Function of the SmpB Tail in Transfer-messenger RNA Translation Revealed by a Nucleus-encoded Form*

Received for publication, August 12, 2004, and in revised form, December 9, 2004
Published, JBC Papers in Press, December 13, 2004, DOI 10.1074/jbc.M409277200

Yannick Jacob, Stephen M. Sharkady, Kanchan Bhardwaj‡, Alina Sanda, and Kelly P. Williams§
From the Department of Biology, Indiana University, Bloomington, Indiana 47405

Stalled bacterial ribosomes are freed when they switch to the translation of transfer-messenger RNA (tmRNA). This process requires the tmRNA-binding and ribosome-binding cofactor SmpB, a β-barrel protein with a protruding C-terminal tail of unresolved structure. Some plastid genomes encode tmRNA, but smpB genes have only been reported from bacteria. Here we identify smpB in the nuclear genomes of both a diatom and a red alga encoding a signal for import into the plastid, where mature SmpB could activate tmRNA. Diatom SmpB was active for tmRNA translation with bacterial components in vivo and in vitro, although less so than Escherichia coli SmpB. The tail-truncated diatom SmpB, the hypothetical product of a misspliced mRNA, was inactive in vivo. Tail-truncated E. coli SmpB was likewise inactive for tmRNA translation but was still able to bind ribosomes, and its affinity for tmRNA was only slightly diminished. This work suggests that SmpB is a universal cofactor of tmRNA. It also reveals a tail-dependent role for SmpB in tmRNA translation that supersedes a simple role of linking tmRNA to the ribosome, which the SmpB body alone could provide.

Translating ribosomes can stall, for example, when they arrive at the 3′-end of an mRNA lacking an in-frame stop codon (nonstop mRNA) (1). Stalling could sequester ribosomes and produce incomplete polypeptides. In bacteria these problems are ameliorated by tmRNA, a specialized RNA with both tRNA-like and mRNA-like properties. The stalled ribosome can switch mRNA templates, leaving nonstop mRNA and resuming translation on the reading frame in tmRNA, where the ribosome is freed upon release at the tmRNA stop codon. The protein encoded by the nonstop mRNA gains a hydrophobic peptide tag encoded by tmRNA that is a signal directing the proteolysis of the entire tagged protein.

In model bacteria, the protein SmpB is a requisite cofactor of tmRNA (2, 3). SmpB binds to the tRNA portion of tmRNA in the elbow region on the D-loop face (4), retaining the β-barrel structure found in the free protein (5–7). A C-terminal tail of ~30 amino acids, emerging from the β-barrel opposite to the tmRNA-binding face, appears unstructured in both free and tmRNA-bound SmpB.

SmpB has been found to improve aminoacylation of tmRNA and to allow simultaneous tmRNA binding by EF-Tu-GTP (4, 8). SmpB is further required for interaction of tmRNA with the ribosome (9, 10). It can bind the ribosome directly in the absence of tmRNA, and its position in the complex with the tRNA domain of tmRNA suggests that its tail might contact the ribosome in the vicinity of the decoding center (7, 11).

Both ssrA (tmRNA) and smpB genes have been found in all fully sequenced bacterial genomes (12). ssrA has also been identified in certain whole plastid genomes (12, 13) producing the expected RNA (14), but smpB has not been identified in these same plastid genomes, raising the possibility that tmRNA in plastids might function without its usual cofactor.

In this article we show that for two eukaryotes with a plastidial ssrA gene, the diatom Thalassiosira pseudonana and the red alga Cyanidioschyzon merolae, SmpB is encoded in the nucleus with an apparent signal for import into the plastid. The predicted mature T. pseudonana SmpB is active in tmRNA translation with Escherichia coli components in vivo and in vitro. Its lower activity relative to that of E. coli SmpB is ascribed, through the study of chimeric proteins, to its β-barrel domain and not to its C-terminal tail. The latter result does not mean that the tail has no function; indeed, with deletion of just half the tail tmRNA translation was undetectable. The affinity of SmpB for either ribosomes or tmRNA was not significantly affected by tail truncation, indicating that the role of SmpB in tmRNA translation is more complex than providing a simple link between tmRNA and the ribosome.

EXPERIMENTAL PROCEDURES

Construction of the T. pseudonana smpB Gene—A coding sequence was designed for T. pseudonana pre-SmpB residues 52–197 (protein TT0 of Fig. 1; see also Fig. 2), with the codon bias approximating as closely as possible that of E. coli SmpB, and flanked by start and stop codons and NsiI and BamHI restriction sites. The gene was constructed from 16 ~45-mer oligonucleotides sharing ~15-nucleotide sequence overlaps with neighbors by mutually extending eight oligonucleotide pairs hybridized at their 3′-ends with TaqDNA polymerase, followed by three rounds of the following: 1) amplification of one strand of each DNA with TaqDNA polymerase; 2) hybridization at the 3′-end to a neighboring partner; 3) mutual extension with TaqDNA polymerase; and 4) amplification of the product by PCR. The final product was subsequently cloned into the pCR2.1 Topo vector (Invitrogen) and sequenced. The remaining 3′-codons for the tail of the mature SmpB were added with designed oligonucleotides using PCR as above.

SmpB Expression Plasmids—A vector was prepared for an in vivo SmpB assay by amplifying the E. coli smpB promoter and the 5′-untranslated region from genomic DNA, adding an NdeI restriction site overlapping the start codon and a BamHI site further downstream during the PCR, and ligating into the low copy plasmid pBBR-MCS2 (15) at its NsiI and KpnI restriction sites. The E. coli smpB coding region amplified from genomic DNA and the T. pseudonana construct of the previous section were used as starting points to prepare, by PCR methods, various chimeric and truncated smpB genes (Fig. 1), which

* This study was supported by grants from Le Fond Québécois de la Recherche sur la Nature et les Technologies (to Y. J.) and the National Institutes of Health (to K. P. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843-2128.
§ To whom correspondence should be addressed: Dept. of Biology, Indiana University, 1001 E. 3rd St., Bloomington, IN 47405. Tel.: 812-856-5697; Fax: 812-856-6705; E-mail: kelliwill@indiana.edu.

1 The abbreviations used are: tmRNA, transfer-messenger RNA; EF, elongation factor; MES, 4-morpholinoethanesulfonic acid.
prepared from E. coli strains KW1063 and KW2073, the products of transducing either the ssrA-cat or ΔsmpB-ssrA::kan alleles into the strain CAN20–12E (20) deficient in the RNases I, II, D, and BN. SmpB was detected in the preparation from the ssrA-cat strain by quantitative Western analysis at a molar ratio (SmpB/ribosome) of 0.005 ± 0.002. All additional factors were purified as six-histidine fusions of E. coli proteins (21).

Reactions were in 100 μl of translation buffer (95 mm potassium glutamate, 9 mm magnesium acetate, 8 mm putrescine, 5 mm NH₄Cl, 5 mm KPO₄, pH 7.3, 1 mm spermidine, 1 mm diithiothreitol, and 0.5 mm CaCl₂) containing 3.2 μl of storage buffer, 30 pmol of ribosomes, 20 pmol of tmRNA, 0.6 μg of initiation factor (IF) 1, 1.2 μg of IF2, 0.45 μg of IF3, 30 pmol of EF-G, 60 pmol of EF-Tu, 30 pmol of EF-Ts, 0.5 μg of release factor (RF) 1, 0.5 μg of RF3, 0.3 μg of ribosome recycling factor (RRF), 120 μg of creatine kinase (Sigma), 90 ng of myokinase (Sigma), 32 μg of nucleotide diphosphate kinase (Sigma), 2.1 μg of alanyl-tRNA synthetase, 2.5 μg of phenylalanyl-tRNA synthetase, 0.6 μg of asparaginyl-tRNA synthetase, 0.24 μg of aspartyl-tRNA synthetase, 0.39 μg of glutamyl-tRNA synthetase, 0.012 μg of tyrosyl-tRNA synthetase, 0.12 μg of leucyl-tRNA synthetase, 2.5 A₂₆₀ units of total E. coli tRNA (Roche Applied Science), 1 μM ATP, 1 μM GTP, 0.1 μM [³²P]C alanine (Amer sham Biosciences), and 0.1 μM (each) phenylalanine, asparagine, aspartate, tyrosine, and leucine (21). Reactions additionally contained E. coli or T. pseudonana SmpB as specified. Reaction mixtures were incubated for 5 min at 37 °C prior to initiation by the addition of 50 μg poly(U), incubated for 60 min at 37 °C, terminated with 2 ml of 5% trichloroacetic acid, heated to 20 min at 90 °C, and incubated for 30 min on ice; alanine incorporation was then measured by filtering through glass fiber disks (Whatman) presoaked in 5% trichloroacetic acid, washing with 6 ml of 5% trichloroacetic acid and then 2 ml of ethanol, drying, and scintillation counting.

tmRNA Binding Assay—tmRNA was radiolabeled by randomly incorporating [³²P]PCMP during in vitro transcription and purified and folded as described above in binding buffer (0.2 mM KCl, 50 mM MES-KOH, pH 6.5, 5 mM MgCl₂, and 0.5% Nonidet P-40). Reactions in 20 μl of binding buffer containing 25 μt pm RNA (~10000 cpn), 2 μl of SmpB storage buffer, 5 μl of mercaptoethanol, 0.1 mg/ml bovine serum albumin, and varying amounts of SmpB were incubated for 30 min at 22 °C and then filtered through 0.45-μm nitrocellulose disks prewet with binding buffer. Filters were washed three times with 2 ml of binding buffer and air-dried, and the fraction of tmRNA retained was measured by scintillation counting. Data were fit to the formula

\[
K_d = \frac{F}{S},
\]

where \(K_d\) is the dissociation constant for \(F\), and \(S\) is the concentration of SmpB, \(F\) is the fraction of tmRNA that binds tightly, \(K_s\) is the dissociation constant for \(F\), and \(K_d\) is the apparent dissociation constant for the remaining tmRNA. The three fitted values for \(F\) averaged 0.48 with a S.D. of ±0.03.

tmRNA Aminoacylation Assay—Reactions in 70 μl of translation buffer containing 50 μt pm [³⁵S]alanine, 1 μt pm tmRNA, 2 μl of SmpB or various forms of E. coli SmpB (1 μt) were initiated by the addition of 300 μl alanyl-tRNA synthetase and incubated at 37 °C. At various time points 10 μl was removed, quenched in 2 ml of ice-cold 5% trichloroacetic acid, incubated on ice for 30 min; alanine incorporation was then measured as described above.

Ribosome Binding Assay—The ΔsmpB-ssrA E. coli strain described above was transfected with the low copy plasmid encoding either the 000, EEE, or EE0 (Fig. 1) form of SmpB, and grown to an A₆₀₀ of 0.41–0.42 in 800 ml of Luria-Bertani medium supplemented with kanamycin. Cells were pelleted, resuspended in 8 ml of lysis buffer (100 mm NH₄OAc, 25 mM Tris-HCl, pH 7.5, 10.5 mM MgOAc, 2 mM diithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) supplemented with 5 μg/ml DNase I, and lysed using a French press cell. Lysates were centrifuged by centrifugation for 45 min at 26,000 rpm in a Beckman TLA-100.4 rotor, and then ribosomes were pelleted by centrifugation for 90 min at 50,000 rpm in the TLA-100.4 rotor and resuspended in 0.4 ml of lysis buffer. The pellet fraction was subjected to centrifugation in an 11-ml 10–30% linear sucrose gradient in lysis buffer for 90 min at 35,000 rpm in a Beckman SW41 rotor. Gradient fractions (0.5 ml) were concentrated 10-fold and washed with lysis buffer using Microcon YM-10 centrifugal filter devices (Millipore). Samples were subjected to electrophoresis in a protein gel (14% polyacrylamide) followed by semi-dry electroblotting onto a nitrocellulose sheet. The blot was developed using a rabbit antibody raised against wild-type E. coli SmpB and horseradish peroxidase-coupled goat anti-rabbit antibody.
RESULTS

Nuclear smpB in Two Eukaryotes—Both plastidial ssrA and nuclear smpB genes were identified in highly redundant shotgun sequencing data for the diatom *T. pseudonana* (www.jgi.doe.gov) by BLAST searching.

The plastidial location of ssrA is indicated by the match that it and its flanking sequence make to a previously determined plastid sequence from other diatoms. tmRNA is encoded in 341 bp (scaffold 118, 10569–10229) that can be aligned with a few short gaps to the *Thalassiosira weissflogii* plastidial tmRNA sequence (22) with 85% identity. The flanking sequence continues to match the entire 1.8-kbp ssrA-containing sequence from the *T. weissflogii* plastid (GenBank™ accession AF049491) with 82% identity, and the whole 68-kpb scaffold containing the plastid genome of the diatom *Odontella sinensis* (GenBank™ accession Z77753), albeit with re-arrangements. The tmRNA encoded in the *T. pseudonana* plastid would appear to be functional because it contains the key tmRNA features, namely a tRNA-like structure involving the RNA termini and a reading frame encoding a hydrophobic peptide tag.

The nuclear location of *smpB* is indicated by the two typical *Thalassiosira* nuclear introns it contains (Fig. 2)(23). This first example of eukaryotic SmpB shows remarkable similarity to bacterial SmpB, in particular to cyanobacterial *SmpB* (Fig. 3). Like introns identified in *T. weissflogii* nuclear genes (23), the *smpB* introns are short with strong matches to consensus features at the splice sites and branch region. The assigned start codon is the first in its reading frame; the only other in-frame AUG codon in the first exon is absent from one of the two *T. pseudonana* *smpB* alleles (see below). SmpB is much better conserved from bacteria to plastids than is tmRNA. The best BLAST hit at GenBank™ was to *SmpB* from the cyanobacterium *Trichodesmium erythreum* (Fig. 3), in keeping with the cyanobacterial origin of plastids. Identity is 48% over the length of the *T. erythreum* SmpB.

The algal protein is predicted to contain an N-terminal signal peptide for its import into the simple plastid (Fig. 3). Like all but 27 of *C. merolae* nuclear genes, *smpB* has no introns.

*smpB* genes found in other eukaryotic genome projects are best ascribed to bacterial contamination because they are un-

---

**Fig. 2.** *T. pseudonana smpB* allele 2. The nucleotide sequence is indexed at the left, and the protein sequence is indexed at the right with coding and predicted mature sequences in *uppercase*. The stop codon for the adjacent expressed gene is also in *uppercase*. Matches to consensus splice and branch sequences for U2/U6-spicessen (17) are double-underlined, and positions that differ in allele 1 (see Table 1) are double-underlined. Nucleotide sequence data are nucleotides 171710–172548 of scaffold 30 from the United States Department of Energy Joint Genome Institute site (www.jgi.doe.gov).
Fig. 3. SmpB alignment. Pre-SmpBs of T. pseudonana (Tps) and C. merolae (Cme) (nucleotides 295705–296337 of chromosome 15, fragment 5 at the C. merolae Genome project (merolae.biool.s.u-tokyo.ac.jp)) are aligned along their mature sequences (uppercase) with bacterial SmpBs. Aligned residues identical to those of Tps are shaded, α-Helices (A), β-strands (B), and the limits of structural data (pipes) are those assigned by Protein Data Bank for Aquifex aeolicus SmpB in crystal complex with tmRNA chains A (XI-A) and C (XI-C) from Protein Data Bank file 1P6V) and the NMR-based structural models of A. aeolicus (NMR-Aae) and Thermus thermophilus (NMR-Thh) SmpB (5–7); predictions by Jpred (33) and PHD for E. coli SmpB are also shown. The cleavage sites for the signal sequence and the transit peptide predicted by TargetP (34) are marked, as are the positions of the introns in the gene. The Aap-190 residue of E. coli SmpB, altered to Asn in protein TEE*, is underlined. Bacterial SmpB sources and their GenBank accession numbers are as follows: Synechococcus elongatus, Q8HMH0 (Sul); Trichodesmium erythreum, ZP_00072026 (Ter); Fibrobacter succinogenes, (www.tigr.org) (Fsu); E. coli, P32052 (Eco); Bacillus subtilis, CAB15365 (Bsu); Ureaplasma urealyticum, Q8DHM0 (Uur); Mycoplasma fermentans, AAD25739 (Mfe); and A. aeolicus, O66640 (Aae).

Table I  
Two smpB alleles present in the sequenced diatom

| No. shotgun clones impinging on smpB | Variant smpB gene position* |
|-------------------------------------|-----------------------------|
|                                     | No. of clones |
| Allele 1 (22 clones)                |               |
| 4                                   | C             |
| 1                                   | A             |
| 2                                   | G             |
| 3                                   |               |
| 9                                   |               |
| Allele 2 (18 clones)                |               |
| 2                                   | A             |
| 1                                   | A             |
| 7                                   | A             |
| 2                                   | A             |
| 1                                   |               |
| 6                                   |               |
| Recombinant                         | A             |
| 1                                   | G             |
| 1                                   |               |
| Undetermined                        |               |
| 6                                   |               |

Linkage to next variant position  
| Nonrecombinant-1 | 3 | 6 | 8 | 6 |
| Nonrecombinant-2 | 7 | 9 | 3 |
| Recombinant      | 0 | 1 | 0 |

* Dash (—) represents a position not reached by sequence data for clone interrupted by introns, in contigs with other bacterial affiliations, and encode proteins that are especially similar to known bacterial SmpBs. Sequences obtained from the nematode Brugia malayi, known to contain a Wolbachia endosymbiont, encode an SmpB that matches that from the Wolbachia endosymbiont of Drosophila melanogaster with 90% identity. One rice genome project (for Oryza sativa ssp. indica) contains at least four smpB sequences encoding proteins with identities of 76% to Sphingomonas elodea SmpB, 53% to Bacteroides thetaiotaomicron SmpB, 74% to Burkholderia fungorum SmpB, and 75% to Rubrivivax gelatinosus SmpB; such bacterial sequence contamination was identified and purposefully removed from another rice genome project. A segment containing adjacent smpB and ssrA genes, affiliated with Gram-positive bacteria, is found in a contig from the Trypanosoma cruzi genome project and in sequence data from the other kinetoplastids Trypanosoma brucei or Leishmania major.

Diatom SmpB Promotes tmRNA Translation in E. coli—The extensive sequence similarity that eukaryotic SmpB shares with bacterial SmpB, together with its predicted import into plastids that encode tmRNA, strongly suggests that the role of SmpB in plastids is equivalent to its only known role in bacteria, in tmRNA translation. The homology further suggests that the plastid protein might function in bacteria as has been demonstrated for other plastid proteins (27, 28). Very high sequence similarity between the tRNA domains of the E. coli and T. pseudonana tmRNAs, with base identity at all the positions where contacts were observed between SmpB and the tRNA domain (7), encouraged a test for activity of T. pseudonana SmpB in E. coli cells.

An in vivo SmpB assay was developed from an earlier tmRNA assay (16, 17). An E. coli strain with a chromosomal deletion encompassing the neighboring smpB and ssrA genes was transformed with three plasmids; the first plasmid allowed degradation of tmRNAs, with base identity at all the positions where contacts were observed between SmpB and the tRNA domain (7), encouraged a test for activity of T. pseudonana SmpB in E. coli cells.
increased in this assay even though the same promoter as the chromosomal gene was used, perhaps due to a mildly elevated gene copy number on a low copy plasmid.

To assay the predicted mature diatom SmpB in this system, its gene was constructed de novo. The codon bias was matched as closely as possible to E. coli smpB to keep synthesis rates equal. The T. pseudonana SmpB strongly promoted tmRNA translation in E. coli, albeit with some reduction of activity relative to E. coli SmpB (Fig. 4; compare lanes TTT and EEE).

**Diatom SmpB Promotes tmRNA Translation in an In Vitro E. coli System**—SmpB activity was assayed in a recently described in vitro system (9) adapted from an earlier tmRNA assay (29). Ribosomes from ΔsmpB-ssrA E. coli were programmed with poly(U) in the presence of radiolabeled alanine, bulk E. coli tRNA, enzymatically synthesized tmRNA, elongation factors, all required aminocyl-tRNA synthetases, and the test SmpB. Poly(U) serves as a nonstop mRNA that should not promote the incorporation of alanine into polypeptide when SmpB is omitted but, nonetheless, produces a high background. With each round of tmRNA translation, five equivalents of alanine are incorporated.

Preliminary titrations showed that alanine incorporation reached a plateau at 20 μM E. coli SmpB or at 90 μM diatom SmpB (data not shown). Plateau activity was lower with diatom SmpB and yet was distinct from the background of the assay (Table II). The same result was obtained with ribosomes that were purified from ΔsmpB and yet was distinct from the background of the assay for 000, TT0, or EE0.

**SmpB Tail Function**

**SmpB Tail Is Not Required for tmRNA Binding or Enhancement of Aminoacylation**—The reduced activity of T. pseudonana SmpB and inactivity of tail-truncated SmpB in tmRNA translation could be due to lower affinity for E. coli tmRNA. This possibility was tested using a filter-binding assay to measure the affinity of purified SmpB for tmRNA (Fig. 5A). Approximately half of the tmRNA reproducibly showed high affinity binding to E. coli SmpB, and the other half was trapped only at higher SmpB concentrations, which could be explained by low affinity SmpB binding to a substantial fraction of improperly folded tmRNAs. A substantial misfolded fraction of tmRNA is not surprising given that, in our hands and for others (8), alanylation yields for tmRNA are only half of those for similarly prepared tRNA\(^{\text{Ala}}\). The lower affinity binding phase can be explained by the observation that SmpB has an affinity of 20 μM for simple double-stranded RNA (30). Fitting the data for the high affinity phase with the assumption of 1:1 binding stoichiometry yielded a dissociation constant of 0.34 ± 0.18 nM for full-length E. coli SmpB. This \(K_d\) value is much lower than those reported previously (ranging from 20 to 400 nM) from gel retardation and indirect assays that usually have omitted magnesium ions from the binding buffer (2, 4, 30). The \(K_d\) was increased ~2-fold for partially or fully tail-truncated E. coli SmpB, showing that the tail contributes little to tmRNA binding and further suggesting that the β-barrel portions of the truncated proteins were well folded despite their complete inactivity in tmRNA translation in vivo.

Functional binding of tail-truncated SmpB to tmRNA was confirmed by an aminocacylation assay. It has been shown that, although tmRNA alone is a substrate for alanyl-tRNA synthetase, one equivalent of SmpB increases the rate of alanyla-

**SmpB Tail Is Not Required for Stability or for tmRNA-independent Ribosome Binding**—SmpB has recently been shown to bind to ribosomes even in the absence of tRNA, and it has been suggested that tmRNA translation depends on this activity (11). Thus, the inability of the tail-truncated SmpB to promote tmRNA translation might be explained if such ribo-
some binding activity were reduced. We investigated this possibility by fractionating lysates of ΔsmpB-ssrA E. coli expressing either no, wild-type, or truncated SmpB (000, EEE, or EEO of Fig. 1) from the native smpB promoter on a low copy plasmid. Both SmpB forms were found in the pellet with ribosomes after high speed centrifugation and further co-purified with ribosomes in sucrose density gradient centrifugation (Fig. 6). Thus, it does not appear that the defect of truncated SmpB in tmRNA translation is explained by the failure of tmRNA-independent ribosome binding.

Fig. 6 also shows that the tail-truncated SmpB accumulates to approximately the same level as full-length SmpB. Thus, the effect of the tail on tmRNA translation is not due to an effect on SmpB stability.

**DISCUSSION**

This work shows that eukaryotes with a plastidial tmRNA gene also have the smpB gene. Eukaryotic SmpB is encoded in the nucleus, with a peptide presequence expected both to promote import into the plastid and to be removed during the process. The expected mature eukaryotic SmpB promotes tmRNA translation in bacterial *in vivo* and *in vitro* systems. These results suggest not only that plastid tmRNA has the same protein cofactor requirement as in bacteria but also that the tmRNA-SmpB complex has the same function in plastids as in bacteria.

The implication that SmpB is a universal cofactor of tmRNA encourages a search for smpB in the nuclear genome of jakobids, whose mitochondria have no recognizable smpB but do produce a truncated tmRNA homolog that retains features important for SmpB binding (1, 31). Among completed eukaryotic genomes, excluding apparent contamination from bacteria, either the ssrA and smpB genes are both found, or neither is found. An apparent lack of smpB could result from a sufficient divergence such that its sequence is no longer recognizable, but this possibility has become less tenable with the finding that two eukaryotes have a readily recognizable smpB. Thus, the correlation of ssrA and smpB genes appears to be genuine, which is in line with the idea that SmpB has little function beyond its role as a tmRNA cofactor.

The SmpB tail emerges from the β-barrel opposite to the tmRNA-binding face (7), rationalizing our observation that it contributes little either to tmRNA binding or to aminoacylation by the class II alanyl-tRNA synthetase (Fig. 5). Neither does the tail contribute significantly to the stability of SmpB *in vivo* (Fig. 6). We therefore look to a role for the tail within the ribosome during tmRNA translation. One specific suggestion has been that the SmpB tail promotes peptidyl transfer to tmRNA by stimulating the decoding center in the small ribosomal subunit as codon-anticodon pairing does for tRNA (7, 32). Removal of only 15 residues from its C terminus is sufficient to completely block tmRNA translation *in vivo*. Conserved sequence features of the tail suggest that it would form an α-helix (Fig. 3) that would present several positively charged residues on one face, yet the region appears unstructured in solution and in the crystal complex with the tRNA domain of tmRNA (5–7). We propose that the tail does adopt a helical structure during tmRNA translation. The positive face of this helix could be stabilized by a negative charge in ribosomal RNA. It is also possible that even though the SmpB tail does not appear to interact with tmRNA outside of the ribosome, it does so inside the ribosome. It will be of great interest to elucidate the course that SmpB takes in the ribosome during tmRNA translation and the encounters that it makes, perhaps by derivitization with Fe(II) for directed hydroxyl radical probing.

**Acknowledgments**—We thank Meredith Livingston and Keith Obye for technical assistance.

**REFERENCES**

1. Keiler, K. C., Waller, P. R., and Sauer, R. T. (1996) *Science* **271**, 999–993
2. Karzai, A. W., Susskind, M. M., and Sauer, R. T. (1999) *EMBO J.* **18**, 3793–3799
3. Wiegert, T., and Schumann, W. (2001) *J. Bacteriol.* **183**, 3885–3889
4. Barends, S., Karzai, A. W., Sauer, R. T., Wower, J., and Kraal, B. (2001) *J. Mol. Biol.*
5. Dong, G., Nowakowski, J., and Hoffman, D. W. (2002) EMBO J. 21, 1845–1854
6. Someya, T., Nameki, N., Hosoi, H., Suzuki, S., Hatanaka, H., Fuji, M., Terada, T., Shirouzu, M., Inoue, Y., Shibata, T., Kuramitsu, S., Yokoyama, S., and Kawai, G. (2003) FEBS Lett. 535, 94–100
7. Gutmann, S., Haebel, P. W., Metzinger, L., Sutter, M., Felden, B., and Ban, N. (2003) Nature 424, 699–703
8. Barends, S., Wower, J., and Kraal, B. (2002) Biochemistry 314, 9–21
9. Shimizu, Y., and Ueda, T. (2002) FEBS Lett. 514, 74–77
10. Hanawa-Suetsugu, K., Takagi, M., Inokuchi, H., Himeno, H., and Muto, A. (2002) Nucleic Acids Res. 30, 1620–1629
11. Hallier, M., Ivanova, N., Rametti, A., Pavlov, M., Ehrenberg, M., and Felden, B. (2004) J. Biol. Chem. 279, 25978–25985
12. Williams, K. P. (2000) EMBO J. 19, 5423–5433
13. Williams, K. P., and Bartel, D. P. (1998) Nucleic Acids Res. 26, 163–165
14. Gimple, O., and Schon, A. (2001) Biol. Chem. 382, 1421–1429
15. Kovach, M. E., Elzer, P. H., Hill, D. S., Robertson, G. T., Farris, M. A., Roop, R. M., II, and Peterson, K. M. (1995) Gene 166, 175–176
16. Roche, E. D., and Sauer, R. T. (1999) EMBO J. 18, 4579–4589
17. Williams, K. P., Martindale, K. A., and Bartel, D. P. (1999) EMBO J. 18, 5423–5433
18. Datsenko, K. A., and Wanner, B. L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6640–6645
19. Kao, C., Rudisser, S., and Zheng, M. (2001) Methods 23, 201–205
20. Reuven, N. B., and Deutscher, M. P. (1993) FASEB J. 7, 143–148
21. Shimizu, Y., Inoue, A., Tomi, Y., Suzuki, T., Yokogawa, T., Nishikawa, K., and Ueda, T. (2001) Nat. Biotechnol. 19, 754–755
22. Gueneau, P., Loiseux-de Goer, S., and Williams, K. P. (1999) Eur. J. Physiol. 34, 533–535
23. Armbrust, E. V. (2000) J. Physiol. 36, 942–946
24. Apte, K. E., Zaslavkaia, L., Lippneier, J. C., Lang, M., Kilian, O., Wetherbee, R., Grossman, A. R., and Kroth, P. G. (2002) J. Cell Sci. 115, 4061–4069
25. Matsuzaki, M., Muto, A., Shin, I. T., Maruyama, S., Takahara, M., Miyagishima, S. Y., Mori, T., Nishida, K., Yagisawa, F., Yoshida, Y., Nishimura, Y., Nukao, S., Kohayashi, T., Momoyama, Y., Higashi, T., Minoda, A., Sano, M., Nomoto, H., Oshii, K., Hayashi, H., Ohta, F., Nishizaka, S., Haga, S., Miura, S., Morishita, T., Kabe, Y., Terasawa, K., Suzuki, Y., Ishii, Y., Asakawa, S., Takano, H., Ohta, N., Kuriwa, H., Tanaka, K., Shimizu, N., Sugano, S., Sato, N., Nozaki, H., Ogasawara, N., Kohara, Y., and Kuriwa, T. (2004) Nature 428, 653–657
26. Gueneau de Novoa, P., and Williams, K. P. (2004) Nucleic Acids Res. 32, D104–D109
27. Jiang, F., Yi, L., Moore, M., Chen, M., Rohl, T., Van Wijk, K. J., De Gier, J. W., Henry, R., and Dalbey, R. E. (2002) J. Biol. Chem. 277, 19281–19288
28. Wall, M. K., Mitchenall, L. A., and Maxwell, A. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7821–7826
29. Himeno, H., Sato, M., Takagi, M., Fukushima, M., Ushida, C., and Muto, A. (1997) J. Mol. Biol. 268, 803–808
30. Wower, J., Zwieb, C. W., Hoffman, D. W., and Wower, I. K. (2002) Biochemistry 41, 8826–8836
31. Jacob, Y., Seif, E., Paquet, P. O., and Lang, B. F. (2004) RNA (N. Y.) 10, 605–614
32. Haebel, P. W., Gutmann, S., and Ban, N. (2004) Curr. Opin. Struct. Biol. 14, 58–65
33. Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M., and Barton, G. J. (1998) Bioinformatics 14, 892–893
34. Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000) J. Mol. Biol. 306, 1005–1016
