

exchange energy and act like a coupled quantum system. In this strong-coupling regime, the system becomes sensitive to individual photons, that is, the cavity responds differently to an incoming photon depending on whether it is already occupied by a photon or not. This sensitivity opens up another new realm for photon-photon interactions, such as single-photon logic and single-photon emission. For comparison, photon-photon interactions in a conventional nonlinear optical device require  $\sim 10^6$  or more photons.

However, because the cavity  $Q$  factors are so high (meaning very narrow spectral linewidths) and volumes so small, the strong coupling is difficult to control. To overcome this problem, most researchers fabricate so many cavities that the chance of finding one that contains a quantum dot of suitable resonance in the right place is high. Putting the dot in the right place is a rather important aspect that is easily overlooked; even though

the cavity is small, the light is not evenly distributed in it, and for maximum effect, the dot must sit at a peak of the cavity mode. Badolato *et al.* achieve this through the use of “marker dots” that accurately indicate the dot position; with the use of alignment markers, the cavity can then be placed lithographically at the right position (1).

If several such strongly coupled systems can be brought into close proximity, they can interact and “entangle” their states in a quantum system, which forms the basis for quantum computing. Here lies the true strength of the deterministic approach: It is difficult enough in the “chance” approach to find a single cavity and dot in resonance, but the chances of finding two or more next to one another is vanishingly small; controlling the resonances is the only viable option.

Badolato *et al.* have not actually demonstrated strong coupling in their cavities, but they are very close. Much stronger interactions could easily be achieved through the

use of cavity designs with higher  $Q$  (5). The strength of their result is therefore that two major mechanisms for controlling the coupling between quantum dot and cavity have been successfully demonstrated: the placement of the dot with respect to the cavity mode, and the tuning of the cavity resonance.

For the dream of quantum computers based on optical cavities (see the figure) to become reality, further control needs to be exercised, for example, in the creation of quantum dots with deterministic emission wavelength. The work by Badolato *et al.* is nonetheless a major step forward in exercising control over quantum systems in the solid state.

#### References

1. A. Badolato *et al.*, *Science* **308**, 1158 (2005).
2. J. McKeever *et al.*, *Science* **303**, 1992 (2004); published online 26 February 2004 (10.1126/science.1095232).
3. J. P. Reithmaier *et al.*, *Nature* **432**, 197 (2004).
4. T. Yoshie *et al.*, *Nature* **432**, 200 (2004).
5. B.-S. Song *et al.*, *Nat. Mater.* **4**, 207 (2005).

10.1126/science.1113753

## MOLECULAR BIOLOGY

# A Renewed Focus on Transfer RNA

Tina Daviter, Frank V. Murphy IV, V. Ramakrishnan

**T**ranslation of the genetic code occurs through base-pairing interactions between the codon on messenger RNA and the anticodon on transfer RNA (tRNA) that are mediated by the ribosome, the molecular machine that catalyzes protein synthesis. Even before the complete elucidation of the genetic code, it was clear that the accuracy of protein synthesis is too high to be accounted for by codon-anticodon pairing alone (1). The discovery that it could be affected by antibiotics and ribosomal mutations suggested that the ribosome has a decoding site that inspects codon-anticodon interactions (2). Hence, the discovery of a mutant tryptophanyl tRNA (“Hirsh suppressor”) that could suppress the termination of protein synthesis was a puzzle (3). A G24A mutation on this tRNA, although quite distant from the anticodon at positions 34 to 36 (see the figure), nevertheless conferred on the mutant tRNA the ability to pair with the UGA stop codon in addition to the wild-type UGG codon. How does this tRNA recognize UGA and evade proofreading by the ribosome? Now, decades after the discovery of the Hirsh suppressor, its secrets are being

revealed. On page 1178 in this issue, Cochella and Green (4) show how the kinetic properties of the mutant tRNA allow it to decode stop codons.

Aminoacyl-tRNA is initially brought into the ribosome as a complex with elongation factor Tu (EF-Tu) and guanosine 5'-triphosphate (GTP) (see the figure). Upon GTP hydrolysis, EF-Tu is released. In a view of decoding termed kinetic proofreading (5, 6), incorrect tRNAs can dissociate from the ribosome either before or after EF-Tu release, with the overall selectivity being as much as the product of both selection steps. Experimental evidence for proofreading came when it was shown that near-cognate tRNAs (which contain a single subtle mismatch between codon and anticodon) require more GTPs hydrolyzed per amino acid incorporated than do cognate tRNAs (7, 8). In this view, the ribosome merely provided a passive platform for tRNA selection, with mutations and antibiotics altering accuracy by affecting the rate of GTP hydrolysis by EF-Tu. In principle, mutations distant from the codon-anticodon pairing could also affect the rate of GTP hydrolysis.

More recent work points to a direct role for the ribosome and its associated players in tRNA selection. Careful studies on the

stability of RNA helices show that the free-energy differences from a base-pairing mismatch can account for a factor of 5 to 10 in selectivity (9) rather than the factor of 100 assumed previously. This is too low to account for the accuracy of protein synthesis even with proofreading. Pre-steady-state kinetic experiments show that the forward rates of GTPase (guanosine triphosphatase) activation (the rate-limiting step in GTP hydrolysis) and accommodation (movement of tRNA into the peptidyl transferase center) are dramatically higher for cognate tRNA than for near-cognate tRNA (10, 11). Thus, cognate tRNA is likely more efficient at inducing a productive ribosome conformation, consistent with results from nuclear magnetic resonance studies on a portion of the decoding site (12). Crystallographic studies on the 30S ribosomal subunit showed that, in the productive conformation, the ribosome directly monitors the geometry of base pairing at the minor groove of the first two positions (but interestingly not at the wobble position) between the codon and anticodon (13).

Several other key pieces of evidence helped to clarify things further. Kinetic studies showed that intact tRNA is required to transmit the signal from codon recognition in the 30S subunit to the GTPase center in the 50S subunit (14). Cryoelectron microscopy demonstrated that in the ribosome, the tRNA in complex with EF-Tu has a bend in the anticodon stem-loop: The region around the anticodon loop is in nearly the accommodated orientation, while the bend allows the rest of the tRNA to remain in the orientation presented by EF-Tu (15, 16). Crystal structures revealed

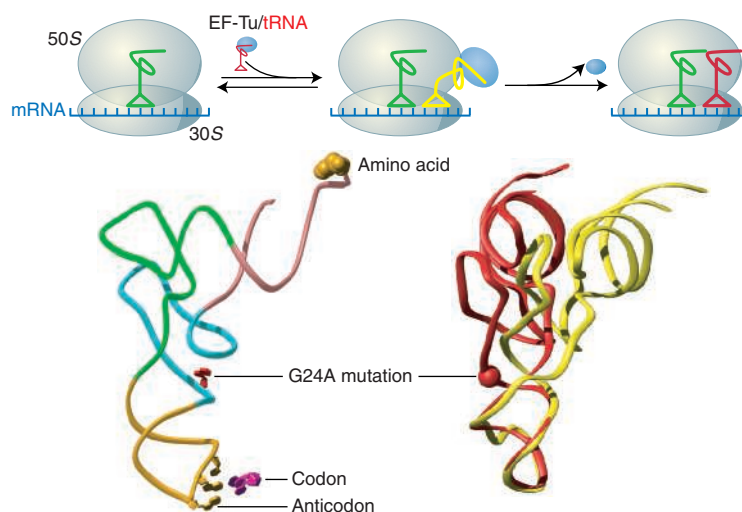
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK. E-mail: ramak@mrc-lmb.cam.ac.uk

that conformational changes seen could only be induced in the 30S subunit by cognate and not near-cognate tRNA (17). Thus, the ribosome uses part of the additional binding energy from its interactions with the minor groove of the cognate codon-anticodon helix to induce a conformational change leading to a productive closed form, which involves a distorted tRNA. After GTP hydrolysis and dissociation of EF-Tu, the distorted tRNA, which has been described as a “molecular spring” (18), would be free to relax into the accommodated state. This would be faster in the cognate case where the anticodon end was constrained in a conformation close to the accommodated state (17).

This view made it possible to rationalize various mutations affecting the accuracy of translation that were spread throughout the ribosome or tRNA (1, 17). These mutations would favor one or the other conformation, thus facilitating or inhibiting the closure of the ribosome or the distortion of the tRNA. A role for the dynamic property of tRNA in decoding was presaged long before the recent structural data (19). But decades after its discovery, there were still no hard data to explain exactly how the Hirsh suppressor tRNA increased the error rate.

Now, using rapid quench methods, Cochella and Green (4) have directly measured the rates of GTP hydrolysis and dipeptide formation for the Hirsh suppressor and its corresponding wild-type tRNA. These rates reflect the rate-limiting preceding step in each case, namely, GTPase activation and accommodation (10, 11). Using a mutant EF-Tu that is defective in GTP hydrolysis, they also measured the rate of dissociation of tRNA from the ribosome. As might be expected for a mutation far from the anticodon, the dissociation rates of the Hirsh suppressor and the corresponding wild type are very similar on the various codons tested. However, the forward rates of both GTPase activation and accommodation are much higher for the Hirsh suppressor than for the wild type when a near-cognate codon is used. The increase in the rate of GTPase activation can be explained if the mutation makes the tRNA more easily deformable into the bent structure in the transition state. But what about the increase in the accommodation rate?

Based on the observation that forward rates of GTPase activation and accommoda-



**An active role for tRNA: (Top)** A new aminoacyl-tRNA is brought into the A site of the ribosome in complex with elongation factor EF-Tu. Upon GTP hydrolysis, the aminoacyl-tRNA is released by EF-Tu, and swings into the peptidyl transferase center of the 50S subunit. The tRNA is distorted when bound with EF-Tu on the ribosome. **(Bottom)** A G24A mutation allows a tryptophanyl tRNA to read the UGA stop codon in addition to the UGG tryptophan codon. Strikingly, this mutation in the Hirsh suppressor tRNA is far from the anticodon, but close to the region of distortion in the tRNA when it first binds to the ribosome. The mutation allows GTP hydrolysis and the movement of tRNA into the peptidyl transferase center to proceed efficiently even on the stop codon (4).

tion were simultaneously affected by various agents (for example, codon-anticodon pairing or antibiotics such as paromomycin) (11), it was proposed that these two steps were both regulated by the domain closure in the 30S subunit (17). That the Hirsh suppressor also affects both these rates might appear to strengthen this notion. However, the Hirsh suppressor is the one case where one might not have expected these two rates to be coupled: If GTPase activation is accelerated because the tRNA is more deformable, then such a tRNA should be slower in relaxing into the undeformed state during accommodation. The “molecular spring” should be less stiff and therefore snap into place more slowly.

So where does this leave the molecular spring idea? Cochella and Green (4) propose that specific interactions between the ribosome and tRNA might be altered for the mutant tRNA. Interestingly, the mutation itself is close to helix 69 of the 50S subunit in both the bent and relaxed forms of tRNA. If the mutant tRNA makes stronger interactions with the ribosome, it could compensate for weaker codon-anticodon interactions. This in turn could help to distort the tRNA and place its anticodon stem-loop in approximately the accommodated orientation before EF-Tu release, and hold it in this orientation during its subsequent movement, just as with cognate tRNA. Perhaps holding the anticodon end in the accommodated orientation and thus restricting the movement of the tRNA (17)

is more important in speeding up accommodation than the “stiffness” of a putative tRNA spring. To resolve these issues, we need similar kinetic data on other mutants, as well as higher resolution structures of wild-type and mutant tRNAs bound to the ribosome in these various states.

In the meantime, after a long period in which the focus has been on the ribosome, Cochella and Green have redirected attention toward the properties of the tRNA substrate itself. Interestingly, tRNA is one of the most heavily modified RNAs in the cell. These modifications may alter the dynamic properties of various tRNAs to ensure roughly similar rates and accuracies despite differences in the strength of the codon-anticodon interactions.

Indeed, recent binding affinity data support this notion (20). In any case, it is becoming increasingly clear that far from being a rigid and passive substrate, tRNA has co-evolved with the ribosome to allow the close and dynamic interplay required for the fidelity and speed of translation.

#### References

1. J. M. Ogle, V. Ramakrishnan, *Annu. Rev. Biochem.* **74**, 129 (2005).
2. J. Davies, W. Gilbert, L. Gorini, *Proc. Natl. Acad. Sci. U.S.A.* **51**, 883 (1964).
3. D. Hirsh, *J. Mol. Biol.* **58**, 439 (1971).
4. L. Cochella, R. Green, *Science* **308**, 1178 (2005).
5. J. J. Hopfield, *Proc. Natl. Acad. Sci.* **71**, 4135 (1974).
6. J. Ninio, *Biochimie* **57**, 587 (1975).
7. R. C. Thompson, P. J. Stone, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 198 (1977).
8. T. Ruusala, M. Ehrenberg, C. G. Kurland, *EMBO J.* **1**, 741 (1982).
9. N. Sugimoto, R. Kierzek, S. M. Freier, D. H. Turner, *Biochemistry* **25**, 5755 (1986).
10. T. Pape, W. Wintermeyer, M. V. Rodnina, *EMBO J.* **18**, 3800 (1999).
11. M. V. Rodnina, W. Wintermeyer, *Annu. Rev. Biochem.* **70**, 415 (2001).
12. D. Fourmy, S. Yoshizawa, J. D. Puglisi, *J. Mol. Biol.* **277**, 333 (1998).
13. J. M. Ogle *et al.*, *Science* **292**, 897 (2001).
14. O. Piepenburg *et al.*, *Biochemistry* **39**, 1734 (2000).
15. M. Valle *et al.*, *EMBO J.* **21**, 3557 (2002).
16. H. Stark *et al.*, *Nat. Struct. Biol.* **9**, 849 (2002).
17. J. M. Ogle, F. V. Murphy, M. J. Tarry, V. Ramakrishnan, *Cell* **111**, 721 (2002).
18. M. Valle *et al.*, *Nat. Struct. Biol.* **10**, 899 (2003).
19. M. Yarus, D. Smith, in *tRNA: Structure, Biosynthesis and Function*, D. Söll, U. RajBhandary, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 443–468.
20. R. P. Fahlman, T. Dale, O. C. Uhlenbeck, *Mol. Cell* **16**, 799 (2004).