SECTION III

EXPRESSION OF PROTEINS IN MAMMALIAN CELLS

Overview of Protein Expression in Mammalian Cells

UNIT 16.12

As described elsewhere in this manual (Chapter 9), mammalian cells are often used as hosts for expression of genes obtained from higher eukaryotes because the signals for synthesis, processing, and secretion of these proteins are usually recognized. The units that follow in Sections III and IV describe three vector systems or strategies for introducing foreign genes into mammalian cells (additional transfection methods can be found in *UNITS 9.1-9.4*). The first method relies upon COS cells for rapid, transient expression of protein from specific vectors (*UNIT 16.13*). The second method relies upon Chinese hamster ovary (CHO) cells; in this procedure, a targeted gene is cotransfected with a selectable marker, becomes stably integrated into the host cell chromosome, and is subsequently amplified (*UNIT 16.14*). The third system relies upon vaccinia viral vectors in a transient expression system (*UNITS 16.15-16.19*). The systems described in these units differ in the ways in which DNA is introduced into the cell, in the particular vectors used with each system, and in their suitability for particular cell types.

The criteria for choosing a certain system include these considerations: whether DNA can be introduced directly by transfection methods or needs to be introduced by viral-mediated transfer, the identity of the control elements that can direct efficient mRNA expression and protein synthesis, and whether a particular host cell is appropriate for expression of the gene of interest. If it is necessary to produce a large amount of protein for a long period of time, the CHO system should be utilized. When transient expression is appropriate, the choice of which system to use depends upon the particular experiment. When a high transfection efficiency is necessary, the vaccinia system is appropriate because every cell can be infected with the virus and gene of interest; however, this system suffers from the disadvantage that the cells die within one to two days. If a lower transfection efficiency is sufficient and if it is desirable that the cells continue to grow for several days, COS cells should be used.

VIRAL-MEDIATED GENE TRANSFER

Viral-mediated gene transfer provides a convenient, efficient means to introduce foreign DNA into most recipient cells. Representative expression levels obtained from SV40 recombinant viruses, retroviruses, and vaccinia viruses are shown in Table 16.12.1 in comparison to other expression strategies. A more detailed review of the different eukaryotic viral vectors can be found in Muzyczka (1989).

UNITS 16.15-16.19 address one viral vector system, vaccinia virus, which has demonstrated success. Vaccinia virus is most useful for the production of proteins (such as regulatory factors) that are potentially toxic to the cell.

TRANSIENT EXPRESSION

The efficiency of expression from transient transfection depends on the number of cells that take up the transfected DNA, the gene copy number, and the expression level per gene. Most methods of DNA transfer allow 5% to 50% of the cells in the population to acquire DNA and express it transiently over a period of several days to several weeks. Transient DNA transfection is most frequently used to: (1) verify the identity of cloned genes based on their ability to express a particular activity, (2) rapidly study the effect of engineered mutations on either gene activity or protein function, and (3) isolate genes from cDNA libraries constructed in mammalian expression vectors based on their ability to express a particular activity in cells. The limitations of transient expression are that it is difficult to scale up for production of large quantities of protein (>1 mg), that it is difficult to study the consequences of gene expression only in the portion of the total population that has been transfected, and that the high copy number is eventually lethal; this lethality may significantly affect results.

16.12

Table 16.12.1 Expression Levels and Uses for Different Mammalian Cell Expression Systems[a]

Cell line	Expression method	Typical expression level (μg/ml)	Primary use
Monkey cells			
CV1	SV40 virus infection	1-10	Expression of wild-type and mutant proteins
COS	Transient DNA/DEAE-dextran transfection	1	Cloning by expression in mammalian cells; rapid characterization of cDNA clones; expression of mutant proteins
CV1	Transient DNA/DEAE-dextran transfection	0.05	
Murine fibroblasts			
C127	BPV stable transformant	1-5	High-level constitutive protein expression
3T3	Retrovirus infection	0.1-0.5	Gene transfer into animals; expression in different cell types.
Other cells			
CHO(DHFR$^-$)	Stable DHFR$^+$ transformant	0.01-0.05	
	Amplified MTX^r	10	High-level constitutive protein expression
Primate	Vaccinia virus infection	1	Production of vaccines; expression of toxic proteins
	EBV vector	n.a.	Cloning by expression

[a]Abbreviations: BPV, bovine papilloma virus; EBV, Epstein-Barr virus; n.a., not applicable.

UNIT 16.13 describes procedures and vectors used for transient expression in COS cells. This cell line is most frequently used for transient expression and is derived from African green monkey kidney cells by tranformation with an origin-defective simian virus 40 (SV40). COS cells express high levels of the SV40 large tumor (T) antigen which is required to initiate viral DNA replication at the origin of SV40. T antigen–mediated replication can amplify the copy number of plasmids containing the SV40 origin of replication to 100,000 per cell, which results in high expression levels from the transfected DNA.

STABLE DNA TRANSFECTION

If a selection procedure is applied after DNA transfection, it is possible to isolate cells that have stably integrated the foreign DNA into their genome (*UNIT 9.5*). Different cell lines exhibit different frequencies of stable transformation and vary in their capacity to incorporate foreign DNA. In most cases, the limiting factor for obtaining stable transformants is the frequency of DNA integration, not the frequency of DNA uptake. Cells selected for by incorporation and expression of one genetic marker will frequently incorporate a second gene provided on an independent plasmid during transfection; this ability to incorporate two separate plasmids into the chromosome has been termed cotransformation. Different cell lines and transfection methods yield varying frequencies of cotransformation.

Many recessive genetic selectable markers encode enzymes involved in the purine and pyrimidine biosynthetic pathways.

AMPLIFICATION OF TRANSFECTED DNA

Frequently, it is desirable to increase expression by selecting for increased copy number of the transfected DNA within the host chromosome. The ability to coamplify transfected DNA has permitted a 100- to 1000-fold increase in the expression of the proteins encoded by transfected DNA. Although there are over twenty selectable and amplifiable genes that have been described (Kaufman, 1990a), the most experience and success has occurred when methotrexate selection has been used for amplification of transfected dihydrofolate reductase genes. *UNIT 16.14* describes the use of dihydrofolate reductase–deficient CHO cells to obtain high-level expression of heterologous genes through coamplification by selection for methotrexate resistance.

EXPRESSION VECTORS

Most mammalian cell expression vectors are designed to accomodate cDNAs rather than large genomic fragments because the small size of cDNA clones makes them more convenient to manipulate. Today most useful vectors contain multiple elements including: (1) an SV40 origin of replication for amplification to high copy number in COS monkey cells, (2) an efficient promoter element for high-level transcription initiation, (3) mRNA processing signals such as mRNA cleavage and polyadenylation sequences, and frequently intervening sequences as well, (4) polylinkers containing multiple restriction endonucleases sites for insertion of foreign DNA, (5) selectable markers that can be used to select cells that have stably integrated

the plasmid DNA, and (6) plasmid backbone sequences to permint propagation in bacterial cells.

CHOICE OF EXPRESSION SYSTEM

In evaluating which approach to take in expressing a gene, it is most important to consider the goals of the expression work. If expression is required to demonstrate that a clone has some functional activity or to characterize this activity, then transient expression in COS cells is often the most convenient approach. If a large quantity of protein (>1 mg) is required, then stable coamplification in CHO cells is generally the most desirable approach. If the gene is potentially cytotoxic, high-level expression may be approached through vaccinia virus vectors or inducible promoter-vector systems. If there is a particular requirement for the host to produce the protein properly then that requirement will dictate the choice of the host.

16.12

TROUBLESHOOTING

If protein expression from the heterologous gene cannot be detected, it is important to examine the vector system in detail. In this sequence, each point should be satisfactorily addressed before proceeding to the next step.

1. Confirm the expected structure of the vector using restriction mapping (*UNITS 3.1 & 3.2*) and, if necessary, DNA sequencing (*UNITS 7.4 & 7.5*).
2. Determine transfection efficiency by including a positive control—e.g., the same vector with another insert.
3. Ensure that the RNA is of the expected size and amount compared to an appropriate control by preparing RNA (*UNITS 4.1, 4.2, & 9.8*) and analyzing it by northern hybridization (*UNIT 4.9*).
4. Use a completely different expression vector or system (*UNITS 16.13-16.18*) if the RNA transcript of the correct size cannot be detected in the transfected cells, as it is always possible that some unforeseen situation may result in aberrant splicing (Wise et al., 1989).
5. Determine if the coding region may contain a point mutation or other lesion that keeps it from encoding a full-length protein by carrying out in vitro translation to produce protein (*UNIT 10.17*) using mRNA isolated from transfected cells and using RNA transcribed by in vitro transcription (i.e., SP6; *UNIT 3.8*) of a vector containing the cDNA insert.

Reference: Kaufman, 1990.

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