with *spoIIR74* we isolated four. The *spoIIR151* suppressor and three of the four *spoIIR74* suppressors were found to map in *prfB* (data not shown). The location of the fourth *spoIIR74* suppressor was not determined. We have named the *spoIIR151* suppressor mutation *prfB2* and the three *spoIIR74* mutations *prfB3*, *-4*, and *-5*. In the presence of these suppressors, sporulation of the *spoIIR* mutants was increased 70- to 450-fold (Table 2). Thus, *spoIIR152* strains with the new *prfB* mutations sporulated at a frequency similar to or less than that of the *spoIIR152* strain with *prfB1*. This indicates that the Spo$^+$ phenotype used to isolate these suppressors did not closely reflect the activity of the suppressors and also indicates the fact that *spoIIR74* and *spoIIR151* were leakier than *spoIIR152*. None of these Spo$^+$ suppressors exhibited the strong Ts$^-$ sporulation phenotype exhibited by *prfB1* (Table 2 and 3).

Mapping and cloning of the gene affected by *prfB1*. By utilizing the Ts$^-$ sporulation phenotype of *prfB1*, a Tn*10* insert was identified that was 65% linked to *prfB1* by transformation. The Tn*10* and its flanking chromosomal DNA were cloned and used to probe the ordered YAC library of *B. subtilis* (6). The DNA hybridized to one YAC clone, 10-119, which carries DNA of the 305° region of the chromosome.

The wild-type gene was isolated from a *B. subtilis* lambda library by using the Tn*10*-flanking region as a probe. A subclone (pMLK262, from a lambda clone) rescued the *prfB1* phenotype, and the insert in pMLK262 was sequenced. Comparison of the resulting sequence with those in GenBank (1, 16) indicated that pMLK262 carried a region internal to the *prfB* gene encoding RF2. This region was originally cloned and sequenced by Sadaie et al. (35) as part of the operon carrying the *B. subtilis secA* gene (GenBank accession no. D90218). Pel et al. (30) later recognized that the gene encoded *prfB* and that it contained a region similar to the frameshift site found in most *prfB* genes, thus extending the predicted open reading frame 5′ to include codons for an additional 27 amino acids. The published sequence did not include the 3′ end of the *prfB* gene. We sequenced the complete *prfB* gene and found that it is followed by a sequence similar to that of Rho-independent terminators, indicating this is most likely the end of the operon. Two parts of the sequence that differed from the published sequence

FIG. 2. Alignment of RF2 amino acid sequences. The amino acid sequences of RF2 from *B. subtilis* (Bs) (this work and reference 35), *S. coelicolor* (Sc) (28), *E. coli* (Ec) (10), *S. typhimurium* (St) (21), and *H. influenzae* (Hi) (GenBank accession no. P43918) are shown. Amino acids that are identical in all five sequences are shown in bold type; the overall identity is 35%. The asterisks with numbers located above the *B. subtilis* sequences identify the amino acids changed in *prfB1*, *-3*, and *-5*. The frameshift region found in all of the sequences except that of *S. coelicolor* is marked with a dashed line above the *B. subtilis* amino acid sequence.

were found. One is an extra G after base pair 3137 (following the numbering of Sadaie et al. [35]) that extends the open reading frame 5′ to include 12 more codons, extending the encoded protein to a length similar to that of *E. coli*, *S. typhimurium*, *Streptomyces coelicolor*, and *Haemophilus influenzae* (Fig. 2). The new suggested AUG initiation codon is preceded by a good ribosome-binding site with a ΔG for binding equal to −17.2 kcal/mol. The other difference is a loss of a G (residue 4176 [35]), changing the reading frame such that the downstream encoded protein is now also similar to other RF2 sequences (Fig. 2).

Sequencing of the *prfB* mutants. To determine the nature of the *prfB* mutations, the mutant *prfB* genes were amplified by PCR and the products were sequenced. The *prfB1* mutant contained a transversion of T to A, changing Tyr (TAT) 325 to Asn (AAT). Both *prfB2* and *prfB4* affected the proposed ribosome-binding site, with *prfB2* containing a T-to-C transition at −13 (with +1 as the A of the ATG translation initiation codon) and *prfB4* containing a G-to-A transition at −12. The *prfB3* mutant contained a G-to-A transition that altered the second amino acid residue of the protein, changing it from Glu (GAA) to Lys (AAA). The *prfB5* mutant contained a G-to-C transversion, changing residue 21 from Arg (AGG) to Thr (ACG). Thus, three of the mutations likely impair the formation of RF2, *prfB2*, and *prfB4* by weakening the ribosome-binding site and *prfB5* by affecting frame shifting. Such impaired RF2 formation would be expected to increase UGA suppression (14).

Nonsense suppression during vegetative growth of the *prfB* mutants. To determine if these suppressors could act under conditions of vegetative growth as well as to quantify misreading by the *prfB* mutants, we used a TGA nonsense mutation that had been constructed in codon 7 of the CAT gene *catA86*. This mutant, named UGA-7, is carried on pPL708C2 UGA-7 (26) and was introduced into the *prfB1* strain MLK1013 and its parent strain BR151. In the presence of *prfB1*, the level of CAT activity from UGA-7-containing bacteria grown at 37°C was increased 6.6-fold over that from the isogenic *prfB*$^+$ strain (Table 4). Analysis of the other *prfB* suppressor mutants indicated that they too increased the level of UGA readthrough from 3.8- to 7.1-fold. This analysis was complicated by an unexpected effect of some of the suppressor mutations on the constitutive expression of wild-type *catA86* from pPL708C2. When this is taken into consideration, the *prfB* mutants increased CAT activity to 19 to 54% of that of the wild type from the readthrough level of 6% of that of the wild type. These results indicate that the suppressors were not acting specifically during sporulation and could also suppress TGA mutations during vegetative growth. The high level of UGA readthrough did not affect the growth rate, even though UGA is used as a stop codon for about 20% of *B. subtilis* genes (36). We also tested the effects of the *prfB1* mutation on readthrough of a UAA codon at position 7 and found no suppression (data not shown), indicating that suppression is limited to UGA codons. To explore the nature of this suppression, we sequenced puri-

TABLE 4. Suppression of *catA86* UGA-7 by *prfB* mutants

prfB[a] mutation	CAT activity[b]		% of suppression
	C2 (WT)[c]	UGA-7[d]	
prfB$^+$	10.8	0.7	6
prfB1	23.9	4.6	19
prfB2	9.5	5.0	53
prfB3	14.7	2.9	20
prfB4	14.5	2.9	20
prfB5	5.1	2.7	54

[a] *prfB*$^+$ is in strain BR151; *prfB* mutations are in derivatives of *B. subtilis* 168 strain BR151.

[b] Specific activity of CAT is expressed as micromoles per minute per milligram of protein. Data are the average of four experiments, with specific activities varying ±10 to 15% between experiments. In each experiment, the relative level of expression between the different samples remained the same.

[c] Strain containing pPL708C2 (see Materials and Methods).

[d] Strain containing pPL708C2 UGA-7.

fied intact protein (UGA-7) from the *prfB1* pPL708C2 UGA-7 strain. The sole residue at position 7 was identified as tryptophan, indicating that $tRNA^{Trp}$ was decoding the UGA codon in the suppressor mutant. The three *spoIIR* UGA mutants that were analyzed (Table 2) were codon changes from UGG, encoding Trp. Thus, if suppression at these UGA codons occurs also by insertions of tryptophan, it would lead not only to the elongation of the protein to its full length but also to the synthesis of a wild-type *SpoIIR* protein.

The nucleotide 3′ to the UGA triplet affects the extent of readthrough in *E. coli* in the order A>G>C>U (23) and may explain the leakiness of *catA86*-UGA7 where the sequence is UG AA (2). The corresponding sequences for *spoIIR74*, *spoIIR151*, and *spoIIR152* are UGAG, UGAU, and UGAU, respectively; the position 3′ to UGA might also explain, at least partly, why the *spoIIR74* mutant exhibits a leakier sporulation phenotype than the *spoIIR151* and *spoIIR152* mutants as well as the relatively tight phenotype the *spoIIR* mutants display compared to *catA86*-UGA7.

To our knowledge, these are the first examples of mutations mapping in a release factor gene in gram-positive bacteria. Identification of UGA-suppressor mutations mapping in *prfB* provides the first experimental support for the supposition that *prfB* codes for RF2. The finding that the majority of the UGA-suppressor mutations obtained (five of six) mapped in *prfB* and not in a *trn* gene was surprising. It contrasts with results for *Enterobacteriaceae* when nearly all nonsense suppressors are altered tRNAs (13, 19). It suggests subtle differences in translational machinery between *B. subtilis* and *Enterobacteriaceae*.

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