Adenosine-to-Inosine RNA Editing: Perspectives and Predictions

Barry Hoopengardner*

Central Connecticut State University, 1615 Stanley Street, New Britain, Connecticut 06050, USA

Abstract: Adenosine-to-Inosine RNA editing introduces changes in RNA transcripts *via* a post-transcriptional mechanism, the hydrolytic deamination of adenosine (A) to inosine (I) which is interpreted as guanosine by cellular machineries. Adenosine deaminases that act on RNA (ADAR) enzymes catalyze editing in double-stranded (ds) RNA substrates.

Key Words: Adenosine, inosine, RNA, editing, drosophila, ion channel, convergence.

It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change.

~Attributed to *Charles Darwin*

INTRODUCTION

As genomic resources accumulate, it is becoming increasingly obvious that genome size is not an accurate predictor of morphological or behavioral complexity. Encoded changes in DNA are not the only source of genetic variation. In particular, RNA editing of a transcript can result in a messenger RNA with changes in coding or secondary structure from the version that is unedited. An understanding of the influence of RNA editing in the creation of transcript diversity is in its infancy. This review will focus upon adenosine-to-inosine (A-to-I) pre-messenger RNA (pre-mRNA) editing.

The basic mechanism of the process of A-to-I pre-mRNA editing relies upon the formation of RNA secondary structures targeted by adenosine deaminases; see Fig.(**1**). A-to-I RNA editing, which occurs following transcription, can result in the recoding of transcripts. This chemical rewriting will be considered here in the context of altered ion channel kinetics and permeability in several recently published examples.

ADENOSINE-TO-INOSINE RNA EDITING

RNA editing was first discovered in the mitochondria of trypanosomes. There are several types of RNA editing, including insertion/deletion editing and substitution editing. Modes of substitution editing include cytidine-to-uridine and adenosine-to-inosine (A-to-I) modification.

A-to-I RNA editing is accomplished through the enzymatic hydrolytic conversion of adenosine to inosine. These conversions are catalyzed by the adenosine deaminases that act on RNA, or ADARs, a family of enzymes characterized by a shared deaminase domain and the presence of RNA-binding motifs and found primarily within the nucleus. A-to-I RNA editing may be promiscuous or specific. Promiscuous editing, which can modify up to fifty percent of transcript adenosines, is thought to be a means of viral defense [1, 2]. This review will focus upon specific editing which has the potential to recode genetic information since inosine is recognized by the ribosome as guanosine.

The identification of targets of RNA recoding has largely been serendipitous. Indeed, until this phenomenon was recognized, many examples of editing may likely have been ignored as artifactual, or attributed to single nucleotide polymorphisms (SNPs).

ADARs bind to regions of duplex RNA. ADARs edit perfectly complementary stretches of duplex RNA promiscuously. Structural models of ADAR targets that are edited selectively show that these targets are imperfectly complementary duplexes. Evidence suggests that ADARs position themselves and act catalytically within the constraints provided by unpaired areas. The synthetic probing of ADAR substrate requirements by Lehmann and Bass [3] discovered that internal loops promote the selectivity of ADAR action by delimiting helical regions; unpaired regions of six bases or more acted as helical ends, while internal loops 4 bases or smaller did not. Co-crystallization of ADARs with target RNAs, using both synthetic and natural substrates, should provide some additional answers to questions of the control of ADAR positioning and catalytic activity, and an understanding of the structural cues that guide ADAR selectivity.

The general mechanistic principles and participants in the process of A-to-I RNA editing have been known for some time [4]. In the archetypal example of specific A-to-I editing, the GluR-B Q/R site, the region of the edited site within exonic sequence participates in binding with a complementary stretch of RNA to form an imperfect duplex. The pairing partner of the sequence surrounding the edited adenosine is located in the downstream intron and is known as an editing site complementary sequence, or ECS. The imperfect RNA duplex is a substrate of ADAR enzyme(s); in the case of the GluR-B Q/R site, ADAR2 is the editing enzyme. The ECS need not reside near the edited adenosine or within an intron. Examples of such substrates have shown that an ECS can be located more than a kilobase from the location of the edited adenosine [5] and can even be located within the same exon

*Address correspondence to this author at the Central Connecticut State University, Department of Biomolecular Sciences, 1615 Stanley Street, New Britain, Connecticut, 06050, USA; E-mail: hoopengardnbam@ccsu.edu

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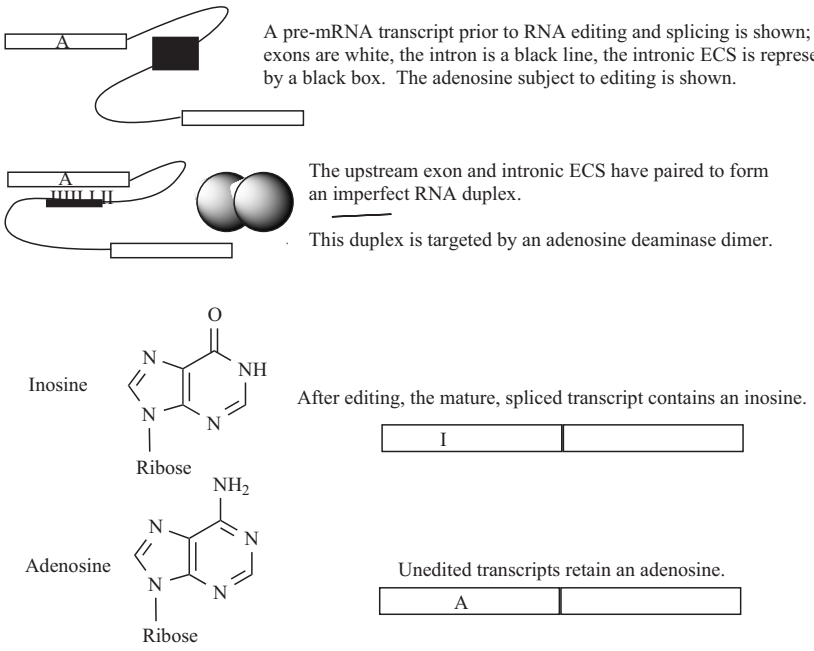

Fig. (1). A-to-I RNA editing substrates are formed by the pairing of complementary sequences.

[6, 7, 8]. The majority of ADAR activity is found in the nucleus, however, an interferon-inducible form of ADAR1 contains a nuclear localization signal and can shuttle from nucleus to cytoplasm [9], possibly functioning as a means of defense against double-stranded RNA viruses.

This mechanism of RNA editing requires complementary pairing partners, and the lack of a particular editing motif frustrates the search for new editing targets. However, the conservation of sequences that form the edited site region and its pairing partner can be used as a screen for editing targets. Hoopengardner *et al.* [7] compared sequences from *D. melanogaster* and *D. pseudoobscura* genes from the annotated genomes of these insects to search for highly conserved exonic regions. Exons that were highly conserved between these species were then analyzed by direct sequencing of RT-PCR products. In this way, 16 new *D. melanogaster* editing targets and one new mammalian target were discovered. The editing targets discovered by this comparative sequence-based approach are nervous system-specific, comprising voltage-gated ion channels, ligand-gated ion channels, and components of the synaptic release machinery. In addition to these gene categories, the study of Hoopengardner *et al.* [7] included the entire set of annotated transcription factors (n=499) and G protein-coupled receptors (n=178) of *D. melanogaster* and over 100 additional targets from various ontological classes; no genes of these various ontological classifications were found to be editing targets.

It should be helpful to include a brief description of the process of the propagation of an action potential to place the editing of specific target transcripts within the context of the communication between pre- and post-synaptic cells. RNA editing modifies numerous targets within this integrated milieu, affecting voltage-gated ion channels, ligand-gated ion channels, and components of the synaptic release machinery.

The nerve impulse is transmitted along the axon of the nerve cell by the coordinated opening and closing of voltage-gated ion channels. Upon stimulation of the neuron, voltage-gated sodium channels respond by opening, depolarizing the membrane by allowing an influx of extracellular sodium. The local influx of sodium in turn causes the opening of adjacent sodium channels, and the signal, an action potential, begins to propagate along the axon. The membrane is repolarized by potassium channels that open soon thereafter in the wake of the depolarization. In this way, an action potential is propagated toward the synaptic terminal.

At the pre-synaptic terminal, the arrival of the action potential depolarizes the membrane, resulting in the influx of calcium ions through voltage-gated calcium channels. Calcium triggers the fusion of synaptic vesicles carrying neurotransmitters, causing the release of neurotransmitters into the synaptic cleft.

At the post-synaptic cell, which can be another neuron, or a muscle or gland, ligand-gated channels respond to the release of neurotransmitters, depolarizing the membrane and propagating the signal, or attenuating the signal by increasing membrane polarization.

The preponderance of nervous system targets suggests that A-to-I RNA editing acts to fine-tune electrical and chemical neurotransmission, affecting channel kinetics and permeability. Brusa *et al.* [10] found that glutamate receptor channels containing the edited, arginine (R)-form of GluR-B are impermeable to calcium. Bhalla *et al.*[8] describe the effect of editing upon the human potassium channel Kv1.1

(the *H. sapiens* homologue of Drosophila *Shaker*); in this case, a single edit results in significantly quicker recovery from inactivation.

BEYOND MECHANISM

After discussions of enzymatic substrate specificity, a basic question remains- why edit RNA? Why is such an investment made in transcript revision when such changes could be encoded, "hard-wired" into the genome. The rationale for the ubiquity of RNA editing in metazoa appears to be its utility. RNA editing may first have arisen as a form of viral defense; the ancestral deaminase, already capable of RNA binding and modification, was exploited by neurons for the alteration of their transcripts. Indeed, RNA editing appears to have retained its ancestral role, but is also maintained by the cell for the protection of host transcripts against RNA interference and to increase diversity. Changes in transcript coding can be tested, and retained when providing a selective advantage. Several recent observations also indicate that the editing of nervous system components in metazoa is common, but the editing of these components can be highly clade-specific ([11]; Hoopengardner, unpublished), implicating RNA editing as a potential agent of speciation.

INCREASING DIVERSITY

Editing results in a more diverse population of protein isoforms. Many editing targets are found in protein families that assemble to form multimeric complexes; edited and unedited isoforms can assemble with different stoichiometries. In populations of proteins active as monomers, editing generates simple diversity; for proteins that assemble as multimers, such as the majority of known ion channels, edited and unedited subunits can assemble in several combinations, each of which may be subtly different or profoundly distinct.

DIVERSITY BEYOND THE GENOME

The editing of a transcript may result in the recoding of that transcript without the necessity of committing to that alteration by genomic change. Furthermore, change at the genomic level results in a novel allele which can occur in a population as homozygous or heterozygous; with editing, the presence of such recoded "alleles" may be represented as a continuum within a transcript population.

Such recoding is subject to regulation at the temporal and spatial level. Changes in amino acid sequence may be regulated not only by levels of ADAR expression, but different ADAR isoforms may have different target specificities. Much evidence suggests that ADARs act as dimers [12], which introduces an additional means for the adjustment of ADAR target specificity by the cooperative action of different ADAR monomers.

The results of RNA editing have been studied in ion channels, including the glutamate receptors and potassium channels. Such studies have shown that the changes generated in these proteins by RNA editing can profoundly alter channel kinetics and selectivity. For instance, inclusion of the edited R-form of GluR-B results in ion channels that are impermeable to calcium. Lower levels of GluR-B editing are implicated in amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease) [13].

Invertebrate ionotropic glutamate receptors are not targets of RNA editing, and possess a glutamine (Q) residue in a receptor region linked to calcium permeability. The transcripts of the mammalian ionotropic glutamate receptors GluR-B, GluR-5, and GluR-6 edit this glutamine to an arginine (R). Glutamate receptors probably assemble as homotetramers and heterotetramers, and glutamate receptors assembling with an edited, R-form subunit lose calcium permeability [14]. GluR-B is edited at >99% frequency in the murine brain; therefore, regulation of calcium conductance can be achieved by the regulation of GluR-B transcription or of RNA editing. Mice which possess an uneditable allele of GluR-B as heterozygotes die by the third week of age [10]. The Q/R editing site is found in the AMPA receptor GluR-B as well as the related but distinct kainate receptors GluR-5 and GluR-6. The presence of the Q/R editing site is likely the result of molecular convergence, rather than conservation of the site within the distinct, but related ionotropic glutamate AMPA and kainate receptors. The Q/R site is not found in other AMPA or kainate receptors. The ECS elements for the GluR-B and for GluR-5/6 are also distinct [4, 5], arguing that editing at this site is not the result of conservation of the site from a common glutamate receptor ancestor, but of genuine convergence.

To address the potential role of the unedited, Q-form GluR-B, Kask *et al.* [15] replaced the glutamine codon of GluR-B with an arginine codon; in this mouse, there were no unedited Q-form GluR-B channels, yet the mouse was healthy and showed no obvious neuropathies. If subpopulations of CNS cells express primarily the unedited GluR-B, their respecification to an edited default state led to no ill effects. However, when a similar experiment was conducted in *C. elegans*, the resultant phenotypes at low penetrance were of worms that were uncoordinated, manifested neuronal degeneration and the dysregulation of development, and lethality. The penetrance of these phenotypes was increased when the ratio of Q:R subunits was 1:3 and suppressed by mutations affecting glutamatergic transmission and intracellular calcium release [16].

The Drosophila *Shaker* potassium channel is a target of editing, as is the human homologue of this channel, Kv1.1 [7]. Editing in Kv1.1 results in a quicker recovery from inactivation than the wild-type potassium channel [8]. Bhalla *et al.* [8] also showed that a related Drosophila potassium channel, *Shab*, was a target of editing, highlighting the importance of editing in this type of channel. Even more intriguingly, this position is also edited in a Kv2 channel of squid (*Loligo pealei)* [17] suggesting that editing of these sites in human, fruit fly, and cephalopod is the result of convergent evolution.

The first A-to-I editing site was discovered in the transcripts of a glutamate receptor in the mammalian brain. The mammalian ionotropic glutamate receptor B (GluR-B) is a target of RNA editing; specific editing in GluR-B transcripts converts a glutamine (Q) (C<u>A</u>G) codon to that of arginine (R) (C<u>G</u>G). Members of at least two families of glutamate receptors are edited, the AMPA receptors (GluR-B,-C,-D) and the kainate receptors (GluR-5,-6). The transcripts of two paralogues of GluR-B, the kainate receptors GluR-5 and GluR-6, are also edited at the Q/R site. However, the tran-

scripts of three other AMPA receptors, GluR-A, -C, -D, and the kainate receptor GluR-7, do not share this Q/R site. Additionally, GluR-B, -C, and -D possess an arginine-to-glycine (R/G) site, and GluR-6 alone has an isoleucine-to-valine (I/V) and a tyrosine-to-cysteine (Y/C) site. GluR-A and GluR-7 have no known editing sites. [4, 5, 10, 14, 15]

In these examples, the flexibility and the multiple levels of regulation of transcript alteration provided by editing appear to act as selective pressures in certain critical residues of related ion channels, leading to the convergence of editing sites in paralogous gene families.

Natural variation in transcript sequences provides a population upon which selection can act. Initially, some transcript sequences capable of forming transient, duplex RNA structures may meet the criteria for minimal ADAR substrates. When the editing of transcripts, however slight, provides a selective advantage, such levels of editing can be supported, maintained, and rapidly optimized. The introduction of small, non-encoded changes in ion channel transcripts by editing can lead to profound consequences in channel function. Ion channels are well conserved in metazoa, and this similarity of structure predicts that similar changes in conserved protein domains and residues *via* mutation or RNA editing will give rise to similar alterations in function. For instance, an alteration of the sequence of a blocking particle or selectivity filter of a potassium channel would be significant in all organisms that produce this channel. While RNA editing might appear wherever the formation of duplex RNA would support deaminase binding, the conserved character of ion channels may predispose the retention of A-to-I RNA editing at similar adenosine positions in divergent species of organisms. The commonality of channel structures suggests that RNA editing might develop de novo at the same sites in transcripts from different organisms, appearing as a convergent character. The alteration of the structure of RNA editing substrates at the single-nucleotide level appears to be an ideal method for detailed, systematic molecular reconstructions of the processes of convergence.

ACKNOWLEDGEMENTS

I wish to thank Dr. Robert Reenan for helpful comments in the preparation of this manuscript.

Received: February 01, 2006 **Revised: May 09, 2006** **Accepted: May 10, 2006**

ABBREVIATIONS

A-to-I	=	Adenosine-to-Inosine
ECS	=	Editing site Complementary Sequence
ADAR	=	Adenosine Deaminase that acts on RNA
ds	=	double-stranded
pre-mRNA	=	pre-messenger RNA
SNP	=	Single Nucleotide Polymorphism
ALS	=	Amyotrophic Lateral Sclerosis
GluR	=	Glutamate Receptor
Q	=	Glutamine
R	=	Arginine

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