

Scientific Correspondence

Dear Editor,

Benjamin Berkhout (1) has recently published a sequence analysis of the primer binding site (PBS) region of the RNA genome of simian and human immunodeficiency viruses (SIV and HIV). He concluded that: (i) the U5 hairpin upstream of the PBS is critical for virus replication, (ii) a proposed interaction between the A-rich loop of the HIV-1 U5 hairpin and the anticodon loop of the primer tRNA may not occur through direct basepairing. The first conclusion is supported by sequence and structure conservation in these viruses and by recent experiments on an avian virus (2). The second conclusion is based on the fact that the proposed interaction is not possible in SIV. However, there is no experimental evidence indicating that the primer/template interactions are similar in HIV and SIV. Indeed, HIV-2 and SIV reverse transcriptases are unable to efficiently initiate reverse transcription of HIV-1 RNA from tRNA₃^{Lys}, even though they use the same primer (3). Moreover, the loop-loop interaction, initially proposed from structure probing (4) and site-directed mutagenesis (5), has been independently supported: (i) a tRNA₃^{Lys} containing a mutated anticodon, although packaged into the virion, is not used as a primer in endogenous reverse transcription (6); (ii) deletion of the A-rich loop causes defects in HIV-1 replication, and an A-rich loop is progressively restored during long-term culture (7); (iii) alternative primer tRNAs are stably used in long-term culture, provided that both the PBS and the A-rich loop are mutated to match these tRNAs (8,9). In this case, an initial replication defect is corrected after prolonged culture by appearance of mutations in the stem of the U5 hairpin. As suggested by B. Berkhout, a structural rearrangement is likely responsible for this phenotypic reversion (1). The proposed rearrangement that restores the transiently destabilized hairpin structure, does not preclude the loop-loop interaction with the tRNA. Furthermore, it does not explain why the A-loop must be mutated to stably maintain an alternative tRNA as primer.

**Chantal Ehresmann*, Bernard Ehresmann,
Roland Marquet and Mark Wainberg¹**

UPR 9002 du CNRS
IBMC, 15 rue R. Descartes
67084 Strasbourg-cedex
France

¹MacGill University AIDS Centre
Jewish General Hospital
3755 Cote Ste-Catherine Rd
Montreal, Quebec H3T 1E2
Canada

*Tel: +33 1 88 41 70 54; Fax: +33 3 88 60 22 18;
Email: ehresmc@ibmc-u-strasbg.fr
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Dear Editor,

All HIV-SIV viruses have conserved the U5 hairpin (1), but the A-rich loop that has been proposed to interact with the U-rich anticodon of the tRNA^{Lys3} primer (2) is not maintained in evolution. In their comment, Ehresmann *et al.* list observations that support the anticodon/A-loop interaction for HIV-1. Mutation of the tRNA^{Lys3} anticodon or the A-rich loop causes a defect (3,4). Although these findings testify to the importance of these sequence motifs, it does not form independent evidence for the anticodon/A-loop interaction. Several studies have suggested that the A-loop sequence must be changed to allow the stable maintenance of a mutant PBS that binds an alternative tRNA primer. However, the 'genetic stability' of HIV-1 PBS mutants in prolonged cultures is not an accurate measurement of their replication potential. Such evolutionary experiments, which are based on random mutation and subsequent selection of faster replicating variants, are rather intricate and depend on multiple parameters, including fitness of the mutant virus. In fact, mutation of the A-rich loop to accommodate a new primer tRNA leads to reduced instead of increased replication (5). When such crippled viruses are used for reversion experiments, it is not surprising to find a 'stabilizing effect' because evolution depends on spontaneous mutation and therefore on the viral replication rate. Furthermore, an unforeseen problem had been introduced by the original A-loop mutations due to stabilization of the U5 hairpin (1). HIV-1 apparently decided to first repair this bottleneck, which will delay other repair processes. It should also be noted that 'stable' HIV loop/PBS mutants do acquire mutations within the new loop sequences (6). Most strikingly, the mutant loop reverts to an A-rich sequence, which supports the idea that this sequence motif plays a critical role in virus replication that is independent of the type of tRNA primer used. Thus, there is some direct evidence against the proposed HIV-1 vRNA-tRNA interaction model. The role of both the U5 hairpin present in all HIV-SIV viruses and the HIV-1 A-rich loop remains to be determined.

Benjamin Berkhout

Department of Human Retrovirology
Academic Medical Center
Meibergdreef 15
1105 AZ Amsterdam
The Netherlands

Tel: +31 20 566 4822; Fax: +31 20 691 6531;
Email: b.berkhout@amc.uva.nl
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