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Autoregulation of the Stability Operon of IncFII Plasmid NR1

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The *stb* locus of IncFII plasmid NR1, which mediates stable inheritance of the plasmid, is composed of an essential *cis*-acting DNA site located upstream from two tandem genes that encode essential stability proteins. The two tandem genes, *stbA* and *stbB*, are transcribed as an operon from promoter P_{AB}. Using P_{AB}-*lacZ* gene fusions, it was found that the *stb* operon is autoregulated. A low-copy-number *stb*$^+$ plasmid introduced into the same cell with the P_{AB}-*lacZ* fusion plasmid repressed β-galactosidase activity about 5-fold, whereas a high-copy-number *stb*$^+$ plasmid repressed β-galactosidase about 15-fold. The details of autoregulation were analyzed by varying the concentrations of StbA and StbB to examine their effects on expression from the P_{AB}-*lacZ* fusion plasmid. StbB protein by itself had autorepressor activity. Although StbA protein by itself had no detectable repressor activity, plasmids that encoded both *stbA* and *stbB* repressed more effectively than did those that encoded *stbB* alone. Plasmids with a mutation in *stbA* had reduced repressor activity. One mutation in *stbB* that inactivated the stability function also reduced, but did not eliminate, repressor activity. Repressor activity of the mutant StbB protein was effectively enhanced by *stbA*. These results indicate that StbB serves two functions, one for stable inheritance and one for autoregulation of the *stb* operon, both of which may be influenced by StbA protein.

IncFII plasmid NR1, like most low-copy-number plasmids that have been examined (1, 4, 23), encodes functions that ensure its stable inheritance in the population of cells (17, 27). The *stb* (stability) locus of NR1 (17, 27), which may be equivalent to the *parA* locus of closely related IncFII plasmid R1 (11), is thought to participate in the partitioning of plasmid molecules to daughter cells during cell division, which is essential for stable plasmid inheritance. Mutations that inactivate the *stb* function result in plasmid instability, so that plasmid-free cells are segregated at a rate consistent with random distribution of plasmid copies at cell division (17, 25).

Within the 95-kb genome of the self-transmissible antibiotic resistance plasmid NR1 (30), *stb* is located approximately at coordinates 24.5 to 26.1 kb and is contained within a 1.7-kb region bounded by *Nae*I and *Taq*I restriction sites (Fig. 1) (27). The three essential elements of the *stb* locus are a *cis*-acting DNA site and two tandem genes, *stbA* and *stbB*. These genes encode *trans*-acting stability proteins of 36,000 and 13,000 Da, respectively (27), and are transcribed together from promoter P_{AB} (19). The *cis*-acting DNA site is located at or near P_{AB} and can stabilize a plasmid if StbA and StbB are provided in *trans* (20, 27). Therefore, the *stb* locus of NR1 is basically similar to the stabilizing loci of plasmids F (*sop*) and P1 prophage (*par*), which also are composed of two genes that encode essential *trans*-acting proteins and a *cis*-acting site (1–3, 6, 12, 15, 21, 22, 24). A primary difference among these plasmid-stabilizing loci is that for F and P1, the *cis*-acting partition sites lie downstream from the two genes, whereas for the *stb* locus of NR1, the site is upstream from the genes and may overlap promoter P_{AB}. This fundamental difference may signal differences in the ways the loci function and in how they are regulated.

Several lines of indirect evidence suggest that the *stb* operon is autoregulated. Mutants that are deleted of the *Bgl*II fragment that contains *stbB* and the 3′ half of *stbA* (Fig. 1) produce an overabundance of truncated StbA protein (11, 27) and have a high rate of transcription of the remaining *stb* sequences (19). Other mutants that have a transposon insertion in *stbA*, which prevents transcription of the region downstream from the insertion including *stbB*, produce an overabundance of mRNA that hybridizes to probes from the upstream region of the operon (19). Also, an excess of StbB provided in *trans* (without additional StbA) can destabilize *stb*$^+$ plasmids (20), which suggests that StbB might play some role in negative regulation. Results reported here demonstrate directly that StbB is an autorepressor of transcription from the *stb* promoter, P_{AB}.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Escherichia coli* K-12 strains JM83 and JM105 (28) were used for plasmid construction. Strains JM109 (31) and SE4006 (10), which are *recA*, were used for β-galactosidase assays. In most experiments, cells were cultured in 2YT medium (18) containing, per liter, 16 g of tryptone, 10 g of yeast extract (both Difco Laboratories), and 5 g of NaCl. 2YT plates contained 15 g of Bacto Agar (Difco) per liter. Screening of clones for β-galactosidase activity was done on B agar (18) containing, per liter, 10 g of tryptone, 8 g of NaCl, 15 g of agar, 20 mg of thiamine, and 50 μg of 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (Research Organics, Inc.). B broth (18) was used for β-galactosidase and β-lactamase assays. Steady-state induction of *lac* transcription by different concentrations of isopropyl-β-D-thiogalactopyranoside (IPTG) was performed as described by Miller (18). The following antibiotics (Sigma Chemical Co.) were included in the medium when appropriate to select for cells harboring various plasmids: tetracycline hydrochloride (30 mg/liter), sodium ampicillin (50 mg/liter), and chloramphenicol (30 mg/liter). Cells were cultured at 37°C, and growth was monitored by turbidity at 600 nm with a Gilford model 260 spectrophotometer.

Plasmids. The plasmids used in this study are listed in Table 1. Various *Taq*I and *Nae*I-*Taq*I fragments that contain

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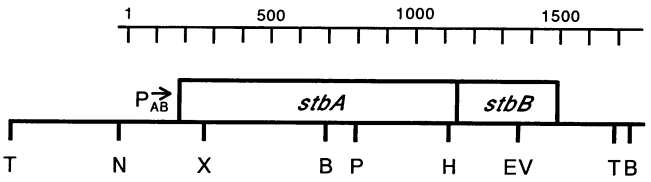

FIG. 1. Map of the *stb* operon of NR1. The scale above the map is in base pairs in reference to the nucleotide sequence of *stb* (27). The *stbA* and *stbB* genes are transcribed from left to right from promoter P_{AB} and encode essential *trans*-acting stability proteins of 36 and 13 kDa, respectively. The translation stop codon of *stbA* overlaps the translation start codon of *stbB*. The *cis*-acting stability site is at or near P_{AB}. The *Taq*I-*Taq*I or *Nae*I-*Taq*I *stb* fragments were cloned into vector plasmid pUC19 to construct pTR1909 and pTR1919, respectively, such that polylinker sites for *Eco*RI and *Bam*HI are located to the left and that for *Hin*dIII is located to the right of the inserted *stb* DNA. Restriction sites: *Taq*I (T), *Nae*I (N), *Xmn*I (X), *Bgl*II (B), *Pst*I (P), *Hin*cII (H), and *Eco*RV (EV).

the wild-type (wt) or mutant *stb* loci (Fig. 1) were inserted into the polylinker sites of pUC19 as described previously (20, 27) to construct pTR1909, pTR1911, pTR1913, and pTR1919, in which the *Eco*RI and *Bam*HI sites of the polylinker are located to the left of the *stb* fragment shown in Fig. 1 and the *Hin*dIII site is to the right. The P_{AB} promoter was deleted from pTR1909, which contains the wt *stb* locus, by digestion with *Eco*RI, which cleaves to the left of P_{AB}, followed by digestion with *Bal* 31 exonuclease and ligation with a *Bam*HI linker (all linkers were obtained from New England Biolabs). The deletion in pTRd603 proceeds from the left through bp 124, thereby removing P_{AB} but not the translation start signals of *stbA* (Fig. 1). The mutations in *stbA* and *stbB* in pMR21 and pFR12 are located at positions 775 and 1439, respectively (27), and therefore are downstream from the *Bgl*II site in *stbA* (Fig. 1). Plasmids having the same P_{AB} deletion as does pTRd603 but with either the *stbB* or *stbA* mutation were constructed by swapping the mutant *Bgl*II-*Hin*dIII fragments from pTR1911 or pTR1913, respectively, with the equivalent fragment from pTRd603. To construct plasmid pTRd501, the *Hin*cII-*Hin*dIII fragment from the right side of *stb* (Fig. 1; the *Hin*dIII site is in the polylinker to the right of *stb*) was deleted from pTRd603 and replaced with a synthetic double-stranded oligomer composed of a *Hin*cII linker, the remaining 24 bp from the 3′ end of *stbA*, and a *Hin*dIII linker. Therefore, pTRd501 contains the same P_{AB} deletion as does pTRd603 and the wt *stbA* gene but not *stbB*.

Plasmid pATRR101 was constructed by inserting the *Bgl*II fragment that contains the wt *stbB* gene from pTR1909 (Fig. 1) into the *Bam*HI site in the *tet* gene of pACYC184. The orientation of the inserted fragment in pATRR101 was such that *stbB* was transcribed by the *tetP* promoter. pATRR110

TABLE 1. Plasmids used

Plasmid	Description[a]	Reference or source
pRR720	wt NR1-derived *repA*$^+$ *stb*$^+$ miniplasmid (24.6 kb); *cat*	27
pFR12	Unstable *stbB* nonsense mutant of pRR720; *cat*	27
pMR21	Unstable *stbA* missense mutant of pRR720; *cat*	27
pRR933	wt NR1-derived miniplasmid without *stb*; *cat*	9
pUC18, pUC19	pBR322-derived cloning vectors; *bla*	31
pTR1909	pUC19 + *Taq*I-*Taq*I *stb*$^+$ fragment from pRR720; *bla*	27
pTR1911	pUC19 + *Taq*I-*Taq*I *stbB* mutant fragment from pFR12; *bla*	27
pTR1913	pUC19 + *Taq*I-*Taq*I *stbA* mutant fragment from pMR21; *bla*	27
pTR1919	pUC19 + *Nae*I-*Taq*I *stb*$^+$ fragment from pTR1909; *bla*	This study
pTRd603	Deletion of P_{AB} from pTR1909; *bla*	This study
pTRd6032	pTRd603 with replaced *Bgl*II-*Hin*dIII *stbB* mutant fragment from pTR1911; *bla*	This study
pTRd6033	pTRd603 with replaced *Bgl*II-*Hin*dIII *stbA* mutant fragment from pTR1913; *bla*	This study
pTRd501	pTRd603 with replaced synthetic *Hin*cII-*Hin*dIII fragment; *bla*	This study
pTH01	pUC18 with replaced *Pvu*II fragment containing additional cloning sites; *bla*	H. Kuramitsu
pACYC184	p15A-derived cloning vector; *cat tet*	5
pATRR101	pACYC184 + *Bgl*II-*Bgl*II *stbB*$^+$ fragment from pTR1909; *cat*	This study
pATRR110	pACYC184 + *Bam*HI-*Hin*dIII *stb*$^+$ fragment from pTR1919; *cat*	This study
pVLRR10	pACYC184 + *Eco*RI-*Eco*RI *lacI*^{q} fragment; *tet*	7
pTHI01	pTH01 + *Eco*RI-*Eco*RI *lacI*^{q} fragment from pVLRR10; *bla*	This study
pSU2718	pACYC184 + *lacP* and polylinker cloning sites	16
pTRI185	pSU2718 + *lacI*^{q} (sense orientation); *cat*	This study
pTRI186	pSU2718 + *lacI*^{q} (antisense orientation); *cat*	This study
pTB1001-5	pTRI185 + *Hin*cII-*Hin*dIII *stbB*$^+$ fragment from pTR1909; *cat*	This study
pTB1001-6	pTRI186 + *Hin*cII-*Hin*dIII *stbB*$^+$ fragment from pTR1909; *cat*	This study
pTB1012-5	pTRI185 + *Hin*cII-*Hin*dIII *stbB* mutant fragment from pTR1911; *cat*	This study
pTR6105	pTRI185 + *Bam*HI-*Hin*dIII *stb*$^+$ fragment from pTRd603; *cat*	This study
pTR6106	pTRI186 + *Bam*HI-*Hin*dIII *stb*$^+$ fragment from pTRd603; *cat*	This study
pTR6205	pTR6105 with replaced *Pst*I-*Hin*dIII *stbA*$^+$ fragment from pTRd501; *cat*	This study
pTR6206	pTR6106 with replaced *Pst*I-*Hin*dIII *stbA*$^+$ fragment from pTRd501; *cat*	This study
pTR612B-5	pTRI185 + *Bam*HI-*Hin*dIII *stbB* mutant fragment from pTRd6032; *cat*	This study
pTR621B-5	pTRI185 + *Bam*HI-*Hin*dIII *stbA* mutant fragment from pTRd6033; *cat*	This study
pFZY1	Mini-F-derived transcriptional *lacZ* fusion cloning vector; *bla*	13
pTRZ1	pFZY1 + *Bam*HI-*Xmn*I P_{AB} fragment from pTR1919; *bla*	This study
pTRZ2	pFZY1 + *Eco*RI-*Bgl*II P_{AB} fragment from pTR1919; *bla*	This study
pTRZ3	pFZY1 + *Bam*HI-*Hin*cII P_{AB} fragment from pTR1919; *bla*	This study

[a] Abbreviations: *bla*, ampicillin resistance; *tet*, tetracycline resistance; *cat*, chloramphenicol resistance.

was constructed by inserting the *Bam*HI-*Hin*dIII fragment that contained the entire wt *stb* locus from pTR1909 between the *Bam*HI and *Hin*dIII sites of pACYC184.

To construct plasmids in which the expression of *stbA* and *stbB* was under control of *lacP* and *lacI*^{q}, vector plasmids pTRI185 and pTRI186 were first constructed as follows. Plasmid pTH01, a pUC18 derivative whose *Pvu*II fragment was replaced by one containing additional cloning sites, was constructed and provided by H. K. Kuramitsu. The *Eco*RI fragment from pVLRR10 that contains the *lacI*^{q} gene was inserted into the *Eco*RI site of pTH01, such that it was between *Bam*HI and *Bgl*II sites, to make pTHI01. Vector plasmid pSU2718 was derived from pACYC184 by replacing the *tet* gene with a polylinker cloning site downstream from *lacP*. There is a single *Xmn*I site in pSU2718 upstream from *lacP* that was replaced by insertion of a *Bgl*II linker, which was followed by insertion of the *Bam*HI-*Bgl*II *lacI*^{q} fragment from pTHI01 at the new *Bgl*II site in pSU2718. In pTRI185, the orientation of the *lacI*^{q} fragment is such that transcription is in the same direction as that from *lacP* (sense orientation), whereas in pTRI186 it is in the antisense orientation. The various fragments that contain *stbA*, *stbB*, or both were then inserted into pTRI185 and pTRI186 as described in Table 1. In these plasmids, the inserted *stb* genes are transcribed from *lacP*, which is repressed by LacI^q. Expression of the *stb* gene products can be induced by including IPTG in the medium.

pFZY1 is a low-copy-number vector plasmid with a polylinker sequence upstream from a promoterless *lac* operon that contains translational stop codons in all three reading frames between the polylinker and *lacZ*, for the purpose of constructing transcriptional *lacZ* fusions (13). Plasmids in which *lacZ* is transcribed from P_{AB} were constructed by inserting *stb* fragments from pTR1919 into pFZY1 as described in Table 1 to make pTRZ1, pTRZ2, and pTRZ3.

DNA isolation and manipulation. DNA isolation, restriction endonuclease digestion, gel electrophoresis, ligation of restriction fragments, and transformation of *E. coli* cells with plasmid DNA were performed as described previously (9, 17, 26). All enzymes were used as recommended by the suppliers. Restriction endonuclease fragments were purified from agarose gels with DEAE-membranes (8). The alkaline minilysate method (26) was used for screening of plasmid sizes and restriction endonuclease analysis of plasmid DNA.

Nucleotide sequence analysis. The extent of the *Bal* 31-induced deletions in plasmids like pTRd603 was determined by DNA sequencing. DNA sequencing kits were purchased from United States Biochemical, and dideoxy sequencing was performed as described by Williams et al. (29), using [α-^{35}S]dATP (New England Nuclear). DNA sequencing was also performed to confirm the presence of mutations in *stbA* or *stbB* in various plasmid derivatives, using synthetic oligonucleotide primers as described previously (27).

Enzyme assays. The β-galactosidase activities of cultures of cells harboring the various P_{AB}-*lacZ* fusion plasmids were assayed by a modification of the method of Miller (18) as described previously (9). The relative copy numbers of the P_{AB}-*lacZ* fusion plasmids that contained the *bla* gene were estimated from gene dosage by assaying their β-lactamase activities as described by Lupski et al. (14), using cephaloridine (Sigma) as the colorigenic reagent. Protein concentrations were determined with Bio-Rad protein assay kits (Bio-Rad Laboratories) by following the instructions supplied with the kits.

TABLE 2. Regulation of promoter P_{AB} in transcriptional *stb-lacZ* fusion plasmid pTRZ2[a]

Coresident plasmid	Replicon	*stb* protein(s) encoded	β-Galactosidase activity[b] (Miller units)
pRR933	NR1	None	410 ± 46
pRR720	NR1	StbA$^+$ + SbtB$^+$	81 ± 15
pFR12	NR1	StbA$^+$ + StbB$^-$	183 ± 27
pMR21	NR1	StbA$^-$ + StbB$^+$	190 ± 5
pACYC184	p15A	None	398 ± 106
pATRR110	p15A	SbtA$^+$ + StbB$^+$	27 ± 3
pATRR101	p15A	StbB$^+$	82 ± 15

[a] Host strain JM109 contained P_{AB}-*lacZ* fusion plasmid pTRZ2 plus the indicated coresident plasmid to supply *stb* proteins in *trans*. StbA$^+$, wt StbA protein; StbA$^-$, mutant StbA protein from pMR21; StbB$^+$, wt StbB protein; StbB$^-$, mutant StbB protein from pFR12.

[b] Averages from at least four experiments.

RESULTS

Autoregulation of *stb* transcription. To test the activity of the *stb* promoter, P_{AB}, transcriptional fusions were constructed in which P_{AB} was inserted upstream of a promoterless *lacZ* gene in low-copy-number vector plasmid pFZY1. These fusion plasmids contained the region of *stb* DNA between the *Nae*I site located to the left of P_{AB} and the *Xmn*I (pTRZ1), *Bgl*II (pTRZ2), or *Hin*cII (pTRZ3) site located within *stbA* (Fig. 1). The level of β-galactosidase activity expressed from each of the fusion plasmids was about 440, 400, or 330 Miller units, respectively, in host strain JM109, which indicated that P_{AB} was active in the transcriptional fusions. When a second compatible plasmid containing the wt *stb* locus was introduced into the same cell with the P_{AB}-*lacZ* fusion plasmids, the level of β-galactosidase activity was repressed. For pTRZ2, the repression was about 5-fold if the *trans stb*$^+$ plasmid was of low copy number (pRR720) and about 15-fold if the *trans stb*$^+$ plasmid was of high copy number (pATRR110) (Table 2). The repression was less if the coresident plasmids contained an ochre point mutation in *stbB* (pFR12) or a missense point mutation in *stbA* (pMR21) (Table 2). Each mutation results in unstable inheritance (20, 27). Coresident plasmid pATRR101 contains only the *stbB* gene, which is transcribed from the *tet* promoter of the vector. The repression of β-galactosidase activity from pTRZ2 by pATRR101 indicated that StbB protein by itself could repress transcription from P_{AB} (Table 2). Similar results for the high-copy-number coresident plasmids were obtained with pTRZ1 and pTRZ3 (data not shown). Together, these results indicate that the *stb* operon is autoregulated and that StbB protein by itself has autorepressor activity.

Autoregulation of *stb* transcription as a function of repressor protein concentration. To examine the details of autoregulation of the *stb* operon, plasmids were constructed in which the expression of various combinations of the *stbA* and *stbB* genes was under control of the *lacP* promoter and *lacI*^{q} repressor, such that the levels of StbA and StbB proteins could be varied by varying the concentration of IPTG inducer. These plasmids were constructed with a pACYC184-based vector plasmid that contains a polycloning site downstream from *lacP*, with the *lacI*^{q} gene inserted upstream from *lacP* in either orientation to form plasmids pTRI185 and pTRI186. The *stbA* and *stbB* genes lacking their own promoters were inserted into pTRI185 and pTRI186 downstream from *lacP*. Those plasmids were then introduced into the same cell with the P_{AB}-*lacZ* fusion

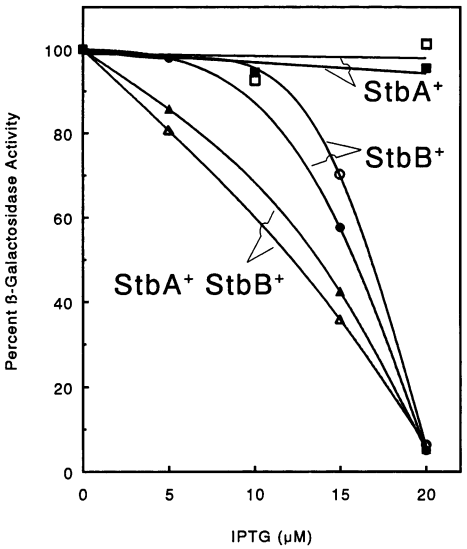

FIG. 2. Repression of P_{AB}-*lacZ* fusion in plasmid pTRZ2 by different concentrations of StbA, StbB, or both. The host strain was SE4006 containing pTRZ2 plus a coresident plasmid that can supply *stb* proteins in *trans* under control of *lacP*, *lacI*^{q}, and IPTG. The cells were cultured in medium containing various concentrations of IPTG inducer. The β-galactosidase activity of each culture was measured and normalized to the 100% level in the absence of inducer. The coresident plasmids (and their 100% activity levels and plotting symbols) were pTB1001-6 (2,601; ○, StbB$^+$), pTB1001-5 (2,466; ●, StbB$^+$), pTR6106 (1,822; △, StbA$^+$ StbB$^+$), pTR6105 (2,059; ▲, StbA$^+$ StbB$^+$), pTR6206 (1,408; □, StbA$^+$), and pTR6205 (1,652; ■, StbA$^+$). The data points are averages from multiple experiments.

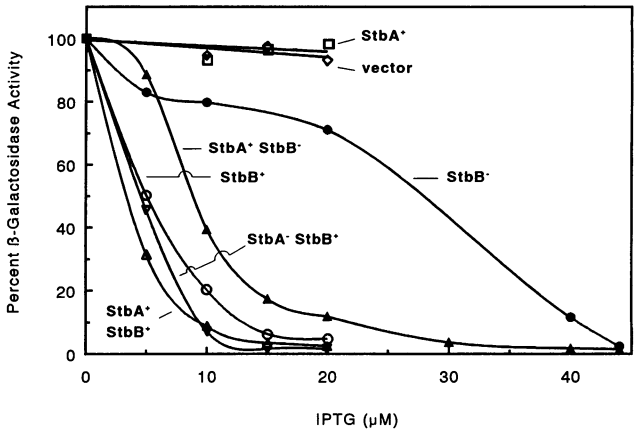

FIG. 3. Repression of the P_{AB}-*lacZ* fusion in plasmid pTRZ1 by wt and mutant *stb* proteins. The host strain was SE4006 containing pTRZ1 plus a coresident plasmid that can supply *stb* proteins in *trans* under control of *lacP*, *lacI*^{q}, and IPTG. The cells were cultured in medium containing various concentrations of IPTG inducer. The β-galactosidase activity of each culture was measured and normalized to the 100% level in the absence of inducer. The coresident plasmids (and their 100% activity levels and plotting symbols) were pTB1001-5 (1,900; ○, StbB$^+$), pTB1012-5 (2,116; ●, StbB$^-$), pTR6105 (1,262; △, StbA$^+$ StbB$^+$), pTR621B-5 (1,523; ▽, StbA$^-$ StbB$^+$), pTR612B-5 (1,570; ▲, StbA$^+$ StbB$^-$), pTR6205 (1,764; □, StbA$^+$), and pTRI185 (1,203; ◇, vector). The data points are averages from multiple experiments.

plasmids to examine their effects on regulation of *stb* expression. Cells that contained both types of plasmid were cultured in medium containing different concentrations of IPTG to induce different amounts of StbA and StbB and examine their effects on transcription from P_{AB}. The results with *lacZ* fusion plasmid pTRZ2 indicated that StbB protein had autorepressor activity, whereas StbA protein did not (Fig. 2). In addition, at any given concentration of IPTG, there was more repression by plasmids that contained both *stbA* and *stbB* than by those that carried only *stbB*. This finding suggested that StbA protein, although having no repressor activity by itself, might augment the repressor activity of StbB protein. The orientation of the *lacI*^{q} gene, which might have affected the copy number or levels of expression from the pACYC184 derivatives, was not important, since plasmids derived from either pTRI185 or pTRI186 gave similar results (Fig. 2). The copy numbers of the pFZY1-derived fusion plasmids also were monitored at different levels of IPTG and found to be unaffected, so that changes in plasmid copy number are not responsible for the differences in β-galactosidase activity at different concentrations of IPTG (data not shown).

Effects of mutation on autoregulation of *stb* transcription. In experiments similar to those presented in Fig. 2, plasmid derivatives that produce wt or mutant StbA and StbB proteins under control of *lacP* and *lacI*^{q} were introduced into the same cell with *lacZ* fusion plasmid pTRZ1. By itself, StbB protein from a plasmid with a mutation in *stbB*, in which the last 19 amino acids have been truncated by a nonsense mutation, retained repressor activity, although much higher levels of IPTG were required to obtain repression than for the wt StbB protein by itself (Fig. 3). The augmentation of repressor activity by StbA protein was particularly evident for the plasmid that made both wt StbA and mutant StbB proteins, since much lower IPTG concentrations were required to achieve repression than for the plasmid that produced mutant StbB alone (Fig. 3). In general, repression of β-galactosidase activity from P_{AB}-*lacZ* fusion plasmid pTRZ1 (Fig. 3), in which the fusion is at the *Xmn*I site, was achieved at levels of IPTG lower than those required for repression of pTRZ2 (Fig. 2), in which the fusion is at the *Bgl*II site (Fig. 1). Nevertheless, the augmentation of repressor activity by StbA protein was still evident in comparison of the data curve for wt StbB alone with that for the plasmid that made both wt StbA and wt StbB (Fig. 3). For the plasmid with a missense mutation in *stbA* that caused unstable inheritance, there appeared to be a small but reproducible decrease in repressor activity compared with the plasmid that made both wt StbA and wt StbB (Fig. 3). Wild-type StbA protein by itself had no repressor activity, giving results similar to those with the vector plasmid alone (Fig. 3).

DISCUSSION

When excess StbB protein is provided in *trans*, NR1-derived *stb*$^+$ plasmids become unstable (20). Therefore, excess StbB protein behaves like a plasmid incompatibility factor (4). When StbA and StbB are provided in *trans* together, coresident *stb*$^+$ plasmids remain stable (20, 27), which suggests that it is not the high concentration of StbB protein alone that causes the destabilization. Additional StbA protein in the absence of additional wt StbB protein also does not cause destabilization (20). The results presented here explain these earlier observations. The *stb* operon is autoregulated by StbB protein (Table 2; Fig. 2 and

3). Because both StbA and StbB are required for stability (20, 27), an excess of StbB protein represses the *stb* operon and prevents synthesis of the essential StbA protein, which causes the plasmid to be unstable. Because both StbA and StbB are *trans* acting (20, 27), *stb*$^+$ plasmids remain stable when StbA and StbB are provided together, even if the operon is repressed. Having no repressor activity by itself (Fig. 2 and 3), additional StbA protein does not cause destabilization (20, 27).

These results may also explain the lack of a destabilizing incompatibility effect when the *cis*-acting site of *stb* is introduced into the same cell with a *stb*$^+$ plasmid (20, 27). The *cis*-acting stability site of NR1 overlaps, or is coincident with, promoter P_{AB} (20, 27). Since StbB is the autorepressor of the *stb* operon, any StbB protein that was titrated in *trans* by the additional *cis*-acting sites would be replenished by the autoregulatory system. This is a fundamental difference from the stabilizing loci from plasmids F and P1, for which the *cis*-acting sites are downstream from the tandem stability genes (6, 12, 15, 24). Although the *sop* and *par* loci of F an P1 are also autoregulated (10, 22), the protein that binds to the downstream *cis*-acting partition site, at least for P1, is not the autorepressor protein (6). That could explain why the *cis*-acting sites of F and P1 destabilize *sop*$^+$ and *par*$^+$ plasmids, respectively, present in the same cell, since the protein that binds to the additional partition sites would be titrated and would not be replenished by the autoregulatory system. The *parA* locus of plasmid R1, which is homologous to *stb* of NR1 and differs at only a few nucleotide positions (27), was reported to exhibit incompatibility with other *parA*$^+$ plasmids (11). The only incompatibility element found associated with the *stb* locus of NR1 is StbB protein, which is explained by its autorepressor activity. The lack of competition between multiple copies of the NR1 partition site within the same cell may suggest that partition is connected to plasmid replication, although other explanations are still possible.

Although StbB protein by itself had autorepressor activity, plasmids that encoded both *stbA* and *stbB* repressed P_{AB} more effectively than did those that encoded *stbB* alone (Fig. 2 and 3). By itself, StbA did not repress (Fig. 2 and 3), which indicates that the presence of *stbA* somehow enhances the repressor activity of StbB or that StbB imparts repressor activity to StbA. The latter seems less likely, since additional StbA provided in *trans* does not destabilize *stb*$^+$ plasmids (20). It is not clear whether the augmentation of StbB repressor activity by *stbA* is direct or indirect. One possibility is that StbA protein interacts with StbB protein to increase its activity, perhaps by increasing its affinity for DNA. However, in vitro binding studies have not revealed any effect of StbA protein on the binding of StbB to DNA (unpublished data). A second possibility is that the presence of *stbA* upstream from *stbB* enhances production of StbB protein, such that more repressor is synthesized at any given concentration of IPTG. The translation stop codon of *stbA* overlaps the translation start codon of *stbB* (27), and frameshift mutations in *stbA* are polar on *stbB* (19, 20, 27). This finding suggests that normally, expression of *stbA* and *stbB* may be translationally coupled, which could influence the level of synthesis of StbB protein. However, a missense mutation in *stbA* that causes loss of stability (27) also reduced repressor activity (Table 2 and Fig. 3). The mutation in *stbA* had little influence on the overall rate of transcription of *stb* mRNA from an intact *stb* operon (19). Although it cannot be ruled out that the amino acid substitution in StbA somehow altered the expression of the *stb* operon, it seems likely that the reduction in autorepressor activity from the *stbA* mutant indicates some direct influence of StbA on StbB. It is possible that both the direct and indirect effects apply.

The *stbB* mutation in pFR12 causes loss of stability (27). By itself, the mutant StbB protein from pFR12 had much reduced autorepressor activity (Fig. 3). However, in the presence of *stbA*, the mutant StbB protein was quite effective at repression (Fig. 3). This finding suggests that the two functions of StbB protein, stability and autorepression, can be separated by mutation. It seems likely that both StbB protein and the *cis*-acting site serve dual roles for the stability locus of NR1. Since StbB represses P_{AB}, it is most likely that it binds to an operator sequence near the promoter. The DNA sequence surrounding P_{AB} has a strong A-plus-C strand bias (27), and this unusual sequence might serve both as the operator for control of *stb* mRNA transcription and as the *cis*-acting site. The binding of StbB to this DNA might both repress transcription of *stb* and provide the first step in the plasmid partitioning mechanism. Later steps might involve StbA protein somehow interacting with StbB and some host-provided machinery. Those later steps might augment the autorepressor activity of StbB.

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