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Plant Mitochondrial RNA Editing

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Abstract. RNA editing affects messenger RNAs and transfer RNAs in plant mitochondria by site-specific exchange of cytidine and uridine bases in both seed and nonseed plants. Distribution of the phenomenon among bryophytes has been unclear since RNA editing has been detected in some but not all liverworts and mosses. A more detailed understanding of RNA editing in plants required extended data sets for taxa and sequences investigated. Toward this aim an internal region of the mitochondrial nad5 gene (1104 nt) was analyzed in a large collection of bryophytes and green algae (Charales). The genomic nad5 sequences predict editing in 30 mosses, 2 hornworts, and 7 simple thalloid and leafy liverworts (Jungermanniidae). No editing is, however, required in seven species of the complex thalloid liverworts (Marchantiidae) and the algae. RNA editing among the Jungermanniidae, on the other hand, reaches frequencies of up to 6% of codons being modified. Predictability of RNA editing from the genomic sequences was confirmed by cDNA analysis in the mosses *Schistostega pennata* and *Rhodobryum roseum*, the hornworts *Anthoceros husnotii* and *A. punctatus*, and the liverworts *Metzgeria conjugata* and *Moerckia flotoviana*. All C-to-U nucleotide exchanges predicted to reestablish conserved codons were confirmed. Editing in the hornworts includes the removal of genomic stop codons by frequent reverse U-to-C edits. Expectedly, no RNA editing events were identified by cDNA analysis in the marchantiid liverworts *Ricciocarpos natans*, *Corsinia coriandra*, and *Lunularia cruciata*. The findings are discussed in relation to models on the phylogeny of land plants.

Key words: RNA editing — Pyrimidine exchange — Plant mitochondria — Bryophytes — Liverworts — Hornworts — Mosses — cox3 — nad5

Introduction

RNA editing processes modify the genetic information on the transcript level by exchanging, inserting, or deleting standard nucleotides of the genetic alphabet in very diverse groups of eukaryotes (Benne 1996; Smith et al. 1997). While the RNA editing systems discovered in protists and metazoa at least in some cases appear to be of regulatory use to the organism, no such obvious advantage is as yet recognized for the editing systems in plant organelles. The frequent replacement of cytidine bases by uridines in plant mitochondria and chloroplasts (Maier et al. 1996) is mandatory for the correct expression of the genetic information contained in mRNAs and tRNAs. However, it remains unclear why a corrected base replaced by RNA editing in the transcript is not correctly coded in the gene itself in the first place. While essentially every protein-encoding mRNA in higher plant mitochondria is subject to RNA editing, the phenomenon is significantly less frequent in chloroplasts, where it affects only some transcripts.

To approach the question of its potential biological meaning, we have begun to analyze whether the occur-

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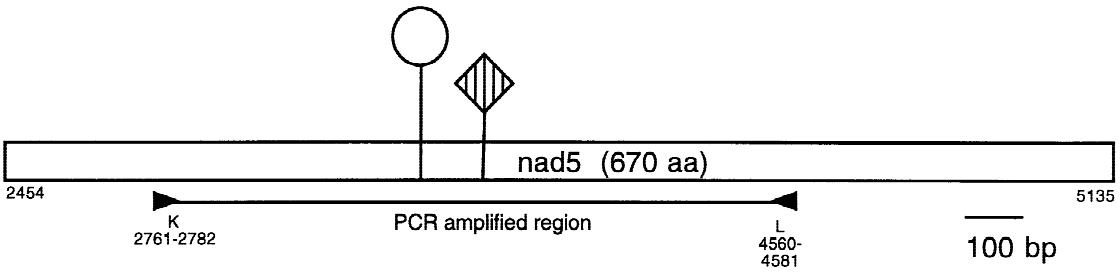

Fig. 1. The nad5 reading frame is interrupted by a single group I intron in *Marchantia polymorpha*, which is positionally conserved in all bryophytes included in this study to the exclusion of hornworts (*open circle*). Only *Anthoceros punctatus* carries a group II intron (*hatched square*) at a different position. Functional splicing of the introns in bryophytes is confirmed in cDNA analysis of the selected species (Table 1). No introns are located in the amplified region of *Anthoceros husnotii*, the algae and seed plants. The nad5 coding sequence of the latter is interrupted by cis- and trans-splicing group II introns in different positions (Knoop et al. 1991; Pereira de Souza et al. 1991). The indicated region of 368 codons under investigation in this study is bordered by the two oligonucleotides K and L used for PCR amplification. Numbers indicate nucleotide coordinates of the *Marchantia polymorpha* chondriome sequence (M68929) for reference.

rence of RNA editing is correlated with major events in the evolution of land plants. Extending initial results (Hiesel et al. 1994), the presence of mitochondrial RNA editing has been detected among all vascular plants (seed plants, ferns, fern allies) and also in representatives of the three conventionally distinguished classes of bryophytes: the hornworts, liverworts, and mosses (Malek et al. 1996; Sper-Whitis et al. 1996). The regions of the cox3 and cox1 genes, encoding subunits of the cytochrome oxidase, hitherto investigated are too short to give reliable estimates about the absence of editing in some bryophytes and gave skewed impressions about a low frequency of editing in extant representatives of early branches in the plant phylogeny. The absence of editing in a given taxonomic group can of course never be ultimately shown but is, at best, reliably predicted from a sufficiently large and comparative data set. To define more clearly the dividing line between editing and nonediting plant species, we have now analyzed an internal region of the nad5 gene (1104 nt) encoding 368 amino acids in bryophytes. Evidence for RNA editing is found in all bryophyte groups except the subclass of complex thalloid liverworts (Marchantiidae).

RNA editing in plant organelles is of particular evolutionary interest since the available data suggest the simultaneous existence of this phenomenon in land plant chloroplasts and mitochondria. As in mitochondria, RNA editing was recently shown to exist in chloroplasts of a hornwort (Yoshinaga et al. 1996) as well as in certain liverworts and mosses (Freyer et al. 1997).

Materials and Methods

Nucleic Acid Preparation. Plant material was either collected in the field or cultivated on sterile agar. Total nucleic acids were extracted in the presence of cetyltrimethylammonium bromide (CTAB). The CTAB extraction method (Doyle and Doyle 1990) was modified by adding 1% polyvinylpyrrolidone (PVP 40), incubating at room temperature (RT) for 15 min, and extracting once with phenol–chloroform. DNA and RNA were differentially precipitated in the presence of 2 *M* lithium acetate. For cDNA analysis RNA was treated with RNAase-free DNAase (Boehringer Mannheim). Commercially available kits to extract plant nucleic acids (Qiagen) were alternatively used in some cases. First strand cDNA was synthesized with AMV reverse transcriptase in the presence of random hexamer primers or the specific downstream primer used in the PCR (polymerase chain reaction).

PCR Amplification, Cloning, and Sequencing. The upstream primer K (5′-ata tgt ctg agg atc cgc ata g-3′) and the downstream primer L (5′-aac ttt ggc caa gga tcc tac aaa-3′) were routinely used for amplification of the nad5 gene region (see Fig. 1). Primer K2 (5′-agt agc ttr gty cat mtt tat tc-3′), located 31 nt upstream of K, was used as an alternative upstream primer in some cases when mispriming was observed with oligonucleotide K. The PCR amplification assays contained 1 μl template DNA (approximately 10 ng–1 μg), 10 μl 10× PCR buffer (100 m*M* Tris/HCl, pH 8.85, 250 m*M* KCl, 50 m*M* $(NH_4)_2SO_4$, 20 m*M* $MgSO_4$), a 250 m*M* concentration of each dNTP, 0.25 μg of each primer, 2.5 U of DNA polymerase, and double-distilled water to 100μl. Different commercially available thermostable DNA polymerases were used, e.g., a mixture (90:1) of *Taq* DNA Pol (GIBCO BRL) and *Pwo* DNA Pol (Boehringer Mannheim). The addition of 0.1% skim milk powder (1μl of a 10% aqueous solution) improved the PCR results in some cases (DeBoer et al. 1995). A typical amplification assay included an initial denaturation (5 min, 94°C), followed by 35 cycles with 1 min of denaturation at 94°C, 1 min of annealing at 50–55°C, and 2 min 30 s of synthesis at 72°C and a final step of synthesis for 6 min at 72°C. PCR fragments were blunt-end ligated into pBlueskript II SK+ (Stratagene). Positive clones were sequenced with a Thermosequenase kit (Amersham) using Cy5 fluorescence-labeled oligonucleotides and run on an Alf Express sequencer (Pharmacia). At least two clones were sequenced, and additional clones where ambiguities had to be resolved. Three clones each from spliced and unspliced transcripts, respectively, were analyzed for *Moerckia flotoviana.* Sequencing primers were universal and reverse primers of the polylinker sequence and three primers matching internal sequences of the cloned nad5 fragment (Li, 5′-gct gca tga atc raa gcr gat act gg-3′; Le, 5-cat atc ttg ctc atc cga cat ggc atg-3′; and Ki, 5′act ygg tta ccy gat gca atg gag ggt-3′). A detailed phylogenetic analysis of the nad5 exon and intron sequences will be presented elsewhere.

Results

Prediction of C-to-U RNA editing sites in plant mitochondrial sequences is generally feasible, with a high reliability by comparison to homologous genes of other

species, most notably to the gene sequences of the liverwort *Marchantia polymorpha*, a species with an apparent lack of RNA editing in its organelles (Oda et al. 1992; Ohyama et al. 1993). Translation of the genomic DNA into protein sequences highlights codons in which a C–U exchange can reestablish codon identity in *Marchantia* or other species. These predicted editing positions are generally confirmed by subsequent cDNA analysis, and additional silent edits in third or first codon positions that do not affect amino acid identity are occasionally identified. The inferences about the occurrence of editing among land plants predicted from genomic cox1 sequences (Sper-Whitis et al. 1996) were thus in accord with comparative DNA and cDNA analysis of the cox3 gene (Hiesel et al. 1994; Malek et al. 1996). Not every prediction of RNA editing sites is, however, confirmed by cDNA analysis. Particularly, reverse edits (U to C), which occur very rarely in seed plants, cannot be predicted with similar reliability. An example is a site recently observed in the cox3 sequence of *Chara corallina* (Malek et al. 1996), a green alga of the Charales, which are discussed as being most closely related to land plants.

No Evidence for RNA Editing in Charales

The suspicious tyrosine codon (UAU) in the cox3 gene of *Chara* that could be reverse edited to a well-conserved histidine (CAU) codon (Malek et al. 1996) prompted us to investigate other members of this order. The same cox3 region was therefore analyzed in three other species of the Charales: *Nitella flexilis*, *Nitellopsis obtusa*, and *Lamprothamnium papulosum*. The UAU tyrosine codon in question was identified in the genomic sequences in all three algae and no evidence for editing was found in cDNA analysis for this or any other position in the analyzed region of the cox3 gene.

Lamprothamnium papulosum and *Nitella flexilis* were also included as representatives of the Charales in the analysis of the nad5 gene presented here (Fig. 1). No editing sites are predicted from the alignment of this nad5 region of *Lamprothamnium* and *Nitella* with the other species in accord with the earlier observation about absence of RNA editing among green algae.

RNA Editing Occurs in All Mosses

The distribution of RNA editing within the bryophytes has been unclear. Among the mosses (Bryopsida) editing was identified in the cox3 gene of *Ceratodon* and *Tetraphis* (Malek et al. 1996) but not in *Physcomitrella* and *Sphagnum* (Hiesel et al. 1994). Analysis of the cox1 gene predicted editing in the moss genus *Hypnum* (Sper-Whitis et al. 1996). *Physcomitrella patens* and *Sphagnum fallax* were included in the present survey of 47 bryophytes including 30 mosses, 15 liverworts, and 2 hornworts. Taxon sampling is intended to give a representative coverage of species divergence based on classic taxonomy (Table 1).

RNA editing events are predicted for the nad5 sequence in all mosses (Fig. 2) including *Sphagnum fallax* and *Physcomitrella patens*, for which previously no evidence of RNA editing has been found in the investigated cox3 region. Predicted editing frequencies within the mosses range from a single affected amino acid codon in 368 nad5 codons in *Diphyscium sessile* to nine codons in *Hookeria lucens*. Significantly, editing sites are seen in all orders of mosses including the basal-most-branching orders Sphagnales and Andreaeales.

The predictive value of RNA editing prognosis among mosses was confirmed by examplary cDNA analysis in *Schistostega pennata* and *Rhodobryum roseum*. RNA editing from C to U was found in *Rhodobryum* indeed to introduce all the postulated codon changes: twice S to F, twice S to L, and once R to C and R to W (Fig. 3). Three additional silent exchanges are observed in the *Rhodobryum* cDNA (see corresponding database entry). Similarly, the two predicted RNA editing events to introduce codon changes are identified in *Schistostega pennata* cDNA. A potential reverse U-to-C editing event in *Rhodobryum* to reconstitute a proline from a serine codon (position 338) was not identified in the cDNA sequence, underlining the restricted predictability of this editing type in some plant lineages (see below).

U-to-C RNA Editing Can Be the Dominating Exchange in Hornworts

The hornworts are a unique group among the bryophytes and recent phylogenetic analysis corroborates their isolated position in relation to the other bryophytes (Beckert et al. 1998). Alignment of the genomic nad5 sequences from *Anthoceros punctatus* and *Anthoceros husnotii* displays the highest number of nucleotide exchanges in a mitochondrial sequence ever observed between two species of a single plant genus (not shown). Most strikingly, in the genomic nad5 sequences of both species, reading frames are interrupted by six stop codons in *A. punctatus* and nine in *A. husnotii*, respectively (Fig. 2). Analysis of cDNAs of *Anthoceros husnotii* identified a total of 38 edited positions affecting 33 codon identities. All genomic stop codons are removed in the transcript by U-to-C edits reconstituting five conserved glutamine and four arginine codons (Fig. 3). A further 12 reverse U-to-C edits and 16 conventional C-to-U edits similarly reconstitute conserved codon identities. In four instances two edits in single codons establish phenylalanine or leucine codons, and in position 166 a CUU leucine codon is converted by both types of editing (C to U and U to C) side by side to a serine UCU codon. Only a single third codon position is edited by a C-to-U exchange in this nad5 region of *A. husnotii*, two in *A. punctatus*. In *A. punctatus* the cDNA analysis likewise confirms the re-

Table 1. Species list with sequence accessions and the number of codons predicted to be edited by pyrimidine exchanges within the 368-amino acid stretch of the nad5 gene under investigation[a]

Order	Species	Editing, C>U/U>C	Accession No.
Anthoceropsida			
Anthocerotales	***Anthoceros husnotii***	**16/21**	AJ000697
	Anthoceros punctatus	**32/18**	AJ000698
Marchantiopsida			
Sphaerocarpidae			
Sphaerocarpales	*Sphaerocarpos donnellii*	0	AJ001033
Marchantiidae			
Marchantiales	*Bucegia romanica*	0	AJ001031
	Corsinia coriandra	**0**	AJ001034
	Lunularia cruciata	**0**	AJ001002
	Marchantia polymorpha	0	M68929
	Targionia hypophylla	0	AJ001001
Ricciales	***Ricciocarpos natans***	**0**	AJ001032
Jungermanniidae			
Jungermanniales	*Jamesoniella autumnalis*	5/0	AJ000700
	Lejeunea cavifolia	2/0	AJ000701
	Plagiochila asplenioides	0/0	AJ000704
	Scapania nemorea	7/0	AJ000706
	Trichocolea tomentella	5/1*	AJ000707
Metzgeriales	*Fossombronia pusilla*	13/0	AJ000699
	Metzgeria conjugata	**11/0**	AJ000703
	Moerckia flotoviana	**21/0**	AJ223717
Bryopsida			
Buxbaumiales	*Diphyscium sessile*	1/0	Z98972
Tetraphidales	*Tetraphis pellucida*	6/0	AJ224855
Schistostegales	***Schistostega pennata***	**2/0**	AJ224856
Syrrhopodontales	*Calymperes erodes*	1/0	Z98952
	Syrrhopodon spec.	2/0	Z98953
Sphagnidae			
	Sphagnum fallax	7/2*	AJ001225
Andreaeidae			
	Andreaea nivalis	6/0	AJ001226
	Andreaea rupestris	7/0	AJ001227
Bryidae			
Polytrichanae			
Polytrichales	*Atrichum undulatum*	5/0	AJ001229
	Polytrichum formosum	2/0	AJ001228
Dicrananae			
Dicranales	*Ceratodon purpureus*	3/0	Z98955
	Dicranum scoparium	6/0	Z98956
Fissidentales	*Fissidens cristatus*	1/0	Z98954
Grimmiales	*Schistidium apocarpum*	2/0	Z98958
Pottiales	*Pottia truncata*	4/0	Z98957
Bartramianae			
Bartramiales	*Bartramia halleriana*	2/2*	Z98961
	Plagiopus oederi	2/3*	Z98962
Timmiales	*Timmia bavarica*	6/0	Z98963
Funarianae			
Funariales	*Funaria hygrometrica*	3/0	Z98959
	Physcomitrella patens	2/0	Z98960
Bryanae			
Bryales	***Rhodobryum roseum***	**6/0**	Z98964
	Plagiomnium cuspidatum	4/0	Z98965
Hypnanae			
Neckerales	*Hedwigia ciliata*	3/2*	Z98966
	Pterogonium gracile	5/1*	Z98968
	Rhacocarpus purpurascens	2/2*	Z98967
Hookeriales	*Hookeria lucens*	8/1*	Z98969
Hypnales	*Brachythecium rutabulum*	6/0	Z98970
	Rhytidiadelphus triquetrus	5/1*	Z98971
	Jaegerina stolonifera	4/1*	AJ224854
	Thuidium tamariscinum	3/1*	AJ004809

Table 1. Continued

Order	Species	Editing, C>U/U>C	Accession No.
Algae			
Charales	*Lamprothamnium papulosum*	0	AJ000702
	Nitella flexilis	0	X

[a] Bryophytes are grouped according to a taxonomy by Frahm and Frey (1991). Codon changes by RNA editing are exemplarily investigated by cDNA analysis for the species underlined. In *Metzgeria conjugata, Moerckia flotoviana, Schistostega pennata,* and *Rhodobryum roseum,* all predictions of C-to-U but not of U-to-C edits are confirmed in the cDNA. Prediction of U-to-C editings in other liverworts and mosses thus appears unreliable and is designated with an asterisk. All codon changes in both directions were, however, identified by cDNA analysis in the hornworts *Anthoceros punctatus* and *Anthoceros husnotii* (except a possible but not observed V-to-A codon exchange in position 93 in *A. punctatus;* see Fig. 3). Additional silent nucleotide exchanges observed are documented in the corresponding database entries. No editing events are detected in cDNA analysis of *Ricciocarpos natans, Lunularia cruciata,* and *Corsinia coriandra.*

constitution of conserved codon identities by RNA editing (Fig. 3) and also splicing of the unique group II intron in this species (Fig. 1).

The type of codons affected are similar to those in other plant species, while the extraordinary high levels of U-to-C vs. C-to-U edits (55 and 35%, respectively) is unique to the hornworts. *Anthoceros* thus questions the designation of ''reverse'' vs. ''conventional'' editings. The frequent removal of stop codons is also required in mitochondrial gene sequences of ferns (Malek et al. 1997). The faithful prediction of reverse editing events in general is apparently restricted to ferns and hornworts.

RNA Editing in Liverworts

The prediction about the general absence of RNA editing deduced from the entire *Marchantia polymorpha* chondriome sequence (Oda et al. 1992) was supported by selective cDNA analysis (Ohyama et al. 1993). On the other hand, RNA editing has recently been observed in the liverwort *Pellia epiphylla* (Malek et al. 1996). To address whether the absence of RNA editing among liverworts is unique to *Marchantia*, 14 additional species of this group were investigated. Conservation of the deduced nad5 amino acid sequences is extreme among the seven complex thalloid liverworts (Marchantiidae). Only one single amino acid deviation (L/V in position 135) is observed in *Targionia hypophylla* in the protein alignment, which cannot be converted by RNA editing. To reduce the risk that unconventional RNA editing, which has occasionally been observed in angiosperms (Schuster and Brennicke 1991), or rare silent editing events not expected from the genomic sequences may be overlooked, we have analyzed cDNAs from *Ricciocarpos, Corsinia,* and *Lunularia.* Except for the splicing of the group I intron, no sequence differences between DNA and cDNA were observed. The inference about the absence of RNA editing in *Marchantia* may thus tentatively be extended to all members of this subclass of complex thalloid liverworts (Marchantiidae).

A completely different picture emerges for the simple thalloid and leafy liverworts treated taxonomically as Jungermanniidae. Frequent predictions can be made for RNA editing sites from the protein alignment. *Moerckia flotoviana* was selected for cDNA analysis as species with the highest frequency of predicted editing sites and *Metzgeria conjugata* because of a strikingly divergent pattern of presumed edits. In both species all of the predicted C-to-U edits were confirmed, changing 11 codon identities in *Metzgeria conjugata* and 21 codons in *Moerckia flotoviana* (Figs. 2 and 3). Seven additional silent edits are identified in analysis of the *Moerckia* cDNAs, and none in *Metzgeria.* As in higher plants, the silent sites are edited to a much lesser extent in eight independent cDNA clones than in those changing codon identities. Full editing of all nonsilent sites was found in cDNA clones derived from spliced transcripts and variable editing in those derived from unspliced precursors, thus reflecting the parallel maturation of RNAs with respect to splicing and editing as commonly observed for angiosperms. Neither a phenylalanine codon at position 268 in *Metzgeria* nor a valine codon at position 93 in *Moerckia* is converted by reverse editing (Fig. 3). Notably, no editing at the latter position has been identified in *Anthoceros punctatus*.

RNA Editing in Plant Phylogeny

No generally accepted phylogeny of land plants is as yet available that clearly defines the topology of the earliest branches leading to recent lineages of ferns, fern allies, seed plants, hornworts, mosses, and liverworts (Fig. 4). Different suggestions have been made on the monophylies of these groups and their sister-group relationships. Mitochondrial sequences have only recently contributed to the ongoing debate (Malek et al. 1996), but no data set (including nad5) can as yet reliably resolve the branching patterns of the lineages mentioned above. A detailed consideration of the bryophyte phylogeny based on the nad5 gene that takes the conserved intron sequence into account will be presented elsewhere (Beckert et al. 1999).

The phylogenetic distribution of RNA editing would be most easily explained by the Marchantiidae being a sister group to all other land plants and a unique gain of

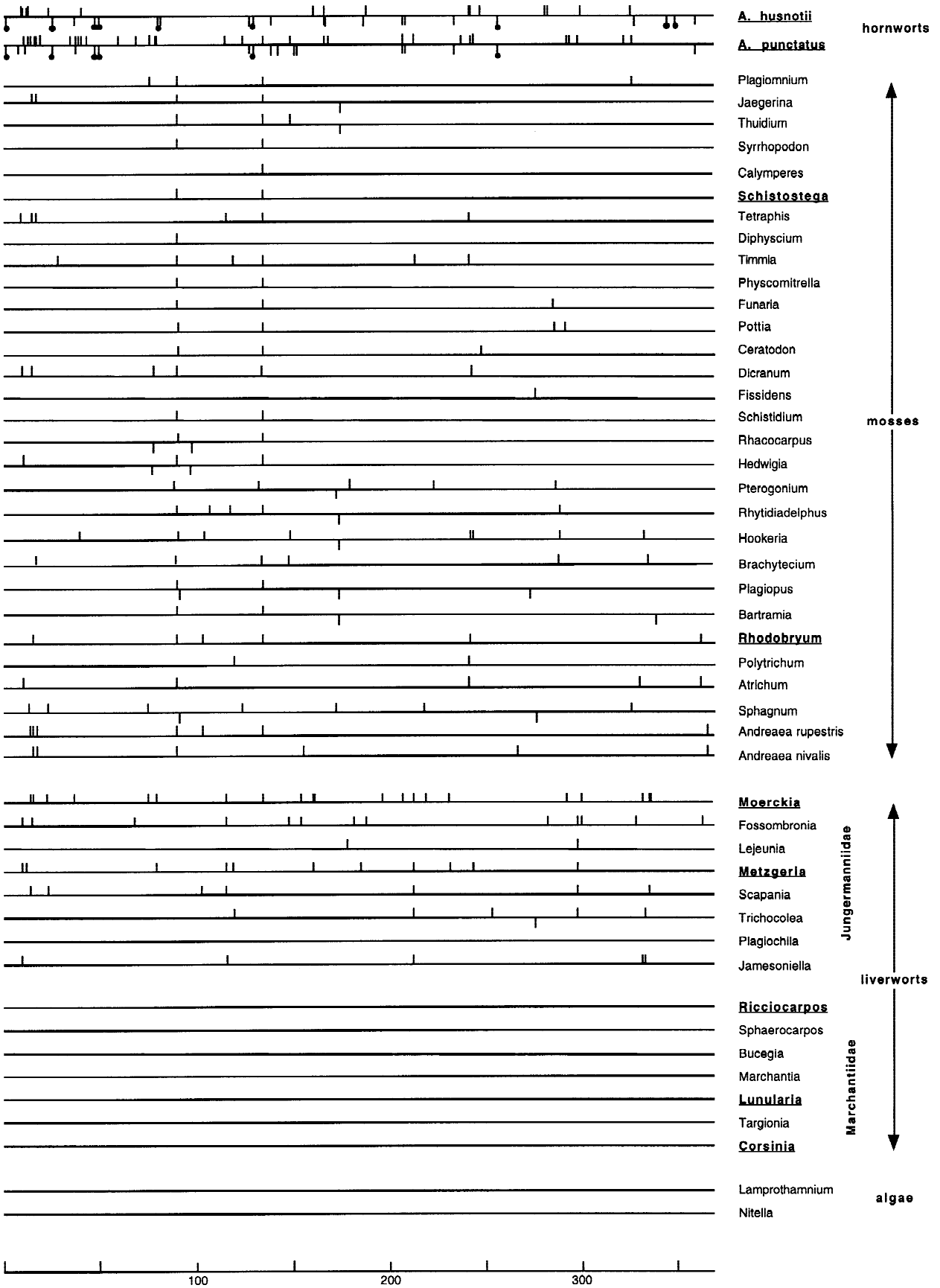

Fig. 2. Distribution of editing sites within a region of the mitochondrial nad5 gene coding for 368 amino acids in bryophytes (367 amino acids in the Charales algae). Drawing is approximately to scale, with codon changes by C-to-U edits shown as *vertical lines above* and those by U-to-C edits shown *below* the sequence, respectively. Stop codons in the reading frames are denoted by a *dot* at the end of the vertical line. Prediction of U-to-C edits among mosses and liverworts must be considered with caution since similar sites were not confirmed in cDNA analysis of *Moerckia, Metzgeria, Rhodobryum,* and *Schistostega* (see text and Fig. 3).

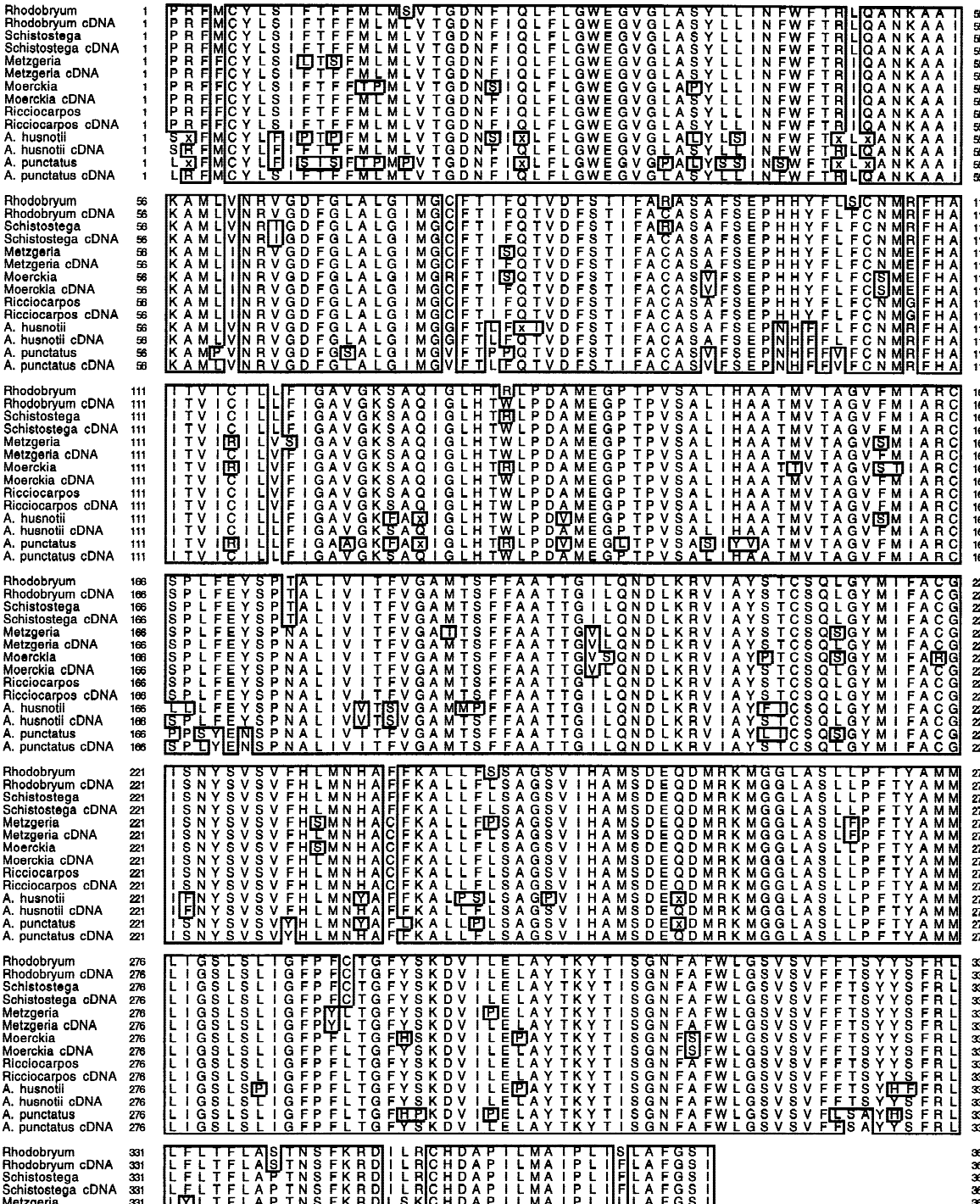

Fig. 3. Amino acid sequences of the nad5 region derived from DNA and cDNA sequences of the liverworts *Moerckia flotoviana* and *Metzgeria conjugata* (Jungermanniidae), the mosses *Rhodobryum roseum* and *Schistostega pennata*, and the hornworts *Anthoceros husnotii* and *A. punctatus*. Only the sequence of *Ricciocarpos natans* is shown as representative of the marchantiid liverworts, where cDNA and DNA sequences are identical except for splicing of the intron. Stop codons in the genomic *Anthoceros* sequences are indicated by x. The alignment was produced with the SeqVu 1.0.1 software by J. Gardner (Garvan Institute, Sydney, Australia).

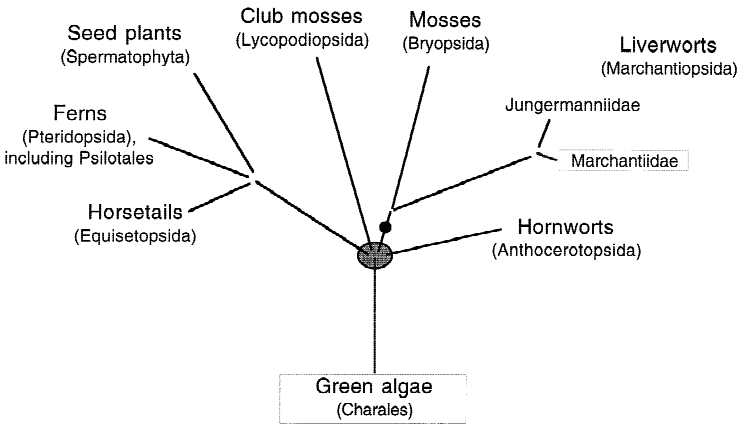

Fig. 4. The phylogenetic relationships of the earliest branching lineages of land plants are currently unresolved and a matter of debate (gray circle). The seven recent lineages shown are probably monophyletic. Molecular data support club mosses as the earliest branching vascular plants (e.g., Raubeson and Jansen 1992; Malek et al. 1996) and include the whisk ferns (Psilotales) among eusporangiate ferns. RNA editing has not been identified in algae and marchantiid liverworts (*shaded boxes*). A sister-group relationship of mosses and liverworts is suggested by gain of the common group I intron (*black dot*; see Fig. 1). With liverworts alternatively placed as sister group to all other land plants, a separate gain of RNA editing among Jungermanniidae would be an alternative parsimonious explanation.

RNA editing after their branching. The marchantiid liverworts have indeed been found as a sister group of all other bryophytes in analysis of the nuclear 18S rRNA gene (Bopp and Capesius 1996; Capesius and Bopp 1997) but this has not been corroborated by other molecular analyses, including those based on the same gene (Hedderson et al. 1996). According to the nad5 data, liverworts as a whole appear strongly supported as a monophyletic group (Beckert et al. 1999) and a sister-group relationship of liverworts and mosses is suggested by the shared group I intron sequence (Fig. 4). This observation together with the unequivocal presence of editing in all land plant lineages may suggest a secondary loss or at least extremely reduced frequency of RNA editing in the Marchantiidae liverworts. A highly reduced frequency of RNA editing will certainly always remain a distinct possibility to be considered even after very extensive cDNA analysis as is also underlined by the apparent lack of RNA editing in the investigated nad5 region of the jungermanniid liverwort *Plagiochila.* With the liverworts as the earliest branching lineage and the absence of RNA editing among Marchantiidae, an independent gain of RNA editing among the Jungermanniidae may be an alternative explanation.

Discussion

The general presence of editing in all recent lineages of land plants (except marchantiid liverworts) makes the hypothesis that RNA editing arose together with the first terrestrial plants an attractive idea. Even assuming that liverworts must be considered the sister group to all other plant lineages would postulate one separate gain of editing for Jungermanniidae liverworts, if their placement as sister group to Marchantiidae as suggested by classical taxonomy and the nad5 sequences is confirmed.

The absence of RNA editing for any given genome is hard to establish with ultimate confidence and the ever-increasing number of novel examples for RNA editing in the living world (for a recent example see Petschek et al. 1996) reflect how long these phenomena can remain undetected. It can consequently not be ruled out that the informations collected for the nad5 gene are locus-specific and some editing positions may be hidden in other sites of marchantiid liverwort chondriomes. The availability of the full *Marchantia polymorpha* genome (Oda et al. 1992) is a strong point but we will continue our phylogenetic analyses of bryophytes in general and the occurrence of RNA editing in particular by investigation of other mitochondrial gene loci. As in angiosperms, RNA editing frequencies are likely gene specific in bryophytes. The observation of six and three editing sites observed per 1104 nt in the nad5 genes of *Tetraphis* and *Ceratodon*, respectively, correlate well with one site each observed in 384 nt of the cox3 gene. Nine potential editing sites versus none in the respective regions of the *Sphagnum* chondriome may, on the other hand, hint at locus differences in this species, but this remains as yet clearly without statistical confirmation.

Evidently, it remains to be clarified whether the extremely reduced primary sequence divergence among the Marchantiidae liverworts and the apparent lack of RNA editing are unique to the nad5 gene or possibly even functionally related. A slowly ticking molecular clock is interestingly not a typical hallmark of Marchantiidae per se since the evolution of the nuclear 18S rRNA gene is rather accelerated in comparison to the other liverworts (Bopp and Capesius 1996). A correlation of GC content and RNA editing frequency found earlier for a wider taxonomic but narrower sequence sampling (Malek et al. 1996) cannot be confirmed by the nad5 data due to a lack of GC content variation among all bryophytes investigated (40 ± 1.5%).

Comparing the mitochondrial and chloroplast type of pyrimidine exchange editing in plants yields two striking observations besides the significantly higher editing frequence (on average about 30-fold) in mitochondrial transcripts. Both the phylogenetic distribution of RNA editing in general and the tendency for an increase in U-to-C vs. C-to-U edits in hornworts is congruently observed in both organelles at the same time. Although for chloro-

plast RNA editing in Marchantiidae no data other than for *Marchantia polymorpha* itself are available, the obvious absence of editing deduced from its endosymbiont genomes (Ohyama 1996) may be taken as significant indicator for the absence of editing in this liverwort subgroup as a whole. RNA editing was, on the other hand, recently found in chloroplasts of a Jungermanniidae liverwort species (Freyer et al. 1997). Even more strikingly, the extraordinary frequency of (reverse) edits in the U-to-C direction is observed in both mitochondria and chloroplasts (Yoshinaga et al. 1996) of hornworts. This particular type of editing was not seen in a recent extensive analysis of chloroplast transcripts in the black pine *Pinus thunbergii* (Wakasugi et al. 1996). In conclusion, these observations argue in favor of the concerted appearance/disappearance of the biochemical activities responsible for the observed base exchanges in both organelles. The exchanges of uridine to cytidine, designated reverse editings for historical reasons, have lately been observed at a high frequency in hornworts and ferns (Hiesel et al. 1994; Malek et al. 1996, 1997; Yoshinaga et al. 1996; this study). Their occurrence may be taken as evidence for the existence of an additional mechanism complementing the base deamination process hitherto postulated for plant mitochondrial RNA editing (Blanc et al. 1995; Yu and Schuster 1995).

The coincidence in chloroplast and mitochondrial RNA editing rather supports a singular biochemistry (e.g., transamination) with a unique bias for the direction of pyrimidine exchanges in both plant organelles. The genetic information in plant mitochondrial and chloroplast genomes precludes that the editing activity is organellar encoded and postulate the import of the corresponding nuclear gene product(s) after synthesis in the cytosol. The striking difference in editing frequency is presumably associated with separately encoded specificity factors unique for each organelle that likewise have to be imported from the cytosol (Bock and Koop 1997). The recent elegant approaches to decipher the biochemistry of RNA editing using chloroplast transformation techniques in tobacco (Bock et al. 1996; Chaudhuri and Maliga 1996) may thus at the same time identify enzymatic activities which operate in the mitochondrion. The requirement of RNA editing in the moss *Physcomitrella patens* reported here may make this species a complementary system for biological analysis of RNA editing. *Physcomitrella* is being established as a novel model organism in plant molecular biology (Cove et al. 1997; Reski 1998) due to its high frequency of homologous recombination after nuclear transformation (Schaefer and Zryd 1997).

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References

Beckert S, Steinhauser S, Muhle H, Knoop V (1999) A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial nad5 gene. Plant Syst Evol (in press)

Benne R (1996) RNA editing: How a message is changed. Curr Opin Genet Dev 6:221–231

Blanc V, Litvak S, Araya A (1995) RNA editing in wheat mitochondria proceeds by a deamination mechanism. FEBS Lett 373:56–60

Bock R, Koop HU (1997) Extraplastidic site-specific factors mediate RNA editing in chloroplasts. EMBO J 16:3282–3288

Bock R, Hermann M, Kössel H (1996) In vivo dissection of cis-acting determinants for plastid RNA editing. EMBO J 15:5052–5059

Bopp M, Capesius I (1996) New aspects of bryophyte taxonomy provided by a molecular approach. Bot Acta 109:368–372

Capesius I, Bopp M (1997) New classification of liverworts based on molecular and morphological data. Pl Syst Evol 207:87–97

Chaudhuri S, Maliga P (1996) Sequences directing C to U editing of the plastic psbL mRNA are located within a 22 nucleotide segment spanning the editing site. EMBO J 15:5958–5964

Cove DJ, Knight CD, Lamparter T (1997) Mosses as model systems. Trends Plant Sci 2:99–105

DeBoer SH, Ward LJ, Li X, Chittarajan S (1995) Attenuation of PCR inhibition in the presence of plant compounds by addition of BLOTTO. Nucleic Acids Res 23:2567–2568

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Frahm JP, Frey W (1991) Moosflora. Ulmer, Stuttgart

Freyer R, Kiefer-Meyer MC, Kössel H (1997) Occurrence of plastid RNA editing in all major lineages of land plants. Proc Natl Acad Sci USA 94:6285–6290

Hedderson TA, Chapman RL, Rootes WL (1996) Phylogenetic relationships of bryophytes inferred from nuclear-encoded rRNA gene sequences. Pl Syst Evol 200:213–224

Hiesel R, Combettes B, Brennicke A (1994) Evidence for RNA editing in mitochondria of all major groups of land plants except the Bryophyta. Proc Natl Acad Sci USA 91:629–633

Knoop V, Schuster W, Wissinger B, Brennicke A (1991) Trans splicing integrates an exon of 22 nucleotides into the nad5 mRNA in higher plant mitochondria. EMBO J 10:3483–3493

Maier RM, Zeltz P, Kössel H, Bonnard G, Gualberto JM, Grienenberger JM (1996) RNA editing in plant mitochondria and chloroplasts. Plant Mol Biol 32:343–365

Malek O, Lättig K, Hiesel R, Brennicke A, Knoop V (1996) RNA editing in bryophytes and a molecular phylogeny of land plants. EMBO J 15:1403–1411

Malek O, Brennicke A, Knoop V (1997) Evolution of trans-splicing plant mitochondrial introns in pre-Permian times. Proc Natl Acad Sci USA 94:553–558

Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi T, Ohyama K (1992) Gene organization deduced from the complete sequence of liverwort Marchantia polymorpha mitochondrial DNA. A primitive form of plant mitochondrial genome. J Mol Biol 223:1–7

Ohyama K (1996) Chloroplast and mitochondrial genomes from a liverwort, Marchantia polymorpha—gene organization and molecular evolution. Biosci Biotechnol Biochem 60:16–24

Ohyama K, Oda K, Ohta E, Takemura M (1993) Gene organization and

evolution of introns of a liverwort, Marchantia polymorpha, mitochondrial genome. In: Brennicke A, Kück U (eds) Plant mitochondria. Verlag Chemie, Weinheim, pp 115–129

Pereira de Souza A, Jubier MF, Delcher E, Lancelin D, Lejeune B (1991) A trans-splicing model for the expression of the tripartite nad5 gene in wheat and maize mitochondria. Plant Cell 3:1363–1378

Petschek JP, Mermer MJ, Scheckelhoff MR, Simone AA, Vaughn JC (1996) RNA editing in Drosophila 4f-rnp gene nuclear transcripts by multiple A-to-G conversions. J Mol Biol 259:885–890

Raubeson LA, Jansen RK (1992) Chloroplast DNA evidence on the ancient evolutionary split in vascular land plants. Science 255: 1697–1699

Reski R (1998) Development, genetics and molecular biology of mosses. Bot Acta 111:1–15

Schaefer DG, Zryd JP (1997) Efficient gene targeting in the moss Physcomitrella patens. Plant J 11:1195–1206

Schuster W, Brennicke A (1991) RNA editing makes mistakes in plant mitochondria: editing loses sense in transcripts of a rps19 pseudogene and in creating stop codons in coxI and rps3 mRNAs of Oenothera. Nucleic Acids Res 19:6923–6928

Smith HC, Gott JM, Hanson MR (1997) A guide to RNA editing. RNA 3:1105–1123

Sper-Whitis GL, Moody JL, Vaughn JC (1996) Universality of mitochondrial RNA editing in cytochrome-c oxidase subunit I (coxI) among the land plants. Biochim Biophys Acta 1307:301–308

Wakasugi T, Hirose T, Horihata M, Tsudzuki T, Kössel H, Sugiura M (1996) Creation of a novel protein-coding region at the RNA level in black pine chloroplasts: The pattern of RNA editing in the gymnosperm chloroplast is different from that in angiosperms. Proc Natl Acad Sci USA 93:8766–8770

Yoshinaga K, Iinuma H, Masuzawa T, Uedal K (1996) Extensive RNA editing of U to C in addition to C to U substitution in the rbcL transcripts of hornwort chloroplasts and the origin of RNA editing in green plants. Nucleic Acids Res 24:1008–1014

Yu W, Schuster W (1995) Evidence for a site-specific cytidine deamination reaction involved in C to U RNA editing of plant mitochondria. J Biol Chem 270:18227–18233