The data showing that the *nef* product of one virus (HIV-1_{SF2}) can affect the replication of other HIV-1 as well as HIV-2 strains (Fig. 4 and Table 1) suggest that common sequences in the LTR of these viruses are responsive to the *nef* protein. In this regard, the observed lack of effect of HIV-1_{SF2} *nef* on the highly cytopathic and fast replicating HIV-1_{SF33} strain and the later sequential isolates from infected individuals (HIV-1_{SF13}, HIV-1_{SF216}, and HIV-1_{SF665}) is important. Since a functional *nef* is supplied in these infections by the plasmid, these more pathogenic HIV-1 variants that emerged over time in individuals may have mutated in the LTR sequences responsive to *nef*. Alternatively, other positive regulatory elements might be more potent in these strains and outweigh the *nef*-mediated suppression of replication. A comparison between the LTR regions of responsive and nonresponsive isolates should provide insight into this possible mechanism of HIV pathogenesis.

In summary, our observation on the differential effect of cell lines expressing the HIV-1_{SF2} *nef* protein on replication of HIV strains strongly suggest a key role for this viral gene in the establishment and maintenance of latent viral infection and in HIV pathogenesis. Further studies with these lymphoid cell lines should allow us to determine the mechanism by which *nef* exerts its negative effect. Moreover, they provide valuable cell culture systems for defining factors involved in activating latent infections. Finally, these results suggest that manipulation of *nef* in early stages of HIV infection may prove effective in therapeutic approaches.

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9. In plasmid pAM3, the SV40 early promoter and leader region cloned into the vector pML (*10, 11*), was positioned upstream of the HIV-1_{SF2} *nef* gene (*11*). A synthetic oligonucleotide adapter (GATCCAAGGCTTTTCCTATAA) joins the SV40 leader to the start codon for *nef*. Another synthetic oligonucleotide adapter encodes sequences from the start codon for *nef* to the Xho I site within the gene (*12*). The remainder of *nef* is contained in a DNA fragment derived from the biologically active, molecularly cloned proviral form of HIV-1_{SF2} (p9B-18) (*12, 13*). This DNA fragment has sequences from the Xho I site in *nef*, through the rightward LTR and into cellular flanking sequences up to an Eco RI site. The plasmid p3R has the HIV-1_{SF2} LTR positioned upstream from the *nef* gene. The DNA fragment bounded by BAM HI and Eco RI sites from pAM3 was cloned into a polylinker downstream from the LTR in the plasmid pLTR-1 (*14*).
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25. We thank A. Murgia, P. Barry, and R. Unger for technical assistance, D. Wong for preparation of the manuscript, and B. M. Peterlin for helpful comments. The polyclonal rabbit anti-p27 antiserum and the purified yeast p27 were provided by I. Bathurst and P. Feucht (Chiron Corp., Emeryville, CA). This work was supported by grants from NIH (RO1 AI-25284 and RO1 AI-24499) and the California State Universitywide Task Force on AIDS.

6 July 1989; accepted 31 October 1989

RNA Editing in Plant Mitochondria

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Comparative sequence analysis of genomic and complementary DNA clones from several mitochondrial genes in the higher plant *Oenothera* revealed nucleotide sequence divergences between the genomic and the messenger RNA–derived sequences. These sequence alterations could be most easily explained by specific post-transcriptional nucleotide modifications. Most of the nucleotide exchanges in coding regions lead to altered codons in the mRNA that specify amino acids better conserved in evolution than those encoded by the genomic DNA. Several instances show that the genomic arginine codon CGG is edited in the mRNA to the tryptophan codon TGG in amino acid positions that are highly conserved as tryptophan in the homologous proteins of other species. This editing suggests that the standard genetic code is used in plant mitochondria and resolves the frequent coincidence of CGG codons and tryptophan in different plant species. The apparently frequent and non–species-specific equivalency of CGG and TGG codons in particular suggests that RNA editing is a common feature of all higher plant mitochondria.

SEQUENCE ANALYSIS OF GENOMIC and cDNA clones from the mitochondrially encoded cytochrome oxidase subunits II (*coxII*) and III (*coxIII*), the cytochrome b (*cytb*), and the reduced nicotinamide adenine dinucleotide–dehydrogenase subunit I (*nadI*) loci revealed a number of discrepancies between the two respective sequences. One of these instances within the coding region of *coxIII* was reported earlier (*1*). Several independently derived cDNA clones contained two adjacent T residues where the genomic DNA encodes C's. The cDNA specifies phenylalanine instead of the proline specified by the genomic DNA, where phenylalanine is conserved in the human, yeast, and *Neurospora* proteins (Fig. 1).

Further analysis has now shown that such nucleotide exchanges are not a singular event at this locus, but occur at many positions in several different genes. Three additional nucleotide changes are found in the analyzed coding region of the *coxIII* cDNA sequence, all involving C to T transitions (Fig. 1). All four events are nonsilent and specify amino acids in the cDNA sequence that are better conserved in other species at the respective positions than the genome-encoded amino acids (Fig. 1C).

Artifacts of cDNA cloning had been assumed when the first sequence differences between genomic and the mRNA-derived DNAs were observed. This explanation now seems unlikely since a number of such events have been observed in apparently physically "normal" sequence surroundings that give no indication as to why reverse transcriptase or the bacterial amplification processes should introduce these particular modifications repeatedly. The conservation of the cDNA-specified amino acids between different species also indicates that the cDNA sequence is correct.

These nucleotide divergences between genomic and mRNA-derived sequences are not restricted to coding regions, but are also found, for example, in the trailer sequence of the *coxIII* locus (Fig. 1B), which presumably is not translated. The effect of these untranslated alterations is as yet unclear and needs further experimental evaluation.

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The functional consequences of the nucleotide identity changes within the coding region can be deduced from comparative analysis of the altered codon and amino acid identities in the deduced sequences. Two of the nucleotide changes alter the second nucleotide of TCA and TCG serine codons, leading to TTA and TTG leucine codons, respectively, where leucine is dominantly specified in other species (Fig. 1B).

Another of the nucleotide alterations modifies the genomic arginine triplet CGG in the first position to the tryptophan codon TGG at amino acid position 252, where tryptophan is conserved in human, *Saccharomyces*, and *Neurospora* polypeptides. The genomic CGG codon has been found in a number of different plant mitochondrial genes at conserved tryptophan positions, and it has been suggested that CGG actually specifies tryptophan in a modified decoding system in plant mitochondria that diverges from the standard genetic code of translation (*2, 3*). To further investigate the identity of this codon in other mRNAs, we analyzed a number of independent cDNA and genomic clones of different genes in *Oenothera* mitochondria, notably the *coxIII* (Fig. 2), *cytb*, and *nad1* loci.

Differences in the investigated coding region of *coxII* (Fig. 2) include another example of the alteration of an arginine CGG codon to TGG at a conserved tryptophan position. A genomic CGG is found only in *Oenothera* at this site, whereas standard tryptophan TGG codons are encoded in the genomic sequences in other higher plants—for example, maize (*2, 3*). Additional nucleotide changes in this region of the *coxII* locus involve two more second position changes in TCA and TCG serine to TTA and TTG leucine codons, respectively, and two different alterations of proline CCT codons to serine TCT in one instance and to leucine CTT in the other.

Further examples of CGG to TGG alterations are found in the cDNA sequences of the cytochrome b locus, which, like most other alterations in this locus, also improve the degree of conservation between the deduced plant protein sequence and the homologous polypeptide sequences from other organisms (*4, 5*).

The overall frequency of nucleotide exchanges is similar in the loci compared here. Of 651 analyzed nucleotides of the *coxIII* locus, 11 are altered in the cDNA, equivalent to 1.7% on the average. Changes in 5 of the 276 nucleotides compared for the *coxII* cDNA represent a 1.8% sequence modification. The 16 alterations in the total of 927 nucleotides we analyzed give a divergence of 1.73% in the mRNA-derived sequence from the genome-specified nucleotide identities, equivalent to one nucleotide alteration for every 58 nucleotides.

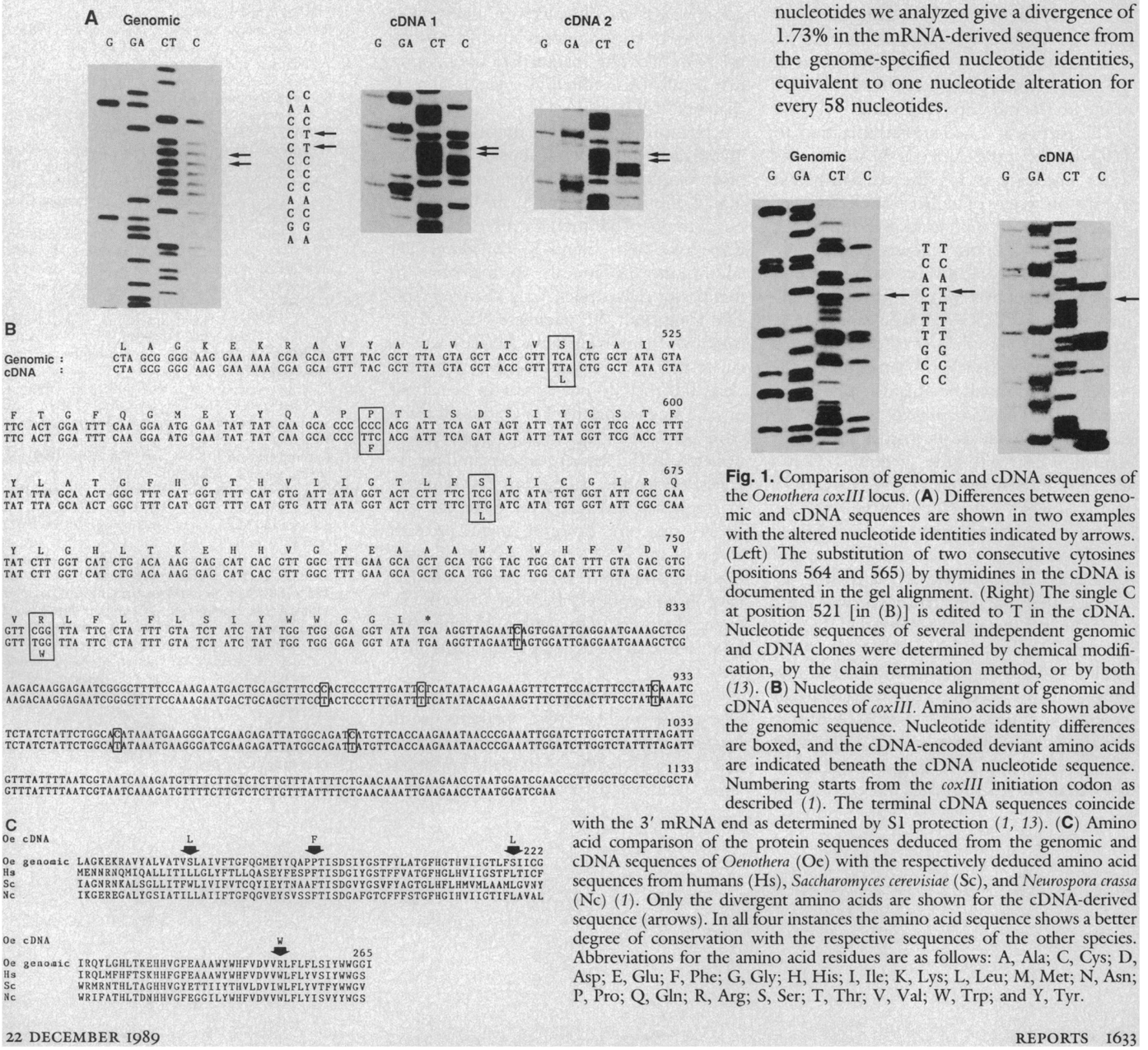

Fig. 1. Comparison of genomic and cDNA sequences of the *Oenothera coxIII* locus. (**A**) Differences between genomic and cDNA sequences are shown in two examples with the altered nucleotide identities indicated by arrows. (Left) The substitution of two consecutive cytosines (positions 564 and 565) by thymidines in the cDNA is documented in the gel alignment. (Right) The single C at position 521 [in (B)] is edited to T in the cDNA. Nucleotide sequences of several independent genomic and cDNA clones were determined by chemical modification, by the chain termination method, or by both (*13*). (**B**) Nucleotide sequence alignment of genomic and cDNA sequences of *coxIII*. Amino acids are shown above the genomic sequence. Nucleotide identity differences are boxed, and the cDNA-encoded deviant amino acids are indicated beneath the cDNA nucleotide sequence. Numbering starts from the *coxIII* initiation codon as described (*1*). The terminal cDNA sequences coincide with the 3′ mRNA end as determined by S1 protection (*1, 13*). (**C**) Amino acid comparison of the protein sequences deduced from the genomic and cDNA sequences of *Oenothera* (Oe) with the respectively deduced amino acid sequences from humans (Hs), *Saccharomyces cerevisiae* (Sc), and *Neurospora crassa* (Nc) (*1*). Only the divergent amino acids are shown for the cDNA-derived sequence (arrows). In all four instances the amino acid sequence shows a better degree of conservation with the respective sequences of the other species. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

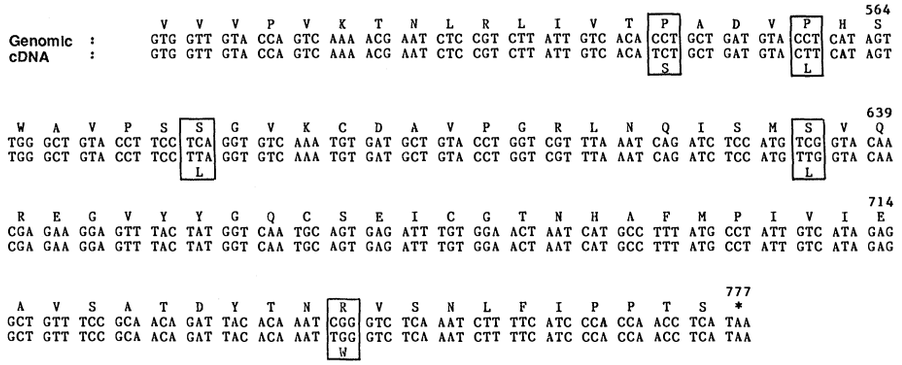

Fig. 2. Genomic and cDNA sequences of the *Oenothera coxII* coding region. Both DNA sequences are shown aligned with the sequence divergences indicated by boxes. The corresponding amino acids are shown above and below the nucleotide sequences. Numbering starts from the first coding nucleotide (*2*). The derived amino acids show that the cDNA-encoded sequence is better conserved in evolution than the polypeptide specified by the genomic DNA. The asterisk indicates the termination codon.

The consequences of alterations in nucleotides from coding regions that lead to amino acid alterations differ from the silent exchanges in coding regions of the *nad1* (*6, 7*) and *cytb* loci (*4, 5*) and the alterations in the presumably untranslated trailer sequence of the *coxIII* transcript (Fig. 1B).

The particular CGG triplet alteration to TGG at the mRNA level, however, has wider implications for the decoding and translation system of higher plant mitochondria. This specific modification in the cDNA sequence of *Oenothera* suggests that in these instances the only tRNA-Trp so far found in plant mitochondria (*8*), which specifically recognizes the UGG codon, is sufficient to incorporate tryptophan in those positions highly conserved as tryptophan between different species of higher plants, fungi, and mammals. It thus seems likely that the standard genetic code is used in higher plant mitochondria with the genomic CGGs changed in those instances to UGG codons in the mRNA (*3*). Not all CGG codons appear to be altered, however, since highly conserved arginines are found in several instances of genomic CGG codons (*9*).

The numerous differences between the genomic and the mRNA-derived cDNA sequences are best explained by an RNA editing process in plant mitochondria that introduces the specific sequence alterations described in this report. Other RNA editing processes have been observed; the most extensive example is in trypanosome mitochondria, where U residues not encoded by DNA are inserted with varying frequencies into the mRNA (*10*). RNA editing in *Physarum polycephalum* involves the insertion of cytosines in fairly uniform spacing into the mRNA (*11*). The mammalian apolipoprotein B mRNA is edited by a single C to U change (*10, 11*).

RNA editing in plant mitochondria as investigated in *Oenothera* appears to involve most frequently the change (or chemical modification) of cytosine to uridine. So far no examples of additionally inserted nucleotides have been found in *Oenothera*. The editing process appears to maintain the number of nucleotides, only changing specific identities. The parameters that determine which individual nucleotides will be edited in higher plant mitochondria are unclear, but they do not appear to be simple, linear sequence motifs. No obvious features potentially acting as editing signals can be detected in the sequences surrounding the edited nucleotides at the primary sequence or secondary structure level. Extensive secondary structure, however, might preclude access of the RNA editing activity in plant mitochondria, since no nucleotide changes have been reported between directly sequenced termini of the wheat mitochondrial 18*S* ribosomal RNA and the respective gene (*12*).

One of the selection criteria of the editing specificities might involve pressures on the incorporation of evolutionarily conserved and thus supposedly functionally important amino acids at particular positions. Other determinants, however, are needed for silent exchanges, the consequences of which remain to be investigated.

The presumably post-transcriptional transformation of CGG codons to TGG suggests that the standard genetic code may be used in plant mitochondria. The RNA editing process we described could resolve the uncertainty about the suggested divergence from the standard genetic code with CGG coding for tryptophan (*2–4*). The apparent free exchangeability of CGG and TGG codons in different plant species at a given triplet position conserved as tryptophan in the evolution of several different genes indicates that RNA editing might be a general feature of higher plant mitochondria.

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14. Supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

24 August 1989; accepted 19 October 1989