TOOL BOX 9.1

Production of recombinant proteins

Many proteins which may be used for medical treatment or for research are normally expressed at very low concentrations. Through recombinant DNA technology, a large quantity of a protein of interest can be produced. This is called "overexpression" of the recombinant protein. Production of recombinant proteins involves the cloning of the cDNA encoding the desired protein into an "expression vector." The expression vector contains a promoter so that the cDNA can be expressed. Next, the recombinant expression vector is introduced into a bacterial or eukaryotic host cell to allow overexpression of the protein. There are also systems available for *in vitro* translation.

Overexpression of recombinant proteins in bacteria

A commonly used bacterial expression vector contains the *lac* promoter upstream of a multiple cloning site (see Section 10.5 for details). The lactose analog isopropylthiogalactoside (IPTG) stimulates the expression of a cloned cDNA encoding the protein of interest (Fig. 1A). Depending upon the particular application, cloned genes can be expressed to produce native proteins (in their natural state) or fusion proteins, where the foreign polypeptide is fused to a vector-encoded epitope tag (e.g. GST, His, or FLAG). Native proteins are preferred for therapeutic use because fusion proteins can be immunogenic in humans.

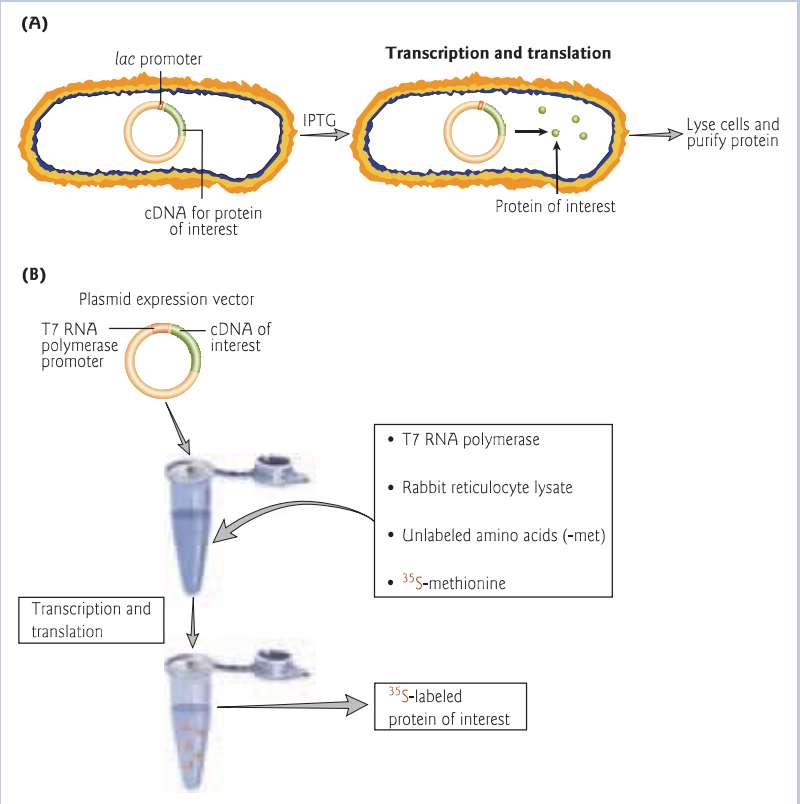

Figure 1 Comparison of the production of recombinant proteins in a bacterial host and by *in vitro* translation. (A) Production of recombinant proteins involves the cloning of the cDNA encoding the protein of interest into an expression vector. The expression vector typically contains a *lac* promoter so that the cDNA can be expressed in the *E. coli* host cell. Upon induction with IPTG the recombinant protein is expressed. (B) Proteins can be translated *in vitro* in a rabbit reticulocyte lysate. The lysate is a cell-free extract made from red blood cell precursors. The lysate contains the cellular components necessary for protein synthesis (tRNA, ribosomes, initiation, elongation, and termination factors). In this system, the cDNA is cloned into an expression vector under control of a phage RNA polymerase promoter, such as SP6, T3, or T7. Upon addition of the appropriate phage polymerase to the cell lysate, transcription of an mRNA transcript occurs, followed by translation. If a radioactively labeled amino acid, such as ^{35}S-methionine, is included in the reaction mixture, then a labeled protein is produced.

Production of recombinant proteins

TOOL BOX 9.1

However, fusion proteins are usually more stable in *E. coli* because they resemble endogenous proteins, whereas native proteins can be targeted for degradation. Fusion proteins also offer another advantage: they can be easily purified (e.g. by affinity chromatography). It is sometimes possible to cleave the vector-derived polypeptide from the fusion protein using specific proteases, to yield native protein.

Although the overexpression of cloned genes in *E. coli* has facilitated the industrial-scale synthesis of many prokaryotic and eukaryotic proteins, there are a number of problems associated with this system. Overexpressed foreign proteins often can form insoluble inclusion bodies which must be broken up by harsh chemical treatments that denature proteins. *E. coli* often fails to fold and process eukaryotic proteins properly, probably because it lacks the molecular chaperones present in eukaryotic cells. For example, post-translational modification such as cleavage or glycosylation does not take place, and correct disulfide bonds do not form.

Overexpression of recombinant proteins in eukaryotic cells

Where bacterial cells fail to process expressed proteins correctly, eukaryotic cells may be used as alternative expression hosts. Three types of eukaryotic hosts are used for protein overexpression: yeast, insect cells, and mammalian cells. The insect cell system involves a baculovirus expression system where foreign genes are overexpressed from the strong promoter of a nonessential polyhedrin gene in baculovirus-infected insect cells. Mammalian cell systems are the least efficient of the three; however, sometimes they are the only option for producing recombinant protein if processing or post-translational modification does not occur correctly in other cells.

***In vitro* translation of recombinant proteins**

In vitro translation in a cell-free extract can be used to produce small quantities of labeled protein for analysis from purified mRNA or from a DNA template (Fig. 1B). If a radioactively labeled amino acid is included in the reaction mixture, then a labeled protein is produced.