

ABSTRACT

There is currently very few information about the translation of linear cytoplasmic plasmids occurred in yeast cells *Kluyveromyces lactis*. However, there is a relatively well developed information about their transcription apparatus. A study of transcript linear plasmids revealed an atypical organization at the 5' end. Those ends contain nontemplate polyadenylation and they are missing the N⁷ methylguanosine hat. Because of the presence of this structure, which is localized at 5' end of plasmids specific mRNA, raised a question regarding the initiation of the translation.

The present thesis is focused on the preparation of reporter system suitable for studying the influence of a number of the nontemplate adenosins, which were added at the 5' ends of mRNA linear plasmids. The first step was making a construction of dual yeast cell plasmids carrying two reporter genes, which are under the control of two different promoters. After a successful construction, the activity of promoters TEF1 and PGK1 was measured, whereby the promoter TEF1 proved twice stronger. The transcription start site of both promoter was determined. The second step was the construction of a reporter system directly in yeast cell plasmid pGKL. Reporter genes were under the control of two promoters originating from the pGKL plasmids. Those plasmids are the initiators of the creation of transcripts with a different structure. After a measuring of promoters strength, it was showed that their strength in comparison with the promoters TEF1 and PGK1 is very low.

Key words: translation initiation, regulation of gene expression, reporter system, luciferase, yeast, linear plasmids