A Complementary Approach to Studying Eukaryotic Glutaminyl-tRNA Synthetase: A Combination of SAXS, Crystallography and Molecular Modeling

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In all organisms, aminoacyl tRNA synthetases covalently attach amino acids to their cognate tRNAs. Many eukaryotic tRNA synthetases have acquired appended domains, whose origin, structure and function are poorly understood. The domains are predicted to be linked to the main protein by highly disordered regions. The N-terminal appended domain (NTD) of glutaminyl-tRNA synthetase (GlnRS) is intriguing since GlnRS is primarily a eukaryotic enzyme, whereas in other kingdoms Gln-tRNA(Gln) is primarily synthesized by first forming Glu-tRNA(Gln), followed by conversion to Gln-tRNA(Gln) by a tRNA-dependent amidotransferase. We crystallized the full length *Saccharomyces cerevisiae* GlnRS (Gln4), but the NTD was not visible in the structure. SAXS was used to examine the protein revealing an N and C-terminal domain connected by a flexible linker. We report a functional and structural analysis of the NTD and place this into the context of the complete protein aided by SAXS, computational modeling and knowledge of the NTD tertiary structure. The NTD consists of two subdomains, each exhibiting an extraordinary structural resemblance to adjacent tRNA specificity-determining domains in the GatB subunit of the GatCAB amidotransferase, which forms Gln-tRNA(Gln). These subdomains are connected by an apparent hinge comprised of conserved residues. Mutation of these amino acids produces Gln4 variants with reduced affinity for tRNA(Gln), consistent with a hinge-closing mechanism proposed for GatB recognition of tRNA. Our results, combining crystallography, SAXS and molecular modeling suggest a possible origin and function of the NTD that would link the phylogenetically diverse mechanisms of Gln-tRNA(Gln) synthesis. This specific example of the synergy between crystallographic and SAXS techniques is one of many studies performed by us developing high-throughput SAXS applications in collaboration with Hiro Tsuruta at SSRL.