In living organisms, the translation of the genetic code into proteins uses transfer RNA (tRNA) as adaptor molecule. This nucleic acid is highly modified in all domains of life. Over a 100 chemically distinct modifications are known to occur and influence the stability, structure and decoding ability of tRNA. One of the most common modification in tRNA is the addition of a methyl group. The enzymes catalysing these methylation reactions can be divided in 4 structural families, of which the SPOUT family is the second largest group. At present the mechanism by which these proteins recognise and select their target nucleotide on tRNA is not well understood. Interestingly, some SPOUT proteins were shown to have expanded specificity, modifying more than one positions on the tRNA or several nucleotides at one position. In this PhD thesis we shed light on the latter case by investigating homologous SPOUT proteins displaying a different specificity for their substrate nucleotide. Structural and biochemical analysis were performed on two SPOUT proteins: one which methylates the ribose moiety of a nucleotide in the anticodon loop of tRNA and one which methylates the base of a nucleotide in the core of the tRNA structure. The molecular structures of homologues of these proteins were determined at high resolution and together with mutagenesis data helped us identify the specificity determinants which govern whether a nucleotide will be substrate for the protein.

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