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The First Strand Transfer during HIV-1 Reverse Transcription Can Occur either Intramolecularly or Intermolecularly

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Reverse transcription is a complicated process that involves at least two cDNA transfer reactions to produce a full-length copy DNA of the retroviral RNA genome. Because one retrovirus particle contains two identical genomic RNA molecules, the transfers can occur in an intramolecular or intermolecular manner. The mechanism of the first transfer step (minus-strand strong-stop cDNA transfer) has been studied previously in detail in transduction experiments with spleen necrosis virus vectors containing genetic markers. Different results have been reported with respect to the type of strand transfer mechanism. In this study, we analyzed the first strand transfer for human immunodeficiency virus type 1 (HIV-1). Two genetically marked genomes were copackaged into virions and reverse transcription was initiated within these particles upon permeabilization by NP-40 and addition of dNTPs. To test whether intrastrand or interstrand transfer had occurred, the cDNA products of this endogenous reverse transcription reaction were extracted from the virions and analyzed for the presence of restriction enzyme recognition sites provided by the genetic markers. The results of this analysis demonstrated that the first DNA transfer reaction occurs in a random manner, with approximately the same contribution of intrastrand and interstrand transfers. The ability to perform intermolecular strand transfer was lost upon extraction of the dimeric RNA template from the virion particle.

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The experimental system

Reverse transcription is initiated from a cellular tRNA primer bound at the PBS site and two cDNA transfer reactions are required to produce a full-length DNA copy with two long terminal repeat (LTR) segments. There is ample evidence that reverse transcription is initiated within extracellular particles prior to infection of new cells. For instance, most of the genome-associated tRNA\(^{Lys} \) primer in HIV-1 virions is in an extended form (16, 17). However, reverse transcription is apparently aborted before synthesis of the minus-strand strong-stop cDNA is completed (18, 19, 20). This failure to elongate reverse transcription is most likely because of a limitation in the concentration of available deoxypentanucleotides (dNTPs) (21, 22, 15). Reverse transcription can be reinitiated within these particles by permeabilization of the virion membrane with detergent and addition of a high concentration of dNTPs. This reaction is termed the endogenous reverse transcription assay and was used in this study to examine the first DNA transfer event. The major advantage of this intravirion reverse transcription assay compared with the transduction experiments used previously (6, 7) is that it is a more direct method to measure reverse transcription. For instance, the transduction type of experiments cannot rule out effects because of differences in the completion of reverse transcription, integration, or subsequent steps.

To study the first strand transfer mechanism for the HIV-1 virus, we used a replication-competent mutant of the pLAI molecular clone with nucleotide substitutions in the U3 and R regions of both 5’ and 3’ LTR elements (Fig. 1A). The mutation within the U3 element introduced an Aval or XhoI restriction enzyme recognition sequence, and the mutation within R inserted a BglII site. The rationale for using this mutant was as follows. If reverse transcription is performed within heterozygous virions that contain both a wild-type and mutant genome, the genetically marked R and U3 segments will become mixed upon interstrand transfer. Thus, a simple restriction analysis of the cDNA would allow one to distinguish between the two types of first strand transfer. Both the mutations in the U3 and R region were previously demonstrated to have no major effect on the virus replication rate (11, 23). This may be surprising because the mutation within R changes the bulge element of the TAR RNA hairpin. This hairpin is critically involved in binding of the Tat trans-activator protein (24). However, bulge sequence variation (UCU and UUU) is observed frequently in natural HIV isolates (25) and was demonstrated to fully support Tat-mediated activation of the viral LTR promoter (26). Furthermore, we measured no major contribution of the TAR element to packaging of the RNA genome into virus particles (Das and Berkhout, unpublished results). Another consideration in using this R region point mutation instead of a larger substitution in this study was to minimize any deleterious effects on the reverse transcription process. In particular, sequence differences between the donor and the acceptor R regions may negatively affect the strand transfer efficiency, although there is evidence that HIV-1 RT can efficiently translocate with an acceptor R region that is much smaller than the wild-type HIV-1 R element of 97 nucleotides (27).

The first strand transfer can occur either intramolecularly or intermolecularly

The experimental protocol is illustrated in Fig. 2. The cervix carcinoma cell line C33A was cotransfected with the two HIV-1 molecular clones. To maximize the production of heterozygous virus particles (Fig. 2), we used the calcium phosphate transfection method that allows for efficient cotransfection of cells with multiple DNA molecules. The two HIV-1 plasmids were also transfected individually to provide appropriate control samples. Viruses were harvested 3 days posttransfection and reverse transcription was performed within these particles in the so-called endogenous assay. The viral cDNA was subsequently extracted from the virions and the U3/R region was PCR amplified and analyzed by diagnostic restriction enzyme digestions. This direct PCR-mediated analysis of the progeny DNAs allowed us to examine large populations of reverse transcription products and avoid sampling biases.

The HIV-1 DNA products that had completed the first DNA transfer were selectively amplified by a set of primers located in the nef gene and the PBS site. PCR amplifications were performed, and the resulting products were analyzed by agarose gel electrophoresis and ethidium bromide staining to visualize the DNA product (results not shown). When the DNA from dNTP-treated virions was used as template for PCR amplification, a discrete 827-bp DNA product was observed, which is the size expected for a translocated DNA. Control virion samples that were incubated without dNTPs did not produce this DNA signal, indicating that the first DNA transfer reaction was accomplished within the extracellular particles upon the addition of dNTPs. A longer DNA product of approximately 3000 bp could be detected in the PCR samples. This product results from PCR amplification on the HIV-1 plasmids that were used for transfection. Although the set of primers was designed to be specific for translocated minus strand, amplification of the input HIV-1 plasmids is possible because the 3’ and
LTR sequences are connected over the plasmid backbone. To exclude this plasmid-derived signal from the subsequent restriction enzyme analysis, we eluted the 827-bp fragment from the gel and performed five additional PCR cycles in the presence of \( \alpha-\text{32P} \)dCTP.

We next estimated what portion of progeny DNAs arose from intramolecular and intermolecular strand transfer by digesting the \( \text{32P} \)-labeled 827-bp fragment with the appropriate restriction enzymes. This approach is complicated by the presence of a mixture of homozygous and heterozygous virus particles (illustrated in Fig. 2). As discussed above, both HIV-1 genomes are transcribed at similar levels, even though a single nucleotide was substituted in the mutant TAR element (26). Thus, approximately 50% of the virion will be heterozygous, and the two homozygous virion forms will each constitute 25% of the virus population. If the first strand transfer is purely intrastrand, even the heterozygous particles will

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### Table A

<table>
<thead>
<tr>
<th>U3 region</th>
<th>R region</th>
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<tr>
<td><strong>wild-type</strong></td>
<td></td>
</tr>
<tr>
<td>CAGCTGCTTTTGGCTGTACTG</td>
<td>GTCTCTCTGTAGACCAGATTGA</td>
</tr>
<tr>
<td>+1</td>
<td></td>
</tr>
<tr>
<td><strong>mutant</strong></td>
<td></td>
</tr>
<tr>
<td>CAGAGTTAGCTGACTGAGATG</td>
<td>GTCTCTCTGTAGACCAGATCTGA</td>
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</tbody>
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\( \text{AvaI/XhoI} \)

\( \text{BglII} \)

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### Table B

<table>
<thead>
<tr>
<th>U3 region</th>
<th>R region</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AvaI/BglII</strong></td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>152</td>
</tr>
<tr>
<td>+/−</td>
<td>152</td>
</tr>
<tr>
<td>−/+</td>
<td>178</td>
</tr>
<tr>
<td>−/−</td>
<td></td>
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**FIG. 1.** Constructs used to analyze the first strand transfer mechanism. (A) Part of the HIV-1 U3-R sequences of the wild-type and mutant HIV-1 construct (+1 denotes the transcription start site). A stretch of 17 nucleotides was mutated in the U3 region, thereby creating the AvaI/Xhol restriction site. A BglII restriction site was introduced by single nucleotide substitution in the R region. (B) An outline of the restriction enzyme analysis of cDNA products. HIV-1 cDNA products that performed the minus-strand DNA transfer and subsequent elongation were selectively PCR-amplified by a set of primers located in the nef gene and the PBS site. This product, which encompasses the U3-R region, is shown on top. The presence of AvaI and BglII restriction sites is indicated. Please note that upstream AvaI and BglII sites are present in the U3 region of all constructs, but the downstream sites are diagnostic for the +/+ mutant. The size of the DNA digestion products is indicated in nucleotides. Upon denaturing gel electrophoresis, some fragments will show two DNA strands of different size due to the staggered cut by the restriction enzymes used. The bottom panel illustrates the AvaI/BglII digestion pattern for all U3-R combinations (+/+ , +/− , −/+ , and −/− ). Each genotype will produce one unique DNA fragment, which is marked by an asterisk. For instance, intrastrand transfer will lead to conservation of the wild-type and mutant genotypes (represented by the −/− and +/+ signs), with unique DNA fragments of 361/357 and 26 nt, respectively. The +/− and −/+ cDNA sequences, which result from interstrand transfer, produce unique fragments of 209/205 and 178 nt, respectively. The ratio of these four unique fragments was analyzed in the subsequent analyses (Fig. 3 and Table 1). The unique 178-nt fragment of the −/+ genome was difficult to quantitate because of the overlap with the 179 fragment that is produced by both the +/+ and −/+ genotypes. To resolve this, we included the HindIII restriction enzyme in some of the subsequent analyses. This enzyme cleaves the 183/179 fragment at position +77 of the R region, such that the unique 178-nt fragment can be detected more easily on the gel. Because the introduced U3 restriction site is recognized by both Aval and Xhol, we also used the latter enzyme in some of the assays. This produces a different pattern of DNA fragments because Xhol does not recognize the upstream Aval site.

5′ LTR sequences are connected over the plasmid backbone. To exclude this plasmid-derived signal from the subsequent restriction enzyme analysis, we eluted the 827-bp fragment from the gel and performed five additional PCR cycles in the presence of [\( \alpha-\text{32P} \)]dCTP.
not produce U3/R recombinants. Thus, the total progeny will have either both diagnostic U3/R restriction sites (50% +++) or no sites (50% --/__). On the other hand, pure interstrand transfer will produce a progeny with a fully mixed genotype (25% each of ++/+, +/−, −/+ and −/−). If strand transfer occurs randomly, that is, 50% interstrand and 50% intrastrand, we expect the following ratio of genotypes (37.5% of both input forms ++/+ and −/− and 12.5% of the mixed genotypes +/− and −/+).

The cleavage products for all four possible U3/R combinations upon Aval/BglII double digestion are illustrated in Fig. 1B. Several fragments are produced by all U3/R combinations and are therefore noninformative (e.g., the fragments of 138/142 and 329 nt). However, each U3/R combination encodes one unique DNA fragment that was labeled by an asterisk in Fig. 1B (e.g., the U3 genome produces the unique 209/205 signal). For a quantitative analysis, we performed several restriction analyses and focused on the ratio of these four unique DNA products. The results of four independent restriction enzyme digestion assays are summarized in Table 1. This table also lists the expected ratios for the three hypothetical strand transfer mechanisms.

An example of a quantitative analysis of the DNA digestion products is presented in Fig. 3. The digestion pattern was compared for DNA derived from the two control transfections with the individual plasmids (lanes marked a and b) and the cotransfection (lanes marked c). To comprehend the complex pattern of digestion products, we included control digestions with a single restriction enzyme. Furthermore, we used both Xhol and Aval enzymes to screen for the presence of the U3 mutation. Although several batches of the Xhol restriction enzyme from different commercial sources were tested, a significant proportion of the DNA reproducibly failed to be cleaved to completion. Obviously, incomplete digestion

### Table 1

<table>
<thead>
<tr>
<th>DNA Fragments</th>
<th>Ratioa of DNA Fragments</th>
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<tr>
<td>++</td>
<td>96</td>
</tr>
<tr>
<td>+/−</td>
<td>504</td>
</tr>
<tr>
<td>−/+</td>
<td>66</td>
</tr>
<tr>
<td>−/−</td>
<td>592</td>
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a Ratio of the four specific DNA fragments (marked with an * in Fig. 1B) that distinguish among the different DNA transfer mechanisms. The hypothetical ratio is provided for three types of DNA transfer reactions. The experimental ratio was determined in reverse transcription reactions performed within virion particles (endogenous assay).

b Random means 50% intrastrand and 50% interstrand transfer.

c Specific DNA fragments were quantitated on a Phosphor Image.

The relative counts were divided by the number of dCTP residues in that particular DNA fragment (PCR primer sequences not included).

d Aval/BglII digest with specific fragments of 26, 178, 209/205, and 361/357 nt.

e Aval/BglII/HindIII digest with specific fragments of 26, 507, 209/205, and 690/686 nt.

f Not determined because of overlap of the specific 178-nt fragment with the 179-nt internal fragment.
will impede a careful analysis of the undigested products that have lost the U3/R restriction sites. We therefore performed all subsequent assays with the Aval enzyme. Another technical difficulty was the quantitation of the unique 178-nt fragment, because of the presence of an internal 179-nt signal (Fig. 3, lane 15). To separate these two signals, we included the HindIII restriction enzyme in some digestions, which truncates the 179/183 internal fragments (Fig. 1B).

Most importantly, we found evidence for interstrand transfer by the appearance of unique DNA fragments in the progeny of the cotransfection experiment. For instance, the Aval/BglII analysis of the cotransfected sample produces the unique 209/205 signal (Fig. 3, lane 15), which was absent from the two single transfections (lanes 13 and 14). However, this signal was not as intense as several of the control fragments (e.g., the 138/142 and 329-nt fragments), indicating that transfer did not occur in a purely interstrand manner. The different signals were quantitated and corrected for the number of dCTP incorporated per fragment. Although considerable variation was observed among different digestion experiments, this endogenous reverse transcription revealed a ratio of the unique products that resembles the hypothetical 3:1:1:3 ratio (Table 1). These results strongly suggest that the first DNA transfer in HIV-1 occurs in a random fashion, with an approximately balanced contribution of the intramolecular and intermolecular transfer. In a separate set of experiments, we first extracted the RNA genome from HIV-1 particles and then performed reverse transcription by addition of dNTPs and the HIV-1 RT enzyme. This assay yielded no fragments that are indicative of an interstrand strand transfer mechanism, even though the RNA genome was still in the native dimer conformation (results not shown).

Previous studies with the SNV used constructs with genetic markers to distinguish between interstrand and intrasranch transfers of the minus-strand strong-stop cDNA. It was initially reported that this first strand transfer occurred exclusively between the two packaged RNA genomes in an interstrand manner (6). However, another group observed intrasranch as well as intertransfert first transfer (7). Both groups observed only intramolecular transfer in the second transfer reaction, which was not addressed in the current study with the HIV-1 retrovirus. We used a more direct assay to study reverse transcription in extracellular virions and found that both type of transfers occur during minus-strand strong-stop cDNA translocation. Furthermore, we determined the relative frequency of the two sorts of strand transfer to be approximately 50% each. Because of this dramatically high recombination efficiency, one can disregard a possible contribution of DNA recombination that can occur between two cotransfected plasmids in our experimental system.

Several obvious differences exist between our experimental system and that used by others to analyze the cDNA transfer reaction (6, 7; see also the detailed discussion in (5)). These differences include: (i) the type of retrovirus; our study represents the first analysis with HIV-1, previous studies were performed with vectors based on SNV (6, 7); (ii) we used infectious HIV-1 virions with a full-length RNA genome, whereas the other studies used transduction-competent retroviral vectors with large insertions for selection markers; (iii) the reverse transcription reaction was followed within virus particles in our study, whereas a more indirect screening system was used in the other studies, including the selection of drug resistance in the transduced cells; (iv) unlike in our study, one of the previous studies (7) used vectors with inactivating mutations, such that recombination during
reverse transcription was required to produce progeny; (v) unlike in our experiments, the other study (6) used a 1000-fold excess of one of the two genomes. Such experimental differences may explain the observation of purely interstrand transfer in the initial study (6). More importantly, the SNV study of Hu and Temin combined with the current HIV-1 analysis strongly suggest that all retroviruses use both intramolecular and intermolecular first strand transfers. The observation that the isolated RNA genome does not support an interstrand jump suggests a facilitating role of the virion architecture.

It can be argued that mutation or reassortment of the U3-R elements occurred during PCR amplification of the progeny DNA. It seems impossible that spontaneous errors by Taq DNA polymerase could have generated these specific mutations at this high frequency. However, it cannot be excluded that reassorted/hybrid genomes were generated by PCR-mediated recombination between the wild-type and mutant DNA. In fact, previous studies suggest that recombination during PCR is a fairly common phenomenon (28, 29). To minimize the chances for PCR-mediated recombination we used a PCR protocol with a minimal number of cycles and maximal extension times. To further rule out that the +/− and −/+ genomes were generated during PCR amplification, we performed one additional experiment. The cDNA products of endogenous reverse transcription within wild-type and mutant virions (derived from separate transfections) were mixed prior to PCR amplification. The subsequent restriction enzyme analysis revealed no recombinant DNA products (not shown). Furthermore, PCR analysis of the reverse transcription products of the extracted HIV-1 RNA genome did not result in the appearance of recombination products (not shown). These combined results rule out PCR artifacts.

A unique property of retroviruses is the presence of a dimeric RNA genome, and an interstrand first transfer may boost the recombination rate of these viruses. An alternative mechanism was originally proposed to explain the extraordinary high recombination rate in retroviruses. It was suggested that retroviral RNA genomes are frequently broken and that the RT enzyme, upon encountering a break in the RNA template, can jump onto the other RNA strand of the dimeric complex (30, 31). This 'forced copy choice transfer' may be mechanistically similar to the first strand transfer, and both mechanisms will lead to frequent recombination during retrovirus replication. These mechanisms may increase the genetic diversity in the virus population and may repair lethal mutations (reviewed in (32, 33)). In addition, such strand transfers were shown to increase the frequency of base misinsertions (34, 35), which may provide the virus with an extraordinary evolutionary capacity.

HIV-1 constructs and DNA transfection

The full-length infectious molecular clone pLAI was kindly donated by Keith Peden (10). Introduction of the Xho-10 mutation within the U3 region of both 5′ and 3′ LTRs was described previously (11). Because the mutant LTR segment that was introduced into the pLAI clone was derived from a different viral background (12), an additional mutation was present in both LTRs at position +23 of the R region. These mutations in the U3 and R regions create diagnostic XhoI/Aval and BglII restriction enzyme recognition sequences (illustrated in Fig. 1A). C33A cervix carcinoma cells were grown in Dulbecco's modified Eagle's medium supplemented with glucose, MEM amino acids, and 10% fetal calf serum at 5% CO2 and 37°C. These cells were cultured to a subconfluent monolayer in a 75-cm2 flask and transfected by the calcium phosphate method as described previously (13). We used 40 μg DNA per transfection (wild-type or mutant pLAI molecular clone) and cotransfection was performed with 20 μg of each plasmid.

Endogenous reverse transcription

The culture supernatant of transfected C33A cells was harvested at day 3 posttransfection and used as a source of virions for the endogenous RT assay. This assay allows reverse transcription to proceed within permeabilized virus particles and was performed according to Schwartz et al. (14) with some minor deviations (15). The synthesized cDNA was extracted from the virion particles, precipitated with NaAc/ethanol, and dissolved in 20 μl TE buffer (10 mM Tris–HCl, pH 8.0, 1 mM EDTA), of which 5 μl was used for PCR amplification of the HIV-1 U3-R region.

PCR amplification of progeny DNA, labeling, and restriction enzyme digestion

DNA products of the endogenous and exogenous assay were amplified by PCR, with a 5′ primer identical to nef sequences located upstream of the U3 region (NEF-SEQ2, positions 8961 to 8970 on pLAI) and a 3′ primer complementary to the PBS site just downstream of the U5 region (Lys21, positions 636 to 656). The PCR was performed in 100 μl standard buffer with 800 μM dNTPs as follows: 25 cycles (1 min at 95°C, 1 min at 55°C, and 2 min 72°C) and final extension for 10 min at 72°C. Ten microliters was analyzed on a 1% agarose gel and the 827-basepair (bp) U3-R product was purified by USBio-Clean MP extraction (USBio Biochemicals). This product was solubilized in 20 μl, of which 5 μl was used for 5 additional PCR cycles in the presence of [α-32P]dCTP (1 μl of specific activity 3000 Ci/mmol, 10 μCi/ml, Amer sham) and 80 μM unlabeled dNTPs. The labeled DNA was purified on an agarose gel, eluted, and used for restriction enzyme digestions in the buffer provided by
the manufacturer. The digestion products were mixed with an equimolar amount of formamide sample buffer, heated at 95°C, and analyzed on a denaturing 6% urea±
polyacrylamide gel. Gels were exposed to X-ray film and
analyzed with a PhosphorImager (Molecular Dynamics) to
quantitate specific DNA bands.

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References

merase in virions of Rous Sarcoma Virus. Nature 226, 1211±
1213.
dependent DNA polymerase in virions of RNA tumour viruses.
Nature 226, 1209±1211.
model of reverse transcription and tests of crucial aspects. Cell 18,
93±100.
Harbor Laboratory Press, Cold Spring Harbor, NY.
intrastrand DNA transfer during reverse transcription. Science
241, 1064±1069.
reverse transcription. Science 250, 1277±1283.
is sufficient for synthesis of viral DNA. J. Virol. 68, 207±216.
Homologous recombination occurs in a distinct retroviral sub-
population and exhibits high negative interference. J. Virol. 71,
6028±6036.
growth properties on passage in tissue culture of viruses de-
rived from infectious molecular clones of HIV-1LAI, HIV-3MAL, and
HIV-1LVI. Virology 185, 661±672.
tants of human immunodeficiency virus type 1 viruses mutated in
the long terminal repeat promoter region. J. Gen. Virol. 76,
845±853.
activates the human immunodeficiency virus through a nascent
the human immunodeficiency virus type 1 RNA genome is essen-
Human immunodeficiency virus type 1 Nef increases the effi-
ciency of reverse transcription in the infected cell. J. Virol. 69,
4053±4059.
tions emphasize the processivity defect of lamivudine-resistant vari-
ants of HIV-1 reverse transcriptase. Antimicrob. Agents Che-
mother. 41, 1248±1249.
transcriptase discriminates against non-self tRNA primers. J.
Mol. Biol. 264, 243±254.
17. Huang, Y., Wang, J., Shalom, A., Li, Z., Khorcid, A., Wainberg, M. A.,
and Kleiman, L. (1997). Primer tRNAly3 on the viral genome
exists in unextended and two-base extended forms within ma-
immunodeficiency and murine leukemia viruses. J. Virol. 66,
4893±4900.
and Gallo, R. C. (1992). Viral DNA carried by human immunode-
ficency virus type 1 virions. J. Virol. 66, 5067±5074.
found in human immunodeficiency virus type 1 particles may not
Incompletely reverse-transcribed human immunodeficiency vi-
rus type 1 genomes in quiescent cells can function as interme-
type 1 reverse transcription in blood mononuclear phagocytes
are slowed by limitations of nucleotide precursors. J. Virol. 68,
1258±1263.
mutated retroviral genomes. Nucleic Acids Res. 21, 5020±5024.
24. Dingwall, C., Emborg, I., Gait, M. J., Green, S. M., Heaphy, S., Kam,
Human Immunodeficiency Virus 1 tat protein binds trans-acti-
Sci. USA 86, 6925±6929.
simian immunodeficiency viruses: A phylogenetic analysis. Nuc-
elic Acids Res. 20, 27±31.
TAR RNA: Critical spacing between the bulge and loop recogni-
tion domains. Nucleic Acids Res. 19, 6169±6176.
DNA strand transfer during reverse transcription in mutant HIV-1
cloning of HLA-AB cDNA by using the polymerase chain reac-
tion: Frequency and nature of errors produced in amplification.
recombination during PCR. Nucleic Acids Res. 18, 1687±1691.
30. Vogt, P. K. (1973). “Possible Episomes in Eukaryotes” (L. Sylvestri,
retrovirus genomes: some unifying hypotheses. J. Gen. Virol. 42,
1±26.
33. Temin, H. M. (1993). Retrovirus variation and reverse transcription:
Abnormal strand transfers result in retrovirus genetic variation.
immunodeficiency virus type 1 reverse transcriptase at RNA
and DNA template ends. Proc. Natl. Acad. Sci. USA 91, 549±
553.
strand transfer reactions catalyzed by HIV-1 reverse transcrip-
tase. Biochemistry 33, 3890±3895.