Protein synthesis: an ancient process with unexpected diversity throughout the living world.

Dieter Söll. Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06520-8114. dieter.soll@yale.edu

The ancient essential process of ribosomal protein synthesis requires twenty sets of aminoacyl-tRNAs, one for each canonical amino acid, for the correct transmission of the genetic information. It was commonly accepted that in order to carry out aminoacyl-tRNA synthesis every organism or organelle possesses twenty aminoacyl-tRNA synthetases. It is now clear that aminoacyl-tRNA formation is far more varied (1), as the biosynthetic routes to asparaginyl-tRNA, glutaminyl-tRNA, lysyl-tRNA and cysteinyl-tRNA vary greatly in nature. For instance, the amide aminoacyl-tRNAs (Asn-tRNA and Gln-tRNA) can be formed by two redundant mechanisms, direct acylation or pre-translational amino acid modification by amidation. Thus, the routes to amide aminoacyl-tRNAs differ not only in the three domains of life (Archaea, Bacteria, Eukaryotes) (2) but also vary among organelles (3). These transamidation enzymes appear to have evolved by recruitment of amino acid metabolizing enzymes. This possible evolutionary link between protein synthesis and amino acid biosynthesis is further highlighted by the discovery that tRNA-dependent amidation of aspartate appears to be the sole route to asparagine synthesis in most bacteria (4).

The discovery of a non-canonical lysyl-tRNA synthetase gave the first clues (5) on the amino-acylation of pyrrolysine, the 22nd cotranslationally inserted amino acid. Formation of pyrrolysyl-tRNA in the *Methanosarcinaceae* is catalyzed by an aminoacyl-tRNA synthetase-like enzyme solely specific for a modified amino acid (6). Similar enzymes carry out important other functions in protein synthesis (A. Sauerwald *et al.*, *Science*, in press).

New challenges to our understanding of tRNA biosynthesis and the role of the RNA intron emerge from the finding that the deep-rooted *Nanoarcheaon equitans* makes functional tRNA from encoded half-genes (7).

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