Figure S1. Magnesium acetate gives higher plateau charging values for hmt tRNA$^{Leu}_{UAA}$ transcript compared to magnesium chloride. Reactions were performed at 1 µM LARS-2 in the presence of either magnesium chloride (red symbols and traces) (or) magnesium acetate (blue symbols and traces) at 37°C at concentrations of either 20 mM Mg$^{2+}$ (open symbols) or 55 mM Mg$^{2+}$ (closed symbols).
Figure S2. Aminoacylation plateau value is low at higher temperature (>37°C) for hmt tRNA\textsuperscript{Leu} because of structural instability and not because of RNA being damaged at these temperatures. Leucylation of hmt tRNA\textsuperscript{Leu\_UAA} was performed at 45°C and at 20 mM Mg(oAc)\textsubscript{2}, which resulted in a low plateau value for aminoacylation (0.25) (Open symbols and trace). A large aliquot was transferred to 21°C and aminoacylation monitored at 21°C (solid symbols and trace). Because the only difference that resulted in higher plateau value was a transfer to a lower temperature, we conclude that the tRNA was not damaged at 45°C but secondary and/or tertiary structure was severely perturbed at 45°C compared to 21°C.
Figure S3. Log-linear plot of the rate constant against $1/T$ (Kelvin) for folding of the hmt tRNA$^{\text{Leu}}_{\text{UAA}}$ transcript (see Figure 4). The slope is equal to $-E_a/R$, where $E_a$ is the activation energy and $R$ is the gas constant (8.314 Jmol$^{-1}$K$^{-1}$). The fit gives $E_a$ of $13.7 \times 10^4$ J-mol$^{-1}$ (32.8 kcal-mol$^{-1}$).
Figure S4. 1M7 SHAPE analysis at different Mg$^{2+}$ concentrations. (A) SHAPE footprinting gel showing DMSO and 1M7 intensities at 0, 12 and 50 mM MgCl$_2$. (B) Absolute SHAPE reactivities at 0, 12 and 50 mM MgCl$_2$. (C) Absolute SHAPE reactivities of a subset of nucleotides at 0, 12 and 50 mM MgCl$_2$. Nucleotides 28, 31, 32, 33, 38, 39 and 40 show clear protections at increasing MgCl$_2$ up to 50 mM.
Figure S5. Predicted optimal (upper left) and sub-optimal secondary structures of hmt tRNA^{Leu}_{UAA} transcript tRNA. Structures were predicted using minimal energy criteria using MC-fold.
Figure S6. Predicted native secondary structures (based on comparative sequence analysis) of human and bovine transcript tRNA$_{\text{Leu}^{\text{UAA}}}$-UAA. Differences in primary sequence are circled in the *Bos taurus* tRNA.