RT-PCR Artifacts from Processed Pseudogenes

Ravi S. Menon, Ying-Fon Chang, Judy St. Clair, and Richard G. Ham

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

Reverse transcription (RT) together with the polymerase chain reaction (PCR) provides a powerful tool for detection of rare mRNAs.^(1,2) The use of primers from separate exons is assumed to eliminate amplification of genomic DNA targets of similar size; however, this assumption overlooks processed pseudogenes, which can yield PCR fragments similar in size to those expected from cDNA. The following example illustrates this problem.

RNA samples used in RT-PCR are usually assayed for cytoplasmic β -actin mRNA. This is essential to verify that absence of an amplification product is caused by absence of the message being sought and not due to degradation of the mRNA. During RT-PCR studies on RNA from SV40-transformed human mammary epithelial cells (FR2), a strong signal of the size expected from β -actin mRNA was obtained both in the positive control (Fig. 1, lane 3) and in a negative control without reverse transcriptase (lane 4). To rule out possible effects of genomic rearrangements in continuous lines, these controls were repeated with RNA from normal human muscle satellite cells, again yielding products of the expected size, both in the positive control (lane 8) and in the negative control without reverse transcriptase (lane 9).

A common source of PCR contamination is a cDNA clone that includes the sequence being amplified.⁽³⁾ However, the only β -actin cDNA in the laboratory was a partial clone lacking the sequence in question. Also, the absence of amplification products in template-free controls (lanes 2 and 7) and with the partial cDNA as template (lane 6) both suggest that the contaminating template was introduced with the RNA samples.

The possibility that human genomic DNA might contain the template was examined by PCR with the β -actin primers. A product identical in size to those obtained from the RT-PCR reactions was obtained (lane 5). Partial sequencing demonstrated that the genomic amplification product differed from the expected β -actin sequence by about 10% (data not shown), several of those resulting in nonsynonymous substitutions. A computer search revealed a processed β -actin pseudogene,⁽⁴⁾ which with the same primers would yield an amplification product of 541 bp that could not be distinguished on gels from the 540-bp product expected from authentic β -actin cDNA. A further literature search revealed the presence of at least 19 processed β -actin pseudogenes scattered among three chromosomes.⁽⁵⁾

Finally, to confirm that genomic DNA was serving as the template in the

FIGURE 1 Agarose gel electrophoresis of β -actin PCR products. Profiles used: A, 1 min at 94°C, 2 min at 55°C, 2 min at 72°C $\times$ 35 cycles; B, 5 min at 94°C, 2 min at 60°C, 3 min at 72°C $\times$ 1 cycle; 2 min at 94°C, 2 min at 60°C, 3 min at 72°C $\times$ 10 cycles; 1 min at 94°C, 1 min at 60°C, 3 min at 72°C $\times$ 20 cycles; 1 min at 94°C, 1 min at 60°C, 5 min at 72°C $\times$ 1 cycle. (Lanes 1 and 13) DNA markers; (lanes 2 and 7) template-free negative controls for profiles A and B, respectively; (lane 3) RT-PCR reaction with FR2 cell RNA, profile A; (lane 4) RT-PCR reaction without reverse transcriptase, profile A; (lane 5) 500 ng of human genomic DNA as template, profile A; (lane 6) partial β -actin cDNA as template, profile B; (lane 8) RT-PCR of human muscle cell RNA, profile A; (lane 9) RT-PCR reaction without reverse transcriptase, profile A; (lane 10) direct PCR of RNA after DNase I digestion, profile A; (lane 11) RT-PCR after DNase I digestion, profile A; (lane 12) RT-PCR after RNase A digestion, profile A. All PCR reactions were done in 0.05 ml volume with 50 pmoles of each primer. Reverse transcription reactions were done as reported earlier.⁽¹⁾

RT-PCR reactions, RNA samples were exhaustively digested with RNase-free DNase I. Direct PCR without RT yielded no product (lane 10). However, RT followed by PCR yielded a product of the expected size (lane 11), confirming the presence of intact β -actin mRNA. RNase digestion of the DNase-treated samples abolished the RT-PCR product (lane 12), verifying that the product in lane 11 was derived from mRNA.

Contaminating cDNA for the target in question is admittedly the most serious source of contamination in RT-PCR reactions. These observations, however, clearly illustrate that processed pseudogenes can also be a serious source of contamination in such studies.

REFERENCES

1. Brenner, C.A., A.W. Tam, P.A. Nelson, E.G. Engleman, N. Suzuki, K.E. Fry, and J.W. Larrick. 1989. Message amplification phenotyping (MAPPING): A technique to simultaneously measure multiple mRNAs from small numbers of cells. *BioTechniques* **7**: 1096-1103.
2. Rappolee, D.A., D. Mark, M.J. Baanda, and Z. Werb. 1988. Wound macrophages express TGF- α and other growth factors in vivo: Analysis by mRNA phenotyping. *Science* **241**: 708-710.
3. Kwock, S. and R. Higuchi. 1989. Avoiding false positives with PCR. *Nature* **339**: 237-238.
4. Moos, M. and D. Gallwitz. 1983. Structure of two human beta-actin-related processed pseudogenes one of which is located next to a simple repetitive sequence. *EMBO J.* **2**: 757-761.
5. Leavitt, J., P. Gunning, P. Porreca, S.-Y. Ng, C.-S. Lin, and L. Kedes. 1984. Molecular cloning and characterization of mutant and wild-type human beta-actin genes. *Mol. Cell. Biol.* **4**: 1961-1969.

Received April 22, 1991; accepted in revised form May 28, 1991.

RT-PCR artifacts from processed pseudogenes.

R S Menon, Y F Chang, J St Clair, et al.

Genome Res. 1991 1: 70-71

Access the most recent version at doi:[10.1101/gr.1.1.70](https://doi.org/10.1101/gr.1.1.70)

References

This article cites 5 articles, 2 of which can be accessed free at:
<http://genome.cshlp.org/content/1/1/70.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

Focused on your science.

USC
SCIENTIFIC
SIDE BY SCIENCE

To subscribe to *Genome Research* go to:
<https://genome.cshlp.org/subscriptions>