The Use of Non-Canonical Amino Acids to Study Protein Function. Green Fluorescent Protein and Rob Protein

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Several strategies now exist for the ribosomal synthesis of proteins containing non-proteinogenic amino acids. These enable the incorporation of one or more modified amino acids into predetermined positions in a protein. While a wide variety of amino acid side chains not found in natural proteins can be incorporated, bacterial ribosomes do not readily incorporate amino acid analogues such as D-amino acids or beta-amino acids.

Over the past several years, we have also developed a strategy for modifying the 23S ribosomal RNA in *E. coli* ribosomes; this is the ribosomal constituent that mediates the peptide bond formation. By the use of structurally modified puromycin analogues, libraries of clones harboring plasmids with modified 23S rRNAs can be screened to identify clones capable of incorporating modified amino acids not normally incorporated by bacterial ribosomes.

Green Fluorescent Protein (GFP) is a naturally occurring protein that has been the source of inspiration for many recent studies. GFP utilizes a cluster of three canonical amino acids to cyclize and oxidize, forming a novel fluorophore within a beta barrel protein conformation. The initial finding has prompted numerous studies of GFP analogues that produce a broad spectrum of colors, with altered amino acid chemistry and facility of amino acid cyclization with concomitant oxidation. Presently, we describe a novel set of synthetic dipeptidomimetic structures that exhibit weak fluorescence in aqueous solution, but whose fluorescence is strongly enhanced in more hydrophobic environments such as beta barrels. They have structures reminiscent of the formed fluorophore in GFP, but are significantly brighter than GFP or any of its synthetic analogues.

In addition, we have studied altered DNA transcription in *E. coli* involving the interaction of Rob protein with RNA stress response gene *micF*, the latter of which encodes a 93-nucleotide antisense RNA that post-transcriptionally controls the expression of the outer membrane porin gene *ompF* by binding to its target ompF mRNA, thus diminishing porin expression. Altered Rob protein interaction with *micF* is increased slightly by altering Arg40 or Arg90 in each of two helix-turn-helix motifs in Rob that are responsible for *micF* binding. Their increased binding produces an increase in the cellular antisense RNA transcript, which diminishes cellular ompF porin and alters cell phenotype, reducing the uptake of some macrocyclic antibiotics and toxic metal cations.