15.3. GENE THERAPY USING VIRAL VECTORS

Gene therapy is the transfer of one or more genes into cells for a therapeutic effect. It can be done *ex vivo* (outside the body) in tissues that are transplanted back into the patient or *in vivo,* where the genes, usually in a delivery vehicle such as a virus, are injected into the patient.

Gene therapy is intellectually connected to metabolic engineering. However, the complexity of humans presents an even greater challenge than the metabolic engineering of a single cell. Gene therapy is a quantitative problem that makes good use of the quantitative skills of engineers. Basically the right gene needs to be delivered to only the right tissue target in the right amount, with the gene products being expressed at the right level at the right time for the right length of time. For gene therapy to be effective many things have to go right. Clinical success with gene therapy has been minimal, as approaches have been qualitative and trial-and-error in nature. A rational analysis would be a useful tool.

Many methods can be used for gene delivery. These involve viral vectors, use of naked DNA, and liposome or particle-mediated gene delivery. In this chapter we will focus only on viral systems. Bioprocess technology is necessary for production of the viral vectors, and analyses arising from bioprocess studies are applicable to gene therapy.

15.3.1. Models of Viral Infection

The three primary virus vectors for gene therapy are retroviruses (derived from a wild-type virus that infects mice), adenoviruses, and adeno-associated viruses. Retroviruses are enveloped viruses, because they are encapsulated in a lipid bilayer membrane. The adenovirus and adeno-associated viruses are nonenveloped viruses. The model of viral trafficking that we discuss below is for enveloped viruses.

Dee et al.[†] proposed a model for the viral trafficking of Semliki Forest virus (SFV), an enveloped RNA virus that has been considered as a vector for large-scale production of heterologous proteins. However, this analysis, which was motivated by a bioprocess application, is applicable to retrovirus vectors for gene therapy.

As indicated in Fig. 15.2, enveloped RNA viruses can enter cells through receptor-mediated endocytosis. The virus binds to specific receptor molecules on the cell's surface. It is assumed (for this analysis) that attachment is irreversible and that the number of receptor molecules is much greater than the number of virus particles present. Under this circumstance:

$$dV_{ex}/dt = -k_a C V_{ex} \tag{15.1}$$

where V_{ex} is the number of extracellular viruses per cell, C is the cell concentration, and k_a is the attachment-rate constant. The value of k_a can be estimated as:

$$k_a = k_f(\alpha R) \tag{15.2}$$

where k_f is the intrinsic forward rate constant for the binding of a single viral attachment protein to a receptor, α is the number of viral attachment proteins per virus, and R is the

[†]K. U. Dee, D. A. Hammer, and M. L. Shuler, *Biotechnology Bioengineering 46:* 485–496 (1995).