In Vivo Incorporation of Multiple Unnatural Amino Acids

Thesis by:

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Dedicated to my Mother and family,
who have supported me throughout my life.

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Abstract

Unnatural amino acid (UAA) incorporation is an invaluable technique that is seeing increased use. THG73 is an amber suppressor tRNA used to incorporate > 100 residues at the UAG, amber stop codon, in *Xenopus* oocytes. We have found that yeast Phe frameshift suppressors (YFFS) can incorporate UAAs at the CGGG and GGGU quadruplet codons *in vitro* and *in vivo*, allowing simultaneous incorporation of three UAAs in the nicotinic acetylcholine receptor (nAChR). The YFFS are more "orthogonal" than the amber suppressor tRNA, THG73, but the frameshift suppressors incorporate UAAs less efficiently than THG73. A library of tRNAs derived from THG73 has produced an amber suppressor that is "orthogonal" and suppresses similarly to THG73. An analogous opal suppressor tRNA allows incorporation of UAAs at the UGA, opal stop codon. The use of the amber, opal, CGGG, and GGGU codons should allow for the simultaneous incorporation of four UAAs *in vivo*. Bioorthogonal labeling of UAAs is useful for the addition of large fluorophores. We incorporated *p*-AcPhe at α70 of the nAChR and labeled with biotin and Cy5.5 hydrazide. Biotin and Cy5.5 hydrazide consistently labeled three proteins on oocytes not expressing α70*p*-AcPhe and isn't useful for site-specific labeling of ketone containing UAAs in oocytes. We explored the known subunit stoichiometry of the nAChR (2α:β:γ:δ) expressed in oocytes and detected each subunit with the HA tag by Western blot. The α-subunit is present in excess of the other subunits in a ratio of ≈ 3:1, which is expected to be 2:1. UAAs are being sold commercially for detection of protein-protein interactions in eukaryotic cells. The UAAs are heterogeneously incorporated and little is known about the effect on protein function and stability. We heterogeneously incorporated UAAs into the nAChR and detected changes in function by shifts in EC_{50}. Many UAAs altered the function of the nAChR. Incorporation of photo-reactive UAAs allowed for detection of cross-linking by Western blot. Heterogeneously incorporated UAAs also altered the functional nAChR expression on the surface of oocytes. Site-specific and heterogeneous incorporation of multiple UAAs are useful techniques for novel experiments to explore protein function, FRET experiments, cross-linking, and protein expression.

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