

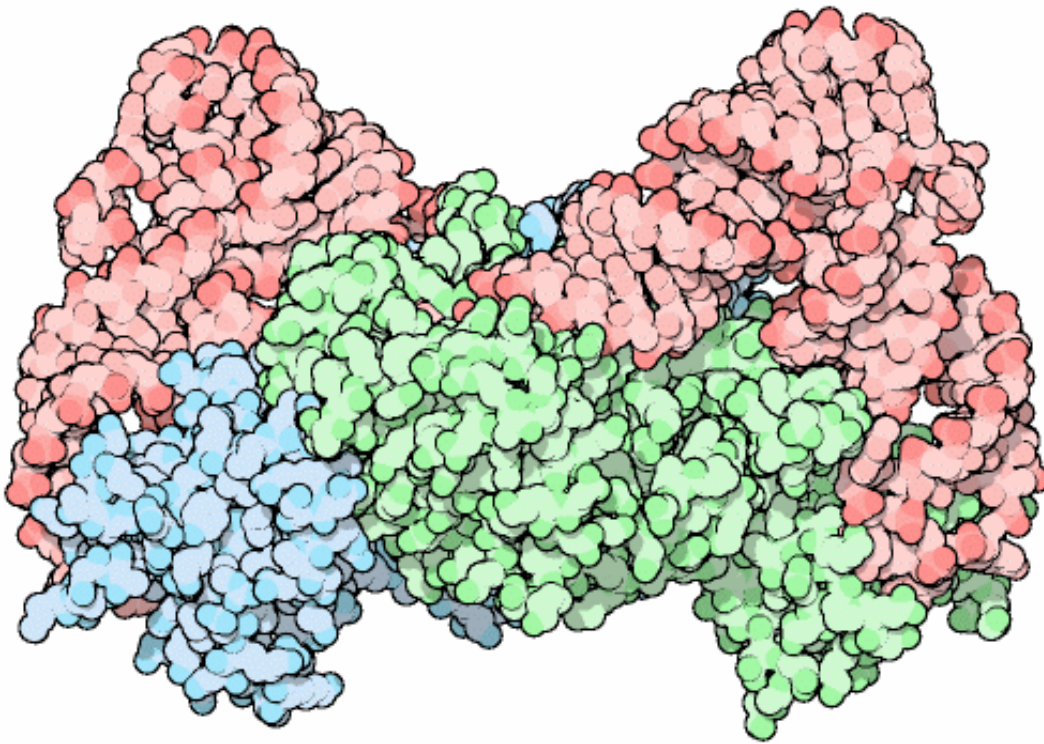
April 2001: Aminoacyl-tRNA Synthetases

When a ribosome pairs a "CGC" tRNA with "GCG" codon, it expects to find an alanine carried by the tRNA. It has no way of checking; each tRNA is matched with its amino acid long before it reaches the ribosome. The match is made by a collection of remarkable enzymes, the aminoacyl-tRNA synthetases. These enzymes charge each tRNA with the proper amino acid, thus allowing each tRNA to make the proper translation from the genetic code of DNA into the amino acid code of proteins.

Twenty Flavors

Most cells make twenty different aminoacyl-tRNA synthetases, one for each type of amino acid. These twenty enzymes are widely different, each optimized for function with its own particular amino acid and the set of tRNA molecules appropriate to that amino acid. The one shown, which charges aspartic acid onto the proper tRNA (entry [1asz](#)), is a dimer of two identical subunits (colored blue and green, the two tRNA molecules are colored red). Others are small monomers or large monomers, or dimers, or even tetramers of one or more different types of subunits.

April 2001: Aminoacyl-tRNA Synthetases



Finding the Proper Mate

As you might expect, many of these enzymes recognize their tRNA molecules using the anticodon. But this may not be possible in some cases. Take serine, for instance. Six different codons specify serine, so seryl-tRNA synthetase must recognize six tRNA molecules with six different anticodons, including AGA and GCU, which are entirely different from one another. So, tRNA molecules are also recognized using segments on the acceptor end and bases elsewhere in the molecule. One base in particular, number 73 in the sequence, seems to play a pivotal role in many cases, and has been termed the discriminator base. In other cases, however, it is completely ignored. Note also that each enzyme must recognize its own tRNA molecules, but at the same time, it must not bind to any of the other ones. So, each tRNA has a set of positive interactions that match up the proper tRNA with the proper enzyme, and a set of negative interactions that block binding of improper pairs. For instance, the aspartyl-

April 2001: Aminoacyl-tRNA Synthetases

tRNA synthetase recognizes the discriminator base and 4 bases around the anticodon. But, one other base, guanine 37, is not used in binding, but must be methylated to ensure that the tRNA does not bind improperly to the arginyl-tRNA synthetase.

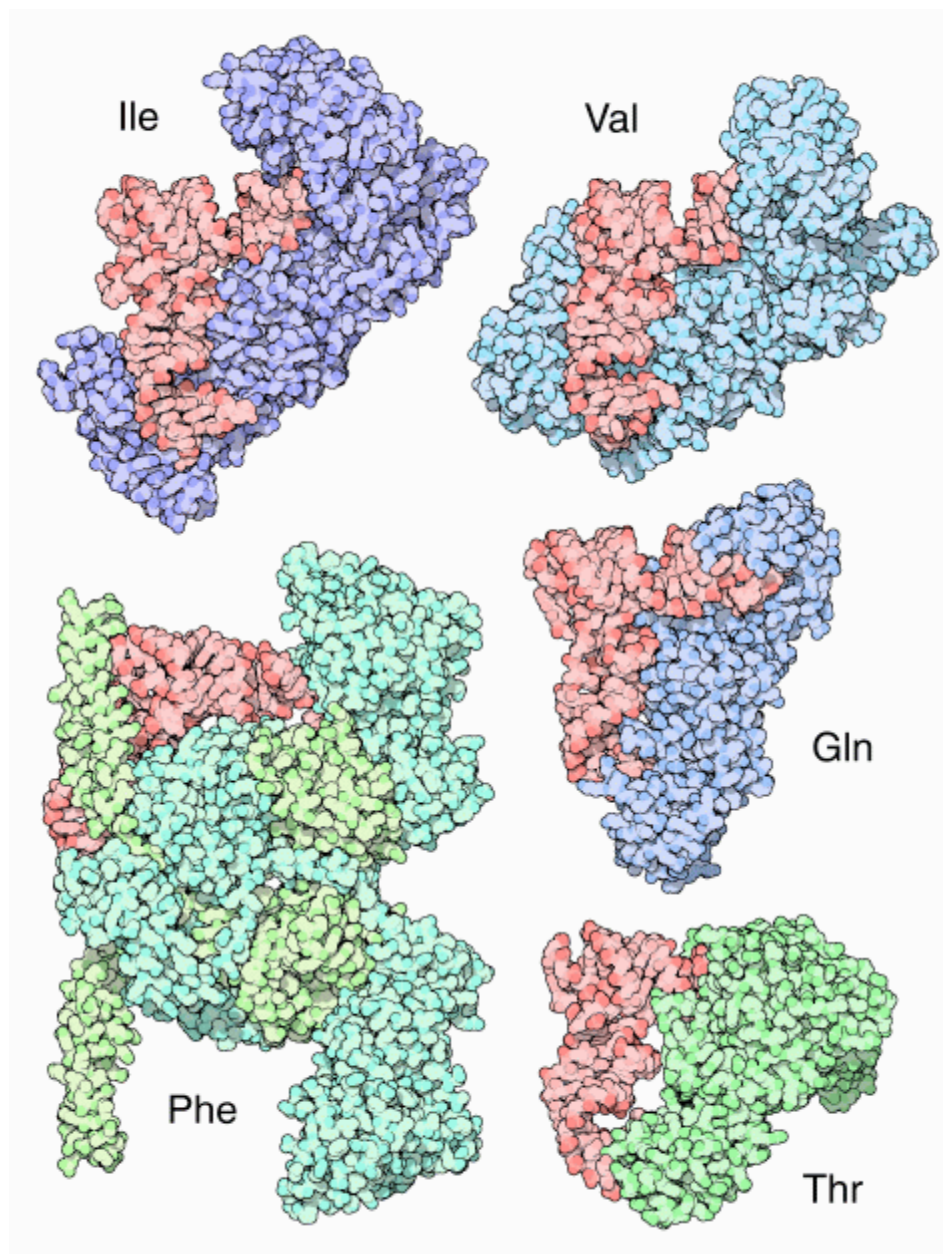
Surprises from Genome Analyses

Recent analyses of entire genomes revealed a big surprise: some organisms don't have genes for all twenty aminoacyl-tRNA synthetases. They do, however, use all twenty amino acids to construct their proteins. The solution to this paradox revealed, as is often the case in living cells, that more complex mechanisms are used. For instance, some bacteria do not have an enzyme for charging glutamine onto its tRNA. Instead, a single enzyme adds glutamic acid to all of the glutamic acid tRNA molecules *and* to all of the glutamine tRNA molecules. A second enzyme then converts the glutamic acid into glutamine on the latter tRNA molecules, forming the proper pair.

Different Approaches to the Same Problem

In the next picture, five complexes of an aminoacyl-tRNA synthetase with tRNA are shown, aligned so that the tRNA molecules (shown in red) are in the same orientation. Notice that the enzymes approach the tRNA from different angles. The isoleucine (entry [1ffy](#)), valine (entry [1gax](#)) and glutamine (entry [1euq](#)) enzymes cradle the tRNA, gripping the anticodon loop (at the bottom in each tRNA), and placing the amino-acid acceptor end of the tRNA in the active site (at the top right in each tRNA). These all share a similar protein framework, known as "Type I," approaching the tRNA similarly and adding the amino acid to the last 2' hydroxyl group in the tRNA. The phenylalanine (entry [1eiy](#)) and threonine (entry [1qf6](#)) enzymes are part of a second class of enzymes, known as "Type II." They approach the tRNA from the other side, and add the amino acid to the other free hydroxyl on the last tRNA base.

April 2001: Aminoacyl-tRNA Synthetases



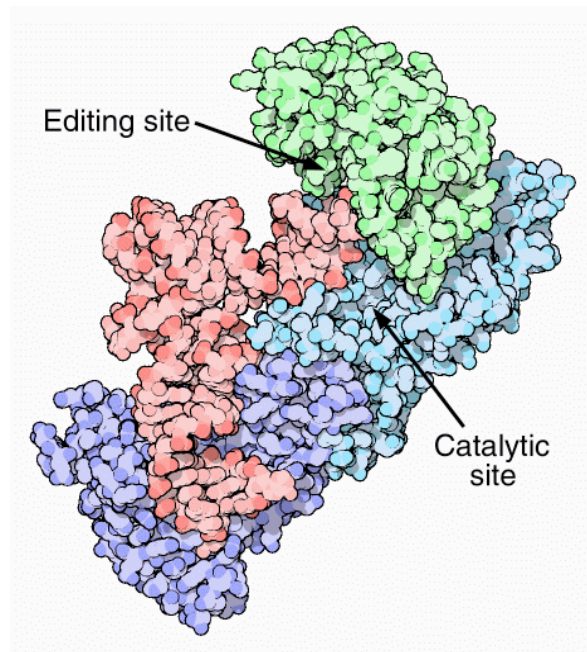
High Fidelity

Aminoacyl-tRNA synthetases must perform their tasks with high accuracy. Every mistake they make will result in a misplaced amino acid when new proteins are constructed. These enzymes make about one mistake in 10,000. For most amino acids, this level of accuracy is not too difficult to achieve. Most of the amino acids are quite different from one another, and, as mentioned before, many parts of the different tRNA are used for accurate recognition. But in a few cases, it is

April 2001: Aminoacyl-tRNA Synthetases

difficult to choose just the right amino acids and these enzymes must resort to special techniques.

Isoleucine is a particularly difficult example. It is recognized by an isoleucine-shaped hole in the enzyme, which is too small to fit larger amino acids like methionine and phenylalanine, and too hydrophobic to bind anything with polar sidechains. But, the slightly smaller amino acid valine, different by only a single methyl group, also fits nicely into this pocket, binding instead of isoleucine in about 1 in 150 times.



This is far too many errors, so corrective steps must be taken. Isoleucyl-tRNA synthetase (PDB entry [1ffy](#)) solves this problem with a second active site, which performs an editing reaction. Isoleucine does not fit into this site, but errant valine does. The mistake is then cleaved away, leaving the tRNA ready for a properly-placed leucine amino acid. This proofreading step improves the overall error rate to about 1 in 3,000.

April 2001: Aminoacyl-tRNA Synthetases

Exploring the Structure

These enzymes are not gentle with tRNA molecules. The structure of glutaminyl-tRNA synthetase with its tRNA (entry [1gtr](#)) is a good example. The enzyme firmly grips the anticodon, spreading the three bases widely apart for better recognition. At the other end, the enzyme unpairs one base at the beginning of the chain, seen curving upward here, and kinks the long acceptor end of the chain into a tight hairpin, seen here curving downward. This places the 2' hydroxyl on the last nucleotide in the active site, where ATP and the amino acid (not present in this structure) are bound.

