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Genomic and functional analysis of non-conventional aminoacylation systems in pathogens

ABSTRACT

The term “unconventional (or unusual) aminoacylation systems” describes biosynthetic pathways that involve aminoacyl-tRNAs as essential factors, not only during protein synthesis but also in alternative processes (tRNA-dependent). During recent years, many functional genomic studies have demonstrated the unconventional role of aminoacyl-tRNAs outside protein synthesis and their participation in many different but equally essential biochemical pathways.

In the first part, the present dissertation deals with the study and biochemical characterization of the role of tRNA molecules during the tRNA-dependent conversion of aspartate to asparagine in the pathogen *Neisseria meningitides*. This pathway is catalyzed by the tRNA-dependent amidotransferase (AdT). This biosynthetic pathway is present in all pathogens with known genome and plays a dual role. It supplies the necessary Asn-tRNA^{Asn} substrates for the incorporation of asparagine into nascent polypeptides, but it also plays critical role on the biosynthesis of asparagine, in the organisms that lack the appropriate biosynthetic enzymes for this specific amino acid. It was found that the crucial determinant elements for recognition by bacterial amidotransferases constitute by the first base-pair U1-A72 of tRNA^{Asn} and the length and the sequence of the variable loop for the archaeal enzymes. In addition, an extra nucleotide in the D-loop of tRNA^{Asp} is the anti-determinant element that prevents the interaction with the amidotransferases.

In the second part, the present dissertation deals with the study and elucidation of the tRNA-dependent synthesis of the cell wall in the pathogen *Staphylococcus aureus*. The peptidoglycan moiety in this specific pathogen is stabilized through characteristic pentaglycine interpeptide bridges, which are synthesized independent of ribosomal activity. As donors of glycine this pathway utilizes Gly-tRNA^{Gly} molecules. However, until today it was not known, how many and which tRNA^{Gly} molecules are actually encoded, expressed and involved in this exo-ribosomal pathway of peptide synthesis. In the present dissertation was determined the exact number of genes encoding for tRNA^{Gly} isoacceptors that are expressed. In addition, biochemical studies lead to the characterization of specific tRNA^{Gly} isoacceptors as proteinogenic (those partipating solely into ribosomal protein synthesis, 2 molecules P1 and P2) and as non-proteinogenic (those devoted to exo-ribosomal bacterial cell wall synthesis, 3 molecules NP1, NP2 and NEW).

The study of both unconventional aminoacylation systems focuses on the essential role of tRNA molecules not only as passive adaptors during the flow of the genetic information but as key-players in essential biochemical pathways that are now considered as novel molecular targets for specific inactivation for the majority of pathogens.