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Biochemistry

Improved gene expression upon transfer of the adenosine deaminase minigene outside the transcriptional unit of a retroviral vector

(gene therapy/double-copy retroviral vectors/promoter interference)

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ABSTRACT **This study describes a type of retroviral vector called double-copy (DC) vector that was designed to improve the expression of transduced genes. The unique feature of DC vectors is that the transduced gene is inserted within the U3 region of the 3′ long terminal repeat (LTR). Consequently, in the infected cell the gene is duplicated and transferred to the 5′ LTR. The important result is that in its new position the gene is placed *outside* the retroviral transcriptional unit, eliminating or at least reducing the negative effects of the retroviral transcriptional unit. The utility of the DC vector design was tested by using a 2.1-kilobase-pair (kbp)-long adenosine deaminase (*ADA*; EC 3.5.4.4) minigene that was inserted into the 3′ LTR of the N2 retroviral vector, generating a 2.7-kbp-long chimeric LTR. DNA blot analysis was used to show that the chimeric LTR was faithfully duplicated in cells infected with the corresponding virus, generating two copies of the *ADA* minigene, one copy in each LTR. Insertion of the *ADA* minigene into the 3′ LTR of the N2 vector led to a 10- to 20-fold increase in *ADA* transcripts and human ADA isozyme synthesized in NIH 3T3 cells as compared to cells harboring the same vector in which the *ADA* minigene was inserted between the two LTRs. A similar increase in *ADA* expression was observed in two human lymphoid cell lines tested, HUT 78 and Raji. These results are consistent with previous observations that upstream promoters exert an inhibitory effect on promoters placed downstream and bear out the predictions used in the design of DC vectors. The use of DC vectors may contribute to the solution of the problems encountered in expressing retrovirally transduced genes in cultured cells and, in particular, when introduced into the live animal.**

The understanding of gene expression has been greatly enhanced by the ability to transfer cloned genes into cells and to study the mechanism of their regulation. Retroviruses are used with increased frequency as vectors to introduce genes into eukaryotic cells that are refractory to other gene transfer techniques (1–3). Retrovirus-derived vectors utilize the biochemical processes unique to this group of viruses to transfer genes with high efficiency into a wide variety of cell types. The special features of retroviral-based gene transfer techniques have provided the opportunity of introducing genes into somatic cells of live animals (4, 5).

We and others have observed that efficient expression of retrovirally carried genes in transduced cells constitutes a major problem in using this gene transfer technique (6–10), and the experiments described here were designed to address this particular issue. N2-derived vectors [N2 is a Moloney murine leukemia virus (Mo-MuLV)-based vector developed in our laboratory (11)] generate high titers of corresponding virus, resulting in improved efficiency of gene transfer *in vitro* and *in vivo*. The main drawback of this strategy of vector design is that the promoter used to express the transduced gene is placed *within* the retroviral transcriptional unit, which interferes with the expression of the transduced gene. This is not unexpected since it has been shown that the activity of promoters is often reduced when placed downstream from an active promoter (12–15). Experience accumulated so far suggests that the outcome of placing promoters internal to a retroviral vector is unpredictable, in some cases leading to efficient expression from the internal promoter (11, 16) and in other cases resulting in little or no expression from the internal promoter (6, 7, 9, 10).

To address the issue of expression from internal promoters we have designed a retroviral vector, called self-inactivating (SIN) vector, in which the long terminal repeat (LTR)-driven transcript is eliminated in the infected cell, allowing for the uninhibited expression of the transduced gene (17), and similar vectors were described by other investigators as well (18–21). Unfortunately, the usefulness of SIN vectors was limited because they yield a low virus titer and a systematic effort to increase virus titers derived from SIN vectors was unsuccessful (S.-F. Yu and E.G., unpublished results).

In this study we describe a type of retroviral vector, called double-copy (DC) vector that, like SIN vectors, is designed to generate an RNA transcript without the interference of a read-through transcript emanating from the viral LTR, albeit by a mechanism different from that of SIN vectors. By using a DC vector that contains the human adenosine deaminase (*ADA*; EC 3.5.4.4) minigene, we show that these vectors function as predicted and lead to efficient expression of the *ADA* gene.

MATERIALS AND METHODS

Vector Constructions. The Mo-MuLV-based N2 retroviral vector contains the bacterial neomycin resistance gene (*Neo*) and was previously described (11). The AAX vector (shown in Fig. 2*A*) was generated by introducing a human *ADA* minigene into the N2 vector downstream from the *Neo* gene. The 2082-base-pair (bp)-long *ADA* minigene consists of the *ADA* promoter, which extends 730 bp upstream from the RNA start site, and the *ADA* coding sequences from which the poly(A) signal sequence, AAUAAA, was removed (22, 23). The *ADA* minigene was inserted into a unique *Xho* I site, present downstream from the *Neo* coding sequences, in a transcriptional orientation that is parallel to the viral transcriptional unit. The DCA (DC ADA) vector is similar to the AAX vector except that the *ADA* minigene was inserted into

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Abbreviations: Mo-MuLV, Moloney murine leukemia virus; ADA, adenosine deaminase; *Neo*, neomycin resistance gene; DC, double-copy; LTR, long terminal repeat; SIN, self-inactivating; cfu, colony-forming units.

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the 3′ LTR of the N2 vector. The N2 vector was first modified by insertion of a 52-bp-long polylinker sequence into a *Nhe* I site present in the 3′ LTR, 30 bp downstream from the 5′ end of the LTR. The polylinker sequence contains five restriction sites that are unique to the N2 plasmid: *Apa* I, *Bgl* II, *Sna*BI, *Sac* II, and *Mlu* I. Since the insertion of the polylinker sequence into the *Nhe* I site may interfere with viral integration, the polylinker sequence was designed to regenerate an additional 16 bp of viral sequence downstream from the *Nhe* I site. Thus, the polylinker-modified N2 vector, called N2A, contains a total of 50 bp of viral sequence downstream from the 5′ end of the LTR before foreign sequence is encountered. The *ADA* minigene was inserted into the unique *Sna*BI site present in the polylinker.

Generation and Characterization of *ADA* Containing Virus. Vector DNA was converted to corresponding virus by calcium phosphate-mediated DNA transfection into the amphotropic packaging cell line PA317 (24). Productively transfected cells were isolated using G418 selection, and individual G418-resistant clones were expanded to cell lines and analyzed for virus production. Producer cell lines that generated high virus titers were used for further analysis. Over 100 G418-resistant colonies derived by infection of NIH 3T3 cells with high-titer virus-containing supernatants were pooled and used to examine the structure of proviral DNA, examine the expression of RNA, and measure human *ADA* activity.

DNA and RNA Analysis. Chromosomal DNA and total cellular RNA (both nuclear and cytoplasmic) were prepared using the guanidinium isothiocyanate extraction procedure (25). DNA was digested with various restriction enzymes, subjected to electrophoresis in 1% agarose gels (10 μg per lane), transferred to a nylon filter (Biotrans, ICN) using an electroblotting apparatus (Bio-Rad), hybridized with a ^{32}P-labeled specific probe, and exposed to an x-ray-sensitive film (Kodak XAR5) in the presence of intensifying screens (Dupont Cronex Lightning Plus). Total cellular RNA was fractionated on oligo(dT)-cellulose columns, the poly(A)$^+$ RNA fraction was subjected to electrophoresis in 1% agarose/formaldehyde gels (26), and vector-specific RNA species were identified after electroblotting to nylon filters, hybridization with ^{32}P-labeled probes, and exposure to x-ray-sensitive film in the presence of intensifying screens.

RESULTS

Principle of DC Vectors. Fig. 1 shows the basic structure and principle of DC retroviral vectors. DC vectors utilize the same principle used in the design of SIN vectors, that the U3 region in the 3′ LTR of a provirus serves as the template for the synthesis of the U3 regions in both the 5′ and 3′ LTRs of the progeny provirus (27). Consequently any modification in the U3 region of the 3′ LTR, provided it does not affect viral replication, will be duplicated in the progeny provirus and will appear in the 5′ LTR as well. SIN vectors are characterized by a deletion in the U3 region of the 3′ LTR that encompasses the viral enhancer and promoter sequences, resulting in the effective elimination of the viral transcriptional unit in the infected cell (17). As shown in Fig. 1, the unique feature of DC vectors is that the transduced gene itself is inserted into the U3 region of the 3′ LTR. Consequently, in the infected cell the transduced gene will be duplicated and transferred to the 5′ LTR generating two copies of the transduced gene, hence its name, DC vector. The important result is that in its new position, in the 5′ LTR, the gene is placed *outside* the retroviral transcriptional unit, eliminating or at least reducing the negative effects of the retroviral transcriptional unit (12–15).

Fig. 2*A* shows the structure of a DC vector called DCA. The DCA vector is composed of the 2.1-kilobase-pair (kbp)-long human *ADA* minigene that was inserted into the 3′ LTR of the

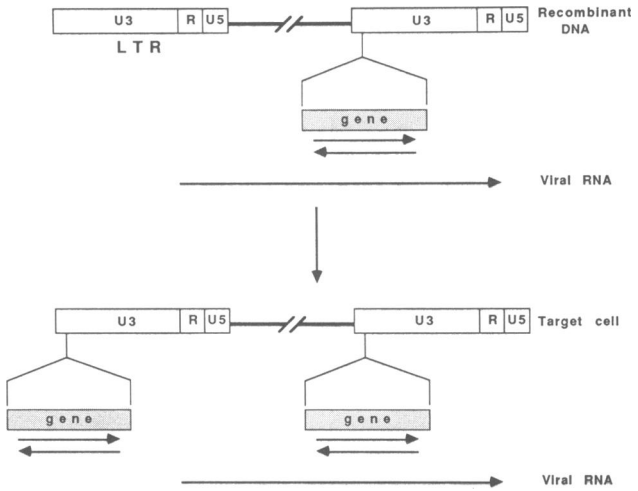

FIG. 1. Structure and principle of DC vectors. A prototype retroviral vector consists of two LTRs and sequences between the two LTRs: (*i*) of viral origin, encoding the packaging signal and other sequences essential for viral replication, and (*ii*) of nonviral origin such as a selectable marker. The retroviral LTR is functionally subdivided into three regions, U3, R, and U5. The U3 region encodes the viral enhancer and promoter functions that are active in the 5′ LTR but not in the 3′ LTR (12). The border between the U3 and R region in the 5′ LTR specifies the viral RNA start site, and the 3′ end of the retroviral RNA is defined by the border between the R and U5 region in the 3′ LTR. In a DC vector the gene of interest is introduced into the U3 region of the 3′ LTR (in either transcriptional orientation as indicated by the arrows). In this position, the transcriptional regulatory sequences of the transduced gene are placed within the retroviral transcript, downstream from the strong viral promoter encoded in the 5′ LTR. Since the U3 region in the 3′ LTR is the template for the synthesis of the U3 regions in both the 5′ and 3′ LTRs of the progeny provirus (27), the transduced gene will be duplicated and also transferred to the 5′ LTR in the infected cell. In its new position, in the 5′ LTR, the gene is situated outside the retroviral transcriptional unit.

N2 retroviral vector, between the 5′ end of the LTR and the viral enhancer sequences, generating a 2.7-kbp-long chimeric LTR structure. Vector DNA was converted into corresponding virus by transfection into PA317 cells, and G418-resistant colonies were isolated, expanded to cell lines, and tested for virus production and *ADA* gene transfer as described in *Materials and Methods*. Seven of 10 cell lines generated virus titers between 0.2 and 0.8 × 10^5 *Neo* colony-forming units (cfu)/ml and virus-containing supernatants from those cell lines were used in subsequent experiments. By comparison, AAX virus producer cell lines, generated and tested concurrently, yielded virus titers between 1 and 2 × 10^5 cfu/ml, only 2- to 4-fold higher than DCA virus producer cell lines.

DNA analysis was used to test whether duplication of the 2.7-kbp hybrid 3′ LTR takes place in cells infected with the DCA virus. As shown in Fig. 2*A*, a single *Bam*HI restriction site is present in the DCA vector DNA, and if the 2.7-kb hybrid LTR is duplicated at the 5′ end of the proviral DNA, a characteristic 5.1-kbp DNA fragment hybridizing to the *Neo* probe will be generated in the infected cells. As shown in Fig. 2*B*, lanes 5–8, infection of NIH 3T3 with four independently derived DCA virus-containing supernatants generates the predicted 5.1-kbp DNA band. Additional bands present in lanes 6 and 8 indicate that the corresponding virus preparations also contain virus that has undergone rearrangements, presumably during transfection of the PA317 cells with the vector DNA. Restriction analysis using restriction enzymes *Stu* I, *Bgl* II, and *Nco* I is also consistent with the accurate duplication of the hybrid 3′ LTR in the infected cells (Fig. 2*B*, lanes 2–4, and accompanying diagram in Fig. 2*A*).

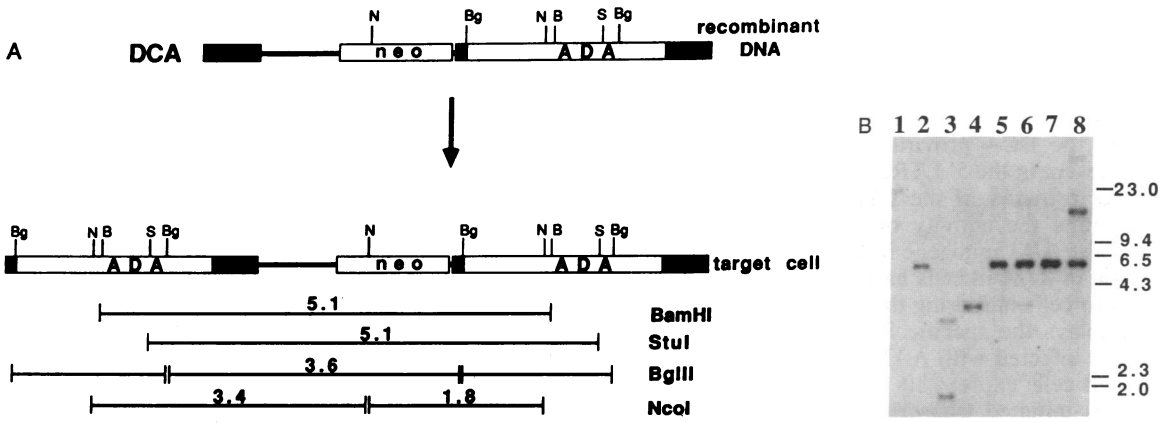

FIG. 2. Structure of the proviral DNA in NIH 3T3 cells infected with a DC vector carrying the *ADA* minigene (DCA). (*A*) Structure of the DCA vector (recombinant DNA) and the predicted structure of the corresponding provirus in the infected (target) cell. Black boxes represent the viral LTRs, solid lines represent unique viral sequences, and open boxes represent the foreign genes introduced into the retroviral vector, the *Neo* and *ADA* minigenes. Restriction sites used in this analysis are also shown. N, *Nco* I; Bg, *Bgl* II; B, *Bam*HI; S, *Stu* I. The predicted DNA fragments generated by digestion of the proviral DNA with each restriction enzyme are shown and the sizes in kbp are indicated only for the DNA fragments that hybridize with the Neo probe. (*B*) Results of a DNA blot analysis of NIH 3T3 cells infected with DCA virus-containing supernatants. Hybridization was performed with a *Neo*-specific probe. Lane 1, uninfected NIH 3T3 cells. Lanes 2–5, DCA-6 virus-containing supernatant infected cells digested with *Stu* I (lane 2), *Nco* I (lane 3), *Bgl* II (lane 4), and *Bam*HI (lane 5). Lanes 6–8, cellular DNA digested with *Bam*HI, derived from NIH 3T3 cells infected with three additional virus-containing supernatants: DCA-3 (lane 6), DCA-4 (lane 7), DCA-9 (lane 8). Migration of DNA fragments generated by *Hind*III digestion of λ DNA is indicated in kbp.

Expression of vector-specific RNA in NIH 3T3 cells infected with DCA virus was determined by using RNA blotting techniques (Fig. 3) and compared to RNA expressed from a "conventional" vector, AAX. AAX is similar to the DCA vector, except that the *ADA* minigene is inserted downstream to the *Neo* gene, 457 bp upstream from the cloning site in the DCA vector. Fig. 3*A* shows the structure of the AAX- and DCA-derived proviruses and the predicted RNA species expressed from the viral LTR and the *ADA* promoter in NIH 3T3 cells. N2-based vectors generate two LTR-derived transcripts, an unspliced RNA species and a spliced RNA form (Fig. 3*A*, virion RNA and *Neo* RNA, respectively). A third RNA species is expressed from the *ADA* promoter in both AAX and DCA vectors and serves as the mRNA for *ADA* synthesis (11). All three RNA transcripts terminate at the poly(A) site present in the viral LTR, the

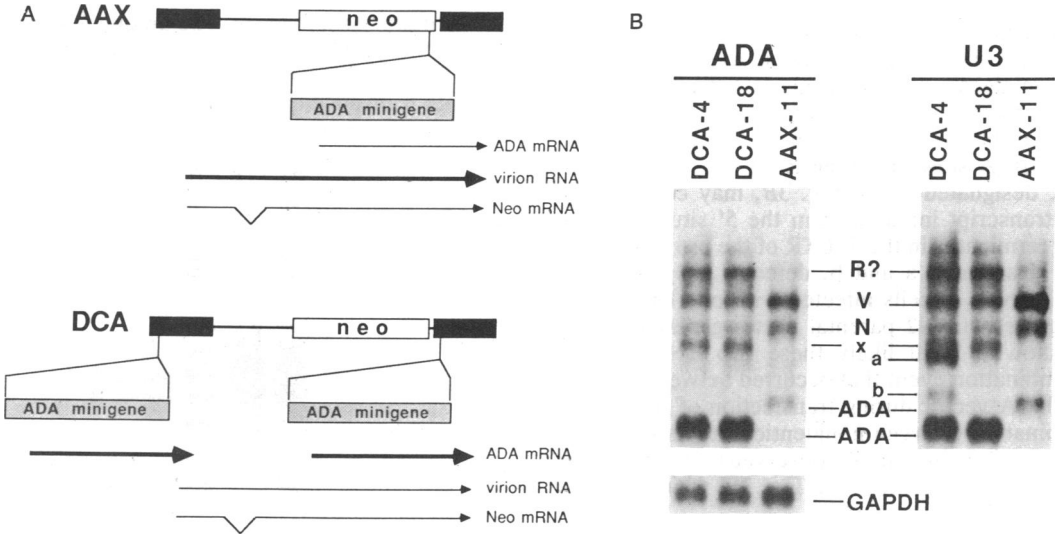

FIG. 3. RNA expression in NIH 3T3 cells infected with AAX and DCA viruses. (*A*) Structure of AAX and DCA proviruses and corresponding RNA transcripts (arrows). In the AAX vector the *ADA* minigene was cloned between the two LTRs (downstream from the *Neo* gene) and therefore the structure of the provirus is identical to the vector DNA. In the DCA vector the *ADA* minigene was inserted into the 3' LTR and therefore the provirus will contain two copies of the *ADA* minigene in each LTR (see text and Fig. 2). Three RNA transcripts are expressed from the AAX provirus, two LTR-initiated RNA forms unspliced and spliced, and a third RNA transcript expressed from the internally placed *ADA* promoter that serves as the mRNA for *ADA* synthesis. The DCA provirus also generates the same three transcripts except that both *ADA* minigenes can serve as templates for *ADA* mRNA synthesis. The predicted (see text) and observed (*B*) levels of RNA transcripts in cells infected with DCA virus are indicated by the thickness of the arrows. (*B*) RNA blot analysis of cells infected with AAX and DCA virus-containing supernatants. Poly(A) RNA, fractionated on formaldehyde/agarose gels (26) and blotted to nylon filters, was first hybridized to a Mo-MuLV U3-specific probe. After hybridization, exposure, and development of the x-ray-sensitive film, the probe was removed and filter was rehybridized with a human *ADA*-specific probe. To obtain a quantitative comparison of RNA loaded in each lane, the same filter was also hybridized to a human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. AAX-11, DCA-18, and DCA-4, three independently derived virus preparations that were characterized by DNA blotting to generate the predicted proviruses in the infected cells. V and N, LTR-initiated transcripts—the unspliced virion RNA (V) and spliced *Neo* mRNA (N). ADA, *ADA* promoter-initiated RNA transcripts. R?, x, a, and b, additional transcripts whose possible origin is discussed in the text.

R/U5 junction. The two LTR-initiated transcripts generated from AAX and DCA vectors are identical in size, whereas the *ADA* promoter-initiated transcript expressed from the DCA vector should be 457 nucleotides shorter than the corresponding transcript synthesized on the AAX template. Fig. 2*A* also shows that in the DCA provirus a second copy of *ADA* minigene is present in the 5′ LTR, thus generating two *ADA* transcripts per provirus. If the LTR-initiated read-through transcript inhibits the activity of internal promoters, the placement of the *ADA* minigene in the 5′ LTR will enhance the expression of *ADA* in cells harboring the DCA provirus as compared to cells harboring the AAX provirus.

Fig. 3*B* displays the specific RNA species expressed in NIH 3T3 cells infected with AAX and DCA viruses. DCA vector-infected cells express 10- to 20-fold higher levels of *ADA* promoter-initiated transcripts as compared to AAX vector-infected cells. (Relative levels of RNA transcripts were determined by exposing the autoradiogram for various times and correcting for the small variations in RNA loaded in each lane.) This difference in RNA expression is consistent with the prediction that placement of one copy of the *ADA* minigene outside the viral LTR will reduce the inhibitory effect of an upstream promoter on the activity of a downstream situated promoter. (It is not possible to determine experimentally whether one or both copies of the *ADA* minigene in the DCA provirus contribute equally to *ADA* expression.) It is also evident from this analysis that in cells infected with DCA virus, expression of the LTR-initiated transcripts (Fig. 3*B*, V and N) is reduced. [The unspliced RNA form (V) is reduced by a factor of 3–5 and the spliced RNA form (N) is reduced by a factor of 10–15.] This is also consistent with the observations that read-through transcripts inhibit the activity of internal promoters since the *ADA* promoter-initiated transcript in the 5′ LTR traverses the viral enhancer/promoter region overlapping with the LTR-initiated transcripts over a short region. Although not possible to test experimentally, reduced expression of the LTR-initiated transcripts should augment transcription from the 3′ situated *ADA* minigene as well. (This is indicated by the thickness of the corresponding arrow in Fig. 3*A*.) Additional RNA species can be also detected in the infected cells as shown in Fig. 3*B*. A slow-migrating RNA species in DCA-infected cells, designated R? in Fig. 3*B*, may constitute a read-through transcript initiated from the 5′ situated *ADA* promoter that terminates in the 3′ LTR of the provirus. Two RNA species designated a and b, detected only with the Mo-MuLV U3 probe in cells infected with one DCA virus isolate, comigrate with N2 parental vector-generated transcripts (not shown). Most likely these two RNA species reflect a recombination event that occurred between the two LTRs of the DCA vector during transfection of the PA317 cells. Recombination between nonidentical LTRs in similar circumstances has been previously observed (17). The nature of the RNA species designated X in Fig. 3*B*, which appears in all DCA-infected cells, is unclear.

To further assess the utility of a DC vector to express the transduced *ADA* gene, the level of human ADA isozyme was measured in cells infected with DCA and AAX viruses. Fig. 4 shows that cells infected with DCA virus preparations contain substantially higher levels of human ADA isozyme activity. Thus, both RNA analysis and histochemical determination of enzyme activity show that DC vectors express higher levels of the transduced *ADA* gene product as compared to previously used vectors.

Expression of internally promoted genes such as the expression of the *ADA* minigene from the AAX vector in NIH 3T3 cells (Fig. 3*B*) may be more the exception than the rule. We have noted that the *ADA* transcript is very poorly expressed in several human lymphoid cell lines transduced with the AAX vector such as HUT 78 and Raji (S.-F. Yu and E.G., unpublished results). Therefore, it was of considerable interest to see whether a DC vector design will be more useful in expressing the *ADA* gene in those cell lines. As shown in Fig. 5, in AAX-infected HUT 78 and Raji cells the internal *ADA* promoter-driven transcripts are barely detectable. On the other hand, substantially higher levels of *ADA* transcripts are present in cells harboring the DCA vector, providing additional evidence for the potential utility of this type of vector design.

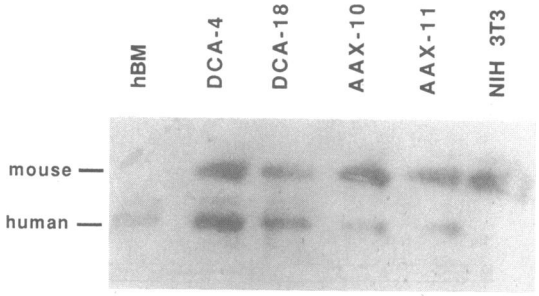

FIG. 4. Human ADA enzyme activity in cells infected with AAX and DCA viruses. Cell extracts prepared from NIH 3T3 cells infected with AAX or DCA virus were subjected to electrophoresis in a Cellogel matrix, and the migration of mouse and human ADA isozymes was determined by a histochemical staining procedure (28). DCA-4, DCA-18, AAX-10, and AAX-11, independently derived virus preparations. Extracts prepared from uninfected NIH 3T3 cells and human bone marrow (hBM) were used to distinguish between the endogenous mouse isozyme and the vector-transduced human isozyme, respectively.

DISCUSSION

DC retroviral vectors were designed in response to the problems we and others have encountered in expressing retrovirally carried genes. The unique feature of DC vectors is that the foreign gene is inserted within the U3 region of the 3′ LTR of the vector resulting in the duplication of the gene and its transposition to the 5′ LTR, outside the retroviral transcriptional unit (Fig. 1). The utility of the DC vector

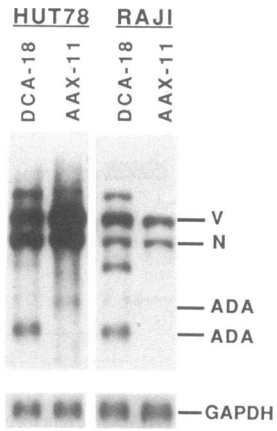

FIG. 5. RNA expression in human lymphoid cells infected with AAX and DCA viruses. HUT 78 or Raji cells (5×10^5) were infected with AAX or DCA virus in 1 ml of culture and grown for 48 hr before G418 was added to a concentration of 0.75 mg/ml; cells were then cultured for about 2 wk until control cultures did not contain live cells. RNA was prepared from the cells and analyzed as described in legend to Fig. 3. A Mo-MuLV U3-specific probe was used to detect vector-specific RNA transcripts. The putative *ADA* transcript present in Raji cells infected with AAX virus can be seen upon longer exposure of the x-ray film. A band migrating at approximately the same position in HUT 78 cells infected with AAX virus does not correspond to the *ADA* transcript. For additional details see the legend to Fig. 3.

design was tested by using a 2.1-kbp-long *ADA* minigene that was inserted into the 3′ LTR of the *Neo*-containing retroviral vector N2. DNA analysis has shown that the 2.7-kbp-long chimeric LTR was faithfully duplicated in the infected cell (Fig. 2). Several studies have described the insertion of short DNA sequences into the 3′ LTR of retroviruses, which did not adversely affect viral functions (29–31). What restrictions may exist on the insertion of foreign sequences in the U3 region of the retroviral LTR? In the course of retroviral replication, duplication of the LTR and its transposition to the 5′ end involves an actinomycin D-sensitive step in which the reverse transcriptase uses double-stranded DNA as template to generate a second copy of LTR (27). There is no evidence to suggest that this step will be significantly affected by the insertion of additional sequences into the LTR. It is tempting to speculate that, with probable exceptions, insertion of foreign sequences into the LTR will be tolerated, provided it does not affect essential viral functions. If so, the limitations on composition and lengths of sequence inserted into the LTR will be the same limitations encountered when foreign genes are inserted into retroviral vectors (see ref. 2 for review), the main limitation being the packagability of the corresponding RNA into virions, and a second possible limitation being the stability of proviruses containing long direct repeats (32). For example, the Mo-MuLV-derived DC vector used in this study should accommodate >6 kb of foreign sequence in the LTR.

The main prediction in the design of DC vectors was that the transposition of the gene to the 5′ LTR, outside the retroviral transcriptional unit, will enhance its expression. The experiments summarized in Figs. 3–5 show that expression of the *ADA* gene from a DC vector is significantly enhanced in the three cell lines tested—NIH 3T3, HUT 78, and Raji. This and the reduction in LTR-initiated transcripts (Fig. 3*B*) are consistent with previous observations that upstream promoters exert an inhibitory effect on promoters placed downstream (12–14) and may explain some of the problems encountered in expressing retrovirally carried genes from internal promoters (6, 7).

Efficiency of gene transfer, the fraction of cells transduced with a retroviral vector, is a function of virus titer. Although the important issue of virus titers has not been fully addressed in these studies, virus titers generated from the DCA vector ($0.2–0.8 \times 10^5$ cfu/ml) were only slightly lower than virus titers generated from the AAX vector ($1–2 \times 10^5$ cfu/ml) and significantly higher than the titer of virus generated from SIN vectors carrying the *ADA* gene ($0.5–2 \times 10^3$ cfu/ml; S.-F. Yu and E.G., unpublished results). Both DCA and AAX vectors are based on the high-titer N2 retroviral vector (11).

The specific DC vector described in this study, DCA, represents an example of several possible configurations of DC vectors that can be generated. cDNAs, minigenes, as well as whole genes can be inserted in either transcriptional orientation, throughout the U3 region, provided it does not interfere with viral functions. Moreover, the principle of SIN and DC vector design can be combined by removing the enhancer/promoter sequences from the chimeric 3′ LTR. Each configuration may have unique advantages and limitations and may serve a particular purpose.

In summary, the utility of DC vectors was demonstrated by using the *ADA* minigene, which was inserted into the 3′ LTR of the Mo-MuLV-based N2 retroviral vector. The general usefulness of this vector design will be determined only from the cumulative experience of many laboratories to introduce and express genes in cells of interest. Based on the studies reported here and additional preliminary experience from our laboratory, it is hoped that DC vectors will improve the ability to express retrovirally transduced genes and contribute to the solution of the problems encountered in expressing the transduced gene in cultured cells and, in particular, when introduced into the live animal.

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