Short Communication

Interruption of env Gene Expression Depending on the Length of the SV40 Early Region Used for the PolyA Signal

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SUMMARY: In order to invent a screening system to check in vivo gene function and the efficiency of gene transfer mediated by a retroviral vector system, we established a novel packaging cell, PacNIH/A8, based on the neuropathogenic retrovirus A8-V. To construct the expression vector, pA8(Ψ–), which expresses the genes gag, pol and env derived from A8-V, the SV40 early region was used for the polyadenylation signal (polyA signal). When a 0.85 kbp fragment in the SV40 early region was employed for the expression vector (pA8(Ψ–)β), env expression was abolished. This abolition was rescued by shortening the SV40 early region to 0.14 kbp (pA8(Ψ–)δ). The NIH3T3 cells transfected with pA8(Ψ–)δ showed expressions of both env and gag genes.

A8-V is a molecular clone of the neuropathogenic variant FrC6-V (1, 2), isolated from Friend murine leukemia virus (Fr-MLV). FrC6-V and A8-V induce spongeform degeneration mainly in the grey matter of the central nervous system (CNS), including the brain cortex, thalamus and brain stem of infected rats (3). Studies using chimeric constructed from the A8 virus and non-neuropathogenic Fr-MLV clone 57 (57-V) revealed that the env gene of A8 is a primary determining factor for the induction of neurodegeneration, and the LTR and/or 5' leader sequence of A8 is also necessary for neuropathogenicity (2,4,5). The efficient entry of these neuropathological viruses into the CNS of rats is mediated by an interaction between the viral surface protein (Env), a product of the env gene, and F10-ecoR, which is a receptor for MLV infection isolated from the rat glial cell line F10. The efficiency of the F10-ecoR-mediated entry of the pseudotyped virus carrying Env protein of A8 was an order of magnitude greater than that of the pseudotyped virus carrying Env derived from the 57-V gene (8). These findings prompted us to construct a retroviral vector system to transfer genes of interest into the rat CNS using the genes of A8-V.

An expression vector for Gag, Pol and the Env protein of A8-V was constructed. The vector contained LTR, gag, pol and the env gene of A8-V and the SV40 early polyA signal, but did not have a packaging signal (pA8(Ψ–)), as shown in Fig. 1. In order to select stable transformants, the blastocidin deaminase (BSD) gene was introduced into the vector. This construct was transfected into the NIH3T3 cell line by calcium phosphate co-precipitation as reported elsewhere (2). First, we obtained pA8(Ψ–)β cells, which employed a 0.85 kbp fragment in the SV40 early region for the polyA signal (Fig. 1). We did not detect transient env expression in pA8(Ψ–)β-transfected cells (data not shown). Next, we employed the shortened SV40 early region (0.14 kbp) for the polyA signal, and pA8(Ψ–)δ was constructed (Fig. 1). The drug-resistant cells were selected in medium containing 1.25 μg/ml of blastocidin (Invitrogen, Carlsbad, Calif., USA) and cloned. Thirty clones from the cells transfected with pA8(Ψ–)β (PacNIH/A8β) and 24 clones from pA8(Ψ–)δ-transfected cells (PacNIH/A8δ) were established (9). The cloned cells were seeded on glass slides, cultured overnight, and then fixed in 100% ethanol for 2 min. Each clone was examined by immuno-histochemistry to determine whether it expressed Env or Gag proteins (Fig. 2).

In PacNIH/A8β cells, most of the clones expressed the Gag protein (Table 1), whereas only 3 clones expressed the Env protein. The ratio of the clones that expressed both Env and Gag proteins (Env+, Gag+)) to the clones that expressed

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Gag protein alone (Gag+) was 3/24 (in other words, 21/24 of the PacNIH/A8β cell clones expressed the Gag protein only, without expressing the Env protein [Env−, Gag+]). No clone expressed the Env protein only (Env+, Gag−). In contrast, all of the 13 Gag-protein-expressing clones of PacNIH/A8δ cells expressed the Env protein as well (13/13). Also, in the PacNIH/A8δ cells, no clone was found to be grouped into the (Env+, Gag−) expression type. These results suggested that Env protein expression is less stable than Gag protein expression. In order to elucidate whether this labile Env expression is due to incomplete DNA insertion, we performed Southern blotting for the PacNIH/A8β cell clones with the (Env−, Gag+) type expression, and all of the 5 clones tested had the expected size of the transfected DNA (data not shown). On the other hand, a shortened mRNA was detected after Northern blotting of 5 PacNIH/A8β cell clones with the (Env−, Gag+) type expression. The mRNA that encodes the Env protein (mRNAenv) is 3.1 kb in length and is yielded after splicing out 5.2 kb of the gag and pol region from the full-length transcripts (8.4 kb) of the A8-V genome. The mRNAenv extracted from the (Env−, Gag+) type PacNIH/A8β cell clones was shorter by 1 kb than the expected size, whereas the full length mRNA, which encodes the gag and pol gene products, retained the expected size (Fig. 3). Thus, the (Env−, Gag+) type PacNIH/A8β cell clones did express the Gag protein, but failed in Env expression. This phenomenon was observed only in PacNIH/A8β cells and not in PacNIH/A8δ cells (Fig. 3). Therefore, the unstable expression of the Env protein observed in the PacNIH/A8β cells was induced by the 0.85 kb fragment of the SV40 early region, which was used for the polA signal of pA8(Ψ−)δ. DNA and pA8(Ψ−)δ DNA, respectively, RNA extracted from the cell clones was electrophoresed and transferred to a membrane. RNA quality was confirmed by visualization of ribosomal RNA on the membrane using a UV lamp. A 32P-labeled 2.2-kb XbaI-BglIII fragment (Fig. 1) containing the entire env region was used as a hybridization probe. In A8-infected NIH3T3 cells, full-length viral transcripts (**) and spliced viral transcripts (***) were detected. The size of the spliced transcripts in PacNIH/A8δ (indicated by arrowhead) was smaller than that in PacNIH/A8δ. The size of the full-length transcripts in PacNIH/A8δ (indicated by arrow) was smaller than that in PacNIH/A8β because a part of the SV40-derived sequences was deleted in the pA8(Ψ−)δ construct (see Fig. 1).
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