Abstract (EN)

A novel type of non-canonical 5′ RNA caps, dinucleoside polyphosphate RNA caps, has recently been discovered in our laboratory. To elucidate the physiological role of these caps, more detailed information about their synthesis is essential. It has been already described that dinucleoside polyphosphate RNA caps (Np_nN-RNA caps) can be accepted by RNA polymerase during transcription as a non-canonical initiating nucleotide (NCIN). However, the possibility that Np_nN-RNA caps are created post-transcriptionally cannot be ruled out. The best candidates for post-transcriptional capping enzymes are aminoacyl-tRNA synthetases, which, besides their crucial role in translation, are responsible for the synthesis of free dinucleoside polyphosphates. In this thesis, four *E. coli* tRNA synthetases have been selected, cloned into plasmids, and purified using fast protein liquid chromatography (FPLC). Subsequently, selected tRNA synthetases have been tested for the production of free dinucleoside polyphosphate. These experiments have identified the optimal conditions for production of free dinucleoside polyphosphates, diadenosine tetraphosphate (Ap_4A) particularly. The tRNA synthetases were then tested for their capabilities to form an RNA cap on *in vitro* transcribed radioactively labelled RNA. We found that tRNA synthetases are unable to form an Ap_4A-RNA cap and, therefore, do not act as an RNA capping enzyme. These results were confirmed by analysis on liquid chromatography coupled with mass spectrometry.

Keywords: Non-canonical RNA caps, dinucleoside polyphosphates, Ap_4A, Ap_4A-RNA, aminoacyl-tRNA synthetases, LysU