Abstract title

tRNA anticodon stem length variations are critical for stop codon reassignment

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Abstract

Transfer RNA (tRNA) delivers a specific amino acid residue to ribosomes. Its anticodon pairs with the complementary mRNA codon according to the universal genetic code, which defines translation termination by three stop codons. Remarkably, some protists have reassigned all three stop codons to sense codons, neglecting this fundamental principle. Performing an across- the-genome analysis of in-frame stops in 7,259 predicted protein-coding genes of the newly isolated trypanosomatid *Blastocrithidia nonstop* we show that their distribution is not random and their representation diminishes with increasing protein abundance. Robust comparisons with other trypanosomatids revealed specific features at the end of the coding regions defining UAA as the only functional termination codon. We demonstrate that while novel tRNAs^{Glu} fully cognate to UAG and UAA evolved to reassign these two stops, recoding of UGA followed an unprecedented path via shortening the anticodon stem-loop of tRNA$^{Trp}_{CCA}$ from 5 to 4 base pairs (bp). While tRNA^{Trp} with a canonical 5-bp long stem-loop recognizes UGG as dictated by the genetic code, its shortened version efficiently incorporates tryptophan also into in-frame UGA to allow translation to continue. Mimicking this radical evolutionary twist by engineering and overexpressing both stem loop variants of tRNA$^{Trp}_{CCA}$ from *B. nonstop*, *Trypanosoma brucei* and *Saccharomyces cerevisiae* in the latter two species, we recorded a significantly higher readthrough for all 4-bp stem-loop variants. The phenomenon is specific for tRNA^{Trp}, since decoding by other two *S. cerevisiae* tRNAs near-cognate to UGA (tRNA^{Cys} and tRNA^{Arg}) was unaffected by alterations in their stem-loop length. Furthermore, we demonstrate that a specific mutation in the release factor 1 (eRF1) of *B. nonstop* specifically restricts UGA recognition and thus robustly potentiates the UGA recoding to tryptophan. Revealing that the same strategy has also been independently adopted by the ciliate *Condylostoma magnum*, we propose that these two key alterations co-evolved synergistically. Altogether, we have defined a novel and universal mechanism underlying the stop codon recognition by specific variants of tRNAs^{Trp} in combination with mutated eRF1, which has been exploited in unrelated eukaryotes with reassigned stop codons.