Talk

Cloning and expression of a Saccharomyces cerevisiae RNA-dependent RNA polymerase

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Keywords: RNA-dependent RNA polymerase, cloning, expression, E. coli

ABSTRACT

RNA-dependent RNA polymerases (RdRp) are ubiquous enzimes first described in RNA viruses and virus-like elements. In Saccharomyces cerevisiae this enzyme can be found, fused with capsid proteins, encoded by the L-A helper virus-like particle. Replication cycle of L-A is coupled to that of M satellite particle, which confers a killer phenotype to the yeast (Schmitt & Breinig, 2002). RdRp recognizes and replicates ssRNA from both L-A and M virus. RdRp recognizes a 3'-Terminal Recognition Element (3'TRE), a small stem-loop 5 bases from the 3' end (Ribas, Fujimuras, & Wickner, 1994), which can be used as an effective tool for primerless replication of RNA molecules. To gain further insight on the virus-like particle replication mechanism and to evaluate its possible biotechnological application, the present project is focused on cloning the RdRp coding sequence in several different expression vectors and expressing the protein using different Escherichia coli strains as hosts. Once RdRp is efficiently expressed, we will proceed to purify it by affinity chromatography and test the protein activity by replicating full length viral RNA´s and other RNA sequences with or without the 3'TRE sequence. In our communication we will present our advances in cloning, expression, purification and activity of the yeast polymerase

REFERENCES

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