L-DOPA Ropes in tRNA^{Phe}

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L-DOPA is the most commonly prescribed drug for the treatment of Parkinson's disease. Here, Moor et al. (2011) report that phenylalanyl-tRNA synthetase catalyzes the misacylation of tRNA^{Phe} by L-DOPA, suggesting that it may contribute to the elevated levels of L-DOPA-containing proteins found in patients treated with this drug.

Aminoacyl-tRNA synthetases catalyze the attachment of amino acids to their cognate tRNA using a two-step mechanism in which the amino acid is first activated by ATP, forming an enzyme-bound aminoacyl-adenylate intermediate, then transferred to tRNA to form the aminoacyl-tRNA product. As misacylation of tRNA results in the incorrect amino acid being incorporated during protein synthesis, aminoacyl-tRNA synthetases must be highly specific, with error rates of less than 3×10^{-4} misacylations/turnover. For some aminoacyl-tRNA synthetases, discrimination by the active site is not sufficient and an editing mechanism is used to hydrolyze either the non-cognate aminoacyl-adenylate intermediate (pre-transfer editing) or non-cognate aminoacyl-tRNA product (post-transfer editing). Phenylalanyl-tRNA synthetases present an interesting case, the bacterial, archaeal, and eukaryotic cytoplasmic enzymes are $(\alpha\beta)_2$ heterotetramers with the active site located in the α-subunit and a post-transfer editing site located in the β-subunit (Roy et al., 2004). In contrast, the mitochondrial and chloroplast phenylalanyl-tRNA synthetases are monomeric enzymes that lack editing activity (Roy et al., 2005). The ability of the active site to discriminate between phenylalanine and tyrosine is ~15-fold higher in mitochondrial phenylalanyl-tRNA synthetase than it is in cytoplasmic phenylalanyl-tRNA synthetase, reflecting both the lack of an editing domain in the mitochondrial isozyme and a lower phenylalanine:tyrosine ratio in the mitochondria (Reynolds et al., 2011). Although misacylation of tRNA^{Phe} by L-tyrosine is minimal, Safro and colleagues have found that both cytoplasmic and mitochondrial phenylalanyl-tRNA synthetases are relatively efficient at catalyzing the misacylation of tRNA^{Phe} by *m*-tyrosine (3-hydroxyphenylalanine) (Klipcan et al., 2009). In this issue, Moor et al. (2011) have solved the structures of the *Thermus thermophilus* and human mitochondrial phenylalanyl-tRNA synthetases bound to L-DOPA (L-3,4-dihydroxyphenylalanine) and show that the *T. thermophilus*, human cytoplasmic and human mitochondrial phenylalanyl-tRNA synthetases all catalyze the misacylation of tRNA^{Phe} by L-DOPA. Both the *T. thermophilus* and human cytoplasmic phenylalanyl-tRNA synthetases also catalyze the hydrolysis of misacylated L-DOPA-tRNA^{Phe}, presumably via their editing domains. Although the efficiency of tRNA^{Phe} misacylation by L-DOPA is less than that for m-tyrosine, it is comparable to the tyrosyl-tRNA synthetase catalyzed misacylation of tRNA^{Tyr} by L-DOPA, suggesting that misacylation of tRNA^{Phe} may play a role in the incorporation of L-DOPA into proteins.

L-DOPA is the immediate biosynthetic precursor of the neurotransmitter dopamine and, unlike dopamine, is able to cross the blood-brain barrier. Co-administration of L-DOPA with a peripheral DOPA decarboxylase inhibitor (e.g., carbidopa) is used to treat Parkinson's disease and dopamine-responsive dystonia. L-DOPA has also been found to reduce transition state metals, resulting in oxidative damage to proteins, DNA, and other biomolecules (Rodgers and Dean, 2000). Oxidation of proteins containing L-DOPA leads to their aggregation and has been postulated to play a role in atherosclerosis, cataract formation, Alzheimer's disease, and Parkinson's disease (Fu et al., 1998; Molnár et al., 2005; Rodgers et al., 2006). Incorporation of L-DOPA into proteins can occur by oxidation of tyrosine residues, catalytic conversion of tyrosine to L-DOPA by tyrosinase, or misacylation of tRNA^{Tyr} (Rodgers and Dean,

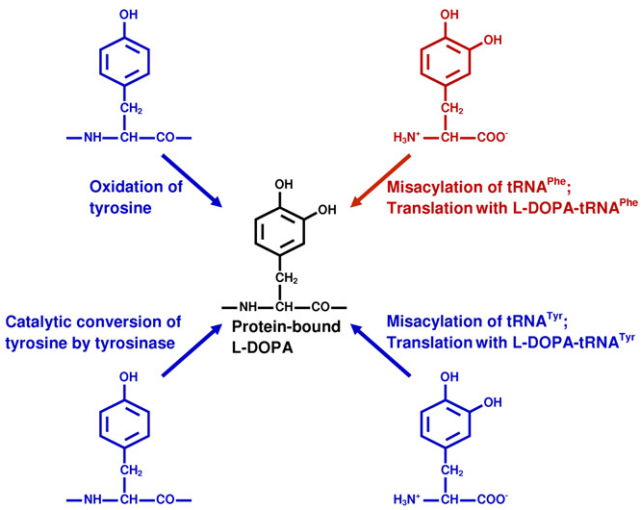

Figure 1. Routes to incorporating L-DOPA into proteins
Four pathways for the incorporation of L-DOPA into proteins are shown. Three of the pathways have previously been identified, while the fourth pathway—misacylation of tRNA^{Phe} by L-DOPA (shown in red)—is described by Moor et al. (2011) in this issue of *Chemistry & Biology*.

2000; Rodgers and Shiozawa, 2008). By demonstrating that phenylalanyl-tRNA synthetases can misacylate $tRNA^{Phe}$ with L-DOPA, Moor et al. (2011) provide a fourth pathway for incorporating L-DOPA into proteins (Figure 1). This new pathway may be particularly relevant to proteins synthesized by the mitochondria, given the inability of the mitochondrial phenylalanyl-tRNA synthetase to hydrolyze misacylated $tRNA^{Phe}$.

Incorporation of L-DOPA through misacylation of $tRNA^{Phe}$ is distinct from the other known mechanisms for incorporating L-DOPA into proteins, as it replaces a phenylalanine residue, whereas in the other three mechanisms L-DOPA replaces tyrosine. As a result, it should be possible to quantify the extent to which misacylation of $tRNA^{Phe}$ results in the incorporation of L-DOPA into proteins in vivo. Furthermore, since the efficiency of tRNA misacylation by L-DOPA appears to be similar for the phenylalanyl- and tyrosyl-tRNA synthetases, misincorporation of L-DOPA in place of phenylalanine may also provide an estimate for the frequency with which L-DOPA incorporation is due to misacylation of $tRNA^{Tyr}$. Given that L-DOPA is currently the most commonly prescribed drug for Parkinson's disease, it is imperative to determine the extent to which tRNA misacylation is responsible for the elevated levels of L-DOPA-containing proteins found in patients treated with this drug (Rodgers et al., 2006). The article by Moor et al. (2011) in this issue of *Chemistry & Biology* provides a way to address this question.

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Green-Red Flashers to Accelerate Biology

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Photoactivatable fluorescent proteins are now widely used for cell and protein tracking and super-resolution optical imaging. In this issue, Adam et al. (2011) report a general approach to introduce photochromism into green-to-red photoconvertible proteins and describe new photoactivatable protein with a complex four-state flasher-like behavior and advanced characteristics.

Green fluorescent protein (GFP) from jellyfish *Aequorea victoria* and its numerous mutants and homologs from diverse marine creatures possess a unique type of posttranslational modifications—a chromophore group formed via self-catalyzed reactions from its own internal amino acids (Chudakov et al., 2010). As a result, GFP-like fluorescent proteins (FPs) become fluorescent by themselves, with no help of external enzymes or cofactors except molecular oxygen. This extraordinary ability makes it possible to use FPs as fully genetically encoded labels to mark specific cells, cell organelles, and proteins and monitor process in live systems. Nowadays, they are an indispensable toolkit in experimental biology, which is used in thousands of studies each year.

A particularly interesting and useful group of FPs is photoactivatable FPs (PAFPs) capable of drastically increasing their fluorescence in a specific spectral region in response to illumination with light of specific wavelength and intensity (Chudakov et al., 2010). There are two main types of such proteins—irreversible and reversible PAFPs. Proteins of the first type photoconvert once from one state to another due to irreversible photochemical reactions of the chromophore (e.g., extension of its conjugated π-system) and/or surrounding amino acids (e.g., decarboxylation of a Glu residue) (Mizuno et al., 2003; Chudakov et al., 2010). PAFPs of the second type can be photoconverted multiple times due to *cis-trans* isomerization of the chromophore and/or reversible reactions such as protonation-deprotonation and hydration-dehydration (Chudakov et al., 2003, 2010; Brakemann et al., 2011).

One of the first irreversible PAFPs was green-to-red photoconvertible FPs called Kaede (after the Japanese word for maple leaf) (Ando et al., 2002). This green FP becomes red after illumination