EXPRESSION OF GENES CLONED IN PLASMID VECTORS IN RECOMBINANT *ESCHERICHIA COLI* STRAINS

Summary

Use of *Escherichia coli* bacteria as a host for high-level expression of cloned genes has become common. The purification of a recombinant protein is greatly accelerated if the protein can be isolated from cells that overproduce it. To maximize expression, the cloned gene must be transcribed and translated as efficiently as possible. This is possible due to the construction of expression vectors, modified plasmids with useful features, which can be propagated and controlled in special hosts (expression systems). Usually, vectors for cloning and expressing target DNA are derived from medium-copy plasmids like pBR322. *E. coli* expression systems should meet several criteria including (*i*) minimal basal expression of the gene to be expressed under repressed conditions, (*ii*) fast and uncomplicated induction of a wide variety of genes to a high level of expression, and, (*iii*) easy cloning and DNA manipulation features. This article describes how the most common T7 expression system, derived from bacteriophage T7, functions. The system consists of a plasmid vector that allows cloning of the target DNA under T7 promoter control, and the T7 RNA polymerase gene borne by the recombinant bacterial host. The system is capable of expressing a wide variety of DNAs from prokaryotic and eukaryotic sources. In principle, the T7 system can be completely selective because host RNA polymerase and phage's polymerase recognize different promoters. However, synthesis of recombinant proteins, especially those that are toxic to the host, must be controlled, being at zero or non-toxic levels in uninduced cells, and high only after induction of expression during the appropriate phase of growth. Basal activity of the T7 RNA polymerase in uninduced cells is lower when the growth medium contains glucose (catobolite repression) and/or its natural inhibitor T7 lysozyme. Another way to reduce the basal expression of the target genes is to use expression vectors with lower or controllable copy numbers. The last possibility is offered by a new single-conditional-medium copy vector, a derivative of two replicons with different functions, *oriS*/RepE from plasmid F and *oriV*/TrfA from RK2 plasmid. The genetic elements from F plasmid enable stable maintenance of a single copy of the plasmid, while the RK2 replicon permits dosage-dependent gene expression and serves as the means for plasmid DNA amplification.