Messenger RNA editing and the genetic code

R. Cattaneo

Institut für Molekularbiologie I, Universität Zürich, Hönggerberg, CH-8093 Zürich (Switzerland)

Summary. Messenger RNA editing is defined as a process leading to predetermined modifications of the coding region of a primary gene transcript. By this definition, splicing processes are special forms of editing; however, they are not dealt with in this review. Editing processes different from splicing have been defined in mammalian cells, in RNA viruses, and in mitochondria of trypanosomes, higher plants and vertebrates. These post- or co-transcriptional processes involve addition, deletion, or modification-substitution of nucleotides, and represent previously unrecognized mechanisms for altering the coding potential of a gene and for modulating gene expression.
Key words. Apolipoprotein B; genetic code; messenger RNA editing; paramyxoviruses; plant mitochondria; RNA modification; trypanosome mitochondria; vertebrate mitochondria.

Introduction

In spite of the existence of an almost universal genetic code, the complete sequence of a gene is not sufficient to deduce a protein sequence since DNA sometimes undergoes programmed rearrangements, and complications arise at and after transcription and translation. Transcriptional complications include splicing and editing processes, whereas translational complications comprise ribosomal frameshifting[31, 54], persistently untranslated sequences[29], and possibly even cross-translation or trans-peptidation[32]. The subject of this review is messenger RNA (mRNA) editing, and in particular the novel, non-splicing forms. For excellent reviews on splicing see Aebi and Weissmann[1] and Smith et al.[47].

Different forms of mRNA editing are presented in the table. In the case of RNA editing by insertion and deletion (top) of uridine (U) nucleotides, defined in transcripts from the mitochondria of trypanosomes, genetic information was initially postulated to be stored partly (and in some cases mostly) in the secondary or higher order structure of these transcripts[8, 23, 43]. RNA editing information was, however, recently discovered in short RNA molecules that base-pair with unedited mRNAs and guide their cleavage, correction and religation[10], but it is not yet clear whether every case of trypanosomal mRNA editing can be accounted for by this mechanism.

Co-transcriptional mRNA editing by nucleotide insertion (table, center) does not require cleavage and religation of RNA molecules. This type of editing was originally described in transcripts of human mitochondria where polyadenylation creates UAA stop codons[4, 37]. Recently in RNA viruses another mechanism, polymerase stuttering, was found to result in the co-transcriptional addition of one or more guanosine (G) residues at a single position of one transcript, allowing the production of proteins with a common amino- and different carboxylterminal domains[15, 49, 51].

Editing by nucleotide modification or substitution (table, bottom) was first described in a cellular transcript, the apolipoprotein B mRNA, where introduction of a stop codon by a cytosine (C) to U (or U-like) base change results in the tissue-specific production of a shorter protein[17, 38]. This form of editing was recently also observed in certain mitochondrial mRNAs of some higher plants[19, 27]. In these transcripts editing has a bearing on the genetic code: arginine (CGG) codons are modified to tryptophan (UGG) codons, an observation that invalidates the previous speculation that CGG in mitochondria of higher plants can code for tryptophan.

RNA processing mechanisms not resulting in the modification of coding regions, such as capping and polyadenylation, should not be considered as RNA editing. The same is true for selective methylation of A residues which concerns mostly untranslated regions and does not alter the coding potential[3, 36], and for double-stranded RNA modification by unwinding that covalently modifies the adenosine (A) residues to inosine (I)[7, 53]. This process

Mechanisms and consequences of different types of mRNA editing. Modified from Cattaneo[14] and Weissmann et al.[55].

Type of mRNA editing	Base	Mechanism	Consequences for protein expression	Gene(s) and organism
Posttranscriptional insertion and deletion of nucleotides	U	Correction by minus strand 'guide' RNAs	Limited or extensive alteration of the reading frame	Mitochondrial genes of trypanosomes
Cotranscriptional insertion of nucleotides	A	Polyadenylation	Introduction of UAA stop codons	Mitochondrial genes of vertebrates
	G	Polymerase stuttering	Additional protein (efficiency: 20–50%)	Phosphoprotein gene of paramyxoviruses
Modification-substitution of nucleotides	C (toU)	Deamination? (tissue-specific)	Shorter protein	Apolipoprotein gene of mammals
	C (to U or U-like)	Unknown	Modified amino acid composition	Mitochondrial genes of plants

usually leads to rapid decay of the modified transcripts[34], but it should be noted that accidental modifications of RNA genomes can favour the rare establishment of persistent infections of a human RNA virus[16]. In this review, the form of mRNA editing documented until the beginning of 1990, and differing from splicing, will be presented, and their mechanisms discussed.

Post-transcriptional insertion and deletion of nucleotides

The most spectacular form of mRNA editing occurs in the single mitochondrion, or kinetoplast, of at least three species of trypanosomes: *Trypanosoma brucei, Crithidia fasciculata*, and *Leishmania tarentolae*. The unusually complex genome of trypanosome mitochondria, known as kinetoplast DNA[44], contains two kinds of circular DNA molecules: about 10,000 more or less heterogeneous minicircles interlocked with about 50 maxicircles of a single type. The function of the minicircles is unknown, but maxicircles are known to encode subunits of the respiratory chain complex and proteins of unknown function (from unassigned reading frames, MURFs), and specify ribosomal RNAs. The first demonstration of editing processes in transcripts of the maxicircle DNA was the finding by Benne et al.[8] that cytochrome c oxidase subunit II (coxII) transcripts contain, within the coding sequence, four closely spaced but non-contiguous U residues not found in the corresponding gene. The analysis by DNA hybridization of the kinetoplast and of the cellular genomes of *T. brucei* and *C. fasciculata* did not reveal any nucleic acid complementary to the edited mRNA, and thus it was proposed that the coxII mRNA itself somehow carries the information for its modification.

Subsequently, it was found that the *T. brucei* apocytochrome b (CYb) mRNA is also edited, in a developmentally regulated fashion, by the addition of several U residues[22]. RNA editing in kinetoplasts was even reported on the cover of 'Cell' when it was demonstrated that addition and deletion of U residues can be extensive: in the extreme case of the cytochrome c oxidase subunit III (coxIII) gene of *T. brucei*, mRNA editing apparently 'creates' over 50% of the coding sequence: in the 712-nucleotide partial sequence available, 398 Us are inserted at 158 sites and 19 Us are deleted at 9 sites[23]. Not less surprisingly, kinetoplast mRNA editing can result in the correction of divergent genes found in different trypanosomal species to generate conserved amino acid sequences[43]. Additional elements of this puzzle were the detection of *T. brucei* coxIII transcripts edited partially and to different extents, but always with an unedited 5′- and an edited 3′-region[2], and of transcripts edited or unedited in the coding region, but with additional U residues in the poly(A) tails[50]. Taken together, these observations indicated that RNA editing in trypanosomes is post-transcriptional and proceeds in the 3′ to 5′ direction. The central problem of how or where information for editing is stored, however, was addressed only theoretically[23,44].

This problem has recently been approached experimentally by Blum, Bakalara and Simpson[10], who found the editing information in small pieces scattered in the 30 kilobase (kb) *L. tarentolae* kinetoplast maxicircle DNA. The case of the MURF3 gene, coding for a peptide homologous to a subunit of a NADH dehydrogenase, is illustrated in figure 1 A, top. The coding region is represented by a white box, and two short regions of complementarity to the edited MURF3 RNA by a grey and a black box. Blum et al. were able to identify these two regions by a computer analysis allowing GU base pairing, known to exist in RNA-RNA hybrids. In this analysis five other regions, which could potentially yield RNAs complementary to three other edited mRNAs (CYb, MURF2 and coxII), were identified. Indeed, short RNAs were then identified on Northern blots, partially sequenced, and christened guide RNAs (gRNAs) in view of their presumed function.

The sequence of the MURF3 'ft' gRNA of *L. tarentolae* is presented in figure 1 B. This 29-base gRNA can pair with 9 bases situated immediately downstream of the MURF3 mRNA editing site and specifies the introduction of five bases (lower case Us in the MURF3 mRNA; the insertion of four Us is directed by As, the fifth by a G). On the basis of similar findings for the seven gRNAs and of the existence of 3′ terminal uridylyltransferase and RNA ligase activities in the *L. tarentolae* kinetoplast[5], the model of RNA editing presented in figure 1 A, center and bottom, was proposed. This model predicts, for example, that the MURF3 'ft' gRNA aligns on the unedited MURF3 mRNA within a ribonucleoprotein particle called an 'editosome' (stippled oval). The MURF3 mRNA is then corrected at the 'ft' site, possibly by five consecutive cycles of RNA cleavage, U insertion, and religation (one cycle is presented in fig. 1 C). The editosome then proceeds towards the 5′ end of the mRNA, where it completes correction with the help of the MURF3 '5' gRNA, which guides the introduction of 20 additional bases.

It should be mentioned that the guide RNA model does not yet account for all the editing events observed. In particular in *L. tarentolae*, completely satisfactory gRNA sequences have not yet been found for the coxIII gene, but the sequence of the maxicircle DNA is still not completely determined and there is only limited information on the heterogeneous minicircle sequences. Moreover, the mitochondrial DNAs of *T. brucei* and *C. fasciculata* have not been screened for gRNAs yet, and the extensive editing of the coxIII gene of *T. brucei* is still unexplained. It has been reported that in this case there is no precursor-product relationship between edited and unedited transcripts, and that the bulk of edited RNA is synthesized de novo as a unit[52], presumably on a complementary RNA template. The data supporting this study, however, are not completely convincing.

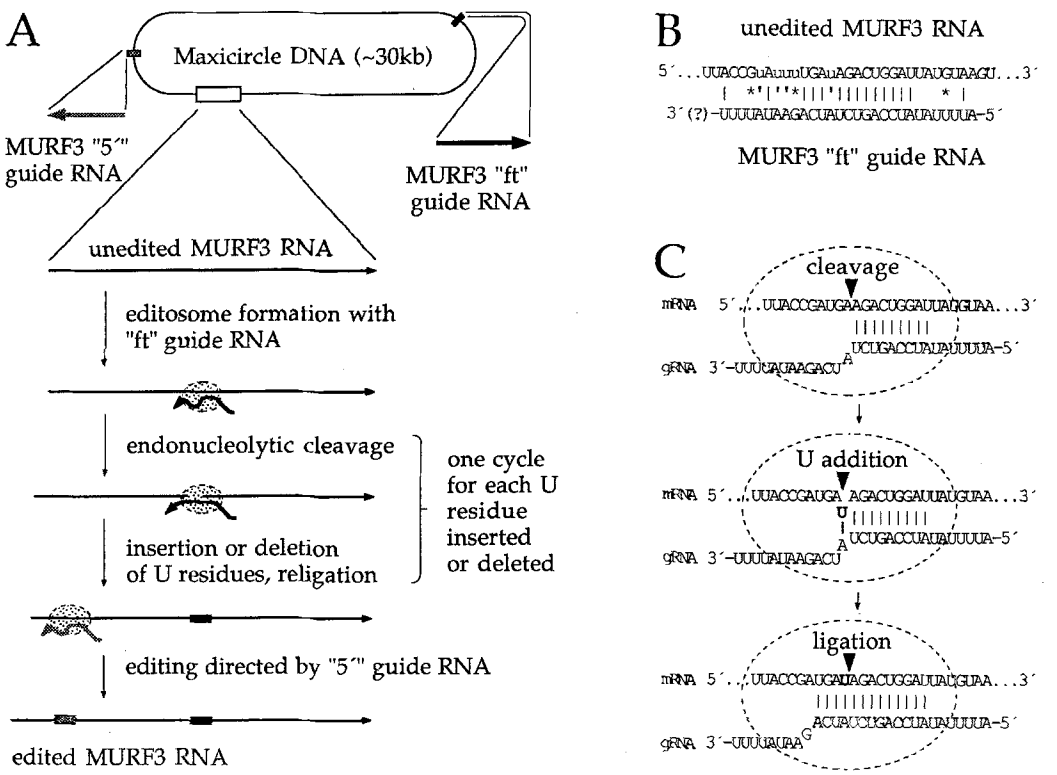

Figure 1. Model for RNA editing in kinetoplasts as proposed by Blum et al.[10]. *A* Top: Location of the sites corresponding to MURF3 mRNA and gRNAs on the maxicircle DNA of *L. tarentolae*. Note that genetic information in this system is tightly packed, and that genes and gRNA complementary regions sometimes overlap. Bottom: Sequential steps in the editing process. *B* Sequences of a segment of the unedited MURF3 mRNA and of the entire 'ft' gRNA. Residues added by editing are indicated as lower case Us in the mRNA. Standard complementarity is indicated by long vertical lines, GU complementarity by asterisks, and base pairs present only after editing by short vertical lines. *C* Detail of one cycle of RNA editing. Drawing reprinted by permission from Nature *343*, p. 698. Copyright 1990 Macmillan Magazines Ltd.

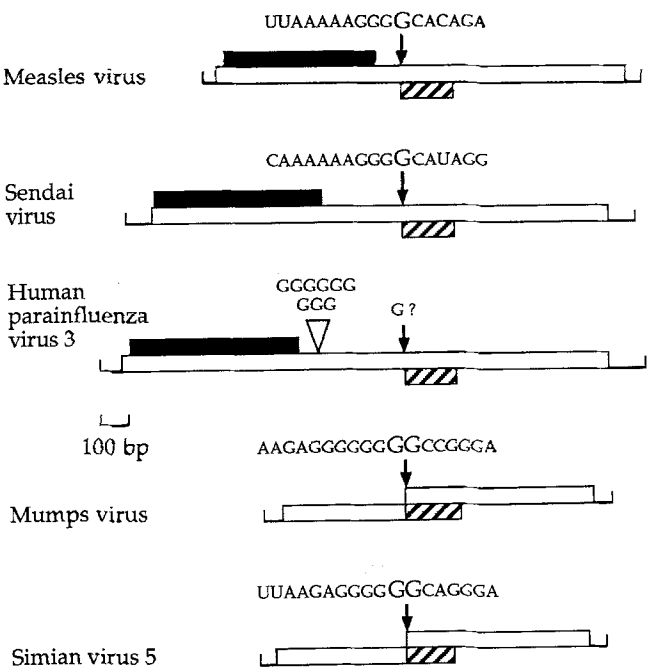

Figure 2. The edited P genes of five paramyxoviruses. Data are from Cattaneo at al.[15] (measles virus), Vidal et al.[51] (Sendai virus), Takeuchi et al.[48] (mumps virus) and Thomas et al.[49] (Simian virus 5). The three genes on the top encode, in addition to a P protein (white boxes) and a V protein (white and hatched boxes), a third protein (C, black boxes). The nucleotides surrounding the G insertion sites are indicated above the genes in the mRNA (plus) sense. For details see text. For the exact location of the reading frames in these genes see[15].

Why are some kinetoplast genes in scrambled pieces? Does this type of editing represent a molecular fossil, recalling RNA processing phenomena used by primitive cells to produce different proteins starting with a limited collection of RNA gene pieces? If this editing mechanism is a molecular fossil, why is such a cumbersome system maintained? Recently, a variety of unorthodox gene arrangements have been discovered: reverse transcribed copies of processed transcripts and reverse transcriptase-like genes have been found in genomes of organelles [12, 40], but not yet in kinetoplast genomes. Scrambled genes encoding proteins are now beginning to be found in different organisms including humans [18, 26, 32], and in the mitochondrion of the unicellular alga *Chlamydomonas reinhardtii* even the DNA segments specifying the ribosomal RNAs are scrambled [11]. Examination of the genomic organization of more organisms could possibly lead to insights into the generality and the evolutionary origin of scrambled genes and of RNA editing by post-transcriptional insertion-deletion of residues.

Co-transcriptional insertion of nucleotides

A form of RNA processing, which in retrospect can be classified as RNA editing, was first described in 1981 for human mitochondria [4, 37] and later for vertebrate mitochondria in general [39]. In these organelles, the transcription stop codons are truncated in most of the genes, retaining only T or TA. During polyadenylation UAA stop codons are reconstituted.

A second form of co-transcriptional mRNA editing was described for paramyxoviruses, a family of negative strand RNA viruses including measles and mumps, where G residues are inserted with a defined frequency at a specific site containing a stretch of purines (as read in the mRNA, or plus, sense; fig. 2). Using this editing mechanism, two proteins with common amino- and different carboxyl-terminal regions are produced. The longer P protein (fig. 2, white boxes) is a polymerase cofactor influencing the efficiency of transcription. The shorter, less abundant and more loosely nucleocapsid-associated V protein [48, 49] has the same amino-terminal region of P, but a different carboxyl-terminal region that encodes a highly conserved cysteine-rich sequence (hatched box). Figure 2 reveals additional features of RNA editing in paramyxoviruses. In measles and Sendai virus (top), P is specified directly by the genome and V can be expressed only from mRNAs with one additional G (larger character above the arrows). In mumps, Simian virus 5 (bottom) and human parainfluenza virus 2 [36a, 45] V is specified by the genome and P is produced from mRNAs in which two Gs are inserted. Editing by addition of one G residue has also been observed in the P gene of rinderpest virus [6], and has been postulated in human parainfluenza virus 3 (center). Moreover, in this virus, P genes of three substrains have been found to differ shortly upstream of a predicted editing site by the number of Gs present, which is 5, 8 or 11, respectively [25, 35, 46]. Another indication that insertion of bases occurs sometimes at 'wrong' positions is the existence of a measles virus fusion gene cDNA containing 19 additional Cs after a stretch of 5 encoded Cs [15]. Paramyxoviral RNA editing lacks precision also with respect to the number of nucleotides inserted: in measles and Sendai viruses many clones with several Gs inserted were found [15, 51], and mumps virus produces not only two but three related proteins by inserting none, two, four (or more) Gs at the same position [21a] (and Reay G. Paterson and Robert A. Lamb, personal communication).

Concerning the editing mechanism, it was recently demonstrated that the activity altering paramyxoviral transcripts cannot act in trans, and that it is likely to be encoded by the virus. Vidal et al. [51] inserted a Sendai virus P gene tagged with a single base mutation into a vaccinia virus vector, and then co-infected cultured cells with Sendai virus and the vaccinia derivative. About 30% of the P mRNAs produced by Sendai in this mixed infection had one or more Gs inserted, whereas none of the tagged P mRNAs produced by vaccinia had inserted G nucleotides. Vidal et al. also used an in vitro transcription system to separate the RNA editing process from the cellular environment, and observed that editing in vitro is almost as efficient as in vivo. Thus, paramyxoviral RNA editing is probably cotranscriptional: the viral polymerase complex stutters on a stretch of pyrimidines on the genomic template, introducing one or more additional G residues in mRNAs. This mechanism seems to be an adaptation of the process resulting in polyadenylation of viral transcripts: the viral polymerase complex is believed to stutter on short poly(U) stretches situated at the 3′ end of all the genes, after having recognized a sequence similar to the editing signal [30, 49].

Other forms of cotranscriptional mRNA editing might exist. In at least one prokaryotic and one eukaryotic DNA virus, polymerase stuttering at the onset of transcription results in addition of As at the 5′ ends of the transcript [33, 42]. These last two processes do not modify the coding regions of the respective genes, and thus should not be considered to be editing processes, but it is conceivable that in other viral or cellular genes predetermined modifications of the coding region are caused by cotranscriptional polymerase stuttering.

Substitution of nucleotides

Substitution of nucleotides, by either modification or exchange mechanisms, might be the type of mRNA editing which has quantitatively the largest impact on coding alterations. Messenger RNA editing by C to U (or U-like) base substitution was initially documented in the apolipoprotein B transcript of mammalian cells [17, 38]. Apolipoprotein B is one of the proteins essential for transport and metabolism of lipid particles. In humans, two major forms are found, one long (apo-B100), and a

shorter one (apo-B48) lacking the carboxyl-terminal half and thus being unable to bind to the low density lipoprotein receptor. Apo-B48 is generated from edited mRNAs in which a genomically templated C is substituted by a U or a functional U-like residue (fig. 3, top). This base substitution converts a glutamine codon (CAA) to a stop codon (UAA) and causes premature termination of translation in a tissue-specific way: in the liver, where the modification does not occur, only apo-B100 is synthetized, whereas in the intestine apo-B48 is the predominant translation product.

Editing by C to U base substitution in the apolipoprotein B system is restricted to a single position of a 16 kb mRNA. The question of the sequence requirements for this strict specificity was investigated in different systems. When cassettes of variable length encompassing the apo-B editing site were transferred into the apo-E gene, shorter proteins resulting from edited mRNAs were obtained with a 354 bp long cassette, but not with a 63 bp long cassette [13]. Studies performed in vitro, on the other hand, indicated that RNAs comprising 2383, 483 or 55 nucleotides around the editing site are modified at levels 5–10 times lower than in vivo, but RNAs comprising 26 nucleotides around the editing site are not modified [21]. Finally, when apo-B cassettes specifying RNAs containing from a maximum of 2385 down to 26 bases were transcribed in cultured cells, editing efficiency was always about 50% [20]. In summary, the minimal sequence requirements for efficient, specific editing by C to U base modification vary in different systems. The mechanism of this type of RNA editing remains elusive. In vitro studies convincingly demonstrate that editing can be posttranscriptional [21], but in vivo editing is linked with polyadenylation: cryptic polyadenylation sites situated in regions that are normally translated are activated by editing [13, 17, 38].

The issue of specificity of editing by C to U conversion was further complicated by findings in transcripts of plant mitochondria. Gualberto et al. [27] reasoned that mRNA editing could be a way to account for the apparent discrepancy of the coding potential of CGG (arginine) codons in some mitochondrial genes of higher plants. These codons are sometimes found at positions where TGG codons (tryptophan) are conserved in the corresponding genes of all animal and of other plant mitochondria [24]. Since no tRNA specific for tryptophan and recognizing CGG was found in these organelles, Gualberto et al. analysed the sequence of part of the cox2, cox3, rps12 and nad3 transcripts, and those of the corresponding genes, of wheat mitochondria. These experiments demonstrated that editing events by C to U base substitutions were indeed taking place, not only at the predicted positions but, surprizingly, also at neighbouring codons. Recently, Grienenberger et al. (personal communication) found that in different transcripts of wheat mitochondria at least 49 different C to U changes occur.

Independently, Covello and Gray [19] compared most of the sequence of the wheat mitochondrial cox2 transcript with the one of the corresponding gene and found, in addition to two C to U modifications resulting in arginine to tryptophan conversion, several other C to U modifications resulting in other amino acid changes. When the data of both groups are compiled (fig. 3, bottom), 11 C to U modifications, all leading to amino acid exchanges, are found over the still incompletely determined sequence of the 800 nucleotide-long coding region. Recently, Covello and Gray (personal communication) also sequenced the cox2 transcript of maize and pea, and found that overall about 6% of the amino acids predicted from the mRNA are different from those predicted by the genomic sequence. Similar observations and conclusions were reported for mitochondrial transcripts of the other higher plant *Oenothera*, where partially edited transcripts were found [28, 41]. Interestingly, partial editing is more frequent in primary, unspliced transcripts than in spliced transcripts, an observation suggesting that editing in this system is also posttranscriptional. Moreover, in *Oenothera* one case of editing by U to C transition was observed [41a].

The most intriguing observation in plant mitochondrial transcripts is the lack of a conserved sequence around the editing sites: does the information necessary for repro-

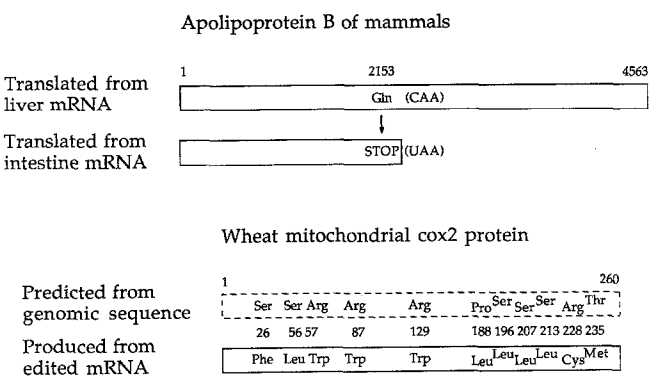

Figure 3. Effects of C to U base modifications on the predicted sequences of human apolipoprotein B (top) and on wheat mitochondrial cox2 protein (bottom). Numbers indicate positions in the amino acid sequences. Top: data from Powell et al. [38], and Chen et al. [17]. Bottom: data from Gualberto et al. [27], and Covello and Gray [19].

ducible sequence alteration reside exclusively in the RNA secondary structure, or is it at least in part coded from outside the gene? It is also interesting to note that in two cases transcripts of the homologous genes (coxII and coxIII) of trypanosome and plant mitochondria are edited by completely different mechanisms.

Knowledge of the mechanism of editing in plant mitochondria and mammalian cells might provide hints about the evolutionary relationship between different types of RNA editing. The chemical nature of the modified base for the apo-B transcript was determined after in vitro modification, and it was demonstrated that it is a U (Boström et al., personal communication). Thus the editing enzyme could be a sequence-specific cytidine deaminase, but two alternative mechanisms are conceivable: the whole base could be exchanged without cleavage of the sugar-phosphate backbone of the RNA chain, or the RNA chain could be cleaved, a C deleted and a U added, in a model similar to the one proposed for editing by U addition and deletion. Examples of enzymatic modification of residues, base exchange, or deletion followed by insertion of residues have been previously described in tRNAs, where more than 50 different types of base modifications are known[9]. It should be noted that tRNA modifying activities are generally very specific, and might only act on mRNAs as a rare exception.

Perspectives

Until 1986, if one ignores the introduction of stop codons by polyadenylation in vertebrate mitochondria, only splicing was known to alter, at the transcriptional or posttranscriptional level, the coding sequence predicted from a gene. Now other mRNA editing systems have been characterized, and more are likely to be discovered. The editing phenomenon described in plant mitochondria has solved the apparent paradox of the genetic code used in these organelles, but opened questions on how its specificity is determined. RNA editing mechanisms might be molecular fossils from a world in which genetic information was as a rule altered at the RNA level. Thus when examining the sequence of a gene, one should always be aware that DNA rearrangements, co- or post-transcriptional RNA modifications, and unconventional translation mechanisms can alter the predicted protein sequence.

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