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RNA Editing in Kinetoplastid Protozoa

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INTRODUCTION

Editing and the Central Dogma

Exceptions often expand rather than disprove elegant concepts and so reveal additional biological diversity and richness. Such is the case with the central dogma: DNA to/from RNA to protein. The discovery of introns revealed the incomplete colinearity between the sequences of some genes and their mature transcripts and amino acid sequences predicted from protein-coding genes. This finding led to the discovery of RNA splicing (intron excision), the spliceosome which catalyzes this process, and differential gene expression by alternative and regulated splicing. Thus, the increased appreciation of the complexity in the storage and processing of genetic information led to an expanded recognition of biological capabilities. The more recent discoveries that the informational content of gene transcripts can be altered during or after transcription by processes collectively termed RNA editing has resulted in a further extension of the central dogma. The first-discovered and perhaps most dramatic example of RNA editing is the subject of this review. It is termed kRNA editing since it occurs in the mitochondrion (mt) of kinetoplastid protozoa and it inserts and deletes uridylates (U's) within mitochondrially encoded pre-mRNAs by a posttranscriptional process. This review emphasizes the biochemistry of the editing process since previous reviews covered earlier work in depth (10, 40, 88, 95, 99, 103).

kRNA Editing Discovered

The first indication of kRNA editing was the discovery that the 3′-coding region of the cytochrome oxidase subunit II (COII) gene is out of frame with its 5′-coding sequence (29, 45, 65). Benne et al. explained this puzzling finding by demonstrat-

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TABLE 1. Edited mRNAs in *T. brucei*

mRNA[a]	Size[b]	Insertions[c]	Deletion[c]	Stage[d]	Reference
Complex I					
ND3[e]	323	210	13	B	79
ND7	1,238	71/482	13/76	B/M	49
ND8	562	259	46	M	101
ND9	649	345	20	M	102
CR3[f]	299	148	13	M	27
CR4[f]	567	325	40	M	25
Cytochromes					
CYb	1,151	34	0	I	31
COII	663	4	0	I	12
COIII	969	547	41	B	30
Others					
A6[e]	811	447	28	B	13
MURF2	1,111	26	4	B	32
RPS12[e]	325	132	28	M	77
Total no. 12		3,030	322		

[a] Abbreviations: ND, NADH ubiquinone oxidoreductase (subunits 3, 7, 8, and 9); CR, G- versus C-strand biased gene (subunits 3 and 4); CY*b* apocytochrome *b*; CO, cytochrome oxidase (subunits II and III); A6, ATP synthase subunit 6; MURF2, maxicircle unidentified reading frame; RPS12, ribosomal protein S12.

[b] Size is in nucleotides excluding the poly(A) tail. Note that NADH dehydrogenase subunit 1, 4, and 5 mRNAs are not edited nor are mRNAs for cytochrome oxidase subunit I or maxicircle unidentified reading frame 1.

[c] Insertions and deletions for the ND7 5′ and 3′ domains are separated by a slash.

[d] M, edited in the mammalian stage of the life cycle; I, edited in the insect stage; B, edited in the mammalian and insect stages.

[e] The assignment of these genes to the respective open reading frames is based on relatively weak DNA homologies.

[f] The assignment of these genes to respiratory complex I is based on their hydrophobicity.

ing that four nonencoded U's are present in COII transcripts of *Crithidia fasciculata* and *Trypanosoma brucei*, thus eliminating the frameshift in the mature transcript (12). Subsequently, 12 of the 17 pre-mRNAs encoded in the mt genome in several kinetoplastid species were shown to be edited by the insertion and deletion of U's at many sites in mt mRNAs (Table 1). Some pre-mRNAs are extensively edited (pan-edited) and are so remodeled that their coding sequence becomes evident only after editing. The kRNA editing process creates the initiation and termination codons and produces or extends the protein-coding sequence. Most of the protein sequences predicted from the edited RNAs have homology to proteins predicted by mt genes in other organisms. However, homology to RPS12 (62, 77), ATPase 6 (13), and ND3 (79) is so low that additional experiments are needed for more confident identification. Edited RNAs derived from GC strand-biased regions CR3 and CR4 predict highly hydrophobic proteins with no homology to proteins in the databases (25, 27). These may encode hydrophobic components of respiratory complex I (NADH dehydrogenase) that are very divergent among species. The protein predicted from MURF2 RNA has no homolog in the databases and may represent a polypeptide peculiar to the mt genome of kinetoplastids (32, 93). Thus, overall, kRNA editing appears to be an essential process in the maturation of mt pre-mRNAs in kinetoplastids. A direct characterization of the translation products of edited RNAs is still eagerly awaited. The lack of direct information about these protein products reflects the technical difficulties inherent in the analysis of extremely hydrophobic proteins.

Other Types of Editing

While kinetoplastids provided the first example of RNA editing, mechanistically unrelated RNA editing processes were subsequently discovered in other eukaryotes (for reviews, see references 8, 11, and 85). These processes fall into two classes, those which alter coding information by nucleotide insertion and/or deletion and those that do so by base modification. Examples of the former include kRNA editing as well as mononucleotide and dinucleotide insertion in the mitochondria of the acellular slime mold *Physarum polycephalum* (58). Examples of the latter include the tissue-specific cytidine-to-uridine deamination at nucleotide 6666 in the apolipoprotein B pre-mRNA by a sequence-specific enzyme complex (23, 73, 98) and the adenine-to-inosine deamination events at various positions in the pre-mRNAs encoding the glutamate-gated receptor subunits by a double-stranded RNA-specific adenosine deaminase (100) (for a review, see reference 9). The mRNAs of the tumor suppressor genes WT1 (92) and NF1 (97) have also been found to be edited, suggesting that editing may be a factor in some diseases. In fact, editing of NF1 mRNA is upregulated in certain tumors (97). In addition to the base modification editing of these nuclear gene encoded transcripts, several mt and chloroplast pre-mRNAs are edited by cytidine-to-uridine deamination or uridine-to-cytidine amination in many species of plants (for a review, see reference 38), and nucleotide replacements also occur in mt tRNAs of *Acanthamoeba castellani* (56). Thus, RNA editing appears to be a widespread phenomenon for both normal and aberrant RNA processing in organelles and nuclei.

Most RNA editing examined to date occurs posttranscriptionally, but some appears to occur cotranscriptionally (for a review, see reference 21). RNA processing such as 3′ CCA addition and 5′ G addition to tRNA, pre-mRNA capping, splicing and polyadenylation, and nucleotide modifications which do not change coding identity is generally not considered RNA editing. However, it may be imprudent to impose a restrictive definition on recently discovered biological processes which may share more mechanistic similarities than we currently imagine or may be more distinct than their phenomenological similarities suggest. Thus, the issue of the definition of RNA editing may be viewed more as an index of biological richness than as a difficulty in classification. Furthermore, various types of RNA processing may have distant evolutionary relationships that are not currently apparent, as discussed in a later section.

gRNAs SPECIFY THE EDITED SEQUENCE

The form and source of the information for the edited sequence were initially perplexing, especially for extensively edited mRNAs. However, the discovery by Blum et al. (15) of small RNAs, called guide RNAs (gRNAs), which are complementary to edited sequences in pre-mRNAs and hence could specify them, revealed that editing was an expansion of the central dogma rather than an exception to it (Fig. 1A). gRNAs are generally 55 to 70 nucleotides (nt) in length, and their sequence characteristics suggest that they have three functional elements. The 5 to 12 nt that are within a few nucleotides of the 5′ end of each gRNA are complementary to the pre-mRNA sequence region immediately 3′ to the portion whose editing the gRNA can specify. This gRNA "anchor" sequence thus provides for specific interaction between the gRNA and pre-mRNA. Adjacent to the anchor sequence, each gRNA contains a "guiding" section of 25 to 35 nt that can specify U insertion and deletion at 1 to 20 internucleotide sites

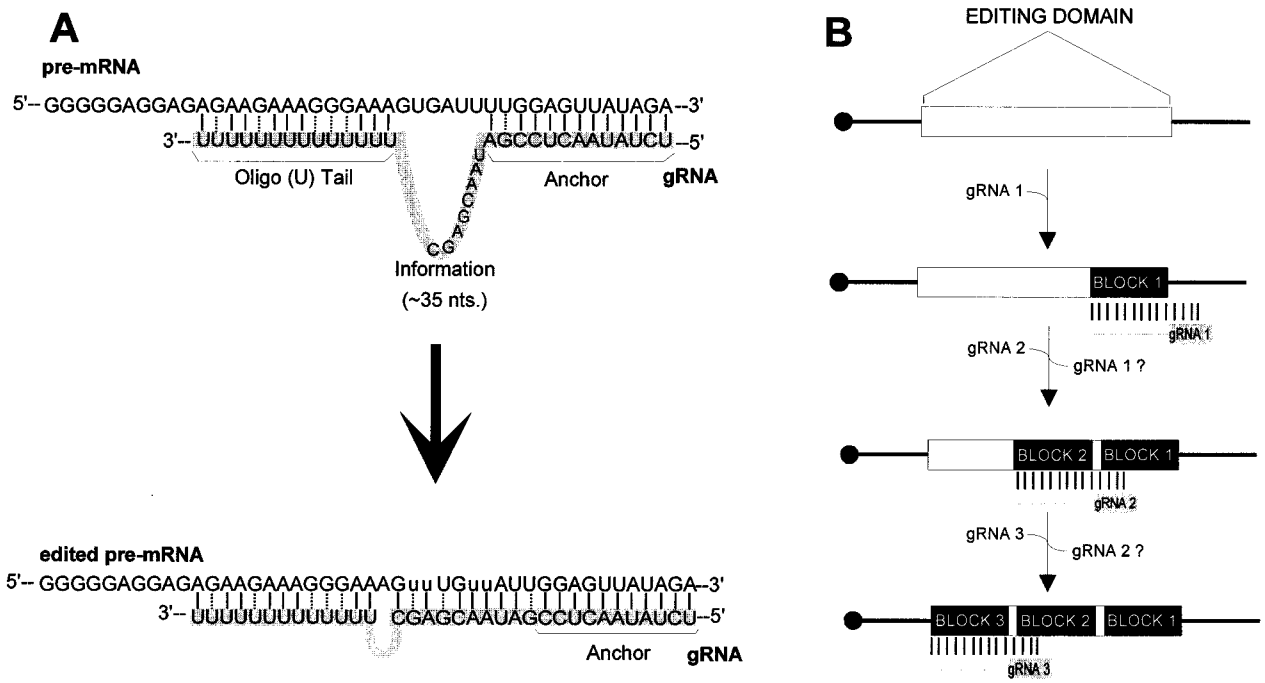

FIG. 1. gRNAs specify edited sequence. (A) Pre-mRNA and gRNA are depicted engaged in an intermolecular duplex with Watson-Crick (vertical lines) and GU (colons) base pairs. The three sequence elements of gRNAs are indicated. (B) Extensively edited pre-mRNAs are processed over a large domain, which may require the action of several gRNAs, each of which directs the remodeling of a sequence block. Only the 3′-most-acting gRNA can associate with the unedited pre-mRNA. More-5′-acting gRNAs must at least partially displace the 3′-acting gRNA before they can associate with partially edited pre-mRNAs. The 5′ ends of the RNAs are indicated by circles in this and subsequent figures.

(editing sites [ESs]). The use of both G · U and Watson-Crick base pairing implies an information transfer process other than conventional template-directed polymerization. The third structural feature of gRNAs is a nonencoded 3′ oligo(U) tail of 5 to 24 nt depending on the species (16). This U tail is presumably added by the mt terminal uridylyltransferase (TUTase). The function of the U tail is not known, but it has been suggested to interact with purine-rich sequences 5′ to regions of editing (16, 90) or to serve as a repository for the inserted and deleted U's (18, 22) as discussed below.

The gRNAs are encoded in the unusual mt genome of kinetoplastids. These organisms have only one mitochondrion, which contains a single DNA network composed of 5,000 to 10,000 heterogeneous minicircles intercatenated with 40 to 50 identical maxicircles. Most gRNAs are encoded in minicircles, but a few are encoded in maxicircles, which primarily encode proteins. Kinetoplastids differ substantially in gRNA coding capacity and amount of editing, as illustrated by comparisons between *Leishmania tarentolae* and *T. brucei*. *L. tarentolae* minicircles are each 0.8 kb in size and encode a single gRNA (108), while *T. brucei* minicircles are each 1 kb in size and encode up to four gRNAs from sequences flanked by inverted 18-bp repeats (24, 72, 80). *T. cruzi* minicircles are 1.45 kb in size and may encode four gRNAs (6), while in *C. fasciculata* the 2.5-kb minicircles each encode single gRNAs (114). *L. tarentolae* UC encodes about 23 gRNAs, which is about the number needed for its lower total editing of 348 U insertions and 56 U deletions. *T. brucei* encodes at least 1,200 different gRNAs, which is more than 10 times the number needed for the 3,030 U insertions and 322 U deletions. Thus, *T. brucei* has redundant gRNA information in the sense that it contains different gRNAs that specify the same or highly overlapping regions of edited sequence (24, 80). Mutant *L. tarentolae* and *T. brucei* strains that lack minicircles have correspondingly less editing, supporting the role of gRNAs in specifying edited sequences (94, 109). The *L. tarentolae* maxicircle is 35 kb in size and encodes a few gRNAs. The 22-kb *T. brucei* maxicircle may encode even fewer gRNAs, but none have actually been shown to be transcribed from this DNA. The maxicircles of *L. tarentolae* and *T. brucei* encode the same genes in the same order, so that the size difference is due primarily to the size of the noncoding variable region. The gRNAs have a 5′-terminal RYAYA sequence and a 5′ di- or triphosphate, which may indicate that gRNA genes are transcribed from individual promoters, but gRNA transcription, like mt transcription in general, remains largely unexplored (16, 70, 72). Nevertheless, the sequence of a mature edited mRNA is dispersed among the pre-mRNA gene and multiple gRNA genes. As a consequence, genetic information is transferred not only from the DNA sequence to its transcript but also from one class of RNAs (gRNAs) to others (pre-mRNA).

gRNA USE IN EDITING

The editing of an entire transcript entails the orchestrated use of multiple gRNAs as well as the transfer of the full sequence information contained in each gRNA to pre-mRNA. Stretches of contiguous sequence which require one or more gRNAs, each of which specifies a block of sequence, are called editing domains (Fig. 1B). Most pre-mRNAs have one editing domain, but ND7 in all kinetoplastids examined, CR6 (GR6) in *L. tarentolae*, and CR5 (GR5) in *T. brucei* are edited in multiple domains (49, 62, 79, 93, 96, 111). Editing of the domains is independent, since molecules that are partially edited in each domain have been observed. The overall editing process involves the interaction between the mRNA, its cognate

gRNAs, and a macromolecular complex that catalyzes the process. The gRNAs are probably selected by their ability to form an anchor duplex. The occurrence of misediting by noncognate gRNAs suggests that short fortuitous duplexes can be sufficient for use by the editing machinery (63, 106). There are many unanswered questions concerning the temporal association of gRNAs with a functional complex and the fate of the gRNAs, mRNAs, and the macromolecular complex when editing is completed. Do gRNAs and pre-mRNAs form duplexes that then bind a complex, do gRNAs or pre-mRNAs bind a complex to which the other is already bound, or do they form separate gRNA and pre-mRNA complexes that then associate? Does a pre-mRNA remain in association with a complex while gRNAs come and go until editing is complete, or do the RNAs dissociate from a complex after each gRNA is used and active complexes reassemble with the next gRNA? Studies on partially edited pre-mRNAs (see below) imply that gRNAs remain associated with the pre-mRNAs and complexes until their complement of sequence information is transferred.

gRNAs Are Used in a 3′-to-5′ Order of the Pre-mRNA

The sequences of numerous partially edited cDNAs from pre-mRNAs revealed a characteristic pattern consistent with an overall 3′-to-5′ direction of editing. Invariably, these cDNAs are edited in the 3′ portions of the editing domains and unedited 5′ to edited sequence (28, 49, 62, 63, 77, 93, 101, 107). Comparisons of gRNA anchor sequences with unedited and with partially and fully edited cDNAs suggest a basis for the sequential formation of anchor duplexes and hence sequential utilization of gRNAs in a 3′-to-5′ order relative to the pre-mRNA (53, 60). Only a small subset of gRNAs can form a substantial anchor duplex with an unedited pre-mRNA, and that duplex is invariably immediately 3′ to the region whose editing the gRNA specifies. The other gRNAs can form a substantial anchor duplex only with edited sequences of the pre-mRNAs. Thus, editing appears able to be efficiently initiated by a select gRNA at the 3′ end of an editing domain. Editing by this gRNA then creates the sequence which can form an anchor duplex with the next gRNA that specifies the editing of the adjacent 5′ region, and this process can be repeated until editing is completed by sequential use of multiple gRNAs (Fig. 1B). It is unknown if the gRNAs are dissociated from the edited mRNA after use and reutilized or degraded or if they remain associated with the mRNA until it is translated.

This simple solution to the polarity of editing requires dissociation of at least part of the downstream gRNA to accommodate the formation of the anchor duplex by the upstream gRNA. Minimally, anchor duplex formation by a more-5′ gRNA would dissociate the ~12-nt U tail of the gRNA and about ~12 nt of the gRNA that specified the new anchor sequence. It might dissociate an additional ~10 nt, since editing always occurs at least this distance 5′ to the 3′ end of a pre-mRNA. Thus, a ~20-bp duplex between the downstream gRNA and pre-mRNA could be retained or the downstream gRNA could be entirely dissociated. An mt RNA helicase activity has been demonstrated (64) and shown to cosediment with in vitro editing activity (26), but it remains to be determined if the helicase activity is in the same complex that performs editing. RNA helicase may function to provide access for incoming gRNAs to sequences for the formation of anchor duplexes, provide access by the catalytic core of the editing machinery to the pre-mRNA site being edited, and/or dissociate the gRNAs from the edited mRNA after utilization. An alternative possibility is that the incoming gRNA may simply displace the downstream gRNA by a process similar to strand invasion. This process may be facilitated by the fact that the anchor duplex of an incoming gRNA is generally composed of Watson-Crick base pairs whereas the duplex formed between the informational part of the downstream gRNA and the edited part of the pre-mRNA frequently contains thermodynamically less stable G · U or other noncanonical base pairs (24, 53, 60). Perhaps the U tail or its associated proteins, which would be adjacent to this region, may also play a role in destabilizing the downstream duplex.

gRNA Choice of Editing Sites

The process by which a gRNA selects an internucleotide site to be edited remains unclear. It was initially proposed that editing proceeds strictly 3′ to 5′, with the site of editing recognized as a mismatched base pair adjacent to the extending RNA helix (15). However, a substantial portion of partially edited pre-mRNAs produced in vivo have sequences at the junction where unedited and edited sequence converge that match neither the edited nor the unedited RNA (28, 49, 62, 63, 77, 93, 101, 105, 107). These junctions contain edited sites 5′ to unedited sites, sites which are edited but are not altered in the mature mRNA, and edited sites with more or fewer U's than in mature mRNA. Since RNA editing frequently produces initiation codons, these RNAs are unlikely to be translated to a substantial extent. But what do these molecules tell us about the process of RNA editing?

It is likely that junctions represent the region of a pre-mRNA undergoing editing at the time of RNA isolation, since most junctions are of a size that could be specified by a single gRNA. Thus, the question becomes that of how a gRNA produces a junction region. Three models have been put forth to explain junction regions. Decker and Sollner-Webb (28) proposed that U insertion and deletion within a pre-mRNA sequence block is random and that gRNAs serve only to protect sites from further processing by sequestering them in an intermolecular duplex. This model requires gRNA-independent endonucleolytic cleavage of the pre-mRNA. An activity has been identified in trypanosome mt which could perform this role (41, 67). However, it has not been established whether this activity plays a role in editing. In vitro analysis of accurate in vitro U insertion (47) and deletion (90) indicates that pre-mRNA cleavage is gRNA directed during RNA editing, and therefore argues against this explanation of junction regions. The two remaining proposals for editing-site selection suggest that gRNAs direct cleavage to mismatched pre-mRNA positions. Sturm et al. (106) proposed that editing proceeds precisely 3′ to 5′. In this model, cleavage always occurs immediately 5′ to the anchor duplex, and this helix is continuously extended. It implies a processivity leading to the complete editing of a sequence block. Junctions containing unexpected sequence result in this model either from misediting by noncognate gRNAs or from the formation of upstream duplexes ("secondary anchors") with cognate gRNAs. As an alternate means to explain junction regions, Koslowsky et al. (50) suggested that junctions reflect a dynamic interaction between the gRNA and pre-mRNA. This model suggests that ESs are selected by endonucleolytic cleavage 5′ to the gRNA/pre-mRNA duplex but that the apparent order of processing is not strictly 3′ to 5′ because the gRNA interacts with the pre-mRNA (or partially edited RNAs) in such a way as to maximize the thermodynamic stability of the duplex. Initial base pairing may not be the same as that predicted by the final sequence, but realignment of the gRNA and partially edited RNAs progressively drive editing to this pairing and consequently to the

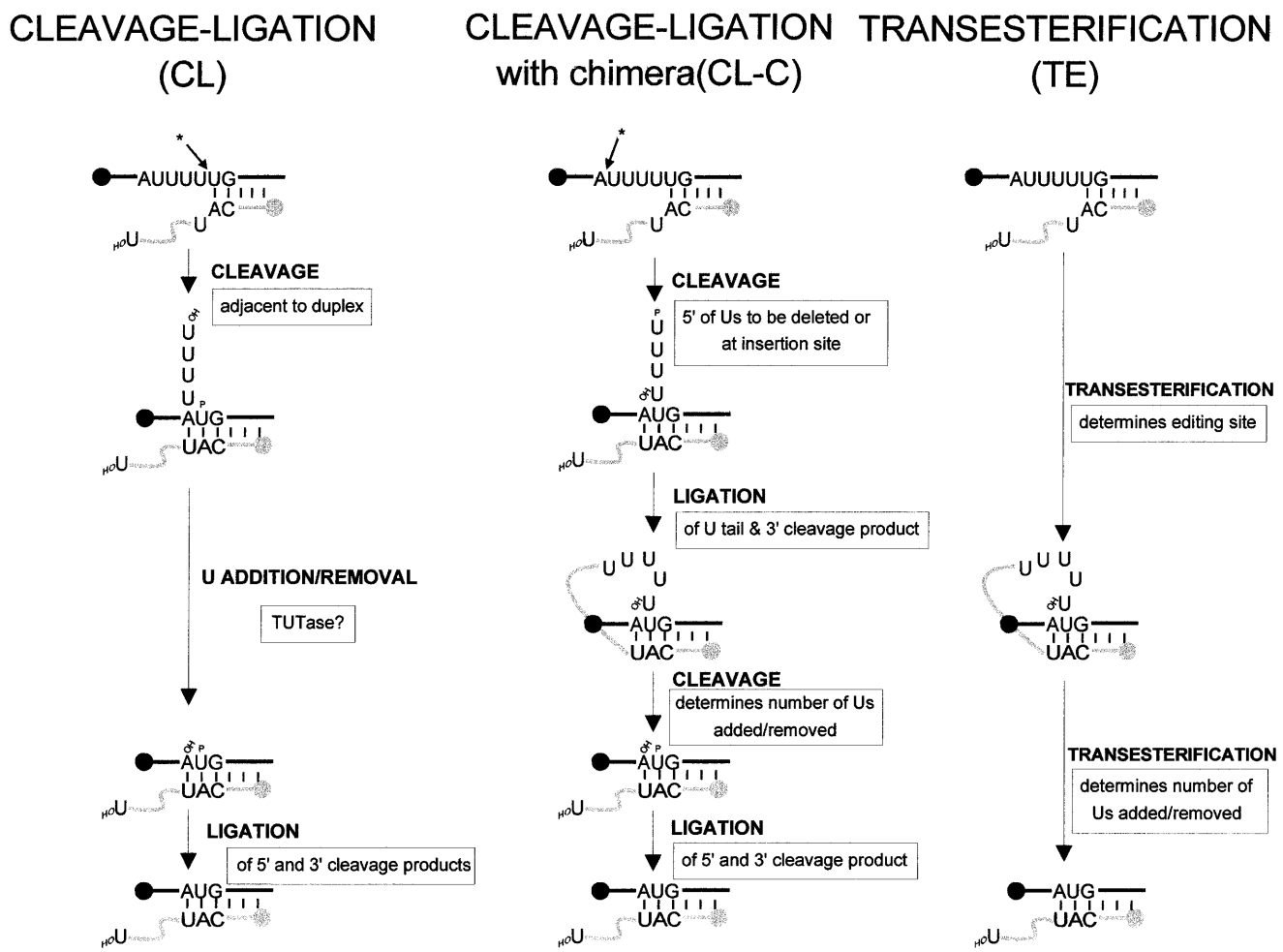

FIG. 2. Theoretical models for the mechanism of RNA editing. Three prevalent models for editing are depicted by using U deletion as an example. Pre-mRNA and gRNA (black and gray lines, respectively), reaction steps (arrows), and endonucleolytic cleavage sites (asterisks) are indicated. Boxes indicate distinguishing characteristics of the various models.

sequence of the mature mRNA. This model is supported by examination of the interactions predicted between partially edited RNAs and their cognate gRNAs. Current in vitro analysis is consistent with both of the last two models for editing site selection. Since these processes are not mutually exclusive, both could operate in vivo.

MODELS FOR RNA EDITING

Overview

Three general models have been proposed for the mechanism of editing (Fig. 2). In the cleavage-ligation (CL) model, Blum et al. (15) proposed a series of steps that are catalyzed by the protein enzymes endoribonuclease, TUTase, and RNA ligase. These activities have been demonstrated in the mt of kinetoplastids (7, 41, 71). Enzyme-catalyzed steps are also part of the cleavage-ligation/chimera (CL-C) model proposed by Sollner-Webb (99), and gRNA/mRNA chimeric molecules (chimeras), which have been detected in cellular RNA, are purported editing intermediates. The transesterification (TE) model, proposed independently by Blum et al. (18) and Cech (22), suggests an entirely different biochemical reaction pathway in which the 3′-hydroxyl groups of the reactant RNAs act as nucleophiles, analogous to the role of hydroxyl groups in intron excision. It suggests that a cycle of editing occurs by two successive transesterifications in which a first reaction forms gRNA/mRNA chimeras, which are resolved by a second transesterification. Each of these three models can readily explain both the U insertion and deletion that occur in RNA editing. Importantly, each model has distinguishing characteristics that allow discrimination among them.

Cleavage-Ligation Models

The CL model proposes that (i) endoribonuclease cleaves mRNA immediately 5′ to the gRNA/mRNA duplex, (ii) U's come from or go to the free nucleotide pool, (iii) U's are added or deleted at the 3′ end of the 5′-cleavage product, which is then (iv) religated with the 3′-cleavage product. This model predicts that the ESs are determined by the site of pre-mRNA cleavage, which is the same regardless of whether editing inserts or removes U's (i.e., immediately 5′ relative to the gRNA/mRNA duplex). It also predicts that the U's are derived from or go to the nucleotide pool, and the number of U's present in the mature mRNA is specified by the gRNA at the time of their insertion/deletion or mRNA religation.

The CL-C model proposes that (i) the mRNA is cleaved 5′ to the gRNA/mRNA duplex when editing inserts U's but is

cleaved 5′ to the U's at the ES when editing removes them, (ii) a first ligation creates a chimeric intermediate, (iii) a second cleavage cuts the chimera, resulting in U addition to the 3′-cleavage fragment of the mRNA or U deletion from this fragment with the uridylates coming from or going to the U tail of the gRNA, respectively, and (iv) a second ligation rejoins the 5′ and 3′ fragments of the mRNA. This model has several predictions that differ from those of the CL model. The ES is determined by the initial cleavage, but, unlike in the CL model, the site of cleavage differs when editing results in U insertion versus deletion. In addition, cleavage of the chimera determines the number of U's inserted or deleted, and the U's are derived from or go to the U tail of gRNA.

Transesterification Model

The TE model proposes (i) that the 3′ hydroxyl of the 3′-terminal U of gRNA attacks the phosphodiester bond at the ES in a first transesterification reaction to produce a gRNA/mRNA chimera and a 5′ cleavage product of the mRNA and (ii) a pseudoreversal of the transesterification reaction occurs in which the 3′-hydroxyl group of the 3′-terminal nucleotide of the 5′ cleavage product attacks a phosphodiester bond in the chimera at a site that results in insertion or deletion of U's and rejoining the 5′ and 3′ portions of the mRNA. The TE model predicts a chimeric intermediate, as does the CL-C model, although transesterification accomplishes in a single step what cleavage-ligation does in two steps. The TE model further predicts that the site of editing is selected by the 3′-hydroxyl attack and that this site would differ between the two types of editing resulting in insertion versus deletion, since the model also postulates that the U's are derived from and go to the U tail of the gRNA. Unlike the CL-C model, the TE model predicts that no 3′-cleavage product of the mRNA would occur. The common feature of both the CL-C and TE models is that chimeras are obligate intermediates. The TE model has the attractive features that it has low energy requirements, the precedent for the transesterification reaction in splicing is well established, and chimeras have been demonstrated in cellular RNA (see below). On the other hand, the detection of RNA ligase activity in kinetoplastid mt, as well as the TUTase and endoribonuclease activities, suggests that these enzymes may play a role in editing. The distinctive characteristics of the models have allowed experimental discrimination among them (see below).

RNAs PRODUCED IN VIVO

Chimeras

The discovery of gRNAs was soon followed by the discovery by Blum et al. (18) of gRNA/mRNA chimeras, which were subsequently proposed as editing intermediates in the CL-C and TE models. Chimeras are molecules in which gRNAs are covalently linked via a stretch of oligo(U) to 3′ regions of mRNA, usually at a site which is edited. The gRNA portion is usually linked to the 5′ end of an mRNA region that is completely edited, although a small fraction of chimeras have one or more sites in the mRNA portion which are not edited to the mature mRNA sequence. The length of the oligo(U) region linking the gRNA and mRNA portions varies between 0 and 15 residues for *T. brucei* (75), between 0 and 19 residues for *C. fasciculata* (5), and between 0 and 26 residues for *L. tarentolae* (18). Many chimeras also have the peculiar characteristic that the gRNA portion of the chimera is truncated with respect to free gRNA (5, 75). Chimeras with gRNAs linked at unexpected sites in mRNAs have also been reported (5).

Chimeras are low in abundance in cellular RNA and have been detected only after amplification by PCR. Chimeras for the most-3′ editing domain of cytochrome *b* (CY*b*) mRNA have been quantified in bloodstream and procyclic forms of *T. brucei*, where the editing of this mRNA is developmentally regulated (32, 81). The steady-state levels of CY*b* pre-mRNA, mature mRNA, and gRNAs were also determined in the same samples. Chimeras are rare, with one molecule per few hundred cells, and they are 3 to 4 orders of magnitude less abundant than the CY*b* gRNAs. Thus, if chimeras are editing intermediates, they must have short half-lives. The gRNAs and CY*b* preedited mRNA are similar in abundance between life cycle stages and similar in abundance to each other. Edited CY*b* mRNA, however, is 1 to 2 orders of magnitude more abundant in procyclic forms than in bloodstream forms, as, interestingly, are CY*b* chimeras. This correlation suggests that chimeras may result from the editing process, but it does not distinguish between their being short-lived intermediates or rare nonproductive end products.

Cleavage Products

Molecules that may represent pre-mRNA cleavage products have been reported (1, 25, 28, 31, 75, 111). These correspond to the 5′-cleavage products that would be expected to occur for any of the mechanisms for RNA editing discussed above and 3′-cleavage products that would result from either the CL or the CL-C models. An obvious concern with such molecules is that they may not represent intermediates in the editing process but may have resulted from shearing, nucleolytic cleavage, or premature termination of reverse transcription that occurred during experimental manipulation. Similarly, partially edited RNAs have been suggested as abortive editing products. While this possibility formally exists, the great abundance of such molecules and the fact that only about 1% of partially edited RNAs in *T. brucei* do not have junctions (i.e., they transit from fully edited to unedited sequence) suggest that such molecules are in the process of being edited.

IN VITRO SYSTEMS

There have been numerous attempts to reproduce kRNA editing in vitro almost from the time of its discovery. These studies explored the overall incorporation of U's into endogenous and exogenous RNAs, pre-mRNA cleavage and chimera formation as potential editing steps, and gRNA-directed U deletion and insertion at specific editing sites.

Early Studies

Harris et al. (43) demonstrated the incorporation of radiolabeled UTP into endogenous mt mRNAs in *T. brucei* by isolated mt vesicles that had been preincubated to deplete the endogenous nucleotide pool. Preincubation apparently blocked transcription since U's, A's, and C's but not G's were individually incorporated. Endogenous RNA which was radiolabelled with UTP preferentially hybridized to DNA encoding preedited RNAs, indicating that the incorporation of U's reflected a posttranscriptional process related to RNA editing. In similar experiments with mt extracts from *L. tarentolae*, Frech et al. (33) observed the incorporation of U's into exogenously added CY*b* and NADH dehydrogenase subunit 7 (ND7) pre-mRNAs but not into several RNAs that are not edited in vivo. Incorporation was primarily at the 3′ end of the RNAs but also occurred at low efficiency at multiple sites within regions of transcripts where editing occurs in vivo. Analysis of the products by oligonucleotide-directed RNase H cleavage allowed for

differentiation between internal and 3′-terminal U incorporation. Nearest-neighbor analysis showed that the internal incorporation was inconsistent with complete precise editing but also implied nonrandom insertion, perhaps reflecting the partially edited junctions observed in partially edited cellular RNAs (see above).

The restriction of the incorporation to specific regions and classes of RNA resembles in vivo RNA editing, but other important features do not. The incorporation is not restricted to U's, which is not a feature of in vivo editing. The incorporation into endogenous RNAs of A's and C's (43) in *T. brucei* mt was only 5% as efficient as U incorporation and may reflect mt mRNA polyadenylation and/or CCA addition to tRNAs (43); however, these possibilities were not assessed. The C incorporation into exogenous pre-mRNAs in *L. tarentolae* (33) may reflect incomplete specificity for U, especially in vitro, of the enzymes catalyzing this process. This could be analogous to poly(A) polymerase, which incorporates nucleotides other than A into RNA with low efficiency in vitro (112). The bulk of the U incorporation at RNA termini is probably due to TUTase activity that is presumed to add the 3′-oligo(U) tail to gRNA (besides its possible role in the actual insertion RNA editing). Low-level U incorporation into ND5 mRNA, which is not edited in vivo, and U insertion into CY*b* pre-mRNAs lacking the anchor sequence at >40% of the level seen with wild-type RNA reveal an important lack of specificity. This incorporation may reflect RNA secondary structures that do not occur in vivo but which may mimic the gRNA/mRNA interaction in vitro, as discussed below.

The utility of the studies described above for biochemical analyses of RNA editing is severely restricted, since in the in organello system (43) only endogenous RNAs are amenable to analysis and the U incorporation in *L. tarentolae* (33) lacks specificity. Furthermore, the latter system is not dependent on exogenous cognate gRNA; rather, equimolar amounts of added gRNA (or, indeed, unrelated RNAs) dramatically inhibit the U incorporation. Thus, it is difficult to use this system to study the mechanism of RNA editing, since only part of the observed activity may represent the genuine RNA-processing pathway.

Partial Reactions

Preedited RNA cleavage. The characterization of the specificity of editing substrate cleavage by mt nuclease and the formation of gRNA/mRNA chimeras have been explored in vitro since they were proposed intermediates in the editing process. Synthetic pre-mRNAs spanning the editing domains of CY*b*, COII, and COIII transcripts were cleaved at or near editing sites upon incubation with *T. brucei* mt extracts, but edited versions of the same RNAs were not so cleaved (41). Importantly, while these cleavages occur in the editing domain, they are not dependent on exogenous gRNA. This led to the suggestion that the initial recognition of editing domains might depend on pre-mRNA secondary structure and not on the cognate gRNA (67). This hypothesis is supported by the production of a similar pattern of nucleolytic cleavages by single-strand-specific mung bean nuclease (68). An alternate view is that the intramolecular structures formed by unedited molecules may mimic the gRNA/mRNA duplexes that direct the specific cleavage of editing substrate RNA (90). Studies with the gRNA-dependent in vitro editing system have shown that nuclease cleavage is specified by the gRNA interaction with mRNA, with the site of cleavage immediately 5′ to the anchor duplex (see below) (47, 90). However, the potential for formation of other gRNA/mRNA duplexes outside the anchor region (50, 106) might direct the cleavage (and hence the editing) at other sites.

Chimeras. Chimeras between exogenous pre-mRNA and exogenous or endogenous gRNA were shown to form in mt extracts from *T. brucei* (42, 51), *L. tarentolae* (17), and *C. fasciculata* (4). Chimera formation requires the ability to form an anchor duplex (4, 17), a characteristic that is expected for editing. Blockage of the 3′-hydroxyl group of the gRNA by pNp or periodate oxidation prevents the formation of chimeras, which requires hydrolysis of the α-β bond of ATP (42, 51, 82, 83). The chimeras that are formed in vitro appear to result from successive cleavage-ligation reactions. Inhibition of pre-mRNA cleavage in the presence of the phosphorothioate Rp stereoisomer at the ES also abolishes chimera formation (82). In addition, the 3′ cleavage product of the pre-mRNA formed in mt extracts carries a 5′ monophosphate and could therefore serve as a substrate for ligation to the 3′ end of gRNAs catalyzed by RNA ligase (67, 89, 90). In fact, the 3′-cleavage product is approximately 60-fold better as a substrate for chimera formation than is intact pre-mRNA (82). Finally, chimera formation cofractionates with RNA ligase activity, and both activities are inhibited by ATP analogs with nonhydrolyzable α-β bonds, indicating RNA ligase involvement (82, 83). While the general mechanism of chimera formation suggests a cleavage-ligation pathway, the relationship of this process to RNA editing is uncertain. Chimera formation is clearly gRNA dependent, but it may just reflect efficient creation of the pre-mRNA 3′-cleavage fragments and their fortuitous ligation to gRNA. This is evident from the ability to form chimeras in vitro with heterologous RNA ligase (68). Moreover, if chimeras are not editing intermediates, all aspects of the mechanisms of their formation in vitro may not be relevant to the mechanism of editing. However, the correlation of their formation with developmentally regulated editing suggests that they may arise from editing-related processes. Interestingly, a small fraction of chimeras formed in vitro from exogenous RNAs have edited sequence (4, 51, 52). This suggests that the extracts used in these studies may be competent to edit, albeit at low efficiency. However, since direct evidence for a conversion from chimeras to edited product is missing, the significance of these molecules as intermediates in the kRNA editing process is not evident.

U insertion by successive steps of cleavage and ligation. The ability of mt extracts to perform successive reactions of pre-mRNA cleavage, chimera formation, chimera cleavage, and ligation of the resultant products was explored by Piller et al. (69). Most chimeras formed after coincubation of CY*b* pre-mRNA and its cognate gRNA, gCY*b*[558], in *T. brucei* mt extract had gRNA/mRNA linkages at ES2 with various numbers of U's, which is consistent with previous in vitro cleavage results (41, 67). A synthetic RNA modeled after such chimeras with 15 U's at the gRNA/mRNA junction at ES2 was found to be cleaved within the oligo(U) tract upon incubation in mt extract. Isolated 3′-cleavage product (the mRNA portion of the chimera) was shown to ligate with isolated 5′-cleavage product of the CY*b* pre-mRNA. The resultant ligated RNAs had about eight or nine U's at ES2 which are derived from the oligo(U) tract of the synthetic chimera. Similar results were obtained when the single-strand-specific mung bean nuclease and T4 RNA ligase were used instead of the mitochondrial extract. The authors suggested that the extract nuclease is structure specific and recognizes single-stranded regions as does mung bean nuclease. Although the final RNAs resemble partially edited molecules found in vivo, these experiments do not mirror some aspects of in vivo editing. The ligation step that produces the final RNA does not require gRNA, and this final product does not contain the number of U's predicted by

the gRNA (i.e., one U) at ES2. The inserted U's are derived from the oligo(U) tract that corresponds to that arising from the gRNA U tail upon chimera formation. Direct analyses of editing, described below, in which gRNA specifies the number of inserted (or deleted) U's in a concerted series of reactions during a single incubation show that added U's are derived from free UTP and that chimeras are probably aberrant side products of editing rather than intermediates (47, 90). Thus, this series of separate in vitro reactions does not appear to parallel essential elements of editing in vivo.

gRNA-Specified In Vitro Editing

A site-specific, gRNA-dependent editing system was recently developed by using exogenous RNAs modeled on *T. brucei* A6 preedited mRNA and the gRNA, gA6[14], for its most 3′ edited region. Seiwert and Stuart (91) initially demonstrated in vitro generation of RNA which was edited by the deletion of two U's as in cellular RNA. They used the indirect analysis of dideoxynucleotide-terminated reverse transcription products and also sequenced cloned cDNAs. Slight modifications of the reaction conditions allowed Seiwert et al. (90) to observe deletion editing at ES1 by direct analysis of the fate of radiolabeled input pre-mRNA. The editing required pre-mRNA, the cognate gRNA, mt extract, magnesium, and ATP with hydrolysis of its α-β bond (89). Mutations of the gRNA (or mRNA) sequence resulted in alterations in the number of U's deleted in a fashion that was predictable from the gRNA pre-mRNA interactions (90, 91). Thus, these experiments showed that the number of U's deleted depended on the sequence of the gRNA, directly demonstrating that gRNAs can specify the edited sequence as previously proposed (15, 16). Kable et al. (47) adjusted this in vitro system so that the pre-mRNA is edited at ES1 and consequently investigated U insertion editing at ES2. This insertion editing had the same requirements as deletion editing but in addition required UTP. Again, mutation of the number of guiding nucleotides in the gRNA resulted in compensatory changes in the number of U's inserted into the editing site. Insertion editing was also observed with a substrate RNA based on the *C. fasciculata* ND7 pre-mRNA (47), showing that in vitro editing is not restricted to a specific gRNA/pre-mRNA pair. Thus, this in vitro editing system meets all criteria expected for RNA editing.

The availability of these in vitro systems allowed the different models for RNA editing to be tested directly for the first time. The in vitro reactions which result in RNA edited by U insertion or deletion generate pre-mRNA cleavage products and chimeric molecules (47, 90). Formation of a 3′-cleavage product itself speaks against the TE mechanism for RNA editing involving chimeras, since the 3′ part of the preedited RNA should never be produced during the course of this pathway (Fig. 2). Time course experiments with 3′-end-labeled pre-mRNA furthermore revealed that the 3′-cleavage products were formed before both edited product and chimeras. Chimeric molecules continued to accumulate during the course of the reactions and therefore behaved as end products rather than as intermediates. The 3′-cleavage product, however, reached steady-state levels early in the incubation and probably represents an intermediate in the formation of both chimeras and edited product. Analysis of the cleavage site in the pre-mRNA provided further insight into the mechanistic background for in vitro RNA editing. Cleavage occurred immediately 5′ to the gRNA/pre-mRNA duplex in reactions leading to U insertion or deletion. A mutant gRNA which predicts an extended anchor duplex consequently resulted in a shifted cleavage position on the pre-mRNA (90). This position of pre-mRNA cleavage is consistent with the CL and the CL-C models in the case of U insertion, but for U deletion the CL-C model predicts that cleavage occurs 5′ to the U's to be deleted and not immediately adjacent to the anchor duplex (see above and Fig. 2). Therefore, the U's destined for deletion do not seem to be transferred to the U tail of the gRNA but obviously have to be removed from the 3′ end of the 5′-cleavage product. Indeed, analysis of 5′-labeled pre-RNA undergoing U deletion revealed a staggered set of RNA species with sizes expected for the initial 5′-cleavage products and incremental deletion of U's from its 3′ end (90).

While an attractive feature of the mechanistic models involving chimeric intermediates is the function of the gRNA oligo(U) tail as a repository for deleted and inserted U's, gRNAs with oligo(U) tails are not sufficient for in vitro editing that results in U insertion. Rather, UTP must be included. The demonstration of the site-specific incorporation of [α-^{32}P]UTP into edited mRNA in a gRNA-dependent manner ruled out the possibility that UTP was only a cofactor (47). Since dynamic exchanges between the gRNA U tail and the nucleotide pool have not been investigated, the remote possibility remains that the U's are shuttled via the gRNA U tail before incorporation into the substrate RNA. This seems unlikely because the investigation of the U insertion reaction with 5′-labeled substrate RNA suggested that the U's are added to the 3′ end of the 5′-cleavage product, just as predicted by the CL model (Fig. 1). Taken together, the mechanistic studies with the in vitro system resulting in U deletion and insertion strongly suggest that editing occurs by the CL mechanism. The significance of chimeras in this context is discussed below.

Studies of chimera formation also provide information relevant to the mechanism of U insertion and deletion, since chimeras are probably formed by the same machinery and processes which catalyze editing. Indeed, chimeras are formed in vitro by successive cleavage-ligation steps rather than transesterification reactions (69, 82, 83). Furthermore, the in vitro U incorporation activity in *Leishmania* mt extracts (33) has stereochemical characteristics which can easily be reconciled only with the CL model (34).

SUMMARY MODEL AND UNANSWERED QUESTIONS

Summary Model

RNA editing appears to occur by the CL mechanism based on the data outlined above (Fig. 3). The preedited RNA and gRNA associate with each other by formation of the anchor duplex (and perhaps other interactions entailing the gRNA U tail) and with components of the editing machinery. Endoribonuclease cleaves the pre-mRNA after this assembly, leaving a 5′ phosphate on the 3′-cleavage product. The cleavage appears to be directed by the gRNA interaction with mRNA and is adjacent to the anchor duplex or the extended anchor duplex in the case of editing of ES1 and ES2 of A6 pre-mRNA, respectively. gRNA-specified cleavages of the pre-mRNA for editing of subsequent sites are directed either adjacent to the extended anchor duplex sequence or at a site further 5′ that is adjacent to a duplex which results from dynamic interactions between the two RNAs (50) and has also been called a secondary anchor (106). U's are added to or removed from the 3′ end of the 5′-cleavage product, potentially by TUTase, although this needs to be investigated. Site-specific labeling of phosphates at or near the ES shows that deleted U's are released to the nucleotide pool (87), and, similarly, studies with radiolabeled UTP show that inserted U's are derived from the

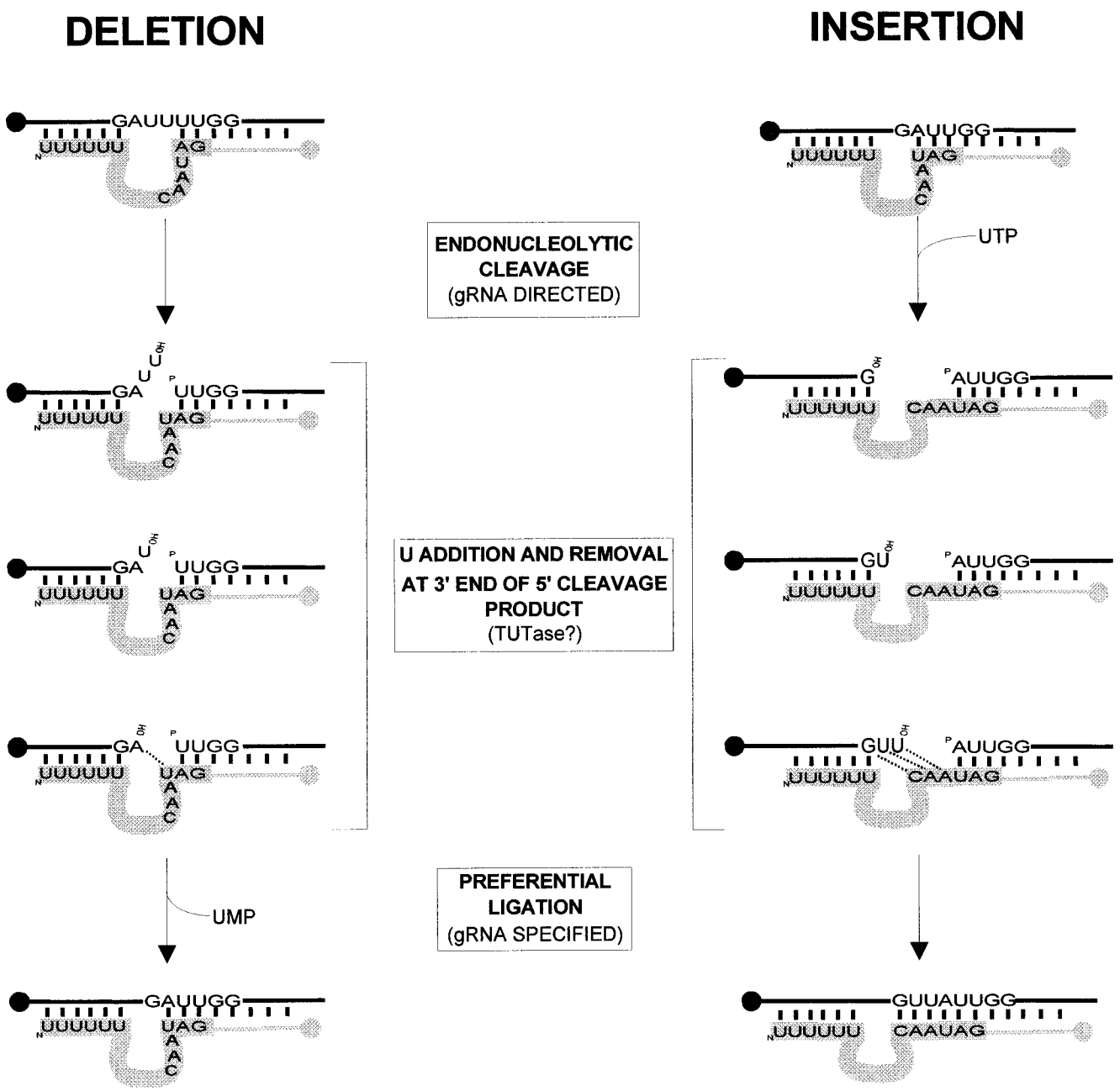

FIG. 3. Experimentally determined models for U insertion and deletion. Pre-mRNA and gRNA (black and gray lines, respectively) and reaction steps (arrows and boxes) are indicated. Cleavage occurs immediately 5′ (with respect to the pre-mRNA) of the anchor duplex and produces a 5′ product with a 3′ hydroxyl and a 3′ product with a 5′ phosphate. TUTase and/or a U-specific 3′ exonuclease adds and removes U's from the 3′ hydroxyl of the 5′-cleavage product. Free UTP is the source of U's during U insertion, while deleted U's are released as free UMP. gRNA does not specify the number of U's added to or deleted from the 5′-cleavage product but preferentially selects the 5′-cleavage product with the specified number of U's for ligation to the 3′-cleavage product. gRNA also appears to interact with, and hold in place, the 5′-cleavage product through an interaction with its 3′ oligo(U) tail.

nucleotide pool (47). The number of U's added or deleted at the 3′ end of the 5′-cleavage product does not appear to be directly specified by the gRNA sequence, since 5′-cleavage products with a range of added or deleted U's, depending on gRNA, are observed (47, 90). Perhaps there is a dynamic equilibrium between U addition and deletion. Furthermore, while most RNAs edited in vitro have the number of U's inserted that are specified by the gRNA, a small fraction have more U's inserted (44). This suggests that the gRNA preferentially directs the ligation of the 5′-cleavage products which have the number of added or deleted U's specified by the gRNA with the 3′-cleavage product of the pre-mRNA. This may occur by creating a ligatable substrate by splinting the two cleavage products and/or positioning in the active site of the RNA ligase.

Significance of Chimeras

Chimeras appear to be products of in vitro editing rather than intermediates, as described above (Fig. 4). The propor-

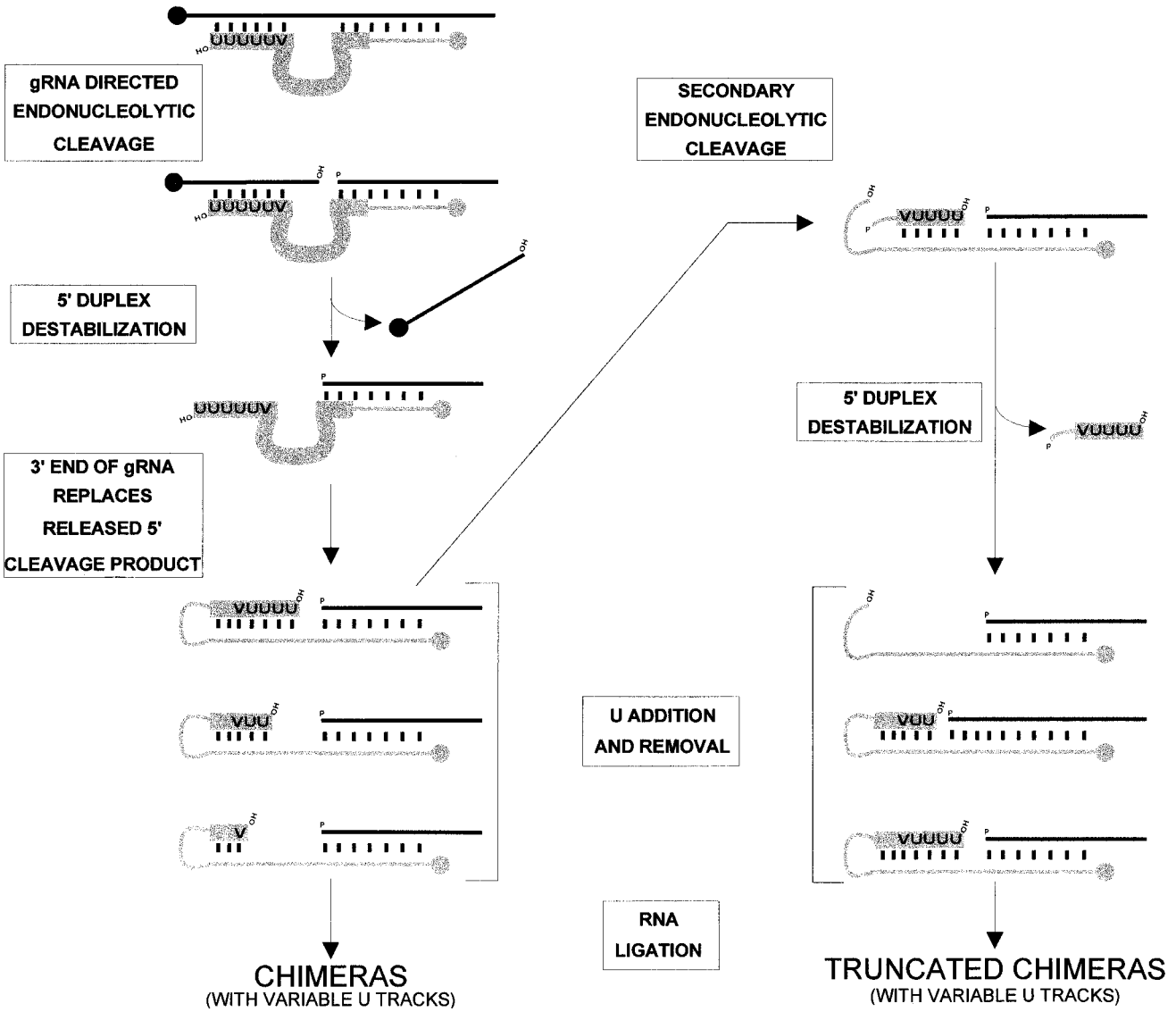

FIG. 4. Experimentally determined model for chimera production. Pre-mRNA and gRNA (black and gray lines, respectively) and reaction steps (arrows and boxes) are indicated. As with U insertion and deletion reactions, chimera formation requires pre-mRNA cleavage. Unlike the case with U insertion and deletion, however, the interaction between the 5′-cleavage product and the oligo(U) tail of the gRNA is destabilized in the pathway leading to chimeras. The 3′ oligo(U) tail of the gRNA then occupies the position of the 5′-cleavage product and could be acted upon by TUTase or 3′ exonuclease. Subsequent RNA ligation produces chimeras with variable numbers of U residues linking gRNA and pre-mRNA. If the gRNA/pre-mRNA duplex undergoes subsequent cleavage in the gRNA portion, followed by TUTase/3′ exonuclease and RNA ligase action, truncated chimeras would be produced.

tion of chimeras relative to edited RNA is a function of the predicted interactions between the 3′ portion of the gRNA and the 5′-cleavage fragment of the pre-mRNA (90). Deletion of the 3′ U tail of the gRNA or additional 3′ sequence enhances the production of chimeras relative to edited RNA. Conversely, a mutation of the 3′ part of the gRNA which predicts a more stable interaction with the 5′ cleavage fragment essentially eliminates the accumulation of chimeras without abolishing the production of edited RNA. Thus, chimeras appear to result from the destabilization of the interaction between the 3′ end of the gRNA and the 5′-cleavage product of pre-mRNA. It has been proposed that during chimera formation the 3′ end of the gRNA assumes the position in the editing machinery normally occupied by the 5′-cleavage product of pre-mRNA and is ligated with the 3′-cleavage product of the pre-mRNA (88, 90). The 3′ region of the gRNA is therefore likely to be accessible to nuclease and U addition and deletion activities, which could account for chimeras with truncated gRNA portions and variation in the length of the oligo(U) stretch linking the gRNA with the pre-mRNA frequently found in vivo and formed in vitro (4, 5, 17, 47, 51, 69, 75). This suggests that the chimeras present in the living cell, which are very low in abundance, are aberrant end products. It also lends support to the proposal by Blum et al. that the U tail of the gRNA might serve to stabilize the interaction between the gRNA and pre-mRNA 5′ to the editing sites (16).

The 3′ Hydroxyl of gRNA

The significance of the 3′-terminal hydroxyl of the gRNA U tail to the RNA editing process is unresolved. Replacement of this hydroxyl with a phosphate by ligation of pUp or the oxi-

dation of both terminal hydroxyls to ketone groups by periodate (along with scission of the 2′-3′ intercarbon bond of the ribose) prevents in vitro deletion editing (90). It also prevents chimera formation, as expected, since RNA ligase requires a 3′-terminal hydroxyl. These results imply that the 3′ hydroxyl of the gRNA can affect RNA editing. There are two general explanations for this blocking effect: the inhibition may reflect direct participation of the 3′ hydroxyl in the editing reactions (e.g., as an attacking group), or it may reflect an indirect effect on editing. A possible indirect effect is interference with binding of a component of the editing machinery to the gRNA. This is conceivable since a 3′ phosphate is bulkier than the hydroxyl and adds two negative charges and since the cleaved ribose with 2′ and 3′ ketones resulting from periodate treatment has large structural differences from the untreated 3′-terminal nucleotide, hence possibly sterically hindering association with a protein factor. In fact, cross-linking of a 124-kDa protein to gRNA has been suggested to require its 2′- and 3′-hydroxyl groups (48). The influence of the 3′ hydroxyl of the gRNA on editing requires further study.

COMPLEXES AND PROTEINS

Complexes

The models for RNA editing all imply the need for a diverse set of molecules for the successive rounds of editing. The CL and CL-C models predict a need for endoribonuclease, exoribonuclease, RNA helicase, RNA ligase, and TUTase activities, as well as molecules for RNA binding and positioning of active sites of the catalysts and perhaps RNA translocation in the complex. The TE model does not require nuclease or ligase but predicts a need for TUTase (to add the U tail to gRNAs), RNA helicase, and the binding and positioning components. Additional molecules may be present to mediate the developmental regulation of RNA editing. Most, if not all, of these components are proteins, since in vitro editing is inhibited by proteases. However, the presence of other components, such as RNA, cannot be excluded despite the insensitivity of in vitro RNA editing to micrococcal nuclease (86).

One approach to search for the components of editing machinery has been sedimentation analysis. Pollard et al. (71) found that in *T. brucei*, the gRNA, preedited mRNA, RNA ligase, and chimera-forming activities sedimented as two broad peaks centered at 19S and 35 to 40S in isokinetic glycerol gradients. TUTase activity and the bulk of the preedited mRNA sedimented primarily with a peak at 35 to 40S. A nuclease activity that cleaved preedited RNA was primarily in the 19S complex (39). Corell et al. (26) also found two broad peaks of editing-associated molecules and activities in *T. brucei* which centered around 20S and 40S. In vitro deletion editing activity sedimented at 20S, along with a portion of the RNA helicase, TUTase, and RNA ligase activities. Cellular A6 gRNA and preedited mRNA sedimented at slightly greater than 20S. The bulk of the RNA helicase, TUTase, and RNA ligase activities was at 40S, where most cellular edited A6 mRNA sedimented. Partially edited A6 RNAs were generally distributed between the two peaks. These studies provided strong circumstantial evidence that editing occurs in a macromolecular complex and that there may be two classes of complexes. Comparison of the results of Pollard et al. (71) and Corell et al. (26) shows differences in the relative proportions of the molecules and activities between the two peaks, which is probably due to the use of different strains, procedural differences, or examination of different cellular RNAs. Nevertheless, both studies detected two peaks of editing-related activities in *T. brucei.*

The relationship between the two complexes in *T. brucei* is unclear. Pollard et al. (71) speculated that the smaller complex functions as a maturation center, perhaps associating gRNA and mRNA, while the larger complex performs the catalysis. One alternative possibility is that the 20S complex performs deletion editing and the 40S complex performs insertion editing. The complexes need not be entirely distinct, since insertion editing may occur in association with a core complex that is capable of deletion editing. Association of an additional component (e.g., TUTase) results in the 40S complex. As another alternative, Corell et al. (26) suggested that the 20S complex may be able to edit exogenously added RNA because it is devoid of cellular gRNA and preedited mRNA. Thus, the larger (~40S) complex may then represent complexes that contain cellular gRNA and mRNA in various stages of editing and the range of sizes of the complexes may reflect the considerable size variation between different edited mRNAs.

Peris et al. (66) also used glycerol gradients as well as native and two-dimensional native and denaturing gel electrophoresis to characterize two classes of complexes that may be involved in RNA editing in *L. tarentolae*. One class (G) sedimented at 25S and contains gRNA and gRNA-independent U addition activity. The other (T) sedimented at 10S and contains gRNA, RNA ligase, and TUTase activity; it consists of about six RNP complexes which can incorporate [α-^{32}P]UTP into bound endogenous RNAs. The labeling of the RNA component in individual members of these classes is dependent on the UTP concentration (20). Peris et al. speculated that the T complexes are involved in 3′-terminal addition of U's to gRNA while the G complex is involved in editing. It remains to be determined if the differences between the complexes observed in *Leishmania* and *Trypanosoma* reflect fundamental differences between species or experimental differences.

Complexes that form in vitro by the association of gRNA and/or preedited mRNA with components of mt extracts have also been investigated. Four specific RNP complexes that form with gRNA (G1 to G4) (37, 76) or with preedited mRNA (M1 to M4) (52) have been characterized by nondenaturing gel electrophoresis. Formation of the G and M complexes is specific to gRNA or mRNA, respectively, since gRNAs (homologous or nonhomologous to the probe) and mRNAs block their formation while a variety of nonspecific RNAs do not. Read et al. (76) also showed that preedited RNA that corresponds to the gRNA is required for only a subcomponent of G1 and enhances the formation of G1 to G4. The authors hypothesized that G1 to G4 represent assembly precursors of the editing machinery. This is based in part on the observation that extract components that sediment at 10S to 20S form the G1 to G3 complexes; interestingly, G4 and fractions capable of forming G4 sedimented at about 40S (26).

Proteins

Proteins that may play a role in editing have been studied by cross-linking, analysis of catalytic activities, and gene cloning and sequencing (Table 2). Read et al. (76) and Köller et al. (48) found that gRNA cross-links with several *T. brucei* proteins upon UV irradiation under conditions that support in vitro editing. Competition studies involving a large molar excess of heterologous RNAs revealed stable cross-linking with 25- and 90-kDa proteins. Cross-linking with the latter protein was eliminated when a gRNA devoid of the U tail was used, indicating a role of this element in the binding of this protein. The 25- and 90-kDa proteins were found by cross-linking and

TABLE 2. Proteins involved in editing

T. brucei proteins			*L. tarentolae* proteins			*C. fasciculata* proteins		
Size (kDa)	Properties[a]	Reference(s)	Size (kDa)	Properties[a]	Reference	Size (kDa)	Properties[a]	Reference
210	a,n	76	110	h	19	88	a,d	55
135	a,m,n	76	94	h	19	65	a,c	55
115–124	a,e,l,m,n	48, 76	75	h	19	30	a,d	55
83–90	a,b,c,f,l,m,n	48, 55, 76	51	j	19			
64–68	a,c,f,n	48, 55, 76	46	h	19			
55–62	a,c,m,n	83	36	h	19			
57	i	83	25	h	19			
50	i	48, 55, 76	18	h	19			
42–45	a,c,l,n,	48, 55, 76						
34–37	a,n	48, 76						
21–25	a,b,g,k,l,m,n	48, 55, 76						
9	a	48						

[a] The properties are as follows: a, UV cross-linking to gRNAs; b, UV cross-linking stable to RNA competition; c, UV cross-linking requires oligo(U) tail; d, UV cross-linking to RNAs with long (>39-nt) tracks of U residues and/or to poly(C) RNA; e, UV cross-linking sensitive to vanadyl ribonucleosides; f, same cross-linking specificity as 65-kDa protein; g, same cross-linking specificity as 88- and 30-kDa proteins; h, UV cross-linking to endogenous RNAs (T complexes); i, autoadenylation with exogenous ATP; j, aldehyde dehydrogenase comigration with T complexes; k, found in native complex G1; l, found in native complex G2; m, found in native complex G3; n, found in native complex G4.

sodium dodecyl sulfate-polyacrylamide gel electrophoresis at just under 20S in glycerol gradients when gRNA was added to the mt extracts before fractionation (26). However, when gRNA was added directly to glycerol gradient fractions, the 25- and 90-kDa proteins were found in <10S and 40S fractions, respectively. In addition, a 65-kDa cross-linking protein, which became prominent after fractionation, was found at ~20S, although its distribution was broad when gRNA was added directly to glycerol gradient fractions. The difference in the location and detection of the cross-linking proteins when gRNA was added before and after glycerol gradient fractionation could reflect various factors. These may include requirements for interactions among molecules, the abundance of gRNA binding proteins, and the presence of molecules that inhibit binding and/or cross-linking. Nevertheless, three proteins appear to form stable specific associations and cross-link with gRNA and thus are candidate editing machinery components. Several other gRNA-cross-linking proteins form less stable interactions but may also be candidate components. A 21-kDa protein that appears to correspond to the 25-kDa gRNA-cross-linking protein has been purified and partially sequenced, and its gene has been cloned (35). The 21-kDa protein could be cross-linked to a variety of gRNAs, while unrelated control RNAs did not show this interaction (48, 76). Unlike the 90-kDa protein, the 25/21-kDa protein does not seem to interact with the oligo(U) tails of the gRNAs and therefore must recognize another feature common to these molecules. gRNAs adopt a simple double stem-loop structure in solution (84), which seems to provide the basis for interaction with the 25/21-kDa protein (35). Further study on this protein is anxiously awaited.

Leegwater et al. (55) found that 88-, 65-, and 30-kDa proteins cross-link to RNAs with poly(U) tracks in *C. fasciculata*. The 65-kDa protein appears to be fairly specific to 3′-terminal poly(U) sequences, while the 88- and 30-kDa proteins have affinity for other U-rich RNAs as well as for poly(C) tracks. Two *L. tarentolae* proteins with molecular masses of 18 and 51 kDa, which were identified as potential editing components due to their comigration with T complexes on native gels, have been purified, and their genes have been cloned (19). The gene for the 51-kDa protein has homology to mitochondrial aldehyde dehydrogenases, but the 18-kDa protein was not identified by database searches. Identification of the 51-kDa protein as a metabolic enzyme does not rule out its possible role in editing, since several other metabolic enzymes, such as glutamate dehydrogenase (74), have RNA binding capability. Further experiments need to be performed to determine the role of these proteins in editing. An overview of proteins suspected to play a role in RNA editing in different kinetoplastid species is given in Table 2.

POTENTIAL EDITING ACTIVITIES

Two catalytic activities that play a potential role in RNA editing are TUTase and RNA ligase. These activities were initially discovered in *T. brucei* by White and Borst (113) and were later shown to be localized in the mt (7). TUTase is likely to be responsible for the addition of the oligo(U) tails to gRNA. There has been no direct analysis of this possibility, let alone determination of how the U tail length is determined, although it is likely that this is not a templated or guided process. TUTase may also add the oligo(U) tails to mt rRNAs (2) and may be responsible for the sporadic presence of U's in the poly(A) tails of mt mRNA, although the latter may be due to poly(A) polymerase (12, 30, 110, 111). TUTase may also be responsible for addition of U's to the 3′ end of the 5′-cleavage product of mRNA that may be an editing intermediate. Recent work suggests that the gRNA does not control this addition step (see above), which is perhaps analogous to gRNA oligo(U) tail addition. TUTase may also remove U's from the 5′-cleavage product of mRNA and thus function in U deletion as well as in insertion in RNA editing. Perhaps the insertion and deletion activities result in a dynamic equilibrium in which the edited sequence is specified by the gRNA at the point where the number of specified added or deleted U's creates a ligatable substrate. TUTase has not been purified, but experiments in progress have substantially enriched the enzyme, and they indicate that the activity occurs as a macromolecular complex that sediments at 20S and 40S in *T. brucei* (3). However, the activity can also sediment as low as 10S after treatments that may strip away components (3), perhaps resembling the T complex described in *L. tarentolae* (66).

The RNA ligase activity generally cosediments with TUTase activity at 20S and 40S. Studies first performed by Sabatini and Hajduk (83) exploited the ATP activation of RNA ligase and demonstrated 50- and 57-kDa proteins in mt extracts that can

be adenylated with [α-^{32}P]ATP in vitro. These proteins cosediment and copurify with RNA ligase activity (26, 83). The proteins are deadenylated coupled with the release of AMP upon incubation with ligatable substrates, but incubation with nonligatable substrates results in the accumulation of adenylated products. Release of AMP was also found to be associated with the production of chimeras. Further studies indicated that α-β bond hydrolysis is required for RNA ligase and chimera-forming activities, as it has been shown to be needed for in vitro editing, supporting the role of this activity in RNA editing (82, 83, 91). An RNA helicase activity has also been detected in mt by Missel and Göringer (64), who hypothesize that the activity plays a role in the RNA unwinding needed for editing. These investigators have also cloned and sequenced an mt RNA helicase (36). Sedimentation studies show that the bulk of the protein is below 10S. Functional studies are in progress.

It is not surprising that nuclease activities are found in mt extracts. Nuclease activity that cleaves preedited RNAs has been reported to sediment below 10S and around 20S, but the actual location of the activities is confounded by the competing RNA ligase activity and variation among laboratories in assay conditions (68, 71). While these localizations examined gRNA-independent cleavage, the gRNA-dependent specific cleavage associated with deletion editing sedimented at 20S, along with the deletion-editing and chimera formation activity (87). This implies that the substrate for this enzyme is the gRNA/pre-mRNA pair. Further analysis of the activity is needed, since it appears essential that a nuclease function in RNA editing to cleave the pre-mRNA and not gRNA. As mentioned above, a 3′ exonuclease activity also appears to remove U's from the 3′ end of the cleaved mRNA.

Definitive identification of the editing machinery is needed. This will probably rely on the development of specific reagents such as cloned and expressed genes and specific antibodies that can be used in functional studies. Many laboratories have initiated the search for specific genes encoding proteins which include the 21-kDa gRNA binding protein (35), RNA helicase (36), and mt proteins selected for copurification with potential editing complexes (19). The likely functional studies will rely on affecting the in vitro editing and/or in vivo studies involving gene replacement or knockout. The genetic approaches of mutant complementation are conceivable, but kinetoplastids are not convenient for such studies.

FUNCTIONS OF EDITING AND EVOLUTIONARY IMPLICATIONS

While the discovery of RNA editing solved the coding peculiarities of kinetoplastid mt DNA, it raised the more puzzling problem of the rationale for its existence, which is counterintuitive. An assessment of the consequences of editing suggests that it may serve to regulate the production of the mt respiratory system during the course of the life cycle. In African trypanosomes, this helps to regulate the alternation between alternate oxidase (α-glycerophosphate oxidoreductase)-mediated terminal electron transport and cytochrome-mediated oxidative phosphorylation. This alternation occurs during the life cycle in which these organisms switch between relying on glycolysis for energy production and preferentially utilizing the Krebs cycle, although the final electron acceptor in both cases is oxygen (for a review, see reference 46). mRNAs encoding components of the cytochrome system are edited in the life cycle stage that employs oxidative phosphorylation, while mRNAs encoding components of respiratory complex I are preferentially edited in the life cycle stage relying on glycolysis (32, 104). This developmental regulation of editing is not apparent in other kinetoplastid genera, such as *Leishmania*, where this dramatic alternation between metabolic modes is not evident. However, laboratory strains of *Leishmania* have lost the ability to edit some mRNAs, including some of those encoding components of respiratory complex I (NADH ubiquinone oxidoreductase) (109). This shows that these mRNAs are not essential, at least in some life cycle stages, and perhaps implies that their production is regulated during the life cycle. Thus, RNA editing may function to regulate gene expression during the life cycle of these organisms and mediate the switch between two alternate modes of terminal electron transport. It may thus represent a primitive genetic regulatory system.

The mechanism by which editing is developmentally regulated is unknown. The gRNAs are present at similar abundance whether or not the corresponding pre-mRNAs are edited (81). There does appear to be a fewfold increase in unedited mRNA accumulation in steady-state RNA when it is preferentially edited, suggesting that transcription or posttranscriptional processes may regulate its abundance and hence its editing. Regulation of edited and unedited mRNA abundance, the potential for regulated cleavage of polycistronic pre-mRNA precursors (especially since many mt genes have overlapping sequences), and the differential poly(A) tail length regulation add complicating factors to determining how editing is regulated (14, 78). One possibility is that editing is regulated at the level of gRNA utilization (i.e., association with or use by the editing machinery). The gRNAs for the first region of editing of the developmentally regulated CY*b* are encoded in peculiar minicircle regions and may have extended sequences that might serve as recognition features for such control (80).

RNA editing appears to be retained by all kinetoplastids. Interestingly, the editing is more extensive in kinetoplastid groups that diverged early (e.g., *T. brucei* and *Trypanoplasma*) than in those that arose later in evolution based on the analysis of mt and nuclear rRNA sequences (54, 57, 59, 61). This suggests selective pressure to diminish the extent of editing. A retrotransposition model for how the extent of editing could be diminished has been proposed (95). The broader taxonomic distribution of RNA editing is unknown. It does not occur in the mt of other lower eukaryotes such as yeasts that have been examined in detail. However, these are very distant by criteria of sequence relatedness from kinetoplastids, as are most groups of lower eukaryotes. Perhaps analysis of more closely related protozoa such as *Euglena* or α-proteobacteria that may have a common ancestral lineage with the hypothetical endosymbiont that gave rise to mt will clarify this issue. An alternative possibility is that editing remains in many organisms but has evolved into very divergent processes. It has superficial similarities to RNA splicing, such as specific RNA interactions that entail mRNA cleavage. An intriguing possibility is that the genes encoding the editing machinery, which must be nuclear, have evolved to perform other RNA-processing functions that have yet to be discovered.

The origin of RNA editing is also a mystery. It is attractive to hypothesize that it originated in the era of the RNA genome. Primitive RNA editing may have played a role in controlling the storage and expression of genetic information prior to the genesis of DNA genomes. Alternatively, RNA editing may have allowed the rapid generation of sequence diversity though the combinatorial use of divergent gRNAs (88). In any event, it is likely that it originated in a much simpler form than currently exists and that it perhaps grew in complexity. Perhaps it achieved greater complexity and extent prior to development of the DNA genome, which introduced substantial evolutionary pressure to diminish the extent of editing. Its retention in

the mt of kinetoplastids may reflect its utility in regulating the expression of the mt genome and the central and perhaps very early importance of the mt respiratory system. The editing does have the property that it buffers mutations in an AT-rich genome, where insertions and deletions of T's, possibly by dimerization, can be overridden by the editing process and where gRNAs, which are multicopy and redundant, can ensure the production of the functional protein. It also allows considerable mt genomic mutation without altering the final protein sequence.

FUTURE WORK

There is still considerable work to be done on RNA editing. The catalysts, their active sites, and the chemistry of editing are yet to be identified and characterized. The process by which the editing of a region specified by a gRNA takes place is unknown. For example, does a gRNA remain associated with a region whose editing it specifies until all sites are edited, or does it dissociate after each site is edited? What is the mechanism for selection of the order of sites to be edited? Is all deletion completed before insertion is initiated? What is the cycle of events, beginning with the association of the mRNA, cognate gRNA, and editing machinery, through the various steps of editing? Are there specific characteristics associated with the initiation of editing of a transcript, and is this coupled to the transcription or processing of the transcript and/or gRNA transcripts? Similarly, are there special characteristics associated with completion of editing, and is this somehow coordinated with translation of the mature mRNA or at least transport to the sites of translation? Indeed, the demonstration that edited mRNA is translated is needed. Perhaps most of all, the editing machinery needs to be identified and characterized to find whether it is a single complex or several complexes that associate with each other and the RNAs and to determine the identity of its components and their specific functions.

PERSPECTIVE

The discovery of RNA editing was as enlightening as it was surprising. It was not intuitive that genetic information can be dispersed and decoded by RNA interactions, although the recognition of this process reveals the existence of a previously unrecognized level for the control of gene expression. The recognition of RNA editing has resulted in an expansion of the central dogma and a realization that there are multiple RNA-editing processes that play a role in normal physiological processes as well as being responsible for some diseases.

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REFERENCES

1. **Abraham, J. M., J. E. Feagin, and K. Stuart.** 1988. Characterization of cytochrome c oxidase III transcripts that are edited only in the 3′ region. Cell **55:**267–272.
2. **Adler, B. K., M. E. Harris, K. I. Bertrand, and S. L. Hajduk.** 1991. Modification of *Trypanosoma brucei* mitochondrial rRNA by posttranscriptional 3′ polyuridine tail formation. Mol. Cell. Biol. **11:**5878–5884.
3. **Allen, T. E.** Unpublished data.
4. **Arts, G. J., P. Sloof, and R. Benne.** 1995. A possible role for the guide RNA U-tail as a specificity determinant in formation of guide RNA-messenger RNA chimeras in mitochondrial extracts of *Crithidia fasciculata*. Mol. Biochem. Parasitol. **73:**211–222.
5. **Arts, G. J., H. Van der Spek, D. Speijer, J. Van den Burg, H. Van Steeg, P. Sloof, and R. Benne.** 1993. Implications of novel guide RNA features for the mechanism of RNA editing in *Crithidia fasciculata*. EMBO J. **12:**1523–1532.
6. **Avila, H. A., and L. Simpson.** 1995. Organization and complexity of minicircle-encoded guide RNAs in *Trypanosoma cruzi*. RNA **1:**939–947.
7. **Bakalara, N., A. M. Simpson, and L. Simpson.** 1989. The *Leishmania* kinetoplast-mitochondrion contains terminal uridylyltransferase and RNA ligase activities. J. Biol. Chem. **264:**18679–18686.
8. **Bass, B.** 1993. RNA editing: new uses for old players in the RNA world, p. 383–418. *In* R. F. Gesteland and J. F. Atkins (ed.), The RNA world. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
9. **Bass, B. L.** 1995. RNA editing: an I for editing. Curr. Biol. **5:**598–600.
10. **Benne, R.** 1994. RNA editing in trypanosomes. Eur. J. Biochem. **221:**9–23.
11. **Benne, R., K. Stuart, L. Simpson, D. A. Maslov, B. Blum, D. Miller, R. Mahendran, M. Spottswood, M. Ling, S. Wang, N. Yang, H. Costandy, D. Kolakofsky, J. Curran, T. Pelet, J. Jacques, P. Hodges, J. Scott, J. M. Grienenberger, U. Kim, and K. Nishikura.** 1993. *In* R. Benne (ed.), RNA editing: the alteration of protein coding sequences of RNA. Ellis Horwood Ltd., Chichester, England.
12. **Benne, R., J. Van den Burg, J. P. J. Brakenhoff, P. Sloof, J. H. Van Boom, and M. C. Tromp.** 1986. Major transcripts of the frameshifted coxII gene from trypanosome mitochondria contain four nucleotides that are not encoded in the DNA. Cell **46:**819–826.
13. **Bhat, G. J., D. J. Koslowsky, J. E. Feagin, B. L. Smiley, and K. Stuart.** 1990. An extensively edited mitochondrial transcript in kinetoplastids encodes a protein homologous to ATPase subunit 6. Cell **61:**885–894.
14. **Bhat, G. J., P. J. Myler, and K. Stuart.** 1991. The two ATPase 6 mRNAs of *Leishmania tarentolae* differ at their 3′ ends. Mol. Biochem. Parasitol. **48:**139–150.
15. **Blum, B., N. Bakalara, and L. Simpson.** 1990. A model for RNA editing in kinetoplastid mitochondria: "guide" RNA molecules transcribed from maxicircle DNA provide the edited information. Cell **60:**189–198.
16. **Blum, B., and L. Simpson.** 1990. Guide RNAs in kinetoplastid mitochondria have a nonencoded 3′ oligo(U) tail involved in recognition of the preedited region. Cell **62:**391–397.
17. **Blum, B., and L. Simpson.** 1992. Formation of guide RNA/messenger RNA chimeric molecules *in vitro*, the initial step of RNA editing, is dependent on an anchor sequence. Proc. Natl. Acad. Sci. USA **89:**11944–11948.
18. **Blum, B., N. R. Sturm, A. M. Simpson, and L. Simpson.** 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.
19. **Bringaud, F., M. Peris, K. H. Zen, and L. Simpson.** 1995. Characterization of two nuclear-encoded protein components of mitochondrial ribonucleoprotein complexes from *Leishmania tarentolae*. Mol. Biochem. Parasitol. **71:**65–79.
20. **Byrne, E., F. Bringaud, and L. Simpson.** 1995. RNA-protein interactions in the ribonucleoprotein T-complexes in a mitochondrial extract from *Leishmania tarentolae*. Mol. Biochem. Parasitol. **72:**65–76.
21. **Cattaneo, R.** 1991. Different types of messenger RNA editing. Annu. Rev. Genet. **25:**71–88.
22. **Cech, T. R.** 1991. RNA editing: world's smallest introns. Cell **64:**667–669.
23. **Chen, S. H., G. Habib, C. Yang, Z. Gu, B. R. Lee, S. Weng, S. R. Silberman, S. Cai, J. Deslypere, M. Rosseneu, A. M. Gotto, Jr., W. Li, and L. Chan.** 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science **238:**363–366.
24. **Corell, R. A., J. E. Feagin, G. R. Riley, T. Strickland, J. A. Guderian, P. J. Myler, and K. Stuart.** 1993. *Trypanosoma brucei* minicircles encode multiple guide RNAs which can direct editing of extensively overlapping sequences. Nucleic Acids Res. **21:**4313–4320.
25. **Corell, R. A., P. J. Myler, and K. D. Stuart.** 1994. *Trypanosoma brucei* mitochondrial gene CR4 encodes an extensively edited mRNA with completely edited sequence only in the bloodstream forms. Mol. Biochem. Parasitol. **64:**65–74.
26. **Corell, R. A., L. K. Read, G. R. Riley, J. K. Nellissery, T. Allen, M. L. Kable, M. D. Wachal, S. Seiwert, P. J. Myler, and K. D. Stuart.** 1996. Complexes from *Trypanosoma brucei* that exhibit deletion editing and other editing-associated properties. Mol. Cell. Biol. **16:**1410–1418.
27. **Corell, R. A., and K. D. Stuart.** Unpublished data.
28. **Decker, C. J., and B. Sollner-Webb.** 1990. RNA editing involves indiscriminate U changes throughout precisely defined editing domains. Cell **61:**1001–1011.
29. **de la Cruz, V. F., N. Neckelmann, and L. Simpson.** 1984. Sequences of six genes and several open reading frames in the kinetoplast maxicircle DNA of *Leishmania tarentolae*. J. Biol. Chem. **259:**15136–15147.
30. **Feagin, J. E., J. M. Abraham, and K. Stuart.** 1988. Extensive editing of the cytochrome c oxidase III transcript in Trypanosoma brucei. Cell **53:**413–422.
31. **Feagin, J. E., D. P. Jasmer, and K. Stuart.** 1987. Developmentally regulated addition of nucleotides within apocytochrome b transcripts in Trypanosoma brucei. Cell **49:**337–345.

32. **Feagin, J. E., and K. Stuart.** 1988. Developmental aspects of uridine addition within mitochondrial transcripts of *Trypanosoma brucei*. Mol. Cell. Biol. **8:**1259–1265.
33. **Frech, G. C., N. Bakalara, L. Simpson, and A. M. Simpson.** 1995. *In vitro* RNA editing-like activity in a mitochondrial extract from *Leishmania tarentolae*. EMBO J. **14:**178–187.
34. **Frech, G. C., and L. Simpson.** 1996. Uridine insertion into preedited mRNA by a mitochondrial extract from *Leishmania tarentolae*: stereochemical evidence for the enzyme cascade model. Mol. Cell. Biol. **16:**4584–4589.
35. **Göringer, H. U.** Personal communication.
36. **Göringer, H. U.** Personal communication.
37. **Göringer, H. U., D. J. Koslowsky, T. H. Morales, and K. D. Stuart.** 1994. The formation of mitochondrial ribonucleoprotein complexes involving guide RNA molecules in *Trypanosoma brucei*. Proc. Natl. Acad. Sci. USA **91:**1776–1780.
38. **Gray, M. W., and P. S. Covello.** 1993. RNA editing in plant mitochondria and chloroplasts. FASEB J. **7:**64–71.
39. **Hajduk, S., and B. Adler.** Personal communication.
40. **Hajduk, S. L., M. E. Harris, and V. W. Pollard.** 1993. RNA editing in kinetoplastid mitochondria. FASEB J. **7:**54–63.
41. **Harris, M., C. Decker, B. Sollner-Webb, and S. Hajduk.** 1992. Specific cleavage of preedited mRNAs in trypanosome mitochondrial extracts. Mol. Cell. Biol. **12:**2591–2598.
42. **Harris, M. E., and S. L. Hajduk.** 1992. Kinetoplastid RNA editing: in vitro formation of cytochrome b gRNA-mRNA chimeras from synthetic substrate RNAs. Cell **68:**1091–1099.
43. **Harris, M. E., D. R. Moore, and S. L. Hajduk.** 1990. Addition of uridines to edited RNAs in trypanosome mitochondria occurs independently of transcription. J. Biol. Chem. **265:**11368–11376.
44. **Heidmann, S.** Unpublished data.
45. **Hensgens, L. A., J. Brakenhoff, B. F. de Vries, P. Sloof, M. C. Tromp, J. H. Van Boom, and R. Benne.** 1984. The sequence of the gene for cytochrome c oxidase subunit I, a frameshift containing gene for cytochrome c oxidase subunit II and seven unassigned reading frames in *Trypanosoma brucei* mitochondrial maxicircle DNA. Nucleic Acids Res. **12:**7327–7344.
46. **Hill, G. C.** 1976. Electron transport systems in kinetoplastida. Biochim. Biophys. Acta **456:**149–193.
47. **Kable, M. L., S. D. Seiwert, S. Heidmann, and K. Stuart.** 1996. RNA editing: a mechanism for gRNA-specified uridylate insertion into precursor mRNA. Science **273:**1189–1195.
48. **Köller, J., G. Nörskau, A. S. Paul, K. Stuart, and H. U. Göringer.** 1994. Different *Trypanosoma brucei* guide RNA molecules associate with an identical complement of mitochondrial proteins *in vitro*. Nucleic Acids Res. **22:**1988–1995.
49. **Koslowsky, D. J., G. J. Bhat, A. L. Perrollaz, J. E. Feagin, and K. Stuart.** 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.
50. **Koslowsky, D. J., G. J. Bhat, L. K. Read, and K. Stuart.** 1991. Cycles of progressive realignment of gRNA with mRNA in RNA editing. Cell **67:** 537–546.
51. **Koslowsky, D. J., H. U. Göringer, T. H. Morales, and K. Stuart.** 1992. *In vitro* guide RNA/mRNA chimaera formation in *Trypanosoma brucei* RNA editing. Nature **356:**807–809.
52. **Koslowsky, D. J., S. M. Kutas, and K. D. Stuart.** 1996. Distinct differences in the requirements for specific RNP formation on differentially regulated pre-edited RNAs in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **80:**1–14.
53. **Koslowsky, D. J., G. R. Riley, J. E. Feagin, and K. Stuart.** 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.
54. **Landweber, L. F., and W. Gilbert.** 1994. Phylogenetic analysis of RNA editing: a primitive genetic phenomenon. Proc. Natl. Acad. Sci. USA **91:** 918–921.
55. **Leegwater, P., D. Speijer, and R. Benne.** 1995. Identification by UV cross-linking of oligo(U)-binding proteins in mitochondria of the insect trypanosomatid *Crithidia fasciculata*. Eur. J. Biochem. **227:**780–786.
56. **Lonergan, K. M., and M. W. Gray.** 1993. Editing of transfer RNAs in *Acanthamoeba castellanii* mitochondria. Science **259:**812–816.
57. **Lukes, J., G. J. Arts, J. Van den Burg, A. De Haan, F. Opperdoes, P. Sloof, and R. Benne.** 1994. Novel pattern of editing regions in mitochondrial transcripts of the cryptobiid *Trypanoplasma borreli*. EMBO J. **13:**5086–5098.
58. **Mahendran, R., M. R. Spottswood, and D. L. Miller.** 1991. RNA editing by cytidine insertion in mitochondria of *Physarum polycephalum*. Nature **349:** 434–438.
59. **Maslov, D. A., H. A. Avila, J. A. Lake, and L. Simpson.** 1994. Evolution of RNA editing in kinetoplastid protozoa. Nature **368:**345–348.
60. **Maslov, D. A., and L. Simpson.** 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.
61. **Maslov, D. A., and L. Simpson.** 1994. RNA editing and mitochondrial genomic organization in the cryptobiid kinetoplastid protozoan *Trypanoplasma borreli*. Mol. Cell. Biol. **14:**8174–8182.
62. **Maslov, D. A., N. R. Sturm, B. M. Niner, E. S. Gruszynski, M. Peris, and L. Simpson.** 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.
63. **Maslov, D. A., O. Thiemann, and L. Simpson.** 1994. Editing and misediting of transcripts of the kinetoplast maxicircle G5 (ND3) cryptogene in an old laboratory strain of *Leishmania tarentolae*. Mol. Biochem. Parasitol. **68:** 155–159.
64. **Missel, A., and H. U. Göringer.** 1994. *Trypanosoma brucei* mitochondria contain RNA helicase activity. Nucleic Acids Res. **22:**4050–4056.
65. **Payne, M., V. Rothwell, D. P. Jasmer, J. E. Feagin, and K. Stuart.** 1985. Identification of mitochondrial genes in *Trypanosoma brucei* and homology to cytochrome c oxidase II in two different reading frames. Mol. Biochem. Parasitol. **15:**159–170.
66. **Peris, M., G. C. Frech, A. M. Simpson, F. Bringaud, E. Byrne, A. Bakker, and L. Simpson.** 1994. Characterization of two classes of ribonucleoprotein complexes possibly involved in RNA editing from *Leishmania tarentolae* mitochondria. EMBO J. **13:**1664–1672.
67. **Piller, K. J., C. J. Decker, L. N. Rusché, M. E. Harris, S. L. Hajduk, and B. Sollner-Webb.** 1995. Editing domains of *Trypanosoma brucei* mitochondrial RNAs identified by secondary structure. Mol. Cell. Biol. **15:**2916–2924.
68. **Piller, K. J., C. J. Decker, L. N. Rusché, and B. Sollner-Webb.** 1995. *Trypanosoma brucei* mitochondrial guide RNA-mRNA chimera-forming activity cofractionates with an editing-domain-specific endonuclease and RNA ligase and is mimicked by heterologous nuclease and RNA ligase. Mol. Cell. Biol. **15:**2925–2932.
69. **Piller, K. J., L. N. Rusché, and B. Sollner-Webb.** 1996. *Trypanosoma brucei* RNA editing: a full round of uridylate insertional editing *in vitro* mediated by endonuclease and RNA ligase. J. Biol. Chem. **271:**4613–4619.
70. **Pollard, V. W., and S. L. Hajduk.** 1991. *Trypanosoma equiperdum* minicircles encode three distinct primary transcripts which exhibit guide RNA characteristics. Mol. Cell. Biol. **11:**1668–1675.
71. **Pollard, V. W., M. E. Harris, and S. L. Hajduk.** 1992. Native mRNA editing complexes from *Trypanosoma brucei* mitochondria. EMBO J. **11:**4429–4438.
72. **Pollard, V. W., S. P. Rohrer, E. F. Michelotti, K. Hancock, and S. L. Hajduk.** 1990. Organization of minicircle genes for guide RNAs in Trypanosoma brucei. Cell **63:**783–790.
73. **Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott.** 1987. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell **50:**831–840.
74. **Preiss, T., A. E. Sang, Z. M. A. Chrzanowska-Lightowlers, and R. N. Lightowlers.** 1995. The mRNA-binding protein COLBP is glutamate dehydrogenase. FEBS Lett. **367:**291–296.
75. **Read, L. K., R. A. Corell, and K. Stuart.** 1992. Chimeric and truncated RNAs in *Trypanosoma brucei* suggest transesterifications at non-consecutive sites during RNA editing. Nucleic Acids Res. **20:**2341–2347.
76. **Read, L. K., H. U. Göringer, and K. Stuart.** 1994. Assembly of mitochondrial ribonucleoprotein complexes involves specific guide RNA (gRNA)-binding proteins and gRNA domains but does not require preedited mRNA. Mol. Cell. Biol. **14:**2629–2639.
77. **Read, L. K., P. J. Myler, and K. Stuart.** 1992. Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. J. Biol. Chem. **267:**1123–1128.
78. **Read, L. K., K. A. Stankey, W. R. Fish, A. M. Muthiani, and K. Stuart.** 1994. Developmental regulation of RNA editing and polyadenylation in four life cycle stages of *Trypanosoma congolense*. Mol. Biochem. Parasitol. **68:**297–306.
79. **Read, L. K., K. D. Wilson, P. J. Myler, and K. Stuart.** 1994. Editing of *Trypanosoma brucei* maxicircle CR5 mRNA generates variable carboxy terminal predicted protein coding sequences. Nucleic Acids Res. **22:**1489–1495.
80. **Riley, G. R., R. A. Corell, and K. Stuart.** 1994. Multiple guide RNAs for identical editing of *T. brucei* apocytochrome b mRNA have an unusual minicircle location and are developmentally regulated. J. Biol. Chem. **269:** 6101–6108.
81. **Riley, G. R., P. J. Myler, and K. Stuart.** 1995. Quantitation of RNA editing substrates, products and potential intermediates: Implication for developmental regulation. Nucleic Acids Res. **23:**708–712.
82. **Rusché, L. N., K. J. Piller, and B. Sollner-Webb.** 1995. Guide RNA-mRNA chimeras, which are potential RNA editing intermediates, are formed by endonuclease and RNA ligase in a trypanosome mitochondrial extract. Mol. Cell. Biol. **15:**2933–2941.
83. **Sabatini, R., and S. L. Hajduk.** 1995. RNA ligase and its involvement in guide RNA/mRNA chimera formation. Evidence for a cleavage-ligation mechanism of *Trypanosoma brucei* mRNA editing. J. Biol. Chem. **270:**7233–7240.
84. **Schmid, B., G. R. Riley, K. Stuart, and H. U. Göringer.** 1995. The secondary structure of guide RNA molecules from *Trypanosoma brucei*. Nucleic Acids Res. **23:**3093–3102.

85. **Scott, J.** 1995. A place in the world for RNA editing. Cell **81:**833–836.
86. **Seiwert, S.** Unpublished data.
87. **Seiwert, S.** Unpublished data.
88. **Seiwert, S. D.** 1995. The ins and outs of editing RNA in kinetoplastids. Parasitol. Today **11:**362–368.
89. **Seiwert, S. D.** 1995. An *in vitro* system to study kinetoplastid RNA editing. Ph.D. thesis. Yale University, New Haven, Conn.
90. **Seiwert, S. D., S. Heidmann, and K. D. Stuart.** 1996. Direct visualization of uridylate deletion in vitro suggests a mechanism for kinetoplastid RNA editing. Cell **84:**831–841.
91. **Seiwert, S. D., and K. Stuart.** 1994. RNA editing: transfer of genetic information from gRNA to precursor mRNA *in vitro*. Science **266:**114–117.
92. **Sharma, P. M., M. Bowman, S. L. Madden, F. J. Rauscher III, and S. Sukumar.** 1994. RNA editing in the Wilms' tumor susceptibility gene, WT1. Genes Dev. **8:**720–731.
93. **Shaw, J. M., J. E. Feagin, K. Stuart, and L. Simpson.** 1988. Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. Cell **53:**401–411.
94. **Shu, H.-H., and K. D. Stuart.** 1994. Mitochondrial transcripts are processed but are not edited normally in *Trypanosoma equiperdum* (ATCC 30019) which has kDNA sequence deletion and duplication. Nucleic Acids Res. **22:**1696–1700.
95. **Simpson, L., D. A. Maslov, and B. Blum.** 1993. RNA editing in *Leishmania* mitochondria, p. 53–86. *In* R. Benne (ed.), RNA editing: the alteration of protein coding sequences of RNA. Ellis Horwood Ltd., Chichester, England.
96. **Simpson, L., and J. Shaw.** 1989. RNA editing and the mitochondrial cryptogenes of kinetoplastid protozoa. Cell **57:**355–366.
97. **Skuse, G. R., A. J. Cappione, M. Sowden, L. J. Metheny, and H. C. Smith.** 1996. The neurofibromatosis type I messenger RNA undergoes base-modification RNA editing. Nucleic Acids Res. **24:**478–486.
98. **Smith, H. C., S.-R. Kuo, J. W. Backus, S. G. Harris, C. E. Sparks, and J. D. Sparks.** 1991. *In vitro* apoliopoprotein B mRNA editing: Identification of a 27S editing complex. Proc. Natl. Acad. Sci. USA **88:**1489–1493.
99. **Sollner-Webb, B.** 1991. RNA editing. Curr. Opin. Cell Biol. **3:**1056–1061.
100. **Sommer, B., M. Köhler, R. Sprengel, and P. H. Seeburg.** 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell **67:**11–19.
101. **Souza, A. E., P. J. Myler, and K. Stuart.** 1992. Maxicircle *CR1* transcripts of *Trypanosoma brucei* are edited, developmentally regulated, and encode a putative iron-sulfur protein homologous to an NADH dehydrogenase subunit. Mol. Cell. Biol. **12:**2100–2107.
102. **Souza, A. E., H. H. Shu, L. K. Read, P. J. Myler, and K. Stuart.** 1993. Extensive editing of CR2 maxicircle transcripts of *Trypanosoma brucei* predicts a protein with homology to a submit of NADH dehydrogenase. Mol. Cell. Biol. **13:**6832–6840.
103. **Stuart, K.** 1993. Introduction: RNA editing. Semin. Cell Biol. **4:**401.1–401.2.
104. **Stuart, K.** 1993. RNA editing in mitochondria of African trypanosomes, p. 26–52. *In* R. Benne (ed.), RNA editing: the alteration of protein coding sequences of RNA, Ellis Horwood Ltd., Chichester, England.
105. **Stuart, K., D. J. Koslowsky, G. J. Bhat, and J. E. Feagin.** 1990. The implications of selective and extensive RNA editing, p. 111–122. *In* N. Agabian and A. Cerami (ed.), Parasites: molecular biology, drug and vaccine design. Wiley-Liss, Inc., New York, N.Y.
106. **Sturm, N. R., D. Maslov, B. Blum, and L. Simpson.** 1992. Generation of unexpected editing patterns in Leishmania tarentolae mitochondrial mRNAs: misediting produced by misguiding. Cell **70:**469–476.
107. **Sturm, N. R., and L. Simpson.** 1990. Partially edited mRNAs for cytochrome b and subunit III of cytochrome oxidase from Leishmania tarentolae mitochondria: RNA editing intermediates. Cell **61:**871–878.
108. **Sturm, N. R., and L. Simpson.** 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.
109. **Thiemann, O. H., D. A. Maslov, and L. Simpson.** 1994. Disruption of RNA editing in *Leishmania tarentolae* by the loss of minicircle-encoded guide RNA genes. EMBO J. **13:**5689–5700.
110. **Van der Spek, H., D. Speijer, G.-J. Arts, J. Van den Burg, H. Van Steeg, P. Sloof, and R. Benne.** 1990. RNA editing in transcripts of the mitochondrial genes of the insect trypanosome *Crithidia fasciculata*. EMBO J. **9:**257–262.
111. **van der Spek, H. J., J. Van den Burg, A. Croiset, M. van den Broek, P. Sloof, and R. Benne.** 1988. Transcripts from the frameshifted MURF3 gene from *Crithidia fasciculata* are edited by U insertion at multiple sites. EMBO J. **7:**2509–2514.
112. **Wahle, E.** 1991. Purification and characterization of a mammalian polyadenylate polymerase involved in the 3′ end processing of messenger RNA precursors. J. Biol. Chem. **266:**3131–3139.
113. **White, T. C., and P. Borst.** 1987. RNA end-labeling and RNA ligase activities can produce a circular rRNA in whole cell extracts from trypanosomes. Nucleic Acids Res. **15:**3275–3290.
114. **Yasuhira, S., and L. Simpson.** 1995. Minicircle-encoded guide RNAs from *Crithidia fasciculata*. RNA **1:**634–643.