31

In Vitro Transcription and Translation

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1. Introduction

Large amounts of active mRNA can be synthesized in vitro. In vitro-transcribed RNA molecules may subsequently be used for various purposes, e.g., in vitro translation. Several plasmids are available that are used to produce transcript molecules from cloned DNA inserts. These contain one or more promoter sequences, recognized by the T3, T7, or SP6 RNA polymerase enzymes, flanking the multiple-cloning site. Once cloned, an insert, e.g., a DNA sequence coding for a viral coat protein (CP), may be transcribed into mRNA in a simple reaction exploiting these enzymes. It is generally thought that a mRNA molecule must contain a 7-methyl guanosine or cap structure at the 5' end to be efficiently translated ***(1)***, but we have found that sufficient quantities of proteins are generated for analysis from uncapped transcripts, at least in vitro.

In vitro translation provides a means of synthesizing proteins from mRNA in a microcentrifuge tube. Reactions are carried out in cell lysates, which contain all the macromolecular components necessary for protein synthesis, e.g., ribosomes, translation factors, tRNAs, and amino acids. Lysates are produced by disrupting the cells and removing the cell debris by centrifugation.

Two of the most useful translation systems are derived from rabbit reticulocytes ***(2)*** and from wheat germ ***(3)***. Both systems provide a reliable, convenient system to initiate translation and produce a full-sized polypeptide. Rabbit reticulocyte lysate (RRL) is favored for translation of larger mRNA molecules; wheat-germ lysate (WGL) is favored when low amounts of double-stranded RNA or oxidized thiols are present, which are inhibitory in RRL. Systems derived from *Escherichia coli* are also widely used ***(4)***.

Artificial translation systems are further supplemented with a chemical method of producing energy, phosphocreatine kinase, and phosphocreatine.

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