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The Reverse-Transcriptase-Like Proteins Encoded by Group II Introns in the Mitochondrial Genome of the Brown Alga *Pylaiella littoralis* Belong to Two Different Lineages Which Apparently Coevolved with the Group II Ribosyme Lineages

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Abstract. The mitochondrial genome of the brown alga Pylaiella littoralis contains two different types of group II introns. They each encode complete complex proteins, i.e., with a reverse transcriptase domain, a maturase or X domain, and an endonuclease or H-N-H/ zinc finger domain. To our knowledge, this is the first example of the presence in the same genome of introns belonging to subgroups IIA and IIB which both contain multidomained RT-like proteins. We describe the group IIA introns that interrupt the cox1 gene. The RT-like proteins contained in these introns were compared to those of the LSU rDNA group IIB introns. The phylogenetic relationships of these intron ORFs were investigated and the possible evolution of group II introns is discussed.

Key words: Brown alga — *cox1* gene — Group IIA and IIB introns — Phylogeny — Reverse transcriptase-like genes

Introduction

Group I and group II introns are RNA enzymes (ribozymes) that catalyze their own splicing by different mechanisms. All group II introns show a characteristic secondary structure, which consists of six double helical domains, numbered from I to VI, radiating from a central wheel (see Fig. 2). The group II introns are classified into two different subgroups, IIA and IIB, and distinguished by specific structural features (Michel et al. 1989). Group II introns often encode open reading frames (ORFs) which consist of several domains, including a reverse-transcriptase (RT) domain related to the RT domains of the ORFs encoded in non-long-terminal repeat (LTR) transposable elements, other cellular transposable elements, retroviruses, and various DNA viruses (Toh et al. 1983). It is not clear how all these complex proteins are related and which are the most ancient (for review see McClure 1991).

The origin of group II introns is unknown and their idiosyncratic distribution seems to reflect efficient mechanisms for dispersal which may have led to lateral transfers from one organism to another. In fact they have been shown to be mobile genetic elements (Yang et al. 1996). The recent discovery of group II introns in cyanobacteria and proteobacteria (Ferat and Michel 1993; Ferat et al. 1994) has raised the hypothesis of a vertical transmission of these introns from prokaryotes to the derived eukaryotic organelles, mitochondria, and plastids.

While studying the mitochondrial genome of the brown alga *Pylaiella littoralis* (L.) Kjellm., we found four group IIB introns in the large-subunit (LSU) rDNA. Two of them contain a reverse-transcriptase-like gene in their region IV (Fontaine et al. 1995a). This was a rather



surprising finding as most group IIB introns have been described in eubacteria (cyanobacteria and γ proteobacteria; Ferat and Michel 1993) and in plastids (Kück et al. 1990; Maier et al. 1995). A few group IIB introns have been described from mitochondrial genomes but these do not contain ORFs, and sometimes some of the helical domains are missing (Kück et al. 1990; Bonitz et al. 1980; Leblanc et al. 1995).

In this paper we describe the *cox1* gene from the mitochondrial genome of *P. littoralis* as well as the three group II introns which interrupt this gene. Based upon their secondary structure they all belong to subgroup IIA and contain RT-related ORFs. The presence in the same mitochondrial genome of both group IIA and IIB introns with RT-like proteins provides the opportunity to compare the domain organization of these ORFs and to investigate their evolutionary history. In contrast with the ORFs found in group I introns, which often are unrelated (see Michel and Ferat 1995), the group II ORFs have a common ancestor and seem to have coevolved with their introns.

Materials and Methods

The BamHI and EcoRI restriction fragments containing part of the cox1 gene came from an organellar gene bank (Loiseaux-de Göer et al. 1988). Missing parts (the 3' end, see Fig. 1) were amplified from organellar DNA extracted from axenic cultures of P. littoralis as in Dalmon and Loiseaux (1981). In a first amplification an intron-specific degenerated intermediate primer, RD1, nearly identical to the RID-1 primer designed by Ferat et Michel (1993), was designed from the conserved domain V (RD1: 5' ACCGAACCGTACGTG(AC)(AGC-T)A(GC)TTTC(AC)C 3'). The other primer (JM1: 5' GAAAAGGG-CACTCGTCGGAAGGCAA 3') consisted of a known sequence from a cloned border BamHI fragment. A second PCR was then performed with the reverse complement of the RD1 degenerate primer (RD1F: 5' G(GT)GAAA(GC)T(AGCT)(GT)CACGTACGGTTCGGT 3') and with a primer derived from the known sequence of a cloned EcoRI fragment (JM2: 5' GCATCTACCGCAATTATAGGACTCA 3'). Cycle times were as follows: denaturation for 5 min at 94°C, followed by 40 thermal cycles (denaturation, 1 min at 94°C; annealing, 2 min at 68°C; elongation, 3 min at 72°C) and a last elongation step of 10 min at 72°C. For the second PCR, the hybridization temperature was 40°C and the duration of elongation was 5 min. The 100µl reaction mixture contained 2.5 units of Taq DNA polymerase (Promega), 0.2 µM of each primer, 2 mM each of dATP, dCTP, dGTP, and dTTP, 10 μl of 10× buffer (Promega), 10 mM of MgCl₂, and two drops of mineral oil (Perkin).

The entire amplified fragments were sequenced in both directions, using a dideoxy sequencing kit (Pharmacia) and synthetic oligonucleo**Fig. 1.** Physical map of the mitochondrial *cox1* gene from the brown alga *P. littoralis.* The *cox1* gene is split into four exons by three group IIA introns, numbered *1–3*. Each of the three introns contains an ORF encoding for an RT-related complex protein. Fragments between *arrows* were amplified by polymerase chain reactions as indicated in Materials and Methods. *E, Eco*RI; *B, Bam*HI.

tide primers (Eurogentec). The sequence of the intron domain V was then verified by amplification and direct sequencing of this region. Nucleotide sequences were analyzed by the McMolly program (Karoi-Kerlag Reiner Bornemann Bielefeld) to search for open reading frames. Secondary structures of group II introns were constructed according to the consensus models of Michel et al. (1989). The amino acid sequences were aligned by eye with those of homologous genes found in group IIA and IIB introns. Alignments were used to generate distance and parsimony phylogenetic trees, as described in Fontaine et al. (1995a).

Results

The cox1 Gene of Pylaiella littoralis

The mitochondrial cox1 gene of the brown alga P. littoralis spans over 8,956 nt. It is interrupted by three introns (Fig. 1). The exons code for a very conserved, 528amino-acid protein. Using the universal genetic code all conserved tryptophans are correctly translated, as for cox3 and other genes from this genome (Fontaine et al. 1995b). The tryptophan codon is generally the first to be modified in mitochondria when modifications do occur in the genetic code; it is the case, for example, of the protozoan Acanthamoeba castellanii (Burger et al. 1995) and of the red alga Chondrus crispus (Leblanc et al. 1995). The only particularity of the P. littoralis cox1 gene is the presence of a long NH₂-terminal region (Fig. 2), 14 amino acids longer than that of A. castellanii, which already is longer than most of the other homologous genes. As can be seen in Fig. 2, most mutations in the cox1 genes are silent or result in conservative substitutions of amino acids, with an overall identity in eukaryote genes of 74-82% (Table 1) and similarity ranging from 92.5 to 97.7%. Insufficient significant lineage signatures are present to build reliable phylogenies from these genes. Nevertheless, as in the case of the LSU rRNA gene (Fontaine et al. 1995a), it appears that, compared to other mitochondrial cox1 genes, the cox1 gene of P. littoralis is slightly more closely related to the homologous α -proteobacterial gene from *Paracoccus* denitrificans (Raitio et al. 1987; Table 1). The COXI amino acid sequences most closely related to that of P. *littoralis* are those from land plants, green and red algae, and from Acanthamoeba castellanii. Surprisingly the COXI amino acid sequence of the oomycete Phytophtora megasperma (Sachay et al. 1993) is more distant even

		1	M.pol 1		P.ans 1	M.pol 2 S.cer 1
S.cer. M.pol. P.wic. A.cas. C.cri. P.meg. P.lit.	M MINRLLI MNFKI MSYSINNIYNKDLVSFFSFS	MVQRWLYSTNAI NNFAQRWLFSTNHI MVTRWLYSTNHI NNLTSFFTDNRWLFSTNHI 2SFFTQWIS-RWIFSTNHI VINKCQL-DWLFSTNHI ITSLSDFCS-RWLFSTNHI	KDI AVLYFI KDM GTLYL KDI GTMYL KDI GTLYL KDI GTLYL KDI GTLYL KDI GTLYL	MLAIFSGMAGTAMSLIII IFCAIAGVMGTCFSVLII IFCAFSGVLGTVFSLLII IFGGFSGIIGTIFSMIII IFCAFSGVLGCMSMLII IFSAFAGIVGTTLSLLII IFGGFSGVLGTAMSVLII	RLELAAPGSQYLH GNSG RMELAQPGNQILG GNHG RMELAQPGNQILN GNHG RLELAAPGSQILS GNSG RMELAQPSNHLLL GNHG RMELAQPGNQIFM GNHG RLQLASPGNQFLG GNHG	DLFN VLVVG DLYN VLMTA DLYN VIITA DLYN VIITA DIYN VLITA DLYN VVVTA DLYN VVVTA
N.	K.lac 1 cra 1 S.cer 2 * * *		A.macr 3 P.ans IA *			
S.cer H M.pol H P.wic H A.cas H C.cri H P.meg H P.lit H	IAVLM IF F LVMPALIGGFG IAFLM IF F MVMPAMMGGFG IAFLM IF F MLMPALMGGFG IAFVM IF F FVMPVMIGGFG IAFLM IF F MVMPVMIGGFG IAFLM VF F LVMPALIGGFG IAFLM IF F MVMPVLIGGFG	YYLLPLMIGATDTAFPRI WFVPILMGSPDMAFPRL WFLPILIGAPDMAFPRL WFVPLMIGAPDMAFPRL WFVPIMIGAPDMAFPRL WFVPLMIGAPDMAFPRM WFVPLMIGAPDMAFPRM	NNIAFW VLI NNISFW LLI NNISFW LLI NNISFW LLI NNISFW LLI NNISFW LLI NNISFW LLI	PMGLVCLVTSTLVESGA PPSLLLLLSSALVEVGCC PPSLLLLVSSALVEVGA PPSLFLLCSSLVEFGA PPSLCLLMSSLVEFGA PPALLLLVSSAIVESGA PPSLILLLASSLVESGA	GTGWTVYPPLSSIQAHSO GSGWTVYPPLSGMTSHSO GTGWTVYPPLASIASHSO GTGWTVYPPLSSIVAHSO GTGWTVYPPLSSIQSHSO GTGWTVYPPLSSVQAHSO GTGWTVYPPLSSIQAHSO	SPSVDLAIFA SGSVDLAIFS SGSVDLAIFS SGSVDLAIFS SGAVDLAIFS SPSVDLAIFS SPSVDLAIFS
	M.pol 5					
S.cer. M.pol. P.wic. A.cas. C.cri. P.meg. P.lit.	LHLTSISSLLGAINFIV TTL LHLSGVSSILGSINFMT TIF LHLAGVSSILGAINFIC TVF LHLAGISSLLGAINFIT TIF LHISGASSILGAVNFIS TIL LHLTGISSLLGAINFIS TIY LHLSGAASILGAINFIT TIF	NMRTNGMTMHKLPLFVWS NMRAPGLTMHRLPLFVWS NMRAPGMSMLDL-LFVWA NMRVPGLSMHKLPLFVWS NMRSPGQSMYRTPLFVWS NMRAPGLSFHRLPLFVWS NMRAPGMTMDRLPLFVWS	IFITAFLLL VLVTAFLLL VFITAWLLL VLITAFLLL ILVTAFLLL VLITAFLLL VLITAFLLL	LSLPVLSAGITMLLLDR LSLPVLAGAITMLLTDR LCLPVLAGGITMLLTDR FSLPVLAGAITMLLTDR LAVPVLAGAITMLLTDR LTLPVLAGAITMLLTDR LSLPVLAGGITMLLTDR	NFNTSFFEVAGGGDPIL NFNTTFFDPAGGGDPIL NFNTSFFDPAGGGDPIL NFNTSFFDPSGGGDPIL NFNTSFFDASGGDPIL NLNTSFYDPSGGGDPVL NFNTFFDPAGGGDPVL	YEHLFWFFGH YQHLFWFFGH YQHLFWFFGH YQHLFWFFGH YQHLFWFFGH YQHLFWFFGH YQHLFWFFGH
	P.lit 1	P.lit :	2			
S.cer. M.pol. P.wic. A.cas. C.cri. P.meg. P.lit.	PEVYI LIIPGFGIISHVVS PEVYI LILPGFGIISHIVS PEVYI LIIPGFGIISHVIA PEVYI LILPAFGIVSQIIG PEVYI LILPGFGMISHIVS PEVYI LILPAFGIISQVAA PEVYI LILPGFGIVSHILS	IYSKKPVFGEISMVY AM. IFSRKPVFGYLGMVY AM. IFSKKPIFGYLGMVY AM. IFSNKSIFGYIGMVY AM. IFSRKPVFGYIGMVY AM. AFAKKNVFGYLGMVY AM. IFARKPVFGYLGMVY AM.	ASIGLLGFL ISIGVLGFI CSIGILGFI LSIAVLGFI VSIGVLGFI LSIGLLGCI LSIGILGFI	VWSHHMYIVGLDADTRA VWAHHMFTVGLDVDTRA VWAHHMYVVGLDIDTRA VWAHHMYTVGLDVDTRA VWAHHMYTVGLDVDTRA VWAHHMFTVGLDVDTRA VWAHHMFTVGLDIDTRA	YFTSATMIIAIPTGIKII YFTAATMIMAVPTGMKII YFTAATMIIAVPTGIKII YFTAATMMIAVPTGIKII YFTAATMIIAVPTGIKII YFSAATMIIAVPTGIKII YFTAATMIIAVPTGIKII	FSWLATIYGG FSWIATMWGG FSWVATMWGG FSWIATLWGG FSWIATIWEG FSWLATLWGG FSWIATLWGG
			S.cer 5*	P.lit 3		
S.cer. M.pol. P.wic. A.cas. C.cri. P.meg. P.lit.	SIRLATPMLYAIAFLFLFTM SMQYKTPMLFAVGFMFLFTV SIELRTPMLFAVGFLFLFTV QIVRKTPLLFVIGFLILFTL SIHLKTPMLFAIGFILLFV SIRLKTPMYFPIGISFLFTI	GGLTGVALANASLDVAFH GGLTGMVLANSGVDIALH GGLTGVVLANSGLDVAFH GGLTGIVLSNAGLDIMLH GGLTGIVLANSGLDISLH GGVTGVAMSNSGLDIAIH GGLTGVVLANSGVDIALH	DTYYVVGHF DTYYVVAHF DTYYVVAHF DTYYVVAHF DTYYVVAHF DTYYIVGHF DTYYVVAHF	HY VLSMGAIFSLFAG HY VLSMGAVFALFAG HY VLSMGAVFALFAG HY VLSMGAVFAFFAG HY VLSMGAVFAFFAG HY VLSMGAVFGIFTG HY VLSMGAAFTMFAA	YYYWSPQILGLNYNEKL FYYWMGKMTGLQYPETIA FYYWIGKITGLQYPETIA FYYWFWKISGYTYNEMY FYYWFGKITGLQYPETIA FYFWIGKISGRKYPEIIA FYFWIGKMTGLAYPEVIA	AQIQFWLIFI SQIHFWITFF SQIHFWLMFL SNVHFWLMFI SQIHFWSTFI SQIHFWLFFI SQIHFWLMFI
S.cer. M.pol. P.wic. A.cas. C.cri. P.meg. P.lit.	GANVIFFPMHFLGINGMPRR GVNLTFFPMHFLGLAGMPRR GVNITFFPMHFLGLAGMPRR GVNLTFFPMHFLGLAGMPRR GVNLTFFPMHFLGLAGMPRR GVNLTFFPMHFLGLAGMPRR	IPDYPDAFAGWNYVASIG IPDYPDAYAGWNAFSSFG IPDYPDCYAGWNAVASYG IPDYPDNYYYWNILSSFG IPDYPDAYAGWNLIASYG IPDFPDAMSGWNAVSSFG IPDYPDSYAGWNGLASLG	SFIATLSLF SYVSVVGIF SYLSITAVL SIISSVSVI SYIALFSTL SYISFFSAL SIMSSLASL	LFIYILYDQ-LVNNK. CFFVVVF-LTLTS-ENK FFFYVVYK-TLTS-NEV VFFYLIY-LAFNNNN FFFYIVF-VSLTSNN FFFYIVY-VTLVYG-KK FFFFVVY-ITLTKGVEE	SVIYAKAP-DFVESI CAPS-PWATLEWM CPRN-PWETLEWM TPKIKLVHSIFAPYI PCTNFPWETLEWI -TEN AN-PWVKG	NTIFNLNTVK VPSPPAFH LPSPPAFH NTLLSKN-LL VTSPPAYH RGLPSPPLPR
S.cer. M.pol. P.wic. A.cas. C.cri. P.lit.	SSSIEFLLTSPPAVHSFNTP TFEELPAIKESI TFEEIQV TFASIKSTSDSSFFKFSK TFEE RS	AVQS FFIFFM				

Fig. 2. Alignment of several *cox1* genes. The amino acid sequence of the *cox1* gene from *P. littoralis* is aligned with those from the oomycete *Phytophthora megasperma*, *P. meg.* (Sachay et al. 1993); the red alga *Chondrus crispus, C. cri* (Leblanc et al. 1995); the protozoan *Acanthamoeba castellanii, A. cas* (Burger et al. 1995); the green alga *Prototheca wickerhamii, P. wic* (Wolff et al. 1994); the land plant *Marchantia polymorpha, M. pol* (Oda et al. 1992); and the yeast

Saccharomyces cerevisiae, S. cer (Bonitz et al. 1980). The locations of all known group II introns which interrupt *cox1* genes are indicated. They all belong to subgroup IIA, with the exception of S. cer. 5, which belongs to subgroup IIB and has no ORF. A. macr. Allomyces macrogynus (Paquin and Lang 1996); K. lac: Kluyveromyces lactis (Hardy and Clark-Walker 1991); P. ans: Podospora anserina (Cumming et al. 1989); N. cra: Neurospora crassa (Burger et al. 1982).

Table 1. Percentage of identical amino acids in cox1 genes (478 amino acids compared)

	М.р.	<i>P.w.</i>	A.c.	C.c.	P.m.	P.1.	P.d.
Marchantia polymorpha	100						
Prototheca wickerhamii	82.2	100					
Acanthamoeba castellanii	75.9	75.9	100				
Chondrus crispus	82.2	79.9	76.8	100			
Phytophthora megasperma	75.1	76.6	75.3	74.3	100		
Pylaiella littoralis	81.2	80.3	77.8	79.3	76.6	100	
Paracoccus denitrificans	60.0	61.1	58.2	58.8	60.5	61.7	100

though oomycetes form a sister group to brown algae (Gunderson et al. 1987; Bhattacharya and Druel 1988). This latter protein, however, shows more random mutations and is equally distant from all of the other COXI sequences (Table 1).

Structural Organization of the Group IIA and IIB Intron ORFs

The three introns of the *cox1* gene are 2,435, 2,530, and 2,405 nt long, respectively. Introns were folded into a characteristic secondary structure (Fig. 3), with a central core from which six major domains radiate, referred to as I-VI (Michel and Dujon 1983). Based upon their secondary structures and tertiary interactions (Fig. 3), the three introns which interrupt the cox1 gene belong to the subgroup IIA. They are delimited by the consensus GT-GCG sequence at their 5' end and by a consensus CYAC tetramer at the 3' end. All of the three introns interrupt a codon specifying a conserved amino acid. In contrast with most known group II introns, which interrupt the cox1 genes within the first 200 amino acids of the sequence, all three P. littoralis introns are located in the second half of the coding *cox1* sequence. This is also the case of the fifth cox1 intron of Saccharomyces cerevisiae, which is one of the group IIB introns found in mitochondrial genomes (Fig. 2). On the whole, cox1 genes contain more group I (see Wolff et al. 1993) than group II introns, but none of these interrupt the gene at the same location as the P. littoralis group II introns.

The three introns of *P. littoralis cox1* gene each contain an open reading frame coding for a multidomained protein, including one domain related to the reverse transcriptase regions of non–long-terminal-repeat transposable elements and of various viruses (Michel and Lang 1985; Doolittle et al. 1989; McClure 1991). In contrast with most group IIA introns, these open reading frames are not in frame with the 5' exons, yet they span most of intron domain I. Interestingly, all three ORFs start in the first stem loop of domain I (see arrows in Fig. 3), referred to as subdomain IA by Michel et al. (1989).

An alignment of the ORFs of the *P. littoralis* mitochondrial group IIA and IIB introns with several homologous ORFs from other organisms is shown in Fig. 4. These ORFs fall into two different lineages, lineage a and lineage b. All of the ORFs encoded in characterized group IIA introns, including those of P. littoralis, belong to lineage a whereas all ORFs encoded in characterized group IIB introns fall into the other lineage, lineage b. The structural characteristics of these two different ORF lineages are delineated in Fig. 5. In the complex RT proteins from lineage b, the 5' end region (noted A in Fig. 5), 50–100 amino acids in length and located in the intron region IV of the introns, is fairly conserved. In lineage a, this region, 180–280 aa long, which spans the intron regions I-III, is not readily alignable. Moreover, in contrast with the ORFs from lineage b, most of the RT amino acid sequences from lineage a are in continuity and in frame with the 5' exons, implying a chimerically translated protein and subsequent processing events (Michel and Ferat 1995). In both ORF types this region is followed by approximately 50 rather well-conserved amino acids (referred to as region B in Fig. 5; the first 50 aa in Fig. 4). This clearly identifiable (conserved) region of the group II intron ORFs perhaps corresponds to the Z region of non-LTR transposable elements, which is said to be "a unique but unidentifiable sequence" (Doolittle et al. 1989). In the reverse transcriptase domain the main difference between the two intron sets is the addition of ca. 60 aa in the lineage a ORFs, between conserved regions RT4 and RT5. This additional segment is poorly conserved and difficult to align, with the exception of the ORFs in the three cox1 introns of P. littoralis, the cox1 intron 2 of Marchantia polymorpha (Oda et al. 1992) and the cox1 intron 1 of Neurospora crassa (Burger et al. 1982). The RT part of the gene is followed by a very poorly conserved region (denoted C in Fig. 5), approximately 50 aa long in the *b* lineage and 75-300 aa long in the a lineage. The subsequent X domain is well conserved in all of the group II ORFs, albeit with an addition of 25-35 well-conserved amino acids in those from lineage a. This domain has been shown to have a maturase function, since the intron no longer splices when this region is mutated (Mohr et al. 1993; Moran et al. 1994, 1995). A very variable region follows, which is longer in the *b* than in the *a* lineage. Finally, the 3' zinc fingerlike/H-N-H domain (3' end of Fig. 4) is fairly well conserved in both types of ORFs. It has been suggested that this region might participate in the endonuclease activity





Fig. 3. The mitochondrial group IIA introns of *P. littoralis*. A group IIB intron from the mitochondrial LSU rRNA of *P. littoralis* (Fontaine et al. 1995a) is shown for the comparison. The tertiary base-pairing sequences are indicated by EBS1-EBS2, IBS1-IBS2, $\alpha - \alpha', \beta - \beta' \gamma - \gamma'$, $\epsilon - \epsilon', \zeta - \zeta'$ (Michel and Jacquier 1987; Michel et al. 1989; Jacquier and Michel 1990; Costa and Michel 1995). *Arrows with a star* indicate the start of the ORF. The main differences between group IIA and IIB introns are as follows: a bulging "A" on the 3' side of domain VI 7 nt (exceptionally 8 nt) upstream of 3' intron-exon junction for the group IIA introns and 8 nt for group IIB introns. Group IIA introns usually

of group II introns (Zimmerly et al. 1995; Shub and Goodrich-Blair 1994; Gorbalenya 1994; Yang et al. 1996).

Phylogenetic Relationships of the Lineage a and b Intron ORFs

As exemplified in Fig. 6, various phylogenetic trees were constructed from the alignment of the group II intron complex RT proteins (complete alignment available upon request). Positions which were difficult to align were suppressed as well as the region D and the H-N-H domain, which is absent in some of the genes. Nearly

end with YAY and group IIB with RAY. In group IIA the tertiary interaction ϵ' is carried by an internal loop of 9/11 nt and of 4/5 nt for group IIB introns. Some exceptions do exist, as can be seen in the *P*. *littoralis cox1* intron 1. The tertiary interaction indicated by a *small star* is potentially present in all group IIA introns whereas it is found only in a minority of group IIB introns. In the group IIB introns the open reading frame is free-standing in domain IV, whereas the ORF in group IIA introns spans domains I–IV, and can even be in frame with the 5' exons. For the other differences see Michel et al. (1989).

identical results were obtained from distance and parsimony trees and whether 267 or 302 amino acid positions were taken into consideration. Two main branches are clearly delineated, separated by a bootstrap value of 100: all characterized group IIA intron ORFs group together in lineage *a*; all characterized group IIB intron ORFs group together in lineage *b*. In each lineage some of the ORFs are inserted in introns which have not been characterized, either because their sequence is incomplete (*S. obliquus*) or their secondary structure is unknown (*M. polymorpha*) or because they are too modified to be classified in group II subgroups (*Pyrenomonas salina cpn60* ORF).

P.11C.	2221	(22)	1101010	o mooninging	2-VIMVV97	JUK I KATADI F		IL2G2ILAGAVI	UUANTEL	S-SPGELRPLGIE
P.lit.	23S2	(58)	AVRAIT	T-NKGKNTPGING	-EIWDTS1	KKLDATH	I-RL-	GRVSNYSCSPV	RVYTPK	SGGKI RPI GTF
S.obl	netD	(70)		SS-KGSPSPGLSE	-FCFKT-N		FOT	CNDUVVVATDI	DTVTD	- PDCC. APDI CTI
0-1-+-	peco	(/0)	AVQIVE		-ESPKI-P	VINI VANDATI	LEQT I	SNPHKIKAIPL	SKIIIPF	-RDGS-ARPLSIE
Carolin	TX .	(59)	AVRKVI	QDNQGKKAAG1DG	WKSLKPS-	-ARL-'I'LVMNM	1-КГ-1	NHKVKATI	RVWIPK	XP-GNVEKRPLGIE
E COli	rhsC	(16)	AYRRVF	TSA-GAAGIDK.	(-QSLADFI	DKRLVDNI	JXKIM	NRLSSGSYFPPAVI	KAVA <u>IP</u> F	<u> KLGGE-RILGIE</u>
N.cra.	coll		AYELIK	SNP-GNMTKGANP.	-ETLD	GMNLKFL	EKIQ	RDLRDGKFEFPPAF	RIQIPK	P-GKKETRPLTIA
M.pol.	<i>co</i> 12	(263)	CYESIR	GKP-GTSGSDA	-KPLD	G-P-EWF	VOVGI	EKLKKGOFEFSPAF	RTTK	P-GKKEKRPLGIN
Plit	co11	(100)	AVECTE	CKD_CNIMTDCANC	TT_			THE RACKERECHAR		
I.I.I.C.	1011	(103)	ALESIN	SKP-GINHIPSANS	-6100	GrGLAWV	VKASI	NINLKAGKF KF SNAF	GRVHIPK	P-GSSKLRPLGVV
P.11C.	COTT	(200)	AYLMVK	-NNRGISAKGVD-	-DSSLE	GISLRT-L	QAMSI	NDTLSGRIKFSPVF	RVYIKK	-EGKTDLRPLGIS
P.lit.	<i>co</i> 13	(202)	AYIKIK	SKP-GNMTKGVDG	-KTLD	GVNVDWL	KSLSI	RDVGSGSYNPYLVE	RLMIPK	RKGRRPLGIF
		*							r	ev trang 1
									-	
P lit	2391	MM-TI	DRALO-A		CDCCCVCF	ד גרוואיזיסכיעס	14 V	TINDADTCVCEDNIT	י דישודים	
D 11+	2202	NDA 371					14 1			2 C-ELIVEAWLIKA
P.11L.	2352	INM-YI	JRGLQ-Y	LWKLALDPIAECR	ADRHSYGF	RKGRSTQD-V	15 W	VLEADIRGFFDNII	NHDWI	7 -KNILREWLKA
S.obl.	petD	SY-TI)RCLQ-A	LYKLAIEPMAEEV	ADLSSYGF	RPMRNVSWAV	15 Y	VVEIDIKGCVDNIN	NHQFI	7 PKKILWAWLKC
Calothi	rix	TM-OI	DRATO-S	LVKLALEPEWEAK	FEPNSYGF	RPGRNAHDA-	13 W	VLDADISKCFDKT	JHEKL 1	1 -RO-TKAWIKA
E coli	rhcC	mv_ ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	DETAO-T	WINT AFFOOTFOU	FLADGVCV	DDMICATIONT	11 147	VI PEDTICI EDNITI		
D 0011	11150	10 01	MINQ I	V VICLAR DE QVIDE III	1.11HD2101	<u>REINGRUDA</u> I	TT W	APEL DIRGELDHII	<u>- nc</u> u i	4 DAMMPARWVAL
N.cra.	co11	SP-RI)KVVOKA	T-OLVMEPVEEKT	FLOCSHOF	RPHROTETAT	11 F	TTFADFSKAFDST	HCKT. 1	
M pol	0012	CDuCI	PUT LOUR	I OLUTENTVEDT	EL DOCUCE	DTIDCOUDAL	44 1.	TIMU DIGI DOLL		
<u>m.por.</u>	COIZ	SPAGE	SVT A OVY	L-QLVLEATIEPI	FLICSHGF	RIHRSCHIAL	TT W	VVEGNIRKFFDSM	PHKVI 1	2 TLELLQRALRA
P.11t.	coll	SP-RI	JKVILTA	VLQ-VLEPFYEKK	FLDISHAF	RPGRGCHTAL	11 W.	AIEGDIARCFDDII	DHDIL 1	2 TIALIKKSLKN
P.lit.	<i>co</i> 12	SP-RO	XIVOKS	I-EMVLTSIFEEI	FLDCSHGS	RIGRSCHTAL	14 W	VVEGDIKGCEDNTI	PH 1	5 TTNI VKKTLDA
P lit	co13	CD_DI	KTVOFS	T-PUNT OCTVEDC	FTACCUCE	DDCDCCUTTAT	11 147	FTECDTEVCEDGT		
1.110.	0010	or id	MI VQES	T KINDQUIDES	TAC <u>SIGE</u>	<u>KFGKSCHIAL</u>	TT VV.	LIEG <u>DIEVCLO21</u>		
					rev	.trans.2		rev.trans.	3	
P.lit.	23S1	PII	7 PSRG	PQGGVLSPLLCNM	ITLNGLE 2	2		VVRYADDFII	23 RGL	EISEKKSRII 5
P.lit.	23S2	GAL	9 GIAG	/POGGPISPLIANM	TLDGLE 2	4		VVRYADDFVV	22 RGI	VINOEKTCTT 4
S obl	notD	CVT		TOOCCTTCDL TMNT		7			at DCL	ENTRY AVOIDTE 7
G-l-th	peco	GII	9 111G	FQGGIISFDIMNE		.,			21 RGL	EVALIANTIAN /
Calothi	rıx 🛛	GVL	8 TE-G	PQGGVISPLLANI	ALHGLE 2	4		IRYADDFVI	21 MGL	ELNPNKTRIV 14
E coli	rhsC	-YI 1	.7 RTMG	<u>POGGVISPLLANI</u>	FMHYVF 1	2		WY <u>RYADD</u> GIL	22 – <u>GL</u>	EMHPEKTRVI 14
N.cra.	coll	GYI	8 LDIG	PQGSILSPLLCNI	FLHRLD 5	6 PV-TKD1	DSYV-	-RVNYVRYADDFII	23 LGL	RLNPDKTGIT 5
M.pol.	<i>co</i> 12	GY- 1	0 LDEG	SOGSVLSPLLCNI	MLHYLD 4	3 LIPSKDPL	DPYFF	RILYVRYADDFVT	23 L.RI	ELSLEKTVVS 5
Plit	co11	DEV	7 POKC	FOCSPI SPFT ONT	VLHEMD A	ATDOKDOU	 ביברסת		22 T.AT	FLOMDATTC F
D 144	12	OUT -				- APPONDEV	DFDFF		23 LAL	ELSIDETTS 5
P.11C.	COTT	GYI 1	7 PDVG	PQGIILSPLFSNI	VLHELD 4	7 AFPSKSIE	DPDFR	(RLFYVRFVDDWVR	22 LGL	ELNMEKTKIT 4
P.lit.	<i>co</i> 13	GYM	7 SDK <u>G</u>	<u>POGSVVSPLL</u> SNI	YLHELD 3	8 - IPSADPL	DPNFK	(RLRYV <u>RYADD</u> FLI	23 L <u>KI</u>	DLNLTKTKLT 6
				rew trang A	1	*		rev.trans.5		
									r	ev.trans.6
				Lev. clamb. 4					r	ev.trans.6
				167.11415.4					r	ev.trans.6
P.lit.	2351	SF-NI	'LGW 52	DLNPVLRGWANY	YRGSYH 4	s GE	IY'	VYO-LFWKWAOKKI	r 1558 74	w.trans.6
P.lit. P.lit	23S1 23S2	SF-NI	7LGW 52	DLNPVLRGWANY	YRGSYH (8GH	IY'	VYQ-LFWKWAQKKI	ro HSSR 74	NKFRCFVCRGS
P.lit. P.lit.	23S1 23S2	SF-NI GF-DI	TLGW 52 TVGF 46	DLNPVLRGWANYY	YRGSYH YKAT-S	8GH 8GK	IY' (Y'	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI	r HSSR 74 H-RQ 83	NKFRCFVCRGS DKYKCKVCNEY
P.lit. P.lit. S.obl.	23S1 23S2 petD	SF-NI GF-DI GF-EI	FLGW 52 FVGF 46 FLSF 45	DLNPVLRGWANYY ELNPILRGWANYY EINAVFRDCGYYY	YRGSYH YKAT-S YRFAHT	8GF 8GK 1FSS-	IY' (Y' LGYW	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI LWKQFYKH-CYKR	r HSSR 74 H-RQ 83 IKDK 94	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN
P.lit. P.lit. S.obl. Calothr	23S1 23S2 petD cix	SF-NI GF-DI GF-EI GF-NI	FLGW 52 FVGF 46 FLSF 45 FLGF 59	DLNPVLRGWANYY ELNPILRGWANYY EINAVFRDCGYYY -LNPVIRGWVNYY	YRGSYH YKAT-S YRFAHT Y-STSV	BGH BGK IFSS- 5SK	IY' (Y' LGYW (LSHL)	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI	FSSR 74 H-RQ 83 FKDK 94 HPDK 86	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL
P.11t. P.lit. S.obl. Calothr E coli	23S1 23S2 petD cix rhsC	SF-NI GF-DI GF-EI GF-NI MF-DI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY -INPKLNGWINY	YRGSYH YKAT-S YRFAHT Y-STSV Y-GRYT	9GH 9GK 9FSS- 5SK 9SK	IY' (Y' LGYW (LSHL)	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK'	FSSR 74 H-RQ 83 FKDK 94 HPDK 86 KMI, 34	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end
P.lit. P.lit. S.obl. Calothr E coli	23S1 23S2 petD rix rhsC	SF-NI GF-DI GF-EI GF-NI MF-DJ	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54	DLNPVLRGWANYY ELNPILRGWANYY EINAVFRDCGYY -LNPVIRGWVNYY -INPKLNGWINYY	YRGSYH YKAT-S YRFAHT Y-STSV Y-GRYT	8GH 8GK 4FSS- 5SK 4YS	IY' (Y' LGYW (LSHL) -VFRY)	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 FKML 34	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end
P.11t. P.lit. S.obl. Calothr E coli N.cra.	23S1 23S2 petD cix rhsC coll	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 92	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY	YRGSYH YKAT-S YRFAHT Y-STSV Y-GRYT YDFTSN 13	9GH 9GK 1FSS 5SK 1YS 9 SCALTLARK	IY' (Y' LGYW (LSHL) VFRY (YKLK)	VYQ-LFWKWAQKKI VWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKI TLSKVFRKFGKDLA	HSSR 74 H-RQ 83 IKDK 94 HPDK 86 /KML 34 GC-D 52	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK- <u>CSHC</u> -GL end A-T-CIICGET
P.11t. P.lit. S.obl. Calothn E coli N.cra. M.pol.	23S1 23S2 petD cix rhsC coll col2	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 92 FLGT 75	DLNPVLRGWANYY ELNPILRGWANYY EINAVFRDCGYYY -INPVIRGWINYY YNSVMRGIYNYY LYNOKVRGIYNYY	YRGSYH & YKAT-S & YRFAHT & Y-STSV 9 Y-GRYT & YDFTSN 13 YSFASN 13		IY' (Y' LGYW (LSHL) VFRY (YKLK)	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKDLA	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end A-T-CIICGET end
P.11t. P.lit. S.obl. Calothr E coli N.cra. M.pol. P lit	23S1 23S2 petD cix rhsC coll coll	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 92 FLGT 75	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVYY -INPKLNGWINY YYNSVMRGIYNY LYNQKVRGTLNY	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 5 Y-GRYT 4 YDFTSN 13 YSFASN 13	 General Content of the second secon	IY' (Y' LGYW (LSHL) VFRY (YKLK' (YKLK'	VYQ-LFWKWAQKKI VWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL/ WD WIDWERC	HSSR 74 H-RQ 83 IKDK 94 HPDK 86 IKML 34 GC-D 52 IC 33	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK- <u>CSHC</u> -GL end A-T-CIICGET end
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GTT-H	FLGW 52 TVGF 46 TLSF 45 TLGF 59 TLGY 54 TLGY 54 TLGT 75 TLGT 75	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY LYNQKVRGILNY	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 5 Y-GRYT 4 YDFTSN 13 YSFASN 13	BGH BGK CFSS SYS S SCALTLARK S SCALTLALK S SCALTLALK S SCALTLALK	IY' 'LGYW 'LSHL 'VFRY 'YKLK' 'YKLK' 'LKLR'	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKI TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ	HSSR 74 H-RQ 83 HPDK 86 KML 34 GC-D 52 IC 33 SCKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI	FLGW 52 FVGF 46 FLSF 45 FLGY 59 FLGY 54 FLGY 92 FLGT 75 FLGT 75 FLGT 79	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWNYY YNSVMRGIYNY LYNQKVRGILNY FYNQKIRGILNY YFNSRIRGILNY	YRGSYH (YKAT-S (YRFAHT (Y-STSV ! Y-GRYT (YDFTSN 13 YSFADN 13 FSCVHN 1		IY' (Y' LGYW LSHL VFRY (YKLK' (YKLK' (LKLR) (FKLK)	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ	ISSR 74 I-RQ 83 IKDK 94 IPDK 86 IKML 34 IC-D 52 IC 33 ICKE 43 ICKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK- <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DI PV-KI GF-HI GIT-I GIT-I GKCRI PYI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 92 FLGT 75 FLGT 75 FLGT 79 FLGT 71	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY FYNQKIRGILNY YFNSRIRGILNY YYNRVYMGLSNY YNRVYMGLSNY	YRGSYH (YKAT-S (YRFAHT (Y-STSV ! Y-GRYT (YDFTSN 13 YSFADN 13 YSFADN 13 YSFADN 13 YSFSDD 13	BGH FSS YS SCALTLARK SCALTLARK SCALTLARK SCALTLARK SCALTLARK SCALTLARK	IY' (Y' LGYW LSHL VFRY (YKLK' (YKLK' (LKLR' (FKLK) (LRLG'	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGRRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNI	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 FKML 34 GC-D 52 FC 33 GCKE 43 EFVN 40 CIKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-NCWICGSI.
P.lit. P.lit. S.obl. Caloth E coli N.cra. M.pol. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GIT-I GKCRI PYJ	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 92 FLGT 75 FLGT 75 FLGT 79 FLGT 7	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY FYNQKIRGILNY YYNSRIRGILNY YYNSRIRGILNY YYNSRIRGILNY YYNSRIRGISNY	YRGSYH YKAT-S YRFAHT Y-STSV Y-GRYT YDFTSN 13 YSFADN 13 FSCVHN 13 YSFSDD 13		IY' (Y' LGYW (LSHL VFRY) (YKLK' (YKLK' (IKLR' (IKLR) (IKLR) (IKLR)	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGRRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGINI(ISSR 74 H-RQ 83 FKDK 94 HPDK 86 FKML 34 SC-D 52 FC 33 SCKE 43 SFVN 40 CIKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK- <u>C</u> SH <u>C</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 54 FLGY 54 FLGY 54 FLGY 54 FLGY 54 FLGY 75 FLGT 75 FLGT 75 FLGT 71 FLOT 7	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWNYY YNSVMRGIYNY YYNSVMRGIYNY FYNQKIRGILNY YFNSRIRGILNY YFNSRIRGILNY YNSVMGLSNY domain X	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 5 Y-GRYT 4 YDFTSN 13 YSFADN 13 YSFADN 13 YSFSDD 13	General Content of the second	IY' (Y' LGYW USHL VFRY (YKLK (YKLK (YKLK) (IKLR) (IKLR) (IRLG) ain X	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNI(HSSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 GCKE 43 GCFVN 40 CIKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C(2-3)C
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GF-HI GIT-I GKCRI PYJ	FLGW 52 FVGF 46 FLGF 45 FLGF 59 FLGY 54 FLGY 54 FLGY 75 FLGT 75 FLGT 71 rt.7 *	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY LYNQKVRGTLNY FYNQKIRGILNY YFNSRIRGILNY YYNRVYMGLSNY domain X	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 5 Y-GRYT 4 YDFTSN 13 YSFASN 13 YSFADN 13 YSFSDD 13	 General Content of the second secon	IY' (Y' LGYW (LSHL) VFRY (YKLK' (YKLK' (LKLR' (FKLK) (LRLG' ain X	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLI TKKKVYSKFGTNI	ISSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 ECKE 43 EFVN 40 CIKE 43 EFVN 40 CIKE 43	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C ₍₂₋₃₎ C finger domain
P.11t. P.11t. S.obl. Calothn E coli N.cra. M.pol. P.1it. P.1it. P.1it.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 75 FLGT 75 FLGT 75 FLGT 71 rt.7 *	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY -INPKLNGWINY YNSVMRGIYNY YNSVMRGIYNY FYNQKIRGILNY YFNSRIRGILNY YNRVYMGLSNY domain X	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 13 YSFADN 13 YSFADN 13 YSFADN 13 YSFSDD 13	 General Content of the second secon	IY' (Y' LGYW LSHL: VFRY (YKLK' (YKLK' (LKLR' (LKLR) (LKLR) (LKLR) (LKLR) (LKLR) (LKLR) (LKLR)	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNI	ISSR 74 H-RQ 83 IKDK 94 HPDK 86 (KML 34 GC-D 52 IC 33 ECKE 43 EFVN 40 CIKE 43 at circ	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C ₍₂₋₃)C finger domain
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ	FLGW 52 TVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 54 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY INPVIRGWNYY YNSVMRGIYNY YYNSVMRGIYNY YYNSVRGIYNY YYNSKIRGILNY YFNSRIRGILNY YFNSRIRGILNY YNRVYMGLSNY domain X HLIA-RKDGG-EY	YRGSYH (YKAT-S (YRFAHT (Y-STSV ! Y-GRYT (YDFTSN 13 YSFADN 13 YSFADN 13 YSFADN 13 YSFSDD 13	 GH <	IY' (Y' LGYW LSHL VFRY (YKLK' (YKLK' (XKLR') (XKLR' (XKLR' (XKLR') (XKLR' (XKLR') (XKLR' (XKLR') (XKLR' (XKLR')	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKI TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGINIG	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FVN 40 CIKE 43 * zinc Y +3	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SHC-GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C(2-3)C finger domain IIB
P.lit. P.lit. S.obl. Calothn E coli N.cra. M.pol. P.lit. P.lit. P.lit. P.lit. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1 23S2	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GKCRI PYJ LYGDI ICGEI	FLGW 52 FVGF 46 FLGF 45 FLGF 59 FLGY 54 FLGY 54 FLGY 75 FLGT 75 FLGT 71 rt.7 * C-PIHLH C-KVEIH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY LYNQKVRGTLNY FYNQKIRGILNY YFNSRIRGILNY YYNRVMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 13 YSFADN 13 FSCVHN 13 YSFSDD 13 TSFSDD 13 TLK-NI	 GHAMMAN Control Contr	IY' IGYW USHL VFRY YKLK' YKLK' IKLR' IKLR' AIRLG' ain X	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKK TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNIC	ISSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 ECKE 43 EFVN 40 CIKE 43 EFVN 40 CIKE 43 H +18	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK- <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C(2-3)C finger domain IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. P.lit. S.obl.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1 23S2 petD	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GKCRI PYJ LYGDI ICGEI LEIN	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 72 FLGT 75 FLGT 75 FLGT 71 rt.7 *	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY YYNSRIRGILNY YYNSRIRGILNY YYNRVYMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD	YRGSYH (YKAT-S (YRFAHT (Y-STSV ! Y-GRYT (YDFTSN 12 YSFADN 12 YSFADN 12 YSFADN 12 YSFSDD 13 * TLK-NI- HAISNNV T-PNNM/-	Gradient Constraints Gradient Constra	IY' IGYU ILSHL: VFRY CYKLK' CY	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL VRAKVFKKFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ HAICH-DSITT HAICH-DSITT KSPCH-OLVG	HSSR 74 H-RQ 83 IKDK 94 HPDK 86 IKML 34 GC-D 52 ICC 33 ECKE 43 EFVN 40 CIKE 43 * zinc X +3 H +18 S +36	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C ₍₂₋₃₎ C finger domain IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. P.lit. P.lit. S.obl. Calothy	23S1 23S2 petD cix col1 col2 col1 col2 col3 23S1 23S2 petD	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ LYGDI ICGEI LEINS	FLGW 52 TVGF 46 FLGF 59 FLGF 59 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH D-KVEIH SIPYELH DULUEUT	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY INPVIRGWNYY INPKLNGWINYY YYNSVMRGIYNYY LYNQKVRGTLNYY FYNQKIRGILNYY YFNSRIRGILNYH YYNRVYMGLSNYY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD	YRGSYH & YKAT-S & YRFAHT & Y-STSV ! Y-GRYT & YDFTSN 12 YSFADN 13 YSFADN 13 YSFSDD 13 TLK-NI AISNNV T-PNMW	GH GH GH GH GH SCALTLARK SCALTLARK SCALTLARK SCALTLARK SCALTLARK SCALTLARK Com	IY' IGYW: (LSHL) VFRY: (YKLK' (YKLK' (TKLK) (FKLK) (LRLG' ain X -VPV -V-L (L-LC)	VYQ-LFWKWAQKKI VWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNIG HAICH-DSIT HAICH-DSIT HAECHKQL-T KSPCH-QLVS: UDUVL	ISSR 74 H-RQ 83 IFKDK 94 HPDK 86 IFKML 34 GC-D 52 IFC 33 GCKE 43 GFVN 40 CIKE 43 * zinc Y +3 H +18 S +36 + 12 K +12 K	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C(2-3)C finger domain IIB IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. P.lit. S.obl. Calothm	23S1 23S2 petD rix col1 col2 col1 col2 col1 col2 col3 23S1 23S2 petD rix	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ LYGDI ICGEI LEINS YFREI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH SIPYELH DULI <u>EIT</u>	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY YYNSRIRGILNY YFNSRIRGILNY YYNSRIRGILNY YYNSRIRGLNY HLIA-RKDGG-EY HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HIIP-KSQG-KD	YRGSYH 4 YKAT-S 9 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 13 YSFADN 13 FSCVHN 13 SSCVHN 13 YSFSDD 13 TLK-NI NISNNV YT-PNNMV VY-D <u>NL</u>	 GHOME 	IY' IGYM LGYM LSHL VFRY CYKLK	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCLI VRAKVFKKFGKNLJ TKKKVYSKFGTNIC HAICH-DSIT HAICH-DSIT HAECHKQL-TI KSPCH-QLVS HRHC <u>H</u> -DVKT	ISSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 GCKE 43 GCKE 43 GCKE 43 EFVN 40 CIKE 43 EFVN 40 CIKE 43 + 18	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>CWIQ</u> GSL C(2-3)C finger domain IIB IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. S.obl. Calothm N.cra.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1 23S2 petD cix col1	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GKCRI PYJ ICGEI LCGEI LCGEI LEINS YFREI	FLGW 52 FVGF 46 FLSF 45 FLGY 54 FLGY 52 FLGT 75 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH D-KVEIH JDLIEID	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY INPKLNGWINY YNSVMRGIYNY YNSVMRGIYNY YNSVRGILNY YNSRIRGILNY YNRVYMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HILP-KSQGQ-KD	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 12 YSFADN 12 YSFADN 12 YSFADN 12 TLK-NI- NAISNNV T-PNNMV- VY-D <u>NL</u>		IY' (Y' LGYWI LSHL VFRY (YKLK' (YKLK' (ILLC' (ILLC' (IKLK' (IKLK' (ILLC' (ILC' (ILLC' (ILLC' (ILLC' (ILLC' (ILC' (ILC'	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGRRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL VRAKVFKKFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ HAICH-DSITT HAICH-DSITT KSPCH-QLVSS HRHC <u>H</u> -DVKTZ	HSSR 74 H-RQ 83 IKDK 94 HPDK 86 IKML 34 GC-D 52 ICC 33 ECKE 43 EFVN 40 CIKE 43 * zinc X +3 H +18 S +36 A +18 E +2	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C ₍₂₋₃₎ C finger domain IIB IIB IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. P.lit. S.obl. Calothm N.cra.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1 23S2 petD cix col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GKCRI PYJ ICGEI LEIN YFREI KD-	FLGW 52 TVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 75 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH D-KVEIH SIPYELH DDLIEIE PCMH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPKLNGWINY YNSVMRGIYNY YNSVMRGIYNY YNSVMRGIYNY YNSVMRGIYNY YNSVMGINY YNSVMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HIIP-KSQGG-KD	YRGSYH & YKAT-S & YRFAHT & Y-STSV ! Y-GRYT & YDFTSN 12 YSFADN 12 YSFADN 12 YSFADN 12 YSFSDD 12 TLK-NI AISNNV T-PNNMV- VY-PNNMV- VY-SKLDFFT	3 GR 3 GR 5 SR 5 SCAL/TLARK 6 SCAL/TLARK 6 SCAL/TLARK 6 SCAL/TLARK 7 GOMA 7 COMAA INFRKC	IY IGYM LSHL VFRY XYKLK XYKLK XKLK XKLK XKLK XFKLK LKLC A VPV - - - - - - - - - - - -	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKNLI TKKKVYSKFGTNIG HAICH-DSIT HAICH-DSIT KSPCH-QLVS: HRHC <u>H</u> -DVKT; KTHHIGLHNNTWSI	ISSR 74 H-RQ 83 IFKDK 94 HPDK 86 IFKML 34 GC-D 52 IFC 33 GCFE 43 IFCN 40 CIKE 43 * zinc X +3 H +18 S +36 A +18 E +2 IFC	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>C</u> SH <u>C</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>C</u> WI <u>C</u> GSL C(2-3)C finger domain IIB IIB IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. S.obl. Calothm N.cra. P.lit.	23S1 23S2 petD cix col1 col2 col1 col2 col1 col2 col3 23S1 23S2 petD cix col1 col2 col1 col1 col2 col1 col1 col1 col1 col1 col1 col1 col1	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GF-HI GIT-I GKCRI PYJ LYGDI ICGEI LEINS YFREI KD	FLGW 52 FVGF 46 FLSF 45 FLGY 54 FLGY 54 FLGY 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH SIPYELH DOLIEIT VEMH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY YYNSKIRGILNY YYNSKIRGILNY YYNRYMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HILP-KRFGG-KD HILP-KSQG <u>-</u> KD HVRKIKDLR-NQE	YRGSYH 4 YKAT-S 9 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 13 YSFADN 13 YSFADN 13 SSCVHN 13 YSFSDD 13 TLK-NI NISNNV Y-PNNMV YT-PNNMV YY-D <u>NL</u> SGCIAFFI SSGLAFFI	3 GF 3 GK 5	IYY IGYW LSHL VFRY CYKLK CYKL CYKLK	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCLI VRAKVFKKFGKNLJ TKKKVYSKFGTNIO HAICH-DSIT HAICH-DSIT HAECHKQL-TI KSPCH-QLVS HRHC <u>H</u> -DVKTI KTHHIGLHNNTWSI KIHHIGLHNNTWSI	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 GCKE 43 GC-D 52 FC 33 GCKE 43 FTVN 40 CIKE 43 * * * * * * * * * * * * *	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GKCSHC_GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-NCWICGSL C(2-3)C finger domain IIB IIB IIB IIB
P.lit. P.lit. S.obl. Calothm E coli N.cra. P.lit. P.lit. P.lit. S.obl. Calothm N.cra. P.lit.	23S1 23S2 petD cix rhsC col1 col2 col1 col2 col3 23S1 23S2 petD cix col1 col1 col2 col3	SF-NI GF-DI GF-EI GF-FI MF-DJ PV-KI GF-HI GKCRI PVJ CGF-HI GKCRI PYJ LYGDI ICGEI LEINS YFREI KD- HDI	FLGW 52 FVGF 46 FLSF 45 FLGF 59 FLGY 54 FLGY 72 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH D-KVEIH JULIEIU VEMH VEMH JIEIH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -LNPVIRGWVNY -INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY YYNSVRGILNY YYNSRIRGILNY YYNRVYMGLSNY domain X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HILP-KRFGG-KD HILP-KSQGQ-KD HVRKIRDLR-NQE HVRKIKDLKSRYD	YRGSYH & YKAT-S & YRFAHT & Y-GRYT & YDFTSN 13 YSFADN 12 YSFADN 12 YSFADN 12 YSFADN 12 YSFADN 12 TLK-NI TLK-NI YSFSDD 13 * TLK-NI SCVHN 12 SGGIAFWI TRTYAQWI	3 GH 3 GK 4	IY' LGYM LSHL VFRY XYKLK XYKLK XYKLK XYKLK XYKLK XYKLK XYKLK XYKL XYKL	VYQ-LFWKWAQKKI WWDKT-WIWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL VRAKVFKKFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKNLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKRFGKLJ SLAKTFKFGKLJ SLAKTFFGKLJ SLAKTFKFGKLJ SLAKTFKFGKLJ SLAKTFKFGKLJ SLAKTFKFGKLJ SLAKTFKFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFFGKLJ SLAKTFGKJ	HSSR 74 H-RQ 83 IKDK 94 HPDK 86 IKML 34 GC-D 52 ICC 33 ECKE 43 EFVN 40 CIKE 43 * zinc X +3 H +18 S +36 S +36 A +18 E +2 H +15 A +23	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>CWICGSL</u> C(2-3)C finger domain IIB IIB IIB IIB IIB IIB IIA IIA
P.lit. P.lit. S.obl. Calothm E coli N.cra. P.lit. P.lit. P.lit. P.lit. S.obl. Calothn N.cra. P.lit. P.lit. P.lit.	23S1 23S2 petD rix rhsC col1 col2 col1 col2 col3 23S1 23S2 petD rix col1 col2 col3 23S1 23S2 petD cix col1 col2 col3	SF-NI GF-DI GF-EI GF-FI GF-NI MF-DJ PV-KI GIT-I GKCRI PYJ ICGEI LEINS YFREI KD- EI	FLGW 52 TVGF 46 FLSF 45 FLGF 59 FLGT 75 FLGT 75 FLGT 75 FLGT 77 FLGT 71 rt.7 * E-PIHLH D-KVEIH SIPYELH DDLIEIE PSEMH VIELH	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPKLNGWINY YNSVMRGIYNY LYNQKVRGTLNY FYNQKIRGILNY YFNSRIRGILNY YFNSRIRGILNY MOMAIN X HLIA-RKDGG-EY HIKP-KSLGG-DD HILP-KRFGG-KD HILP-KRFGG-KD HILP-KSQGG-KD HVRKIRDLR-NQE HVRKIKDLR-NQE	YRGSYH & YKAT-S & YRFAHT & Y-GRYT & YDFTSN 12 YSFADN 13 YSFADN 13 YSFADN 13 YSFSDD 11 TLK-NI YAISNNV T-PNNMV- VY-D <u>NL</u> SGGIAFWI SSGGIAFWI ANS-DYLI	General Content of the second	IY' LGYW LSHL VFRY YKLK' YKLK' LKLRG ain X VPV V-L L-LC IPLC JIPLC JIPLC	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGKRRI I-NKALVRWGRKKT TLSKVFRKFGKDLA TASKTFNRFGKCL/ VRAKVFKKFGKNLI SLAKTFKRFGKNLI SLAKTFKRFGKNLI TKKKVYSKFGINIG HAICH-DSIT HAICH-DSIT HAECHKQL-TI KSPCH-QLVS: HRHC <u>H</u> -DVKTJ KIHHLGLHNNTWSI KIHHLALHRGTLTI RYHHKNLHAGTLSJ KAC <u>H</u> ISI <u>H</u> KGSYS	ISSR 74 H-RQ 83 IFKDK 94 HPDK 86 IFKML 34 GC-D 52 IFC 33 GC-C 43 IFCKE 43 EFVN 40 CIKE 43 * zinc X +3 H +18 S +36 A +18 S +36 A +18 S +26 H +15 S +27 H +12 S +77 H +12 H +12 S +77 H +177 H +1777 H +1777 H +1777	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GK <u>CSHC</u> -GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-N <u>CWIC</u> GSL C(2-3)C finger domain IIB IIB IIB IIB IIB IIB IIA IIA IIA
P.lit. P.lit. S.obl. Calothm E coli N.cra. M.pol. P.lit. P.lit. P.lit. S.obl. Calothm N.cra. P.lit. P.lit. P.lit.	23S1 23S2 petD cix col1 col2 col1 col2 col1 23S1 23S2 petD cix col1 col1 col2 col3	SF-NI GF-DI GF-EI GF-NI MF-DJ PV-KI GIT-II GKCRI PYJ LYGDI ICGEI LEINS YFREI KD EI	FLGW 52 FVGF 46 FLSF 45 FLGY 54 FLGY 54 FLGY 75 FLGT 75 FLGT 75 FLGT 71 rt.7 * E-PIHLH D-KVEIH SIPYELH DLI <u>EIT</u> VEMH PSEMH UIEIH B_(1)HE	DLNPVLRGWANY ELNPILRGWANY EINAVFRDCGYY -INPVIRGWNY 'INPKLNGWINY YYNSVMRGIYNY YYNSVMRGIYNY YYNSKIRGILNY YYNSRIRGILNY YYNSRIRGILNY YYNSKIRGILNY YYNSKIRGILNY HINP-KSLGG-EY HILP-KRGG-KD HILP-KRFGG-KD HILP-KRFGG-KD HILP-KRFGG-KD HILP-KSQGG-KD HILP-KSQGG-KD HYRKIRDLR-NQE HVRKIKDLKSRYD HIKSIKKVRVK HYRHLRKMGNV I(1)P(2-4) G(5-5)	YRGSYH 4 YKAT-S 4 YRFAHT 4 Y-STSV 9 Y-GRYT 4 YDFTSN 13 YSFASN 13 YSFADN 13 YSFADN 13 YSFSDD 13 TLK-NI YSFSDD 13 TLK-NI YSFSDD 13 YSFSDD 13 YSF	General Content of the second	IY LGYW. LLSHL VFRY YKLK' YKLK' FKLK: FKLK: IRLG UFVC V-L L-LC IPLCC IPLCC IPLCC IPLCC	VYQ-LFWKWAQKKI WWDKT-WTWAKRKI LWKQFYKH-CYKR IYQK-LKRWGRRKI I-NKALVRWGRKKI TLSKVFRKFGKDLA TASKTFNRFGKCLI VRAKVFKKFGKYLJ SLAKTFKRFGKNLJ TKKKVYSKFGTNIC HAICH-DSIT HAICH-DSIT HAICH-DSIT KIKKVYSKFGTNIC KIHLGLHNNTWSI KIHHLALHRGTLT RYHHINLHAGTLSI KACHISIHKGSYSC 3 H(3)H	HSSR 74 H-RQ 83 FKDK 94 HPDK 86 KKML 34 GC-D 52 FC 33 GCKE 43 GC-D 52 FC 33 GCKE 43 FVN 40 CIKE 43 * zinc K +3 H +18 S +36 A +18 E +2 H +15 A +23 G +9	NKFRCFVCRGS DKYKCKVCNEY GPC-CGLCRKN GKCSHC-GL end A-T-CIICGET end GCLVCGET SCAICG-T SK-NCWICGSL C(2-3)C finger domain IIB IIB IIB IIB IIB IIA IIA

Fig. 4. Alignment of the deduced complex RT-like proteins encoded by the group IIA and IIB introns of *P. littoralis* with other homologous genes. Only the most closely related ORFs (see tree, Fig. 6) are shown. These ORFs fall into two groups, one group containing RT-like proteins encoded in group IIB introns and the other including the RT-like proteins encoded in group IIA introns. Regions which could not be aligned are indicated by the number of amino acids which have not been figured. *Stars* (*) refer to regions where a large addition (or deletion) exists in only one of the groups. These complex proteins

In the *b* lineage, the intronic ORF 439 from the cyanobacterium *Anabaena* sp. PCC 7120 (U13767) seems to be inserted into a recognizable group II intron. Its relatedness to that of the plastidial *cpn60* gene of the crypcontain a reverse transcriptase domain, a maturase domain (Lambowitz and Belfort), and an endonuclease domain (zinc finger or H-N-H, Shub and Goodrich-Blair 1994), shown under the alignment. The species included in this figure are *P. littoralis* (this work), *M. polymorpha* (Oda et al. 1992), *N. crassa* (Burger et al. 1982), *S. obliquus* (Kück 1989), *Calothrix* PCC7601 (Ferat and Michel 1993), and *E. coli* (Ferat et al. 1994). 23S1, 23S2 refer to the first and second intron of the 23S rDNA; *col* = *cox1* gene. The intron of *Calothrix* sp. is inserted in an unidentified gene (Ferat and Michel 1993).

tophyte *P. salina* (Maier et al. 1995) is probably artefactual even though it is found in all trees and with good bootstrap values (see Fig. 6). Alignments show that these two sequences are more heavily and randomly mutated



RT domain

4 5

3

2

B

Lineage b

67

С

H-N-H D ^{zing finger} Fig. 5. Schematic representation and comparison of the multidomained proteins encoded in group II introns. The lineage *b* ORFs are free-standing in region IV. The lineage *a* ORFs often are in phase with the 5' exon or begin in region I of the intron. They all end in the 3' end of region IV. Regions are *shaded from white to black* to indicate increasing degrees of conservation. Domains are referred to as in Fig. 4. All of the ORFs found in IIB introns which have been characterized by their secondary structure belong to the *b* lineage whereas those found in characterised IIA introns belong to the *a* lineage.

> Fig. 6. Unrooted neighbor-joining tree (Saitou and Nei 1987) for values of divergence between amino acid sequences of group II intron-encoded genes. Twelve lineage a ORFs and all published lineage b ORFs were aligned manually, taking into account their conserved regions only. A Dayhoff distance matrix was generated by the PROTDIST program of the PHYLIP package, v. 3.5c (Felstenstein 1985). The scale bar represents 0.1 (corrected) amino acid substitution per site. Only the bootstrap values above 80 are indicated (100 replicates). Species are as in Fig. 2 and 4, with the addition of RT-like proteins from Schizosaccharomyces pombe (S. pom) in the cob gene (x02819), from Anabaena sp. PCC7120 (Bauer et al. 1994), from cryptophyte, Pyrenomonas salina (P. sal) in the plastid cpn60 gene (Maier et al. 1995), and from E. coli (Ferat et al. 1994).

than the others, and this result is therefore likely to be due to a long-branch attraction phenomenon (see Philippe et al. 1995). The IIB ORF from *Escherichia coli* is the first to emerge in lineage *b*, well separated from all others, as in the tree published by Ferat et al. (1994).

In lineage *a* the intron ORF from the *M. polymorpha atp9* gene also consistently emerges first. The grouping of *cox1* intron 1, 2, and 3 ORFs from *P. littoralis* with those of *M. polymorpha cox1* intron 2 and *N. crassa cox1* intron 1 was found in all trees, consistent with the visual analysis of alignments. In contrast, the *Schizosaccharomyces pombe cob* intron 1 ORF does not appear as closely related to the latter proteins and, in some trees, branches together with the *S. cerevisiae cox1* ORFs.

Discussion

The Group II RT-Like Proteins Probably Coevolved with the Ribozyme Component of the Introns

Unlike other organelles, the *Pylaiella littoralis* mitochondrial genome possesses both group IIA and group IIB introns that contain complete RT complex proteins, i.e., with an RT domain, an X domain, and a zinc-fingerlike domain. The recent discovery of two novel RT-like proteins contained in group IIB introns (Fontaine et al. 1995a) increased the number of available sequences for analysis of the complex proteins from this intron II lineage. As shown in Fig. 6, most of the proteins have coevolved with their respective introns: characterized subgroup IIA introns contain lineage a ORFs whereas known subgroup IIB introns contain lineage b ORFs. The distinctness of the two ORF lineages is well supported by bootstrap values in consensus trees inferred from both distance matrices and parsimony. Since the lineage a and b ORFs all have the same overall organization, they obviously originated from the duplication of a common ancestral multidomained gene. In most domains the lineage *a* ORFs are longer than those of the *b* lineage (Fig. 5), suggesting that they may have retained ancestral characteristics. In contrast with group I introns no shuffling between these ORF lineages and ribozyme components has yet been observed, suggesting that these have coevolved together.



Fig. 7. Schematic drawing showing the expected phylogenetic branching of the RT-like intronic ORFs, according to the period of invasion by group II introns. Subtree *A* shows the consensus branching order of cyanobacteria, proteobacteria, and their derived organelles, mitochondria, and plastids, based upon ribosomal RNA gene sequences. Complete group II introns could have arisen in ancestral eubacteria (*1*), or have been propagated by unknown vectors (viruses?) into ancestral eubacteria (*2*) and then transmitted vertically. In such a case the phylogenetic tree based upon their ORFs should match the phylogeny of the other eubacterial genes, as shown in tree *A*. In contrast, if group II introns were transferred laterally (*3*) after the endosymbioses leading to plastids and mitochondria, one would expect a topology as in *B*. This is what is seen in Fig. 6 and in Ferat et al. (1994). The *E. coli* introns could have arisen from a previous contamination.

Distribution of Group II Introns

The two intron lineages (IIA, IIB) have representatives in both mitochondria and plastids. All of the complete group IIA introns found so far, i.e., containing multidomained ORFs, are inserted in mitochondrial genomes. In contrast, the group IIA known introns found in plastids do not contain complete ORFs. No group IIA intron has yet been found in free-living eubacteria. The complete group IIB introns have been so far identified in eubacteria, and in the P. littoralis mitochondrial LSU rDNA gene. Their ORFs group with those of two unclassified plastid group II introns. The other group IIB introns found in plastids do not contain ORFs and the other IIB introns reported in mitochondria do not contain RT-like complex proteins. This is, for example, the case for the mitochondrial cox1 intron 5 of Saccharomyces cerevisiae (Bonitz et al. 1980), for the LSU rDNA intron of the chlorococcalean green alga Scenedesmus obliquus (Kück et al. 1990), and for the intron in a mitochondrial trn gene of the red alga Chondrus crispus (Leblanc et al. 1995). It is likely, however, that the introns that invaded these organellar genomes initially were mobile and contained the complete transposing machinery, i.e., with a reverse transcriptase, a maturase, and an endonuclease domain, which enabled them to insert at different locations in these genomes. For some unknown reason, the IIA introns have been preserved intact (with their ORFs) in

mitochondria only, whereas intact IIB introns are much more rare in these organelles. As far as we know the only exceptions are the IIB introns from the mitochondrial LSU rDNA gene of *P. littoralis*. Once more, this finding may be thought of as another piece of evidence showing that this mitochondrial molecule has retained ancestral characteristics (Fontaine et al. 1995a; Delaroque et al. 1996).

The tendency of group II introns to invade one gene from place to place (Ferat et al. 1994) is particularly clear in the mitochondrial genome of *P. littoralis*. In this molecule the LSU rDNA gene displays three closely related group IIB introns, the third having lost its ORF while the *cox1* gene is split by three closely related group IIA introns. The fourth intron in the LSU rDNA gene, although belonging to subgroup B, is not of the same immediate origin (Fontaine et al. 1995a). Why these introns multiply in a given gene and do not invade other nearby genes is not known.

Origin of the Group II Introns

The scattered distribution of group II introns throughout various prokaryotic and eukaryotic lineages raises the question of their origin and transmission. Since they have been found in eubacteria (Ferat et al. 1994), a possible origin involves arising in these or from an early retroviral invasion of ancestral eubacteria. This would have been preceded or followed by an intron duplication, leading to complete IIA and IIB introns. Both intron lineages would then have been transmitted through successive endosymbioses of the prokaryotes which gave rise to mitochondria and plastids, then differentially and progressively eliminated from these organelles, losing their ORFs first.

Two findings, however, are inconsistent with the above hypothesis of a vertical transmission of group II introns. First, the detailed topology within both branches of the RT phylogenetic tree (Fig. 6) is not congruent with the 16S phylogeny of eubacteria and of the derived organelles nor of the eukaryotic tree for the *a* lineage (Fig. 7). In particular, with the exception of the E. coli intron, the *b* lineage branch shows a crown diversification of the RT-like complex proteins that encompasses intronic ORFs found in cyanobacteria, plastids, the mitochondria of P. littoralis, as well as in Azotobacter vinelandii, a γ -proteobacterium phylogenetically close to E. coli (see Ferat et al. 1994). Second, group II introns have not been found in the mitochondria of several eukaryotic lineages or groups such as animals and many protists (A. castellanii, Prototheca wickerhamii, Chlamydomonas reinhardtii, Phytophthora infestans, Paramecium aurelia). This distribution could be interpreted either as evidence for differential loss or independent insertion of these introns, but is probably more easily explained by independent insertion as in the case of nuclear spliceosomal introns (Stoltzfus et al. 1994).

If various prokaryotic and eukaryotic organisms were infected with group II introns, this must have occurred more or less at the same time (Fig. 7), i.e., at the period of the diversification of early eukaryotes. Retroviral-like group II introns may have been progressively invaded various cell genomes. Such a hypothesis of a late, lateral transfer of group II introns is consistent with both the distribution and the phylogenetic relationships of the two ORF lineages. Up to now, no group II introns have been found in nuclei. However, based upon similarities in their splicing mechanisms it has been suggested that nuclear mRNA introns may have arisen from organellar group II introns (Jacquier 1990; Sharp 1991), but such splicing similarity may only be due to a convergent evolution (Weiner 1993). It follows that the nuclear introns of direct (viral?) origin would have either been eliminated or transformed into nuclear-type introns which have retained only a very distant relatedness with the group II introns.

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