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# Adenovirus-mediated gene delivery into neuronal precursors of the adult mouse brain

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ABSTRACT **Precursor cells found in the subventricular zone (SVZ) of the adult brain can undergo cell division and migrate long distances before differentiating into mature neurons. We have investigated the possibility of introducing genes stably into this population of cells. Replication-defective adenoviruses were injected into the SVZ of the lateral ventricle of adult mice. The adenoviruses carried a cDNA for the LacZ reporter or the human p75 neurotrophin receptor, for which species-specific antibodies are available. Injection of the viruses into the SVZ led to efficient labeling of neuronal precursors. Two months after viral injection, infected cells were detected in the olfactory bulb, a significant distance from the site of injection. Labeled periglomerular and granular neurons with extensive dendritic arborization were found in the olfactory bulb. These results demonstrate that foreign genes can be efficiently introduced into neuronal precursor cells. Furthermore, adenovirus-directed infection can lead to long-term stable gene expression in progenitor cells found in the adult central nervous system.**

The adult mammalian brain is capable of giving rise to new neurons from progenitor cells located in the subventricular zone (SVZ; ref. 1). Experiments have demonstrated that these precursor cells are mitotically active and multipotent (2–4). Remarkably, neuronal precursors can migrate long distances away from their site of origin and undergo terminal differentiation. For example, adult progenitor cells originating near the lateral ventricle can migrate to the olfactory bulb, where they undergo differentiation into granular and periglomerular neurons (5, 6). The restricted migratory path and the resulting cell specification indicate that highly specific mechanisms dictate the fate of these cells.

As a way to understand molecular mechanisms of adult neurogenesis and to establish an efficient method of gene transfer in the adult brain, we have explored the possibility of using adenovirus to transfer genes into progenitor cells in the SVZ. As a method of *in vivo* gene delivery, adenovirus offers a number of distinct advantages over other available viral vectors. The adenovirus vectors can carry up to 7.5 kb of foreign DNA, and can be easily grown to high titer, in excess of $10^{13}$ plaque-forming units (pfu)/ml, and can achieve 100% infection efficiency in culture. In addition, adenoviral DNA is not incorporated into the chromosome, thereby eliminating the possibility of chromosomal rearrangements that could lead to potential tumor formation. Replication-defective adenoviruses have been successfully applied to a wide variety of cell types including both postmitotic neurons and glia in the nervous system (7–9). The ability to infect both mitotic and postmitotic neurons can offer an advantage over retroviruses that are restricted in their ability to infect only dividing cells.

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In this report, we have applied replication-defective adenoviruses to adult precursor cells in the subventricular zone and have followed the migration and differentiation of these progenitor cells. Using recombinant adenoviruses carrying either the LacZ or the human p75 neurotrophin receptor, the infected precursor cells could be found to be fully differentiated in the olfactory bulb, a considerable distance from the SVZ infection site. Expression of the recombinant genes persisted for at least two months without noticeable cytotoxicity. These results indicate that genes can be effectively introduced into neuronal precursor cells in the adult central nervous system.

## MATERIALS AND METHODS

**Production of pAdCMV-p75 Adenoviral Vector.** An adenoviral p75 expression vector was generated in a two-step process: (*i*) construction of an adenovirus expression plasmid carrying the cDNA of the human p75 neurotrophin receptor, and (*ii*) conversion of the plasmid into an adenovirus expression vector. The human p75 cDNA subclone in pBluescript KS (Stratagene) was digested with *Sma*I and *Sal*I and ligated into pAd5(HS)V vector at its filled-in *Hin*dIII and *Sal*I sites. The p75 cDNA encoded nucleotides 1–1507 of the human gene (10). The expression plasmid contained the adenoviral DNA sequences encoding the left-end replication origin/packaging elements and the overlap-recombination region, which are required for production of the adenovirus vector. The p75 adenovirus expression vector was then generated by cotransfecting the pAd5-hp75 plasmid and the 33-kb *Xba*I fragment of adenovirus dlAd5NCAT into 293 human embryonic kidney cells. The 33-kb fragment corresponds to a region from 7.8–100 map units in the viral genome, lacking the left-end origin/packaging element and the E1 transcription unit. Human 293 cells provide the E1 gene product for complementation. The p75 adenoviral vector was recovered after cotransfection and the viral DNA was verified by restriction enzyme analysis following plaque purification.

The recombinant p75 adenovirus was generated according to published methods (11). The virus titer was $4 \times 10^{10}$ pfu/ml. The verification of a positive virus was initially based on viral DNA analysis from a plaque-purified virus using appropriate restriction enzymes. The recombinant LacZ virus was a generous gift from Burkhard Bewig and Ronald Crystal (12). The titer of the LacZ virus was $1.9 \times 10^{11}$ pfu/ml.

**Stereotactic Adenovirus Injection.** The virus stock was dialyzed against 0.1 M NaCl, 10 mM Tris (pH 7.4), and 20% glycerol to remove CsCl carried over from the viral particle isolation procedure (11). For injection, the virus was diluted by 20:1 with L15 medium. Typically, $1.2 \times 10^6$ pfu of virus was

Abbreviations: SVZ, subventricular zone; pfu, plaque-forming unit(s); β-gal, β-galactosidase; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; PB, phosphate buffer.

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injected in 40 or 200 nl volume into the SVZ (1 mm anterior to bregma, 1 mm lateral, and 2.3 mm deep from the pial surface). For ventricle injection, $12 \times 10^6$ pfu of the virus was injected in 2 μl volume (0.4 mm posterior to bregma, 1.6 mm lateral, and 2.2 mm deep from the pial surface). For all injections, adult female CD-1 mice at 2–4 months of age were used.

**Tissue Processing and Immunohistochemistry.** At 3, 7, 14, 30, and 60 days after the virus injection, animals were deeply anesthetized using Nembutal (Abbott) and perfused transcardially with 3% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Brains were postfixed in the perfusion solution overnight, followed by transfer to 0.1 M PB. The brain sections were prepared on a Vibratome at 50 μm thickness in the sagittal or horizontal plane. Floating sections were incubated in blocking solution containing 10% horse serum and 0.1% Triton X-100 in 0.1 M PB for 1–2 hr, followed by 2-day incubation in ME20.4 monoclonal supernatant at 4°C. ME20.4 is a specific monoclonal antibody that recognizes the human, and not murine, p75 (13). ME20.4 was used without any dilution from the original supernatant. Brain sections were then incubated with the secondary antibody (goat anti-mouse IgG, rhodamine conjugate; Boehringer Mannheim) at 1/200 dilution in 0.1% Triton X-100 and 2.5% horse serum in 0.1 M PB, and subsequently counterstained with Hoechst 33258 at 12.5 μg/ml.

For LacZ immunohistochemistry, animals were perfused in the same manner as above, except that brains were postfixed for 2–3 hr instead of overnight. Brain sections were prepared in the same manner as with p75. For detection of β-galactosidase (β-gal) activity, brain sections were incubated overnight at 37°C in a solution containing 1 mg/ml 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal), 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.01% Nonidet P-40 in 0.1 M PB. Sections were subsequently postfixed in 3% paraformaldehyde and counterstained with Hoechst 33258 at 12.5 μg/ml.

**S1 Nuclease Analysis.** The p75 S1 probe was labeled by filling in the *Bsu*36I site with the Klenow fragment of *Escherichia coli* DNA polymerase I in the presence of $^{32}$P-dNTPs. For hybridization, 100 ng of the labeled probe was precipitated with 30 μg of total RNA, and incubated at 42°C for 3 hr after being heated at 65°C for 10 min in 1× Harts [40 mM piperazine-*N*,*N*′-bis(thanoesulfonic acid) (Pipes), 400 mM NaCl, 1 mM EDTA] plus 80% formamide. S1 digestion was performed for 30 min at 42°C in 10 volumes of S1 nuclease buffer (0.28 M NaCl/0.05 M sodium acetate, pH 4.6/0.5 mM $ZnSO_4$/20 mg/ml salmon sperm DNA) in the presence of 400 unit/ml S1 nuclease. The digested samples were analyzed on urea/polyacrylamide gels.

**Affinity Cross-Linking.** Affinity cross-linking was performed according to Patil *et al.* (13) with $1–2 \times 10^6$ cells per each sample. For detection of p75 receptors on the cell surface, cells were harvested 44–48 hr after viral infection and subjected to binding and crosslinking at 4°C (13). Immunoprecipitation of crosslinked p75 was carried out using an antibody against p75 [9992] directed against the cytoplasmic domain of p75. The amount of p75 receptor protein was determined by

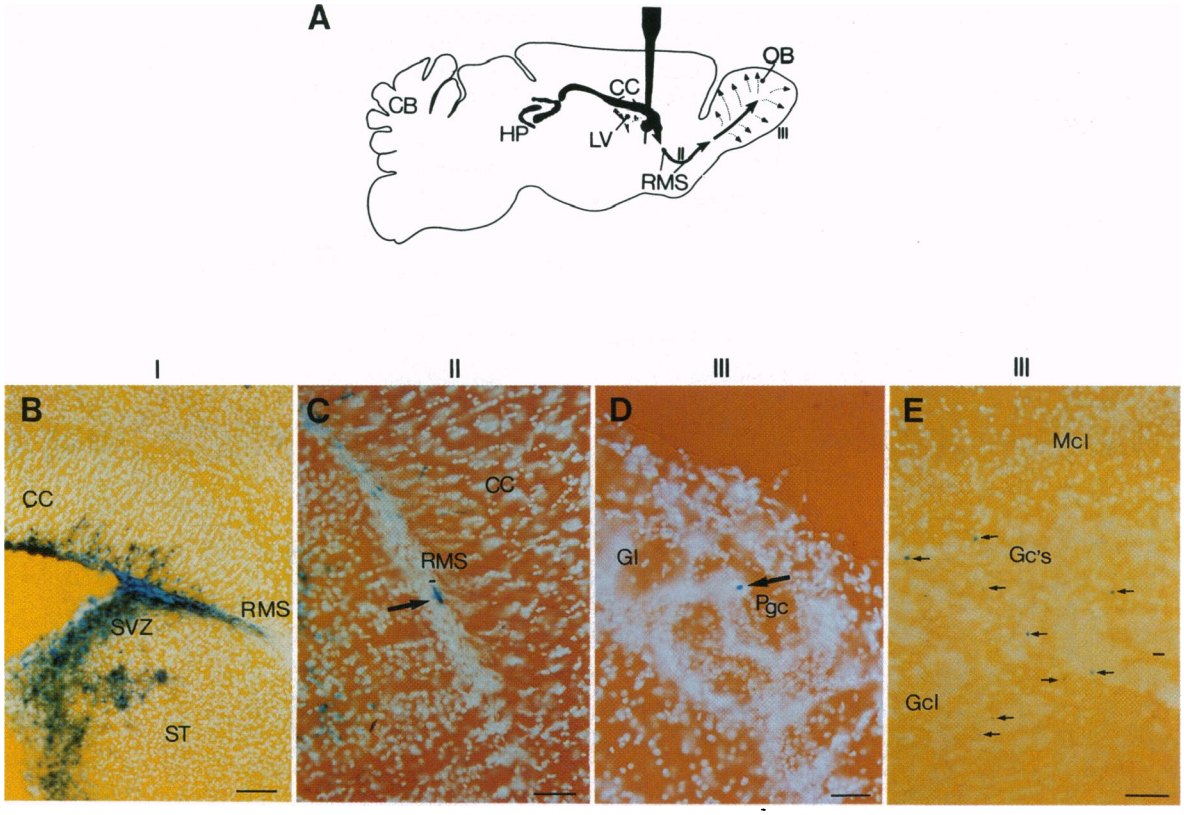


FIG. 1. Infection of the SVZ precursor cells with the Lac Z adenovirus. (*A*) Schematic diagram of the sagittal view of the adult mouse brain showing the site of injection (I) into the SVZ of the anterior part of the lateral ventricle (LV). Cells born here migrate in an anterior direction along the rostral migratory stream (II; RMS, thick arrows) to the olfactory bulb (III; OB), where cells migrate radially (thin dotted arrows) to form new interneurons. (*B*) Expression of β-gal at the injection site. This corresponds to site I at Fig. 2*A*. Note the spread of the virus to the adjacent striatum. The picture was taken one month after injection. Dorsal is up; rostral is right. (*C*) Photograph showing migrating neuroblasts in the RMS (thick arrow), two weeks after injection. Thin arrow points to the viral spread in the striatum. (*D*) Photograph showing a X-Gal-positive periglomerular neuron in the olfactory bulb one month after injection. (*E*) Photograph showing many X-Gal-positive granule neurons in the olfactory bulb two months after injection. In all the photographs, the nuclei stained with Hoechst 33258 are visible as white dots. The pictures were taken from 50-μm sagittal sections. CB, cerebellum; HP, hippocampus; CC, corpus callosum; LV, lateral ventricle; RMS, rostral migratory stream; OB, olfactory bulb; SVZ, subventricular zone; ST, striatum; Gl, glomerular layer; Pgc, periglomerular cell; Gcl, granule cell layer; Gc, granule cell; Mcl, mitral cell layer. [Bars = 500 μm (*B*); 150 μm (*C–E*).]

performing Western blot analysis of the total lysates against p75. Cells were lysed 44–48 hr postinfection, and 100 μg of lysates at each pfu were used for the analysis.

## RESULTS

**Infection of Neuronal Precursors in the SVZ with the LacZ Adenovirus.** Neurogenesis occurs in the adult vertebrate brain (for reviews, see refs. 1 and 14). The presence of neuronal progenitors has been demonstrated in culture (2, 4, 15) and *in vivo*, the major site for these precursors is the SVZ (2, 3). In neonatal and adult mice, the precursor cells divide and migrate to the olfactory bulb where they differentiate into granule and periglomerular neurons (5, 6). To test whether adenoviral injection could label these precursor cells, we injected the recombinant virus carrying a reporter gene, β-gal, into the SVZ of the lateral wall of the lateral ventricle and followed the fate of injected SVZ cells over a two month period (Fig. 1*A*). The recombinant virus was injected bilaterally at a multiplicity of infection -of $1.2 \times 10^6$ pfu (40 nl) into five animals at 2–4 months of age. The mice were then killed at 3 days, 1 week, 2 weeks, 1 month, and 2 months, and the expression of the β-gal gene was assayed for its activity using X-Gal as a substrate.

At the injection site in the SVZ, cells were heavily labeled (Fig. 1*B*; site I in Fig. 1*A*). Cells in the neighboring corpus callosum and caudate nucleus were also labeled due to some injection-related spread of the virus (Fig. 1 *B* and *C*). In the case of the corpus callosum, some cells may correspond to oligodendrocytes migrated from the SVZ, as has been reported in juvenile rats (16). These cells did not migrate away from the injection site to the cortex or striatum. In the SVZ, continued expression of β-gal existed at least two months without any noticeable reduction in the level of X-Gal staining (data not shown). Fig. 1*C* shows a migrating cell found in the rostral migratory stream (site II in Fig. 1*A*; thick arrow). This cell has a spindle shape, an elongated nucleus, and a leading process typical of migrating neuroblasts. Also, some staining was observed in the striatum due to viral spread from the nearby injection site (thin arrow). The spread of these cells, however, was limited to a small radius from the injection site, without migration farther into other regions.

In the olfactory bulb, many X-Gal-positive cells were observed beginning one week after the virus injection (Fig. 1 *D* and *E*). X-Gal-positive cells were observed in the olfactory bulb of all animals that received the LacZ virus into the SVZ. Fig. 1*E* shows a representative section of the olfactory bulb with many X-Gal-positive cells. The position of X-Gal-positive cells suggested that they corresponded to granular (Fig. 1*E*) and periglomerular interneurons (Fig. 1*D*; refs. 6, 17, and 18). The localization of X-Gal-positive cells that have migrated into the olfactory bulb is consistent with previous studies in which a LacZ-retrovirus was injected in neonatal rats (5) and in transplantation experiments of LacZ-positive cells in adult mice (6).

Typically, 200–400 X-Gal-positive cells were observed in the olfactory bulb of each experimental animal. This number is comparable to or greater than the numbers observed after retroviral infection in neonatal animals (5), which harbor a larger number of proliferating precursors in the SVZ than in the adult. Therefore, adenoviral infection resulted in the labeling of numerous precursor cells that migrated appropriately to the olfactory bulb.

**Generation of a p75 Neurotrophin Receptor Adenovirus.** The LacZ adenovirus was effective in labeling SVZ precursors *in vivo* without marked cytotoxicity. However, the expression of β-gal was mostly confined to the cell perikaryon. To

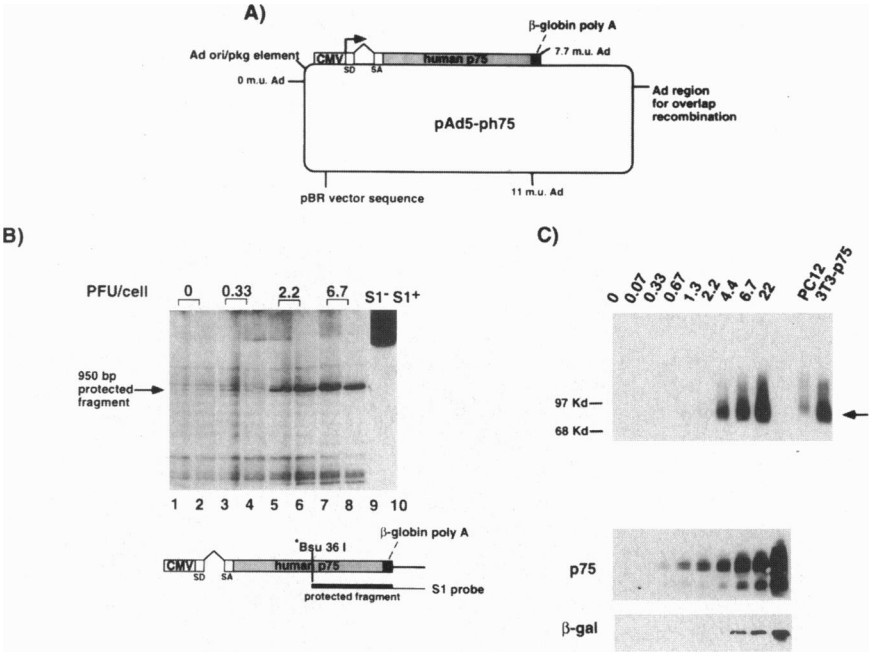


FIG. 2. Expression plasmid pAd5-hp75 used in constructing Ad5-hp75 virus and characterization of the virus in COS-1 cells. (*A*) Expression plasmid pAd5-hp75. The left end of the adenoviral genome contains the origin of replication (ori) and the viral packaging sequence (pkg). Each map unit corresponds to 359 bp. Adenovirus sequences from 1.0–3.8 map units were deleted and replaced with the sequence for the CMV-1 promoter, human p75, and the mouse β-globin polyadenylylation site. Adenovirus sequence from 7.7–11 map units provides DNA sequence for recombination. (*B*) S1 nuclease analysis of the mRNA made upon viral infection of COS-1 cells. Samples from COS-1 cells are shown in duplicates at each viral pfu/cell. Lane 9 shows the probe alone, and lane 10 is the probe subjected to S1 nuclease digestion in the absence of RNA. Shown under the lanes is a schematic of the probe (thin line) and the region of protection upon S1 nuclease digestion (thick line). The size of the correct protected fragment is 950 bp as indicated by an arrow. (*C*) Affinity cross-linking analysis of the protein product upon viral infection in COS-1 cells. Each lane represents $1 \times 10^6$ cells, except for PC12 cells and 3T3-p75 controls that were $2 \times 10^6$ cells. The crosslinked NGF-receptor complex is indicated by an arrow. The number of p75 receptors present in control samples are 120,000/cell for PC12 cells and 200,000/cell for 3T3-p75 cells. Also shown is a Western blot analysis of p75 and β-gal (anti-LacZ antibodies) from cells infected with both viruses at increasing viral dose.

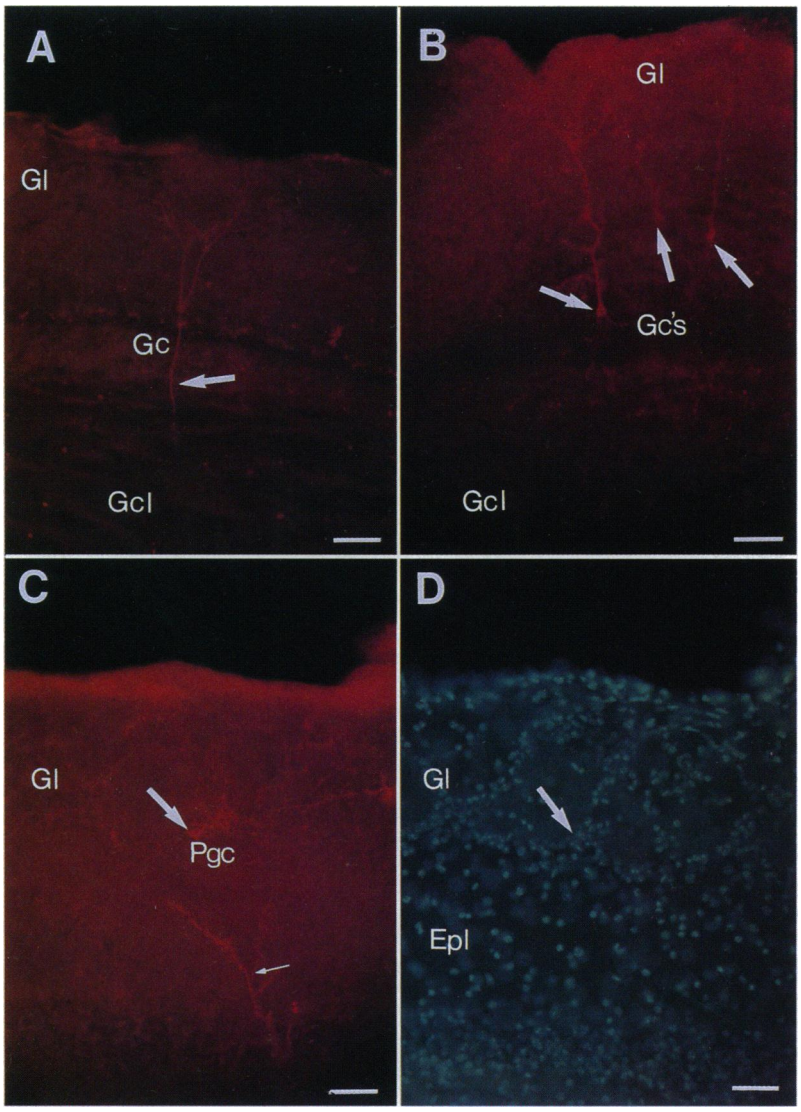


FIG. 3. Infection of the SVZ precursor cells with the p75 adenovirus. (*A*) Expression of p75 in a granule cell whose dendritic processes reached the external plexiform layer 1 month after injection. Note that the cell body is not visible in this plane of picture. (*B*) Photograph of mature granule neurons at 2 month post-injection (arrows). Note that they have extensive dendritic arborization and synaptic boutons. (*C*) Expression of p75 in a periglomerular neuron (thick arrow) at the 2 month postinjection. Note the location of its cell body at the perimeter of a glomerulus (compare with the arrow in *D*). Also shown are the dendritic branches of a granule neuron with synaptic boutons (thin arrow). (*D*) Hoechst counter staining of *C*) showing that the cell body of a labeled cell in *C* resided at the perimeter of a glomerulus. The pictures were taken from 50-$\mu$m sagittal sections. Gl, Glomerular layer; Gc, granule cell; Gcl, granule cell layer; Pgc, periglomerular cell; Epl, external plexiform layer. [Bars = 150 $\mu$m (*A–D*).]

visualize staining of the entire cell, a recombinant adenovirus was generated that contained the human p75 neurotrophin receptor (10). The p75 cDNA was inserted into a multiple cloning site of the expression plasmid pAd5CMV-(H-S)V (19) to produce pAd5-hp75 (Fig. 2*A*). The titer of the p75 recombinant adenovirus was $4 \times 10^{10}$ pfu/ml based on a plaque assay using 293 cells. To evaluate the RNA and protein products produced by the recombinant virus, a series of infections was first carried out in COS-1 cells. Infection of COS-1 cells produced a correct p75 transcript, as indicated by a 950-bp fragment in S1 nuclease experiments (Fig. 2*B*). The 950-bp protected fragment corresponded to the coding region of p75 and the $\beta$-globin poly adenylation signal. In addition, steady state levels of transcript increased with the viral titer, indicating the dose dependency of p75 transcription upon viral infection.

We next determined cell surface expression of the p75 receptor by affinity crosslinking with $^{125}$I-NGF (Fig. 2*C Upper*). The $M_r$ of the crosslinked protein product from infected cells was ≈90 kDa, consistent with previous measurements of the NGF-p75 complex (20, 21). The amount of p75 expressed on the cell surface increased as the viral dose increased, which is reflected in the total amount of p75 detected by Western blot analysis. A similar increase in the total amount of p75 receptor was also observed even when the cells were coinfected with both the p75 and lacZ viruses (Fig. 2*C Lower*). Even at the highest viral dose, COS cells did not display any noticeable cytotoxicity. By extrapolating from the protein crosslinking measurements, it was estimated that viral infection yielded a high level of expression, exceeding 800,000 receptors per infected cell. This number was derived from a comparison with positive control cell lines, PC12 and 3T3-p75 (Fig. 2*C*), which had been evaluated for p75 receptor levels by equilibrium binding and immunoblot measurements (22).

**Infection of Neuronal Precursors with the Recombinant p75 Adenovirus.** The p75 recombinant adenovirus was injected bilaterally into the SVZ of the lateral ventricle at a multiplicity of infection of $1.2 \times 10^6$ pfu (200 nl). Five animals were analyzed at 3 days, 1 week, 2 weeks, 1 month, and 2 months. The pattern of p75 expression was then assessed using a specific monoclonal antibody, ME20.4 (23), which recognizes

the human, and not murine, p75. This antibody was used previously to detect human p75 expression in transgenic mice (13, 24).

At the site of injection, SVZ cells were labeled in a similar manner as with the Lac Z adenovirus. Additionally, expression of the human p75 receptor remained high at the injection site over two months. Beginning one week postinjection, many p75-positive neurons were observed in the olfactory bulb. Fig. 3 shows olfactory neurons detected two months after infection using ME20.4 immunostaining in the olfactory bulb. In contrast to the lacZ virus-infected cells that displayed β-gal expression only around the nucleus, the entire cell body and processes were visible in p75-virus infected cells. Granule neurons exhibited long dendritic processes, which extended to the external plexiform layer (Fig. 3 *A* and *B*; thick arrows). Dendritic boutons were evident among labeled granule neurons, especially in their lateral branches (thick arrow in Fig. 3*B* and thin arrow in 3*C*). At this time, ME20.4-labeled periglomerular neurons were also observed with extensive arborization that reached neighboring glomeruli (thick arrow in Fig. 3 *C* and *D*).

The expression of p75 and migration of infected olfactory neurons were specific to the SVZ injection site. Injection of p75 virus in the lateral ventricle resulted in intense staining for human p75 in ependymal cells lining the lateral ventricle (Fig. 4) at 4 days, 2 weeks, and 1 month postinjection, but did not result in labeled cells in the olfactory bulb. These results indicate that olfactory bulb interneurons are derived from the SVZ and not from ependymal cells.

Infection of the SVZ cells with the p75 adenovirus in adult mice resulted in labeled granular and periglomerular neurons in the olfactory bulb 4–6 mm away from the injection site. The fate of SVZ precursors labeled by the p75 adenovirus is consistent with the findings using the Lac Z adenovirus. In addition, the morphology of small interneurons observed with ME20.4 staining is in agreement with studies in which 1,1′-dioleyl-3,3,3′,3′-tetramethylindocarbocyanine methanesulfonate was used to follow a small population of SVZ derived neurons in the olfactory bulb (6). For these injected cells that migrated and differentiated, the recombinant adenovirus infection did not interfere with the normal neurogenic potential of neuronal precursors in the SVZ, their migration or differentiation *in vivo*. More importantly, β-gal or p75 expression was detected only in small interneurons that do not project outside the olfactory bulb, indicating that the staining did not simply result from retrograde transport of the virus. Therefore, the ME20.4 or X-Gal-positive cells observed in the olfactory bulb originated from virally infected SVZ precursors that followed their natural course of maturation and differentiation to the olfactory bulb 4–6 mm away.

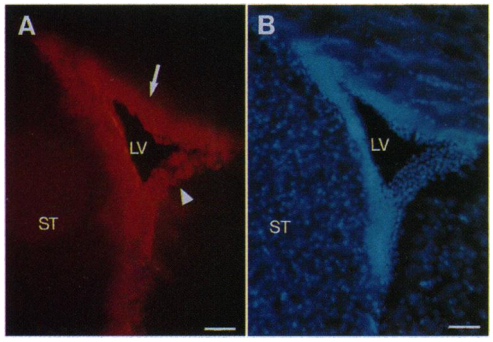


FIG. 4. Control injection of p75 adenovirus virus in the lateral ventricle. (*A*) Expression of p75 in ependymal cells lining the lateral ventricle 2 weeks after injection (arrow). The triangle points to a single labeled cell. Despite the intense labeling in the ependymal layer, no ME20.4-positive cells were found in the olfactory bulb of control animals one month after infection. (*B*) Hoechst staining of the same section as *A*. Dorsal is up; lateral is left. The staining was carried out on frontal sections (50 μm). ST, striatum; LV, lateral ventricle. [Bars = 150 μm (*A–D*).]

## DISCUSSION

Our results demonstrate that adenoviruses provide an efficient way of introducing foreign genes into neuronal precursors in the adult brain. Infected cells were able to maintain recombinant gene expression following proliferation, migration, and differentiation. Since these cells have been shown to divide during their migration (6), cell division appears not to have affected the functional integrity of the viral genome. The adenoviral infection did not affect the migratory route or the eventual fate of SVZ precursors: SVZ cells infected with the adenovirus generated two types of interneurons, granular and periglomerular neurons in the olfactory bulb. Since these neurons do not project outside the olfactory bulb, the labeling detected after viral infection was derived from SVZ precursors that migrated into the olfactory bulb and not by retrograde transport or diffusion. The adenoviral infection also did not interfere with terminal differentiation of SVZ precursors. The infected granular and periglomerular neurons in the olfactory bulb developed a mature neuronal morphology as evidenced by long, branched dendritic processes with synaptic boutons.

A striking result of this study was the long-term expression of recombinant p75 and β-gal genes, which was maintained even 2 months after infection. Other experiments observed a similar long-term β-gal expression after viral infection (9). One potential reason for the persistent recombinant gene expression observed here may be due to continuous arrival of infected cells from the SVZ to the olfactory bulb, thereby replenishing dying cells in the bulb. Two observations suggest that infected cells in the olfactory bulb live to become fully mature: (*i*) the number of infected cells in the olfactory bulb increased over the course of our study; and (*ii*) the p75-positive cells displayed short processes at earlier times and gradually developed a more mature morphology with extensive dendritic branches.

Expression of p75 in the precursors of olfactory interneurons did not disrupt their normal migration and differentiation. It should be noted that a large number of activities have been proposed for the p75 neurotrophin receptor (25). These include increasing neurotrophin responsiveness through the Trk tyrosine kinase receptors (26, 27); production of the second messenger, ceramide (28); increasing rate of apoptosis (29), effects upon cell migration (30) and NF-κB induction (31). There was no apparent enhancement of cell death in this population of cells due to exogenous p75 expression. The numbers of p75-positive neurons observed in the olfactory bulb were similar to those observed by adenoviral LacZ infection, suggesting ectopic expression of p75 in precursor cells does not lead to cell death in this population of cells. It remains to be determined whether expression of p75 in precursor cells displays any detectable phenotype, such as enhanced survival or migratory properties.

Adult neurogenesis in the mammalian SVZ offers an invaluable experimental system for studying the mechanisms of neuronal formation, especially in conjunction with the use of adenovirus as a method of gene delivery. For instance, it has been hypothesized that the SVZ in the adult is comprised of two different populations of mitotic precursors of the same lineage; one, being a stem cell population capable of self-renewal and the other, constitutively proliferating precursors that give rise to olfactory interneurons (3). The evidence for their shared lineage is, however, lacking mainly due to the lack of suitable means to label these relatively quiescent stem cells effectively. Since the adenovirus can infect both the mitotic and postmitotic cells, this method may allow for the fate of these putative stem cells to be followed in the adult brain. In

addition, by introducing genes into the SVZ, the factors that control their migration and differentiation into mature olfactory interneurons can be studied. Genes that encode growth factors and their receptors, cell adhesion molecules, and components of the cell cycle will provide mechanistic clues in the processes of neurogenesis, migration, and differentiation. Furthermore, adenovirus-mediated delivery of genes to the postmitotic target zone may provide a means of introducing therapeutically relevant genes in these cells. This will depend upon overcoming the potential cytotoxic effects from recognition of viral proteins by the immune system. This report demonstrates that the adenovirus system is feasible for studying neurogenesis by targeting neuronal precursors in the adult central nervous system, and also provides an efficient and effective means to introduce genes into both mitotic and postmitotic cells in the central nervous system.

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