RNA editing of a miRNA precursor

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ABSTRACT
Micro RNAs comprise a large family of small, functional RNAs with important roles in the regulation of protein coding genes in animals and plants. Here we show that human and mouse miRNA22 precursor molecules are subject to posttranscriptional modification by A-to-I RNA editing in vivo. The observed editing events are predicted to have significant implications for the biogenesis and function of miRNA22 and might point toward a more general role for RNA editing in the regulation of miRNA gene expression.

Keywords: RNA editing; miRNA; inosine; pri-miRNA; adenosine deaminase; ADAR

RNA editing by adenosine (A) to inosine (I) modification generates RNA and protein diversity in higher eukaryotes, selectively altering coding and noncoding sequences in nuclear transcripts (for reviews, see Bass 2002; Maas et al. 2003). The enzymes responsible for A-to-I editing, the adenosine deaminases acting on RNA (ADARs), are ubiquitously expressed in mammals and specifically recognize partially double-stranded (ds) RNA structures where they modify individual adenosines depending on the local structure and sequence environment (Bass 2002). Long extended dsRNAs undergo massive editing (Morse et al. 2002), whereas RNA duplex structures with bulges and loops are subject to site-selective editing, as observed in several nuclear encoded pre-mRNAs (Bass 2002; Schmauss and Howe 2002). Because inosine acts as guanosine during translation, A-to-I conversion in coding sequences leads to amino acid changes and often entails changes in protein function. The best studied examples are neuronal glutamate receptor (Glu-R) genes where single amino acid changes alter gating behavior and kinetic properties of the ion channels (Seeburg et al. 1998; Seeburg and Hartner 2003) and a serotonin receptor subunit where editing regulates G-protein coupling efficiency (Burns et al. 1997). Furthermore, editing within noncoding regions of pre-mRNAs can lead to alternative splicing (Rueter et al. 1999) or might affect the translation, transport, or stability of RNAs (Morse et al. 2002). Theoretically, any RNA with the ability to form a dsRNA fold-back structure could be a target for editing.

Micro (mi) RNAs (Lai 2003; Bartel 2004) are generated as long pri-miRNA transcripts that are processed into ~60–70-nt pre-miRNAs in the nucleus by the ribonuclease III-like enzyme Drosha (Lee et al. 2003). After nuclear export the pre-miRNAs are further processed by Dicer into ~20–22-nt mature miRNAs (Bernstein et al. 2001). The stem–loop structures predicted for all miRNA precursors are reminiscent of the partially double-stranded fold-back structures of known editing substrates (Bass 2002), raising the possibility that miRNA precursor molecules might be targeted by ADARs in vivo. Because ADARs are predominantly nuclear enzymes, the most likely targets for miRNA editing are pri-miRNA and pre-miRNA precursor molecules prior to nuclear export.

To test this hypothesis, we analyzed brain-specific cDNAs from human and mouse pri-miRNA22 for evidence of A-to-I RNA editing. In contrast to many other known human miRNA genes, miRNA22 is widely expressed in mammalian tissues (Lagos-Quintana et al. 2001, 2002) and sequences that correspond to pri-miRNA22 transcripts can be found in expressed sequence databases (dbEST; Boguski et al. 1993). Interestingly, human and mouse EST sequences corresponding to miRNA22 precursors appear to be spliced from a longer, primary transcript with several exons and show evidence of alternative splicing (Fig. 1A), properties usually associated with RNA polymerase II transcripts (Goldstrohm et al. 2001). We specifically amplified pri-miRNA22 molecules corresponding to two alternative splice forms (see Fig. 1A) and sequence analyzed a 197-nt region including the complete miRNA22 precursor stem–loop of 85 nt, which encompasses 17 adenosines.

In brains from human and mouse, as well as in human lung and testis, low-level editing is detectable at several
positions, including nucleotides within the mature miRNA sequence (Fig. 1B). The occurrence of miRNA22 editing in two mammalian species further suggests a functional relevance for editing. Within our sample the positions of editing events, however, are different for human tissues (nucleotide positions −5,−1,+9,+18) and mouse tissues (−41,+1,+2).

To establish a direct link between RNA editing enzyme activity and base changes in pri-miRNA sequences, the analysis was extended to human cell lines that ectopically overproduce ADAR1 or ADAR2 (Maas et al. 2001). In cells with the highest ADAR1 intracellular activity, 35% of endogenous pri-miRNA22 transcripts are edited at one or more positions (nt −30,−1,+1,+2,+15,+18) within the precursor sequence (see Fig. 1B,C). Interestingly, ADAR2 hyperactivity yields a moderately increased rate of editing (∼10%; nucleotide positions +1, +2), but with high site selectivity for the +1 position (7%).

Taken together, these results show that overexpression of both ADAR enzymes leads to an increase in editing levels at sites that are also subject to editing in mammalian tissues; however, ADAR1 overexpression results in a pattern of modification that includes bases that do not get modified under physiological conditions. In other cases of nuclear RNA editing, ADAR1 and 2 also show overlapping but different site specificities and often additional adenosines are modified in conjunction with ADAR hyperactivity (Bass 2002). In brains from ADAR2 knock-out mice (Higuchi et al. 2000) pri-miRNA22 editing is still detectable at position +1, indicating that the endogenous ADAR1 editing enzymes can target this position (Fig. 1C).

The current data set does not clearly point to the physiological role of miRNA22 editing because of the low levels of editing observed in adult human and mouse tissues and because miRNA22 function is unknown. The editing events might simply represent “background” activity of the editing machinery targeting this miRNA stem-loop without a significant impact on its function. However, considering the transient and regulatory role of characterized miRNAs (Bartel 2004) and the regulatory function of RNA editing, a specific time point or place may exist where editing of pre-miRNA22 is transiently or locally induced to much higher levels. Recently several candidate target genes for miRNA22 have been identified in computational studies (Lewis et al. 2003) and their functional characterization might lead to insights into the role of miRNA22 as well as the biological consequences of its post-transcriptional modification by ADARs.

The editing of miRNA precursors by ADARs could have major implications for miRNA analysis, biogenesis, and function. When cloning mature miRNAs, editing could result in misidentification, and miRNA expression analysis by Northern hybridization could yield incorrect results due to edited RNA/probe mismatches. It is well known that point mutations within the miRNA precursor can strongly influence processing activity by dicer (Zeng and Cullen 2003), and the −1 and +1 positions, both subject to editing in pri-miRNA22, are positioned right around the cleavage site. Therefore, A-to-I modification of miRNA precursors could modulate processing kinetics and outcome, or even prevent...
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