

PROTEIN ENGINEERING

Site-specific incorporation of amino acid analogues

(A general introduction)

C. Koehrer
7.344 2007

Why introduce unnatural amino acids into proteins ?

I. Synthesis of proteins with novel properties (protein engineering)

- Changes in structural properties
 - Changes in physico-chemical properties
 - e.g. improvement in thermostability
 - e.g. improvement in stability in organic solvents
 - Changes in conformation (e.g. α -helix)

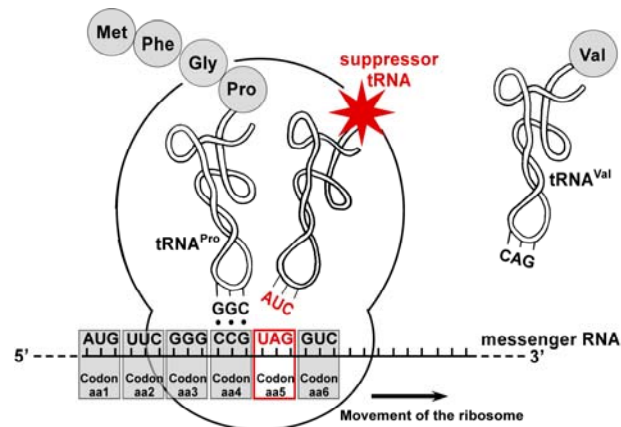
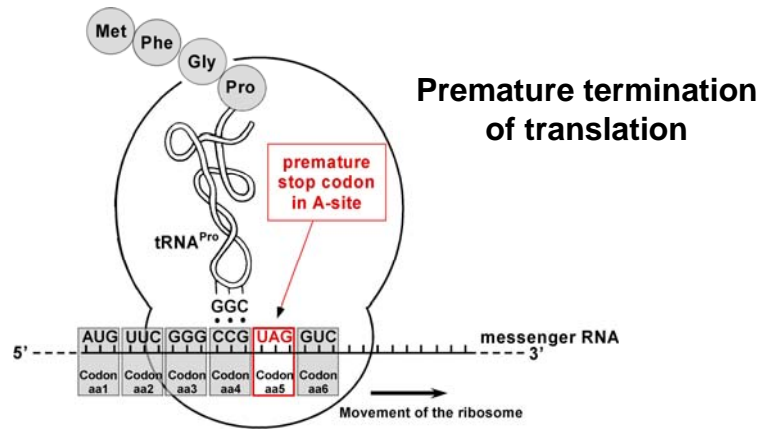
- Changes in catalytic properties
 - Increase in V_{\max}
 - Decrease in K_m
 - Changes in pH & temperature optimum
 - Modification of specificity of reaction

Why introduce unnatural amino acids into proteins ?

II. Analysis of protein structure and function

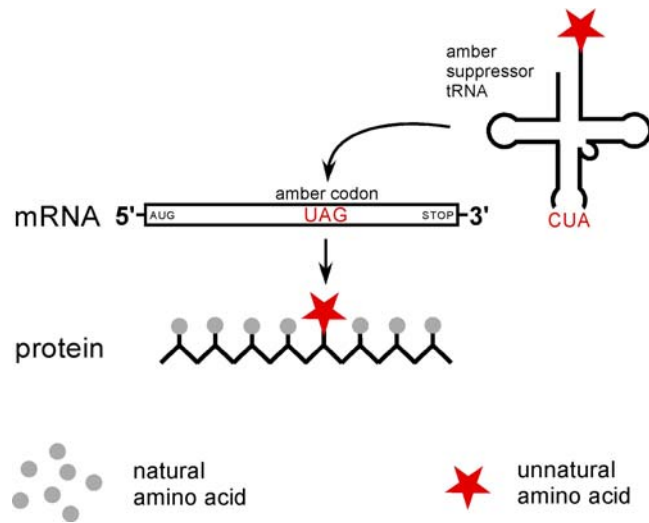
- Fluorescent analogues for studying intracellular protein localization, protein folding, structure, binding to ligands etc.
- Photoactivatable amino acids for protein-protein interactions
- Chemically reactive groups
- Phospho amino acids for studies of signal transduction
- Heavy-atom derivatives for x-ray crystallography
- Spectroscopic probes for NMR, EPR etc.

Role of specialized tRNAs (suppressor tRNAs) in translation of premature termination codons

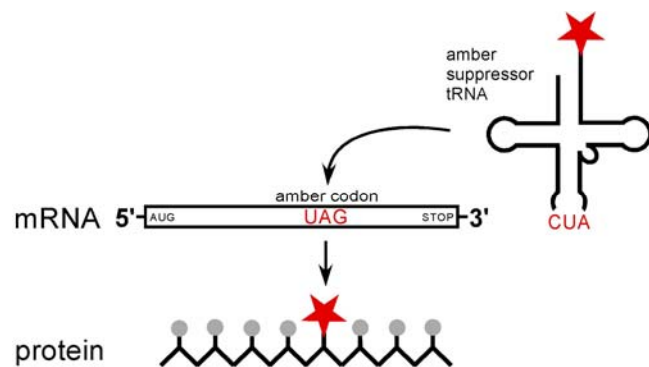


Nonsense Suppression

Strategy for site-specific incorporation of unnatural amino acids into proteins using nonsense suppression



Strategy for site-specific incorporation of unnatural amino acids into proteins using nonsense suppression



Key requirement:

suppressor tRNA carrying the unnatural amino acid **IS NOT** recognized by any of the endogenous synthetases ...'orthogonal' suppressor tRNA

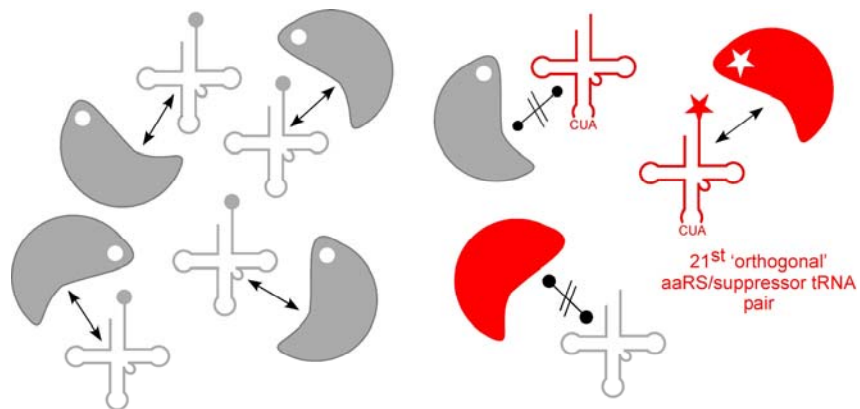
Use of 'orthogonal' suppressor tRNAs in unnatural amino acid mutagenesis

1. *In vitro* protein synthesis



- *E. coli* S30 cell extracts
- Wheat-germ system
- Rabbit reticulocyte systems

21st aminoacyl-tRNA synthetase/suppressor tRNA pairs



Incorporation of unnatural amino acids into proteins

random

- Use of minimal media supplemented with analogue



site-specific

in vitro

- Chemical peptide synthesis
- In vitro protein synthesis systems

in vivo

- Nonsense suppression

Expanding the genetic code

