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Evolution

Evolution of trans-splicing plant mitochondrial introns in pre-Permian times

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ABSTRACT **Trans-splicing in angiosperm plant mitochondria connects exons from independent RNA molecules by means of group II intron fragments. Homologues of trans-splicing introns in the angiosperm mitochondrial *nad2* and *nad5* genes are now identified as uninterrupted group II introns in the ferns *Asplenium nidus* and *Marsilea drummondii*. These fern introns are correctly spliced from the pre-mRNA at the sites predicted from their well-conserved secondary structures. The flanking exon sequences of the *nad2* and *nad5* genes in the ferns require RNA editing, including the removal of in-frame stop codons by U-to-C changes for correct expression of the genetic information. We conclude that cis-splicing introns like the ones now identified in ferns are the ancestors of trans-splicing introns in angiosperm mitochondria. Intron disruption is apparently due to a size increase of the structurally variable group II intron domain IV followed by DNA recombination in the plant mitochondrial genome.**

Group II introns have gained considerable interest as potential progenitors of the widespread eukaryotic nuclear introns and the spliceosome (1, 2). Generally considered organellar introns, group II introns have recently also been discovered in eubacteria related to the prokaryotic ancestors of mitochondria and chloroplasts (3–5). A gap in theories postulating an evolutionary connection is currently the nonoverlapping distribution of nuclear spliceosomal introns and group II introns, the latter being restricted to organelles and eubacteria. Only fragments of organellar group II introns have been reported in nuclear genomes of plants at the sequence level (6), while no intact group II intron structure has been discovered in nuclear sequence data through systematic data base screenings (7).

A major evolutionary event in the postulated transition from group II to nuclear introns is the fragmentation of a single continuous RNA molecule into the several cooperating small RNAs of the spliceosome. The clearly defined secondary and tertiary structure features of group II introns (8, 9) may represent an evolutionary ancestor of the highly ordered small nuclear RNAs (snRNAs) in the spliceosome (1). Assuming such an evolutionary connection between group II and nuclear introns, it may be possible to identify some kind of intermediate in the extant living world.

Group II introns processively disrupted *in vivo* may represent such intermediate stages of early steps in intron evolution. Fragmentation of group II introns is observed in land plant and algal organelles. Examples in chloroplasts include the *rps12* gene in land plants (10, 11) requiring one trans-splicing for mRNA maturation and the *psbA* gene in the alga *Chlamydomonas reinhardtii* (12) requiring two. In mitochondria of angiosperms five group II introns located in the *nad1, nad2*, and *nad5* genes (all of which encode subunits of complex I, the NADH dehydrogenase) are found to connect exons from independent RNA molecules by trans-splicing (13–18). The complex trans-splicing arrangements of these mitochondrial *nad* genes are generally conserved between mono- and dicotyledonous species and consequently indicate a common origin before the establishment of the angiosperm line at least 140 million years B.P. Among flowering plants, variability in cis- versus trans-arrangements has been found only for the last intron of the *nad1* gene, which is cis-arranged in *Oenothera berteriana* (14) and broad bean (19), but disrupted in wheat (13) and *Petunia* (17) at different positions. This intron, however, is unique in being the only example of 25 vascular plant mitochondrial group II introns carrying a maturase-like reading frame (19).

While group II intron distribution is highly conserved among the angiosperms, an entirely different picture emerges from the completely sequenced mitochondrial genome of the liverwort *Marchantia polymorpha* (20). Although similar in number, all but one group II introns occupy different positions, none of them being trans-splicing. Plant mitochondrial genome evolution has thus seen a frequent coming and going of group II introns before the establishment of the angiosperm lineage.

Trans-splicing mitochondrial introns in angiosperms may represent very ancestral gene structures, could be derived from insertions of *a priori* trans-splicing introns, or could be generated by disruption of cis-arranged introns. To resolve this question, we investigated the respective gene structures in the large evolutionary gap between the liverwort and the angiosperms.

MATERIALS AND METHODS

Nucleic Acid Preparation. Approximately 0.5–3 g of plant leaf material was used for total cellular DNA preparation by the cetyltrimethylammonium bromide (CTAB) method (21). Total nucleic acids were fractionated into RNA and DNA by differential precipitation in the presence of 2 M lithium acetate. Subsequently, the crude DNA preparations were treated with RNase A and the RNA fraction was treated with RNase-free DNase. Mitochondrial DNA from *Asplenium nidus* was prepared from organelles purified by differential centrifugation as described earlier (22).

Molecular Biology Techniques. DNAs were cut with restriction enzymes and separated on 0.8% agarose gels prior to Southern blotting onto nylon membranes. Restriction fragments from cloned PCR products were used for radioactive labeling with [α-^{32}P]dCTP. Blot membranes (PALL Biodyne B, 0.45 μm) were used according to recommendations of the manufacturer and washed at 60°C in 0.1× SSC prior to

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Data deposition: The sequences reported in this paper have been deposited in the GenBank data base (accession nos. Y07910–Y07912).
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autoradiography. Locations and extensions of the probes are depicted in Fig. 4. cDNA was synthesized with a kit from Boehringer Mannheim in the presence of random hexamer primers as recommended by the manufacturer. Sequences of the oligonucleotides flanking the trans-splicing sites of angiosperm *nad* genes as schematically outlined in Fig. 1 were as follows (5′ to 3′): 1abup, GTTACAACCTGCAGCAGATGGTTTG; 1abdown, CCATTTGAGCTGCAGATCGTAATGC; 1cdup, GAAACTAATCGAGCTCCGTTTGATC; 1cdown, CTCATTAAGATCTTATTGGCATACTC; 2bcup, ATTGCCATGGATTTAGCTATTGAG; 2bcdown, GAAAAGGAACTGCAGTGATCTT; 5bcup, GTGATTCATGCCATGGCGGATGAGC; 5bcdown, TACCTAAACCAATCATCATATC; 5cdup, GATATGATGATTGGTTTAGGTA; and 5cddown, CAATAGCACCTTTGTCTAAAGCTT.

Oligonucleotide pairs were used for PCR amplification in a Biomed waterbath thermocycler with annealing temperatures of 45–50°C. PCR products were cloned in the Bluescript SKII+ (Stratagene) vector. Sequencing of the cloned products was done by the dideoxynucleotide method in the presence of [α-[^{35}S]thio]dATP.

RESULTS

The primary aim of the experiments reported here was to investigate the presence of possible cis-arranged counterparts to trans-splicing introns in angiosperm mitochondria. If continuous group II introns were the evolutionary progenitors of the contemporary trans-splicing angiosperm introns, some may still survive in early branches of land plant evolution such as bryophytes, fern allies, ferns, and gymnosperms.

Intron Search Logistics. The *nad1, nad2*, and *nad5* genes in plant mitochondria are sufficiently conserved to design oligonucleotides for the exon sequences flanking the known trans-splicing intron insertion sites (Fig. 1). PCR products can be obtained only from genomic DNA, where the respective gene is arranged in a continuous order of exons and cis-splicing introns, and not from trans-splicing arrangements. A size increase in comparison with the corresponding PCR product from angiosperm cDNA or the liverwort *Marchantia polymorpha* genomic DNA should reflect both presence and size of a cis-arranged intron in the amplified region. This increase should be clearly detectable, given the size distribution of some 0.8 to 3.5 kb for the known plant mitochondrial group II introns.

Testing the Strategy. The designed set of five oligonucleotide pairs yielded correct products from a crude DNA preparation of the liverwort *Marchantia polymorpha* and from mitochondrial cDNA of the evening primrose *Oenothera berteriana* (Fig. 1*D*). The oligonucleotides were then used in PCR assays with DNA preparations from selected species covering the extant range of land plant diversity. PCR products of the liverwort species *Pellia epiphylla* and *Frullania tamarisci* corresponded in size to those resulting from amplification of *Marchantia polymorpha* DNA.

No PCR products were obtained from the angiosperm DNA used as control (*Arabidopsis thaliana*). Likewise, no PCR products were observed in any instance with DNA of the gymnosperm *Picea abies*. While generally the absence of a PCR amplification product can indicate an unfortunate choice of primer sequences, we do not consider this a likely possibility here, since the primers work correctly in the evolutionary distant angiosperm and bryophyte species and (except for the *nad2* primer combination) even for the algal species *Chara corallina* (results not shown). Further experiments were performed with species occupying evolutionary positions intermediate between the bryophytes and gymnosperms (fern species *sensu lato).*

A Continuous Homologue of a Trans-Splicing Intron in *nad2*. Amplification products of cDNA size were obtained for the *nad1* c/d connection in the ferns *Asplenium nidus* and *Marsilea drummondii*. However, the *nad2* b/c amplification in these two species yielded PCR products of 2.6 and 1.6 kb, respectively. Cloning and sequencing identified in both instances cis-arranged group II introns at precisely the same

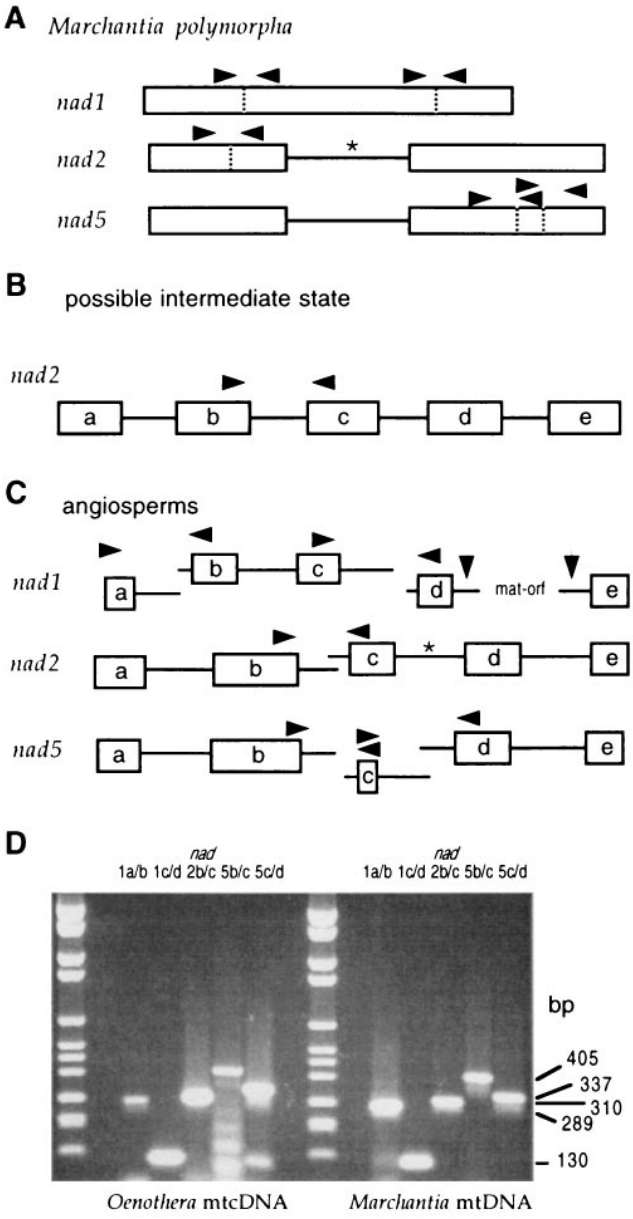

FIG. 1. Arrangements of the genes *nad1, nad2*, and *nad5*, which encode subunits of the NADH dehydrogenase in *Marchantia polymorpha* (*A*) and angiosperm mitochondria (*C*). Coding regions of the *nad* gene reading frames are distributed over 5 exons (boxes) each in angiosperms (*C*). Conventional cis-arranged group II introns (lines) are accompanied by disrupted group II introns (broken lines) requiring one (*nad2*) or two *(nad1, nad5)* trans-splicings. Additional intron disruptions are observed for intron *nad1* d/e in wheat upstream and in petunia downstream of the maturase reading frame, respectively (arrows). The single *nad2* intron in *Marchantia polymorpha* has its homologue in angiosperms as intervening sequence between exons c and d (asterisk). Conserved exon primer sequences (arrowheads) were designed for regions conserved between the angiosperm and *Marchantia polymorpha* nucleotide sequences. Primer design tested in PCR amplification yields the expected products for noninterrupted reading frame fragments from *Marchantia polymorpha* genomic DNA and *Oenothera berteriana* cDNA (*D*). Experiments are designed to search for cis-splicing introns as potential progenitors of the trans-splicing introns as shown for a hypothetical *nad2* gene structure in *B*.

position where the trans-splicing intron is inserted in angiosperms (Fig. 2). The fern introns show approximately 70% nucleotide sequence identity with the *Oenothera* sequences (18) and are highly similar to each other, excluding the loop of domain IV. The domain IV sequence has expectedly tolerated considerable sequence divergence in evolution, since it carries no functionally relevant elements. While structurally relevant intron elements are very similar between *Marsilea* and *Asplenium,* the domain IV loops are only partially conserved (Fig. 2). The deduced intron secondary structure and tertiary interactions conform to the consensus model (8) and are well conserved in the trans-splicing counterpart in *Oenothera*. Extension of the domain IV loop to more than 1.7 kb as seen in *Asplenium* has presumably increased the likelihood to become a target for DNA recombination, ultimately resulting in the disruption of this intron during evolution of the seed plants. Notably, no traces of maturase-like reading frames are observed in the fern introns.

The looped-out guanosine nucleotide in domain VI differs from the consensus model, which features a highly conserved adenosine residue required for 5′–2′ branch site formation at this position (8). Another unusual observation is an in-frame stop codon in the upstream *nad2* exon of *Asplenium*. This codon will have to be removed by a reverse RNA editing event, as observed in other *Asplenium nidus* mitochondrial sequences (23, 24).

Transcription, Editing, and Intron Splicing at the Fern *nad2* locus. To test whether the novel *nad2* locus in the ferns is transcribed, spliced, and edited or just represents a pseudogene, PCR amplification was done with cDNA from both *Asplenium* and *Marsilea*. The PCR products of 310 bp expected for a spliced *nad2* product were indeed obtained. Cloning and sequence analysis confirmed the splice sites predicted from the secondary structure (Fig. 3). Moreover, in the 264 bp of flanking exon sequences 11 RNA editing events of the C-to-U type were observed in *Asplenium*. A reverse exchange of U to C was identified to remove the genomic stop codon and to reconstitute a conserved glutamine residue. On the basis of the secondary structure model the silent nucleotide exchange three nucleotides upstream from the splice site appears to be a prerequisite for a matching IBS–EBS1 interaction and thus maybe also for splicing competence (Fig. 2). Interestingly, all nonsilent RNA editing events are preedited in the genomic *Marsilea* sequence, which shows only a single editing event at a unique position not edited in *Asplenium* (Fig. 3). Analogous to the editing events observed in *Asplenium,* this exchange in *Marsilea* reconstitutes a conserved amino acid codon in comparison with the *Oenothera* sequence. The differences in RNA

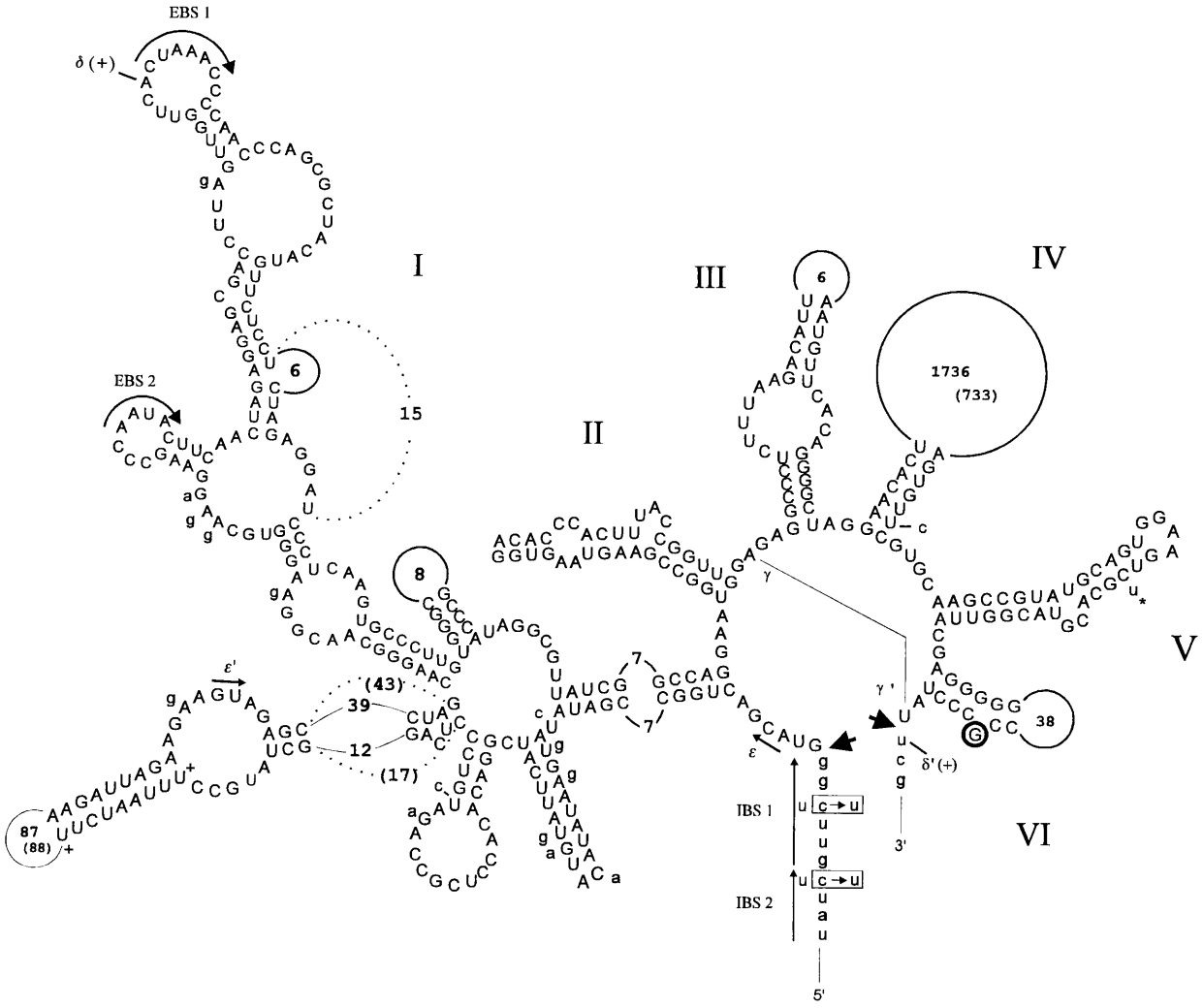

FIG. 2. Group II intron secondary structure of the *Asplenium nidus nad2* intron with the exchanges in *Marsilea drummondii* given next to the model, in parentheses, and with dotted lines, respectively. Roman numerals indicate the six well-defined domains radiating from a central wheel (8). Splice sites are indicated by arrows. Tertiary structure interactions between the exon and intron binding sites (EBS1–IBS1, EBS2–IBS2) and other nucleotides (γ–γ′, δ–δ′) are highlighted. A guanosine residue (encircled) is found at the branch-site position where adenosine is conserved in other introns. Editing is required at the 3′ end of the upstream exon to reconstitute conserved codons and to allow the EBS–IBS interactions.

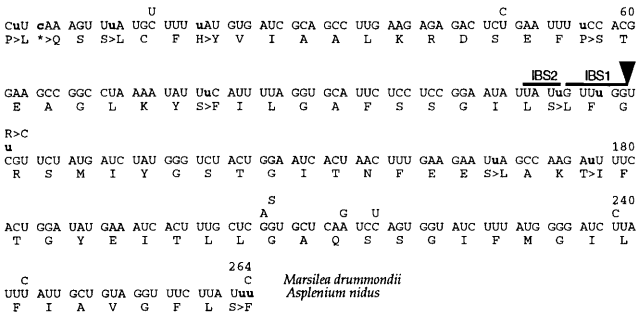

FIG. 3. *Asplenium nidus* and *Marsilea drummondii* cDNA sequences identify the splice sites of the *nad2* introns and show several RNA editing sites in the flanking exon sequences. Location of the group II intron is indicated by an arrowhead. Intron binding sites IBS1 and IBS2 are overlined. A total of 11 C-to-U RNA editing events (**u**) and one reverse exchange (**c**) modify the RNA sequence in *Asplenium* and reconstitute conserved amino acid codons. Nucleotide and amino acid differences in *Marsilea* are shown above the *Asplenium* sequence. The single editing event in *Marsilea* is not observed in *Asplenium,* but reconstitutes a cysteine codon conserved in *Oenothera*. The *Marsilea* and *Asplenium nad2* sequences have been deposited in the database under accession nos. Y07910 and Y07911, respectively.

editing may be due to the phylogenetic distance between *Asplenium* and *Marsilea*, the latter branching off earlier in the fern phylogeny (25, 26). On the other hand, considerable variability in RNA editing patterns is also observed between closely related plant species.

Southern Blot Verification of the Intron Cis-Arrangement. Although it is only a remote possibility, the PCR product obtained from *Asplenium* and *Marsilea* DNAs may be an artifact due to template switching involving repeated sequences associated with two separated (trans-arranged) loci. To verify the nature of this locus, Southern hybridizations were performed against *Asplenium* mitochondrial DNA (Fig. 4). The separate probes for the upstream and downstream parts of the intron detect the same *Bam*HI and *Hin*dIII restriction fragments of 4.0 and 6.5 kb, respectively. An additional *Bam*HI fragment of 1.3 kb identified with the upstream probe is due to a probe internal *Bam*HI site. These results thus confirm the physical linkage of the upstream and downstream gene regions.

A Second Cis-Arranged Homologue to a Trans-Splicing Intron in nad5. The *nad5* b/c primer set amplified in *Asplenium* a PCR product of 3.0 kb. In this instance no PCR product is obtained with *Marsilea* DNA. The *Asplenium nad5* PCR product was cloned and sequenced and found to contain a cis-arranged intron (Fig. 5) homologous to the trans-splicing intron inserted at the same site in the *nad5* gene of angiosperm species (15, 16, 27). This intron in *Asplenium* is 1824 nucleotides in size and, like the fern *nad2* introns, features a rather large domain IV loop of 1137 nucleotides with no traces of a maturase-like reading frame. The trans-splicing *nad5* group II intron fragments in *Arabidopsis thaliana, Oenothera*, and wheat (27) have been found difficult to fold into a secondary structure satisfying all canonical group II intron features. The secondary structure of the *Asplenium* cis-intron in Fig. 5 similarly includes most of the described group II intron features at the expense of others that can be accessed only in alternative foldings. The unusual ACC 3′ terminus of the intron is also present in the trans-splicing *nad5* introns of angiosperms. Sequence similarity between the *Asplenium* and angiosperm introns breaks off abruptly at the base of the domain IV loop. Analogous to the case of the *nad2* intron, this *nad5* intron has apparently also experienced a disruption in the vascular plant line after branching of *Asplenium* facilitated by a preceding domain IV size increase.

The cis-arrangement of the *nad5* intron in *Asplenium* was also verified by Southern blot hybridization (not shown).

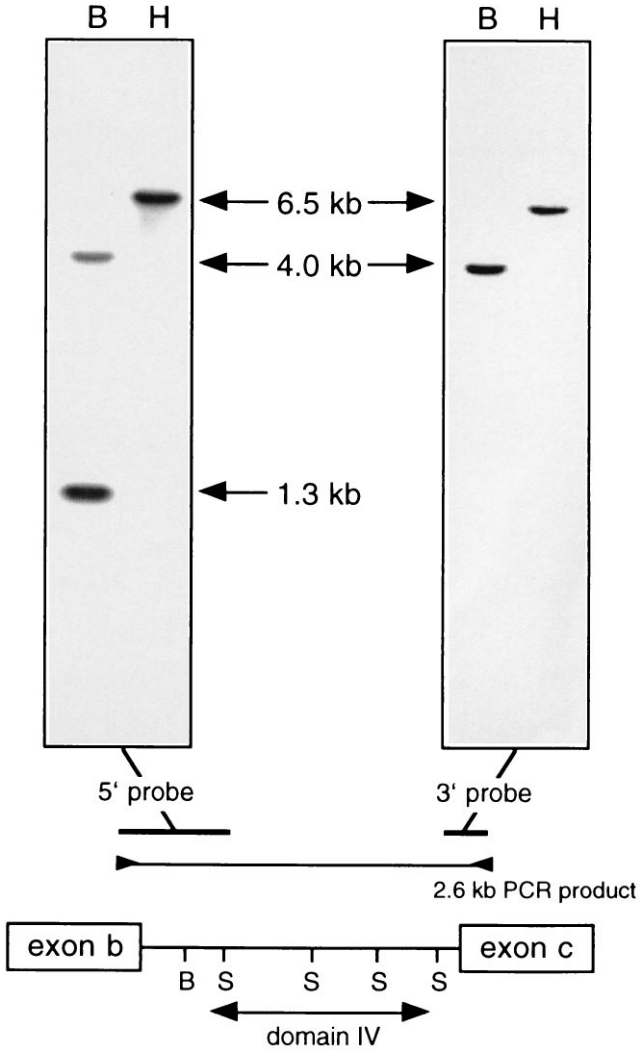

FIG. 4. Southern hybridization verifies the cis-arrangement of the novel *nad2* intron in *Asplenium nidus.* Asplenium mitochondrial DNA was cut with *Bam*HI and *Hin*dIII and size-fractionated on a 0.8% agarose gel prior to Southern-blot transfer. Hybridization probes covered the upstream and downstream regions of the intron together with adjacent exon sequences (terminal *Stu*I fragments of the PCR product). The two detect identical *Bam*HI and *Hin*dIII restriction fragments of 4.0 and 6.5 kb, respectively. The additional *Bam*HI fragment of 1.3 kb is due to a *Bam*HI site in the upstream probe sequence as deduced from the sequence data. The restriction map at the bottom depicts the extension of gene elements with restriction sites indicated for *Bam*HI (B) and *Stu*I (S).

Splicing of this intron in *Asplenium* was investigated by cDNA analysis, and the intron was found to be excised correctly from the RNA at the predicted sites (Fig. 5). As in the *nad2* gene, cDNA analysis identified several editing events in the flanking *nad5* gene regions, which are required to reconstitute conserved codons. Interestingly, the 3.0-kb PCR product obtained from *Asplenium* DNA contains an additional group II intron with similarity to the *rps10* intron of angiosperms (not shown, but refer to data base entry Y07912).

DISCUSSION

In the mitochondrial genomes of the ferns *Asplenium nidus* and *Marsilea drummondii* group II introns in the *nad2* and *nad5* genes have been identified that presumably represent

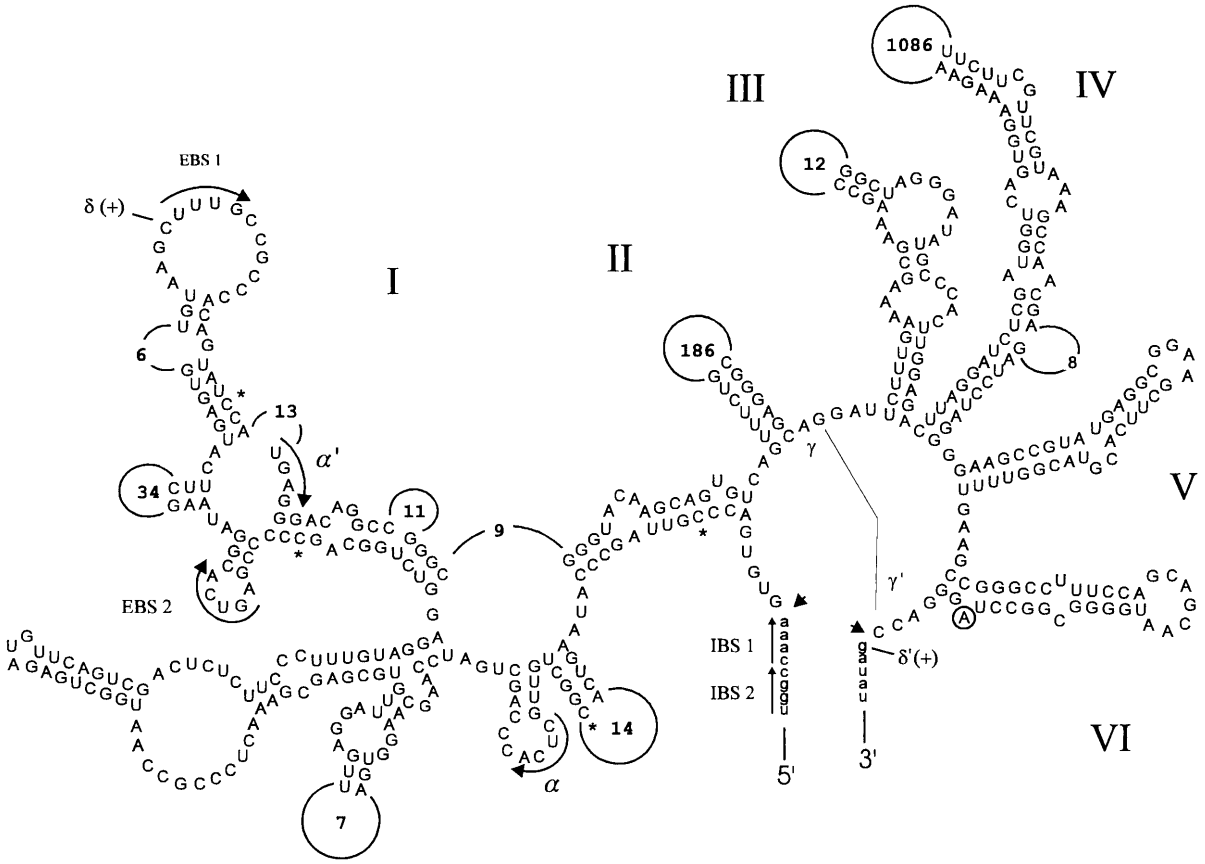

FIG. 5. A cis-spliced group II intron is identified in *Asplenium nidus* by using PCR amplification with the *nad5* b/c primer set. The intron and its trans-arranged homologues in wheat (25), *Arabidopsis*, and *Oenothera* show certain divergences from the group II intron consensus, such as the unusual ACC 3′ terminus, an additional nucleotide (U) between the conserved GA dinucleotide and the domain III stem, and noncanonical domain I substructures. Denotion of intron features is as in Fig. 2. The *nad5* sequence of *Asplenium nidus* is deposited in the data bases under accession no. Y07912.

uninterrupted progenitors of contemporary trans-splicing mitochondrial introns in angiosperms. The presented data furthermore indicate conservation of all five angiosperm trans-arrangements in the gymnosperm *Picea abies* and variable cis- or trans-arrangements among ferns. The appearance of plant mitochondrial trans-splicing thus apparently predates the establishment of seed plants, which has been dated to Pennsylvanian times approximately 285 million years B.P. by molecular methods (28).

The secondary structure models support the idea that intron disruption has occurred in the domain IV loop, which is structurally the most variable loop. As a prerequisite for transition to a trans-splicing arrangement the loop of this domain apparently expanded during mitochondrial genome evolution in plants. This size extension has increased the probability that domain IV will become disrupted by one of the frequent DNA recombinations well documented in angiosperm mitochondria. A domain IV loop size of more than 1.7 kb as in the *Asplenium nad2* intron is extremely large, particularly when no maturase is encoded. Notably, the domain IV size of the homologous intron is significantly smaller in *Marsilea*, a fern branching off much earlier in the vascular plant phylogeny (25, 26). The size increase of angiosperm chondriomes (mitochondrial genomes) in comparison to the *Marchantia* mitochondrial genome may thus tentatively be dated to the evolutionary times of fern diversification. Definite statements, however, have to await the unequivocal placement of the seed plant root in the vascular plant phylogeny, most importantly in relation to recent fern species.

The observations presented here may explain why only certain of the 25 plant mitochondrial group II introns are disrupted in trans-arrangements. Physical breakage may be confined to those introns which experienced a sufficiently large domain IV size increase in evolution. Notably, no trans-splicing introns are present in *Marchantia polymorpha*, a species with an apparent lack of active mitochondrial DNA recombination. Apparently the combination of these two factors, domain IV size increase on the one hand and recombinational activity in the mitochondrial genome on the other, has allowed the genesis of trans-splicing introns in plant mitochondria.

As yet it remains to be established where the now identified presumptive progenitors of trans-splicing introns originated. Although similar in number, the group II introns in the mitochondrial genome of the liverwort *Marchantia* (20) occupy different positions (except for a single *nad2* intron; see Fig. 1) and are generally not very similar to those in vascular plants. An exception is the recently identified *rps10* intron of some angiosperms. This intron is clearly related to both the second intron of the *cox3* gene and the single intron in the *rrn26* gene of *Marchantia* (29). Such observations raise speculations about lateral group II intron transfer in the phylogenetic lines leading to vascular plants or liverworts. *Marchantia* group II introns have been categorized into families whose members display compatibility of EBS–IBS interactions and thus support the idea of recent intron spread by means of reverse splicing mechanisms (30).

The assumption of an evolutionary relation between group II introns and the nuclear spliceosome—with the former being close to the predecessors of the latter—appears to be a valid working hypothesis (1, 2). Similarities of splicing mechanisms between the two intron types, however, must be interpreted

with caution (31). The series of hypothetical evolutionary events includes the random establishment of a group II intron in a eubacterial genome, its spread to new sites (e.g., by means of reverse splicing mechanisms), the uptake of a eubacterial endosymbiont by the urkaryote, the migration of group II introns into the nuclear genome via DNA or RNA, and the transfer of intron-encoded functions to the interacting RNA components of the spliceosome. Support for these ideas comes from the observations (*i*) that group II introns are present in eubacteria related to the endosymbiotic ancestors of chloroplasts and mitochondria and (*ii*) that no introns have as yet been identified in primary amitochondrial protists—e.g., *Giardia lamblia* (2). On the other hand, no group II or definite group II intron derivative has as yet been identified in the nuclear genome of a mitochondrial eukaryote. Whether the highly evolved spliceosome today excludes the parallel existence of this intron type in the nucleus is an open question that could, however, be addressed–e.g., by using plant transformation technology.

At present the trans-splicing introns in plant organelles should be regarded as a separate line of evolution rather than evolutionary intermediates on the way to a spliceosomal type of intron. Although both types have functional elements separated *in trans,* the continuous order of exons is undisrupted in the majority of eukaryotic genes. The transfer of functional elements to novel molecules in the small nuclear RNAs appears as a line of evolution different from the disruption of exon–intron orders in the organelles.

Yet another line of intron evolution is presented by the known examples of nuclear trans-splicing initially identified in trypanosomes and later shown to occur in other protists and primitive invertebrates (32–34). This type of trans-splicing, the addition of SL (spliced leader) sequences, involves components of the small nuclear RNA and therefore appears as a unique line of evolution initiated after establishment of the spliceosome components.

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1. Sharp, P. A. (1991) *Science* **254,** 663.
2. Roger, A. J. & Doolittle, W. F. (1993) *Nature (London)* **364,** 289–290.
3. Ferat, J.-L. & Michel, F. (1993) *Nature (London)* **364,** 358–361.
4. Knoop, V. & Brennicke, A. (1994) *Nucleic Acids Res.* **22,** 1167–1171.
5. Ferat, J.-L., Le Gouar, M. & Michel, F. (1994) *C. R. Acad. Sci. Ser. III* **317,** 141–148.
6. Knoop, V. & Brennicke, A. (1994) *J. Mol. Evol.* **39,** 144–150.
7. Knoop, V., Kloska, S. & Brennicke, A. (1994) *J. Mol. Biol.* **242,** 389–396.
8. Michel, F., Umesono, K. & Ozeki, H. (1989) *Gene* **82,** 5–30.
9. Costa, M. & Michel, F. (1995) *EMBO J.* **14,** 1276–1285.
10. Fukuzawa, H., Kohchi, T., Shirai, H., Ohyama, K., Umesono, K., Inokuchi, H. & Ozeki, H. (1986) *FEBS Lett.* **198,** 11–15.
11. Zaita, N., Torazawa, K., Shinozaki, K. & Sugiura, M. (1987) *FEBS Lett.* **210,** 153–156.
12. Kück, U., Choquet, Y., Schneider, M., Dron, M. & Bennoun, P. (1987) *EMBO J.* **6,** 2185–2195.
13. Chapdelaine, Y. & Bonen, L. (1991) *Cell* **65,** 465–472.
14. Wissinger, B., Schuster, W. & Brennicke, A. (1991) *Cell* **65,** 473–482.
15. Knoop, V., Schuster, W., Wissinger, B. & Brennicke, A. (1991) *EMBO J.* **10,** 3483–3493.
16. Pereira de Souza, A., Jubier, M.-F., Delcher, E., Lancelin, D. & Lejeune, B. (1991) *Plant Cell* **3,** 1363–1378.
17. Conklin, P. L., Wilson, R. K. & Hanson, M. R. (1991) *Genes Dev.* **5,** 1407–1415.
18. Binder, S., Marchfelder, A., Brennicke, A. & Wissinger, B. (1992) *J. Biol. Chem.* **267,** 7615–7623.
19. Wahleithner, J. A., Macfarlane, J. L. & Wolstenholme, D. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 548–552.
20. Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Kanegae, T., Ogura, Y., Kohchi, T. & Ohyama, K. (1992) *J. Mol. Biol.* **223,** 1–7.
21. Doyle, J. J. & Doyle, J. L. (1990) *Focus* **12,** 13–15.
22. Schuster, W., Hiesel, R., Wissinger, B., Schobel, W. & Brennicke, A. (1988) in *Plant Molecular Biology,* ed. Shaw, C. H. (IRL, Oxford), pp. 79–102.
23. Hiesel, R., Combettes, B. & Brennicke, A. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 629–633.
24. Malek, O., Lättig, K., Hiesel, R., Brennicke, A. & Knoop, V. (1996) *EMBO J.* **15,** 1403–1411.
25. Hasebe, M., Omori, T., Nakazawa, M., Sano, T., Kato, M. & Iwatsuki, K. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5730–5734.
26. Pryer, K. M., Smith, A. R. & Skog, J. E. (1995) *Am. Fern J.* **85,** 205–282.
27. Pereira de Souza, A. (1992) Ph.D. thesis (Université de Paris-Sud, Paris).
28. Savard, L., Li, P., Strauss, S. H., Chase, M. W., Michaud, M. & Bousquet, J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5163–5167.
29. Knoop, V., Ehrhardt, T., Lättig, K. & Brennicke, A. (1995) *Curr. Genet.* **27,** 559–564.
30. Ohyama, K., Oda, K., Ohta, E. & Takemura, M. (1993) in *Plant Mitochondria*, eds. Brennicke, A. & Kück, U. (VCH, Weinheim, Germany), pp. 115–129.
31. Weiner, A. (1993) *Cell* **72,** 161–164.
32. Agabian, N. (1990) *Cell* **61,** 1157–1160.
33. Nilsen, T. W. (1996) *Mol. Biochem. Parasitol.* **73,** 1–6.
34. Tessier, L.-H., Keller, M., Chan, R. L., Fournier, R., Weil, J.-H. & Imbault, P. (1991) *EMBO J.* **10,** 2621–2625.