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Does MMLV-RT lacking RNase H activity have the capability of switching templates during reverse transcription?

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The reverse transcription reaction which transcribes mRNA into cDNA is catalyzed by an RNA-dependent DNA polymerase named reverse transcriptase (RT). This enzyme is involved in the infectious cycle of retroviruses and promotes RNA recombination by the strand transfer (also called strand switch) mechanism to expand the genetic variability of viruses. Research shows that the occurrence of strand transfer depends upon both activities carried by RT, that is, its RNase H and polymerase activities. Before switching templates, RNase activity is required for the removal of RNA fragments annealed to the nascent DNA strand, which makes the 3′ end of this DNA available for hybridization to the homologous region of another template RNA [1]. In vitro experiments also suggest that homologous recombination between two distinct RNA templates promoted by RT needs the involvement of RNase H activity [2,3]. For example, Negroni et al. [3] found RNase H^+ RT may produce low recombination frequency (1.4×10^{-3}) from two distinct RNA templates, but RT lacking RNase H activity (RNase H^- RT) cannot recombine two RNA molecules. Moreover, to our knowledge, no one has reported that RNase H^- RT has the capability of switching templates during reverse transcription.

Regarding the argument proposed by Zeng and Wang, the question is whether BmTXKβ–BmKCT is an artifact or a naturally occurring transcript. First we must emphasize that the RT we used in the synthesis of the first strand cDNA is an engineered MMLV-RT provided by Gibco BRL (Superscript RT). This enzyme does not contain RNase H activity. Therefore, this excludes the possibility of template switching. Considering the fact that Zeng and Wang used the same RT as we did for the synthesis of the first strand cDNA, we do not know how they detected a 1% recombination rate in their experiment. And in fact, even with RNase H^+ RT, template switching in vitro is a low frequency event because the nascent DNA must bind to the free homologous region of the second RNA template. In other words, the acceptor RNA must remain uncomplexed to its cDNA during template switching [3]. Obviously, this scenario should be very rare in an in vitro system.

Lastly, the only paper cited by Zeng and Wang for criticizing our work actually does not support their results. Perhaps they neglected the basic conclusion made by Ouhammouch et al., which is that RNase H^+ AMV-RT only forms fusion products at 37°C for much longer incubation times (90 min) [5]. In fact, both Zeng and Wang and our laboratory used RNase H^- MMLV-RT to perform the first strand reaction at 37°C for 60 min [4].

In conclusion, regardless of whether one argues on the basis of reaction parameters or enzymology, BmTXKβ–BmKCT is a naturally occurring transcript, and not an artifact. We feel that Zeng and Wang's argument lacks support in theory and overemphasizes cloning errors derived from RT.

References

[1] Negroni, M. and Buc, H. (2001) Annu. Rev. Genet. 35, 275–302.
[2] Peliska, J.A. and Benkovic, S.J. (1992) Science 258, 1112–1118.
[3] Negroni, M., Ricchetti, M., Nouvel, P. and Buc, H. (1995) Proc. Natl. Acad. Sci. USA 92, 6971–6975.
[4] Zhu, S.Y., Li, W.X., Zeng, X.C., Jiang, D.H., Mao, X. and Liu, H. (1999) FEBS Lett. 457, 509–514.
[5] Ouhammouch, M. and Brody, E.N. (1992) Nucleic Acids Res. 20, 5443–5450.

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