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Conditionally Lethal and Recessive UGA-Suppressor Mutations in the *prfB* Gene Encoding Peptide Chain Release Factor 2 of *Escherichia coli*

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Strains carrying mutations in the *prfB* gene encoding peptide chain release factor 2 of *Escherichia coli* were isolated. *prfB1*, *prfB2*, and *prfB3* were selected as suppressor mutations of a *lacZ* (UGA) mutation at 37°C, one of which, *prfB2*, is temperature sensitive in growth. A *prfB286* strain was selected as a conditionally lethal mutant which grows at 32 but not at 43°C and was shown to have UGA-suppressor activity. All the mutations are recessive UGA-suppressors. These data indicate that release factor 2 is essential to *E. coli* growth and that all mutants isolated here trigger suppression of the UGA codon.

Translation termination requires participation of two peptide chain release factors at specific termination codons in *Escherichia coli*. Release factor 1 (RF1) catalyzes termination at UAA and UAG codons, and release factor 2 (RF2) catalyzes termination at UGA and UAA codons (16). The gene encoding RF1, *prfA*, has been cloned and mapped at 27 min on the *E. coli* genome, and mutations in *prfA* have been shown to cause suppression of UAG and UAA nonsense codons (8, 9, 14, 15, 19). The RF2 gene, *prfB*, was first cloned by Caskey et al. (2), and its nucleotide sequence has been determined (4, 4a). Recently we have mapped the *prfB* gene at 62 min on the *E. coli* chromosome and clarified the structure and expression of the operon encoding RF2 (4a). This operon is composed of two genes, *prfB* and *herC*. The downstream gene, *herC*, encodes a protein of molecular weight 57,603 and has been defined by a suppressor mutation which restores replication of a ColE1 plasmid mutant.

In our previous work we suggested that a *supK* mutation of *Salmonella typhimurium*, a recessive UGA-suppressor mutation which maps in the same region as *prfB* in *E. coli*, affects the *S. typhimurium* RF2 protein (4a). To test directly whether a mutation in the *prfB* gene creates a UGA-suppressor activity in *E. coli*, we attempted to isolate mutations in *prfB* of *E. coli*. This article reports the isolation and the characterization of four RF2 mutants and the demonstration that they suppress the UGA nonsense codon.

Two rationales of selection were used to isolate RF2 mutations. One was to directly select a suppressor mutation of a *lacZ* UGA allele (*lacZ659*), some of which may have a defect in the peptide chain releasing activity of RF2 at the UGA codon. The other was to isolate a temperature-sensitive (ts) mutant which grows at low temperatures but not at high temperatures, because RF2 function may be essential to *E. coli* growth. In both cases, localized mutagenesis was conducted by transduction with mutagenized P1 phages by selecting for a Tn*10* transposon (*zgc*::Tn*10*) located about 7 kilobase pairs downstream of the *prfB* gene (4a) (Fig. 1).

In the first selection scheme, P1 phages grown on the wild-type strain C600 (*prfB*$^+$) carrying the closely linked Tn*10* marker were treated with hydroxylamine as described previously (7) and used to infect *E. coli* LS653, which carries the *lacZ659* (UGA) mutation (17). Tetracycline-resistant (Tet^r) transductants were directly selected at 37°C on lactose-MacConkey plates (MLA plates) containing tetracycline (15 μg/ml). Five "red" colonies, which are due to the increased *lacZ* expression, were isolated among 10^4 Tet^r "white" transductants. The parental strain LS653 also harbors a UGA mutation in *leu*. Four of the five red colonies grew on leucine-free minimal plates, suggesting that these four acquired a UGA-suppressor activity. Linkage of these mutations to Tn*10* was scored by P1 transduction from the mutants to the parental strain (LS653). Mutations in three strains, YN2978, YN2979, and YN2980, were cotransducible with Tn*10* into LS653 (Table 1); half of these Tet^r transductants were red, and red colonies were all phenotypically Leu$^+$. These frequencies of cotransduction are consistent with the distance between *prfB* and Tn*10*. Therefore, the three mutants, YN2978, YN2979, and YN2980, were examined further. (Another mutation was not cotransduced with Tn*10* and was not examined.)

In the second selection scheme, the same P1 phage stock as used in the above experiment was used to infect C600 cells. Tet^r transductants were first selected at 32°C on nutrient YT agar plates and then replica plated and incubated at 43°C to screen for ts mutants. One such mutant (KK539, carrying the mutation later designated *prfB286* [see below]), isolated in 10^4 Tet^r colonies, is unable to grow at 43°C on either minimal E agar and nutrient YT agar. *prfB286* was linked to Tn*10* at frequency of 60% (111/186) by P1 transduction, using KK539 (*thyA*$^+$ Tet^r *prfB286*) as a donor and C600 as a recipient, and then mapped by using LS653 (*thyA*) as a recipient. The deduced distribution of unselected markers suggests that the *prfB286* mutation maps at the side of Tn*10* opposite to that of *thyA*. The LS653 strain carries the *lacZ659* (UGA) and *leu* (UGA) mutations as described above. Surprisingly, all ts transductants formed red colonies on MLA plates and grew on leucine-free minimal plates at 32 and 37°C. These observations can be interpreted as indicating that the *prfB286* mutation is able to suppress UGA mutations.

Reciprocal crosses were conducted between these mutants (*thyA*) as recipients and C600 (*thyA*$^+$) as a donor by P1 transduction (see Table 1). The data clearly indicate that

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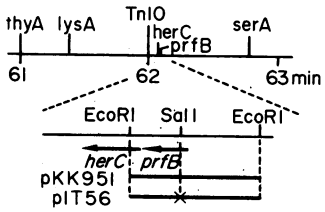

FIG. 1. Genomic structure of the 62-min region of the *E. coli* chromosome and plasmids carrying *prfB-herC*. Bold bars indicate the bacterial DNA cloned in the plasmid, and bold arrows indicate location and orientation of the genes. The pKK951 plasmid carries the 3-kilobase-pair *Eco*RI fragment encoding *prfB* and a part of *herC* in the same restriction site of pACYC184 (4a). The 3-kilobase-pair fragment contains a single *Sal*I site in the coding sequence of *prfB*. It was cleaved by *Sal*I and the ends were repaired with T4 DNA polymerase and ligated. The resulting *Sal*I-filled-in plasmid, pIT56, does not produce an active RF2 protein.

UGA-suppressor mutations in YN2978, YN2979, and YN2980, as well as *prfB286*, are linked to Tn*10* at a frequency of about 50% and map on the other side from *thyA*, which is consistent with the location of *prfB*.

The β-galactosidase levels in these strains were measured in vitro. All mutant strains exhibited β-galactosidase activity of about 200 U/min per mg, which is about 7% (i.e., suppression efficiency) of the wild-type *lacZ* level (i.e., 3,000 U) of activity but sevenfold higher than that of the parental LS653 strain. To test whether these mutations affect RF2, a pACYC184 plasmid carrying the *prfB* gene, pKK951 (4a), was introduced into mutant cells (YN2970, YN2971, YN2972, and KK579; *thyA*$^+$ Tet^s derivatives constructed by the above P1 transductions), and β-galactosidase activities were measured. The pKK951 plasmid clearly reduced expression of β-galactosidase and growth on leucine-free minimal plates (Table 2). To firmly establish that this complementation is due to the *prfB* gene itself, not to some other gene(s) on pKK951, a *prfB*-deficient plasmid, pIT56, was constructed by filling in a unique *Sal*I site in the coding region of *prfB* with DNA polymerase (see Fig. 1). pIT56 did not eliminate the UGA suppression (Table 2). These results, taken together with the mapping data presented above, strongly indicate that these suppressor mutations affect the RF2 protein. Accordingly, they are referred to as *prfB1*, *prfB2*, *prfB3*, and *prfB286*.

Concerning the ts growth of the *prfB2* and *prfB286* mutants, growth was not fully restored by introducing pKK951 or any other plasmids carrying further upstream or downstream chromosomal regions of *prfB*. However, such merodiploid strains containing the *prfB* portion were able to generate temperature-tolerant colonies at high frequencies, presumably owing to recombination between the chromosome and plasmid DNAs. These observations, taken to-

TABLE 1. Transductional mapping of the *prfB* mutations[a]

Donor (relevant markers)	Recipient (relevant markers)	Selected marker	Distribution of unselected markers		
			Class	No.	Frequency (%)
YN2978 (Tet^r *prfB1*)	LS653[b] (Tet^s *prfB*$^+$)	Tet^r	*prfB1*	4	*prfB1*/Tet^r (67)
			prfB$^+$	2	
YN2979 (Tet^r *prfB2*)	LS653 (Tet^s *prfB*$^+$)	Tet^r	*prfB2*	10	*prfB2*/Tet^r (48)
			prfB$^+$	11	
YN2980 (Tet^r *prfB3*)	LS653 (Tet^s *prfB*$^+$)	Tet^r	*prfB3*	11	*prfB3*/Tet^r (46)
			prfB$^+$	13	
KK539 (*thyA*$^+$ Tet^r *prfB286*)	LS653 (*thyA* Tet^s *prfB*$^+$)	Tet^r	*thyA*$^+$ *prfB286*	1	*prfB286*/Tet^r (52)
			thyA$^+$ *prfB*$^+$	5	
			thyA prfB286	16	*thyA*$^+$/Tet^r (18)
			thyA prfB$^+$	11	
C600 (*thyA*$^+$ Tet^s *prfB*$^+$)	YN2978 (*thyA* Tet^r *prfB1*)	*thyA*$^+$	Tet^s *prfB*$^+$	2	Tet^s/*thyA*$^+$ (11)
			Tet^s *prfB1*	3	
			Tet^r *prfB*$^+$	0	*prfB*$^+$/*thyA*$^+$ (4)
			Tet^r *prfB1*	42	
	YN2979 (*thyA* Tet^r *prfB2*)	*thyA*$^+$	Tet^s *prfB*$^+$	3	Tet^s/*thyA*$^+$ (10)
			Tet^s *prfB2*	2	
			Tet^r *prfB*$^+$	0	*prfB*$^+$/*thyA*$^+$ (6)
			Tet^r *prfB2*	43	
	YN2980 (*thyA* Tet^r *prfB3*)	*thyA*$^+$	Tet^s *prfB*$^+$	5	Tet^s/*thyA*$^+$ (17)
			Tet^s *prfB3*	3	
			Tet^r *prfB*$^+$	0	*prfB*$^+$/*thyA*$^+$ (10)
			Tet^r *prfB3*	40	
	KK573 (*thyA* Tet^r *prfB286*)	*thyA*$^+$	Tet^s *prfB*$^+$	4	Tet^s/*thyA*$^+$ (17)
			Tet^s *prfB286*	4	
			Tet^r *prfB*$^+$	0	*prfB*$^+$/*thyA*$^+$ (8)
			Tet^r *prfB286*	40	

[a] Recipient cells were infected with phage P1 *vir* grown on donor cells, incubated for 20 min, and plated on L agar (5) containing 15 μg of tetracycline per ml for Tet^r selection or on minimal ME agar (5) with appropriate supplements (except thymine) for *thyA*$^+$ selection. After incubation for 2 days at 30°C, colonies were picked, purified, and scored for unselected markers. UGA-suppressor activity was scored by colony color on MLA plates and growth on leucine-free minimal plates; ts growth was scored on YT agar plates (6).

[b] The full genotype is *leu* (UGA) *lacZ659* (UGA) *trpA9605* (UAG) *his29* (UAG) *ilv thyA metB argH rpoB rpsL* (17).

TABLE 2. Complementation analysis of UGA-suppressor activity of the *prfB* mutations

Strain[a]	β-Galactosidase synthesis[b] (U/min per mg of protein)			Growth on leucine-free minimal plates[c]		
	pACYC184	pIT56	pKK951	pACYC184	pIT56	pKK951
YN2970 (*prfB1*)	154	171	3	+	+	−
YN2971 (*prfB2*)	152	201	5	+	+	−
YN2972 (*prfB3*)	173	209	18	+	+	−
KK579 (*prfB286*)	194	217	10	+	+	−
LS653 (*prfB*$^+$)	35	45	8	−	−	−

[a] The strains indicated are *prfB* Tet^s derivatives of LS653. They were constructed by P1 transduction as described in Table 1. The cells were transformed with the indicated plasmids and selected for Tet^r.

[b] Transformant cells were grown at 37°C in Q broth (6) supplemented with 0.5% glycerol, 15 μg of tetracycline per ml, 1 mM isopropyl-1-thio-β-D-galactoside, and 20 μg of required amino acids per ml. Portions of the exponentially growing cultures were withdrawn, and the β-galactosidase activity was determined as described previously (6).

[c] +, Growth; −, no growth.

gether with the P1 mapping data, suggest that the ts phenotype conferred by the *prfB* mutations may be (partially?) dominant to the wild-type *prfB* allele, although involvement of a second ts mutation, closely linked to *prfB*, cannot be completely excluded at present. Such dominance may be explained by assuming that a ts RF2 protein causes ribosomal stalling at UGA and interferes with the action of the wild-type RF2 protein.

The wild-type *S. typhimurium* strain shows a leakiness of the UGA codon, which is eliminated upon introducing an F′ plasmid (KLF16) encoding the wild-type *supK* gene but not a *supK* mutant allele (12). The UGA codon is also leaky in the wild-type *E. coli* strain LS653 (Table 2), since significantly higher expression of β-galactosidase was observed compared with the background level in deletion or amber *lacZ* mutant strains. This increased expression was abolished when pKK951, but not pIT56, was transformed into the strain. Therefore, we infer that increased levels of RF2 stimulate translation termination at the UGA codon and that the leakiness of the UGA codon is due at least in part to the limiting intracellular concentration of RF2 under normal conditions.

It is noteworthy that two independent selection procedures yielded RF2 mutations with the same mutant phenotypes. The *prfB2* strain selected as a UGA-suppressor mutant exhibits ts growth, and the *prfB286* strain isolated as a ts lethal mutant shows a UGA-suppressor activity. They are both recessive UGA-suppressor mutations. A similar mutation has been isolated in *S. typhimurium, supK599*, which shows ts growth and recessive UGA suppression (12). These data strongly support our previous suggestion that the *supK* gene, a UGA-suppressor gene of *S. typhimurium*, encodes the *S. typhimurium* RF2 protein (4a).

Two specific questions remain to be solved. First, the *supK* strain of *S. typhimurium* has two properties: suppression of UGA and a low level of tRNA methyltransferase (10, 11, 13). To explain this phenomenon, we have suggested that the *supK* gene encodes RF2 and that its mutant form affects the expression of the methyltransferase (4a). In this regard, the level of tRNA methyltransferase in the *E. coli prfB1, prfB2, prfB3,* and *prfB286* mutants remains to be determined. Second, RF2 also is involved in ribosomal frameshifting. The *prfB* coding region contains an intragenic UGA stop codon at amino acid position 26, and a natural +1 frameshift occurs at this site to allow complete translation of RF2, presumably when the intracellular concentration of RF2 is low (3, 4). Recently Weiss et al. have found that the specific sequence context immediately upstream of the UGA codon is necessary to shifting; a Shine-Dalgarno-like sequence base pairs with the 3′ end of the 16S rRNA during elongation of protein synthesis, and a string of repetitive nucleotides directly preceding the stop codon causes tRNA decoding the string to shift at a high rate (18). This would be a natural mechanism of autotranslational control of RF2 expression. Another example comes from *S. typhimurium*, where *supK* mutants suppress both UGA and frameshift mutations (1). This cross-suppression suggests that the altered RF2 protein in the *supK* mutant triggers not only UGA suppression but also ribosomal frameshifting. It is not certain that these *supK* and *prfB* mutations inactivate the activity of RF2. Therefore, it is necessary to define the actual defect(s) in the catalytic activity of the mutant RF2 proteins in vitro and to determine whether or not these mutations cause frameshifting or stalling of ribosomes.

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