RNA editing in kinetoplastid mitochondria

STEPHEN L. HAJDUK,[1] MICHAEL E. HARRIS,[2] AND VICTORIA W. POLLARD

Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA

ABSTRACT **RNA editing in the mitochondrion of kinetoplastid protozoa results in the posttranscriptional addition and deletion of uridine residues in mRNAs. Editing of mRNAs can lead to the formation of initiation codons for mitochondrial translation, the correction of frame-shifted genes at the RNA level, and in extensively edited mRNAs, the formation of complete reading frames. Kinetoplastid RNA editing requires that genetic information from two or more separately transcribed genes be brought together to form the mature, edited mRNA. The information necessary for the proper insertion or deletion of uridines in the mRNA is present in small mitochondrial transcripts termed guide RNAs (gRNAs). Editing of mRNAs appears to be associated with a high molecular weight complex, called the editosome, containing specific gRNAs, unedited mRNAs, and proteins. Editing is likely a two-step process involving first the breakage of a phosphodiester bond at the editing site and formation of a chimeric molecule with a gRNA covalently joined to the 5' end of the 3' portion of an mRNA. The chimera is resolved by the rejoining of the 5' end of the mRNA to the 3' portion of the mRNA with the addition or deletion of a uridine at the junction point. Two models are proposed for the biochemical mechanism of RNA editing. The first is an enzymatic cascade of cleavage and ligation while the other supports successive rounds of transesterification. The obvious functional necessity for editing in kinetoplastid mitochondria is the formation of translatable mRNAs. Far less clear is the evolutionary origin of editing and the role editing plays in regulating mitochondrial gene expression.—Hajduk, S. L., Harris, M. E., and Pollard, V. W. RNA editing in kinetoplastid mitochondria. *FASEB J.* 7: 54–63; 1993.**

Key Words: trypanosome · RNA processing

SHEER DISBELIEF PERHAPS BEST DESCRIBES the reaction of most molecular biologists to the initial reports of RNA editing in kinetoplastid mitochondria (1–3). Some 5 years later, after the discovery of small RNA templates that seem to "guide" the editing process and the identification of editing intermediates, kinetoplastid RNA editing has been elevated to a level of respectability in the scientific community. No longer do cries of heresy abound at the notion that RNA editing might violate the "central dogma" of molecular biology.

RNA editing has now been added to an expanding list of molecular processes that alter the flow of genomic information at the RNA level, and is defined as any RNA processing event that results in the production of an mRNA that differs in nucleotide sequence from its gene (4). Excluded from this definition is the removal of introns and joining of exons by conventional splicing mechanisms. A wide array of examples of RNA editing, in a wide range of organisms, has been described during the past 5 years.

Two nuclear encoded mRNAs have been shown to be edited. The mammalian mRNAs for apolipoprotein B (5, 6) and the brain glutamate receptor (7) are altered by single base changes in long mRNA sequences. Editing of the apolipoprotein B mRNA results in a single C to U change, leading to the formation of a UAA termination codon and tissue-specific expression of a shorter apolipoprotein B molecule. The agent of nucleotide change in the apolipoprotein B mRNA is a site-specific deaminase that selectively modifies a single C within the mRNA (9). Editing of the glutamate receptor mRNA results in an A to G change, which also leads to a single amino acid change from a glutamine (CAG) to an arginine (CGG). A similar site-specific nucleotide deaminase has been suggested to mediate the editing of the glutamate receptor. In this case, adenosine deamination would first result in a CIG codon that might encode an arginine. The inosine residue would also appear as G in the coding strand of the cDNA (7).

Plant mitochondria and chloroplast mRNAs are also modified by C to U changes (8). Unlike mammalian RNA editing, numerous C to U changes occur in a single transcript. mRNA are also edited in the mitochondrion of the slime mold *Physarum polycephalum*, which results in the addition of nonencoded nucleotides to mRNAs (10). These added nucleotides are generally, but not exclusively, cytosines. The mRNAs produced by several RNA viruses (paramyxoviruses) differ from their genes by the addition of one or more guanosine residues. This is accomplished by a RNA polymerase stuttering mechanism, which results in the production of mRNAs with shifted reading frames (11).

Editing of kinetoplastid mitochondrial mRNAs is posttranscriptional and results in the addition and removal of hundreds of uridines. The information for the correct editing of kinetoplastid mRNAs is encoded in small "guide" RNAs (gRNAs)[3], which are complementary to the mRNAs, when G-U base pairing is allowed (12). Although all forms of RNA processing described above are termed RNA editing, the mechanism of kinetoplastid mitochondrial RNA editing clearly differs from editing of mRNAs in mammals, paramyxoviruses, slime molds, and plants. The focus of this review will be on the mechanisms involved in the editing process in kinetoplastid mitochondria.

---

[1]To whom correspondence should be addressed, at: Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

[2]Current address: Department of Biology, Indiana University, Bloomington, IN 47401, USA.

[3]Abbreviations: kDNA, kinetoplast DNA; COI, cytochrome oxidase I; CR2, G-C rich region 2; ND1, NADH dehydrogenase 1; A6, ATPase subunit 6; VR, variable region; gRNA, guide RNA; S12, ribosomal protein S12; CYb, cytochrome *b;* MURF, maxicircle unidentified reading frame; Ori, replication origin; TUTase, terminal uridylyltransferase; PCR, polymerase chain reaction.

0892-6638/93/0007-0054/$01.50. © FASEB

KINETOPLASTID MITOCHONDRIAL GENOMES AND RNA EDITING

With the recent excitement over RNA editing in kinetoplastid mitochondria, it is easy to overlook another unique feature of the mitochondrion of trypanosomes that first attracted the attention of molecular biologists almost 25 years ago. The mitochondrial genome of trypanosomes contains thousands of minicircles and approximately 50 maxicircles catenated together into a single large network structure, called the kinetoplast DNA (kDNA) (**Fig. 1**). The kinetoplast is, without doubt, the most unusual DNA structure ever described (13, 14). Although we now know a great deal about the function of the minicircles and maxicircles of the kDNA, the function of the catenated network structure remains unresolved. Recently, Borst (14) suggested that the network functions in maintaining the heterogeneity of the mitochondrial genomes of trypanosomes. He argues that the organized network structure reduces the risk of mitotic segregation of the minicircle sequence classes and the maxicircles. Even though it is untested, this model is appealing because the segregation of these DNA populations would rapidly lead to nonfunctional mitochondria. Other mechanisms for maintaining heterogeneous DNA populations within mitochondria must also exist, as the kDNA of the nonparasitic members of the order Kinetoplastidae is composed of thousands of heterogeneous, but noncatenated, circles (15).

Kinetoplast DNA maxicircles range in size from approximately 40 kb in *Crithidia fasciculata* to about 20 kb in *Trypanosoma brucei*. The maxicircles contain the genes for mitochondrial proteins and mitochondrial rRNAs (13) (**Fig. 2**). There is considerable variation in the size and sequence heterogeneity of the minicircles from different species of trypanosomatids. For example, the kDNA of *T. brucei* contains approximately 300 different minicircle sequence classes all about 1 kb in length. Minicircles of *C. fasciculata* are larger, 2.5 kb, and show less sequence heterogeneity. The kDNA of *T. equiperdum* contains homogeneous minicircles. The genetic function of minicircles was unknown until recently. Minicircles of all trypanosomatids studied encode some or all of the gRNAs required for the editing of maxicircle transcripts (16–18). The extent of the minicircle sequence heterogeneity correlates with the number of gRNAs required for complete editing of the maxicircle encoded mRNAs. Thus *T. brucei*, which has the most extensive editing of maxicircle transcripts, also has the largest number of minicircle sequence classes. Guide RNAs can also be encoded by maxicircles (12). In *Leishmania tarentolae*, seven maxicircle gRNA genes have been identified whereas only three maxicircle sequences in *T. brucei* have been identified as potential gRNA genes.

The general organization of the minicircle and maxicircle genomes of *T. brucei* is shown in Fig. 2. The maxicircles contain eight edited and five nonedited protein coding genes. In addition, several G-C rich maxicircle sequences have been identified as potential genes for an additional four extensively edited mRNAs. The extent of editing varies not only from gene to gene within a species but also within the same gene in different species (**Table 1**).

Each minicircle of *T. brucei* contains a constant region, representing about 20% of the minicircle sequence, and a variable region. Sequence heterogeneity within the variable region results in the approximately 300 different sequence classes. gRNA genes are positioned within the variable region of the minicircle between imperfect 18-bp inverted repeats (Fig. 2) (16, 19). gRNA transcription for several minicircle-encoded gRNAs has been shown to initiate at the sequence 5'-RYAYA-3', 31–32 nucleotides downstream of an inverted repeat sequence (16, 20). This may be a general feature of minicircle gRNA transcription in *T. brucei*. The function of the 18-bp repeats is unknown but the fixed distance of the upstream repeat from the start site of transcription suggests a role in transcription initiation. Alternatively, the inverted repeats may be sites of recombination serving an important, yet unproven, role in gRNA gene amplification and the generation of rapid sequence diversity. In the

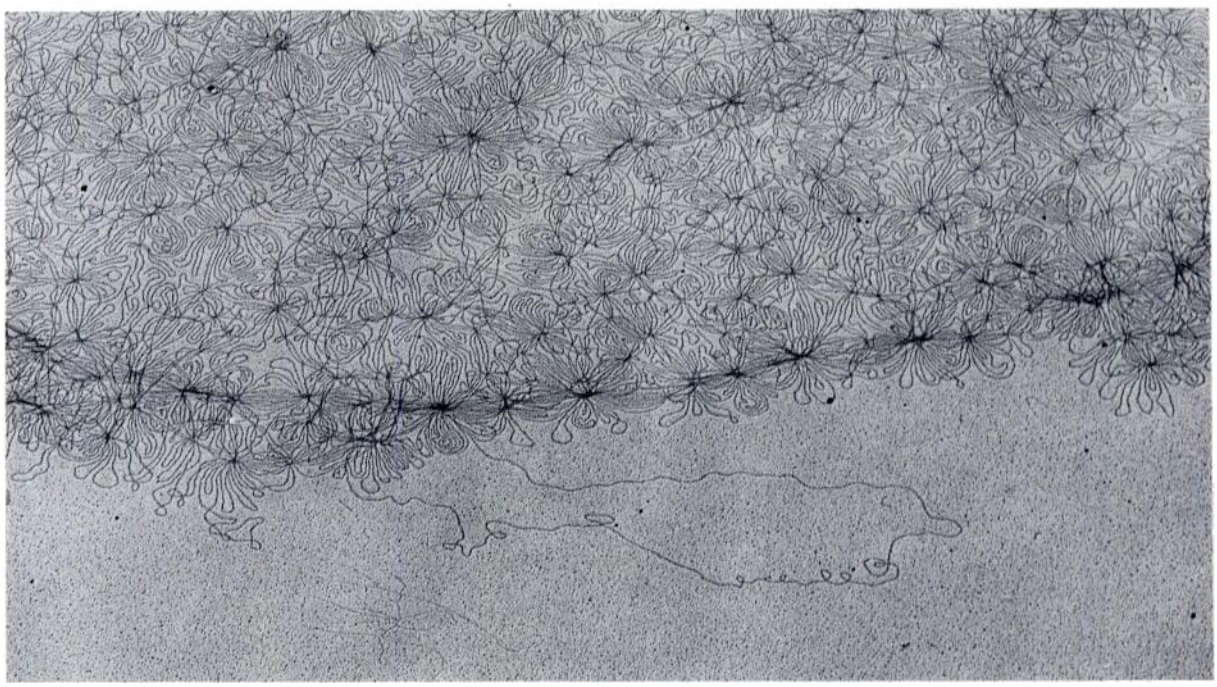

Figure 1. Kinetoplast DNA network. Electron micrograph showing a small portion of a kDNA network isolated from *C. fasciculata*. The small loops are catenated 2.5-kb minicircles. The long extended edge loop is part of a 40-kb maxicircle.

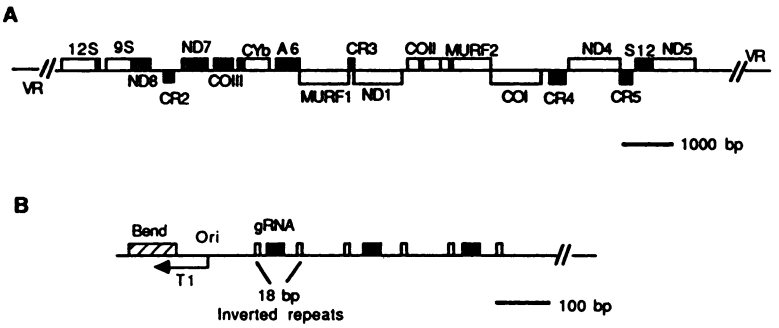

Figure 2. Transcription maps of the maxicircle and minicircle genomes of *T. brucei*. *A)* Linear map of the 22-kb maxicircle of *T. brucei*. The genes on the top of the line are transcribed from left to right, whereas the genes beneath the line are transcribed from right to left. The ribosomal RNAs (12S, 9S) have added uridines at their 3' ends (black box). Transcripts from cytochrome *b* (CYb), cytochrome oxidase II (COII), and MURF2 have limited amounts of internal editing (black boxes). The transcripts from the NADH dehydrogenase 7 and 8 (ND7, ND8), cytochrome oxidase III (COIII), ATPase subunit 6 (A6), ribosomal protein S12 (S12), and G-C rich 2, 3, 4, 5 (CR2, CR3, CR4, CR5) genes are all extensively edited (shaded boxes). The variable region of the maxicircle is indicated (VR). *B)* Linear map of the 1-kb minicircle of *T. brucei*. The bent helical region of the minicircle (hatched box) and the origin of replication (Ori) are within the conserved region of the minicircle. The transcript T1 is probably a primer for DNA replication. The gRNA genes (black boxes) are flanked by 18-bp inverted repeat sequences.

mitochondrion of *L. tarentolae*, editing is less extensive and minicircle heterogeneity is reduced to approximately 20 sequence classes with each minicircle encoding a single gRNA (21). In addition, *L. tarentolae* minicircles lack the 18-bp inverted repeats.

There are several features of gRNAs that are important in our discussion of RNA editing (see Fig. 4). First, gRNAs contain 30–40 nucleotides of continuous sequence complementary to an edited mRNA. These complementary sequences contain G-U base pairs in addition to conventional Watson and Crick A-U and G-C base pairing. This sequence, within the gRNA, is likely to provide the information needed to properly edit mRNAs. Though an appealing idea, this remains unproven. Second, each gRNA contains a short sequence of 7-10 nt which is complementary to unedited or edited sequences immediately 3′ to the editing site on the mRNA. This sequence is believed to "anchor" the gRNA to unedited mRNAs and play a role in the initial recognition and binding of gRNAs to unedited mRNAs. Third, all gRNAs contain a 5-15 nt, nonencoded, oligo (U) tail. This oligo (U) tail is thought to donate or accept Us during editing. Finally, gRNAs are primary transcripts and have unprocessed 5' ends. Currently, no function has been assigned to the 5' nucleotide triphosphate of gRNAs, although one seems likely as these are the only stable *T. brucei* mitochondrial RNAs that exhibit them.

TABLE 1. *Kinetoplastid mRNA editing*

Gene	Organism	Number of uridines added-deleted	Size of edited mRNA, bp	Reference
CYb	*T. brucei*	34–0	1151	2
	C. fasciculata	39–0	ND	22
	L. tarentolae	39–0	1150	22
COII	*T. brucei*	4–0	663	1
	C. fasciculata	4–0	643	1
	L. tarentolae	4–0	670	53
MURF 2	*T. brucei*	26–4	1111	54
	C. fasciculata	28–0	1117	3
	L. tarentolae	28–4	1099	3
COIII	*T. brucei*	547–41	969	23
	C. fasciculata	32–2	912	3
	L. tarentolae	29–15	904	3
ND7 (MURF 3)	*T. brucei*	553–88	1238	25
	C. fasciculata	27–0	1198	28
	L. tarentolae	25–0	1199	53
A6 (MURF 4)	*T. brucei*	447–28	811	27
	C. fasciculata	ND–ND	ND	–
	L. tarentolae	106–5	746	12
CR 6 (S12)	*T. brucei*	132–28	325	47
	C. fasciculata	ND–ND	ND	–
	L. tarentolae	107–32	320	24
CR1 (ND8)	*T. brucei*	259–46	574	26
	C. fasciculata	ND–ND[a]	ND	–
	L. tarentolae	ND–ND	ND	–

[a]ND, not determined.

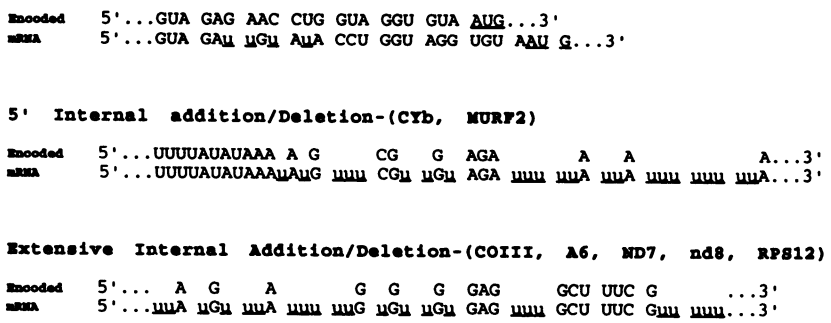

Figure 3. Three general types of mRNA editing in *T. brucei. A)* Limited internal uridine additions lead to the correction of frame-shifted transcripts. The unedited example given is COII. The termination codon UAA is underlined on the top line. Uridines added by editing are lowercase and underlined. *B)* Uridine addition and deletion to 5' regions. The 5' end of the CYb mRNA is shown. The CYb initiation codon AuG is formed by editing. *C)* Extensive internal editing leads to the creation of entire reading frames. The sequence for a portion of COIII is illustrated. Only short segments of the sequence for each of these unedited (encoded) and edited mRNAs are shown.

Although the maxicircle of trypanosomes contains the genes for typical mitochondrial proteins, the sequences of these genes (**Fig. 3**) are often unusual in that they do not code for functional proteins. The explanation for these unusual sequences first came for the COII gene when Benne and co-workers (1) reported that the mRNA from the COII gene contained four uridines not encoded in the genome. The addition of four uridines at three sites in the COII mRNA during editing corrects a frameshift and results in the production of in-frame mRNA. Editing of the CYb, ND7, COIII, and MURF2 mRNAs near their 5′ ends results in the formation of an AUG initiation codon and several additional downstream codons (22). The most impressive examples of RNA editing are seen in the extensively edited COIII (23), RPS12 (24), ND7 (25), ND8 (26), and A6 (27) mRNAs, where as much as 60% of the nucleotides in the mature mRNA are added uridines. In addition, several uridines encoded by these genes are deleted from the mRNA. Thus, the coding region as well as the initiation and termination codons are formed by addition of uridine to the purine rich gene sequence (Table 1).

These examples of editing described involve uridine addition or deletion within the coding region of mitochondrial mRNAs. Kinetoplastid mitochondrial mRNAs can also be modified by uridine addition within untranslated 5′ and 3′ regions. However, neither the position nor the number of added uridines is phylogenetically conserved, casting some doubt on the functional significance of these modifications. Another poorly understood form of RNA processing in kinetoplastid mitochondria is the addition of uridines within the poly A tail of many mRNAs (28). These uridines might be added by editing or by the mitochondrial poly A polymerase or a mitochondrial terminal uridylyltransferase (TUTase) (29). The mitochondrial rRNAs (30) and the gRNAs (20, 31) are also modified by the addition of a oligo (U) tail at the 3′ terminus. The 9S rRNA differs from its gene by the presence of at least seven nonencoded uridines at their 3′ termini. The 3′ terminus of the 12S rRNA has 2–17 nonencoded uridines. Although the function of the added uridines is untested, it is possible that the oligo (U) tail is required for the formation of structural elements of the mitochondrial rRNAs. The oligo (U) tails of the gRNAs contain 5 to 15 nonencoded uridines (20, 31). The mechanism of 3′ uridine addition to gRNAs and rRNAs may be analogous to mRNA editing. For example, uridine addition might occur internally on a precursor rRNA or gRNA, followed by endonuclease cleavage within the oligo (U) region to generate the mature 3′ terminus. Alternatively, the 3′ termini of gRNAs may be a substrate for the mitochondrial TUTase.

GENERAL FEATURES OF THE EDITING PATHWAY

All models for RNA editing in trypanosomes must take into account the overall directionality of the process, the characteristics of editing intermediates identified in the mitochondrion and the complementarity of gRNAs to edited mRNA sequences (**Fig. 4**). Sequence analysis of trypanosome mitochondrial cDNAs has suggested that editing occurs in a post-transcriptional fashion, as both unedited and partially edited transcripts were found (32–36). Direct evidence that editing was posttranscriptional came from studies using an isolated mitochondria system from *T. brucei* (36). It was shown that CYb mRNA is synthesized as an unedited primary transcript. When minicircle and maxicircle transcription was arrested by depleting the mitochondria of CTP, it was found the UTP continued to be incorporated into some maxicircle transcripts. Furthermore, the UTP incorporation was specific for edited mRNAs; it occurred internally and at editing sites.

The majority of the transcripts of COIII, ND7, and A6 genes of *T. brucei* are partially edited (32, 34, 35). This suggests that editing of these transcripts might be slow and inefficient. The sequence of these incompletely edited mRNAs reveals several interesting features. All of the partially edited mRNAs contained edited 3′ regions and unedited 5′ regions. Thus editing proceeds in a 3′ to 5′ direction along the mRNA.

A high percentage of partially edited mRNAs for *T. brucei* ND7, A6, and COIII genes contain sequences in the region between the 3' edited and 5' unedited regions that appear to be either incompletely or incorrectly edited. Within these junction regions, uridines are added or deleted at incorrect sites and the incorrect number of uridines is added at editing sites (32–35). There is only one junction region per mRNA or editing domain of a transcript; therefore, it is very likely that the junctions are the sites where editing is actively occurring. These results also suggest that re-editing of sequences occurs; otherwise these mRNAs would be nonfunc-

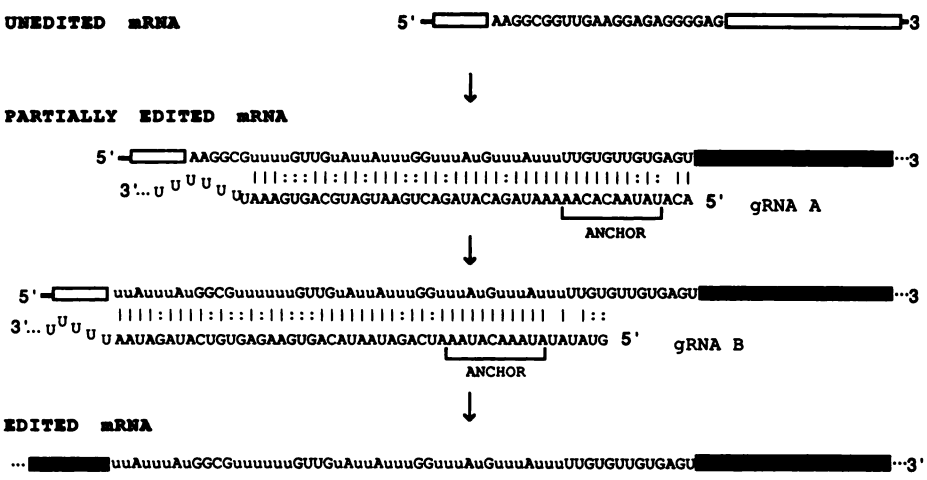

Figure 4. Schematic drawing showing the involvement of multiple gRNAs in the editing of an mRNA. Unedited portions of the mRNA (*T. brucei,* COIII) are shown in uppercase letters and as open boxes. Edited sequences are indicated by lowercase uridines and black boxes. gRNA A base pairs with the unedited mRNA immediately 3' to the first uridine addition site to form the anchor region. Editing of this portion of the mRNA forms a sequence that is complementary to gRNA B (15). The overlapping gRNA sequences can direct overall editing of the mRNA in a 3' to 5' direction.

tional products. In contrast, the junction of edited and unedited sequences in the cytochrome *b* transcripts of *L. tarentolae* suggests a more precise editing mechanism (33).

The interaction of gRNAs and mRNAs is important in the editing mechanism. To explain the partially edited mRNAs described, several models have been proposed. Blum et al. (37) first proposed that editing of the mRNA begins at a mismatch immediately 5' to the gRNA-mRNA anchor duplex. The addition or deletion of a uridine in the mRNA results in the formation of the next base pair (either an A-U or G-U) of the gRNA/mRNA duplex. Editing of the mRNA proceeds in a precise fashion 3' to 5', with mismatches between the gRNA and mRNA being recognized by the editing machinery of the trypanosome. Misediting of junction sequences could be explained in this model by the use of incorrect gRNAs at these sites (33). These mRNA molecules are not necessarily destined for degradation, as incorrectly edited sites could be re-edited by the appropriate gRNA. An alternative mechanism was proposed by Decker and Sollner-Webb (34) to account for the imprecise nature of editing in the junction regions. In their study of incompletely edited cytochrome *b* and cytochrome oxidase III mRNAs of *T. brucei,* the junction regions contained sequences in which the addition and deletion of uridines was fairly random. This led them to propose that the role of the gRNA is not to direct uridine addition and deletion to a specific site in the mRNA, but rather to form a duplex with the correctly edited sequences formed by random addition and deletion of uridines, which would protect the mRNA from further modification. The model predicts that an editing complex associates with the unedited mRNA and moves along the RNA until an editing domain is recognized. Recognition might involve the gRNA-mRNA duplex. The editing region would then act as a substrate for more or less indiscriminate rounds of cleavage, uridine addition, and ligation. Correctly edited mRNA sequence would base pair with the gRNA and be protected from subsequent modification. As pointed out by Stuart and co-workers (35), neither of these models can be completely correct because they fail to explain how a U can be added to or deleted from an mRNA immediately 3' to a C. gRNAs with G at the corresponding position would base pair with the encoded C and not direct the addition or deletion of a uridine. They propose a third model, called the "dynamic interaction" model, in which interaction of the gRNA and mRNA by base pairing of the anchor sequence initiates the process. The selection of the sequence to be edited is based on thermodynamic stability. The initial structure of the gRNA/mRNA hybrid is not stable. Editing within a domain would allow successive realignment of the gRNA/mRNA structure. Editing would be driven toward the lowest energy structure, which would be the completed gRNA/mRNA duplex. This model does not require that editing within an editing domain be strictly in a 3' to 5' direction, and accounts for many of the incompletely edited mRNAs identified.

Editing domains in COIII and other extensively edited transcripts are larger than the region that could be edited by a single gRNA. The identification of overlapping gRNAs provides a mechanism for the editing of large mRNAs by a number of gRNAs (16, 38). The overall directionality of editing is maintained by the formation of new anchor sequences by the editing at 3' sites (Fig. 4).

BIOCHEMICAL EVENTS AT THE EDITING SITE

The identification of partially edited mRNAs, and the complementarity of gRNAs and edited mRNAs, have provided the basis for editing site selection and a general pathway of editing. The detailed biochemical mechanism for addition and deletion of uridines at an editing site is unknown at this time. As editing reactions occur at internal sites, there is a requirement for strand breakage, nucleotide insertion, or deletion and rejoining of a phosphodiester bond in the mRNA. Editing reactions are specific with regard to the selection of particular mitochondrial mRNA to be modified and the site on the selected mRNA. In addition, only uridines are added or deleted. Finally, it is very likely that gRNAs play an important role in the editing site selection and nucleotide specificity of the reactions, and could be directly involved in the chemical process.

On theoretical grounds alone, two fundamentally different mechanisms for editing can be proposed. The first is mediated by sequential enzymatic reactions akin to tRNA splicing. The second possible mechanism resembles mRNA splicing and involves successive rounds of transesterification between a uridine donor molecule and the unedited mRNA substrate.

The original enzymatic cascade model (37) for editing must now be reevaluated. This model predicted that an editing site-specific endoribonuclease must recognize either a sequence or structure at the editing site in the mRNA or the intermolecular duplex formed by the gRNA and the mRNA. After cleavage, uridines could be added to the newly formed 3' hydroxyl of the 5' mRNA cleavage fragment by the mitochondrial TUTase. Rejoining of the cleavage products, now with an added uridine (or uridines), by an RNA ligase would complete one round of editing. The enzyme cascade mechanism is supported by detection of all of the predicted enzymatic activities in mitochondrial extracts. An editing site-specific endoribonuclease activity (39, 40), a nonspecific RNA ligase, and TUTase activities have been demonstrated in trypanosome mitochondria (29, 41). However, based on recent in vitro and in vivo studies, it is unlikely that editing proceeds precisely by this mechanism. Polymerase chain reaction (PCR) has been used to selectively amplify gRNA covalently joined to the 3' fragment of an edited mRNA (42). The observed chimeric gRNA-mRNA molecules are linked at editing sites of COII, COIII, and ND 7 mRNAs by the oligo (U) tails of the specific gRNAs.

The detection of gRNA-mRNA chimeric molecules supports another model for editing in which uridines are inserted and deleted from the mRNA either by a variation on the enzymatic cascade described previously or by multiple rounds of transesterification (**Fig. 5**). The transesterification model was originally proposed on theoretical grounds by Cech (43), and independently by Blum et al. (42) on the basis of the detection of chimeric gRNA-mRNA molecules. The model proposes that the gRNAs serve a dual role in editing, providing both the information in their sequence to direct the editing process and the added nucleotides via the oligo (U) tail. This model predicts that the first step in the editing reaction is a nucleophilic attack by the 3' hydroxyl of the gRNA oligo (U) tail at a phosphodiester bond within the editing site of the mRNA. This results in the formation of a chimeric molecule containing the gRNA joined to the 3' fragment of the mRNA by the oligo (U) tail. The other product of this reaction is the 5' mRNA fragment. The positioning of the nucleophilic attack could be directed by the mismatch between the gRNA-mRNA at the editing site. Alternatively, additional secondary and tertiary interactions between the mRNA and gRNA might come into play. The gRNA-mRNA chimeric molecules are resolved in the second step of the editing reaction. The 3' hydroxyl on the 5' mRNA fragment generated in the first step attacks a phosphodiester bond within the oligo (U) tail of the gRNA to generate a gRNA with a shorter oligo (U) tail and an mRNA with added U (or U's) at the editing site. The proposed transesterifications could be catalyzed by the gRNA, as in the group I intron self-splicing (43), or could be protein catalyzed with the gRNA merely acting as a donor for the added uridines and the template for correct addition (43). In vitro formation of chimeric gRNA-mRNA molecules has now been reported (44, 45). Chimera formation requires the presence of exogenously added mRNA, corresponding gRNA, and trypanosome mitochondrial extract. The chimera-forming activity in the mitochondrial extracts is sensitive to proteinase K digestion (44). Chimeric cytochrome *b* gRNA-mRNA molecules are very similar to chimeric molecules seen in vivo, with gRNA joined to the mRNA by the oligo (U) tail at the editing site (44).

The detection of chimeric gRNA-mRNA molecules seems to support the transesterification mechanism for editing. However, it should be kept in mind that there is no biochemical evidence for transesterification, only for chimera formation. It is possible that the formation of the gRNA-mRNA chimeric molecules is more similar to enzymatic splicing reactions, and involves endonuclease cleavage and RNA ligase rejoining reactions between the 3' hydroxyl of the oligo (U) tail of the gRNA and the 3' mRNA cleavage fragment (Fig. 5*A*). The presence of editing site-specific endoribonuclease, TUTase, and RNA ligase circumstantially argues for such an enzymatic cascade model.

Perhaps these two observations are not mutually exclusive. A parallel has been drawn between editing and group I intron splicing (42, 43). Although there is no evidence that this parallel extends to self-catalysis, other aspects of the reaction could mimic splicing by trans-esterification. Splice sites in catalytic introns are especially susceptible to hydrolysis, leading to the assertion that "not all phosphodiester bonds are created equal" (46). It may be that the endonuclease and RNA ligase activities detected in the kinetoplastid mitochondrial extracts are similar to the half-reactions catalyzed in the *Tetrahymena* catalytic intron. No in vitro system has been reported that faithfully completes the editing process. The development of such a system will allow the examination of the second step in the editing pathway, the resolution of chimeric gRNA-mRNA molecules to the edited product.

ORGANIZATION OF THE EDITING MACHINERY: EDITOSOMES

Editing of trypanosome mitochondrial mRNAs is a multistep process requiring the direct interaction of at least two RNA components: the gRNA and unedited mRNA precursor. Several properties of RNA editing suggest the involvement of protein-RNA complexes as either catalytic or accessory components in the editing machinery. At this stage, it is difficult to predict the nature of the catalytic components that might make up such a complex. However, there are several properties of kinetoplastid RNA editing which indicate that multicomponent complexes, termed editosomes, might be required for editing. The initial step in editing requires the recognition of an editing domain, in the unedited mRNA, by the correct gRNA. The short "anchor" regions of complementarity between the gRNA and the unedited mRNA are likely to participate in this step. However, the complementarity of the anchor sequences can be as few as 5 bp. This seems inadequate to ensure the specificity needed at this stage of the editing reaction and argues that the process requires additional factors to establish specific gRNA/mRNA interactions.

The involvement of proteins in the chimera-forming reactions has been demonstrated experimentally. The formation of chimeric cytochrome *b* gRNA-mRNA molecules, in vitro, requires a protease sensitive factor from mitochondrial extracts (44). In addition, when mitochondrial extracts from *T. brucei* were fractionated on glycerol gradients, the chimera-forming activity sedimented as two complexes of 19S and 35-40S (41). The particles in the 19S fraction also contained gRNAs, RNA ligase, and TUTase activities. The 35-40S particles contained unedited and partially edited mRNAs in addition to the gRNAs, RNA ligase, and the

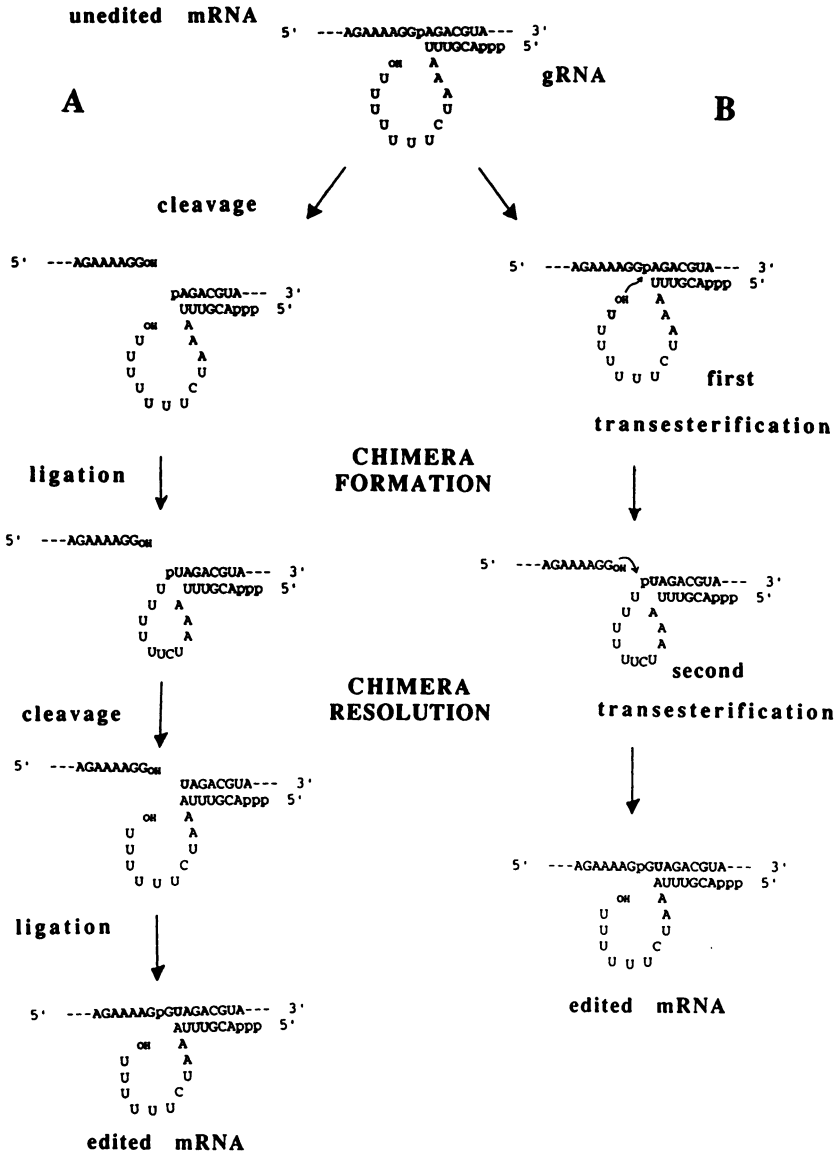

Figure 5. Schematic drawing showing two possible mechanisms for kinetoplastid mRNA editing. *A)* Modified enzymatic cascade model. A gRNA-mRNA chimeric molecule is formed by the sequential cleavage of the unedited mRNA at the editing site, followed by ligation of the oligo (U) tail of the gRNA to the 3' cleavage product. The chimera is resolved by a second round of cleavage within the oligo (U) tail and ligation of the 5' fragment of the mRNA. *B)* The transesterification model. The 3' hydroxyl of the oligo (U) tail of the gRNA attacks the phosphodiester bond at the editing site, resulting in the formation of a gRNA-mRNA chimeric molecule. The chimera is resolved by a second round of transesterification, with the 3' hydroxyl of the 5' cleavage product attacking within the oligo (U) tail of the gRNA. Both models propose the formation of the same intermediate chimeric gRNA-mRNA molecule and the transfer of uridines from the gRNA to the editing site of the mRNA.

chimera-forming activities. The 19S native complexes do not contain unedited mRNA, yet appear to be capable of converting exogenously added unedited mRNA substrate and gRNA to a chimera. The simplest interpretation of these results is that gRNAs are present within 19S protein/RNA complexes, which can recognize and bind specific unedited mRNA. Once the mRNA is bound, the complex is capable of carrying out the first step in mRNA editing leading to the formation of gRNA-mRNA chimeric molecules. A model showing the possible relationship of the 19S and 35-40S complexes is shown in **Fig. 6.**

The production of gRNA-mRNA chimeric molecules, either by cleavage ligation or transesterification mechanisms, produces a free 5' fragment of the mRNA (44, 45). The later steps in editing require that this fragment be kept close to the chimeric molecule. Similar problems in ordered recognition, binding, and processing of most eukaryotic nuclear mRNAs require multicomponent complexes.

REGULATION OF MITOCHONDRIAL GENE EXPRESSION BY RNA EDITING

RNA editing is of obvious importance in the formation of translatable mRNAs in trypanosome mitochondria. Beyond the necessities of initiation and termination codons and of open reading frames within these mRNAs, editing may have an important regulatory function in some and maybe all kinetoplastids.

Recent studies (47) have shown that the processing of polycistronic precursor mRNA may be affected by the edit-

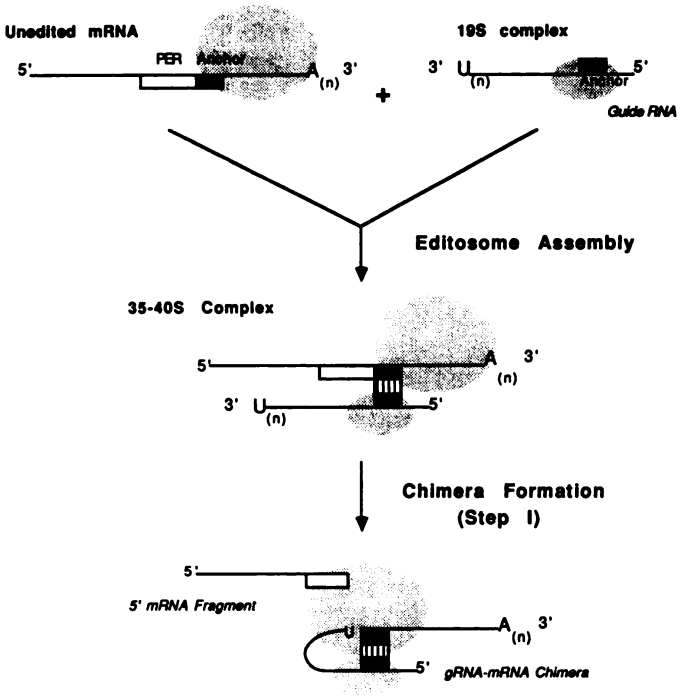

Figure 6. Proposed model for assembly of editing complexes. gRNAs are associated with specific proteins in a 19S particle that can bind to unedited mRNAs. The assembly of the "editosome" probably requires base pairing of the anchor region of the gRNA and mRNA (black box) immediately 3' to the preedited region (PER) (open box) of the mRNA. The complex may function to hold the 5' mRNA fragment in position for the second step in editing.

ing of the precursor transcripts. It has been noted that a number of maxicircle genes are organized with overlapping reading frames. This presents a possible regulatory pathway by which the production of function mRNA is dependent on the timing of 3' or 5' end formation. Editing may play an important role in the expression of at least one pair of overlapping transcripts (47). The 3' end of the extensively edited S12 (CR6) gene overlaps the 5' end of the ND5 transcript by 37 nucleotides. cDNAs spanning the S12 and ND5 gene revealed precursor transcripts containing edited S12 sequences and the downstream ND5 sequences. The mature, functional ND5 mRNA can be made only if cleavage of the precursor transcript forms the ND5 5' terminus S12 editing. Thus it is likely that editing influences which mature mRNA is made from a single polycistronic precursor.

Many trypanosomatids have a mammalian host and insect vector. The parasites undergo dramatic morphological and biochemical changes during their life cycles. Among the best-studied of these is the developmental regulation of mitochondrial activities in the African trypanosomes—in particular, *T. brucei* (48). Two distinct developmental stages are found within the bloodstream of the mammal. During the early stage of infection, *T. brucei* populations consist of rapidly dividing cells that are long and slender in appearance. During later stages of the bloodstream infections, the long, slender trypanosomes differentiate to a nondividing form, which is morphologically short and stumpy. The mitochondrion of the long, slender forms lacks detectable cytochromes and Krebs cycle enzymes. The Krebs cycle and cytochrome respiratory chain are still incomplete in the short, stumpy trypanosomes, but several enzymes begin to accumulate. ATP production in the bloodstream trypanosomes is solely by glycolysis, with glucose from the mammalian bloodstream being metabolized at high rates. When the bloodstream trypanosomes differentiate to the procyclic developmental stage, found in the midgut of the tsetse fly, a complete cytochrome-mediated electron transport system and functional Krebs cycle assemble. As several components of the electron transport system are encoded in mitochondrial genes, it is likely that mitochondrial gene expression is developmentally regulated in African trypanosomes.

When the steady-state amounts of the maxicircle transcripts were examined in the bloodstream and procyclic populations, it was found that the amounts of several of the transcripts varied in the different developmental stages. The levels of rRNAs, CYb, COI, COII, and COIII were low in the long, slender forms and increased in the short, stumpy bloodstream forms and the procyclic insect stage (49). The levels of rRNA have been shown to be regulated posttranscriptionally at the level of RNA stability (50). The developmental regulation of the steady-state levels of mitochondrial mRNAs is transcript specific. The steady-state amounts of several other mitochondrial transcripts for the mitochondrial NADH dehydrogenase complex are unregulated during development (49). This is consistent with the proposed role of the NADH dehydrogenase (complex 1) in ATP production in the bloodstream trypanosomes.

Editing is also regulated during the developmental cycle of *T. brucei*. The transcripts for cytochrome oxidase II and cytochrome *b* are edited in the procyclic forms and short, stumpy bloodstream forms but not in long, slender bloodstream trypanosomes (12, 51, 52). On the other hand, the ND8 mRNA is highly edited in the long, slender bloodstream trypanosomes but not in the procyclic forms. Because the ND8 protein contains an apparent iron-sulfur core based on cDNA sequence, it is possible that it is part of complex 1 and has a role in bloodstream energy production (26). The mitochondrial transcripts MURF 2, ATPase 6, and COIII are edited throughout the developmental cycle of *T. brucei*.

The developmental regulation of editing is not only transcript specific but also domain specific within a transcript. The mRNA for NADH dehydrogenase 7 is edited in the 5' domain in both the bloodstream and procyclic developmental stages, whereas the 3' domain is edited only in the bloodstream forms (25). This suggests the interesting possibility that differential editing of transcripts can lead to the production of structurally and functionally distinct proteins (24).

Nothing is known about the mechanisms involved in the developmental regulation of editing. Clearly, the amount of specific gRNAs could affect the editing of specific transcripts as well as domains within a transcript. Preliminary studies fail to reveal correlations between the amount of editing of an RNA and the steady-state amount of gRNA present (52). This suggests that the regulation and specificity of editing might be controlled by unidentified editing factors present within the editing complexes.

CONCLUSIONS

The process of RNA editing adds a new dimension to the regulation of gene expression in trypanosomes and other organisms. Elucidation of the chemical mechanism for kinetoplastid mRNA editing will require the development of accurate in vitro editing systems. This is currently the focus of intense investigation. The function of editing in kinetoplastid mitochondria is both intriguing and perplexing. Surely the formation of translatable mRNAs could be handled in a more efficient manner. It is difficult to imagine

that editing in the form seen in trypanosomes is simply a relic of an RNA repair or modification system that preceded polymerase enzymes. It seems more likely that the individual chemical reactions that together make-up the process called editing have common origins with other RNA processing reactions, such as splicing. The form that these processing reactions take, whether in the removal of introns or the insertion of uridines, may be influenced by the needs of the organism. Thus, trypanosomes may use editing to modulate the developmentally regulated mitochondrial activities in much the same way that other organisms use differential splicing of introns to regulate the expression of a number of genes. FJ

The authors would like to thank all of the members of the Hajduk laboratory for many lively and insightful discussions on RNA editing. In particular, we thank Jeff Priest for his careful and critical reading of this manuscript. The work on RNA editing in the Hajduk laboratory is supported by Public Health Service grant AI 21401. S. L. H. is a Burroughs Wellcome Fund Scholar in Molecular Parasitology.

REFERENCES

1. Benne, R., Van Den Burg, J., Brakenhoff, J. P. H., Sloof, P., Van Boom, J. H., and Tromp, M. C. (1986) Major transcript of the frameshifted *coxII* gene from trypanosome mitochondria contains four nucleotides that are not encoded in the DNA. *Cell* **46,** 819–826
2. Feagin, J. E., Jasmer, D. P., and Stuart, K. (1987) Developmentally regulated addition of nucleotides within apocytochrome *b* transcripts in *Trypanosoma brucei*. *Cell* **49,** 337–345
3. Shaw, J. M., Feagin, J. E., Stuart, K., and Simpson, L. (1988) Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. *Cell* **53,** 401–411
4. Simpson, L., and Shaw, J. (1989) RNA editing and the mitochondrial cryptogenes of kinetoplastid protozoa. *Cell* **57,** 355–366
5. Powell, L. M., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J., and Scott, J. (1987) A novel form of tissue specific RNA processing produces apolipoprotein-B-48 in intestine. *Cell* **50,** 831–840
6. Driscoll, D. M., Wynne, J. K., Wallis, S. C., and Scott, J. (1989) An in vitro system for the editing of apolipoprotein B mRNA. *Cell* **58,** 519–525
7. Sommer, B., Köhler, M., Sprengel, R., and Seeburg, P. H. (1991) RNA editing in brain controls a determinant of ion flow in glutamate-grated channels. *Cell* **67,** 11–19
8. Gray, M. W., and Covello, P. S. (1993) RNA editing in plant mitochondria and chloroplasts. *FASEB J.* **7,** 64–71
9. Bostrom, K., Garcia, Z., Paksay, K. S., Johnson, D. F., Lusis, A. J., and Innerarity, T. L. (1990) Apolipoprotein B mRNA editing. *J. Biol. Chem.* **265,** 22446–22452
10. Mahendran, R., Spottswood, M. R., and Miller, D. L. (1991) RNA editing by cytidine insertion in mitochondria of *Physarum polycephalum*. *Nature (London)* **349,** 434–438
11. Vidal, S., Curran, J., and Kolakofsky, D. (1990) A stuttering model for paramyxovirus P mRNA editing. *EMBO J.* **9,** 2017–2022
12. Simpson, L. (1990) RNA editing—a novel genetic phenomenon? *Science* **250,** 512–513
13. Simpson, L. (1987) The mitochondrial genome of kinetoplastid protozoa: genomic organization, transcription, replication and evolution. *Annu. Rev. Microbiol.* **41,** 363–382
14. Borst, P. (1991) Why kinetoplast DNA networks? *Trends Genet.* **7,** 139–141
15. Hajduk, S. L., Siqueira, A. M., and Vickerman, K. (1986) Kinetoplast DNA of *Bodo caudatus:* a noncatenated structure. *Mol. Cell. Biol.* **6,** 4372–4378
16. Pollard, V. W., Rohrer, S. P., Michelotti, E. F., Hancock, K., and Hajduk, S. L. (1990) Organization of minicircle genes for guide RNAs in *Trypanosoma brucei*. *Cell* **63,** 783–790
17. Sturm, N. R., and Simpson, L. (1990) Kinetoplast DNA minicircles encode guide RNAs for editing of cytochrome oxidase subunit III mRNA. *Cell* **61,** 879–884
18. Stuart, K. (1991) RNA editing in trypanosomatid mitochondria. *Annu. Rev. Microbiol.* **45,** 327–344
19. Jasmer, D. P., and Stuart, K. (1986) Sequence organization in African trypanosome minicircles is defined by 18bp inverted repeats. *Mol. Biochem. Parasitol.* **18,** 321–331
20. Pollard, V. W., and Hajduk, S. L. (1991) *Trypanosoma equiperdum* minicircles encode three distinct primary transcripts which exhibit guide RNA characteristics. *Mol. Cell. Biol.* **11,** 1668–1675
21. Sturm, N. R., and Simpson, L. (1991) *Leishmania tarentolae* minicircles of different sequence classes encode single guide RNAs located in the variable region approximately 150 bp from the conserved region. *Nucleic Acids Res.* **19,** 6277–6281
22. Feagin, J. E., Shaw, J. M., Simpson, L., and Stuart, K. (1988) Creating of AUG initiation codons by addition of uridines within cytochrome *b* transcripts of kinetoplastids. *Proc. Natl. Acad. Sci. USA* **85,** 539–543
23. Feagin, J. E., Abraham, J. M., and Stuart, K. (1988) Extensive editing of the cytochrome c oxidase III transcript in *Trypanosoma brucei*. *Cell* **53,** 413–422
24. Maslov, D. A., Sturm, N. R., Niner, B. M., Gruszynski, E. S., Peris, M., and Simpson, L. (1992) An intergenic G-rich region in *Leishmania tarentolae* kinetoplast maxicircle DNA is a pan-edited cryptogene encoding ribosomal protein S12. *Mol. Cell. Biol.* **12,** 56–67
25. Koslowsky, D. J., Bhat, G. H., Perrollaz, A. L., Feagin, J. E., and Stuart, K. (1990) The MURF3 gene of *T. brucei* contains multiple domains of extensive editing and is homologous to a subunit of NADH dehydrogenase. *Cell* **62,** 901–911
26. Souza, A. E., Myler, P. J., and Stuart, K. (1992) Maxicircle *CR1* transcripts of *Trypanosoma brucei* are edited and developmentally regulated and encode a putative iron-sulfur protein homologous to an NADH dehydrogenase subunit. *Mol. Cell. Biol.* **12,** 2100–2107
27. Bhat, G. J., Koslowsky, D. J., Feagin, J. E., Smiley, B. L., and Stuart, K. (1990) An extensively edited mitochondrial transcript in kinetoplastids encoded a protein homologous to ATPase subunit 6. *Cell* **61,** 885–894
28. Van der Spek, H., Van den Burg, J., Croiset, A., van den Broek, M., Sloff, P., and Benne, R. (1988) Transcripts from the frameshifted MURF3 gene from *Crithidia fasciculata* are edited by U insertion at multiple sites. *EMBO J.* **7,** 2509–2514
29. Bakalara, N., Simpson, A. M., and Simpson, L. (1989) The *Leishmania* kinetoplast-mitochondrion contains terminal uridylyltransferase and RNA ligase activities. *J. Biol. Chem.* **264,** 18679–18686
30. Adler, B. K., Harris, M. E., Bertrand, K. I., and Hajduk, S. L. (1991) Modification of *Trypanosoma brucei* mitochondria rRNA by post-transcriptional 3' polyuridine tail formation. *Mol. Cell. Biol.* **11,** 5878–5884
31. Blum, B., and Simpson, L. (1990) Guide RNAs in kinetoplastid mitochondria have a nonencoded 3' oligo (U) tail involved in recognition of the preedited region. *Cell* **62,** 391–397
32. 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
33. Sturm, N. R., and Simpson, L. (1990) Partially edited mRNAs for cytochrome b and subunit III of cytochrome oxidase from *Leishmania tarentolae* mitochondria: RNA editing intermediates. *Cell* **61,** 871–878
34. Decker, C. J., and Sollner-Webb, B. (1990) RNA editing involves indiscriminate U changes throughout precisely defined editing domains. *Cell* **61,** 1001–1011
35. Koslowsky, D., Bhat, G. J., Read, L. K., and Stuart, K. (1991) Cycles of progressive realignment of gRNA with mRNA in RNA editing. *Cell* **67,** 537–546
36. Harris, M. E., Moore, D. R., and Hajduk, S. L. (1990) Addition of uridines to edited RNAs in trypanosome mitochondria

occurs independently of transcription. *J. Biol. Chem.* **265,** 11368-11376
37. Blum, B., Bakalara, N., and Simpson, L. (1990) A model for RNA editing in kinetoplastid mitochondria: "Guide" RNA molecules transcribed from maxicircle DNA provide the edited information. *Cell* **60,** 189-198
38. Maslov, D. A., and Simpson, L. (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. *Cell* **70,** 459-467
39. Simpson, A. M., Bakalara, N., and Simpson, L. (1992) A ribonuclease activity is activated by heparin or by digestion with proteinase K in mitochondrial extracts of *Leishmania tarentolae*. *J. Biol. Chem.* **267,** 6782-6788
40. Harris, M. E., Decker, C., Sollner-Webb, B., and Hajduk, S. L. (1992) Specific cleavage of pre-edited mRNAs in trypanosome mitochondrial extracts. *Mol. Cell. Biol.* **12,** 2591-2598
41. Pollard, V. W., Harris, M. E., and Hajduk, S. L. (1992) RNA Editing Complexes from *Trypanosoma brucei* mitochondria. *EMBO J.* **12,** 4429-4438
42. Blum, B., Sturm, N. R., Simpson, A. M., and Simpson, L. (1991) Chimeric gRNA-mRNA molecules with oligo (U) tails covalently linked at sites of RNA editing suggest that U addition occurs by transesterification. *Cell* **65,** 543-550
43. Cech, T. R. (1991) RNA editing: world's smallest introns? *Cell* **64,** 667-669
44. Harris, M. E., and Hajduk, S. L. (1992) Kinetoplastid RNA editing: In vitro formation of cytochrome *b* gRNA-mRNA chimeras from synthetic substrate RNAs. *Cell* **68,** 1091-1099
45. Koslowsky, D. J., Göringer, H. U., Morales, T. H., and Stuart, K. (1992) In vitro guide RNA/mRNA chimaera formation in *Trypanosoma brucei* RNA editing. *Nature (London)* **356,** 807-809
46. Cech, T. R. (1990) Self-splicing of group I introns. *Annu. Rev. Biochem.* **59,** 543-568
47. Read, L. K., Myler, P. J., and Stuart, K. (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. *J. Biol. Chem.* **267,** 1123-1128
48. Hajduk, S., Adler, B., Bertrand, K., Fearon, K., Hager, K., Hancock, K., Harris, M., LeBlanc, A., Moore, R., Pollard, V., Priest, J., and Wood, Z. (1992) Molecular biology of African trypanosomes: development of new strategies to combat an old disease. *Am. J. Med. Sci.* **303,** 258-270
49. Michelotti, E. F., and Hajduk, S. L. (1987) Developmental regulation of trypanosome mitochondrial gene expression. *J. Biol. Chem.* **262,** 927-932
50. Michelotti, E. F., Harris, M. E., Adler, B., Torri, A. F., and Hajduk, S. L. (1992) *Trypanosoma brucei* mitochondrial ribosomal RNA synthesis, processing and developmentally regulated expression. *Mol. Biochem. Parasitol.* **54,** 31-42
51. Feagin, J. E. (1990) RNA editing in kinetoplastid mitochondria. *J. Biol. Chem.* **265,** 19373-19376
52. Koslowsky, D. J., Riley, G. R., Feagin, J. E., and Stuart, K. (1992) Guide RNAs for transcripts with developmentally regulated RNA editing are present in both life cycle stages of *Trypanosoma brucei*. *Mol. Cell. Biol.* **12,** 2043-2049
53. Shaw, J., Chambell, D., and Simpson, L. (1989) Internal frameshifts within the mitochondrial genes coding for cytochrome oxidase subunit II and maxicircle unidentified reading frame 3 of *Leishmania tarentolae* are corrected by RNA editing: evidence for translation of the edited cytochrome oxidase subunit II mRNA. *Proc. Natl. Acad. Sci. USA* **86,** 6220-6224
54. Feagin, J. E., and Stuart, K. (1988) Developmental aspects of uridine addition within mitochondrial transcripts of *Trypanosoma brucei*. *Mol. Cell. Biol.* **8,** 1259-1265

RNA editing in kinetoplastid mitochondria.

S L Hajduk, M E Harris and V W Pollard

FASEB J 1993 7: 54-63