Supporting Online Material

Materials and Methods

Plasmids containing the wild-type HIV-1 sequence (pNL4-3) or a variant in which a portion of the *vif* gene had been deleted (Δ*vif*) (A. M. Borman, et al., *J. Virol*. **69**, 2058, 1995) were used. H9 cells were obtained from the American Type Culture Collection.

Intracellular reverse transcription

Viral stocks in complete medium were mixed with an equal volume of 100 mM Tris-HCl (pH 8.0) containing 0.2 mM $CaCl_2$, 19.2 mM $MgCl_2$ and 340 units/ml DNase I, and incubated (37°C; 30 min). P4 cells were infected by spinoculation (860 x g; 120 min; 22°C). Medium was removed, the cells rinsed once with PBS, and 0.1 ml complete medium was added. Cells were either processed immediately (t=0) or cultured at 37°C for 5 hours. Medium was removed, cells were detached with proteinase K, lysed and DNA purified using the QIAquick 8 PCR purification kit (Qiagen, Valencia, CA). The amount of DNA detected in samples processed prior to incubation (t=0), was always <2% of that detected after the 5 hour incubation at 37°C, as measured by real-time PCR (n=8).

Endogenous reverse transcription

Viral stocks (30 µl) were mixed with an equal volume of 100 mM Tris-HCl (pH 8.0) containing 2 mM $CaCl_2$, 10 mM $MgCl_2$ and 340 units/ml DNase I, and incubated (37°C; 30 min). Forty µl of 7.5 mM EGTA, 0.25% (v/v) NP-40 and 1.25 mM dNTPs was added, and the mixture incubated (39°C; 120 min.). DNA was purified as above. In the absence of dNTPs, the amount of DNA detected in the samples (both wild-type and Δvif virions) was <1% of that present in samples incubated with 500 µM dNTPs (n=6).

Amplification and cloning of viral sequences

RNA was purified from virions produced by H9 cells. Control experiments involving treatment with protease inhibitors indicated that residual HeLa cell virus was not detectable in

these samples (data not shown). Viral cDNA was synthesized using the following reaction conditions: 10 mM DTT, 60 U RNaseOUT ribonuclease inhibitor (Invitrogen), 500 μM dNTPs, 1.25 μM random hexamers, 450 U Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen) and 1X reaction buffer; 42°C for 1 hour. Mock reactions (in which the M-MLV reverse transcriptase was omitted) were performed in parallel. Quantification of viral DNA in these samples indicated that contaminating plasmid DNA or DNA associated with viral particles represented <0.1% of total sequences present after the reverse transcription reaction.

DNA or cDNA was amplified using the following primer pairs: U5+ (5'-RACCCACTRCTTAARCCTCAATA) and U5-rev (5'-YAYTGAYTAAAAGGGTYTGAGGGA) or ENV+ (CACARACCCCAACCCACAA) and ENV-rev (5'-AAYTAAYAYAGAGTGGGGTTAATTTTA). Reaction conditions were: 1X buffer, 2.5 mM $MgCl_2$, 400 μM each dNTP, 200 nM each primer, 1.25U of AmpliTaq Gold *Taq* polymerase (Applied Biosystems). Cycling parameters were 95°C for 10 min, 40 cycles at 95°C for 30 s, 54°C for 30 s; and 72°C for 60 s each; 72°C for 10 min. The PCR products were cloned into pCR2.1-TOPO (Invitrogen) and used to transfect *Escherichia coli*. Plasmids were purified from individual colonies and the insert was sequenced. Unless indicated, PCR reactions were performed using DNA from two or more independent samples, and equal numbers of colonies from each reaction (usually five) were evaluated.

Figure Legends

Figure S1. Sequence analysis of the *env* region for viral DNA synthesized by infected P4 cells. DNA was isolated from P4 cells cultured for 5 hours after spinoculation with virions produced by: (A) H9 cells infected by co-cultivation with transfected HeLa cells for 24 hours and then cultured separately. (B) transfected HeLa cells, or (C) P4 cells infected with cell-free virions produced by transfected HeLa cells. DNA was amplified using degenerate primers (see above), and the amplification products were cloned and sequenced. The sequence of pNL4-3 for the amplified fragments is shown (annotated as per GenBank M19921), and only bases different from these sequences are shown for the other sequences. The numbers preceding the sequences (2x, 3x, …) indicate the number of clones with the given sequence.

Figure S2. Sequence analysis of the *U5* region for DNA synthesized by infected P4 cells. DNA was isolated from P4 cells cultured for 5 hours after spinoculation with virions produced by: (A) H9 cells infected with cell-free virions produced by transfected HeLa cells, (B) H9 cells infected by co-cultivation with transfected HeLa cells for 24 hours and then cultured separately. DNA was amplified using degenerate primers (see above), and the amplification products were cloned and sequenced. The sequence of pNL4-3 for the amplified fragment is shown (annotated as per GenBank M19921), and only bases different from these sequences are shown for the other sequences. The numbers preceding the sequences (2x, 3x, …) indicate the number of clones with the given sequence. $p<0.01$ comparing number of wild-type and Δvif sequences in A and B with G→A changes using Fisher's exact test. †clones from a single amplification were evaluated.

Figure S1

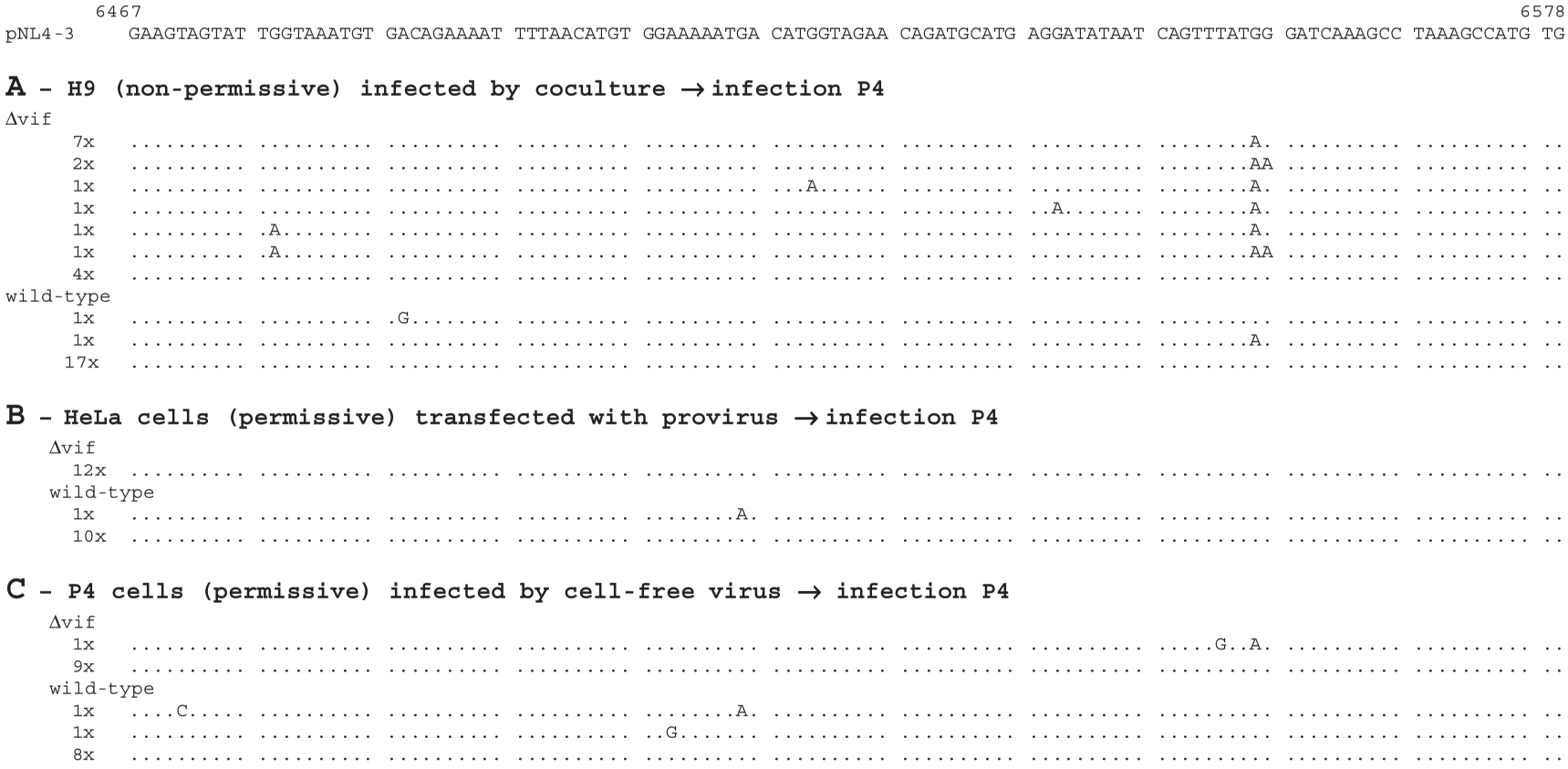
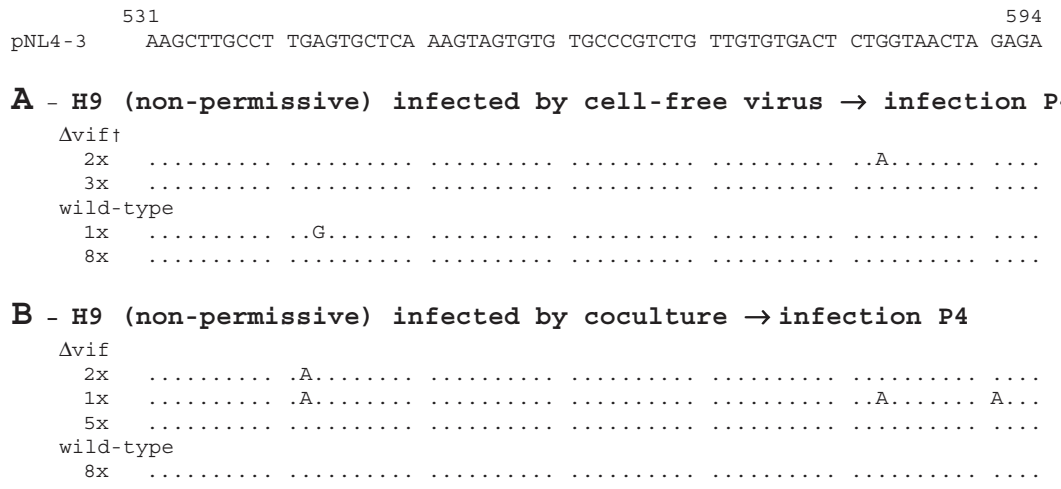

6467
6578
pNL4-3 GAAGTAGTAT TGGTAAATGT GACAGAAAAT TTTAACATGT GGAAAAATGA CATGGTAGAA CAGATGCATG AGGATATAAT CAGTTTATGG GATCAAAGCC TAAAGCCATG TG
A - H9 (non-permissive) infected by coculture → infection P4
Δvif
7x
2x
1x
1x
1x
1x
4x
wild-type
1x
1x
17x
B - HeLa cells (permissive) transfected with provirus → infection P4
Δvif
12x
wild-type
1x
10x
C - P4 cells (permissive) infected by cell-free virus → infection P4
Δvif
1x
9x
wild-type
1x
1x
8x

Figure S2


531
594
pNL4-3 AAGCTTGCCT TGAGTGCTCA AAGTAGTGTG TGCCCGTCTG TTGTGTGACT CTGGTAACTA GAGA
A - H9 (non-permissive) infected by cell-free virus → infection P4
Δvif†
2x ....A..... ....
3x ....
wild-type
1x ....G..... ....
8x ....
B - H9 (non-permissive) infected by coculture → infection P4
Δvif
2x ..... .A..... ....
1x ..... .A..... ....A..... A...
5x ....
wild-type
8x ....