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Generation of Unexpected Editing Patterns in Leishmania tarentolae Mitochondrial mRNAs: Misediting Produced by Misguiding

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Summary

We have analyzed the generation of unexpected patterns of RNA editing, i.e., those not following a strict 3′ to 5′ progression, which occur in junction regions between fully edited and preedited sequences. Evidence is presented that these patterns are generated by misediting due to specific events of misguiding. Misediting can occur through the interaction of inappropriate gRNAs with mRNAs or appropriate gRNAs in an incorrect fashion. Four possible mechanisms for the generation of misedited sequences are presented. Chimeric molecules have been detected in steady-state mitochondrial RNAs that are composed of misguiding gRNAs covalently linked to mRNAs at misediting sites by the 3′ oligo(U) tail. We propose that misediting within junction regions can be corrected by appropriately acting gRNAs.

Introduction

RNA editing in mitochondria of kinetoplastid protozoa involves the addition and deletion of uridine residues (U's) within coding regions of maxicircle DNA transcripts (Benne, 1989; Simpson and Shaw, 1989; Simpson, 1990). The information for the exact sites and the precise number of U's is provided by specific guide RNAs (gRNAs) that can form perfect hybrids (allowing G–U and occasional A–C pairing) with the mature edited mRNAs (Blum et al., 1990; Van der Spek et al., 1991). The 5′ portions of specific gRNAs also have complementary sequences of variable length to the mRNA sequence just 3′ of the preedited region (PER) of the specific target mRNA. These anchor duplex regions are thought to provide the specificity for initiation of the editing process at the 3′ end of the PER, and the process then continues in a 5′ direction. The gRNAs are encoded both in the maxicircle (Blum et al., 1990; Van der Spek et al., 1991) and the minicircle (Sturm and Simpson, 1990a, 1991; Pollard et al., 1990) components of the kinetoplast DNA.

Two specific hypotheses—the enzyme cascade (Blum et al., 1990) and the transesterification (Blum et al., 1991; Cech, 1991) models—have been proposed for gRNA-mediated RNA editing, and the available evidence is not sufficient to decide between them. In both models, however, base pairing of the edited mRNA with the gRNA provides the driving force for the 3′ to 5′ editing that occurs within a single editing block, which is defined as the edited sequence determined by a single gRNA. The overall 3′ to 5′ polarity of editing observed within editing domains (Abraham et al., 1988; Sturm and Simpson, 1990b; Decker and Sollner-Webb, 1990), which are defined as edited sequences determined by multiple overlapping gRNAs, is due to the fact that the anchor duplexes of gRNAs other than the first require edited mRNA sequences (Maslov and Simpson, 1992 [this issue of *Cell*]).

An analysis of a large collection of partially edited mRNAs for the cytochrome b (*CYb*) gene in Leishmania tarentolae agreed well with the mandatory processivity within a block and a domain as suggested by these models (Sturm and Simpson, 1990b). However, 42% of cloned partially edited mRNAs for the cytochrome oxidase subunit III (*COIII*) gene of L. tarentolae (Sturm and Simpson, 1990b) and the majority of a library of partially edited mRNAs for *CYb* and *COIII* genes of Trypanosoma brucei (Decker and Sollner-Webb, 1990) exhibited unexpected editing patterns at junction regions between fully edited and unedited sequences. We have suggested that the generation of these unexpected editing patterns is due to 3′ to 5′ misediting by inappropriate gRNAs (Sturm and Simpson, 1990a, 1990b; Maslov et al., 1992). We have also shown an example of misediting in a *COIII* mRNA caused by the formation of a secondary anchor by the g*COIII-I* gRNA just upstream of a single nucleotide loopout (Blum et al., 1991).

Alternative explanations for misediting within junction regions have also been proposed. Decker and Sollner-Webb (1990) have suggested that editing occurs randomly at multiple sites within a defined region, with hybridization of gRNA to correctly edited mRNA protecting the mRNA from further random editing. The unexpected patterns that occur within junction regions represent sequences that are not yet complementary to the gRNA guide sequences. Koslowsky et al. (1991) have proposed that regions of lower thermodynamic stability in the initially imperfect gRNA–mRNA hybrids are targets for editing. Cycles of progressive realignments and editing are continued until perfect correspondence between mRNA and gRNA is achieved. Like random editing, this model suggests that misedited junction sequences represent natural intermediates of the editing process.

In this paper, we present additional experimental and analytical evidence for the hypothesis in which misediting is mediated by misguiding gRNAs that mediate editing in a strict 3′ to 5′ direction. These incorrectly edited sequences require reediting with the correct gRNA in the correct context and guiding frame to produce a mature mRNA.

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Results

Several Misediting Patterns in Partially Edited *COIII* mRNAs Correlate with Specific *RPS12* and *MURF4* gRNAs

The gRNA transcribed from minicircle Lt154 was first identified as being complementary to unexpected editing pattern 4E-2 of a partially edited *COIII* mRNA (Sturm and Simpson, 1990b). This gRNA was then shown to mediate correct editing of sites 24–32 in the *RPS12* mRNA (g*RPS12-IV*) (Maslov et al., 1992). The g*RPS12-IV* gRNA was the first example of a gRNA that has the potential for specifying both a mature editing pattern for the corresponding mRNA and a misedited pattern for a heterologous mRNA.

The misediting patterns in junction regions of partially edited L. tarentolae *COIII* mRNAs could be separated into subgroups according to the extent of the 3′ to 5′ progression of editing (see Figures 4 and 5 in Sturm and Simpson, 1990b). As shown in Figures 1A and 1B, several of these misediting patterns correspond well with specific *RPS12* and *MURF4* gRNAs. This evidence suggests that these *RPS12* and *MURF4* gRNAs may be responsible for the generation of at least some of the observed *COIII* misediting patterns by the formation of false anchors and subsequent misguiding.

Additional correlations of misedited sequences and specific gRNAs were observed for several partially edited sequences from G-rich region 5 (Simpson and Shaw, 1989) and the gRNAs for *ND7-I*, *MURF2-II*, and *MURF4-IV* (D. A. M., E. S. Gruszynski, N. R. S., and L. S., unpublished data).

Confirmation of an Interaction between the Misguiding g*RPS12-IV* gRNA and the *COIII* mRNA by Detection of Chimeric Molecules

Direct evidence for the involvement of g*RPS12-IV* gRNA in misediting of *COIII* mRNA was obtained by polymerase chain reaction (PCR) amplification of the corresponding gRNA–mRNA chimeric molecules, which are predicted by the transesterification model of editing. We selected chimeric molecules composed of misguiding gRNAs covalently linked to mRNAs, using a *COIII* mRNA-specific 3′ primer and a g*RPS12-IV*-specific 5′ primer. Chimeric molecules were obtained in which g*RPS12-IV* was attached to *COIII* mRNA at several sites. The chimeric molecules in Figure 2A are shown in foldback configurations indicating the most likely local base pairing as determined by the local alignment GCG BESTFIT program (see Experimental Procedures). In each case, the base pairing between the gRNA and mRNA included a potential duplex anchor, which could have resulted in an editing event leading to the attachment of the misguiding gRNA. In some cases, prior editing by g*COIII-I* (molecules 1 and 4) or another putative misguiding gRNA (molecule 5) would be required to form a stable anchor. In molecules 2, 3, and 6, the

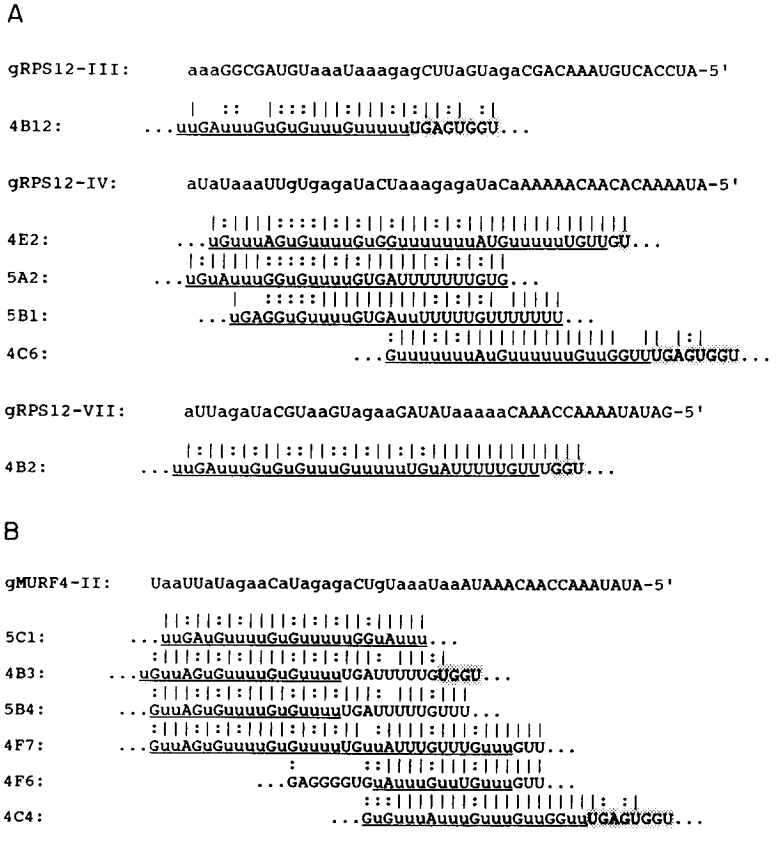

Figure 1. Correlation of *COIII* Misedited Sequence Patterns with *RPS12* and *MURF4* gRNA Sequences

The *COIII* sequences represent unexpected editing patterns of partially edited *COIII* mRNAs obtained by Sturm and Simpson (1990b). The clones are designated by the original figure and clone numbers in Sturm and Simpson (1990b). The patterns were grouped according to shared sequence motifs. Only single representatives of such groups are shown here matching with the putative misguiding gRNAs. gRNA sequences are shown 3′ to 5′. G–C and A–U base pairs are indicated by a vertical line, G–U base pairs by a colon. Uridines added by editing are shown as u's. Edited mRNA sequences are indicated by stippling. Misedited sequences are underlined.

(A) gRPS12-III, gRPS12-IV, and gRPS12-VII gRNAs.

(B) gMURF4-II gRNA.

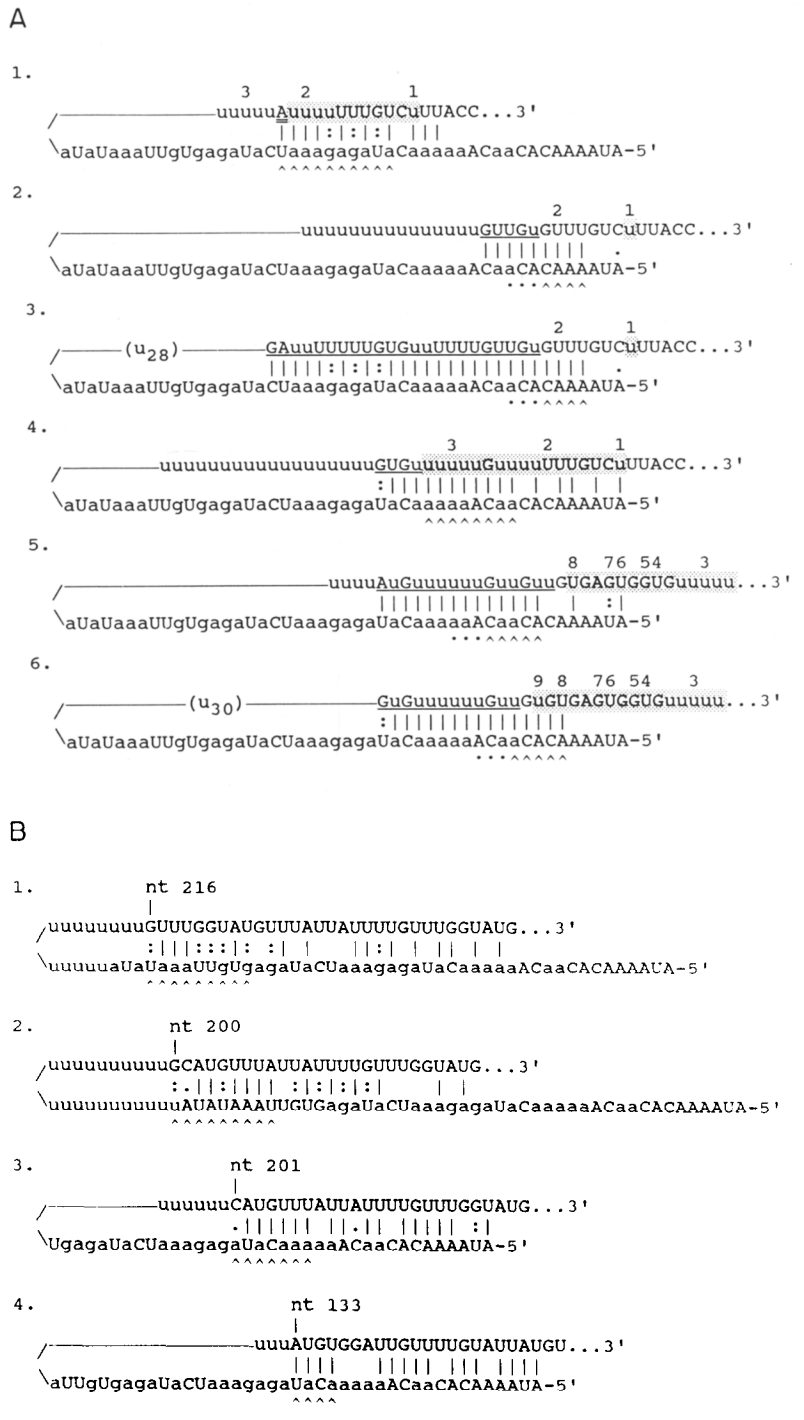

Figure 2. Misguiding Chimeric Molecules

g*PS12-IV–COIII* mRNA chimeric molecules were amplified and cloned as described in Experimental Procedures. The sequences are shown in foldback configuration so as to indicate the complementary regions. The mRNA is above and the gRNA is below. Putative anchor hybrids are indicated by carets, with the uncertainty of the right border location indicated by elipses. Edited mRNA sequences are indicated by stippling, with the editing sites numbered. Lowercase u's refer to uridines added by editing or constituting the 3′ oligo(U) tail of the gRNA. See legend to Figure 1 for symbols.

(A) Chimeric molecules in which the gRNAs are attached within the preedited region. Double-underlined A represents a possible PCR-generated mutation since G is normally present in this position. Guiding nucleotides in g*RPS12-IV* are shown as lowercase letters.

(B) Chimeric molecules in which the gRNAs are attached outside the preedited region. The location of the attachment site is shown by the nucleotide number within the mRNA (5′ end = 1).

anchor duplexes were formed with unedited sequence and probably with a portion of misedited sequence.

Four of the ten g*RPS12-IV–COIII* chimeric molecules obtained had the gRNA attached outside of an editing domain, as shown in Figure 2B. However, in all of these cases, appropriate anchor sequences that could have given rise to the attachments shown did not represent the best local gRNA–mRNA base pairing. In addition, no editing had occurred in these mRNAs.

A New Maxicircle-Encoded gRNA Involved in Misguiding

Two of the chimeric molecules that were amplified using the g*RPS12-IV–COIII* primers contained a previously undescribed gRNA molecule in association with *COIII* mRNA (Figure 3). This gRNA shares a 10 bp absolute match with the g*RPS12-IV*-specific PCR primer that was probably responsible for the amplification. The site of attachment of this gRNA to the *COIII* mRNA is upstream of the preedited

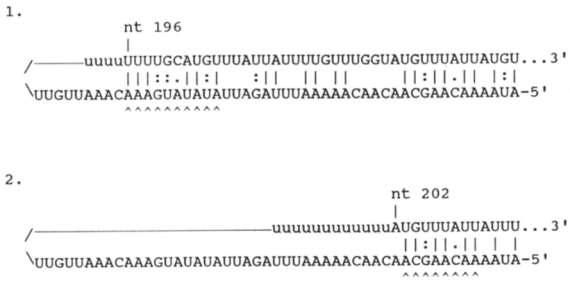

Figure 3. New Maxicircle-Encoded gRNA Found in Chimeric Association with *COIII* mRNA

g*M150–COIII* chimeric molecules. The mRNA portion is shown above, and the gRNA portion is shown below. See legends to Figures 1 and 2 for symbols.

region, in the identical location at which the g*RPS12-IV* gRNAs were attached in the unusual chimeric molecules in Figure 2B. The new gRNA was shown by computer analysis to be derived from a gRNA gene localized in the maxicircle genome at nucleotides 150–102 in the LEIKPMAX sequence, which is transcribed from the opposite strand as the rRNAs. Northern analysis demonstrated the existence of this gRNA as a low abundance transcript (data not shown). The 5′ end was mapped by primer extension sequencing (data not shown). This transcript has been labeled g*M150* in view of the maxicircle localization; it brings the total number of known maxicircle-encoded gRNAs to nine. The corresponding cryptogene transcript for which g*M150* gRNA normally mediates editing has not yet been identified.

The g*M150* gRNA could potentially act as a misguiding gRNA for two unexpected editing patterns previously observed in a collection of partially edited mRNAs from the *RPS12* cryptogene (see Figure 2 in Maslov et al. [1992], clones 25 and 26). As shown in Figure 4, the misedited patterns in clones 25 and 26 both correlate well with the g*M150* pattern. The indicated potential anchor with clone 25 is with the sequence edited correctly up to site 21. The anchor with the clone 26 sequence is with preedited sequence at sites 22, 23, and 24.

Long Misediting Patterns in Junction Regions Can Be Produced by Consecutive Action of Several Misguiding gRNAs

The long unexpected editing pattern of clone 26 shown in Figure 4 could not be produced by g*M150* alone. Consecutive misediting performed by three gRNAs, two of which are identified in Figure 4, could have generated this pattern. The 3′ proximal g*M150* gRNA would initially produce an unexpected pattern that then inadvertently acted as an anchor sequence for the putative second misguiding gRNA, which has not yet been identified. This gRNA would extend the misediting of this junction region and create an anchor sequence for g*RPS12-I*, which would complete this misediting pattern.

The proposed scenario of consecutive anchor formation is similar to the normal process of editing by multiple gRNAs within a domain (Maslov and Simpson, 1992). The probability is low of accidentally creating by misediting a stable anchor sequence suitable for use by the second misguiding gRNA, and this probability should dramatically decrease for each successive misguiding gRNA. This agrees with the observed relatively rare occurrence of long misediting patterns in junction regions.

Some Misediting Can Be Caused by the Formation of a Secondary Anchor by a Normally Editing gRNA

All examples of the misguiding process given above involved the editing events triggered by a single anchor formation followed by the strict 3′ to 5′ progression of editing. However, if a loopout or even a single nucleotide bulge occurs after the formation of the anchor hybrid either in the mRNA or the gRNA, this would produce a guiding frameshift, destroying the original alignment of the gRNA and mRNA. If a secondary anchor can be formed upstream of the bulge, this gRNA could then mediate the creation of an unexpected editing pattern upstream of the anchor. A possible example of this was reported previously in Figure 4B of Blum et al. (1991) for a chimeric *COIII* molecule containing an unexpected editing pattern.

The CU Paradox: Creation of Misediting Patterns at Deletion Sites 3′ of Cytidine

As pointed out by Feagin (1990), the presence of a U residue that must be deleted just 3′ of a C residue leads to a problem for the strict 3′ to 5′ editing mechanism since the guide G residue in the gRNA could easily base pair with the U instead of the C. This would lead to a guiding frameshift and subsequent misediting in a junction region if editing proceeded strictly in a 5′ direction. In mature edited *RPS12* mRNA, there are three sites at which the U's

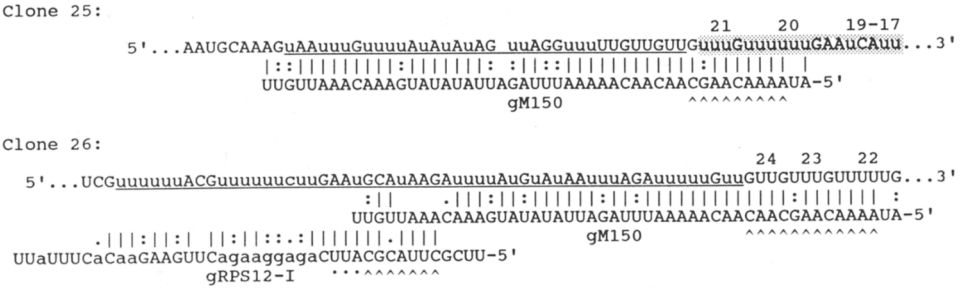

Figure 4. Several *RPS12* Unexpected Editing Patterns Correlate with the g*M150* Sequence

Sequences of two partially edited *RPS12* mRNAs (clones 25 and 26 in Maslov et al. [1992]) are shown in alignment with the putative misguiding gRNA. The symbols are described in the previous legends. The lowercase c in clone 26 probably represents a PCR-generated transition.

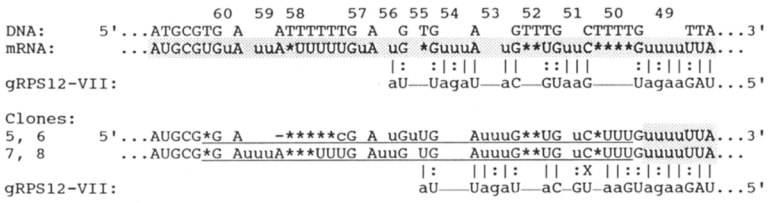

Figure 5. CU Paradox: Creation of Misedited Sequences at Sites of U Deletions 3′ of C Residues

The genomic sequence (DNA) and the correctly edited sequence (mRNA) of *RPS12* mRNA are shown together, with the editing sites numbered. The deleted U's from the mRNA are indicated by asterisks. The match with g*RPS-VII* leading to the correct editing pattern is given underneath the mRNA sequence. The sequences of four clones (5–8) of partially edited RNAs, including the misedited sequence (underlined), are shown below. The misguiding match with g*RPS-VII* is shown underneath the misedited sequences. A single mismatch is indicated by X.

to be deleted are located 3′ of a C residue (sites 16, 18, and 50). Misediting patterns were observed in several partially edited clones associated with these sites. In the four clones shown in Figure 5, the 3′ portions of the observed misediting patterns could be produced by the guide G base pairing with the U that is 3′ of the C, resulting in a 3 nt frameshift combined with a single C–U mismatch of the g*RPS12-VII* gRNA. The misediting pattern, which is common for all four clones, could possibly serve as an anchor for an unidentified misguiding gRNA that would extend misediting further upstream in clones 5 and 6 and act as an anchor for another unidentified gRNA in clones 7 and 8.

The Presence of an Internal Anchor Sequence within an Edited Region Can Lead to Short Upstream Misedited Regions

If an anchor sequence for the normal upstream gRNA is created internally within a block of editing, this implies that the sequence determined by the 3′ portion of the gRNA just upstream of the newly created internal anchor sequence can be misedited. This misediting would then be corrected by hybridization of the upstream gRNA to the internal anchor sequence and standard 3′ to 5′ editing.

Several of the short *RPS12* misediting patterns previously observed coincide in their location with the 3′ ends of gRNAs and represent possible examples of this type of misediting. One example is shown in Figure 6A, in which the short misedited sequence present in clone 18 (see also Figure 6 of Maslov et al., 1992) could be the result of a limited 3′-end heterogeneity of the corresponding gRNA and the involvement of the oligo(U) tail of the gRNA in determining the edited sequence. We have suggested an involvement of the 3′ oligo(U) tail in mediating editing events for the terminal U deletions of *MURF4* mRNA in L. tarentolae (Maslov and Simpson, 1992). The short misedited sequences seen in clones 20, 23, and 24 in Figure 6 of Maslov et al. (1992) could have been generated by a similar mechanism.

If the misedited pattern created by a 3′ extended end of a gRNA by chance forms an anchor with an incorrect gRNA, this can also lead to further misediting of the

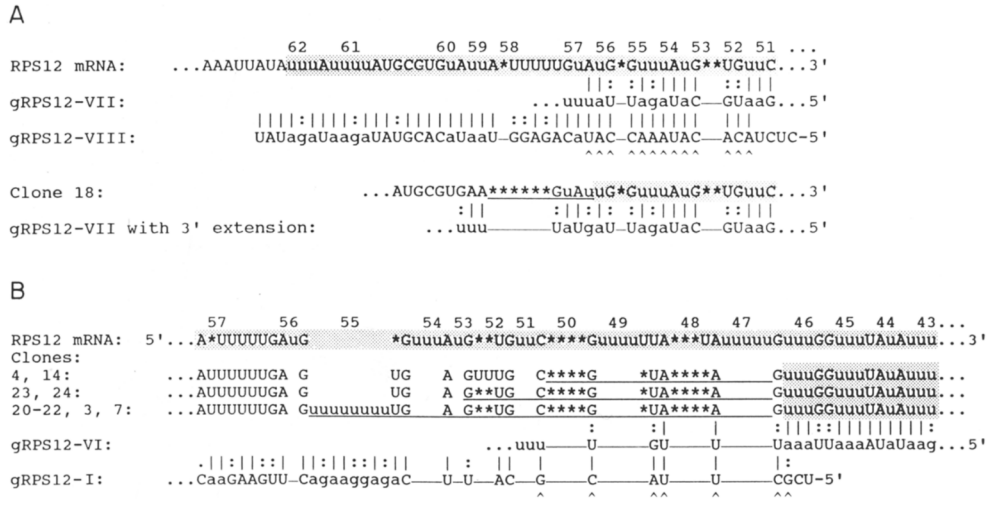

Figure 6. Generation of Misedited Sequences by the 3′ Ends of gRNAs (Internal Anchor Mechanism)

(A) The 3′ extension of g*RPS12-VII* together with the oligo(U) tail may be responsible for a short misedited sequence. The correctly edited *RPS12* mRNA is shown aligned with the g*RPS12-VII* and g*RPS12-VIII* sequences. The putative 3′ end of g*RPS12-VII* shown was determined by alignment with edited mRNA (Maslov and Simpson, 1992). The sequence of the partially edited mRNA clone 18 is shown below, matching the 3′-extended sequence of g*RPS12-VII*, which was obtained from the corresponding minicircle sequence. The exact localization of the 3′ end is unknown.
(B) Creation of a false anchor by a 3′ extension of g*RPS12-VI*. The correctly edited sequence of *RPS12* mRNA and several partially edited clones grouped according to their patterns are shown aligned with g*RPS12-VI*. A 3′ extension of the gRNA beyond editing site 46 would result in the unexpected pattern of clones 4 and 14. The false anchor for g*RPS12-I* is indicated by carets.

mRNA. Figure 6B shows that the sequence of g*RPS12-VI* corresponds to that of edited mRNA up to editing site 46. The exact location of the 3′ end of this gRNA is unknown, but if the 3′ end extends beyond this site, the last several bases would result in generation of a short misedited sequence covering sites 47–50, a situation that was actually observed in clones 4 and 14 (see also Figure 6 of Maslov et al., 1992). A 7 bp anchor then could be formed between this sequence and g*RPS12-I* gRNA, which normally edits sites 1–6. The subsequent misediting could create the unexpected patterns seen in a number of *RPS12* clones.

Discussion

We have proposed four basic mechanisms by which misedited sequences could be generated by misguiding in a strict 3′ to 5′ fashion, as shown schematically in Figure 7. Possible examples of each type of misguiding are presented. The correlations of misedited patterns and gRNAs provide strong, though indirect, evidence in favor of the misguiding hypothesis. If unexpected editing patterns were derived from random editing, as suggested by Decker and Sollner-Webb (1990), or from the reediting–realignment cycles of the dynamic interaction model of Koslowsky et al. (1991), the probability of finding any pattern correlations at all between unexpected patterns and gRNA sequences would be extremely low.

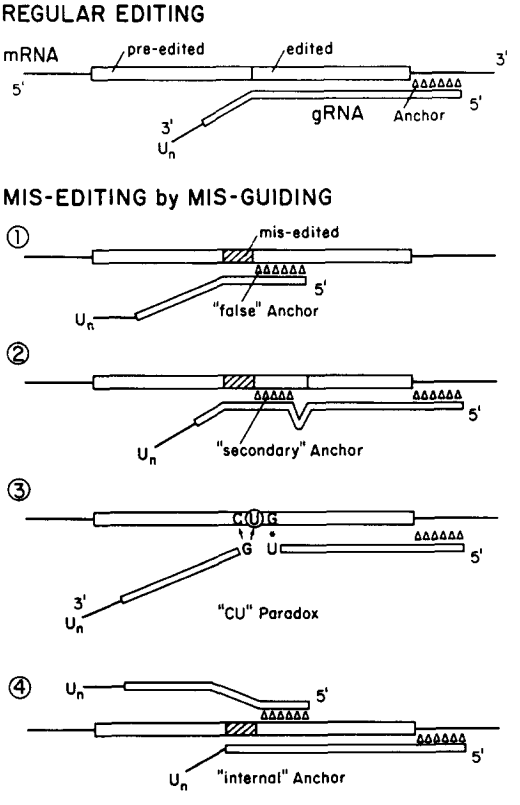

Figure 7. Schematic Diagrams of Normal Editing and Misediting Produced by Misguiding

The anchors are indicated by carets, and misedited sequences are indicated by cross-hatching. See text for explanation of models.

The formation of anchor duplexes (i.e., false anchors) by inappropriate gRNAs represents one type of initiating event in misediting (Figure 7, misguiding type 1). In addition to the indirect evidence for this mechanism by correlating misedited sequences with known gRNA sequences, some direct evidence has been obtained in the form of the occurrence of chimeric molecules with inappropriate gRNA–mRNA pairs.

Wobble G–U base pairing is the major factor responsible for the appearance of spurious anchor formations. The presence of more G and U residues in the L. tarentolae *COIII* preedited region than in the *CYb* preedited region and the subsequent greater potential for wobble base pairing was suggested to be responsible for the observed higher frequency of misediting in the former (Sturm and Simpson, 1990b). In this regard, Maslov and Simpson (1992) have shown that a characteristic feature of many proper anchors is the virtual absence of G–U base pairs.

The frequency of occurrence of a particular misediting pattern should also depend on the relative abundance of a corresponding misguiding gRNA. In the case of *RPS12* (Figures 2 and 6 in Maslov et al., 1992), many misedited patterns were associated with the 5′ domain of the molecule, where a false anchor for g*RPS12-I* gRNA can be created by the putative 3′ extended end of g*RPS12-VI* gRNA. g*RPS12-I* is one of the most abundant gRNAs in steady-state kinetoplast RNA (Maslov and Simpson, 1992).

The misediting produced by the formation of a secondary anchor that is created independently or as a result of a loopout or bulge in either the gRNA or the mRNA (Figure 7, misguiding type 2) is due to a shift in the gRNA–mRNA guiding frame. This mechanism, which was first observed in a gRNA–mRNA chimeric molecule by Blum et al. (1991), has been expanded in the dynamic interaction model of Koslowsky et al. (1991) to include multiple misalignments and misediting occurring as normal intermediates of the editing process.

The CU paradox (Feagin, 1990), misediting produced by mishybridization of a gRNA guide G residue to a U in the mRNA 3′ of a C (Figure 7, misguiding type 3), is another example of frameshift misediting. We have proposed that deletions of U's are caused either by a 3′ exonuclease trimming of the unpaired terminal U in the cleaved mRNA (Blum et al., 1990) or by an initial transesterification 5′ of the unpaired U residue rather than 3′ and a subsequent incorporation of the U into the gRNA tail after the second transesterification (Blum et al., 1991; Cech, 1991). In either case, base pairing of the guide G to the U would prevent the deletion and initiate misediting. We speculate that there is an equilibrium between base pairing of the G to the U or to the C, which is perhaps driven toward the latter by the greater free energy of the G–C base pair. This would allow U deletion and correct editing or reediting of the misedited sequence.

Finally, some other misediting events tend to occur at the locations of the 3′ ends of gRNAs, possibly reflecting gRNA 3′ heterogeneity or misediting mediated by the oligo(U) tail (Figure 7, misguiding type 4).

Misedited patterns produced by misguiding events can

not be extended unless a false anchor sequence is inadvertently formed for a misguiding gRNA. A possible example of this has been presented in the case of *RPS12* editing.

A characteristic feature of misedited patterns is a preferential localization to a fairly well-defined junction region between completely edited and preedited sequences within an editing domain in partially edited molecules. This is functional, for misediting outside of an editing domain could not be repaired by reediting. Limitation to a junction region is a natural consequence of the misguiding mechanisms illustrated as types 2, 3, and 4 in Figure 7. In the case of false anchor formation (misguiding type 1), the limitation to sequences within an editing domain could be a result of the same mechanism that limits normal editing to a junction region. Bakker et al. (A. Bakker, M. Peris, and L. S., unpublished data) have shown recently that gRNAs are bound to a 200–700 kd complex containing terminal uridylyltransferase (Bakalara et al., 1989) and several other proteins and have suggested that this complex is involved with the specific presentation of gRNAs to the editing site. In addition, Harris and Hajduk (1992), Koslowsky et al. (1992), and B. B. and L. S. (submitted) have shown that in vitro formation of gRNA–mRNA chimeric molecules requires the addition of mitochondrial extract, presumably owing to a requirement for similar if not identical protein factors. Limitation of misediting to the junction region that would result from the presentation by such a complex of an inappropriate gRNA could form a false anchor in that region and initiate misediting in a 5′ direction.

We have shown that multiple examples of misediting can be explained by a local 3′ to 5′ processive model involving a misguiding mechanism, and we suggest that this could provide a general explanation for the occurrence of unexpected editing patterns in partially edited RNAs. Misguiding represents mechanically correct RNA editing, but misedited mRNAs must always be corrected by in-frame editing mediated by the appropriate gRNA to obtain the mature mRNA.

Experimental Procedures

Cell Culture and Kinetoplast RNA Isolation

L. tarentolae cells (UC strain) were grown as described previously (Simpson and Braly, 1970). Cells were harvested at mid-log phase and used for mitochondrial isolation for the preparation of kinetoplast RNA as described (Braly et al., 1974; Simpson and Simpson, 1978), except that kinetoplast RNA was isolated from the enriched kinetoplast fraction without centrifugation through Renografin.

Chimeric Amplification, Cloning, and Sequencing

The chimeric molecules were amplified by RNA PCR using a *COIII* mRNA–specific 3′ primer, S-71, which is downstream of the preedited region, and a *Lt154* gRNA (g*RPS12-IV*)–specific 5′ primer, *S-397*, as described (Blum et al., 1991). The products of the amplification were ligated directly from the PCR reaction mix using the TA Cloning Kit (Invitrogen). Colonies were screened by colony hybridization with an internal g*RPS12-IV*-specific primer (*S-252*) or selected on the basis of color and screened for inserts by sizing on agarose gels. Sequencing was performed on plasmid DNA extracted by the boiling method miniprep procedure with the Sequenase Kit (US Biochemical Company).

Oligonucleotides

Oligonucleotides were synthesized by standard phosphoramidite methods on an ABS 341A DNA Synthesizer. The following is a list of oligonucleotides used in this study.

S-71 (*COIII* mRNA 3′ PCR primer, nucleotides 4708–4692 in LEIKPMAX): GTCTACAAATAAGTCAC.
S-252 (g*RPS12-IV* probe): ACACTCTATGATTTCTCTAT.
S-397 (g*RPS12-IV* 5′ PCR primer): ATAGAATTCATAAAACACAACAAAAAA.
S-466 (g*M150* probe, nucleotides 102–120 in LEIKPMAX): AACAATTTGTTTCATATA.

Computer Analysis

To search for the most stable gRNA–mRNA anchor sequences, the UWGCG BESTFIT program was used with a modified data file, SWGAPDNA.CMP, in which mismatches were given a value of −0.9; A–C base pairs, 0.01; G–C base pairs, 1.0; A–U base pairs, 0.5; and G–U base pairs, 0.25. The gap weight was 100, and the gap length weight was 2.

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