

GUEST COMMENTARY

The Ribosome: a Metabolite-Responsive Transcription Regulator[∇]

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Diverse mechanisms for transcription antitermination are widespread (24, 47). Recently, a new type, in which the ribosome and the TnaC leader peptide control Rho-dependent transcription termination upstream of the *tnaAB* operon, was characterized for *Escherichia coli* (Fig. 1) (1, 34, 49). Remarkably, the nascent leader peptide directs formation of a stereospecific tryptophan-binding site near the peptidyl transferase center. Bound tryptophan inhibits peptidyl transferase activity, preventing release factor cleavage of TnaC-peptidyl-tRNA^{Pro} (TnaC-tRNA^{Pro}). The stalled ribosome blocks the RNA entry site for Rho factor, allowing transcription readthrough. In this issue, Luis Rogelio Cruz-Vera and Charles Yanofsky show that conserved TnaC residues Asp-16 and Pro-24 are required for tryptophan inhibition of ribosome release (9). This paper exhibits the in vitro methods developed in the Yanofsky laboratory for studying nascent peptide control of ribosome function.

Those attending Stanford's graduate course "Gene Action" learned a four-step approach to studying regulation. First, understand the physiology by measuring gene expression during culture in different media and in response to metabolic shifts. Second, understand the genetics by characterizing mutants in which regulatory responses are altered. Third, understand the biochemistry by reconstituting regulation in vitro. Fourth, with accumulated information, materials, and assays in hand, return to in vivo studies to try to validate hypotheses. I think of these as "Yanofsky's Postulates." The study of *tnaAB* regulation exemplifies this approach.

Physiology. Tryptophanase (tryptophan-indole lyase), which catalyzes reversible hydrolysis of tryptophan to indole plus ammonium pyruvate (40), allows *E. coli* to use tryptophan as the sole carbon and nitrogen source, excreting indole. Indeed, tryptophan is one of the few amino acids efficiently catabolized from complex media (4, 38).

The *tnaAB* operon encodes tryptophanase (10) and a tryptophan permease essential for catabolism (39, 51). Expression is induced by extracellular tryptophan (51) and is subject to strong catabolite repression (2). This indicates that catabolism is the primary physiological function, although additional roles have been suggested (3, 41; reviewed in reference 49). *tnaAB* is the only operon whose expression is induced by tryptophan (29). Expression is not subject to general nitrogen regulation (15).

The 319-nucleotide (nt) transcribed leader region (*tnaL*)

encodes the 24-residue TnaC regulatory peptide and contains sites for Rho-dependent transcription termination (Fig. 1) (43, 44). Catabolite repression acts exclusively at the cyclic AMP-activated promoter (11, 18, 26), whereas tryptophan induction acts exclusively by transcription antitermination within the leader region (18, 44). Expression is induced efficiently by 1-methyl-tryptophan, which is not charged onto tRNA^{Trp} (51). Thus, tryptophanyl-tRNA^{Trp} is not the inducer.

Rho-dependent transcription termination. Rho factor catalyzes transcription termination, both in vitro and in vivo, at discrete sites within the 220-nt region between *tnaC* and *tnaA* (Fig. 1) (19, 43). A *boxA*-like element overlaps the carboxyl-terminus-coding region of the *tnaC* gene (44), and a *rut* (Rho utilization) element lies immediately downstream (16). *boxA*-like sequences are auxiliary elements for some Rho-dependent terminators (5).

Constitutive *tnaAB* expression results from substitutions and deletions altering the *boxA-rut* element, lesions in the *rho* structural gene, and treatment with the Rho inhibitor bicyclomycin (14, 16, 32, 44, 50). Similarly, *boxA-rut* alterations and bicyclomycin reduce Rho-dependent transcription termination in vitro (19, 43). Thus, tryptophan induction results from antitermination control within the leader region.

TnaC leader peptide. Alterations that block or prematurely terminate *tnaC* translation result in low, uninducible *tnaAB* expression (14, 16, 45), whereas alterations that extend *tnaC* translation beyond the native UGA termination codon result in high constitutive expression (16, 44). Mutant *tnaC* alleles act in *cis*, demonstrating that the TnaC peptide is not a diffusible regulator (14, 44).

Findings to this point suggested a concrete hypothesis: a critical region of nascent TnaC "may act on its translating ribosome and, by affecting elongation, stalling, or release of the ribosome, modulate access of Rho factor to the nascent transcript" (14). This hypothesis was enabled by the work of Paul Lovett and colleagues, who characterized leader peptide control of antibiotic resistance enzyme synthesis (33). Since the *boxA-rut* element overlaps the *tnaC* carboxyl-terminus-coding region (Fig. 1), a ribosome stalled at the *tnaC* UGA termination codon will block Rho entry and thereby prevent transcription termination.

Attention was focused on the single Trp residue at position 12, initially to determine if tryptophanyl-tRNA^{Trp} is involved in regulation (14, 16) and subsequently to characterize its essential structural role in ribosome pausing, as summarized below. Further analysis of *tnaC* missense alterations identified the TnaC carboxyl-terminal half as important for regulation (14, 16), and TnaC sequences from other species share simi-

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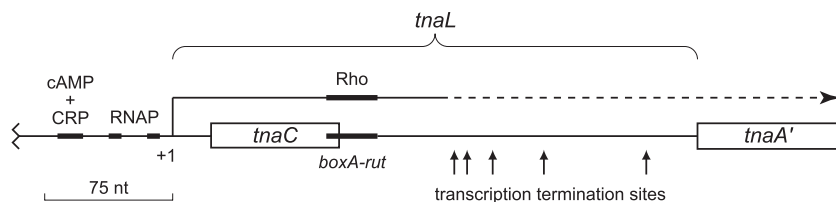


FIG. 1. *tnaAB* operon control region, drawn to scale. The transcription initiation site (11) is indicated as +1. The 319-nt transcribed leader region is denoted as *tnaL* (44). Coding regions for the leader peptide (*tnaC*) and for the amino-terminal portion of tryptophanase (*tnaA'*) are shown as open boxes (truncated for *tnaA'*). DNA sites for binding cyclic AMP (cAMP) receptor protein (CRP) and RNA polymerase (RNAP) are depicted as filled boxes (11). The *boxA-rut* element (16, 44) in RNA serves as an entry point for Rho factor, and sites for Rho-dependent transcription termination (43) within the leader region are indicated as vertical arrows. The nascent TnaC leader peptide causes conformational shifts within the ribosome to form a tryptophan-binding site. Bound tryptophan inhibits peptidyl transferase activity, preventing release of TnaC-peptidyl-tRNA^{Pro} and thereby stalling the ribosome at the *tnaC* termination codon. Rho factor, blocked by the ribosome from the *boxA-rut* element, is unable to load onto the RNA to catalyze transcription termination. In the absence of tryptophan, TnaC-peptidyl-tRNA^{Pro} is cleaved and released, allowing Rho to terminate transcription prior to *tnaAB* transcript synthesis.

larity in this region (14, 27). Indeed, parallel experiments with the *Proteus vulgaris tna* regulatory region have reinforced several observations made with *E. coli* (27, 28).

Leader peptide release in vivo. Polypeptide release is catalyzed by termination codon-specific release factors RF1 (UAG and UAA) and RF2 (UGA and UAA), the activities of which are enhanced by RF3 (36). Loss of RF3 increases uninduced levels of *tnaAB* expression, whereas RF3 overexpression inhibits expression (52). When the UGA codon is converted to UAG, a mutant allele of the *prfA* gene encoding RF1 increases the uninduced expression level (31). Furthermore, tryptophan inhibits LacZ synthesis from a construct in which the *tnaC* UGA termination codon abuts the *lacZ* gene (30). Thus, tryptophan regulates *tnaAB* expression by inhibiting ribosome release from the *tnaC* termination codon.

Regulation reconstituted in vitro. Physiology and genetics produced a solid hypothesis. For its test, Feng Gong and Charles Yanofsky developed an assay based on the cell-free coupled transcription-translation (S-30) system of Geoffrey Zubay (53). Added tryptophan or 1-methyl-tryptophan induces reporter synthesis from a wild-type template but not from *tnaC* mutant templates in which the codon for residue Trp-12 is replaced by one for Arg (W12R) or in which the initiation codon is destroyed. The Rho inhibitor bicyclomycin increases the uninduced level of expression, and antiterminated transcript accumulates in the presence of tryptophan or bicyclomycin (18). Thus, the cell-free system mimics the essential regulatory features as deduced from in vivo analyses.

For a stalled ribosome to inhibit transcription termination, leader peptide translation must coordinate with transcription elongation (47, 49). Experiments with the S-30 system show that this coupling results from a transcription pause just past the *boxA*-like element (21).

Leader peptide release in vitro. In the absence of the inducer, the free TnaC leader peptide accumulates in cell-free reactions. In the presence of the inducer, TnaC-tRNA^{Pro} is detected (18) and remains bound to the ribosome (17). TnaC-tRNA^{Pro} is hydrolyzed upon addition of RF2, but only when the inducer is removed (17). Mutant TnaC(W12R)-tRNA^{Pro} is not detected even in the presence of the inducer (19). In S-30 extracts depleted of RF2, both wild-type and mutant TnaC-tRNA^{Pro} are detected in the absence of the inducer, and antiterminated transcript accumulates (19). Thus, the inducer

inhibits RF2-mediated cleavage of TnaC-tRNA^{Pro}. The ribosomal inducer-binding site is stereospecific, because TnaC transfer to puromycin, an analog of A site-bound tRNA, is blocked only by L-tryptophan and closely related compounds (17).

TnaC residues Trp-12 and Pro-24 are essential for tryptophan-induced accumulation of TnaC-tRNA^{Pro}, as is their spacing (20). However, deletions, insertions, or missense alterations in the amino-terminal half have little effect on tryptophan induction (20), in accordance with earlier mutational analyses (14, 16).

Remarkably, insertion of a Trp codon between those for Pro-24 and termination causes accumulation of TnaC-tRNA^{Pro} even in the absence of the inducer, whereas insertion of a Phe codon blocks accumulation even in the presence of the inducer (20). Thus, tryptophanyl-tRNA^{Trp} delivers the inducer to the binding site, whereas phenylalanyl-tRNA^{Phe} delivers a competitive inhibitor. This indicates that the leader peptide-directed inducer-binding site is near the A site in the ribosome, close to the peptidyl transferase center (20). This region binds several antibiotics, many of which, like tryptophan, contain aromatic groups (e.g., puromycin and chloramphenicol).

Leader peptide-ribosome interactions. The exit tunnel accommodates at least 30 residues of nascent polypeptide in alpha-helical conformation (34, 42, 46). The SecM polypeptide undergoes a regulatory translation arrest at residue Pro-166, in which the essential residue Trp-155 lies near a constriction in the exit tunnel formed by protrusions of proteins L4 and L22 and domain II of 16S rRNA (1, 34, 37).

To determine where TnaC interacts, TnaC-tRNA^{Pro}-ribosome complexes were purified from S-30 reactions (8). Isolated complexes contain RF2, and tryptophan removal releases cleaved TnaC. In complexes depleted of RF2, TnaC residue Lys-11 cross-links to residue A750 in domain II of 23S rRNA, showing that residue Trp-12 is near the exit tunnel constriction. Wild-type TnaC but not TnaC(W12R) protects residue A788 from methylation, demonstrating that TnaC induces a conformational change displacing 23S rRNA domain II (8).

Tryptophan-binding site. Next, RF2-depleted complexes were used to study interactions of the inducer tryptophan (6). Free tryptophan inhibits both methylation of residue A2572,

which is required for peptidyl transferase activity, and TnaC transfer from tRNA^{Pro} to puromycin, which requires peptidyl transferase activity. Sparsomycin, a peptidyl transferase inhibitor, enhances methylation of residue A2059; this methylation is also inhibited by tryptophan. Tryptophan has none of these effects in complexes formed with the TnaC(W12R) mutant (6) or with mutant ribosomes with exit tunnel substitutions in 23S rRNA or L22 (7). This reinforces the conclusion that the nascent peptide-induced tryptophan-binding site is at or near the peptidyl transferase center (6).

Leader peptide residues Asp-16 and Pro-24. This brings us to the featured work (9). Among available TnaC sequences, the critical residues Trp-12 and Asp-16 (14) are invariant, and Pro-24 is strongly conserved (9). Strikingly, arginine attenuator peptides from fungi share invariant residues Asp-12, Asp-16, and Ala-24 (12, 25). Thus, residues corresponding to these positions appear important in pause-directed formation of a metabolite binding site.

Results from the *in vitro* assays described above reveal that residues Asp-16 and Pro-24 are required both for tryptophan-induced TnaC-tRNA^{Pro} accumulation in S-30 extracts (shown previously for Pro-24 [20]) and for tryptophan-inhibited peptidyl transfer to puromycin in isolated, RF2-depleted ribosome complexes (9). Thus, like invariant residue Trp-12, these positions are critical for the TnaC leader peptide to induce formation of the ribosomal tryptophan-binding site.

Unlike ribosome complexes with wild-type or W12R mutant TnaC (6), those with D16A or P24A mutant TnaC do not exhibit sparsomycin-enhanced methylation of 23S rRNA residue A2059 (9). Sparsomycin does bind, because it inhibits peptidyl transfer to puromycin in D16A and P24A complexes. Thus, the locations of the D16A and P24A peptides in the exit tunnel appear to differ from those of the wild-type and W12R forms, indicating that these residues contribute to proper TnaC-exit tunnel interaction.

Finally, P24A mutant TnaC-tRNA^{Ala} exhibits increased transfer to puromycin, indicating that the carboxyl-terminal Pro residue may reduce peptidyl transferase activity, reduce the rate of transfer to RF2 (9), and increase ribosome stalling (35). In this scenario, tryptophan binding to the site induced by the TnaC leader peptide acts by further inhibiting an already compromised peptidyl transferase activity.

Broader implications. The study of tryptophan metabolism has done much to advance our knowledge in biochemistry, genetics, and microbiology (48). Here, it has illuminated our understanding of ribosome structure, function, and regulation. The single-minded pursuit of mechanism for *tnaAB* control has generated an experimental and conceptual model for nascent peptide control in other contexts (1, 12, 33, 34, 37).

Back to the whole cell. Overexpressed TnaC inhibits *tnaAB* induction (13) and inhibits growth (23), because accumulated TnaC-tRNA^{Pro} depletes this tRNA species (23). Changing codon Pro-24 from CCU to CCG results in depletion of the isoaccepting species tRNA^{Pro}, whereas overproduction of the appropriate tRNA relieves inhibition in a codon-specific manner (23). Overexpressed RF3 and ribosome recycling factor inhibit chromosomal *tnaAB* induction (22, 52) and induce dropoff of overexpressed TnaC-tRNA^{Pro} (22). Further analysis employed ribosomal mutants screened for altered *tnaAB* expression or identified by others. These changes, in L22, L4, and

23S rRNA, all lie near the exit tunnel constriction and all influence *tnaAB* induction (7, 8). Thus, key elements of the hypothesis have been validated *in vivo*, thereby fulfilling the “fourth postulate.”

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