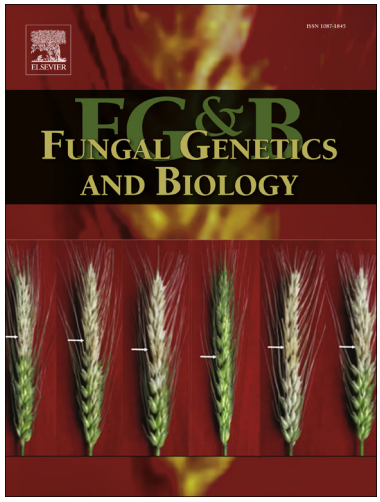

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Incorporation of non-canonical amino acids into proteins in yeast

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Abstract

Non-canonical amino acids add extraordinary chemistries to proteins when they gain access to translation. In yeast, they can be incorporated into proteins by replacing a canonical amino acid or in a site-specific manner in response to an amber stop codon. The first approach simply exploits the natural substrate tolerance of the aminoacyl-tRNA synthetases in an auxotrophic host. The latter requires the co-expression of an orthogonal aminoacyl-tRNA synthetase that is specific for the non-canonical amino acid together with an amber suppressor tRNA. This review briefly recaps the residue- and site-specific incorporation techniques for non-canonical amino acids in yeast. It describes the selection system for orthogonal aminoacyl-tRNA synthetase/suppressor tRNA pairs and compares the different expression systems for these pairs. Numerous examples illustrate the application of non-canonical amino acids for protein engineering in yeast. The compilation includes the chemical structures of the amino acid analogs, the orthogonal pairs that were used for their incorporation and the titers of the labeled variant proteins.

Keywords

genetic code expansion

non-canonical amino acid

orthogonal pair

protein engineering

Saccharomyces cerevisiae

Pichia pastoris