$$V_{cyt} = \frac{k_{fus}\eta k_e k_a C V_{exo}}{k_e - k_a C}\left\{\frac{1}{k_{fus}\eta + k_{tran} - k_a C}\left[\frac{1}{k_a C}(1 - e^{-k_a C t}) + \frac{1}{k_{fus}\eta + k_{tran}}(e^{-(k_{fus}\eta + k_{tran})t} - 1)\right]\right.$$
$$\left. - \frac{1}{k_{fus}\eta + k_{tran} - k_e}\left[\frac{1}{k_e}(1 - e^{-k_e t}) + \frac{1}{k_{fus}\eta + k_{tran}}(e^{-(k_{fus}\eta + k_{tran})t} - 1)\right]\right\} \tag{15.12}$$

For wild-type SFV infecting baby hamster kidney (BHK) cells the rate parameters have been estimated as: $k_a = 5.2 \times 10^{-9}$ ml/cell-min.; $k_e = 0.039$ min^{-1}; $k_{fus}\eta = k_{tran} = 0.14$ min^{-1} (based on 50% of internalized virus being uncoated). Such a model with these parameters fits time-course measurements from actual experiments and can also be used to make estimates of response if mutant virus forms are used.

These same equations can easily be applied to an *ex vivo* system for gene therapy. For *in vivo* use they apply in principle, but the presence of various natural compounds in the bloodstream can alter k_a (and potentially other parameters), and multiple cell types are present which may bind, but not endocytose virus. A pharmacokinetic model (to predict time-dependent virus distribution at different positions in the body) would need to be coupled with virus/cell interaction models (of the type developed here) for each major cell type. The reader can easily see why *ex vivo* therapy would be much easier to design than an *in vivo* protocol. For either the *ex vivo* or *in vivo* therapies to be successful, there must be a method to efficiently produce virus.

15.3.2. Mass Production of Retrovirus

The efficient production of a highly concentrated solution of retroviruses that have been genetically modified to deliver a gene is difficult. Genetically engineered retroviruses, like all recombinant viruses, are produced by a two-part system consisting of a packaging cell line and the recombinant vector. This two-part system is necessary because the recombinant virus is unable to replicate itself. The recombinant virus is derived from a wild-type virus in which essential viral genes have been deleted and replaced with the therapeutic gene of interest. The enzyme reverse transcriptase converts RNA-encoded genes into DNA, which is transported into the nucleus and integrated into the cell's chromosomal DNA.

The packaging cell line is genetically engineered to produce the essential, structural viral genes that have been deleted from the viral genome. Because the viral genes are encoded in the packaging cell's chromosome, the resulting viral particles are incapable of causing disease, but act as carriers for the desired, therapeutic genes. The resulting retrovirus vector can be used only with dividing cells, since cells must undergo mitosis before gene integration can occur. This feature limits *in vivo* use to cases such as cancer suppression but is not a limitation on *ex vivo* systems.

Another limitation on the retrovirus system is a bioprocess limitation; the production of high-titer virus and subsequent purification and concentration without loss of infectivity have been difficult. A major limitation to the effectiveness of many gene-therapy approaches, including the use of retroviruses, is that the average number of genes delivered to target cells is too low to achieve a beneficial therapeutic effect. This low efficiency is due to the bioprocess limitation on production of a concentrated, highly infectious retrovirus preparation.