tRNA-mediated transcription antitermination *in vitro*: Codon–anticodon pairing independent of the ribosome

Frank J. Grundy, Wade C. Winkler*, and Tina M. Henkin†

Department of Microbiology, Ohio State University, Columbus, OH 43210

Communicated by Charles Yanofsky, Stanford University, Stanford, CA, June 19, 2002 (received for review May 23, 2002)

Uncharged tRNA acts as the effector for transcription antitermination of genes in the T box family in *Bacillus subtilis* and other Gram-positive bacteria. Genetic studies suggested that expression of these genes is induced by stabilization of an antiterminator element in the leader RNA of each target gene by the cognate uncharged tRNA. The specificity of the tRNA response is dependent on a single codon in the leader, which was postulated to pair with the anticodon of the corresponding tRNA. It was not known whether the leader RNA–tRNA interaction requires additional factors. We show here that tRNA-dependent antitermination occurs *in vitro* in a purified transcription system, in the absence of ribosomes or accessory factors, demonstrating that the RNA–RNA interaction is sufficient to control gene expression by antitermination. The tRNA response exhibits similar specificity *in vivo* and *in vitro*, and the antitermination reaction *in vitro* is independent of NusA and functions with either *B. subtilis* or *Escherichia coli* RNA polymerase.

The T box transcription termination control system is widely used in Gram-positive bacteria to regulate expression of aminoacyl-tRNA synthetase, amino acid biosynthesis, and transporter genes (1). The transcripts of genes regulated by this mechanism contain a 200- to 300-nt untranslated leader that includes a factor-independent (intrinsic) transcription termination signal and a competing antiterminator structure (2). Read-through of the leader region terminator occurs when the charged to uncharged ratio of the corresponding tRNA isoacceptor is decreased, signaling a deficiency in that aminoacyl-tRNA synthetase or amino acid. Sequence comparisons and mutational studies identified a single codon, displayed at a precise position within the leader RNA structure, that determines the specificity of the amino acid response, presumably by pairing with the anticodon of the cognate tRNA (2). The acceptor end of uncharged tRNA apparently makes a second interaction with a bulged region in the antiterminator, based on genetic analyses as well as gel-shift assays using a model 39-nt antiterminator RNA (3, 4); this interaction is proposed to stabilize the antiterminator, preventing the formation of a competing terminator helix (Fig. 1). Mutational studies of the *Bacillus subtilis* *tyrS* leader and tRNA^{Tyr} indicated that read-through requires additional conserved features of both RNAs (5–7).

Although it has been established that a tRNA is essential for antitermination, it was not known whether other factors are also required to mediate the tRNA–leader interaction. There was no evidence of translation of the leader RNA (2, 3, 8), but *in vivo* assays could not rule out the possibility that the tRNA–leader interaction occurs within the context of a ribosome. In this study, we demonstrate specific tRNA^{Gly}-dependent antitermination of the *Bacillus subtilis* *glyQS* leader in a purified *in vitro* transcription system.

Materials and Methods

Bacterial Strains and Growth Conditions. *B. subtilis* strain BR151MA (*lys-3 trpC2*) was used as the source of chromosomal DNA for amplification by PCR. Strains 1A5 (*glyB133 metC3 tre-12 trpC2*; *Bacillus* Genetic Stock Center) and KS115 (*cysA14*

Fig. 1. Proposed T box antitermination mechanism. The arrow indicates the transcription initiation site. The black rectangle represents the coding region of the regulated gene. Uncharged tRNA is postulated to interact with the nascent transcript at both the specifier sequence and the antiterminator bulge, stabilizing the antiterminator and preventing formation of the competing terminator. RNA polymerase (RNAP) then continues past the terminator region, and the full-length transcript is synthesized. "Factor?" indicates putative factor(s) that could modulate the leader RNA–tRNA interaction *in vivo*.

hisA1 leuA8 metC3 trpC2; K. Sandman, Ohio State Univ., Columbus) were used for amino acid limitation experiments for glycine and cysteine, respectively. Cells were propagated in 2× YT medium (9) or in Spizizen minimal medium (10) for measurements of *lacZ* fusion expression. Cells containing *lacZ* fusions were grown in the presence of chloramphenicol at 5 μg/ml.

In Vitro Transcription Assays. The template for *glyQS* transcription was a 440-bp PCR fragment that included sequences from 135 bp upstream of the *glyQS* transcription start site to position 305 of the transcript; the termination site is predicted to be around position 220 (ref. 11; Fig. 2). The template for *tyrS* transcription was a 420-bp PCR fragment including sequences from 85 bp upstream of the transcription start site to position 335 of the transcript; the termination site is predicted to be around position 270 (12). PCR products were purified by a Qiagen PCR cleanup kit. Template DNA (10 nM) was incubated in 1× transcription buffer (13) with His-tagged *B. subtilis* RNA polymerase (RNAP) (6 nM) purified as described by Qi and Hulett (14). Halted complex transcription assays were carried out essentially as described by Landick *et al.* (15). The dinucleotide ApU (150 μM, Sigma) was used to initiate *glyQS* transcription. ATP and GTP were added to 2.5 μM, UTP was added to 0.75 μM, and [α -³²P]UTP (800 Ci/mmol; 1 Ci = 37 GBq) was added to 0.25 μM. Transcription of *glyQS* was initiated in the absence of CTP;

Abbreviation: RNAP, RNA polymerase.

*Present address: Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520.

†To whom reprint requests should be addressed. E-mail: henkin.3@osu.edu.

Fig. 2. Secondary structure model of the *B. subtilis* *glyQS* leader RNA. Sequence is shown from the transcription start site (+1) through the end of the leader region terminator; the alternate antiterminator is shown above the terminator. The structure is based on the covariation model of T box family leaders (2, 5). Major conserved features are labeled, and conserved primary sequence elements are denoted with asterisks. The specifier sequence residues are boxed. The *glyQS* sequence was obtained from the *B. subtilis* genome sequence (11); DNA sequencing of this region revealed a substitution of A for U at position +6. The residues in brackets (113–122) are replaced by the stem II and IIA/B elements in most T box family leaders, including *B. subtilis* *tyrS*.

for the *glyQS* gene, the first C is at position +17 so that the transcription elongation complex halts after synthesis of 16 nt under these conditions. The dinucleotide ApG was used for initiation of *tyrS* transcription, and GTP was omitted from the initiation reaction, resulting in a halt at position +11. The initiation reaction mixtures were incubated at 37°C for 15 min and were then placed on ice. Heparin (20 μ g/ml, Sigma) was added to block reinitiation, and elongation was triggered by the addition of NTPs to 10 μ M final and other reagents as indicated. *B. subtilis* NusA protein (25 nM), purified as described previously (13), was included in the elongation reaction as indicated. *Escherichia coli* RNAP, prepared as described (16), was provided by I. Artsimovitch (Ohio State Univ.) and was used at 10 nM. Transcription reactions were terminated by extraction with phenol, and the products were resolved by denaturing 6% polyacrylamide gel electrophoresis and visualized by Phosphor-Imager analysis.

Unmodified *B. subtilis* tRNA^{Gly} and tRNA^{Tyr} were made by T7 transcription using a PCR fragment generated with a 5' oligonucleotide primer that included a T7 promoter sequence, positioned so that the first base of the transcript is the first position of the tRNA, and the final position of the PCR product corresponds to 3' position of the tRNA. T7 transcription was carried out by using an Ampliscribe T7 transcription kit (Epicentre Technologies, Madison, WI). The tRNA transcripts were purified on a 6% denaturing polyacrylamide gel, visualized by using UV shadowing, and eluted into 300 mM NaOAc, pH 4.5/1 mM EDTA. The tRNA was purified by extraction with phenol, precipitated with ethanol, and suspended in water. The resulting tRNA was refolded by incubation at 80°C for 2 min and slow cooling to room temperature before use in the transcription assays at 70 nM. Modified *E. coli* tRNA^{Tyr} was purchased from Sigma.

Mutations in the *glyQS* template DNA and tRNA^{Gly} were introduced by PCR, using oligonucleotide primers containing the desired alterations.

β -Galactosidase Measurements. The *glyQS* DNA fragment used for *in vitro* transcription was inserted into the *lacZ* fusion vector pFG328 (17) and integrated in single copy into the *B. subtilis* chromosome by recombination into a bacteriophage SP β prophage. Cells were grown in minimal medium containing all required amino acids at 50 μ g/ml until mid-exponential growth phase and were then collected and divided into two cultures, containing all required amino acids or with one amino acid at 5 μ g/ml. Growth was continued for 4 h, and cells were harvested and assayed for β -galactosidase activity, expressed as Miller units (9). Glycine starvation experiments were carried out in strain 1A5 (Gly[−]), and cysteine starvation experiments were carried out in strain KS115 (Cys[−]). All samples were assayed in duplicate, and growth experiments were carried out at least twice; variation was <10%.

Results

tRNA-Directed Antitermination *in Vitro*. Previous attempts to replicate tRNA^{Tyr}-directed antitermination of the *B. subtilis* *tyrS* leader *in vitro* by using multiround transcription assays under a variety of conditions with purified *B. subtilis* RNAP were unsuccessful (13). We observed efficient termination at the leader region terminator and no response to tRNA^{Tyr}. Luo *et al.* (18) similarly failed to demonstrate tRNA^{Thr}-directed antitermination of the *B. subtilis* *thrS* gene *in vitro*. To increase the range of experimental conditions that could be tested, we moved to halted complex assays (15) so that transcription initiation and elongation could be carried out separately under different sets of conditions. Because correct folding of the nascent transcript was likely to be essential to antitermination, we tested the *B. subtilis* *glyQS* leader, which (like all glycyl leaders) lacks the

Fig. 3. *In vitro* transcription of the *glyQS* and *tyrS* leader regions. Lanes 1–5, *glyQS* DNA; lanes 6–11, *tyrS* DNA. Lanes 1, 3, and 6, no tRNA added; lanes 2, 4, and 7, tRNA^{Gly} (T7 transcript); lanes 5, 8, and 11, tRNA^{Tyr} (T7 transcript); lane 9, *E. coli* tRNA^{Tyr} (modified, purchased from Sigma); lanes 3–9, NusA added; lanes 1, 2, 10, and 11, no NusA. T, terminated transcript; RT, read-through transcript. Percent read-through is indicated at the bottom of each lane.

complex stem II and stem IIA/B pseudoknot elements present in most other leaders, including *B. subtilis* *tyrS* (Fig. 2; unpublished results).

In the presence of 30 mM MgCl₂ and low NTP concentrations (10 μ M) during the elongation reaction, the *glyQS* leader region terminator was highly efficient (Fig. 3). Addition of a T7 RNAP-generated transcript of *B. subtilis* tRNA^{Gly} dramatically increased read-through from 5% (lane 1 or 3) to approximately 60% (lane 2 or 4), with a corresponding decrease in the amount of the terminated transcript. No tRNA-dependent read-through was observed at lower MgCl₂ concentrations or at higher NTP concentrations (data not shown). Mg²⁺ plays a crucial role in RNA folding reactions (19) and may facilitate folding of the nascent transcript into the correct conformation for interaction with the tRNA and antitermination. The rate of transcription elongation is decreased at low NTP concentrations (20), which may favor formation of the correct RNA structure or facilitate pausing by RNAP.

Antitermination of the *glyQS* leader responded specifically to tRNA^{Gly}; addition of tRNA^{Tyr} had no effect (Fig. 3, lane 5). Neither tRNA^{Gly} nor tRNA^{Tyr} addition increased read-through of the *B. subtilis* *tyrS* leader region terminator (Fig. 3, lanes 6–11). Therefore, either the *tyrS* and *glyQS* leaders have different requirements for the tRNA–leader interaction or the *tyrS* leader fails to fold properly under the *in vitro* conditions used. The stem II and stem IIA/B pseudoknot elements present in *tyrS* but absent in *glyQS* may be responsible for this difference. Single nucleotide substitutions that disrupt conserved elements of the stem IIA/B region in the *tyrS* leader result in loss of read-through *in vivo*, indicating that this region is functionally required in the *tyrS* context (5). A variant of the *tyrS* leader in which the specifier sequence and antiterminator were changed to match the anticodon and acceptor end of tRNA^{Gly} has been constructed, and expression was shown to be induced *in vivo* in response to limitation for glycine (21). This leader variant failed to respond to tRNA^{Gly} *in vitro* (data not shown), indicating that the codon–anticodon interaction (GGC–GCC for *glyQS* vs. UAC–GUA for *tyrS*) is not sufficient to explain the lack of tRNA^{Tyr}-directed antitermination of the *tyrS* leader *in vitro*.

***glyQS* Antitermination *in Vitro* Is Independent of NusA and Functions with *E. coli* RNAP.** The NusA protein affects transcription elongation rates and sensitivity of RNAP to pause and termination sites, and participates in a number of transcription termination control systems, including phage λ N and Q antitermination (22). Addition of *B. subtilis* NusA to the *glyQS* antitermination reaction resulted in a small increase in termination in the absence of tRNA, especially at high NTP concentrations (ref. 13;

Fig. 4. Specificity of the *glyQS*–tRNA^{Gly} interaction. (A) Interaction of the *glyQS* leader (black) in the antiterminator conformation with tRNA^{Gly} (green). Substitutions at the specifier sequence and antiterminator regions of the leader, and at the anticodon and acceptor end of the tRNA, are shown with arrows. (B–E) *In vitro* transcription reactions using different combinations of variants of *glyQS* templates and tRNA^{Gly}. (B) Wild-type *glyQS* template DNA (GGC specifier sequence, A158 antiterminator). (C) *glyQS*–UGC template DNA (cysteine specifier sequence, A158 antiterminator). (D) *glyQS*–A158→U template DNA (GGC specifier, U158 antiterminator). (E) *glyQS*–UGC/A158→U template DNA (cysteine specifier sequence, U158 antiterminator). Lane 1, no tRNA; lane 2, wild-type tRNA^{Gly} (GCC anticodon, U73 discriminator); lane 3, tRNA^{Gly}–GCA (GCA anticodon, U73 discriminator); lane 4, tRNA^{Gly}–U73→A (GCC anticodon, A73 discriminator); lane 5, tRNA^{Gly}–GCA/U73→A (GCA anticodon, A73 discriminator). T, terminated transcript; RT, read-through transcript. Percent read-through is indicated at the bottom of each lane.

Table 1. Expression of *glyQS-lacZ* fusions *in vivo*

Fusion	Glycine starvation			Cysteine starvation		
	+Glycine	−Glycine	Ratio	+Cysteine	−Cysteine	Ratio
GGC-A158	42	130	3.1	34	29	0.85
UGC-A158	58	42	0.72	31	90	2.9
GGC-U158	24	11	0.45	ND	ND	ND
UGC-U158	20	6.0	0.30	14	9.1	0.65
ΔTerm	350	360	1.0	ND	ND	ND

Expression was measured by activity of β -galactosidase, in Miller units (9). ND, not determined; ΔTerm, terminator deleted.

data not shown). However, the tRNA^{Gly}-dependent increase in read-through occurred both in the presence and absence of NusA (compare Fig. 3, lanes 1 and 2 with lanes 3 and 4), indicating that NusA is not required for *glyQS* antitermination under these conditions.

The ability of *E. coli* RNAP to replace *B. subtilis* RNAP in the tRNA-dependent transcription antitermination assay was tested to determine whether this activity was sensitive to the source of RNAP. RNAP from *E. coli* and *B. subtilis* has been shown to exhibit different patterns of recognition of pausing and termination signals (ref. 23; unpublished results). *E. coli* RNAP exhibited tRNA^{Gly}-dependent read-through similar to that observed with *B. subtilis* RNAP (data not shown), indicating that the antitermination event is dependent on features of the transcript, but not on the enzyme that generates the transcript. Introduction of T box leaders, including *glyQS*, into *E. coli*, from which this antitermination system is absent, generally resulted in a high level of read-through of the leader region terminator independent of amino acid limitation (data not shown), suggesting that the leader region terminators function poorly in the heterologous host and that the proper leader–tRNA interaction does not occur.

Specificity Determinants for Antitermination *in Vitro* and *in Vivo*. The specificity of the tRNA effect was further tested by using *glyQS* leader variants and corresponding tRNA^{Gly} variants (Fig. 4A). The GGC glycine specifier sequence was changed to a UGC cysteine codon, and position A158 of the *glyQS* antiterminator bulge was changed to a U; this position is a secondary determinant of the specificity of the tRNA–leader interaction (3). The mutations were tested separately and in combination, both *in vitro* and *in vivo*. The wild-type *glyQS* template exhibited efficient antitermination *in vitro* only in combination with the corresponding wild-type tRNA^{Gly} (Fig. 4B, lane 2). The UGC specifier mutation in the *glyQS* leader resulted in decreased antitermination by wild-type tRNA^{Gly}; this effect was suppressed by a variant of tRNA^{Gly} with an anticodon complementary to UGC (Fig. 4C, lane 3). The A158→U substitution in the antiterminator also decreased antitermination directed by wild-type tRNA^{Gly} and was suppressed by a corresponding change in the tRNA discriminator base (Fig. 4D, lane 4). The specificity pattern also held for the UGC/A158→U double mutant, with the most efficient antitermination occurring in response to the matching tRNA (Fig. 4E, lane 5). The native GGC–GCC codon–anticodon pairing consistently resulted in more efficient antitermination than the UGC–GCA cysteinyl combination; this could be due to the extra G–C pair or to other features of the *glyQS* leader that are adapted to the native glycyI combination.

In agreement with the *in vitro* results, a wild-type *glyQS-lacZ* transcriptional fusion exhibited induction *in vivo* in response to

limitation for glycine, but it failed to respond to limitation for cysteine (Table 1). Replacement of the GGC glycine specifier sequence with a UGC cysteine codon resulted in loss of the response to glycine and induction in response to limitation for cysteine; both tRNA^{Gly} and tRNA^{Cys} contain a U at the discriminator position. The A158→U mutation, either alone or in combination with the UGC cysteine specifier sequence mutation, abolished the response to either glycine or cysteine, consistent with the loss of a match with the U discriminator position. The maximum expression observed under glycine limitation conditions was approximately one-third of that observed in a construct from which the terminator was deleted, indicating that full induction was not observed under these conditions.

Discussion

The ability of tRNA mutants to suppress *tyrS* leader mutations provided strong evidence for the role of uncharged tRNA as the effector for transcription antitermination in the T box system (2, 3). However, it was unknown whether the tRNA acted alone or in conjunction with trans-acting factors required to mediate the leader RNA–tRNA interaction. The demonstration of specific tRNA^{Gly}-directed antitermination in a purified *in vitro* transcription system provides clear evidence that the leader–tRNA interaction is sufficient, at least in the case of the *glyQS* leader.

Recognition of the tRNA by the leader RNA seems to mimic recognition by an aminoacyl-tRNA synthetase, which often exploits the anticodon and discriminator positions as specificity determinants (24). As is also true for tRNA charging, the leader RNA–tRNA interaction *in vivo* probably involves additional determinants (6, 21). Other systems in which uncharged tRNA is monitored, such as the yeast GCN2 system (25), require a protein component; tRNA mimics such as the *E. coli* *thrS* regulatory target site are also recognized by a protein (26). In translationally coupled transcription attenuation systems such as the *E. coli* *trp* operon, tRNA charging is monitored by a translating ribosome (27). The T box system is unique in that uncharged tRNA is recognized directly by an RNA, in the absence of protein factors or a ribosome. This may represent a more ancient regulatory mechanism, a vestige of the RNA world, and provides a further example of the ability of RNA to carry out complex interactions in the absence of protein cofactors. Peptidyltransferase activity is RNA-catalyzed (28), and codon–anticodon recognition requires specific interactions between residues in the decoding site of the ribosome and the mRNA and tRNA (29). The *glyQS* *in vitro* antitermination system exhibits codon–anticodon recognition mediated by RNA alone.

The demonstration of tRNA^{Gly}-dependent *glyQS* antitermination *in vitro* does not rule out the participation of proteins in the T box system *in vivo*, in particular for the more structurally complex leaders, like *tyrS*, which are the predominant class of leaders found in nature (unpublished results). The RNA component of RNase P similarly has catalytic activity *in vitro*, but it requires a protein component for function *in vivo* (30), and certain ribozymes use facilitator proteins to promote proper folding (31). The *in vitro* transcription conditions used in this study may have served to replace functions provided by other factors within a cell, but these factors are clearly not essential for the RNA–RNA interaction itself.

We thank F. M. Hulett for providing the strain for production of *B. subtilis* RNAP; I. Artsimovitch for providing *E. coli* RNAP and advice on halted complex transcription conditions; T. R. Moir for technical assistance; and M. Ibba, I. Artsimovitch, and J. R. Reeve for comments on the manuscript. This work was supported by National Institutes of Health Grant GM47823.

- Henkin, T. M. (2000) *Curr. Opin. Microbiol.* **3**, 149–153.
- Grundy, F. J. & Henkin, T. M. (1993) *Cell* **74**, 475–482.

- Grundy, F. J., Rollins, S. M. & Henkin, T. M. (1994) *J. Bacteriol.* **176**, 4518–4526.

4. Gerdeman, M. S., Henkin, T. M. & Hines, J. V. (2002) *Nucleic Acids Res.* **30**, 1065–1072.
5. Rollins, S. M., Grundy, F. J. & Henkin, T. M. (1997) *Mol. Microbiol.* **25**, 411–421.
6. Grundy, F. J., Collins, J. A., Rollins, S. M. & Henkin, T. M. (2000) *RNA* **6**, 1131–1141.
7. Winkler, W. C., Grundy, F. J., Murphy, B. A. & Henkin, T. M. (2001) *RNA* **7**, 1165–1172.
8. Grandoni, J. A., Fulmer, S. B., Brizzio, V., Zahler, S. A. & Calvo, J. M. (1993) *J. Bacteriol.* **175**, 7581–7593.
9. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
10. Anagnostopoulos, C. & Spizizen, J. (1961) *J. Bacteriol.* **81**, 741–746.
11. Kunst, F., Ogasawara, N., Moszer, I., Albertini, A. M., Alloni, G., Azevedo, V., Bertero, M. G., Bessieres, P., Bolotin, A., Borchert, S., Borriss, R., *et al.* (1997) *Nature (London)* **390**, 249–256.
12. Henkin, T. M., Glass, B. L. & Grundy, F. J. (1992) *J. Bacteriol.* **174**, 1299–1306.
13. Grundy, F. J., Moir, T. R., Haldeman, M. T. & Henkin, T. M. (2002) *Nucleic Acids Res.* **30**, 1646–1655.
14. Qi, Y. & Hulett, F. M. (1998) *Mol. Microbiol.* **28**, 1187–1197.
15. Landick, R., Wang, D. & Chan, C. L. (1996) *Methods Enzymol.* **274**, 334–353.
16. Hager, D. A., Jin, D. J. & Burgess, R. R. (1990) *Biochemistry* **29**, 7890–7894.
17. Grundy, F. J., Waters, D. A., Allen, S. H. G. & Henkin, T. M. (1993) *J. Bacteriol.* **175**, 7348–7355.
18. Luo, D., Condon, C., Grunberg-Manago, M. & Putzer, H. (1998) *Nucleic Acids Res.* **26**, 5379–5387.
19. Treiber, D. K. & Williamson, J. R. (2001) *Curr. Opin. Struct. Biol.* **11**, 309–314.
20. Rhodes, G. & Chamberlin, M. J. (1974) *J. Biol. Chem.* **249**, 6675–6683.
21. Grundy, F. J., Hodil, S. E., Rollins, S. M. & Henkin, T. M. (1997) *J. Bacteriol.* **179**, 2587–2594.
22. Friedman, D. I. & Court, D. L. (2001) *Curr. Opin. Microbiol.* **4**, 201–207.
23. Artsimovitch, I., Svetlov, V., Anthony, L., Burgess, R. R. & Landick, R. (2000) *J. Bacteriol.* **182**, 6027–6035.
24. Giege, R., Sissler, M. & Florentz, C. (1998) *Nucleic Acids Res.* **26**, 5017–5035.
25. Qiu, H., Dong, J., Hu, C., Francklyn, C. S. & Hinnebusch, A. G. (2001) *EMBO J.* **20**, 1425–1438.
26. Sankaranarayanan, R., Dock-Bregeon, A.-C., Romby, P., Caillet, J., Springer, M., Rees, B., Ehresmann, C., Ehresmann, B. & Moras, D. (1999) *Cell* **97**, 371–381.
27. Landick, R., Turnbough, C. L., Jr., & Yanofsky, C. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Curtis, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaecter, A. & Umberger, H. E. (Am. Soc. Microbiol., Washington, DC), pp. 1263–1286.
28. Ban, N., Nissen, P., Hansen, J., Moore, P. B. & Steitz, T. A. (2000) *Science* **289**, 905–920.
29. Ogle, J. M., Brodersen, D. E., Clemons, W. M., Jr., Tarry, M. J., Carter, A. P. & Ramakrishnan, V. (2001) *Science* **292**, 897–902.
30. Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N. & Altman, S. (1983) *Cell* **35**, 849–857.
31. Weeks, K. M. & Cech, T. R. (1995) *Cell* **82**, 221–230.