Retraction

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Alternative splicing produces high levels of noncoding isoforms of bHLH transcription factors during development
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"We are writing to clarify the interpretation of the results from our above-mentioned paper. In this study, we used two methods to examine the structure of RNA from the mouse Math5 (Atoh7) locus. Our initial characterization was an analysis of the Math5 RNA structure using RT/PCR. We designed several sets of primers to interrogate the 5′ untranslated region (UTR) and 3′ UTR, as well as the coding region (CDS). The RT/PCR gave a surprising result. It appeared that the majority of the Math5 RNA molecules did not contain a CDS, but were short transcripts with the 5′ and 3′ UTRs joined together. We were surprised by this finding, and were aware that there are many artifacts created by RT and/or PCR. Rather than alter the conditions for these reactions, we chose to verify this finding using a different method, one that we thought would not be susceptible to the same artifacts as RT and PCR. We thus examined Northern blots using retinal RNAs and probes made for the CDS and each of the UTRs. The results from the Northern blot seemed to confirm that there were two Math5 RNA species: a larger one that had the CDS, and a smaller one that contained the UTRs but not the CDS. These data were consistent with the RT/PCR results, and we took these data to mean that the majority of the Math5 RNA species did not contain the CDS.

"Prasov et al. (2010) have recently published an examination of the Math5 RNA structures using the same RT/PCR primers as used in our study. They can find the same RT/PCR products using the primers and conditions that we described. However, they were able to show that the short products that appear to be missing the CDS are due to a very tight secondary structure, which causes RT to switch strands or otherwise skip the CDS, likely due to an 85% GC domain in the CDS. After they published their findings, we understood how we incorrectly interpreted the RT/PCR products. We have since gone back and probed Northern blots using the same RNA probes used in our original study. We have now purified the probes using two different protocols. In addition, we washed the Northern blots using different levels of stringency. The probe preparation method and the washing conditions were found to change the hybridization results with the CDS probe.

"The fact that two independent methods appeared to reinforce each other, to give the interpretation of two different RNA species, was quite unfortunate. It is likely that both the variability in the behavior of the probes on the Northern blot and the RT skipping of the CDS were due to the region of high GC content. In fact, the very high GC content might indicate that this mRNA is regulated by this structure, which may lead to poor, or at least regulated, translation of this protein. However, we no longer believe that the majority of the RNA is spliced such that the CDS is eliminated."

Reference

Prasov L, Brown NL, Glaser T. 2010. A critical analysis of Atoh7 (Math5) mRNA splicing in the development mouse retina. *PloS One* **5:** e12315. doi: 10.1371/journal.pone.0012315.