Safety aspects related to recombinant protein expression from Semliki Forest virus vectors

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Abstract
Semliki Forest virus vectors (SFV) have been developed for efficient transgene expression to result in high receptor yields (50–200 pmol receptor/mg protein) in a variety of mammalian host cells. Transfer of the SFV technology to mammalian cells growing in suspension cultures has made it feasible to produce hundreds of milligrams of receptor proteins in a short time. Large-scale production, however, raises the questions of the safety of handling virally infected cells for down-stream processing. Analysis of cell culture medium and SFV-infected cells revealed that some infectious particles were still present. Replacement of virus-containing medium at 2 h post-infection efficiently removed the majority of infectious replication-deficient SFV particles. Washes with PBS further reduced the number of infectious particles significantly both in the medium and associated with cells to levels that allowed safe handling of SFV-infected cells outside the cell culture facility for biochemical, pharmacological, or electrophysiological assays or down-stream processes in connection to receptor purification. Furthermore, engineering of novel temperature-sensitive mutant SFV vectors resulted in temperature-controlled transgene expression, which completely eliminates the risk of contaminating laboratory personnel.

Introduction
Viral vectors have played an important role in recombinant gene expression because of their high capacity of gene delivery and strong promoter activity. On the other hand, viral vectors capable of infecting mammalian cells, and particularly human cells, have always posed a threat concerning safety issues, especially when applied for large-scale recombinant protein production, where large volumes of infected cells are handled. It is therefore of utmost importance that the highest possible safety standards are met when viral vectors are employed.

The Semliki Forest virus (SFV) expression system is based on an expression vector containing the viral nonstructural genes and the foreign gene(s) of interest and a helper vector supplying the structural genes in trans. The replication-deficient recombinant SFV particles generated from co-transfections of RNA from the expression and helper vectors are capable of one round of infection of host cells. After infection the viral recombinant RNA will be amplified directly in the cytoplasm due to the presence of the replicase complex (encoded by the nonstructural genes), which will lead to extreme overexpression of the foreign gene of interest. SFV vectors have earlier demonstrated high-level expression of topologically different (nuclear, cytoplasmic, membrane and secreted) proteins (Liljestrom and Garoff, 1991; Lundstrom, 1999). The broad host range of SFV has allowed expression studies in a variety of cell lines (mammalian, insect, amphibian, reptilian cells) and in primary cell cultures (fibroblasts, hepatocytes, neurons). Particularly, G protein-coupled receptors (GPCRs), which due to their seven transmembrane domain topology have been difficult to express from many vectors, were efficiently expressed from SFV vectors (Lundstrom et al., 1994). It was also possible to transfer the SFV technology to mammalian cells adapted to growth in suspension cultures (Blasey et al., 1997). This has sub-
Figure 1. Infection kinetics of SFV-GFP on adherent BHK cells. BHK cells on 6-well plates were infected with SFV-GFP at a multiplicity of infection (MOI) of 10 for 2, 5, 15, 30, 60 and 120 min. Virus-containing medium was carefully removed and the cells washed with PBS. GFP expression was microscopically visualized at 24 h post-infection.