

## **Adenosine-to-inosine editing of miRNAs during zebrafish development**

Diana Brauswetter, Elizabeth Ferrick, Ronald B. Emeson

Center for Molecular Neuroscience, Vanderbilt University, Tennessee, USA

The conversion of adenosine-to-inosine (A-to-I) by RNA editing is a post-transcriptional processing event catalyzed by a small family of adenosine deaminases that act on RNA, referred to as ADARs. ADARs convert A-to-I in double-stranded regions of RNAs formed by intramolecular base-pairing. Most modifications are found within non-coding sequences, such as 5'- and 3'-untranslated regions or introns, and may play a role in alternative splicing, nuclear retention, non-sense-mediated mRNA decay, RNA interference or in interactions with miRNAs. Since miRNAs are processed from primary (pri-miRNA) and precursor (pre-miRNA) transcripts containing extended regions of double-stranded RNA, they also can serve as targets of the ADAR family. Modification of pri- or pre-miRNAs can regulate the expression of the mature miRNAs by affecting the site-specific cleavage or efficiency of the two key endonucleases involved in miRNA expression, Drosha and Dicer. If the site of A-to-I editing occurs within the 21-23 nucleotide long mature miRNA sequence itself, such modifications can interfere with base-pairing to the mRNA target, particularly if they occur within the 5'-proximal "seed" region of miRNA. In this study, we initially focused upon the editing of small RNAs during zebrafish development using a deep-sequencing-based approach in which twenty-four A-to-G discrepancies (an index of A-to-I editing) between genomic DNA and miRNA-derived cDNA sequences were identified.

To validate the identified editing sites, we have employed a new biochemical method, referred to as inosine chemical erasing (ICE), in which inosine moieties within miRNAs were selectively modified by cyanoethylation (CE) after treatment with acrylonitrile [*Nat Chem Biol.* 6:733-40 (2010)]. This technique is of high reliability and readily distinguishes real editing sites from SNPs or sequencing errors. The ICE method uses a combination of inosine-specific cyanoethylation, reverse transcription, PCR amplification and direct sequencing to detect A-to-I editing sites. Sequence analyses of RT-PCR amplicons from edited RNAs contain a dual adenosine/guanosine peak in sequence electropherogram traces resulting from both edited and non-edited transcripts. After treatment with acrylonitrile, inosine moieties are covalently modified, consequently a reduction (or absence) of the guanosine peak can be observed at the editing site due to the inhibition of cDNA synthesis for inosine-containing RNAs. To optimize this approach, we used transcripts for CAPS1 (Ca<sup>2+</sup>-dependent activator of protein secretion) which contain a known A-to-I editing site. Sequence analyses of RT-PCR amplicons revealed the expected reduction in the guanosine peak for the CE+ derived samples, thus allowing future studies to confirm the editing sites in miRNAs that have been identified using high-throughput sequencing and *in silico*-based approaches. It is anticipated that the identification and validation of editing sites with miRNAs will provide considerable insights into the cellular mechanisms modulating both the processing and target-selectivity of miRNAs and their roles in the regulation of gene expression.