Repair of essential terminal sequences by a negative stranded RNA virus.

Cheryl Walter and John Barr

The genomic termini of RNA viruses contain essential *cis*-acting signals for such diverse functions as packaging, genome translation, mRNA transcription and RNA replication, and thus preservation of their sequence integrity is critical for virus viability. Sequence alteration can arise due to cellular mechanisms that add or remove nucleotides from terminal regions, or alternatively from introduction of sequence errors through nucleotide mis-incorporation by the error-prone viral RNA dependent RNA polymerase (RdRp). To preserve template function, many RNA viruses utilize repair mechanisms to prevent accumulation of terminal alterations. Here we show that *Bunyamwera virus* (BUNV), the prototype of the *Bunyaviridae* family of segmented negative-sense RNA viruses, also can repair its genomic termini. When an intact non-translated region (NTR) was added to the anti-genomic 3' end, it was precisely removed, to restore both length and RNA synthesis function of the wild-type template. Furthermore, when up to 15 nucleotides were removed from the anti-genome 3' end, and replaced with a duplicate and intact NTR, both the external NTR was removed, and the missing nucleotides were restored, thus indicating that the BUNV RdRp can both remove and add nucleotides to the template. We show that the mechanism for removal of terminal extensions is likely that of internal entry of the viral RdRp during genome synthesis. In contrast we propose that repair of missing terminal nucleotides occurs through a RdRp-mediated homologous recombination event.

This mechanism is particularly interesting in the context of a segmented RNA virus such as BUNV, as it is possible that sequences from one segment may be used to repair the damaged sequences of another.

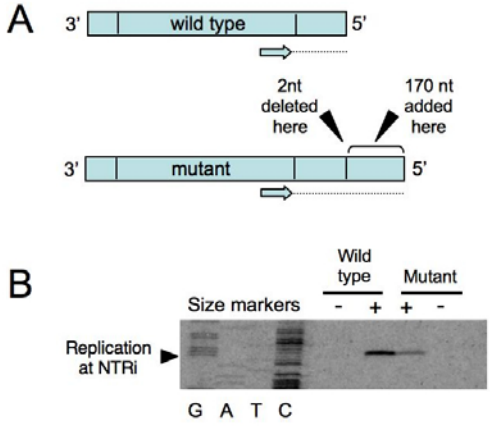

Figure 1: (a) schematic of wild-type and mutant Bunyamwera virus RNA templates. The mutant template has an additional 170 nucleotides at its 5' end, as well as 2 nucleotides deleted, as indicated. Primer extension analysis using a primer that anneals as indicated by the arrow can determine the length of the replicated RNA. **(b)** Primer extension analysis shows that following replication, the wild type and mutant templates are the same length, indicating both the removal additional nucleotides, and repair of the deleted nucleotides.

Publications

Walter, C. and Barr, J. (2010) Bunyamwera virus can repair both insertions and deletions during RNA replication. *RNA*, **16**:1138-1145.

Barr, J. and Fearns, R. (2010) How RNA viruses maintain their genome integrity. *J Gen Virol*, **91**:1373-1387.

Funding

Work in the Barr laboratory is funded by The Wellcome Trust, and the Health Protection agency, and is gratefully acknowledged.