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Improved amber and opal suppressor tRNAs for incorporation of unnatural amino acids in vivo. Part 2: evaluating suppression efficiency.

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The incorporation of unnatural amino acids into proteins is a valuable tool for addition of biophysical probes, bio-orthogonal functionalities, and photoreactive cross-linking agents, although these approaches often require quantities of protein that are difficult to access with chemically aminoacylated tRNAs. THG73 is an amber suppressor tRNA that has been used extensively, incorporating over 100 residues in 20 proteins. In vitro studies have shown that the Escherichia coli Asn amber suppressor (ENAS) suppresses better than THG73. However, we report here that ENAS suppresses with <26% of the efficiency of THG73 in Xenopus oocytes. We then tested the newly developed Tetrahymena thermophila Gln amber suppressor (TQAS) tRNA library, which contains mutations in the second to fourth positions of the acceptor stem. The acceptor stem mutations have no adverse effect on suppression efficiency and, in fact, can increase the suppression efficiency. This study shows that the TQAS tRNA library is an averaging of suppression efficiency, and increased suppression efficiency does not correlate with increased DeltaG of the acceptor stem. The TQAS tRNA library, composed of functional suppressor tRNAs, has been created and will allow for screening in eukaryotic cells, where rapid analysis of large libraries is not feasible.

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