

## Shifting receptor sensitivity through RNA editing

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### Introduction

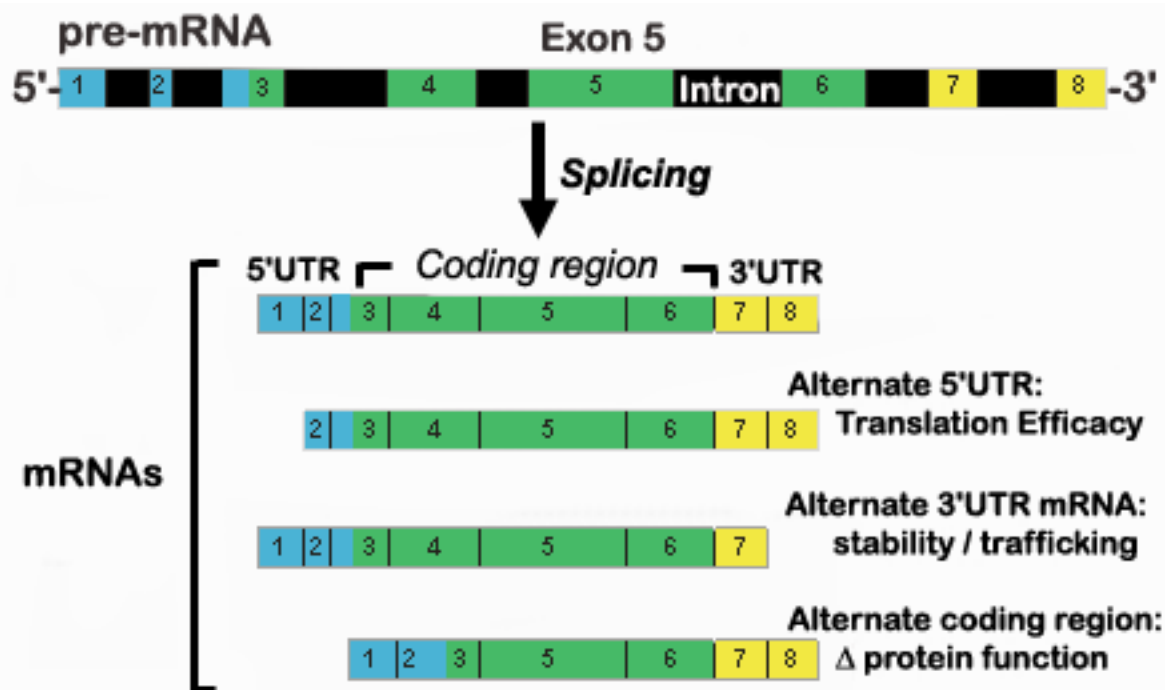
The completion of the human genome has heralded a new era of molecular genetics that promises major advances in understanding human health and disease. However, it also confronted us with a humbling statistic: the genetic blueprint for a human being contains the same number of genes (20-25,000, [1]) as that for the rat [2]. Equally embarrassing for both rodents and us is that the genome of the worm *C. elegans* contains only slightly fewer genes (19,000, [3]). Can this subtle disparity in gene number explain the vast differences in structural and functional complexity between the nervous systems of mammals and worms, which contain 100 billion and 302 neurons, respectively? And how do we explain the divergence in cognitive abilities between mice and man, when their number of genes appears to be the same? A multi-faceted answer to these questions has been emerging. There are a number of distinct mechanisms at work that may increase the information stored in a genome beyond the

complexity suggested by the total number of genes alone. Many of these processes have been known for some time, but new possibilities for increasing molecular diversity in a limited genome are still being discovered.

## **Alternative Splicing**

The number of genes in a particular genome does not accurately reflect the total number of proteins it encodes. Genes in higher organisms are typically organized into distinct exons, that encode the messenger RNA (mRNA), interspersed by non-coding introns. Transcription of such a gene by RNA-polymerase II results in a precursor mRNA (pre-mRNA) that still maintains the exon-intron structure (Fig. 1). Mature mRNAs are produced by removing the introns in a process termed *splicing*. By selectively including only a subset of exons, splicing can give rise to multiple mRNAs from a single pre-mRNA (Fig. 1). The mechanism of alternative splicing allows a single gene to encode many functional variants of a protein, in which specific domains are included or excluded. In addition, mRNAs can be generated with alternate 5' - and 3' -untranslated regions (UTRs), which differ in stability, translatability and trafficking properties. Alternative splicing thus allows a precise control over where, when and at what level proteins are expressed [4]. Alternative splicing affects 40-60% of all human genes [5], and is particularly widespread among proteins expressed in the central nervous system [6]. The average human gene contains 10 exons, compared to 7 for the worm, suggesting that evolution has seized upon the alternative splicing mechanism for generating additional complexity, with most of the effort apparently going into improving the brain.

Figure 1.



**Figure 1. Alternative splicing can produce multiple mRNAs from a single gene**

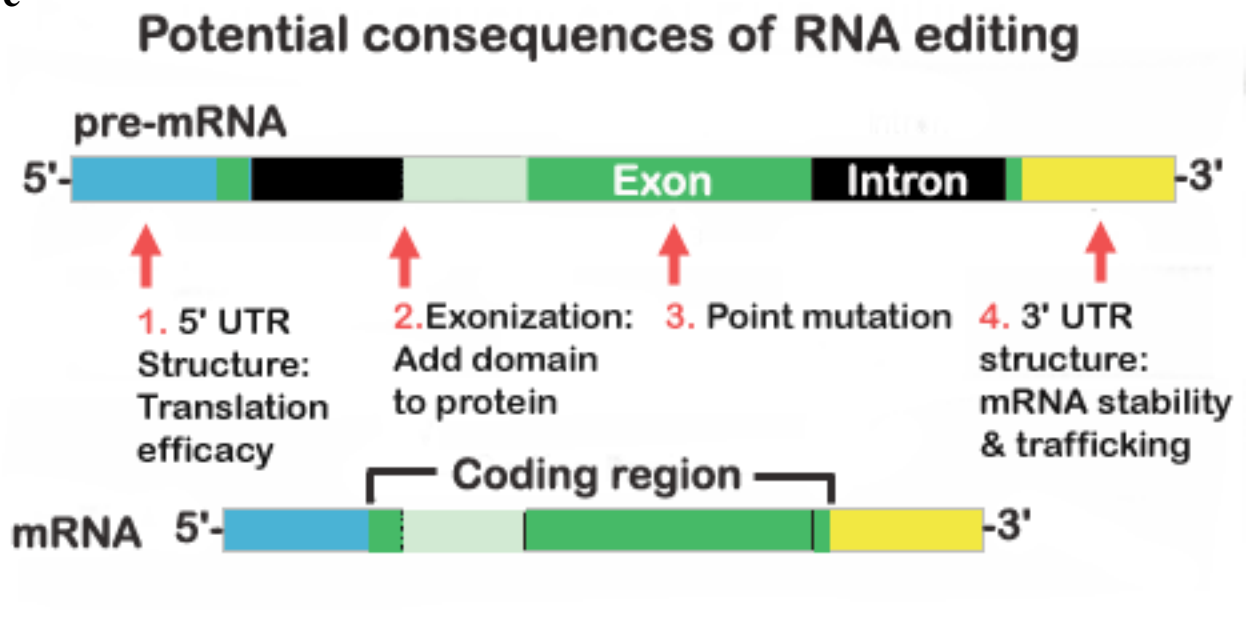
Alternative splicing can produce multiple mRNAs from a single gene. A precursor mRNA transcript is shown containing 8 exons (colored regions) interspersed by non-coding introns (black). Green domains represent the coding region of the mRNA, while blue and yellow domains encode the 5'-, 3'-untranslated regions (UTRs). Alternate coding regions provide functional variants of the encoded protein, while modifying UTRs can be used to affect protein expression levels or control where in the cell mRNAs are translated.

## RNA editing

After the pre-mRNA has been generated by transcription of a gene, its nucleotide sequence can still be altered by the activity of RNA-editing enzymes. The best characterized family of RNA-editing enzymes are the ADARs (adenosine deaminases that act on RNA), which convert adenosine (A) to inosine (I) in their dsRNA targets [7-9]. The ribosome, which is in charge of mRNA translation, recognizes an inosine as guanosine (G). A-to-I editing in the coding region of the target mRNA therefore results in an A→G mutation, which may alter a

single amino acid. RNA-editing of the untranslated regions of the target mRNA has the potential to change mRNA stability, translatability or trafficking. Finally, ADAR-mediated RNA–editing can affect splice site selection, and shorten or extend existing exons (Fig. 2). A-to-I editing has been described for mRNAs encoding glutamate receptors [10-17], K channels [18-21], Na channels [22], nicotinic acetylcholine receptors [23], and the serotonin 5-HT<sub>2C</sub> receptor [24]. In all cases, RNA-editing resulted in a single amino acid substitution that significantly affected the function of the receptor or channel (e.g. calcium permeability; gating properties; subunit assembly). In the serotonin receptor, editing of an intron was shown to affect splice-site selection [25]. A comparative genomics study in the fruitfly has identified an additional 16 targets for A-to-I editing, all of which encode proteins that play a role in neuronal excitability and neurotransmission [26].

**Figure 2.**



**Figure 2. The effect of A-to-I editing depends upon the location in the pre-mRNA**

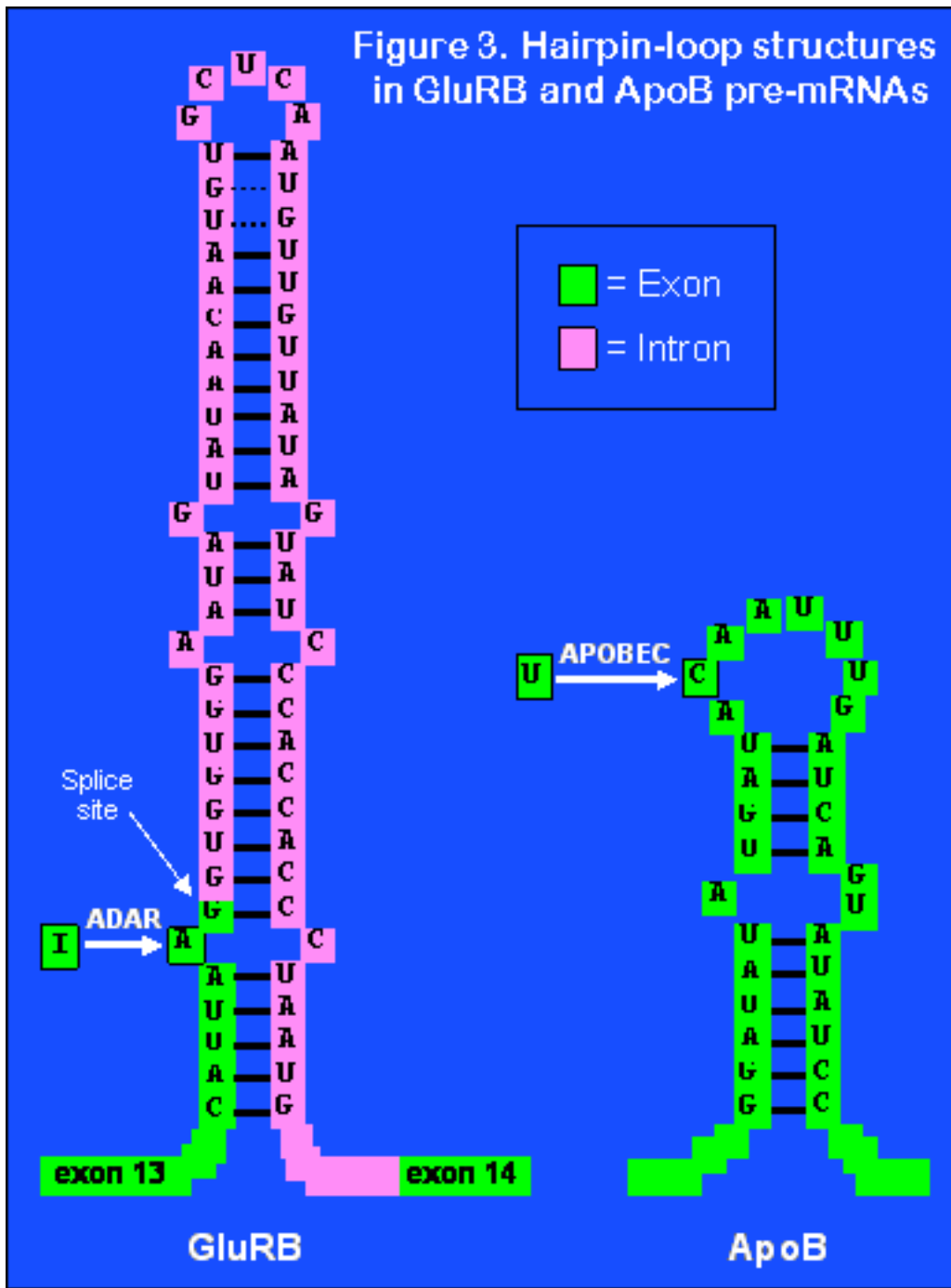
The effect of A-to-I editing depends on the location in the pre-mRNA. Potential consequences of RNA editing are:

1. A nucleotide substitution in the 5'UTR region of the target mRNA may:
  - a. alter its structure and thereby affect translation efficacy
  - b. introduce a novel in-frame start codon (AUA→AUG): extend N-terminus
  - c. remove the original start codon ((AUG→GUG): shorten N-terminus
  
2. Editing in an intron may:
  - a. introduce a new splice donor site, converting part of the intron to an exon
  - b. alter the structure of the intron, which may affect splice site selection
  - c. remove existing splice donor/acceptor site: eliminate exons
  
3. A nucleotide substitution in the coding region may:
  - a. result in a point mutation (amino acid substitution)
  - b. introduce a premature stop-codon, truncating the protein
  
4. Editing of a residue in the 3'UTR may:
  - a. alter mRNA stability, which depends on AU-rich elements
  - b. affect mRNA trafficking by mutating the 'ZIP-code'

The examples of RNA-editing by ADARs given above were identified as discrepancies between cDNA sequences, which are complementary to cellular mRNAs, and the corresponding genomic data. Such anecdotal evidence does not allow us to estimate how widespread RNA editing is in the human genome. However, four reports were published in 2004 that evaluated A-to-I editing in the human genome and unanimously concluded that editing is very abundant indeed [27-30]. Approximately 1 in 2000 bases are edited [30], resulting in an estimation of more than a million edited sites in the human genome. One study identified 1637 edited genes [29], while a second study reported 1445 new human mRNAs that are subject to multiple editing events [28]. The large majority of pre-mRNAs (>85%) are edited, mostly in intronic regions [28]. One potential function for this widespread editing of introns in pre-mRNAs is to counteract the deleterious effects of double-stranded RNA, including activation of the PKR kinase and the interferon response [30]. In addition, RNA-editing of introns may play a critical role in evolution (see Herbert, 1996, [31]).

## **C-to-U editing of a Glycine receptor**

Whereas the ADAR family of adenosine deaminases is responsible for A-to-I recoding of pre-mRNAs, another class of enzymes, the APOBEC cytidine deaminases, performs C-to-U editing. Unlike the promiscuous ADARs, pre-mRNA editing by APOBEC1 is highly selective, since its targets seem to be limited to two genes: apolipoprotein B (ApoB, [32-37]) and neurofibromin [36,38]. Another family member, APOBEC3, has received a large amount of attention since its deaminase activity disables the HIV retrovirus, which has prompted the virus to evolve Vif, a virulence factor that degrades APOBEC3 [39-42].



A recent paper by Meier *et al.* [43] identifies the  $\alpha 3$  subunit of the glycine receptor (GlyR- $\alpha 3$ ) as a novel target for mRNA recoding by a cytidine deaminases. GABA<sub>A</sub> and glycine receptors together take care of inhibitory neurotransmission in the central nervous

system. Using a clever pharmacological separation of GABA and glycine conductances in patch clamp experiments, Meier *et al.* uncovered a tonic glycine current in slices of midbrain neurons that was activated by unusually low amounts of glycine. RT-PCR using primers directed against the predominant  $\alpha 3$  subunits using mRNA isolated from dorsal midbrain neurons uncovered a point mutation (C554T) in a small subset of the cDNA clones. This base substitution results in a mutation in the N-terminal ligand binding domain, altering

the proline at position 185 to leucine. Heterologous expression of GlyR- $\alpha$ 3-P185L receptors in hippocampal neurons and *Xenopus* oocytes indeed confirmed that the mutant glycine receptors were 15-fold more sensitive to glycine than the wildtype controls and somewhat more resistant to desensitization, indicating that an  $\alpha$ 3-P185L mutant could very well underlie the tonic glycine conductance that was characterized electrophysiologically. The prevalence of the C554T mutation in mRNA samples depended on brain region and age, and also changed over time as the brain slices were maintained in culture medium. The significant increase in C554T mutation levels in embryonic slices was completely prevented by *zebularine*, a potent inhibitor of cytidine deaminases or the transcription inhibitor actinomycin-D, indicating that the mutation resulted from C-to-U editing of the pre-mRNA by a cytidine deaminase. Meier *et al.* therefore conclude that the RNA-editing is responsible for producing a mutant glycine receptor that is highly sensitive to agonist. The mutant receptor may generate a tonic inhibition through the effect of ambient glycine present in the brain.

Meier *et al.* also addressed the question which enzyme may be responsible for the C-to-U editing of GlyR- $\alpha$ 3 mRNA. The only enzyme with known cytidine deaminase activity towards mRNA is APOBEC1. Like the ADAR that edits the GluRB glutamate receptor, APOBEC1 recognizes its target by a hairpin loop structure (Fig. 3). Whereas the hairpin in GluRB is formed largely by an intronic region, the ApoB hairpin that binds APOBEC1 is located in the middle of an exon. Because there is very little homology between the nucleotide sequence surrounding the C554T mutation in GlyR- $\alpha$ 3 and the APOBEC1 hairpin, Meier *et al.* suggested that another (as yet unknown) cytidine deaminase may be responsible.

## Outlook

From the above discussion it is clear that we have to look beyond the number of genes to fully appreciate the complexity and information content of a genome. It appears that evolution has come up with mechanisms that supercede the linear DNA→RNA→protein paradigm. Two of these were discussed in some detail, alternate splicing and RNA editing. Interestingly, both of these processes appear to target mainly proteins that play roles in controlling excitability and neurotransmission in the central nervous system (CNS). Recent surveys of the human genome indicate that RNA editing may be much more abundant than ever appreciated, with the large majority of pre-mRNAs being targeted. It appears that the collection of known targets discussed above represent the proverbial tip of the iceberg. The discovery of Meier *et al.* that the glycine receptor, a critical component of the inhibitory drive in the CNS, is functionally altered by RNA recoding through an unknown cytidine deaminase certainly supports this view.

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