Molecular evolution of mitochondrial introns in the liverwort *Marchantia polymorpha*

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Abstract: We here describe in detail the characterization and molecular evolution of group II introns in the mitochondrial genome of the liverwort *Marchantia polymorpha*. We find that 18 introns of the 25 group II introns can be assigned by their similarities to six clusters, indicating an intra-genomic propagation of one ancestral intron each into the respective clusters in the liverwort mitochondrial genome. Interestingly, the intra-genomic propagation of some of these introns occurred only after the evolutionary separation of the bryophytes from the other clades of plants. Finally we report that the maturase-like sequences in the liverwort group II introns have further evolved by horizontal and independent transposition and substitution by analogous sequences from other fungal introns.

Keywords: mitochondrial introns, *Marchantia polymorpha*, intron evolution, maturase-like ORFs, intra-genomic propagation of introns

Introduction

On the basis of structural features, in a combination of conserved nucleotide sequences and potential secondary structures, two types of introns can be classified in organelles, namely, group I and group II introns.[1)] Group I and group II introns were originally described as two families of introns that are distinguished by unique secondary structures.[2)] Group I introns are widely distributed over the genomes of bacteriophages,[3)] prokaryotes,[4)] organelles,[5)] and nuclei.[6)] Group II introns are present in the mitochondrial genomes of fungi[5)] and plants,[7)] and in chloroplast genomes.[8),9)] The complete nucleotide sequence of the liverwort mitochondrial DNA reveals 94 possible genes in the total length of 186,608 basepairs.[10)] Seventeen of these genes are interrupted by a total of 32 introns (Fig. 1). Based on their sequence and structural features, twenty-five of these introns can be assigned to the group II, the remaining seven qualify as *bona fide* group I introns. Here we describe the detailed characterization of group II introns and derive the molecular evolution of the introns in the mitochondrial genome of the liverwort *Marchantia polymorpha*.

Materials and methods

Computer aided analysis. The complete nucleotide sequence of the liverwort mitochondrial DNA was determined in the laboratory of Plant Molecular Biology, Kyoto University. Computer aided analysis was carried out against the sequence database in the GenBank (accession number M68929) using the Hitachi DNASIS program, and the BLAST, FASTA and ODEN programs (DNA Data Bank of Japan, National Institute of Genetics, Japan). Phylogenetic analysis was performed with the CLUSTALW program.

Results and discussion

Six clusters of homologous group II introns. In the complete nucleotide sequence of the liverwort mitochondrial DNA twenty-five group II introns can be identified by their consensus sequences and secondary structures.[10)] Sequence comparison of these group II introns reveals six clusters of highly similar introns.[11)] In order to identify the relative timing of the evolutionary processes having led to the respective clusters of group II introns, we

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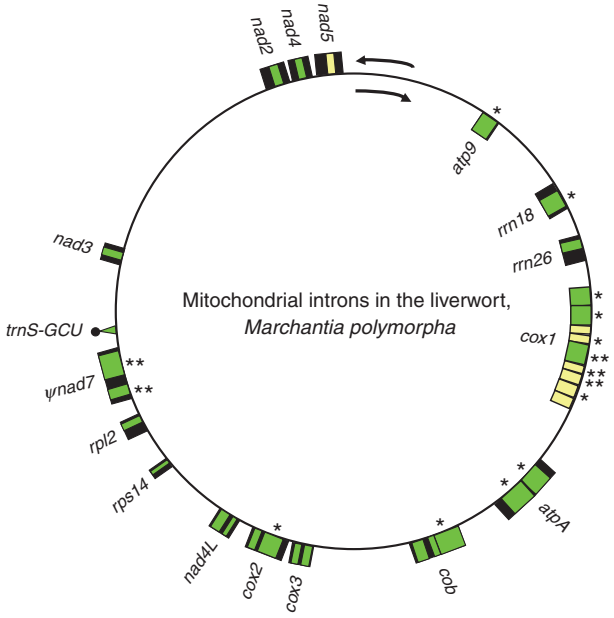

Fig. 1. Genes with group I and group II introns in mitochondrial genome of the liverwort *M. polymorpha*.
Only genes with intron(s) are shown. Black boxes indicate exons. Yellow boxes and green boxes indicate group I and group II introns, respectively. Introns including intact and frame-shifted ORFs are indicated by a single asterisk (*) and a double asterisk (**), respectively. Arrows indicate the direction of transcription.

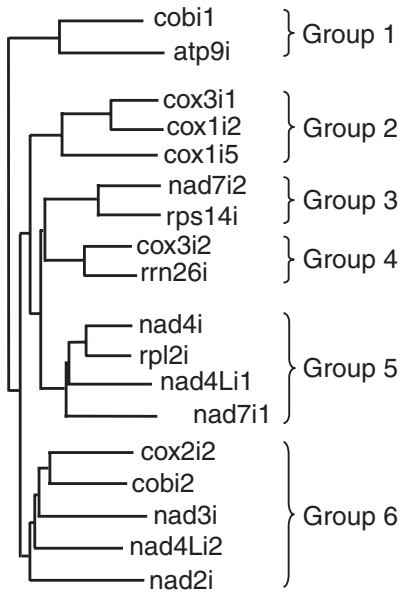

Fig. 2. Phylogenetic tree of the six clusters of group II introns with sequence similarities in the mitochondrial genome of *M. polymorpha*. Abbreviation for introns: First intron of *cox1* gene is abbreviated as cox1i1, second intron as cox1i2 and so on. The rest of introns in the corresponding genes follows as same description manner through the text.

derived the phylogenetic tree of the introns within and between the six clusters. The resulting tree not only confirms the high similarity of the introns within the respective clusters, but also suggests that intra-genomic propagation has played a role in the evolution of the different members of each intron cluster (Fig. 2). As an example of a flowering plant species, *Oenothera berteriana* separated and evolved from a common ancestor with the liverwort about 400 million years ago. Accepting this time scale, the mitochondrial *nad2* gene of the higher plant *O. berteriana* and the liverwort mitochondrial *nad2* gene can be assumed to have evolved independently for 400 million years or more.[12] Taking this time scale into account and considering the nucleotide sequence differences accumulated in the *nad2* gene since this separation, the high similarity between the liverwort cox1i2-cox3i1, rrn26i-cox3i2, nad7i2-rps14i, and rpl2i-nad4i introns implies that these liverwort introns arose by intra-genomic propagation within the liverwort mitochondrial genome after the separation from the evolutionary line of the higher plants (Fig. 3). The mechanism and direction of this intra-genomic propagation will be discussed below.

Intra-genomic propagation of the liverwort group II introns via an RNA intermediate into genomic sequences with splice site similarity. The high similarity of these introns suggests that they are derived from a common ancestor by duplication and insertion into another site. Similar to retrotransposons, group II introns multiply by reverse transcription of the RNA, in this case the excised intron, and subsequent insertion of the cDNA into a new genomic locus. To insert the DNA fragment generated from the RNA intermediate by a reverse splicing reaction, base pairing interactions between the insertion site which interacts as an intron binding sequence (IBS) in the exon and the exon binding sequence (EBS) in the intron are required.[13] This necessary compatibility between the EBS sequence of the moving intron and the IBS-like sequence of a novel insertion locus in a different exon will subsequently ensure the correct insertion of the complete new intron into the previously intron-less mRNA sequence during the reverse splicing step.

Consequently, we searched the splice sites of

Group 1

	cobi1
*atp9i	424

Group 2

	*cox1i2	*cox1i5
cox3i1	196	724
*cox1i2		935

Group 3

	rps14i
*nad7i2	260

Group 4

	cox3i2
rrn26i	277

Group 5

	nad4i	rpl2i	*nad7i1
nad4Li	467	506	485
nad4i		277	400
rpl2i			400

Group 6

	nad3i	nad4Li2	*cox2i2	cobi2	O.b.
nad2i	573	550	620	620	400
nad3i		573	528	620	903
nad4Li2			528	596	1006
*cox2i2				573	870
cobi2					809

Fig. 3. Divergence times of different pairs of homologous introns in millions of years.
Search for intron sequence similarities within the liverwort mitochondrial sequence was performed by using the FASTA and ODEN programs (gap penalty: a = 4) on a FACOM-M-77/10UTS computer (DNA Data Bank of Japan, National Institute of Genetics, Mishima, Japan). Introns which encode RNA maturase like ORFs are indicated by asterisks. Intron *O. berteriana* is the third intron in the *nad2* gene of *O. berteriana*.[12] The rate of substitution per site (K) is calculated by the Jukes-Cantor method: $k = -3/4 \ln(1 - 4p/3)$ where p is the fraction of observed substitutions.[16] The rate of substitutions per site per year is calculated as follows: $k = K/(2t)$, with $t = 400$ Myr. Dates of divergence are calculated as follows: $T = (K/2)/(0.395 \times 10^{-9})$. 0.395×10^{-9} is the rate of substitution per site per year for introns, estimated from the comparison of the nad2i sequence in the liverwort and in the flowering plant *Oenothera*.

the various group II introns in the mitochondrial mRNAs for similarities in the surrounding exon sequences for compatible IBS-like motifs. Indeed, evaluation of these IBS-similarities does indicate the direction of the intra-genomic propagation of various intron pairs in different liverwort mitochondrial genes: Intron cox1i2 has duplicated and homed into the *cox3* mRNA to become cox3i1 (Fig. 4), rrn26i amplified into cox3i2, and nad4i has duplicated and evolved one copy to rpl2i. This trend is especially prominent in those introns which propagated intra-genomically after the evolutionary separation from the line of the higher plants. Between these intron pairs, the potential base-pairings of the deduced EBS-IBS sequences are particularly high probably because of the short time scale since the duplication and insertion of these intron copies from the corresponding ancestral introns.[11] The direction of the propagation of the nad7i2 and rps14i introns is not clear, because the *pseudonad7* gene has several stop codons in its coding region and the IBS-EBS sequences show the same degree of matching base-pairs in either direction.

Intron propagation via an RNA intermediate requires the activity of a reverse transcriptase, which is often supplied by the ORFs sometimes encoded in group II introns.[14] As the final step of amplification and transposition, homologous recombination with an endonuclease or integrase activity is needed to insert the DNA copy of the intron into the new genomic locus.[15] However, these endonucleases or integrases can also act in *trans* and can thus be encoded by other introns. For example, as is common for group II intron ORFs, some of the ORFs encoded by the group II introns in the *M. polymorpha* mitochondrial genome do not code for proteins with endonuclease or integrase domains. The required enzymatic activity may be supplied by one or more of the proteins encoded by group I introns in the liverwort mitochondrial genome, such as the cox1i4 or cox1i8 introns. The ORFs encoded by these two introns do contain such motifs typical for an endonuclease activity,[16] suggesting that they might enable or enhance homologous recombination. Thus the ORFs encoding proteins in the group I introns in liverwort mitochondria might participate in the intra-genomic propagation of the group II introns in the genome. Interestingly, these intra-genomic propagations in the liverwort mitochondrial genome are only seen for group II introns, but not for group I introns. The reason for this bias remains unclear at present. Possibly one of the three main requirements for frequent intra-genomic propagation events (reverse splicing, reverse tran-

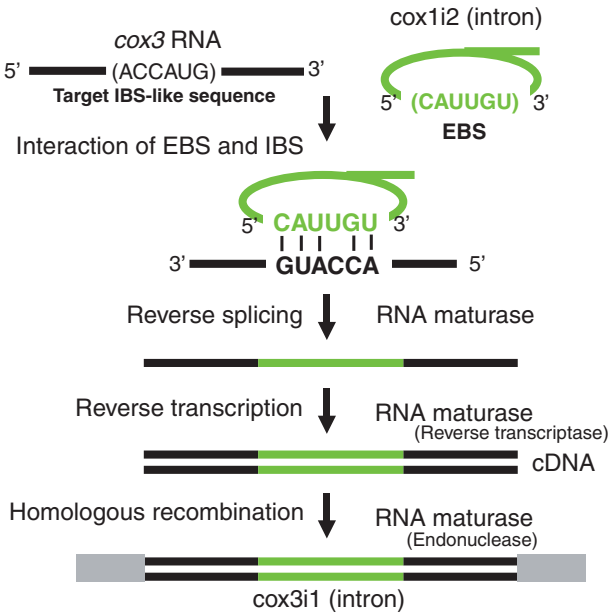

Fig. 4. Schematic presentation of the proposed process of intra-genomic propagation of group II introns.
As an example, the EBS of the cox1i2 intron (lariat green lines) binds to the IBS-like sequence of the *cox3* mRNA and inserts by reverse splicing into the *cox3* mRNA and then the cDNA was synthesized by the reverse transcriptase activity of the intron-encoded RNA maturase. Finally, in separate and independent steps, the cDNA with the cox3i1 intron (copied and propagated from the original cox1i2) eventually replaced by homologous recombination all the copies of the genomic *cox3* gene without intron on other mitochondrial DNA molecules.

scription, and homologous recombination) as depicted in Fig. 4 is not met by the group I introns and consequently no successful amplifications have survived the evolutionary selection to be still detectable today.

Evolutionary origin of five *cox1* introns inserted at the same sites as those of their fungal counterpart. While the *cox1* genes of higher plants contain no introns at all, there are nine introns in the liverwort mitochondrial *cox1* gene coding for cytochrome *c* oxidase subunit 1.[10] We have previously described that six of these introns, the 3rd, 4th, and 6th to 9th introns show all the characteristics of group I introns, while the rest of the *cox1* introns, the 1st, 2nd, and 5th introns, can be clearly classified as group II introns. Five of these *cox1* introns, cox1i2, cox1i4, cox1i6, cox1i7, and cox1i8 are inserted at the same sites where introns have been reported in the genes of fungal mitochondrial *cox1* genes.[10),16)]

To analyze the timing of the evolutionary events leading to this distribution of introns in the mitochondrial *cox1* gene of the liverwort relative to the intron evolution in fungi, we constructed a phylogenetic tree of the mitochondrial *cox1* gene from the liverwort and four species of fungi. As a reference time scale and evolutionary marker we used a nuclear gene, the ribosomal 5.8S rDNA sequences[17)–20)] from the same species for an analogous phylogenetic tree. Comparison of the two derived trees shows that the mitochondrial *cox1* genes of *Saccharomyces cerevisiae*,[5)] *Schizosaccharomyces pombe*,[21)] *Neurospora crassa*[22)] and *Podospora anserina*,[23)] respectively, show less sequence divergence and have thus evolved somewhat slower between *M. polymorpha* and the four fungi than have the nuclear encoded 5.8S rDNA gene sequences (Fig. 5A and 5B).

In order to analyze the possibility of the liverwort *cox1* introns inserted at the same sites as the respective *cox1* introns in the fungal genes being derived from the same ancestral intron, we compared the amino acid sequences of the ORFs encoded by the liverwort cox1i4 and cox1i8 group I introns to the respective ORFs in the analogous introns in fungi. Total database searches with the putative proteins encoded in the cox1i4 and cox1i8 introns in the liverwort indeed identified as closest matches the intron counterparts inserted at the same sites in the fungal mitochondrial genes.[16)] The *S. pombe* ORF in cox1i1 intron is most similar to

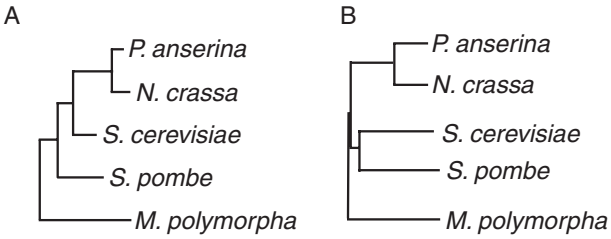

Fig. 5. Phylogenetic trees of the liverwort and fungal nuclear-coded ribosomal 5.8S rDNA genes and the mitochondrial *cox1* genes.
(A) Phylogenetic tree of the nuclear-encoded ribosomal 5.8S rDNA sequences. GenBank accession numbers are: *P. anserina* (AE388930); *N. crassa* (X02447); *S. cerevisiae* (D89866); *S. pombe* (AB054041, unpublished); *M. polymorpha* (AB021684).
(B) Phylogenetic tree of the mitochondrial *cox1* nucleotide sequences from the same species. GenBank accession numbers are: *P. anserina* (X55026); *N. crassa* (X14669); *S. cerevisiae* (YSCMTOC1); *S. pombe* (X54221).

the liverwort ORF in cox1i4 intron, and the liverwort ORF in cox1i8 intron identified as closest relatives the ORFs in the introns cox1i15 in *P. anserina*, cox1i5B in *S. cerevisiae*, and cox1i3 in *S. pombe*, respectively, as described previously.[16] For these introns, the relationships and thus evolutionary origins and pathways correlate for the three features splice site (i.e. EBS/IBS), intron sequence and structure, and the encoded ORF.

An analogous observation is made for the structures and EBS/IBS interaction sites of the liverwort group II intron cox1i2 and the *S. cervisiae* intron cox1i1, which is inserted at the same site. However, when analyzing the ORF encoded by this intron cox1i2 in the liverwort *cox1* gene, similarity was found to be higher to the ORF in an intron in the fungus *N. crassa* intron cox1i1 (41% similarity) than to the cox1i1 intron in *S. cerevisiae*, which is inserted at the genomic site homologous to liverwort (35% similarity; Fig. 6). This finding suggests that the ORF in the liverwort cox1i2 intron has been horizontally replaced by the respective ORF from *N. crassa*. On the other hand, the lower sequence similarity observed between the respective ORFs in the liverwort cox1i2 and cox1i5 introns is probably due to frameshift of the ORF in cox1i5 (Fig. 6). Since the respective introns were classified to same group (group 2 in Fig. 2), the intron cox1i2 (or part of it) subsequently propagated intragenomically and invaded the liverwort cox1i5 intron where it also replaced the previous ORF during the evolution (Fig. 7).

A somewhat different scenario is deduced for the origin and evolution of the introns nad7i1 and rrn18i in the liverwort mitochondrial genome. These two introns most likely diverged independently and at different times from the same intron in fungi (see Fig. 2). Although intron rrn18i, unlike nad7i1, does not belong to any of the six groups by its overall sequence similarity pattern, the two ORFs encoded by the liverwort nad7i1 and rrn18i introns show higher similarity with the ORF encoded in the *P. anserina* intron cox1i1 (42% and 44%, respectively) (Fig. 8). This observation implies that this ORF transposed horizontally and separately into each of the two liverwort introns from the ancestral fungal intron (Fig. 9). We have previously identified an analogous evolutionary pathway for the liverwort cox1i6 and cox1i7 introns and their encoded frame-shifted ORFs.[16] As de-

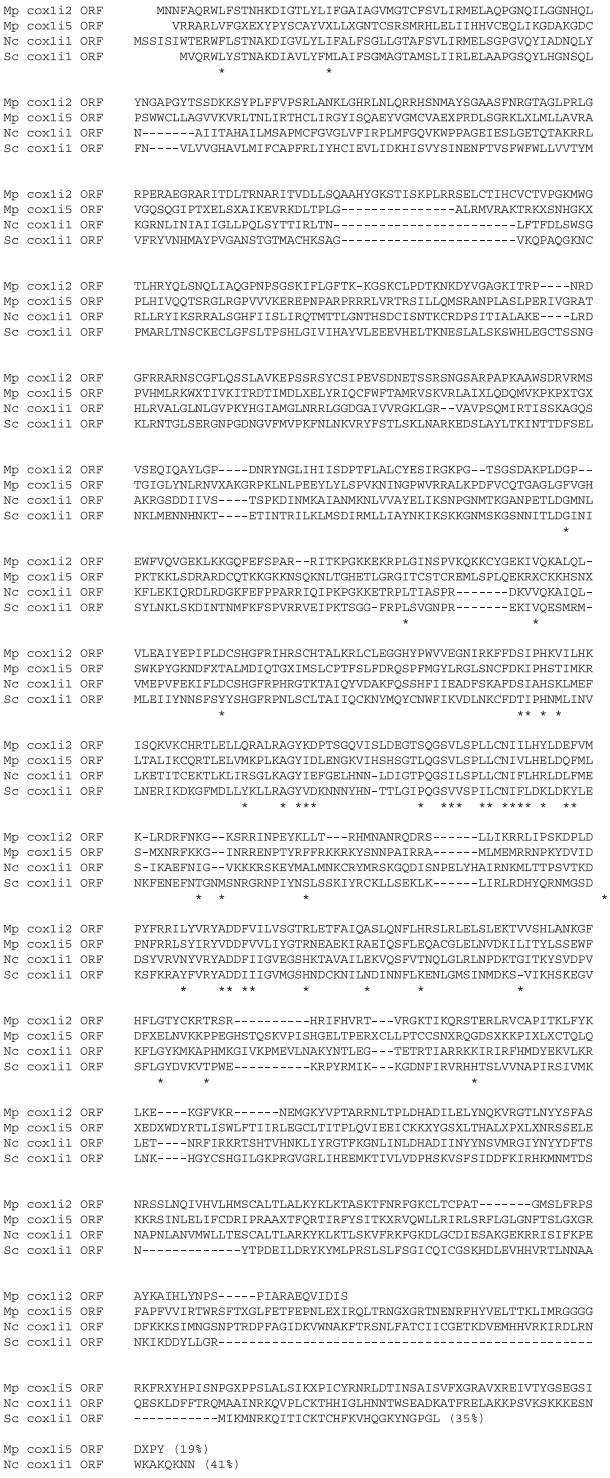

Fig. 6. Sequence comparison of the ORF sequences in the liverwort cox1i2, cox1i5, *N. crassa* cox1i1, and *S. cerevisiae* cox1i1 introns.

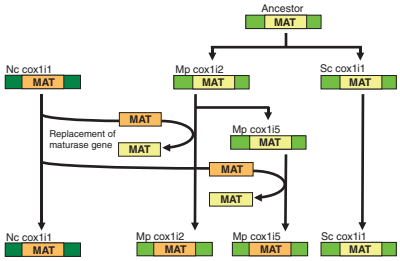

Fig. 7. Possible propagation pathways of ORFs (yellow and orange boxes with MAT) encoded by the liverwort introns cox1i2, cox1i5 (light green boxes), and *N. crassa* intron cox1i1 (dark green boxes).

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Pa cox1i1 ORF                                  GAPFNVRFKSGELDPISYCCCLLNLLTYLMTKGLR
Mp nad7i1 ORF    APFKQSMCSRRADCLISITLSLKGKPKGYCSMWGKGEPGVKPIALCSTSYSSMDLSSSSG

Pa cox1i1 ORF    ECSMSVNPYLTIAIKSVESGEVKASYVLRLLTMVGLCVSIGIKIAIALYWVLIKISASLI
Mp nad7i1 ORF    VIRLRLVGLTNTRGCDWGQNHYVVSGHCDLLQATASNLRVRRKNGISWGQLPYKYGHGSE

Pa cox1i1 ORF    KNSYSFTTSERGYIVYN-----AKGRRRLNVGNSGLPKGRNSYGNGSVVVGVTSG----K
Mp nad7i1 ORF    PTEQQMTHKEKWNCLKPPSPKTRAGQRDIPISSPSPDVSLEREGDGGFAVMAPTGDGSER

Pa cox1i1 ORF    WIHTKVRKSKVSPKSEARKGQGSLGEMLMYNERGQCINAYEVICKLEALYTAYMNIKSE-
Mp rrn18i ORF                                          MTKCNYEQLLDPEIFRLAYELKKSK-
Mp nad7i1 ORF    QGTPSPHASSWPPAAGRRKRATSARTLSTTTIGGMRERPNKSLEHLRKDREGTQNDLSPS
                                                                             *

Pa cox1i1 ORF    PGNMTPRVDSETLDGISKEWFEKISEQLKSE-----------------------------
Mp rrn18i ORF    SGNMKPGADKETLDGFSQAYVEKVVRQLKDE-----------------------------
Mp nad7i1 ORF    HDKLRPYTDHHSMGAFSPLKAGSETQNSKPPGLPMHQMGPDTVRTYGLYAVSPQGPETPN
                      *  *       *          *

Pa cox1i1 ORF    QFRFRPTRRVYIPKANGKMRPLGIASPRDKIVQEVFRAILEQVLEPRFHSSSHGFRPGRG
Mp rrn18i ORF    SFQFRPSRREFIPKADGKLRSLGIPSPRDKIVQEVMRRILEPVFEPRFLDSSHGFRPHRS
Mp nad7i1 ORF    SRALRGLHRSDFGLQTIKRKRLHELPISYKIATHISNRTYIPATKSKSFTIDRSERIILT
                      *   *         *   *      **                        *

Pa cox1i1 ORF    CHSALATIRYWNGIKWFIEGDIKGFFDNIDHHILELKLLVKHFQ-----------------
Mp rrn18i ORF    PHTALRQIRRWTGTSWMIEGDIKGYFDNIDHHLLAGFIAELVK-----------------
Mp nad7i1 ORF    IRHKLTHNNLWTEIKAGKHDSGTADSQNLDRMGFEKTIARLKDKSFQFKTPLGEPLFPNQ
                      *    *                * *

Pa cox1i1 ORF    ------------------------------DQRFIDLYWKMVKAGYVEFDKDK--------
Mp rrn18i ORF    ------------------------------DQRLLALYWKLVRAGYVNQGKAE--------
Mp nad7i1 ORF    MEINVPWVYHLYAIKLYKKLYRQLSNPLSNENFCPLVMGSVQAGLLVLHEKEPMEECKLG
                                                  *    * **

Pa cox1i1 ORF    -------------SSIIG-VPQGGIASPILSNLVLNELDEFVQNIVDEFNEKLKGGKHTS
Mp rrn18i ORF    -------------PHLLTGVPQGRILSPLLSNIYLHQFDLFMEEIKVKYTTTG-------
Mp nad7i1 ORF    NRRRHQRILLQYQPPQTSFVPRCGTSRPIAFTTLLETSRGAGYGLKKEPQTVVGLRGVLS
                                 **      *      *

Pa cox1i1 ORF    KNPAYVVIDSRIGKITRLERKLKSKGQELDSGRKLERMKLIKVRATMPSMIP-NPDLAK-
Mp rrn18i ORF    ----------ALSKNNPIYLKARNKYYKLVKSLKASSAEIIRARRDMLKMTYGIQTGSR-
Mp nad7i1 ORF    PMLPNIYLGPLDQFCEELKIRYKAPTSLITRTIQTVQKIRGRPPESTTGMAKINREEGSK
                                                                        *

Pa cox1i1 ORF    --IYYVRYADDWLIGVAGSSETARAIKERIAAYLKDILKLELSMEKTLITNASEDKAYFL
Mp rrn18i ORF    --VRYVRYADDWVIGVTGPKALAVQIKEEVSTFLQEKLKLSLQAEKTRITNLSRSEALFL
Mp nad7i1 ORF    NLLPTICKRLDRRGGVVGSKKVALAIRAEIASFLKLHLLN---WDNTKITHISSQLALFL
                          *   ** *    *  *       *   *        * **  *   * **

Pa cox1i1 ORF    GTEIQRISSVKGEIKRFKNIKGHPQRIPTTSTVMNAPISKLVTKLADKGIVIWKSKALNE
Mp rrn18i ORF    GTLISITTRKYVQSQKVG---GGHRRASLGRIRLCIPIDILIGKLSQMGACDEK------
Mp nad7i1 ORF    GTHIKVLRAESIRNHRIL-VVGQRTRSATFRLHLLAPIERIVKHLHGKGLCTPTG-----
                 ** *                  *   *          **       *   *

Pa cox1i1 ORF    DNLIPQPILKWVNLPIRDIILRYKMIWNGYINYYSFADNKPRLVLIYWILRKSLAKTLAT
Mp rrn18i ORF    --GTPKAVTKWIFLNVGEIINKYMAVFRGYYNYYSFADDIHHLLQIIYILRYSAINTVAR
Mp nad7i1 ORF    ----KPKPVRWIFLDHHELIFRYQDIMSGYMNYYSFVDNYGMLKRVAYIVRFSAAGTLKR
                        *  *     *  *     ** ***** *    *     * * *   *

Pa cox1i1 ORF    KLKLGTVRKVYLKFGVNLRFEILGTDNKSIEFTKGNLLPTPKNFKGKTNFVDNLKVVEWS
Mp rrn18i ORF    KLGLN-TAKVIKRFGVDLIFRDHTNEIKHKLNFPRSLPNKRMNPALSP----------P
Mp nad7i1 ORF    KFKMLSVASVFKGSGTGRGKELAATPLNLEKRCFAINNKKIMEFSARIR----------
                 *       *    *                                  *

Pa cox1i1 ORF    LRTVSFPNYVCASCGASDNLQVHHVKHIRTIDVKLSGFDKQLAAINRKQVTLCISCHNKV
Mp rrn18i ORF    SDPRVLFDTSCARIQC (44%)
Mp nad7i1 ORF    LRTHPVLTSPCCICRNQENREMQHVKHLR--KSKANGLTKLMVQLNRKQIPVCRTCHLKI
                                *

Pa cox1i1 ORF    HTGKYDGMSLKYMKDISKPELNQQ
Mp nad7i1 ORF    HQGKYDGKKSLFNRKVPGLPASYVEENPVGLSLDCIDPKLTHPLERSGRQGEGKATDSPG

Mp nad7i1 ORF    QKLGPKGLRESEDSLGKPYARKSCTSGSEEGHYLTKGSQLHNWAWPISY (42%)
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Fig. 8. Sequence comparison of the ORFs encoded by the rrn18i and nad7i1 introns with the *P. anserina* cox1i1 intron.

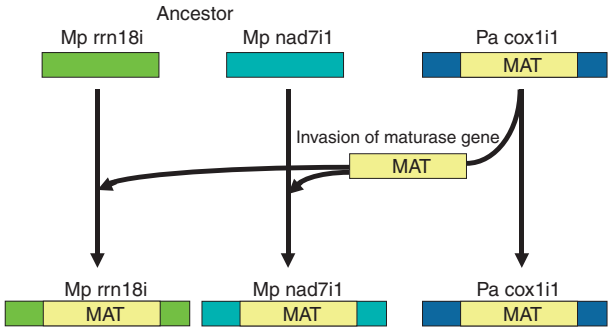

Fig. 9. Possible transposition pathways of the ORFs (yellow boxes) encoded by the rrn18i (green boxes) and nad7i1 (light blue boxes) introns from the *P. anserina* cox1i1 intron (dark blue boxes).

scribed above for the liverwort introns cox1i4 and cox1i8, the liverwort intron cox1i6 is also a cognate homolog of the *N. crassa* intron cox1i3 and the *P. anserina* intron cox1i7A. Likewise, liverwort cox1i7 is related to the *S. cerevisiae* cox1i4 and the *P. anserina* cox1i9 introns, which are apparently all derived from the same ancestral intron and encoded ORF.

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References

1) Michel, F. and Dujon, B. (1983) EMBO J. **2**, 33–38.
2) Michel, F., Jacquier, A. and Dujon, B. (1982) Biochimie **64**, 867–881.
3) Shub, D.A., Gott, J.M., Xu, M.Q., Lang, B.F., Michel, F., Tomaschewski, J., Pedersen-Lane, J. and Belfort, M. (1988) Proc. Natl. Acad. Sci. USA **85**, 1151–1155.
4) Kuhsel, M.G., Strickland, R. and Palmer, J.D. (1990) Science **250**, 1570–1573.
5) Bonitz, S.G., Coruzzi, G., Thalenfeld, B.E., Tzagoloff, A. and Macino, G. (1980) J. Biol. Chem. **255**, 11927–11941.
6) Wild, M.A. and Gall, J.G. (1979) Cell **16**, 565–573.
7) Fox, T.D. and Leaver, C.J. (1981) Cell **26**, 315–323.
8) Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H.,

Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z. *et al.* (1986) Nature **322**, 572–574.
9) Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K. *et al.* (1986) EMBO J. **5**, 2043–2049.
10) Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Kanegae, T., Ogura, Y., Kohchi, T. *et al.* (1992) J. Mol. Biol. **223**, 1–7.
11) Ohyama, K., Oda, K., Ohta, E. and Takemura, M. (1993) *In* Plant Mitochondria (eds. Brennicke, A. and Kuck, U.). Verlagsgesellchaft/VCH Publishers, Weinheim, pp. 115–129.
12) Binder, S., Marchfelder, A., Brennicke, A. and Wissinger, B. (1992) J. Biol. Chem. **267**, 7615–7623.
13) Augustin, S., Muller, M.W. and Schweyen, R.J. (1990) Nature **343**, 383–386.
14) Michel, F. and Lang, B.F. (1985) Nature **316**, 641–643.
15) Dujon, B. (1989) Gene **82**, 91–114.
16) Ohta, E., Oda, K., Yamato, Y., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Ohyama, K. and Michel, F. (1993) Nucl. Acids Res. **21**, 1297–1305.
17) Sone, T., Fujisawa, M., Takenaka, M., Nakagawa, S., Yamaoka, S., Sakaida, M., Nishiyama, R., Yamato, K.T., Ohmido, N., Fukui, K. *et al.* (1999) Plant Mol. Biol. **41**, 679–685.
18) Dettman, J.R., Harbinski, F.M. and Taylor, J.W. (2001) Fungal Genet. Biol. **34**, 49–61.
19) Kelly, J.M. and Cox, R.A. (1981) Nucl. Acids Res. **9**, 1111–1121.
20) Oda, Y., Yabuki, M., Tonomura, K. and Fukunaga, M. (1997) Yeast **13**, 1243–1250.
21) Lang, B.F. (1984) EMBO J. **3**, 2129–2136.
22) Field, D.J., Sommerfield, A., Saville, B.J. and Collins, A. (1989) Nucl. Acids Res. **17**, 9087–9099.
23) Cummings, D.J., Michel, F. and McNally, K.L. (1989) Curr. Genet. **16**, 381–406.

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