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REVIEW

When you can't trust the DNA: RNA editing changes transcript sequences

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Abstract RNA editing describes targeted sequence alterations in RNAs so that the transcript sequences differ from their DNA template. Since the original discovery of RNA editing in trypanosomes nearly 25 years ago more than a dozen such processes of nucleotide insertions, deletions, and exchanges have been identified in evolutionarily widely separated groups of the living world including plants, animals, fungi, protists, bacteria, and viruses. In many cases gene expression in mitochondria is affected, but RNA editing also takes place in chloroplasts and in nucleocytosolic genetic environments. While some RNA editing systems largely seem to repair defect genes (cryptogenes), others have obvious functions in modulating gene activities. The present review aims for an overview on the current states of research in the different systems of RNA editing by following a historic timeline along the respective original discoveries.

Keywords RNA maturation · Base deamination · Editosomes · PPR proteins · Cryptogenes · Pan-editing

Introduction

The genetic language of life has become common knowledge more than 50 years after the discovery of the now famous DNA double helix structure. The four nucleotide letters of the DNA alphabet—adenosine (A), cytidine (C), guanosine (G), and thymidine (T)—are familiar to every high school student. Most students will also remember that these four chemically stable deoxyribonucleotides of DNA chains are transcribed into copies of corresponding ribonucleotide chains of RNA with the important exception of thymidine, which is replaced by uridine (U) in living cells. Many RNA copies are made for the purpose of protein biosynthesis in which nucleotide triplets, read as codons, are translated into the 20-amino-acid alphabet of proteins that make up the larger part of a cell's functionality. This process of translation, protein biosynthesis, takes place at the cells' ribosomes, which are themselves to a large part made up of one dominating type of RNA in the cell, the ribosomal RNAs (rRNAs). Ribosomes use transfer RNAs (tRNAs) carrying amino acids by pairing their anti-codons to the codon triplets in messenger RNA (mRNA) encoding proteins. This is the simple version to understand life and, by and large, understanding the functions of rRNAs, mRNAs, and tRNAs to this day is a very good approximation for protein biosynthesis in the bacterial world of prokaryotes.

When living cells become more complicated and sophisticated and, as eukaryotes, develop a membrane-bound nucleus to store their DNA, things become more complicated. The concept of a strict 1:1 parallel co-linearity of a gene's DNA sequence and its RNA copy received a first major blow when introns were discovered in the late 1970s. For an RNA to become functional, these noncoding intron stretches have to be properly spliced out and flanking exons have to be joined; only the matured RNA can be used for translation. Introns continue to complicate the prediction of gene products when new genome data are produced and alternative splicing adds another layer of complexity.

Another major blow to the predictive power of DNA sequences came with the discovery of RNA editing—you

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can't always confidently trust gene sequences. The term RNA editing was introduced by Rob Benne and colleagues in Amsterdam in 1986 when they reported that four uridine nucleotides were inserted into specific sites of the mitochondrial *cox*2 mRNAs (encoding cytochrome oxidase subunit 2) of trypanosome species to reconstitute the reading frame [1]. This major discovery in molecular biology turned out to be the proverbial tip of an iceberg. RNA sequence alterations due to different types of RNA editing were subsequently identified in organisms separated over wide phylogenetic distances. A timeline for these discoveries is given in Table 1.

Difficulties of terminology: where to draw the line for RNA editing

RNAs are subject to several further biochemical alterations in the cell. The terms "maturation," "processing," "modification," and "editing" describe these phenomena (Fig. 1). Similarly used in everyday language, the terms are frequently also used interchangeably in the scientific literature—most notably, RNA processing in a wider sense is used instead of RNA maturation as an umbrella term for the different biochemical processes with overlapping functionality. In a narrower sense, RNA processing may be restricted to describe those processes of RNA maturation that invoke deletion (and occasionally also ligation) of longer RNA sequence stretches. The term modification in contrast is best reserved for biochemical alterations introducing nonstandard nucleotides into RNAs, extending the four-letter standard alphabet of A, C, G, and U. The typical examples are dihydrouridine and pseudouridine in tRNAs. Novel forms of such unique chemical modifications continue to be identified: a recently analyzed base-modified cytidine in the first anticodon position of a tRNA for isoleucine in archaea has been christened agmatidine [2].

RNA editing in contrast describes sequence changes introduced through selective nucleotide insertions, deletions, and substitutions, which could alternatively be directly encoded by the four standard nucleotides in the gene. The term RNA editing was initially introduced to label the uridine insertion type of RNA editing discovered in trypanosomes but has subsequently been used to describe all sequence modifications affecting the four nucleotides of the standard RNA alphabet. Semantic overlaps with processing and modification exist, however. The polyadenylation of eukaryotic mRNAs, generally considered as a processing event (Fig. 1), normally does not interfere with genetic information but is involved in termination of transcription, terminal intron splicing, and nuclear mRNA export, and confers transcript stability. In animal mitochondria, polyadenylation of 3′ truncated transcripts may alternatively serve to introduce stop codons only by adding adenosines to their end. As another example, the deamination of the adenine base in adenosine nucleotides results in the nonstandard hypoxanthine base of inosine nucleotides and is technically a modification. Given that inosine, however, is subsequently read like guanosine in the cell, this process is considered a phenomenon of RNA editing, as I will discuss below.

1986: The kinetoplastid case—adding and deleting uridines in mitochondrial transcripts

Trypanosomes belong to the kinetoplastid protozoa (Excavata, Euglenozoa). Wide interest in this protist group comes from the fact that many are pathogenic parasites. Most widely known are the human pathogens *Trypanosoma brucei*, the causative agent of sleeping sickness, *T. cruzi*, causing Chagas disease, *Leishmania spp*., causing leishmaniasis, and *Crithidia spp*., parasites of arthropods. The kinetoplast is a defining cell biological feature of this protist clade—a disk-shaped assembly of concatenated mitochondrial DNA rings, the so-called maxicircles and minicircles, in the single mitochondrion at the base of their flagellum.

As in many of the subsequent cases, the initial discovery of an RNA editing process came with the observation that something is wrong with a gene. The mitochondrial *cox*2 gene in the trypanosomes *Crithidia fasciculata* and *Trypanosoma brucei* showed reading-frame shifts, and it turned out that these were corrected only at the transcript level by inserting four uridine residues into the *cox*2 mRNAs [1]. This discovery opened a Pandora's box of genetic complexities to be elucidated over the next nearly 25 years (Table 1). Uridine nucleotides are not only inserted into pre-mRNAs but also deleted in other instances. The extent of the RNA editing process in kinetoplastids can be so immense that more than 90% of codons within a reading frame are established through editing. The long elusive *cox*3 gene of *Trypanosoma brucei* is recognizable at the RNA level because editing affects nearly 60% of the nucleotide positions through insertion of 550 uridines and deletion of 41 others [3]. The terms pan-editing and cryptogenes were introduced for such excessive RNA editing of the transcripts of genes that can hardly or not at all be identified at the DNA level [4, 5]. Not only the obligate parasitic trypanosomatoid taxa but also those of the sister group of free-living bodonid taxa perform RNA editing [6].

A major early step to elucidate the mechanisms of kinetoplastid RNA editing was the discovery of a new type of small antisense RNA species, appropriately termed

Table 1 RNA editing in different genetic systems in the chronological order of their discoveries

Date	Organisms	Type of RNA editing	Affecting	In genetic system	Original discoveries
1986	Kinetoplastids	U insertions, U deletions	Diverse mRNAs	Mitochondrial	Four uridines inserted into the mitochondrial *cox*2 mRNA of *Trypanosoma brucei* and *Crithidia fasciculata* to reconstitute the reading frame [1]
1987	Mammals	C to U	Some mRNAs and viral RNAs	Nuclear	Stop codon introduced by conversion of glutamine codon in *apo*B mRNA in human intestine [41–43]
1988	Paramyxoviruses	G and A insertions	P (phosphoprotein) and glycoprotein mRNAs	Viral	Insertion of two guanosine nucleotides in Sendai virus 5 (SV5) phosphoprotein (P) mRNAs establishing a new reading frame [64]
1989	Plants	C ↔ U	mRNAs, tRNAs (rRNAs)	Mitochondrial	Multiple codon sense changes by C-to-U conversions observed in mitochondrial mRNAs in wheat *Triticum aestivum* [72, 73] and in the evening primrose *Oenothera berteriana* [74]
1991	Plants	C ↔ U	mRNAs, tRNAs (rRNAs)	Chloroplast	The start codon of the maize *Zea mays* chloroplast *rpl*2 gene introduced via C-to-U RNA editing of a genomically encoded ACG threonine codon [92]
1991	Myxomycota	C insertions, U insertions, dinucleotide insertions, C to U	mRNAs, rRNAs, tRNAs	Mitochondrial	Multiple C insertions in the mitochondrial *atp*1 mRNA in *Physarum polycephalum* [165]
1991	Metazoa	A to I (G)	Many mRNAs, tRNAs, miRNAs, and viral RNAs	Nuclear	An apparent glutamine CAG to arginine CGG codon change in the mRNA of glutamate-gated ion channels in mouse brain [187]
1993	*Acanthamoeba* and chytridiomycete fungi	N to N′	tRNA 5′ acceptor stem	Mitochondrial	Different nucleotide exchanges in the first three nucleotides of tRNAs initially found in *Acanthamoeba castellanii* [210], later in *Spicellomyces punctatus* [214]
1993	Marsupials	C to U	tRNA anticodon	Mitochondrial	The genomically encoded GCC anticodon sequence of *trn*D, the tRNA for aspartate is corrected to the GUC anticodon in 50% of tRNAs in mitochondria of the opossum *Didelphis virginiana* [219]
2002	Dinoflagellate	A ↔ G, C ↔ U, U to R, G to C	mRNA	Mitochondrial	Nucleotide substitutions in mitochondrial *cob* and *cox*1 mRNAs of *Pfiesteria piscicida*, *Prorocentrum minimum*, and *Crypthecodinium cohnii* [237]
2004	Dinoflagellate	A ↔ G, C ↔ U, R to C, U to G	mRNA, rRNA	Chloroplast	Nucleotide substitutions in several chloroplast mRNAs and in the small rRNA in *Ceratium horridum* [238]
2009	Archaeae	C to U	tRNAs	Bacterial	Conserved uridine in tRNA consensus position 8 re-established via deamination in tRNAs of the thermophilic archaeon *Methanopyrus kandleri* [245]
2009	Placozoa	U to C	mRNA	Mitochondrial	A U-to-C conversion transforming a tyrosine into a histidine codon in the mitochondrial *cox*1 mRNA in *Trichoplax adhaerens* [244]

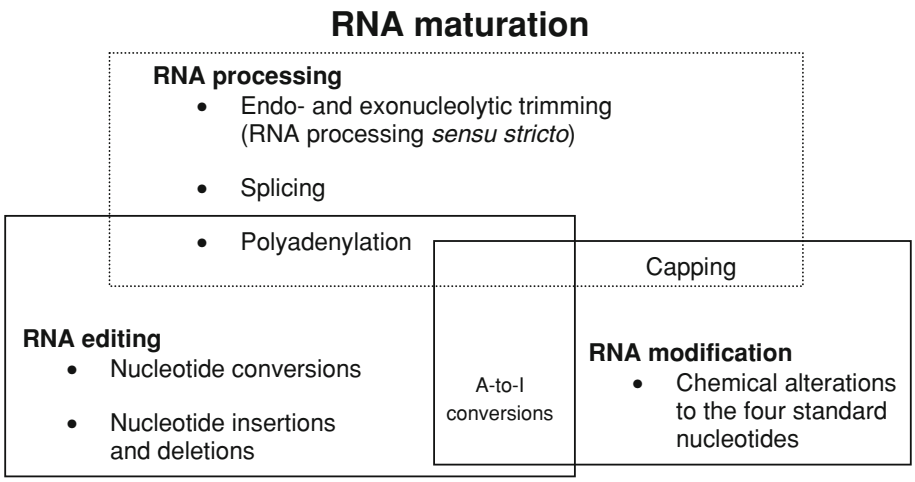

Fig. 1 The term "RNA maturation" is used as an umbrella term for different phenomena of biochemical transformations of RNA transcripts. "RNA processing" is mostly used to describe changes affecting sequence stretches of variable length through cutting and rejoining processes such as intron splicing. It is generally also used for the capping and polyadenylation processes at the 5′ and 3′ ends, respectively, of eukaryotic nucleus-encoded mRNAs. The term "modification" in contrast is best reserved for biochemical alterations resulting in nonstandard nucleotides (mostly identified in tRNAs and rRNAs) such as pseudouridine, dihydrouridine, methylated nucleotides, and many more. Finally, "RNA editing" comprises all sequence changes in the four-letter RNA alphabet relative to the gene template other than splicing and polyadenylation that could in principle be encoded in the DNA directly. Overlaps in terminology exist, however: the capping nucleotide is a methylated guanosine added in inverted orientation, stop codons may emerge only after polyadenylation, and inosine nucleotides (read as guanosine) result from deamination of adenosines

guide RNAs (gRNAs), which direct the editing process as templates [7, 8]. Guide RNAs pair with small segments of pre-mRNAs, and unpaired A (or G) residues in gRNAs lacking complementary bases in the pre-mRNA provide the information for the locations and the numbers of uridines to be inserted at a given site (Fig. 2). Alternatively, missing complementary bases in the gRNAs direct the deletions of uridines at other sites. In fact, the discovery of guide RNAs encoded on the kinetoplast minicircles [9, 10] finally provided an explanation for the existence of these smaller DNA circles accompanying the kinetoplast maxicircles, which represent the kinetoplastid equivalent of the mitochondrial genome in other eukaryotes. The successive pairing of gRNAs progresses from the 3′ to the 5′ end of the maturing pre-mRNA. Stretches of non-encoded uridines at the end of gRNAs were initially suspected of directly providing uridines for insertion into editing sites [11–13]. However, the oligo-U ends instead turned out to participate in the editing process by less specific pairing with the pre-mRNA around the editing site, whereas the substrate for insertions is free UTPs (uridine triphosphates) added to the 3′OH end of the cleaved pre-mRNA [14]. The *trans*-acting guide RNAs provide specificity in the case of kinetoplastid RNA editing, but alternatives can exist, as has been shown for *cox*2 editing, where the 3′-UTR (untranslated region) of the mRNA serves as a guide in *cis* [15].

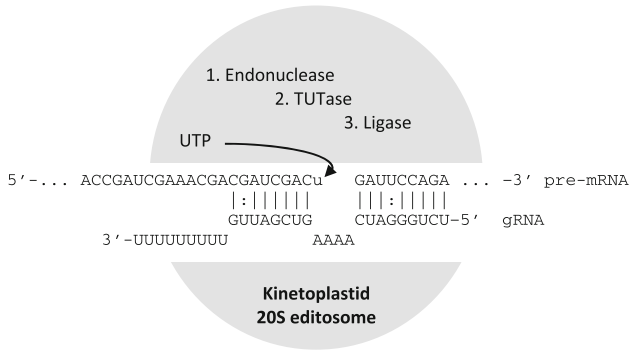

Fig. 2 A highly simplified view of the uridine insertion type of RNA editing in kinetoplastid mitochondria. Small, 3′-oligo-uridylated antisense guide RNAs (gRNAs) pairing with a given pre-mRNA carry information on location and number of uridines to be inserted. The (entirely hypothetical) example shown displays a case for insertion of four uridines into the pre-mRNA that are ultimately complementary to the initially unpaired adenosines of the gRNA. The different enzymatic activities for RNA editing are assembled in 20S editosome multi-protein complexes, which come in at least three different variants of protein composition, also depending on the location and mode (U insertion vs. deletion) of editing. The three major biochemical activities for uridine insertion are an endonuclease activity cleaving the pre-mRNA at the site of editing, a terminal uridylyl transferase adding uridylates from UTPs to the free 3′OH end of the upstream part of the pre-mRNA, and a ligase rejoining the transcript ends after editing. In the case of uridine deletion editing, a 3′-uridine-exonuclease of the ~20S editosome comes into play that removes unpaired "extra" uridines from the pre-mRNA, which remained unpaired in the hybrid with the respective gRNA

Admirable progress to identify the details of the biochemical machinery inherent in RNA editing in kinetoplastids has been made in several laboratories worldwide since the first in vitro systems were established [13, 16–18]. Numerous studies involving the in vitro assay systems, biochemical purifications, mass spectrometry, crystallographic studies, and reverse genetics using RNA interference have elucidated numerous facets of the kinetoplastid editing process [19–25]. Over the years an ever clearer picture of a kinetoplastid 20S "editosome" has been drawn, which has grown from a biochemical concept into a macromolecular structure described in increasing detail [26–31]. Among the major biochemical activities involved in kinetoplast RNA editing are an endonuclease, a terminal uridylyl transferase (TUTase), and a ligase activity operating on the mitochondrial pre-mRNAs (Fig. 2). A uridyl-specific 3′–5′ exonuclease [32] is important for uridine deletions, and other additional or accessory biochemical activities are also present, such as a 3′ nucleotidyl phosphatase activity [33] or accessory RNA-binding proteins [34]. More than a dozen core proteins exist in an RNA editing core complex (RECC, also labeled L for ligase complex). Three biochemically distinct editosome types can be distinguished [35] in which, for example, different types of RNA ligases are used after insertions or deletions of uridylates have taken place [36, 37].

Observations that trypanosome RNA editing depends on the developmental stage were made very early [38]. As in the other editing systems outlined below, the issue of regulation of gene activity is frequently discussed but often remains problematic with respect to cause and effect. A second issue generally associated with RNA editing is the one of protein diversity created through differential editing. The potential of creating protein diversity through alternative editing in kinetoplastids has recently been emphasized [39, 40].

1987: The first metazoan case—C-to-U conversion in mammals

The molecular explanation for two different forms of apolipoprotein B being produced in the human body from only one *apo*B gene—a long version of a 100 kDa protein in the liver and a carboxy-terminally shortened version of 48 kDa in the intestine—was provided in 1987. A cytidine-to-uridine substitution in the *apo*B mRNA converts a CAA glutamine codon into a UAA stop codon in a tissue-specific manner [41–43]. Biochemically, this type of RNA editing is much simpler than the kinetoplastid type of editing, given that the RNA polynucleotide backbone can remain intact and base conversion from cytidine to uridine can simply be achieved through a deamination reaction. Similar to the kinetoplastid editing system, in vitro assays played a major role for clarifying the biochemical mechanisms [44]. Given that *apo*B mRNA editing also occurs in many well established mammalian model organisms such as rats and mice [45], the enzymatic machinery was quickly elucidated by the mid 1990s. It turned out that a zinc-dependent cytidine deaminase named APOBEC-1 for *apo*B editing catalytic subunit 1 played the central role [see 46]. In contrast to the many different *trans*-acting guide RNAs necessary to supply information on the numerous editing sites in kinetoplastids, the sequence specificity for *apo*B editing is supplied by the 11 nt. mooring sequence (UGAUCAGUAUA) in the vicinity of the edited site that is recognized by the editing machinery. The RNA-binding "APOBEC-1 complementing factor" ACF plays the major role in editing site recognition and together with APOBEC-1 forms the core editosome for *apo*B editing.

In contrast to the kinetoplastid type of RNA editing discussed above or the one operating in plant organelles to be discussed below, the discovery of *apo*B editing in mammals was not a finding with many more of its type to be subsequently discovered in the nuclear genetic system of mammals or other metazoa. Only a few additional RNA targets for C-to-U editing have been identified in mammals. An interesting case for functional modulation similar to the *apo*B case is a proline-to-leucine codon change in a glycine receptor modifying its properties as a chloride ion channel [47, 48]. Other previously identified examples of this type of editing are somewhat less clear and include an additional downstream site in the *apo*B mRNA itself, changing an ACA threonine codon into an AUA isoleucine codon at low efficiency [49]. The NAT1 (novel APOBEC target) mRNA [50] and the neurofibromatosis NF1 mRNA [51] are edited as well. The precise roles of RNA editing in these targets and potential correlations with pathological phenotypes remain somewhat unclear [52]. Similarly, a direct involvement of APOBEC-1 activity with tumor formation could not be identified in many different carcinoma samples [53].

The APOBEC-1 protein is one member of the small, vertebrate-specific gene family of APOBEC-related/AID (activation-induced deaminase), zinc-dependent cytidine deaminases [54–56]. Interestingly, at least some of the members (also) act on single-stranded DNA and perform dC-to-dU "DNA editing" of the corresponding deoxynucleotides [57]. The crystal structure of APOBEC-2, which is differentially expressed in muscle tissue and necessary for normal muscle development, has been determined [58], but its precise mode of action is unclear at present [59]. The APOBEC-3 subfamily in its diversity (members A through G) is unique to primates and its members appear to have antiviral activity, notably also the ability to suppress

retrovirus replication and retrotransposon mobility, e.g., by editing of the DNA provirus [60–63].

1988: The viral cases—stuttering polymerases adding purine nucleotides in paramyxovirus mRNAs

The introduction of additional nonencoded guanosine nucleotides in P (phosphoprotein) transcripts of paramyxoviruses was initially demonstrated in 1988 for the simian virus SV5 [64] and subsequently in 1989 for the measles virus [65] and in 1990 for the Sendai virus [66]. Either one (e.g., Sendai, measles) or two guanosine nucleotides (e.g., SV5, mumps) were found inserted in the respective viral mRNAs. The degree of editing determines the expression ratio of protein P versus the alternative reading frame expressing protein V. Soon after the discovery, the mechanism for this co-transcriptional RNA editing phenomenon was characterized as a stuttering of the viral RNA-dependent RNA polymerase on extended oligocytidine sequence stretches in the template [67]. Paramyxoviruses belong to the group of nonsegmented negative strand RNA viruses (=NNV or order Mononegvirales). Interestingly, NNV generally polyadenylate their mRNAs by stuttering on a stretch of oligouridine sequences in the template RNA. The occurrence of G insertion RNA editing in the paramyxovirus subgroup, however, is enigmatically restricted to "polyhexameric" ($6n + 0$) virus genome sizes, i.e., those that are a multiple of six nucleotides [68]. A closely related viral editing process was identified in the Ebola virus, in which a single adenosine nucleotide is added to a run of seven adenosines in the mRNAs encoding the Ebola virus glycoprotein in the middle of its coding region [69]. RNA editing is the prerequisite for translation into the full length structural glycoprotein but takes place in only some 20% of transcripts whereas an excreted soluble version is produced from the unedited mRNA due to premature stop. It is tempting to speculate that shifts in the degree of RNA editing are related to virus infection cycles. An artificial Ebola virus mutant constitutively expressing only the full-length version of the structural glycoprotein reveals increased cytotoxicity [70]. Studies on a set of different measles virus strains, however, could not point out a relation between the degree of editing and consequently the variable protein P/V expression ratios and the viral infection cycles [71]. Whereas the stuttering type of virus RNA editing is clearly inherent in the viral RNA-dependent RNA polymerase and primary sequence features, other types of viral RNA editing, e.g., of hepatitis delta virus (HDV) or human immunodeficiency virus (HIV) RNA, rely on host-encoded ADAR-type adenosine deaminases targeting RNA secondary structures (see below).

1989–1991: The plant organelle cases—exchanging hundreds of pyrimidines in mitochondria and chloroplasts

Discovery of RNA editing in plants came in 1989 with research groups from Canada, France, and Germany reporting on C-to-U exchanges in mitochondrial mRNAs of wheat (*Triticum aestivum*) and the evening primrose *Oenothera berteriana* [72–74]. The pyrimidine exchanges in the majority of cases affect first and second codon position and change codon meaning (e.g., proline to leucine or serine, serine to leucine, arginine to tryptophan) to reconstitute evolutionarily conserved amino acid positions. With the investigations of larger cDNA samples, it soon became obvious that some sites were only partially edited in mRNA populations [75, 76]. Frequently these were "silent" exchanges affecting third codon positions, i.e., unnecessary synonymous editing events leaving codon meaning unchanged. Apparent similarities of editing site environments that could suggest common mechanisms were reported occasionally [e.g., 77], but these similarities were weak and encompassed only a few sites. In contrast to the kinetoplastid RNA editing system, no directionality of editing could be observed in plants, suggesting that RNA editing sites are recognized and converted independently. Very similar to the kinetoplastid case, however, RNA editing largely seemed to serve the purpose of repairing gene functions. This is also apparent given that plant RNA editing occasionally also affects tRNAs and intron sequences.

From a phylogenetic perspective (Fig. 3), it was immediately suggestive that plant RNA editing would affect at least the majority of flowering plants

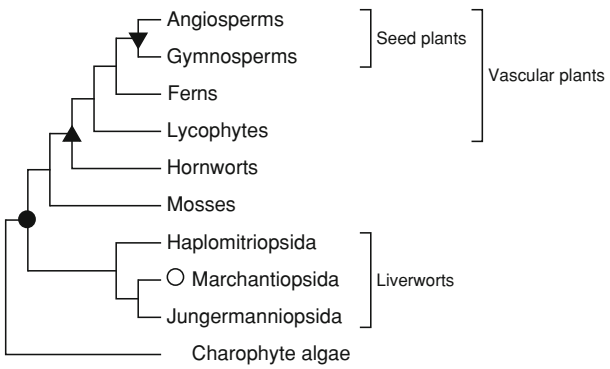

Fig. 3 Modern insights on plant phylogeny place liverworts as the sister clade to all other land plants (embryophytes) and hornworts as the sister group to vascular plants. A most parsimonious explanation for the evolution of the C-to-U type of plant organelle RNA editing postulates a single gain in the ancestor of all embryophytes (*filled circle*) and a secondary loss in the marchantiid liverworts (*open circle*). The reverse type of U-to-C editing arises in the common ancestor of hornworts and tracheophytes (*upward triangle*) and is strongly decreased in frequency in the seed plant lineage (*downward triangle*)

(angiosperms) given the deep phylogenetic split between the monocot wheat and the dicot *Oenothera*. It finally turned out that RNA editing not only operates in flowering plants but also in representatives of all other land plant (embryophyte) clades, i.e., gymnosperms, ferns and fern allies, mosses, hornworts, and (some) liverworts [78–82]. RNA editing has to date not been identified in green algae including taxa closely related to land plants and is also suspiciously absent from the complex thalloid liverworts, the Marchantiopsida [82]. Ironically, the mitochondrial genome of *Marchantia polymorpha* was the first plant mtDNA to be completely determined [83] soon after the discovery of plant mitochondrial RNA editing and remained the only one for 5 more years before the complete mtDNA sequence of *Arabidopsis thaliana* became available [84]. The apparent exclusive absence of editing in the liverwort subclade of marchantiid taxa remained puzzling and suggested either two independent gains or alternatively a secondary loss after a unique primary gain of RNA editing with the emergence of land plants. The second alternative turned out to be correct after unequivocally finding the unique clade of haplomitriid liverworts as sister clade to all other liverworts (including the "nonediting" marchantiids and "editing" jungermanniids) and the observation of highly frequent C-to-U editing in *Haplomitrium* [85]. Similarly, another aspect in the molecular evolution of plant RNA editing can now be plotted rather confidently onto the phylogeny of land plants. Whereas "reverse" uridine-to-cytidine editing is only very rarely observed in seed plants [77, 86] and not in mosses and liverworts, reverse U-to-C editing is strongly increased in frequency in hornworts, ferns, and fern allies [82, 87–89]. Given that hornworts are now reasonably well supported as sister clade to the vascular plants [90, 91], this suggests a gain/increase of reverse editing in the common ancestor of hornworts and tracheophytes and a subsequent decrease in the ancestor of spermatophytes (Fig. 3).

Reports on the same type of pyrimidine exchange RNA editing also operating in the other endosymbiotic organelle in the plant cells, the chloroplasts, followed shortly after the discovery in mitochondria [92]. The features of RNA editing in chloroplasts seemed largely identical to the ones in mitochondria, i.e., mainly serving to correct genetic information at the RNA level. Similarly, the phylogenetic distribution of RNA editing appears to be the same across 500 million years of land plant evolution with chloroplast editing being identified in all groups except the marchantiid liverworts [93]. The one major difference is that only some 20–50 RNA editing sites are identified in angiosperm chloroplast transcriptomes [94–99], whereas an order of magnitude more, i.e., around 400 RNA editing sites, are found in the mitochondrial transcriptomes [100–104]. However, this rule of thumb for angiosperms cannot be generalized for all land plants: some taxa showing high amounts of mitochondrial editing such as the hornworts and ferns may show similarly high amounts with hundreds of instances of editing in their chloroplasts, too [105, 106].

The need for RNA editing to correct codon identities may affect some 1,000 sites in taxa such as the gymnosperm *Cycas taitungensis*, a "fern palm" [107, 108], and more than 1,500 sites in a quillwort, the lycophyte *Isoetes engelmannii* [88]. The task of analyzing and cataloguing vast numbers of editing sites identified in plant organelles called attention to the need for a unifying nomenclature to designate RNA editing sites as recently proposed [108, 109]. Ongoing transcriptome analyses actually suggest that lycophytes hold the record for the amount of RNA editing, exceeding 2,000 sites in a mitochondrial transcriptome (J. Hecht, F. Grewe, S. Herres, and V. Knoop, unpublished observation). On the other end of the spectrum (and not considering the complete absence in marchantiid liverworts), the model moss *Physcomitrella patens* shows only 11 sites of RNA editing in its mitochondrial [109] and 2 sites in its chloroplast [110] transcriptome.

Discovery of pyrimidine exchange RNA editing not only taking place in plant mitochondria but also in chloroplasts had a major impact on subsequent research given the amenability of the plastid genome for genetic transformation and the phenotypes of plastome mutations affecting photosynthesis [111, 112]. Early experiments using transplastomic engineered tobacco lines confirmed that editing is essential for protein functionality [113], as had previously been shown for a mitochondrial protein expressed in the nucleus and targeted to mitochondria [114]. Further experimentation using chloroplast DNA manipulation has shown that RNA editing site recognition mainly relies on recognition sequences located 5′ of the editing sites extending for some 20 or more nucleotides whereas sequences 3′ of the editing site play a minor role, although this may differ depending on the sites in question [115–120]. By and large, the data from transplastomic studies correspond excellently to the findings for mitochondrial editing based on circumstantial evidence [121], experimentation with mitochondrial *in organello* electroporation [122, 123], and in vitro systems [124–130] that have been established in the meantime.

Given the parallel progress in the kinetoplastid RNA editing field, a search for gRNAs in plant organelles was started in some labs but this was to no avail. The breakthrough report on identification of a *trans*-acting factor targeting a specific editing event came with the identification of a so-called PPR protein labeled CRR4, which is responsible for an editing event introducing the start codon of *ndh*D in *Arabidopsis thaliana* chloroplasts [131]. The RNA-binding PPR (pentatricopeptide repeat) proteins are encoded by vastly extended gene families exceeding 400

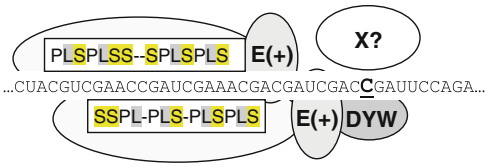

Fig. 4 Several plant-specific pentatricopeptide repeat (PPR) proteins of the PLS subgroup with variable repeat motif lengths including short (*S*) and long (*L*) variants of the classic 35-amino-acid-long PPR motif (*P*) and carboxy-terminal protein domain extensions E, E+, and DYW have been shown to be involved in plant organelle RNA editing (*bottom*). The DYW domain has been proposed to carry the editing (cytidine deaminase) activity, but some PLS proteins without the DYW domain (*top*) have also been identified as RNA editing site recognition factors. The PPR motifs are assumed to recognize RNA primary sequences, presumably on a one-PPR-repeat-per-nucleotide basis, although the exact binding code is currently unclear. Perhaps more than one PLS protein and/or additional, currently uncharacterized, factors ("X?") are involved to provide sequence specificity and/or enzymatic activities

members in flowering plants, the majority of which are targeted to the organelles [132, 133]. Moreover, plants exclusively also encode specific PPR proteins labelled PLS type, not present in other eukaryotes, which are characterized by large (L) and small (S) variants of the 35 amino acid PPR (P) motif [134–136]. Yet more importantly, PLS proteins may carry carboxyterminal domain extensions named the E, E+, and DYW domains (Fig. 4). The DYW domain in particular (so labelled due to the highly conserved terminal aspartate-tyrosine-tryptophan tripeptide at the protein end) has been suggested to play a particularly important role in RNA editing given its weak similarity to cytidine deaminases and, equally importantly, due to its exclusive presence in plant taxa that show RNA editing versus its absence in those where editing appears to be absent [137, 138]. Moreover, there is evidence for a correlation in the number of organellar editing sites and the diversity of the DYW subfamily of PPR proteins in a given taxon [109, 135, 138]. Indeed, several DYW-type PPR proteins were subsequently identified to be specifically responsible for RNA editing events both in chloroplasts and in mitochondria [139–150]. On the other hand, some others, including the initially discovered CRR4, are PLS proteins lacking the DYW-domain extension and ending with the E or E+ domain only [131, 151–154]. These cases certainly shed doubt on the idea of the DYW domain directly providing de- (or *trans*-)amination functionality. Further functional studies have similarly provided an as-yet-inconclusive picture. In one case, a DYW domain has been shown to have endonucleolytic rather that cytidine deamination activity [155], but in this study His_6 tags had been added to the highly conserved DYW protein ends, which may have interfered with its proper function. In another study, DYW domains were shown to be dispensable for editing [146]. However, it is noteworthy that the genome of the moss *Physcomitrella patens* showing RNA editing (albeit at low amounts) encodes only DYW-type but no E or E+ type PPR proteins [109, 135, 156].

Several of the identified editing factors target multiple editing sites. The recently described mitochondrial editing factor 11 (MEF11) mutant is an example where a single amino acid exchange (L48F) in the second of its 14 PPR motifs abolishes editing at sites cox3eU422PL, nad4eU124LL, and ccb203eU344PL [for nomenclature see 108, 149]. Interestingly, a second mutant allele of the protein in which the terminal 25 amino acids are replaced by 11 amino acids of a T-DNA insertion has a slightly different molecular phenotype as this alteration abolished editing of the former two sites but showed 60% residual activity at ccb203eU344PL [157]. One possible model accounting for the observations made thus far is that more than one PPR protein may act simultaneously on certain RNA editing sites (Fig. 4), which may also explain the divergent and extended sequence requirements for recognition of some editing sites. There is evidence that PPR proteins bind to RNA on a one-repeat-per-nucleotide basis, but the code for recognition is not yet identified [158, 159]. It is interesting to note that such a sequence recognition code has recently been deciphered for a very similar case of a protein repeat motif and nucleic acid binding. The phytopathogenic bacterial transcription activation-like (TAL)-III effectors active in plant cells carry highly conserved tandem 34 amino acid motif repeats and a variable dipeptide motif within each of these individually recognizes DNA base pairs [160, 161].

Some publications in recent years have occasionally pointed to potentially regulatory roles of RNA editing in plant organelles [e.g., 162,163] or have reported on a variation of RNA editing in response to environmental changes [e.g., 164]. As with the case of the lavish kinetoplastid editing, the question of cause and effect arises and the aspect of correcting genetic information appears overwhelming in any case.

1991: The myxomycete case—many types of RNA editing coexisting in mitochondria

RNA editing in the plasmodial slime mould (myxomycete) *Physarum polycephalum* was originally discovered in the form of numerous cytidines inserted into the mitochondrial *atp*1 pre-mRNA encoding subunit 1 of the ATPase [165]. Not only mRNAs but also rRNAs [166] and tRNAs [167, 168] were subsequently identified as substrates for RNA editing. Like kinetoplastid mitochondria and plant organelles, myxomycete mitochondria proved to be similarly vast in the extent of RNA editing in the transcriptome. Yet more impressive, there appear to

be four different and independent types of RNA editing co-existing in myxomycetes [169, 170]. A single mRNA such as the one encoding *cox*1 is affected by numerous C insertions (59) similar to the originally discovered *atp*1 editing, but additionally also by one U insertion, three mixed dinucleotide insertions, and four C-to-U conversions [171]. Additionally a single A insertion has recently been identified in the related myxomycete *Didymium iridis* [172]. The different processes can be separated not only phylogenetically according to their occurrence in different myxomycete taxa but also functionally [173, 174]. Most interestingly, guide RNA-like molecules responsible for the kinetoplastid type of insertional editing seem to be absent, and in vitro studies clearly show that the insertional type of editing occurs cotranscriptionally [173, 175–177]. This makes the myxomycete insertional type of editing the only non-viral cotranscriptional RNA editing process hitherto identified, but interestingly not relying on RNA polymerase stuttering on runs of identical nucleotides. The mitochondrial RNA polymerase has been cloned and shown to indeed add nonencoded nucleotides to the 3′ end of nascent RNAs [178, 179]. Investigating the mechanisms that guide and control the cotranscriptional insertions of nucleotides has revealed that approximately 9 bp flanking the insertion sides on both sides of the DNA template play important roles [180–184]. How many further biochemically distinct and independent activities of RNA editing (e.g., of a deamination type for the C-to-U conversions) actually exist in myxomycetes is currently an open question addressed with different in vitro systems [185]. From a phylogenetic perspective, it is interesting to see that a dynamic evolution of editing sites among myxomycetes points to a gain in editing activity with the diversification of this clade. Exactly the opposite is true for editing among the kinetoplastids where editing is more pronounced in ancient lineages [169, 170, 186].

1991: The second metazoan case—A-to-I editing

The discovery of A-to-I editing in mRNAs of nuclear genes in metazoa was first documented with the description of apparent CAG (glutamine) to CGG (arginine) codon changes in glutamate receptors (i.e., glutamate-gated ion channels) of mice [187]. Subsequently, it was found that the adenosines are in fact not exchanged for guanosines in the mature RNAs. Instead, a simple deamination of the adenine base to hypoxanthine results in the corresponding (nonstandard) inosine (I) nucleotide, which is read as G upon translation. In fact, the process of adenosine-to-inosine conversion had actually been discovered before as an activity of unwinding RNA duplexes, and the A-to-I base change had at that time been referred to as a modification rather than editing [188]. The recognition of sites for A-to-I RNA editing relies on intramolecular base pairing in RNA secondary structures of the respective target transcripts. The biochemical reaction of A-to-I conversion is carried out by the so-called ADAR enzymes, the "adenosine deaminases acting on RNA" present in all metazoa. Possibly, the ADAR enzymes have evolved from the ADATs, the "adenosine deaminases acting on tRNA" [189], which will be discussed below. Whereas a single ADAR gene is encoded in the fruit fly *Drosophila melanogaster*, three different ADARs are present in mammalian genomes [190]. The different ADARs of mammals obviously have different RNA targets and a knockout of a single ADAR is incompatible with life in the mouse model [191].

Somewhat in contrast to the C-to-U RNA editing of the *apo*B type, A-to-I editing was found to take place in numerous transcripts in the nucleocytosolic genetic systems of animals, in fact possibly affecting literally thousands of sites in transcriptomes [192–195]. A-to-I editing also appears to have a wider phylogenetic distribution in the animal kingdom, and this opened up a yet wider array of experimentally amenable model organisms in which to investigate this RNA editing system, e.g., *Drosophila* [196]. The initial discovery of glutamate channel A-to-I editing was seminal for many more editing events identified subsequently, which similarly affect mRNAs encoding neurologically important membrane channels and receptors. Hence, the investigation of A-to-I editing has in fact become an important component of neurobiological studies on nervous functions and psychological disorders [197–202]. Strikingly, some of these editing sites are conserved across very wide phylogenetic distances, e.g., ranging from insect to squid in the case of a potassium channel [203]. Among the notable other targets of the ADAR-based A-to-I editing machinery are the transcripts of the primate-specific Alu repeats [204] and genes of the immune system [205], as well as viral RNAs, e.g., of HDV or HIV [206, 207]. Even micro RNAs (miRNAs) may be edited, hence suggesting a "crosstalk between editors and silencers" for gene regulation via the RNA interference machinery [208]. Hence, the A-to-I type of RNA editing affecting a wide spectrum of RNAs in metazoa is not only the one showing the most obvious signs of massive regulatory influence on gene activities but at the same time also the one with the most immediate impact on human life and health. As a very interesting addendum in the light of the multifarious roles of RNA editing in modulation of neuronal channel activities, it was recently shown that adenosine-to-inosine editing is strongly increased in humans versus nonhuman primates [209].

1993: The case of mitochondrial 5′ tRNA editing—*Acanthamoeba* and chytridiomycete fungi

Acanthamoeba castellanii is an amoeboid protozoon. The initial discovery of RNA editing in this protist reported U-to-A, U-to-G, and A-to-G exchanges in one or more of the first three nucleotides in the 5′ half of the acceptor stems of mitochondrial tRNAs (Fig. 5), all of which restored canonical base pairings [210]. It was shown that 13 out of a total of 16 tRNAs encoded in the complete mtDNA of *Acanthamoeba castellanii* require a total of 23 RNA editing events of this type to reconstitute proper base pairing in tRNAs [211, 212]. As expected, these nucleotide exchanges cannot be accomplished by simple biochemical transformation of the bases but require nucleotide replacement and indeed such a novel biochemical activity for nucleotide incorporation (operating 3′–5′ instead of the canonical 5′–3′ direction) could be identified [213]. Very similar to the *Acanthamoeba* case, RNA editing of this type was also identified in mitochondrial tRNAs of the chytridiomycete fungus *Spizellomyces punctatus* [214]. Congruently, the editing events were identified among the first three nucleotides of the 5′ half of the mitochondrial tRNA acceptor stems. Chytridiomycetes represent the most ancient lineages of the fungal kingdom predating the split of the evolutionary younger clades ascomycetes, basidiomycetes, and zygomycetes. Subsequently, the 5′-acceptor stem type of tRNA editing was also discovered in *Hyaloraphidium curvatum* [215], a previously enigmatic taxon believed to be a colorless alga but now clearly placed among the chytridiomycetes [216]. The biochemical activities of tRNA editing in *Spizellomyces punctatus* have recently been characterized using an in vitro system and proved to be remarkably similar to the *Acanthamoeba* system [217]. The phylogenetic distribution of the acceptor stem 5′ nucleotide exchange type of tRNA editing may suggest this to be an ancient mechanism, possibly to be identified in many more protist and basal multicellular eukaryote lineages although independent origins of the tRNA editing activity can equally well be considered [218].

1993: The marsupial case—editing the anticodon in a mitochondrial tRNA

When the mtDNA sequence of several marsupials (Metatheria) was analyzed, it became clear that the apparent mitochondrial *trn*D gene encoding the tRNA for aspartate carried a GCC, instead of the expected GUC anticodon, which would decode GGY glycine instead of GAY aspartate codons. Sequencing the corresponding cDNAs revealed that about 50% of the tRNAs carry the appropriate GUC anticodon sequence, obviously introduced by a C-to-U

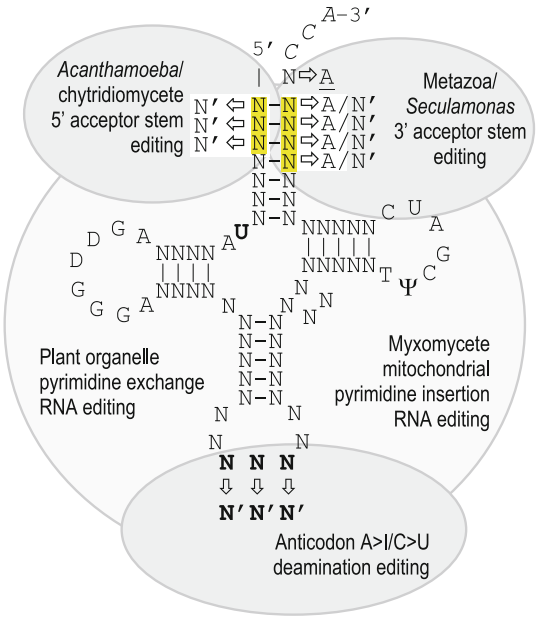

Fig. 5 In the different domains of life, several different types of RNA editing act on tRNA molecules, here shown in the general tRNA consensus structure featuring the acceptor stem (*top*), the dihydrouridine (*D*) arm (*left*), the anticodon arm (*bottom*), the pseudouridine (*Ψ*) arm (*right*), and the size-variable arm between the latter two. Pseudouridine and dihydrouridine are only two examples of the more than 100 different types of nucleotide modifications, besides frequent base methylations, described for tRNAs (and rRNAs); many of these also occur in the tRNA acceptor stems [264]. The deamination type of C-to-U and A-to-I nucleotide conversions obscures the boundary in the definition of modification versus editing. Anticodon positions in the tRNA consensus are 34, 35, and 36; the 3′ terminal (discriminator) nucleotide is number 73 to which the CCA (*italics*) end for subsequent aminoacylation is normally added after tRNA 3′-processing. Main size variations are introduced by the size-variable arm and occasional additional nucleotides in the dihydrouridine arm. The nucleotide replacement type of editing in the 5′ half of the acceptor stem has been identified in the protist *Acanthamoeba castellanii* and in chytridiomycete fungi. RNA editing of the A-to-I deamination type in the anticodon (*bold*) altering codon recognition appears common; the C-to-U deamination type of editing was initially observed in marsupials. Cytidine-to-uridine base exchanges of the deamination type may affect many different tRNA positions in plant organelles, the most prominent example being 18 C-to-U exchanges in the mitochondrial tRNA-Pro in the lycophyte *Isoetes engelmannii* [88]. Similarly, the cytidine or uridine insertion type of editing in myxomycetes can affect all regions of a tRNA molecule. Correction of acceptor stem base pairing in the 3′ half of the stem seems to involve different biochemical activities in different organisms, including oligo-adenyl-transferase, terminal CCA-transferase, or RNA-directed RNA polymerase. Editing of cytidine to the universally conserved uridine nucleotide in position 8 (*bold underlined*) has recently been shown for the majority of tRNAs in the archaeon *Methanopyrus kandleri*

editing event [219]. Interestingly, the unedited version of tRNA-Asp was proven to be charged with glycine to read, as may be expected, GGY glycine codons [220]. Hence, one mitochondrial tRNA gene serves to produce two differently aminoacylated tRNA species. Strikingly, a second *trn*G

gene exists in the marsupial mtDNAs with a UCC anticodon potentially reading all GGN codons. This tRNA species, however, is restricted to decode only GGR codons due to a C-to-U mutation two nucleotides upstream of the anticodon (position 32 of the tRNA consensus structure, see Fig. 5). The example of the marsupial tRNA anticodon editing was hence taken as an example to explain evolutionary fixation of RNA editing [221].

1995: The cases of mitochondrial tRNA acceptor stem 3′ editing in metazoa and a protist

Yet a different type of tRNA editing in metazoan mitochondria was discovered after base-pairing mismatches in the acceptor stems and a substitution of the adenosine discriminator nucleotide at the tRNA 3′-end (see Fig. 5) were observed in several tRNA genes of land snail mitochondria [222]. As shown by cDNA analysis of tRNAs for glycine (G), tyrosine (Y), and lysine (K) in the Japanese land snail *Euhadra herklotsi*, these mismatches turned out to be corrected by base exchanges among the five terminal acceptor stem bases and the discriminator nucleotide on the 3′ side of the acceptor stem [223]. All nucleotide exchanges were conversions of C, G, or U into adenosines to reconstitute U–A base pairs with the single exception of an A–C mismatch converted into an A–A mismatch in tRNA-Tyr (Fig. 3). The process seems to be the outcome of the densely packed mitochondrial genomes, which result in overlaps with the respective downstream genes (4 bp in *trn*G, 6 bp in *trn*K). Obviously, very similar or even identical mechanisms seemed to operate in the squid *Loligo blekeri*, where the terminal guanosine nucleotides of tRNA-Tyr are replaced by adenosines during maturation [224]. Again this phenomenon is obviously a result of gene overlap, in this case of the terminal 2 bp of *trn*Y with the downstream *trn*C gene. The 5′ processing of tRNA-Cys leaves the two terminal nucleotides lacking in tRNA-Tyr. The same observation was made in chicken mitochondria (*Gallus gallus*) although the *trn*Y-*trn*C overlap is only 1 bp [225]. The observations suggested a possibly rather unspecific terminal adenylyltransferase (poly-A-polymerase) activity introducing the adenosines after a 3′–5′ exonuclease activity had removed the mismatched bases as the underlying mechanism [226]. The case of 3′ acceptor stem tRNA editing in the platypus *Ornithorhynchus anatinus* (Monotremata) appeared to be slightly different, given that not only adenosines but also cytidines replaced mismatched bases [227], possibly suggesting that the CCA-adding terminal nucleotidyl transferase activity (the "CCAse") normally producing the CCA acceptor ends of tRNAs is involved.

A much more extensive editing process correcting tRNA ends was observed in mitochondria of the centipede *Lithobius forficatus*, where a full 21 of 22 mtDNA-encoded tRNAs need correction at their 3′ ends. Confirmation of the expected changes at the cDNA level included the introduction of all four RNA nucleotides [228] and suggested that this process relies on an RNA-dependent RNA polymerase activity using the 5′ acceptor stem as a template. Finally, a process of 3′ tRNA editing similar to the one in *Lithobius*, although not as extensive, was described in the jakobid protist *Seculamonas ecuadoriensis* [229]. A total of seven nucleotides in the 3′ acceptor stem half of two tRNAs (for glutamate and serine) are affected, mostly introducing A or C but partially also U and G. Whether the *Seculamonas* editing more closely resembles the one in *Lithobius* or the ones in the other metazoa including the platypus is unclear at present. The wide phylogenetic distance between the jakobid protist and the metazoa suggests convergent evolution in any case.

1999: The case of A-to-I tRNA anticodon editing—kinetoplastids linking the purine and pyrimidine deamination types of RNA editing?

All mitochondrial tRNAs are imported from the cytosol in kinetoplastid mitochondria. The standard tRNA for tryptophan carries a CCA anticodon to decode the UGG tryptophan codon, but in kinetoplastid mitochondria the standard UGA stop codon exceptionally also encodes tryptophan and a corresponding tRNA is missing. It turns out that the imported tRNA for tryptophan is specifically altered in the wobble anticodon position 34 (Fig. 5) in kinetoplastid mitochondria to yield a UCA anticodon able to decipher both tryptophan UGR codons [230]. Hence, a second RNA editing activity (most likely cytidine deamination similar to the plant organelle or marsupial case) exists besides the frequent U insertion and deletion type of editing in kinetoplastid mitochondria [231]. Kinetoplastids also turned out to reveal the first event of C-to-U RNA editing in a nonmitochondrial, nucleocytoplasmic genetic environment. The tRNA for threonine shows C-to-U editing in position 32 of the anticodon arm, 2 nt. upstream of the anticodon (Fig. 5). This editing event is not necessary but is stimulating for a second deamination type of editing event taking place in the same tRNA, an A-to-I editing event in the wobble position 34 [232]. Interestingly, the C-to-U event appears to be a nuclear editing event whereas A-to-I editing takes place in the cytosol [233].

Adenosine deaminations introducing inosine in the first anticodon position of tRNAs have been known for a long time in many organisms as an essential aspect of the wobbling concept (increasing freedom for third-codon position recognition). The A-to-I base conversion has largely been considered as one of the many chemical base modifications

occurring in tRNAs without labeling the phenomenon as RNA editing (Fig. 1). This has changed with the increased awareness of the other phenomena of RNA editing affecting tRNAs and notably also of the A-to-I editing in nuclear mRNAs of metazoa. Consequently, the recently cloned plant chloroplast adenosine deaminase acting on tRNAs (ADAT) and the *Escherichia coli* equivalent identified earlier are now considered editing enzymes [234–236]. In bacteria and plant chloroplasts the enzyme converts only the ACG anticodon of the tRNA for arginine into ICG, whereas more such conversions exist in tRNAs of the nucleocytoplasmic genetic systems of eukaryotes.

2002: The dinoflagellate cases—weird editing in weird mitochondrial and chloroplast DNAs

Yet another nucleotide exchange type of mitochondrial RNA editing that features diverse co-existing nucleotide changes was initially discovered in the dinoflagellate species *Pfiesteria piscicida*, *Prorocentrum minimum*, and *Crypthecodinium cohnii* [237]. Not only pyrimidine transitions, similar to the plant organelle case, but also purine transitions in both directions of exchange and the transversions of guanosine to cytidine as well as singular examples for conversions of uridine into the purine nucleotides were observed. RNA editing of the transcripts of genes encoded in the dinoflagellate minicircles (representing chloroplast DNA) was demonstrated shortly thereafter in *Cerratium horridum* [238]. The DNA minicircles normally carrying only one gene each, which only in their entity represent a chloroplast genome equivalent, are a striking peculiarity of dinoflagellates [239]. RNA editing in the *C. horridum* chloroplast mRNAs and the small (16S) chloroplast rRNA was, as in the mitochondrial transcripts, shown to include many pyrimidine (C-to-U) exchanges in both direction, i.e., analogous to plant organelles. Even more purine exchanges were found in both directions and additionally, the transversions of both purine nucleotides into cytidines and, in the case of the 16S rRNA, of a uridine into a guanosine. As expected, the many types of editing were also identified in other dinoflagellates [237, 240–242] but interestingly not in *Oxyrrhis marina* representing a very basal lineage [243]. The transition types of editing (A-to-G, C-to-U) dominate in both organelles, possibly accompanied by a superset of U-to-R and R-to-C transversions (Table 1). Hence, very similar to the observations in land plants, congruent RNA editing processes seem to operate in both endosymbiotic organelles in parallel. It is obvious to speculate that the (many) transition types of RNA editing may be introduced by base conversions through de- or *trans*-amination processes similar to other eukaryotes and that the rarer transversions are introduced through processes rebuilding phosphodiester bonds similar to the kinetoplastid or *Acanthamoeba*/chytridiomycete cases. However, experimental data are lacking so far [242].

2009: The placozoa case—a U-to-C pyrimidine exchange in a basal metazoan

The placozoa (genus *Trichoplax*) are currently understood to represent one of the most basal lineages in the phylogeny of metazoa, if not in fact representing the sister lineage to all other animals. The mitochondrial DNA of *Trichoplax adhaerens* featuring a complex *cox*1 gene structure and *cox*1 mRNA maturation was deciphered only recently. The *Trichoplax cox*1 gene turned out to possess a *trans*-splicing group I intron [244], interestingly only the second known example of such an intron, with the other just discovered in parallel in the mtDNA of the lycophyte *Isoetes engelmannii* [88]. Furthermore, an editing event converts a genomically encoded tyrosine UAU codon into an evolutionarily conserved CAU histidine codon in the cox1 mRNA. This is a very striking observation, simultaneously as a first editing event in an animal mitochondrial mRNA and a first event of U-to-C exchange in metazoan mitochondria. It will be highly interesting to see whether RNA editing events of C-to-U (similar to the metazoan mitochondrial tRNA editing) and U-to-C (similar to the one now identified in *Trichoplax*) exist in other basal metazoan lineages.

2009: The archaeal case—true RNA editing in prokaryotes

As outlined above, the line between RNA editing and RNA modification may be hard to draw. The deamination of adenine to hypoxanthine in nucleotides leading to the corresponding conversion of adenosines to inosine is a case in point. Technically a modification given that a nonstandard nucleotide is created, the A-to-I conversions today qualify as RNA editing although the base modification process has long been known to operate in tRNAs also of bacteria including *Escherichia coli*. This semantic transformation is certainly associated with the many A-to-I conversions identified in metazoan nuclear genetic systems affecting mRNAs that more clearly qualify as RNA editing. A novel case of tRNA editing in the stricter sense was described recently in Archaea [245]: A universally conserved uridine in position 8 of tRNAs between the acceptor and the dihydrouridine stems is a hallmark of the tRNA consensus structure but is lacking in 30 out of 34 tRNAs in the archaeon *Methanopyrus kandleri*, where a cytidine is found in this position in the corresponding genes (Fig. 5). The cytidines were found to be converted to uridines and the simple genetic system allowed for straightforward

identification of the responsible enzyme named CDAT8 for cytidine deaminase type 8. This enzyme essentially only needs the acceptor stem of tRNAs for recognition and biochemical transformation of its target.

Further cases with less clear status

Some further phenomena of RNA editing in addition to those discussed above have been reported in the literature but not confirmed or followed up with subsequent publications. These include an enigmatic U-to-A conversion in a human α-galactosidase mRNA [246], a C-to-U transition in the small subunit mitochondrial rRNA of the cellular slime mold (mycetozoa) *Dictyostelium discoideum* [247], and a guanosine introduced (or converted) at the beginning of a poly-A-tail in the 3′-UTR of a cytochrome b5 gene in the fungus *Mortierella alpina* [248]. Similarly, a unique U-to-C editing has been reported in the mRNA of the Wilms' tumor susceptibility gene WT1 [249]. However, this apparent U-to-C transition could only be reproduced at very low levels in different samples and using different techniques [250] and had previously also not been identified in 15 independent tumor samples [251]. Possibly, the editing of interleukin IL-12 introducing an alanine-to-valine codon change is a similar case [252] given that it could not be re-identified in a subsequent study [253]. In other cases, claims for RNA editing phenomena have been explicitly refuted with subsequent work, e.g., for a selenocysteine tRNA [254] or the tRNA-Asp in rats [255].

Summary

Speaking in analogies one could state that RNA editing may be for transcriptomes what epigenetic mechanisms are for genomes. The vastly expanding field of epigenetics currently delivers an ever increasing understanding of the added layer of complexity between the genes and their expression, introduced via biochemical modification of DNA by cytidine methylation of target motifs and of DNA-binding histones, largely by acylation or alkylation (dominantly methylation) but also other modifications.

Shortly after the seminal discovery of RNA editing by Benne and colleagues [1], RNA editing made it to the cover of the March 1988 issue of *Cell*, when the overwhelming pan-editing of the *cox*3 cryptogene in *Trypanosoma brucei*, creating more than half of the open reading frame in the matured transcript, was reported [3]. Many other processes of RNA editing have been discovered over the more than 20 years since then (Table 1), and it is more than likely that even those that are mechanistically analogous have arisen independently in evolution, given their disjunct occurrence over the broad spectrum of genetic systems that have evolved over 3.5 billion years on earth—ranging from archaea to plants and including viruses, animals, and fungi. Explanations as to why RNA editing has come into being may appear evident for some genetic systems due to the obvious regulatory effect as, e.g., the C-to-U and A-to-I editing in animals, but remain largely elusive for others where literally thousands of RNA editing events mainly serve to re-establish proper reading frames and evolutionarily conserved functions such as the trypanosome or plant mitochondrial types of RNA editing.

Evolutionary thinking is misunderstood by many to address the "why" questions in biology, hoping for answers pointing out functional gains or apparent advantages. Concepts of neutral evolution with a less prejudiced look into things, not necessarily asking for meaning or regulation, are frequently overlooked. Rare articles such as one recently published on the two evolutionarily separate protist groups showing heavy RNA editing and pointing out quite some convergent evolution are highly recommended in this respect [256]. Several interesting speculations about the evolution of RNA editing have been proposed and discussed previously. Some of these contributions have focused on particular editing systems [257–259], whereas some have explicitly highlighted general concepts of neutral evolution [260–262]. It will be very interesting to see whether a universal model on the evolution of RNA editing will explain not only the many disparate systems of editing already known but also those yet to be discovered. The extra efforts for a genetic system to assemble elaborate editosomes and the many factors providing specificity very obviously outweigh the gain in regulatory potential that could have been achieved by other means much more easily. Francois Jacob nicely pointed out long before the discovery of RNA editing [263] that evolution is more of a tinkerer than an engineer (or a designer, for that matter, one may add).

Acknowledgments I apologize that, owing to space constraints, numerous other important publications elucidating the many facets of RNA editing in all the disparate genetic environments could not be cited. I am grateful for financial support from the Deutsche Forschungsgemeinschaft and to Julia Hecht, Teresa Knoop, Mareike Rüdinger, and Felix Grewe for comments prior to initial submission. I wish to thank two anonymous reviewers for several very valuable comments to improve the manuscript.

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