ABSTRACT

Transcription is an essential step in the expression of genetic information. This process depends on protein complex of multisubunit RNA polymerases that are exceptionally conserved among all cellular organisms. These enzymes together with eukaryotic RNA-dependent RNA polymerases involved in gene silencing form a monophyletic protein family whose members contain two double-ψ β-barrel structural motifs in their active center. This family also includes a group of mainly *in silico* predicted non-canonical DNA-dependent RNA polymerases which differ from multisubunit RNA polymerases in reduced composition. Putative non-canonical RNA polymerase consisting of two subunits is also encoded by cytoplasmic linear plasmids of the yeast *Kluyveromyces lactis* and highly likely transcribes genes of these plasmids. Characterization of a unique transcription machinery of *Kluyveromyces lactis* plasmids with major emphasis on non-canonical RNA polymerase has become the aim of this work.

Bioinformatic analysis *in silico* was used to examine the evidence leading to an assumption of existence of specific RNA polymerase. Subsequent genetic and biochemical methods were used for: 1) production of putative RNA polymerase subunits in several expression systems; 2) testing interaction between several components of transcription apparatus; 3) epitope tagging of large subunit of RNA polymerase by recombination *in vivo*.

This work extended amino acid sequence similarity between putative RNA polymerase and other multisubunit RNA polymerases. Production and partial purification of putative RNA polymerase subunits was also achieved.

Keywords: transcription, multisubunit RNA polymerase, isolation, characterization, pGKL plasmids