Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
RNA Editing and the Mitochondrial Cryptogenes of Kinetoplastid Protozoa

Larry Simpson* and Janet Shaw*
*Department of Ringly
University of California
Los Angeles, California 90024
†Molecular Biology Institute
University of California
Los Angeles, California 90024

The term "RNA editing" was initially introduced to describe a process resulting in the addition of nongenomically encoded uridine residues (Us) to mitochondrial rRNAs in the kinetoplastid protozoa (Benne et al., 1986), but has recently been used to describe other processes involving modification of RNA in other organisms (Powell et al., 1987; Chen et al., 1987; Thomas et al., 1988). We would like to define RNA editing in a broad sense as any process that results in the production of an RNA molecule which differs in nucleotide sequence in coding regions from the DNA template, provided that the difference is not the result of the removal of introns by a classic splicing mechanism. In this review we will examine RNA editing mainly in kinetoplastids but also in other systems and will critically evaluate the central issues and concepts related to this phenomenon.

The discovery of RNA editing was the direct result of a series of investigations on the genomic organization and transcription of the unusual mitochondrial genome of the kinetoplastid protozoa. Since the initial report, several other examples of kinetoplastid RNA editing have been discovered (Foagin et al., 1987; Foagin et al., 1988a; Foagin et al., 1988b; van der Spek et al., 1988a; Abraham et al., 1988). To provide a biological perspective on this phenomenon, we will first review selected aspects of the molecular biology of kinetoplastid protozoa.

The Mitochondrial DNA of Kinetoplastids Is Present as a Nucleoid Body and Consists of a Single Catenated Network of Minicircles and Maxicircles

Kinetoplastid protozoa, which are also known as trypanosomatids or trypanosomes, consist of eight genera belonging to a family of lower eukaryotic cells (Trypanosomatidae) distinguished by the presence of a single, structurally complex mitochondrion containing an unusual genome known as kinetoplast DNA (kDNA) (see Simpson, 1972, Simpson, 1986, and Simpson, 1987, for reviews). These protozoa are parasitic either in a single invertebrate host (monogenetic) or in both an invertebrate and a vertebrate host (digenetic). An analysis of nuclear small rRNA sequences led to the conclusion that these cells represent a deep branch of the eukaryotic line (Sogin et al., 1989).

The kDNA nucleoid body is found in the mitochondrial matrix at the base of the flagellum and consists of two types of circular DNA molecules, minicircles and maxicircles. The genetic role, if any, of the minicircle DNA is still enigmatic; although small minicircle transcripts have been identified in both T. brucei (Rohrer et al., 1987) and L. tarentolae (Simpson and Simpson, unpublished results).

The Kinetoplastid Mitochondrial Maxicircle Genes

The maxicircle DNA molecules encode an incomplete set of standard mitochondrial genes (Simpson, 1987). This sequence of 21 kb of the 30 kb maxicircle molecule of L. tarentolae is known, as is the sequence of almost the entire 22 kb maxicircle molecule of T. brucei and approximately 6 kb of the 32 kb maxicircle of Crithidia fasciculata. The identified genes of L. tarentolae encode the two small mitochondrial rRNAs and components of the respiratory chain, including cytochrome b (Cyb), cytochrome oxidase subunits I, II, and III (COI, COII, and COIII), and NADH dehydrogenase subunits 1, 4, and 5 (ND1, ND4, and ND5) (Figure 1). In addition, there are also four unidentified open reading frames (MUPF1-4) (MUPF = maxicircle unidentified reading frame, Simpson et al., 1987), one of which (MIRF3) has sequence homology with ORF99 from Marchantia polymorpha chloroplast DNA (Ohyama et al., 1986). In comparison, the mitochondrial genomes of mammals and yeast both encode two mitochondrial rRNAs, the structural genes for COI, COII, COIII, and Cyb, and at least two subunits of ATP synthetase (6 and 8). Genes for seven subunits of NADH dehydrogenase (ND) are in addition present in the human mitochondrial genome, whereas genes for a small mitochondrial ribosomal subunit protein (Var-1) and a third subunit of ATP synthetase (9) are present in the yeast mitochondrial genome.

A large portion of the maxicircle (13 kb in L. tarentolae and 7 kb in T. brucei) is apparently noncoding, and consists of tandem repeats of varying complexities (Muhich et al., 1985). Since this region varies in size and sequence between species, it has been termed the variable or divergent region; a lack of open reading frames, rapid changes in sequence between species, and a low abundance of steady-state transcripts (de Vries et al., 1988; Tarazona et al., 1987) make it difficult to assign a genetic function to this portion of the maxicircle.

The maxicircle genes appear normal in terms of conserved functional amino acids, conserved hydropathy patterns, and statistically significant alignments, but several unusual features are apparent: there is an extremely high abundance of cysteine residues in the predicted products, several genes lack ATG initiation codons, three genes (COII, MURF2, and MURF3) encode internal reading frameshifts, and several normally conserved genes such as subunits 6, 8, and 9 of ATP synthetase are apparently absent (de la Cruz et al., 1984; Simpson et al., 1987).

In addition, no mitochondrial rRNA genes have been identified by hybridization studies in either minicircle DNA or maxicircle DNA (Hoeijmakers et al., 1981; Benne et al., 1983; Benne and Slooff, 1987; Suyama, Campbell, Simpson, and Simpson, unpublished data), although an appa-
A complete set of functional tRNAs exists in the kinetoplastid mitochondrion (Suyama et al., unpublished data). The mitochondrial genomes of mammals and yeast, on the other hand, encode a complete degenerate set of 23–24 tRNAs (Barrell et al., 1980; Bonitz et al., 1980), but another protozoan, Tetrahymena, is thought to encode only 8 tRNAs, with the remainder apparently imported into the organelle (Suyama, 1986; Suyama and Jenney, 1989).

Figure 1. Comparative Maxicircle Genomic Maps—Localization of Edited Regions
Conserved homologous genes from L. tarentolae, C. fasciculata, and T. brucei are indicated by cross-hatching. C-rich intergenic regions are shown by stippling. Genes with no (pre-edited) sequence similarity are indicated by open boxes. Pre-edited regions of cryptogenes are indicated by horizontal black boxes or vertical dark lines, and the number of additions, deletions, and affected sites (in parentheses) is shown for each transcript. A–D, different adjacent regions of the maxicircle genomes.

Figure 2. Dragon Dot Matrix of a Comparison of Portions of the L. tarentolae and T. brucei Maxicircle Genomes
A window of 31 nucleotides with a "proportional" match criterion of 21/31 (Staden, 1982) was used. Conserved homologous genes that produce diagonal lines are connected by lines to the axes for ease of visualization. Reprinted from Simpson et al., 1987, with permission.
Comparison of Mitochondrial Genomes of Three Kinetoplastid Species

A comparison of the informational maxicircle DNA sequences from L. tarentolae and T. brucei (Simpson et al., 1987) and a partial sequence from C. fasciculata (Sloof et al., 1987) revealed that the T. brucei maxicircle lacks sequences homologous to the COIII, MURF3, and ORF12 (=MURF4) genes (Figures 1 and 2). In place of these genes, shorter GC-rich sequences are substituted (Figure 3). All other maxicircle genes in T. brucei and L. tarentolae are present at equivalent relative locations and with identical polarities. The C. fasciculata sequence clearly belongs to the L. tarentolae class, in that homologous genes for MURFB and COIII are found immediately upstream of the CYb gene. The other significant observation was that the internal frameshifts present in the COII and MURF3 genes are conserved in the homologous C. fasciculata (Benne et al., 1986; van der Spek et al., 1988) and T. brucei (Payne et al., 1985) gene sequences and therefore are not likely to be due to DNA sequencing errors.

Discovery of RNA Editing

The presence of a conserved frameshift in the COII genes from all three species was intriguing and prompted the investigation of the COII transcripts. It was found that there were four extra uridine nucleotides at this position in the mRNA sequence (Benne et al., 1986) which eliminated the 1 frameshift encoded by the gene. Hybridization studies indicated that a DNA template encoding the modified sequence was not present in the mitochondrial or nuclear genomes of these organisms (Benne et al., 1986). Soon thereafter, additional examples of RNA editing were reported, including 34 added U's in the 5' end of the CYb mRNA of T. brucei (Feagin et al., 1987), 39 added U's in the CYb mRNAs of L. tarentolae and C. fasciculata (Feagin et al., 1988a), and multiple added U's in mRNAs for three other maxicircle genes of all three species (Feagin and Stuart, 1988; Shaw et al., 1988; van der Spek et al., 1988). The deletion of genome-encoded U's was also found to occur in several mRNAs (Shaw et al., 1988). The importance of comparative studies was underscored by the observation that the new methionine initiation codons and the N-terminal amino acid sequences created by this process were almost completely conserved in the three species, which are thought to have been separated by at least 100 million years (Lake et al., 1988).

A dramatic confirmation of the existence of this phenomenon was provided by the discovery that the missing COIII gene in T. brucei was actually present in an abbreviated, encrypted form and that RNA editing acted as a decoding device to expand the DNA sequence into a translatable mRNA (Feagin et al., 1988a). In this case, a transcript encoding the COIII protein was identified in T. brucei, although a DNA sequence that exactly matched...
Figure 4. Summary of All Reported Kinetoplastid Edited Sequences

Added Us are indicated as - T's deleted from the DNA sequence as -- , and gaps introduced for the purpose of aligning sequences from different species as ---. For the T brucei COII DNA sequence, deletions of Ts are indicated by open arrowheads. +, the L. arenaleae MURF3 edited region is identical. ++, the L. tarentolae COII edited region is identical (Shaw et al., unpublished data). References: 1. Benne et al., 1986. 2. van der Spek et al., 1968. 3. Feagin et al., 1987. Feagin et al., 1988a. 4. Shaw et al., 1988. 5. Shaw et al., 1988; van der Spek et al., 1988. 6. Shaw et al., 1988. 7. Feagin et al., 1988b.
this RNA could not be found. However, the COII transcript sequence could be aligned with a region of the maxicircle genome immediately upstream of the CYb gene, provided that multiple U's were added to and deleted from the RNA: in the 712 nucleotide sequence obtained thus far, 308 U's are added at 158 sites and 19 U's are deleted at 9 sites. The relative location of the DNA sequence in the genome was identical to that of the COII gene sequence in L. tarentolae and C. fasciculata. This was interpreted as an example of massive RNA editing in which more than 50% of the nucleotides in the edited mRNA were contributed by this process, resulting in an RNA sequence that had no obvious similarity with the DNA sequence.

Summary of Editing Events in Kinetoplastids

In the interest of clarity, we would like to introduce some new terminology which is generally applicable to discussions of RNA editing. First, we shall call the incomplete genes whose transcripts are edited to yield translatable sequences, "cryptogenes." The region of the cryptogene or the putative primary, unedited RNA that can be aligned (via the addition and deletion of U or T residues) with its corresponding edited sequence will be referred to as a "pre-edited" sequence. At least three major types of kinetoplastid cryptogenes have been identified, based on the pattern of editing events found in the transcripts:

Type I: internal-edited cryptogenes
Type II: 5' edited cryptogenes
Type III: pan-edited cryptogenes

Type I cryptogenes (e.g., COII in all three species) yield RNA molecules that are edited at internal positions within protein coding regions (Figure 4-1). Type II cryptogenes (e.g., CYb in all three species) yield transcripts that are edited at the 5' ends within protein coding regions (Figure 4-3). Some genes have both Type I and Type II cryptogene properties, as, for example, the C. fasciculata and L. tarentolae MURF2 genes (Figure 4-5). Finally, Type III cryptogenes (e.g., T. brucei COII) yield transcripts that are extensively edited (pan-edited) over their entire length to generate RNA nucleotide sequences that encode completely new proteins (Figure 4-7). The use of this terminology is not meant to imply that these divisions represent three distinct types of editing. In fact, as new examples of RNA editing are described, we may find a continuum of cryptogene types. All edited and pre-edited regions described to date are presented in Figure 4 and the equivalent maxicircle genomic locations are shown in Figure 1. CYb, MURF2, COII, and COII transcripts are edited in all three kinetoplastid species, and MURF3 transcripts are edited in two species. It should be noted that the T. brucei MURF3 and MURF4 genes appear on the basis of preliminary RNA sequences (Feagin et al., 1986a, Feagin and Stuart, personal communication) to represent Type III pan-edited cryptogenes also.

At the present time, pan-edited cryptogenes have not been identified in the L. tarentolae or C. fasciculata maxicircles. There are, however, several intergenic regions in the maxicircle DNAs of all three species (shaded regions in Figure 3 and Figure 1) which produce G-rich transcripts of heterogeneous size (Jasmer et al., 1987). Although these regions do not exhibit sequence similarity (Figure 2), there is nevertheless a conservation of the relative location of regions of G versus C strand bias (Figure 3). We speculate that these sequences may represent cryptogenes that could give rise to pan-edited transcripts. The low abundance transcripts of the AT-rich divergent region, which are heterogeneous in size (Tarassoff et al., 1987; de Vries et al., 1988), represent additional candidates for pan-editing events. The 18S and 12S rRNAs have not been examined for edited regions, except for the 5' ends, which were found to be unedited by primer extension sequencing (Simpson et al., 1985; Shurekian and Simpson, unpublished data).

Unedited and Partially Edited Transcripts

The mitochondrial steady-state RNA population contains both completely edited, completely unedited, and partially edited forms of each gene transcript. Unedited transcripts were detected in RNA sequencing experiments as specific extension products of predicted sizes which disappeared when primers specific for edited RNAs were used (Feagin et al., 1987; Shaw et al., 1988). Both unedited and edited forms of transcripts were also detected in Northern blot analysis using specific oligonucleotides as probes (Bene et al., 1986; Feagin et al., 1987; Feagin et al., 1988a; van der Spek et al., 1988). In addition, the amount of edited transcripts in the steady-state RNA population varies from gene to gene. Primer extension assays have been used to measure the ratio of edited to unedited RNA for several genes, and these studies indicate that, in L. tarentolae, 0.9% of the COII and 36% of the MURF3 transcripts are edited (Shaw, Campbell, and Simpson, unpublished data).

Analysis of cDNA sequences has identified three types of partially edited forms of transcripts for several genes (Figure 6). Several T. brucei CYb cDNAs and one T. brucei COII cDNA have been identified that contain edited 3' sequences but terminate at a 5' editing site in a long stretch of U residues. In most cases, the 5' run of Us is longer than the number of added residues present in the mature transcript. For example, in one CYb cDNA (Figure 6-1), the 5' terminus has 36 Us although the fully edited CYb transcript has only 8 added Us at this position (Feagin et al., 1987). However these types of intermediates have thus far been found only in T. brucei. Although analysis of C. fasciculata MURF3 editing events identified a high propor-
Figure 5. Summary of cDNA Sequences Showing the Presence of U's in the 3' Poly(A) Tails of Maxicircle mRNAs

It is not known if the L. major ND1 gene is edited in coding regions also, since the genomic sequence is not known.

A second type of intermediate was characterized for the C. fasciculata MURF3 editing events. In this case, 1 cDNA out of 11 was edited at an internal site but was not edited at all in the 5' region (Figure 6-6). Seven of the 11 cDNAs were fully edited in both the 5' and internal regions. It is possible that the 5' edited sequences reported for both the C. fasciculata MURF3 mRNA and the L. tarentolae MURF3 mRNA represent partially edited sequences, as the created N-terminal amino acid sequences are only similar in the 3' edited region (Figure 4).

A third type of partially edited mRNA was found in the case of the T. brucei COIII pan-edited cryptogene (Abraham et al., 1988). Edited antisense probes detected a smear of partially edited COIII transcripts on Northern blots which ranged in size from 450 to 1200 nucleotides.

Figure 6. Summary of All Reported cDNA Sequences Showing Partially Edited mRNAs

5' poly(U) sequences in 1 and 2 are indicated by underlining. Junction regions are indicated in 3, 4, and 5 by boxes. Arrows mark the 5' unedited/3' edited borders. Extra U's at normally nonedited sites are indicated in 3, 4, and 5 by larger boxes. Deleted T residues are shown as * The 5' edited region and the internal-edited region are indicated in 6 by dashed underlines. References: 1. Feagin et al., 1987. 2, 3, and 4. Abraham et al., 1988. 5. Benne et al., 1986. 6. van der Spek et al., 1988.
the length of the fully edited transcript. Analysis of cDNAs identified COII transcripts that contained progressively more editing events moving in a 3' to 5' direction. PCR amplification, using combinations of unedited and edited oligonucleotide primers, confirmed the presence of 5' unedited/3' edited molecules, but did not detect 5' edited/3' unedited molecules. The sequences at the unedited/edited junctions in these molecules are complex and can cover up to six editing sites (see boxed regions in Figure 6). Moreover, the sequences at the junctions often differ from the sequences found in the DNA or the fully edited RNA at these positions. In several cases the number of Us at an editing site differs from that found in the mature transcript, and in one case, two Us are added at a site that is not edited in the mature RNA. If we assume that the non-editing of Type III cryptogene transcripts is mechanistically identical to the editing of Type I and Type II cryptogene transcripts, then these results suggest that kinetoplastid editing in general may occur in a 3' to 5' direction. However, this model assumes that partially edited cDNAs represent editing intermediates, but a precursor-product relationship between unedited, partially edited, and fully edited RNA has not yet been demonstrated.

RNA Editing as a Translational Control Mechanism RNA editing events in all three kinetoplastid species generate translatable transcripts. Editing of Type I COII and MURF3 transcripts allows translational readthrough of genomically encoded frameshifts. In several species, the Type II cryptogene, CYb, MURF2, and COII, lack AUG codons for the initiation of translation. RNA editing events in the 5' ends of these transcripts create in-frame AUG codons and thereby provide a translational control mechanism for the production of mitochondrial proteins. However, noncanonical initiation codons may also be used to translate some maxicircle mRNAs, since transcripts of several genes lack AUG initiation codons. In some cases, the 5' ends of transcripts are edited but an in-frame AUG is not generated (e.g., L. tarentolae and C. fasciculata MURF3), while in other cases, transcripts that do not contain AUG initiation codons are not edited at all (L. tarentolae MURF4 and MURF1, C. fasciculata ND1, and T. cruzi ND1 and MURF1) (Shaw et al., 1988; van der Spek et al., 1988; Feagin and Stuart, personal communication). Two additional functions of RNA editing are the creation of new N-terminal amino acids in Type II editing and the apparent absence of a conventional DNA or RNA template encoding the fully edited region. The evidence is mainly derived from hybridization studies using oligonucleotide probes complementary to either the edited or the unedited sequence for each cryptogene. These experiments did not detect edited templates in the nuclear or mitochondrial genomes of these cells. Moreover, edited templates could not be detected by several alternative methods, including direct sequencing of uncloned maxicircle DNA (Shaw et al., 1988) and PCR amplification of total kDNA using primers flanking the edited regions (Sturm, Shonekan, and Simpson, unpublished data).

Developmental Regulation of RNA Editing in Kinetoplastids The African pathogenic trypanosomes undergo a biphasic life cycle involving the periodic repression (bloodstream forms) and derepression (procyclic forms) of mitochondrial respiratory activity. Changes in the abundance of certain cryptogene RNAs are correlated with these different life cycle stages. In T. brucei, editing of COII and CYb cryptogene transcripts appears to be developmentally regulated in that only unedited RNAs are found in slender bloodstream forms that lack cytochromes, whereas both edited and unedited RNAs are found in stumpy bloodstream forms and procyclic insect forms. Editing of the MURF2 and COII transcripts, however, occurs during both lifecycle stages. These results suggest that RNA editing is involved in regulating the expression of some maxicircle genes during the lifecycle of the parasite.

Evidence for the Absence of Edited DNA or RNA Template The most startling aspect of the phenomenon of kinetoplastid RNA editing is the apparent absence of a conventional DNA or RNA template encoding the fully edited region. The evidence is mainly derived from hybridization studies using oligonucleotide probes complementary to either the edited or the unedited sequence for each cryptogene. These experiments did not detect edited templates in the nuclear or mitochondrial genomes of these cells. Moreover, edited templates could not be detected by several alternative methods, including direct sequencing of uncloned maxicircle DNA (Shaw et al., 1988) and PCR amplification of total kDNA using primers flanking the edited regions (Sturm, Shonekan, and Simpson, unpublished data).

It has been suggested that edited RNAs might self-replicate (Maizels and Werner, 1988). If it can be shown by kinetic experiments that the partially edited molecules...
represent true editing intermediates, then the existence of a continuum of such molecules is strong theoretical evidence against an RNA template hypothesis since separate templates would be required for each type of intermediate. However, the partially edited molecules could also possibly represent bizarre recombinants between edited and nonedited RNA and not necessarily editing intermediates. The fully edited antisense RNAs predicted by the Weiner model have not been detected by Northern hybridization using sense edited oligonucleotide probes (Feagin et al., 1988a) or by PCR experiments designed to amplify antisense edited RNAs (Sturm, Shonekan, Balakara, and Simpson, unpublished data). However, PCR amplification studies have provided preliminary evidence for the presence of antisense unedited RNA for the L. tarentolae MURF2 gene (Sturm and Simpson, unpublished data), the origin and significance of which is not understood.

Hypotheses for the Apparent Template-Independence of Kinetoplastid RNA Editing

Several hypotheses have been suggested to account for the apparent template-independence of kinetoplastid RNA editing:

—The basic "pseudogene" hypothesis maintains that the cloned and sequenced maxicircle molecules are actually pseudogenes, and that edited versions of the cryptogenes are present on one or a few copies of the 50 maxicircle genomes present in each organelle (Benne, 1985). As discussed above, there is no evidence for this type of maxicircle heterogeneity or for the presence of single copies of edited genes in mitochondrial or nuclear DNA.

—A variant of the pseudogene hypothesis states that there are small edited RNAs which are transcribed from small edited DNA sequences scattered throughout the genome (mitochondrial or nuclear) which could give rise to mature edited RNA molecules by a process of trans-splicing, such as that which occurs for the cytosolic mRNA population in these cells. This model has been tested with negative results (Shaw et al., 1988) by hybridization of Northern blots containing total mitochondrial RNA with labeled antisense oligomers. In addition, the presence of small edited sequences of 20 bp or greater in mitochondrial or nuclear DNA would have been detected by oligonucleotide probes used in the DNA hybridization experiments described above.

—A third hypothesis suggests that the edited template is somehow refractive to hybridization, perhaps as a result of nucleotide modifications, or that the template is easily lost during standard purification procedures (Downs and Roth, 1987). For example, a template that is tightly bound to protein might be resistant to proteases or deproteinization procedures. This hypothesis, however, is difficult to test experimentally.

—Some investigators have suggested that cryptic signals sequence might become exposed in incompletely edited molecules and that these molecules could then proceed to become fully and precisely edited species (Stuart, 1989). This argument does not address the issue of what determines that a pre-edited region is to be edited in the first place, nor the issue of the multiplicity of signals that would be required to generate the multiplicity of editing patterns.

—The information determining the final edited sequences may be encoded in the DNA sequences of the pre-edited or flanking regions of the cryptogenes in the form of secondary structures or primary sequence motifs, which could somehow guide a multienzyme editing complex in making the precise additions and deletions of uridines. However, a detailed analysis of these regions did not reveal any structures or sequences that might function in this manner (Shaw et al., 1988). Nevertheless, this hypothesis must remain as a viable one.

—The edited RNA might self-replicate and produce an edited antisense RNA, which could then act as a template for editing by a mechanism involving direct base-pairing, or by a process involving asymmetric replication, such as that which occurs in certain bacteriophage (Maizel and Weiner, 1988). As stated previously, there is no evidence for the presence of edited antisense RNA in the mitochondrial compartment. Furthermore, the existence of a pre-edited DNA sequence identical to the edited RNA sequence except for the presence and absence of Ts at precise sites argues against a self-replicating RNA model, since there would be no reason to maintain pre-edited sequences in the DNA.

—We would like to suggest an alternative RNA/RNA heteroduplex model based on our preliminary evidence for the presence of antisense unedited RNA for the L. tarentolae MURF2 gene. Ignoring the problem for the moment of the origin of this species, we propose that antisense unedited RNA might hybridize with preexisting sense edited RNA, forming a heteroduplex with mismatch loops at edited sites. This heteroduplex then might associate with the hypothetical editing complex and somehow guide the precise modification of a separate sense unedited RNA. This model does not address the question of the mechanism involved in transfer of the information from the heteroduplex to the editing machinery. However, the model does maintain the necessity for a pre-edited DNA sequence as well as a preexisting edited RNA sequence.

What Do We Really Know about the Mechanism of RNA Editing?

The cumulative frequency distribution pattern of U additions (and deletions) for all edited cryptogenes is shown in Figure 7. Most edits involve the addition of single U's 3' of purines, with decreasing numbers of multiple additions up to 8-mers. U deletions occur 3' of purines and only occur in transcripts that also contain U additions, suggesting that the process of U deletion is mechanistically related to the process of U addition.

RNA editing is probably a posttranscriptional process. The evidence for posttranscriptional editing includes the fact that poly(A) tails contain Us which most likely are inserted by the same mechanism that adds Us to coding regions, and polyadenylation is known to be a posttranscriptional enzymatic process (Humphrey et al., 1987; Conway and Wickens, 1987; Zarkower and Wickens, 1987). The fact that completely unedited and partially
within a pre-edited sequence covering up to six editing sites (and in the poly(A) tail), a uridylyl transferase then edited region and produces multiple random cleavages.

Specific ribonuclease recognizes some aspect of the editing does not occur on the ribosome, as the ribosome enzyme U-addition enzyme that adds multiple U's to the nucleotide that lacks specificity and a terminal transferase U-addition enzyme that adds multiple U's to the 3'-OH of both endogenous mitochondrial RNAs in intact mitochondria and exogenous RNA molecules in Triton lysates of mitochondria (Bakalara et al., unpublished data). Although this activity could not directly produce the subclass of truncated 5'poly(U)-partially edited molecules (Figures 6-1 and 6-2), it is possible that the 5' poly(U) tails on these cDNAs are artifacts of cloning and are not true intermediates since they were not observed on truncated MURF3 cDNAs (van der Spek et al., 1988). Work is in progress to determine if these two enzymes are involved in the editing of maxicircle transcripts and are present in the form of editing complexes.

Two Possible Candidates for Editing Enzymes

Purified mitochondria from L. tarentolae have been found to contain two enzyme activities (Bakalara, Simpson, and Simpson, unpublished data) similar to those previously reported in total cell extracts of T. brucei (White and Borst, 1987), that are possible candidates for participation in editing reactions: a 3' terminal uridylyl transferase (TU-Tase) and an RNA ligase. These activities comigrate with the kinetoplast-mitochondrion fraction in isopycnic Haplografin (Braly et al., 1974) and Percoll density gradients, and can be released by Triton lysis of the organelle (Bakalara et al., unpublished data). The TU-Tase activity adds several U's to the 3'-OH of both endogenous mitochondrial RNAs in intact mitochondria and exogenous RNA molecules in Triton lysates of mitochondria (Bakalara et al., unpublished data). Although this activity could not directly produce the subclass of truncated 5'poly(U)-partially edited molecules (Figures 6-1 and 6-2), it is possible that the 5' poly(U) tails on these cDNAs are artifacts of cloning and are not true intermediates since they were not observed on truncated MURF3 cDNAs (van der Spek et al., 1988). Work is in progress to determine if these two enzymes are involved in the editing of maxicircle transcripts and are present in the form of editing complexes.

Are There Other Examples of RNA Editing?

In the case of the kinetoplastids, the absence of recognizable genes for ATP synthetase subunits in the mitochondrial genome is intriguing, and we speculate that it may in part be due to as yet undescribed events of pan-editing of G-rich tRNAs transcribed from GO-rich intergenic regions. Although there is evidence that most kinetoplastid mitochondrial tRNAs are transcribed from nuclear genes (Suyama, Campbell, Simpson, and Simpson, unpublished data), the possibility remains that a small subset of tRNAs is produced by extensive editing of mitochondrial transcripts. Finally, editing of small minicircle DNA transcripts (Rohrer et al., 1985; Simpson and Simpson, unpublished data) to produce translatable or structural RNAs that have not yet been identified may occur, and this may finally provide a rationale for the existence of this unusual DNA.

Another possible example of RNA editing is a tissue-specific posttranscriptional, non-gonomo onodoxod C to U conversion in mammalian apolipoprotein B mRNA (Powell et al., 1987; Chen et al., 1987; Thomas et al., 1988; Tennyson et al., 1989a; Tennyson et al., 1989b). This modification changes a glutamine codon (CAA) into a translational stop codon (UAA) and thereby generates a shortened protein product, apo-B48. This situation, however, may repre-
sent a more traditional modification of an existing residue, similar to the type of posttranscriptional nucleotide modification that occurs in RNA biosynthesis (Elliot and Trewyn, 1984).

In this regard, an unwinding/modifying activity was recently described in Xenopus which produces covalent modifications in double-stranded RNA by conversion of adenosine to inosine (Bass and Weintraub, 1988), and it was suggested that this type of activity could be responsible for the nucleotide modifications observed in the apo-B situation. Another example of a posttranscriptional change in RNA that could be produced by an unwinding/modifying activity is the so-called "biased hypermutation" phenomenon in the RNA genome of a defective measles virus (Cattaneo et al., 1988; Bass et al., 1989; Lamb and Dreyfuss, 1989), in which 132 of 266 A residues in the matrix gene were found to be mutated to G residues.

Late transcripts of Vaccinia virus contain a fairly discrete length (35 nucleotides) of 5' poly(A) sequence that is not encoded in the viral genome (Schwer and Stunnenberg, 1988). The mechanism in this case appears to involve a slippage or stuttering of the RNA polymerase at an encoded T residue. A similar cotranscriptional RNA modification phenomenon appears to be occurring in negative strand RNA viruses, both in the synthesis of 3' poly(A) tails by transcriptional stuttering on a terminal homopolymer stretch of U residues, and within coding regions. The latter was first described in the paramyxovirus, SV5, in which the presence of two nontemplated G residues in a subpopulation of mRNAs for the P gene overcomes a frameshift and joins two open reading frames to produce the P protein (Thomas et al., 1986). The mRNAs lacking these extra G residues code for the V protein which is amino coterminial with the P protein. Since the nonencoded G's are added after four encoded G's, a stuttering mechanism is also thought to be responsible for this phenomenon. However, out of 22 cDNAs, 10 were found to be modified by the addition of 2 G residues at the same location and the remainder were not modified, which suggests that the mechanism may be precise in this case (Thomas et al., 1988). A similar phenomenon was described for the case of measles viruses, and it was suggested that similar events might occur in other paramyxoviruses (Cattaneo et al., 1989). The main difference in the measles virus situation is that the G addition is imprecise with respect to the number of G's added (Cattaneo et al., 1989).

Another mechanism for modification of RNA sequences is the "jumping RNA transcription" model developed to explain unusual features of coronavirus mRNAs (Makino et al., 1988a). A free leader RNA molecule is synthesized, dissociates from template RNA, and then reassociates with template at downstream initiation sites to act as a primer for transcription. A defective interfering mouse hepatitis viral RNA comprised of three non-contiguous genomic regions fused into a single open reading frame appears to have been formed by this discontinuous type of RNA synthesis (Makino et al., 1988b).

Finally, the generation of TAA termination codons by polyadenylation of processed mammalian mitochondrial UNA transcripts could also be classified as RNA editing, since translatable mRNAs are produced as a result of RNA modifications (Attaeri and Schatz, 1989).

**Evolutionary Implications and Speculations**

Mitochondrial cryptogenes and the accompanying RNA editing decoding mechanism are present throughout the Trypanosomatidae family of kinetoplastid protozoa, occurring in the primitive monogenetic genus, Crithidia, as well as in the more recently evolved digenetic genus, Trypanosoma (Lake et al., 1988), and may also occur in the slime mold, Physarum. The paramyxoviral type of RNA editing also involves a modification of the primary sequence of coding regions of mRNA molecules, but clearly involves a different mechanism and has a different evolutionary origin, as does the coronaviral type of RNA editing. Assuming tentatively that the RNA editing phenomena in kinetoplastids and Physarum are mechanistically similar and are derived from a common ancestor of these primitive lower eukaryotes, this argues for an ancient origin of this process and therefore a possibly wider distribution in present day organisms. In general, RNA editing has a clear selective advantage in evolution in addition to a possible role in regulation of translation, in that genetic information can be maintained at the RNA level in the face of sequence drift and even sequence compression at the genomic level. The presence of abundant noncoding DNA sequences and pseudogenes is a hallmark of the genomic organization of higher eukaryotic cells, and the existence of a process such as the kinetoplastid type of RNA editing might make it worthwhile to reevaluate the significance of some of these sequences.

The most extreme form of kinetoplastid RNA editing, pan-editing, has thus far been found only in transcripts of three genes in T. brucei. In an attempt to determine whether pan-edited genes exist in other kinetoplastid species, we screened kDNAs from a variety of species and genera with restriction fragments containing the unedited portions of the L. tarentolae COIII gene (Simpson et al., 1967; de la Cruz, Shaw, and Simpson, unpublished data). The results indicated that COIII genes cannot be detected in the mitochondrial genomes of two Herpetomonas species. This led to the tentative conclusion that pan-edited cryptogenes may also be present in Herpetomonas. This conclusion is consistent with previous suggestions that Herpetomonas is an evolutionary precursor of Trypanosoma, since Herpetomonas sp. are one of the two monogenetic kinetoplastids that undergo morphogenetic changes resembling those occurring in the digenetic Trypanosoma (Hoare, 1972).

**Conclusions**

RNA editing in the broad sense involves the modification of the primary sequences of coding regions of mRNAs and provides yet another method of modulating genetic activity. There appear to be different mechanisms for RNA editing in different systems. The kinetoplastid and perhaps the Physarum type of editing involves the precise addition and deletion of nucleotides within coding regions of mRNA molecules and differs from the paramyxoviral type.
of editing, which involves a cotranscriptional stutting of the RNA polymerase on homopolymer stretches. Both types differ from the mammalian apolipoprotein B type of editing which is thought to involve a chemical nucleotide modification. Since the kinetoplastid type of editing is probably of ancient origin, RNA editing activities are likely to be found in other organisms also. The major puzzle that the discovery of this phenomenon in the kinetoplastids has presented is the mechanistic nature of the information for the precise modifications of coding sequences of mRNAs in the apparent absence of templates encoding this information. The question of what encodes the specificity of edited RNA has direct relevance to the basic tenets of the central dogma of information transfer in molecular genetics, and it is likely that the solution to this problem may prove to be of general biological significance.

Acknowledgments

We would like to thank N. Sturm, O. Shonekan, H. Avila, and Drs. A. Simpson, N. Bakalar, D. Campbell, V. de la Cruz, and Y. Suyma for discussion and permission to quote unpublished data. We also thank Drs. D. Miller, R. Cattaneo, K. Stuart, and R. Benne for their kind communication of preprints and unpublished data for this review. This work was supported by National Institutes of Health grant AI 09102 to L. S. J. S. was a predoctoral fellow on an NIH training grant (GM07104).

References

Abraham, J. M., Feagin, J. E., and Stuart, K. (1988). Characterization of cytochrome c oxidase III transcripts that are edited only in the 3' region. Cell 55, 267–272.

Attardi, G., and Schatz, G. (1989). Biogenesis of mitochondria. Annu. Rev. Cell Biol. 4, 289–333.

Bass, B. L., and Weintraub, H. (1986). An unwinding activity that covalently modifies its double-stranded RNA substrate. Cell 55, 1089–1098.

Bass, B. L., Cattaneo, R., and Benne, R. A. (1987). cis-acting hypermutation of viral RNA genomes could be due to unwinding/modification of double-stranded RNA. Cell 55, 51–68.

Benne, R. (1985). Mitochondrial genes in trypanosomes. Trends Genet. 1, 117–121.

Benne, R. (1989). RNA editing in trypanosome mitochondria. Biochim. Biophys. Acta, in press.

Benne, R., and Stolf, P. (1987). Evolution of the mitochondrial protein synthetic machinery. Nucleic Acids Res. 15, 51–68.

Benne, R., Agostinelli, M., De Vries, B., van den Burg, J., Klaver, B., and Borst, P. (1989). Gene expression and organization in Trypanosoma mitochondria. In Mitochondria 1983: Nucleo-Mitochondrial Interactions, R. J. Schween, K. Wolf, and F. Kaudewitz, eds. (Berlin: De Gruyter), pp. 258–302.

Bonec, K., van den Burg, J., Brakenhoff, P. J., Stolf, P., Van Boom, J. H., and Tomp, M. C. (1989). Major transcript of the frameshifted 3'-untranslated region from Trypanosoma brucei contains four nucleotides that are not encoded in the DNA. Cell 46, 619–826.

Bonitz, S., Bocar, R., Coruzzi, G., Li, M., Macino, G., Nobrega, M., Nobrega, M., Thalendri, B., and Tzagoloff, A. (1988). Codon recognition rules in yeast mitochondria. Proc. Natl. Acad. Sci. USA 87, 3165–3170.

Brady, R., Simpson, L., and Kretzer, F. (1974). Isolation of kinetoplast mitochondrial complexes from Leishmania tarentolae. J. Protozool. 21, 782–790.

Campbell, D., Spithill, T., Samaras, T., Simpson, A., and Simpson, L. (1989). Apparent uridine addition in the poly A tail of Leishmania major NDI transcripts. Mol. Bioch. Parasitol., in press.

Cattaneo, R., Schmid, A., Eschke, D., Baccio, K., ter Meulen, V., and Billeter, M. A. (1988). Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. Cell 55, 265–265.

Cattaneo, R., Kaelin, K., Barshou, K., and Billeter, M. A. (1989). Measles virus editing provides an additional cytokine-rich protein. Cell 55, 759–764.

Chen, S.-H., Habib, G., Yang, C.Y., Gu, Z.-W., Lee, B. R., Weng, S.-A., Silverman, S. R., Cai, S.-J., Destyrene, J., Rosseneu, M., Gotto, A. M., Jr., Li, W.-H., and Chan, L. (1987). Apolipoprotein D40 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science 238, 363–366.

Conway, L., and Wicken, M. (1987). Analysis of mRNA 3' end formation by modification interference: the only modifications which prevent processing lie in AAAA and the poly(A) site. EMBO J. 6, 4177–4184.

de la Cruz, V., Neckelmann, N., and Simpson, L. (1984). Sequences of six structural genes and several open reading frames in the kinetoplast maxicircle DNA of Leishmania tarentolae. J. Biol. Chem. 259, 1516–1517.

de la Cruz, V. F., Lake, J. A., Simpson, A. M., and Simpson, L. (1985a). A minimal ribosomal RNA: sequence and secondary structure of the 9S kinetoplast ribosomal RNA from Leishmania tarentolae. Proc. Natl. Acad. Sci. USA 82, 1401–1403.

de la Cruz, V., Simpson, A., Lake, J., and Simpson, L. (1985b). Primary sequence and partial secondary structure of the 125S kinetoplast (mitochondrial) ribosomal RNA from Leishmania tarentolae: conservation of peptide-transferase structural elements. Nucl. Acids Res. 13, 2333–2366.

de Vries, B. M., Mulder, E., Brakenhoff, J., Stolf, P., and Benne, R. (1988). The variable region of the Trypanosoma brucei kinetoplast maxicircle: sequence and transcript analysis of a repetitive and a non-repetitive fragment. Mol. Biochem. Parasitol. 27, 71–82.

Downs. D. and Roth. V. L. (1987). A novel P22 prophage in Salmonella typhimurium. Genetics 117, 367–380.

Elliott, M., and Trewyn, R. (1984). Inosine biosynthesis in transfer RNA by an enzymatic insertion of hypoxanthine. J. Biol. Chem. 259, 2407–2410.

Epper, I., Janssen, J., Hoeijmakers, J., and Bori, P. (1985). The major transcripts of the kinetoplast DNA of T. brucei are very small ribosomal RNAs. Nucl. Acids Res. 13, 105–125.

Feagin, J. E., and Stuart, K. (1988). Developmental aspects of uridine addition within mitochondrial transcripts of Trypanosoma brucei. Mol. Biol. Evol. 5, 1259–1265.

Feagin, J. E., Jayser, D. P., and Stuart, K. (1987). Developmentally regulated addition of nucleotides within apocytochrome b transcripts in Trypanosoma brucei. Cell 49, 337–345.

Feagin, J. E., Shaw, J. M., Simpson, L., and Stuart, K. (1988a). Creation of AUG initiation codons by addition of uridines within cytochrome b transcripts of kinetoplastids. Proc. Natl. Acad. Sci. USA 85, 539–543.

Feagin, J. E., Abraham, J. M., and Stuart, K. (1988b). Extensive editing of the cytochrome c oxidase III transcript in Trypanosoma brucei. Cell 53, 413–422.

Hsiao, C. (1972). The Trypanosomona of Mammals (Oxford: Blackwell Scientific).

Hoeijmakers, J., Snijders, I., Janssen, J., and Borst, P. (1981). Transcription of kinetoplast DNA in Trypanosoma brucei bloodstream and culture forms. Plasmid 5, 329–350.

Humphrey, T., Christofori, G., Lecuajec, V., and Kelle, W. (1987). Osteovit and polyadenylation of messenger RNA precursors in vivo occurs within large and specific 3' processing complexes. EMBO J. 6, 4195–4198.

Jaeger, D., Feagin, J., Payne, M., and Stuart, K. (1987). Variation of G-rich mitochondrial transcripts among stocks of African trypanosomes. Mol. Bioch. Parasitol. 22, 259–272.

Lake, J. A., de la Cruz, V. F., Ferreira, P. C. G., Morel, C., and Simpson, L. (1989). Evolution of parasitism: kinetoplastid protozoan history.
reconstructed from mitochondrial rRNA gene sequences. Proc. Natl. Acad. Sci. USA 85, 4779–4783.

Lamb, R., and Dreyfuss, G. (1989). Unwinding with a vengeance. Nature 337, 19–20.

Maihels, N., and Weiner, A. (1988). In search of a template. Nature 334, 469–470.

Makino, S., Soe, L. H., Shieh, C.-K., and Lai, M. M. C. (1988a). Discontinuous transcription generates heterogeneity at the leader fusion sites reconstructed from mitochondrial rRNA gene sequences. Proc. Natl. Acad. Sci. USA 85, 4779–4783.

Makino, S., Shieh, S., Soe, L., Baker, S., and Lai, M. (1988b). Primary structure and translation of a defective interfering RNA of murine coronavirus. Virology 166, 550–560.

Muhirih, M., Neckelmann, N., and Simpson, L. (1985). The divergent region of the Leishmania tarentola mitochondrion (mt) genome contains a diverse set of repetitive sequences. Nucl. Acids Res. 13, 5241–5260.

Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Anta, S., Inokuchi, H., and Ozeki, H. (1986). Chloroplast gene organization deduced from complete sequence of liverwort Marchantia polymorpha chloroplast DNA. Nature 322, 572–574.

Payne, M., Rothwell, V., Jasmer, D., Feagin, J., and Stuart, K. (1985). Identification of mitochondrial genes in Trypanosoma brucei and holomycin to cytochrome c oxidase III in two different reading frames. Mol. Biochem. Parasitol. 15, 159–170.

Powell, L. M., Wallis, S. C., Paepe, R. J., Franks, Y. H., Knott, T. J., and Scott, J. (1987). A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell 50, 831–840.

Hohner, D. P., Micheloth, E. F., Iorn, A. F., and Hajduk, S. L. (1987). Transcription of kinetoplast DNA minicircles. Cell 49, 625–632.

Schwarz, R., and Stumne, H. G. (1988). Vaccinia virus late transcripts generated in vitro have a poly(A) head. EMBO J. 7, 1183–1190.

Shaw, J. M., Feagin, J. E., Stuart, K., and Simpson, L. (1988). Editing of kinetoplast mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. Cell 53, 401–411.

Simpson, L. (1972). The kinetoplast of the hemoflagellates. Int. Rev. Cytol. 32, 139–207.

Simpson, L. (1986). Kinetoplast DNA in trypanosomid flagellates. Int. Rev. Cytol. 99, 119–179.

Simpson, L. (1987). The mitochondrial genome of kinetoplastid protozoa: genomic organization, transcription, replication and evolution. Annu. Rev. Microbiol. 41, 363–382.

Simpson, L., Neckelmann, N., de la Cruz, V., Muhirih, M., and Simpson, L. (1985). Mapping and 5' end determination of kinetoplast maxicircle gene transcripts from Leishmania tarentola. Nucl. Acids Res. 13, 5977–5993.

Simpson, L., Neckelmann, N., de la Cruz, V. F., Simpson, A. M., Feagin, J. E., Jasmer, D. P., and Stuart, K. (1987). Comparison of the maxicircle (mitochondrial) genomes of Leishmania tarentola and Trypanosoma brucei at the level of nucleotide sequence. J. Biol. Chem. 262, 6182–6196.

Sloof, P., van den Burg, J., Voodt, A., Benne, R., Agostinelli, M., Borst, P., Gutell, R., and Noller, H. (1985). Further characterization of the extremely small mitochondrial ribosomal RNAs from trypanosomes: a detailed comparison of the 9S and 12S RNAs from Crithidia fasciculata and Trypanosoma brucei with RNAs from other organisms. Nucl. Acids Res. 13, 417–419.

Sloof, P., van den Burg, J., Voodt, A., and Benne, R. (1987). The nucleotide sequence of a 3.2 kb segment of mitochondrial maxicircle DNA from Crithidia fasciculata containing the gene for cytochrome oxidase subunit III, the N-terminal part of the apocytochrome b gene and a possible frameshift gene; further evidence for the use of unusual initiator triplets in trypanosome mitochondria. Nucl. Acids Res. 15, 51–65.

Sogin, M., Gunderson, J., Elwood, H., Alonso, R., and Peattie, D. (1989). Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from Giardia lamblia. Science 243, 75–77.

Spithill, T., Shimer, S., and Hill, G. (1981). Inhibitory effects of chloramphenicol isomers and other antibiotics on protein synthesis and respiration in procyclic Trypanosoma brucei. Mol. Biochem. Parasitol. 2, 235–256.

Staden, R. (1982). An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. Nucl. Acids Res. 10, 2951–2961.

Stuart, K. (1989). RNA editing: new insights into the storage and expression of genetic information. Parasitol. Today 5, 5–8.

Suwara, Y. (1986). Two-dimensional polycrylamide gel electrophoresis analysis of Tetrahymena mitochondrial rRNA. Curr. Genet. 10, 411–420.

Suwara, Y., and Yenney, P. (1986). The rRNA17 (anticodon TGT) gene and its upstream sequence coding for a homolog of the E. coli large ribosomal-subunit protein L14 in the Tetrahymena mitochondrial genome. Nucl. Acids Res. 17, 803.

Tarassoff, I., Lovenchenko, I., and Zaitseva, G. (1987). Transcripts of the maxicircle kinetoplast DNA of Crithidia oncopelti. Mol. Biochem. Parasitol. 26, 235–246.

Tennyson, G., Stbatos, C., Higuchi, K., Meglin, N., and Brewer, H. (1989). Expression of apolipoprotein B mRNAs encoding higher- and lower-molecular weight isoproteins in rat liver and intestine. Proc. Natl. Acad. Sci. USA 86, 500–504.

Tennyson, G. E., Stbatos, C. A., Eggerman, T. L., and Brewer, H. B. Jr. (1989). Characterization of single base substitutions in edited apolipoprotein B transcripts. Nucl. Acids Res. 17, 891–898.

Thomas, S. M., Lamb, R. A., and Paterson, R. G. (1988). Two mRNAs that differ by two non-templated nucleotides encode the amino-terminal proteins P and V of the Paramyxovirus SV5. Cell 54, 891–902.

van der Spek, H., van der Burg, J., Cioseit, A., van den Broek, M., Sloof, P., and Benne, H. (1988). Transcripts from the frameshifted MURF3 gene from Crithidia fasciculata are edited by U insertion at multiple sites. EMBO J. 7, 2509–2514.

White, T., and Borst, P. (1987). RNA end-labeling and RNA ligase activities can produce a circular ribosomal RNA in whole cell extracts from trypanosomes. Nucl. Acids Res. 15, 3275–3290.

Zarkower, D., and Wickens, M. (1987). Specific pre-cleavage and post-cleavage complexes involved in the formation of SV40 late mRNA 3' termini in vitro. EMBO J. 6, 4185–4192.