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The role of aminoacyl-tRNA synthetases as the enzymes responsible for attaching amino acids to their cognate tRNAs has been appreciated for more than 50 years. In the two step aminoacylation reaction, amino acid is first condensed with ATP to form an on enzyme adenylate intermediate [1]. Following the binding of tRNA, the amino acid is subsequently transferred to the 3' end of the tRNA. Given the requirement for high specificity for both the cognate amino acid and the tRNA, it is not surprising that there is a specific enzyme family associated with each amino acid. However, the partitioning of these families into two distinct structural classes of 10–11 families was unexpected [2], and motivated a longstanding research effort to discover the molecular basis of specificity, as well possible scenarios to explain the evolution of two distinct classes [3]. Prior to the discovery of the two classes, Linus Pauling noted the particularly challenge of discriminating between two amino acids differing only by a single methyl group [4]. The finding that some families of ARSs possess specific editing mechanisms to discourage the formation of misacylated tRNAs was therefore quite satisfying, but it would take many years of dedicated research to identify the kinetic principles underlying this mechanism [5, 6].

Since the millennium, the AARS field has been transformed by numerous reports describing functions of AARS and tRNA that extend beyond the canonical aminoacylation [7]. Among these many alternative functions include splicing of introns, immune cells, stimulation cellular chemotaxis, promotion of angiogenesis and regulation of immune cell functions [8]. Moreover, there is evidence to suggest that some of these alternative functions may involve relocation of ARS to compartments other than the cytoplasm, including the nucleus or the plasma membrane. In some cases, ARSs may need to be secreted from the cell in order to execute chemokines or angiocrine function [9, 10]. The description of alternative ARS functions has been accompanied by an accumulation of reports directly linking mutations in AARS genes to human diseases, particularly those affecting sensory or motor neuron function [7, 11]. With these new functions and new connections to human disease, a new volume describing tools and methods to studying aminoacyl-tRNA function is a timely development.

The ARS field features both a comprehensive monograph covering each of the ARS families in depth [12] and a number of different volumes that address techniques associated with reactions involving tRNA [13–15]. In particular, an earlier *Methods* volume on Aminoacyl-tRNA Synthesis covered many techniques associated with the aminoacylation reaction, and additional reactions where tRNA is a key substrate. In this volume of *Methods*, the emphasis is broadened to include approaches associated with our evolving view of AARS function, rather than just the more standard biochemical assays involving single enzymes and tRNAs in isolation. In the first set of contributions (First [16], Cvetesic [17], and Schwartz [18]) methods are described for continuous analysis of the aminoacylation and editing reactions

(First)[16], in vitro /in vivo analysis of AARS fidelity and editing (Cvetesic [16], Schwartz [18]), and the interaction of AARSs with inhibitors (Saint-Leger)[19]. This is followed by new approaches for synthetase structure, including AARS complex formation analysis by SAXS (Cantara)[20] and NMR (Cho)[21]; stability analysis by thermal shift profiles (Abbott)[22]; phosphorylation status of AARSs (Fox)[23]; and X-ray crystallography (Fang) [24]. Next are featured contributions examining methods for localizing AARS functions in cells, including the various compartments of yeast (Debard)[25]; in the nucleus (Shi)[26], in the mitochondria (Carapito)[27], and in various domains of the bacterial cell (Zhao)[28]. The functions of AARS in vivo are addressed in contributions from Mohler [29] and Miranda[30], which examine tRNA acylation in vivo and the remarkable ability of some AARSs to promote angiogenesis, respectively. Finally, we include a contribution (Oprescu) [31] describing the complex problem of assessing the pathogenicity of identified mutations in AARS genes and their relationship to human disease.

The growing reach of these classic enzymes of the translation system into distant corners of prokaryotic and eukaryotic cellular physiology suggests that the survey of methods provided here represents but a small part of the complete toolbox required to fully unlock the complete range of functions and activities. We hope that this volume of *Methods* proves valuable to workers in the field in facilitating that mission, and ultimately fill out the functional landscape of this diverse and distinctive enzyme superfamily.

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