Correspondence

Template switching generated during reverse transcription?

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It is with a lot of interest that I have followed the recent correspondence in this Journal on whether the reverse transcription process has the capacity to generate artifacts by template switching [1,2]. In this context I would like to provide some experimental evidence that may shed light into this possibility.

A 5 kb region immediately downstream of the human *CYP3A4* gene, a major player in the metabolism of clinically administered drugs [3], was PCR amplified from genomic DNA extracted from HepG2 cells. Using Expand polymerase (Roche) this 5 kb fragment was readily produced. To check whether run-off transcripts of this gene, that escape canonical polyadenylation, are also generated, total HepG2 RNA was reverse-transcribed with and without MMLV RNase H^- reverse transcriptase (RT) (Promega) and subjected to PCR with the same primers and under the same conditions as for the genomic amplification. Although the minus RT amplification was negative, the plus RT revealed, instead of the anticipated fragment of 5 kb, an unexpected band of about 2 kb. Cloning and sequencing established that the 2 kb band is contiguous with the 5 kb genomic region for about 1 kb from each end but the 3 kb intermediate sequence was missing. Interestingly this 3 kb genomic sequence is flanked on both ends by a pentanucleotide repeat, GCTCA, however only a single repeat was present in the 2 kb fragment.

To check the reproducibility of the observed RT-PCR product the same RNA preparation was subjected to an additional RT-PCR amplification assay. Again a fragment of about 2 kb was observed in the plus RT experiment. However, sequencing of this band revealed that the 3 kb missing region has now shifted by a few nucleotides. Interestingly this 3 kb genomic sequence was still flanked by pentanucleotide repeats, this time a GCCTG sequence, and again only a single repeat was present in the 2 kb fragment.

The most plausible interpretation of these observations may be outlined as follows:

1. The observed irreproducibility of the RT-PCR products is not likely to be due to artifacts that are caused by the thermostable polymerase used, as amplification of genomic DNA consistently yields the 5 kb band.
2. Thus the other enzyme employed in this assay, RT, is apparently involved in the observed template switching at the repetitive pentanucleotide sequences. This may depend on the microenvironment of the reverse transcription, thus the different products observed in the two assays. Possibly the Alu repeats present in the run-off RNA may allow a semistable secondary structure that could facilitate such events.

In summary caution should be exercised in interpreting products of RT-PCR amplifications that deviate from the anticipated ones, as there is a possibility that these may not represent pre-existing RNA molecules but are simply in vitro generated recombinants. It is known in the scientific community that thermostable polymerases can generate artifactual products by template switching ([4] and references therein). RTs have apparently the same ability and such recombinants may require more effort to unambiguously disregard than the PCR-generated ones.

References

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