

Exogenously delivered messenger RNA (mRNA) is a promising therapeutic agent with potential uses in cancer immunotherapies, gene therapies, and cell reprogramming. Despite clear advantage of using mRNA over DNA (e.g. mRNA does not integrate with the genome or has not to be delivered to the cell nucleus) several limitations must be overcome before mRNAs can be considered as therapeutic agents. Recently, much effort has been made either to improve cellular instability and to increase translational properties of in vitro transcribed (IVT) mRNA in complex cellular environment. However, there is also additional issue which is currently intensively studied, how immunogenic properties of IVT mRNA could modulate cell immune response. It is well known that exogenous RNA (e.g. RNA viruses) is recognized as non-self RNA and triggers cell immune defence. Key for this recognition are differences in methylation status of 5' end of mRNA. Cellular mRNAs are co-transcriptionally modified by addition of N7-methylated guanosine joined by 5'-5' triphosphate bridge to the first transcribed nucleotide (cap-0). Subsequently, in higher Eukaryotes 5' end of mRNA undergoes further modification, the 2'-O position of the ribose of the first transcribed nucleotide is methylated (cap-1). This 2'-O methylation is necessary for further processing and export into the cytoplasm and importantly distinguish cellular mRNAs from viral ones. Moreover, in the cytoplasm cellular mRNA could undergo also further methylation at 2'-O position of the ribose of the second transcribed nucleotide (cap-2), however exact role of this methylation is still enigmatic.

Studies conducted so far on the cell immune response to exogenous RNA pointed out that immunogenic properties of IVT mRNA could be of great importance in its usage as therapeutic agent. It is believed that in some applications (e.g. in cancer immunotherapy or in allergy prevention) stimulation of innate immune system by IVT mRNA could be advantageous for directing immune responses against the encoded protein. However, strong immune response could lead to reduction of cell global translation rate or even could induce cell apoptosis. Only recently, the possibility to produce IVT mRNA with non-immunogenic cap structures (cap-1 and cap-2) became available. The methods of synthesis of cap-1 and cap-2 analogues were established in Laboratory of Bioorganic Chemistry (CeNT UW) and by others (TriLink BioTechnologies). Therefore, we propose to decipher the principles that govern recognition of IVT mRNA as non-self RNA by cell immune system. Moreover, we plan to study interplay between methylation status of mRNA 5' end and factors responsible either for sensing or for degradation of exogenous RNA. We expect that realization of the proposed project allows for a more conscious approach to mRNA design for therapeutic applications in the future.