Aminoacyl-tRNA synthetases: catalysis and antibiotic (hyper)resistance

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Accurate protein synthesis relies on aminoacyl-tRNA synthetases (AARS), which translate the genetic code by linking cognate amino acid–tRNA pairs. Isoleucyl-RNA synthetase (IleRS) is an excellent model enzyme for studying the trade-offs between catalysis and antibiotic (hyper)resistance. In bacteria, two types of IleRS coexist, each capable of performing a housekeeping function. IleRS1, which is inhibited by the clinically relevant natural antibiotic mupirocin, and resistant IleRS2 with a lower affinity for the antibiotic. We discovered that some naturally occurring IleRS2 enzymes harbour a non-canonical version of the key catalytic motif, the HIGH motif. The motif alteration increases an inhibitory constant for mupirocin by 1000-fold, and, surprisingly, does not interfere with the housekeeping function of the hyper-resistant variant. Notably, no IleRS1 variants with the non-canonical HIGH motif have been found in nature while the laboratory-engineered versions are inactive. Structural data revealed a distinct conformation of the HIGH motif in IleRS2, allowing it to function with the non-canonical sequence. Interestingly, we found that IleRS1, although antibiotic-sensitive, is widely distributed in bacteria and is prevalent in the rapidly growing species. This aligns with our data showing that *Priestia megaterium* IleRS1 facilitates faster translation than IleRS2, possibly due to their different recognition of the tRNA substrate. It appears that the requirement for fast turnover shaped the evolution of IleRS1, likely limiting a rise of resistance. Our results pointed to the intriguing possibility that variations in the key catalytic motifs may serve as a strategy for the evolution of novel AARS features.