Supplementary Information

A sequence element that tunes *E. coli* $tRNA_{GGC}^{Ala}$ to ensure accurate decoding

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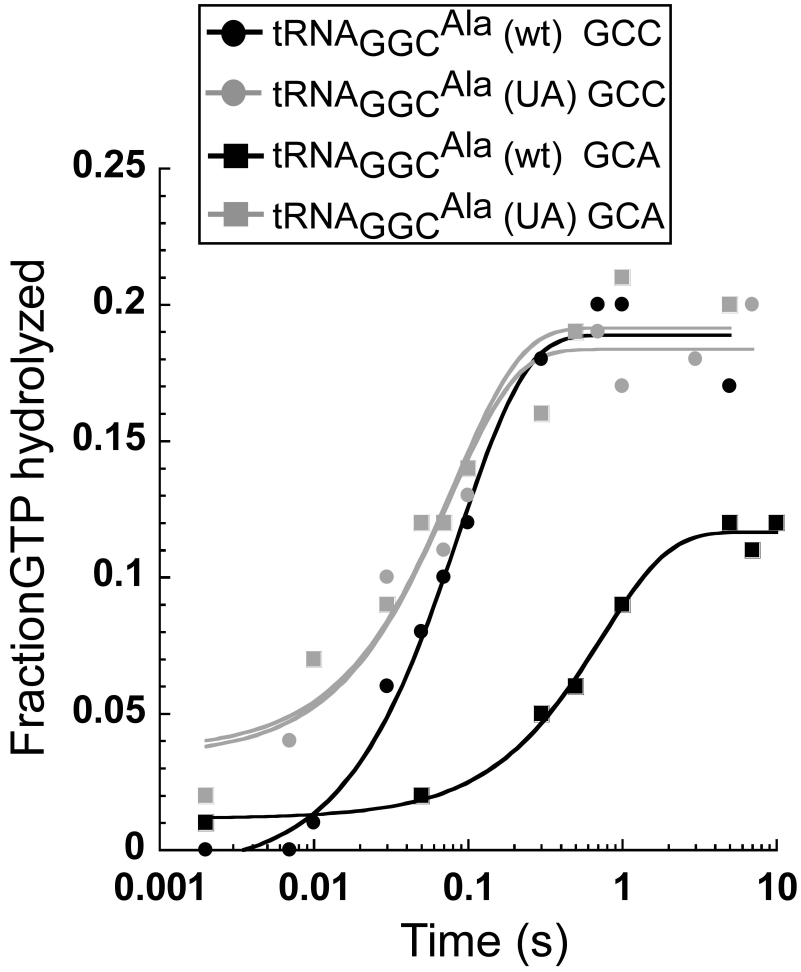
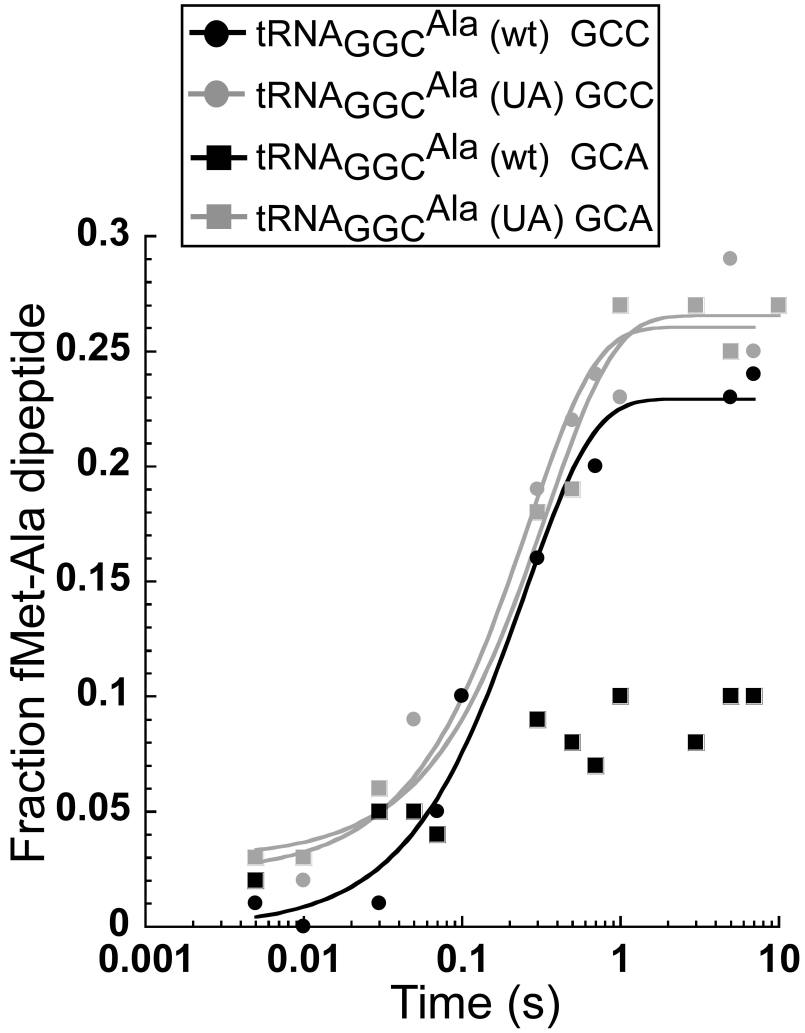
Supplementary Text

Since experiments were performed using tRNA transcripts which lack posttranscriptional modifications, a buffer containing 10 mM $MgCl_2$ was used for most experiments. However, since initial selection steps of decoding have been shown to be affected by the buffer[1,2], tests were also performed in a "high fidelity" buffer which contains polyamines and less $MgCl_2$ (50 mM HEPES [pH 7.0], 30 mM KCl, 70 mM NH_4Cl, 3.5 mM $MgCl_2$, 0.5 mM spermidine, 8 mM putrescine, and 1 mM DTT)[1]. Determination of K_d was not possible due to the filter instability of the complexes in the low $MgCl_2$ buffer. Previous determinations of K_d in the high fidelity buffer have necessitated the addition of excess $MgCl_2$[3]. The rate of GTP hydrolysis at one concentration of ribosomes was determined for both $tRNA_{GGC}^{Ala}$ (wt) and $tRNA_{GGC}^{Ala}$ (UA) on the cognate GCC and near-cognate GCA codons (Supplementary Fig. 1). However, the extent of hydrolysis achieved was much lower than observed in the 10 mM $MgCl_2$ buffer, possibly due to the dissociation of the unstable tRNA transcripts from EF-Tu/GTP during the course of the experiment. The rate of peptide bond formation was also determined (Supplementary Fig. 2). In

both cases, the data supports that collected with the 10 mM $MgCl_2$ buffer: $tRNA_{GGC}^{Ala}$ (wt) is capable of decoding only the cognate codon while $tRNA_{GGC}^{Ala}$ (UA) is capable of decoding both the GCC and GCA codons.



Supplementary Figure 1: $k_{GTPapparent}$ of $tRNA_{GGC}^{Ala}$ (wt) and $tRNA_{GGC}^{Ala}$ (UA) in "high fidelity" buffer. The rate of GTP hydrolysis was measured as in Figure 2b with the exceptions that the ribosome concentration was 2 μM and the buffer was "high fidelity". The rates were determined by fitting the data to a single exponential curve. $tRNA_{GGC}^{Ala}$ (wt) had a rate of 11 s^{-1} on the cognate GCC codon and 1.3 s^{-1} on the near-cognate GCA codon. $tRNA_{GGC}^{Ala}$ (UA) had a rate of 13 s^{-1} on the GCC codon and 12 s^{-1} on the GCA codon.



Supplementary Figure 2: k_{pep} of tRNA$_{GGC}^{Ala}$ (wt) and tRNA$_{GGC}^{Ala}$ (UA) in "high fidelity" buffer. The rate of peptide bond formation was determined as in Figures 2d and 3 with the exception of the use of "high fidelity" buffer. The rates were determined by fitting the data to a single exponential curve. tRNA$_{GGC}^{Ala}$ (wt) had a rate of 4.0 s^{-1} on the cognate GCC codon. Since the rate for the wild type tRNA on the near-cognate GCA codon could not accurately be determined, no line was drawn. tRNA$_{GGC}^{Ala}$ (UA) had a rate of 3.9 s^{-1} on the GCC codon and 5.0 s^{-1} on the GCA codon.

Supplementary References

1. Gromadski, K.B. & Rodnina, M.V. Kinetic determinants of high-fidelity tRNA discrimination on the ribosome. *Mol. Cell,* **13**, 191-200 (2004).
2. Pape, T., Wintermeyer, W. & Rodnina, M. Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *Embo J.,* **18**, 3800-7 (1999).
3. Cochella, L. & Green, R. An active role for tRNA in decoding beyond codon:anticodon pairing. *Science,* **308**, 1178-80 (2005).