'Distorted' RNA helix recognition

SIR — Ibba and Söll have proposed a resolution for an apparent controversy over the basis for recognition of a critical G–U wobble base pair in an RNA helix[1]. We wish to point out that their proposal is not supported by extensive published literature and that more plausible explanations are available.

The controversy has surrounded the question of how the G–U pair at the third position in the acceptor helix of alanine transfer (t)RNAs is recognized by its cognate synthetase. *In vitro* experiments with a multitude of natural bases and non-natural base analogues narrowed down the functional site to the free 2-amino group contributed by G of the G–U pair[2–4]. (Unlike the G–C Watson–Crick pair, the G–U wobble pair places the 2-amino group of G in the RNA minor groove as an unpaired functional group.) These experiments included a demonstration that simple removal of the 2-amino group (using an I–U pair), with no other change, was sufficient to abolish aminoacylation with alanine. Restoration of the free 2-amino group by substitution of a non-natural base pair (2-amino adenosine paired with isocytidine) restored aminoacylation with alanine[4].

Non-natural base analogues cannot be used in experiments that attempt to assess the state of alanylation *in vivo*. However, substitution for G–U of mismatched base pairs (such as A–C) is possible and, when used in specially constructed genetic strains, some of these substitutions have created molecules that are charged *in vivo* with alanine[5]. Because some of these constructions lack the free 2-amino group of the G–U pair, McClain and co-workers suggest that, in these instances, the signal for alanylation is due to indirect recognition of a distortion of the RNA helix[5]. They propose that indirect recognition of a distorted helix accounts for recognition of the G–U pair as well.

Ibba and Söll speculate that differences in conditions used in the *in vivo* and *in vitro* experiments can explain the different results[1]. They suggest that, because the concentrations of alanine used in the *in vitro* experiments were lower than those present *in vivo*, the *in vitro* studies would miss an effect on aminoacylation of a linkage between the tRNA and amino-acid interactions that could enhance aminoacylation at higher amino-acid concentrations.

Their proposal is mostly based on their studies of glutaminyl-tRNA synthetase, a class I tRNA synthetase that requires $tRNA^{Gln}$ as a cofactor for amino-acid activation. However, in contrast to glutaminyl-tRNA synthetase, alanyl-tRNA synthetase is a class II enzyme which can activate its amino acid in the absence of tRNA. Indeed, no significant linkage between tRNA and alanine has been seen when concentrations of alanine and tRNA are varied during *in vitro* aminoacylation experiments, even at concentrations of the amino acid that match those seen *in vivo* (estimated to be about 200 μM)[6,7]. Although a small effect of alanine on the K_m for tRNA was observed[6], this effect was opposite to that required by the proposal of Ibba and Söll.

Ibba and Söll also imply that, if higher amino-acid concentrations were used, mutant substrates containing, for example, the I–U pair would be charged with alanine. But we find that even high alanine concentrations do not result in charging of non-G–U-containing substrates (P. Buening, K. M.-F. and P. S., unpublished data). Nor does the use of Michaelis–Menten or non-Michaelis–Menten conditions make any difference. The problem with those inactive position 3–70-mutant RNAs that have been further characterized (including the I–U mutant) is that they do not detectably bind to the synthetase, in the presence or absence of ATP and alanine[2,8,9]. The disruption of the synthetase-binding interaction is itself sufficient to explain the inactivity of these mutant RNAs.

The question, therefore, is whether the charging of tRNAs with mismatched base pairs *in vivo* reveals anything about charging of the wild-type substrate that has a G–U pair. McClain and co-workers consider that the inactive G3–C70-containing substrates have normal, non-distorted helical stems and, for that reason, are not charged[5]. However, a G3–C70 containing alanine tRNA (with an operationally 'undistorted' helical stem) is made active *in vivo* if a distal mutation is present in the anticodon stem — far removed from, and in a different domain from, where the G–U pair is located[10]. This variant illustrates how a new, serendipitous protein–RNA interaction can be recruited to compensate for a missing normal interaction. At face value, it says nothing about how G–U is normally recognized.

This consideration, together with the known tendency of *in vivo* measurements of charging to distort or amplify weak signals that are difficult to detect *in vitro*[1,11], and the relatively minor differences found by nuclear magnetic resonance between the active G–U and inactive I–U helices[12], suggest to us that the charging *in vivo* of substrates with mismatched base pairs should be interpreted with great caution.

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IBBA AND SÖLL REPLY — Schimmel and Musier-Forsyth raise various relevant points, the most pertinent of which is the requirement of tRNA for aminoacyl-adenylate formation by glutaminyl-tRNA synthetase, but not by alanyl-tRNA synthetase. This, we fully accept, makes extrapolation of results from one synthetase to the other difficult. However, it was never our intention to question the validity of their earlier results. Rather, we had hoped to put forward a model that could account for the discrepancy between the existing *in vivo* and *in vitro* data.

As Schimmel and Musier-Forsyth rightly state, the charging *in vivo* of certain substrates should be interpreted with great caution, a proviso that could be extended to *in vitro* substrates. Indeed, the disparity in size between the RNA substrates themselves represents an important difference between the two experimental approaches.

In vivo, the role of G–U was studied in the context of a full-length tRNA molecule containing modified nucleotides, whereas *in vitro*, RNA minihelices were used which only reiterate a fragment of $tRNA^{Ala}$ and in some cases do not contain a stem–loop structure. Although solution studies suggest that such mini-substrates are comparable in structure to the portion of the tRNA that they mimic[12], functional comparisons suggest that this may not always be the case during interaction with synthetases.

It has recently been shown[13], for example, that certain base pairs in the acceptor stem of $tRNA^{Ser}$ make substantial contributions to aminoacylation specificity in the context of a minihelix but not a full-length tRNA. Although this is not the case for the G3–U70 base pair of $tRNA^{Ala}$, whose function has been extensively documented in both RNA contexts, this example clearly demonstrates the need for caution when attempting to reconcile *in vitro* and *in vivo* data based on divergent substrates.

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