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Comparative Analysis of RNA Editing Sites in Higher Plant Chloroplasts

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Abstract. Transcripts of land plant chloroplast genomes undergo C-to-U RNA editing. Systematic search disclosed 31 editing sites in tobacco, 27 in maize, and 21 in rice. Based on these identified sites, potential editing sites have been predicted in the transcripts from four angiosperm chloroplast genomes which have been completely sequenced. Most RNA editing events occur in internal codons, which result in amino-acid substitutions. The initiation codon AUG was found to be created from ACG by RNA editing in the transcripts from *rpl2*, *psbL*, and *ndhD* genes. Comparison of editing patterns raises a possibility that many editing sites were acquired in the evolution of angiosperms.

Key words: Angiosperm — Chloroplast — Initiation codon — RNA editing — Tobacco — Transcript

Introducton

Chloroplasts are intracellular organelles present in higher plants and algae, which contain the entire machinery for the process of photosynthesis and their own genetic system. The chloroplast genome from higher plants consists of multiple copies of homogeneous circular double-stranded DNA molecules of 120 to 160 kbp in size. The gene content and arrangement of chloroplast DNAs in higher plants are relatively uniform from species to species. For example, the tobacco chloroplast genome comprises 155.939 bp, in which 79 different protein-coding genes and 35 different genes encoding stable RNA species have so far been identified (Shinozaki et al. 1986; Wakasugi et al. 1998). These genes fall into two main classes: genes involved in photosynthesis, and those related to transcription and translation.

In higher plant chloroplasts, many chloroplast genes are initially transcribed as polycistronic forms, and these pre-RNA are processed into complex sets of overlapping transcripts, during which some of the transcripts are edited and/or spliced (e.g. Sugiura et al. 1998; Bock 2000). This review focuses on RNA editing, a process altering genomic information at the level of transcripts in chloroplasts from higher plants.

RNA Editing in Chloroplast Transcripts

Genetic information in land plant chloroplast DNAs is sometimes altered at the transcript level by a process known as RNA editing (mostly C to U conversion). RNA editing is defined as the post-transcriptional modification of precursor RNAs to alter their nucleotide sequences through the insertion and deletion of nucleotides, or specific nucleotide substitution, so as to yield functional RNA species.

The first evidence for RNA editing in chloroplasts came from the maize *rpl2* transcript in which an ACG codon changes to a start codon AUG (Hoch et al. 1991). RNA editing was then observed in four internal codons of a maize *ndhA* transcript; UCG (ser) to UUG (leu), UCA (ser) to UUA (leu), UCA (ser) to UUA (leu), and UCC (ser) to UUC (phe) (Maier et al. 1992). All the

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		tobacco			spinach		*Arabidopsis*		*Oenothera*	
GENE		position	genome	edited	position	genome	position	genome	position	genome
rpoA	1	277	* S(uCa)	L(uUa)	276	L(uua)	279	L(uua)	278	L(uua)
rpoB	1	113	* S(uCu)	F(uUu)	113	F(uuu)	113	* S(ucu)	114	* S(ucu)
rpoB	2	158	S(uCa)	L(uUa)	158	S(uca)	158	L(uua)	159	L(uua)
rpoB	3	184	S(uCa)	L(uUa)	184	S(uca)	184	S(uca)	185	S(uca)
rpoB	4	189	L(uug)	---	189	○ S(uca)	189	L(uug)	190	○ S(ucg)
rpoB	5	208	L(cug)	---	208	L(cug)	208	L(uug)	209	L(cug)
rpoB	6	667	* S(uCu)	F(uUu)	667	F(uuu)	669	F(uuu)	668	F(uuu)
rpoC1	1	21	* S(uCa)	L(uUa)	14	* S(uca)	14	L(uua)	14	* S(uca)
rpoC2	1	778	L(uua)	---	768	L(uug)	776	L(uug)	776	L(uua)
rpoC2	2	1248	○ S(uca)	L(uUa)	1232	L(uug)	1239	L(uua)	1243	L(uua)
rps2	1	83	* S(uCa)	L(uUa)	83	* S(uca)	83	L(uua)	83	L(uua)
rps8	1	61	L(uua)	---	61	L(uua)	61	L(uua)	61	L(uua)
rps14	1	27	S(uCa)	L(uUa)	27	S(uca)	27	S(uca)	27	S(uca)
rps14	2	50	* P(cCa)	L(cUa)	50	* P(cca)	50	* P(cca)	50	* S(uca)
rpl2	1	1	M(aug)	---	1	M(aug)	1	M(aug)	1	M(aug)
rpl20	1	103	S(uCa)	L(uUa)	103	L(uua)	103	L(uua)	103	L(uua)
atpA	1	264	* P(cCc)	L(cUc)	188	L(cuu)	264	L(cuu)	264	* P(ccc)
atpA	2	265	* S(ucC)	S(ucU)	189	S(ucc)	265	S(ucc)	265	S(ucc)
atpA	3	383	L(uua)	---	307	L(uua)	383	L(uua)	383	L(uua)
atpF	1	31	* P(cCa)	L(cUa)	31	L(cua)	31	* P(cca)	31	* P(cca)
psbE	1	72	* P(Cca)	S(Uca)	72	S(ucu)	72	* P(ccu)	72	S(ucu)
psbL	1	1	* T(aCg)	M(aUg)	1	* T(acg)	1	M(aug)	1	M(aug)
petB	1	204	P(cCa)	L(cUa)	204	L(cua)	204	L(cua)	204	L(cua)
ndhA	1	17	L(uug)	---	19	L(uug)	17	L(uug)	17	L(uug)
ndhA	2	114	* S(uCa)	L(uUa)	116	L(uua)	114	* S(uca)	114	* S(uca)
ndhA	3	159	L(uua)	---	161	L(uua)	159	L(uua)	159	L(uua)
ndhA	4	189	L(uua)	---	191	○ S(uca)	189	L(uua)	189	L(uua)
ndhA	5	358	S(uCc)	F(uUc)	360	S(ucc)	355	F(uuc)	258	S(ucc)
ndhB	1	50	* S(uCa)	L(uUa)	50	* S(uca)	50	* S(uca)	50	* S(uca)
ndhB	2	156	P(cCa)	L(cUa)	156	P(cca)	156	P(cca)	156	P(cca)
ndhB	3	196	H(Cau)	Y(Uau)	196	H(cau)	196	H(cau)	196	H(cau)
ndhB	4	204	S(uCa)	L(uUa)	204	S(uca)	204	S(uca)	204	S(uca)
ndhB	5	235	F(uuc)	---	235	F(uuc)	235	F(uuc)	235	F(uuc)
ndhB	6	246	P(cCa)	L(cUa)	246	P(cca)	246	L(cua)	246	P(cca)
ndhB	7	249	* S(uCu)	F(uUu)	249	* S(ucu)	249	* S(ucu)	249	* S(ucu)
ndhB	8	277	S(uCa)	L(uUa)	277	S(uca)	277	S(uca)	277	S(uca)
ndhB	9	279	S(uCa)	L(uUa)	279	S(uca)	279	S(uca)	279	S(uca)
ndhB	10	494	P(cCa)	L(cUa)	494	P(cca)	494	P(cca)	494	P(cca)
ndhD	1	1	* T(aCg)	M(aUg)	1	* T(acg)	1	* T(acg)	1	* T(acg)
ndhD	2	128	* S(uCa)	L(uUa)	128	L(uua)	128	* S(uca)	128	* S(uca)
ndhD	3	293	L(uua)	---	293	○ S(uca)	293	○ S(uca)	293	○ S(uca)
ndhF	1	21	L(uua)	---	21	L(uua)	21	L(uua)	21	L(cua)
ndhF	2	97	* S(uCa)	L(uUa)	97	* S(uca)	97	* S(uca)	97	L(uua)
matK	1	420	Y(uau)	---	416	Y(uau)	437	Y(uac)	419	○ H(cac)
ycf3	1	15	F(uuu)	---	12	F(uuu)	gap	---	5	F(uuc)
ycf3	2	62	M(aug)	---	59	M(aug)	20	M(aug)	52	M(aug)
5'UTR of *ndhG*	1	-10bp	(u)	---	-10bp	(u)	-10bp	(u)	-10bp	(u)

Fig. 1. RNA editing sites in chloroplast transcripts from seven angiosperms. Editing sites were experimentally identified from tobacco (Hirose et al. 1999), maize (Maier et al. 1995; Bock et al. 1997), and rice (Corneille et al. 2000). Based on these identified sites, editing sites were predicted in spinach, *Arabidopsis*, *Oenothera*, and wheat. Position is given with reference to the first base of the initiation codon as 1. T is replaced by U in codons of genomes. Black blocks indicate the sites identified in both tobacco and monocot (maize or rice). Asterisks and circles denote the sites identified in tobacco and in monocot (maize or rice), respectively.

edited codons restore amino acids that are conserved in the *ndhA*-encoded peptides of other chloroplast species. RNA editing occasionally creates stop codons, either UAA from CAA or UGA from CGA, which shortens the size of translation products (Wakasugi et al. 1996; Yoshinaga et al. 1996, 1997). A striking case was reported in which two editing events resulted in the creation of a start codon and a stop codon within the same

GENE		wheat position	wheat genome	rice position	rice genome	rice edited	maize position	maize genome	maize edited
rpoA	1	277	L(uua)	277	L(uua)	---	277	L(uua)	---
rpoB	1	111	F(uuu)	111	F(uuu)	---	111	F(uuu)	---
rpoB	2	156	S(ucg)	156	S(uCg)	L(uUg)	156	S(uCa)	L(uUa)
rpoB	3	182	S(uca)	182	S(uCa)	L(uUa)	182	S(uCa)	L(uUa)
rpoB	4	187	○ S(ucg)	187	○ S(uCa)	L(uUa)	187	○ S(uCg)	L(uUg)
rpoB	5	206	○ P(ccg)	206	P(ccu)	---	206	○ P(cCg)	L(cUg)
rpoB	6	676	F(uuu)	675	F(uuu)	---	675	F(uuu)	---
rpoC1	1	14	L(cuc)	14	L(cuc)	---	14	L(cuc)	---
rpoC2	1	888	○ S(ucc)	906	L(uug)	---	925	○ S(uCa)	L(uUa)
rpoC2	2	1344	○ S(ucg)	1369	○ S(uca)	nd	1381	L(uua)	---
rps2	1	83	L(uua)	83	L(uua)	---	83	L(uua)	---
rps8	1	62	○ S(uCa)	61	S(uca)	---	61	○ S(uCa)	L(uUa)
rps14	1	27	L(uua)	27	S(uCa)	L(uUa)	27	S(uCa)	L(uUa)
rps14	2	53	L(cua)	53	L(cug)	---	53	L(cua)	---
rpl2	1	1	○ T(acg)	1	○ T(aCg)	M(aUg)	1	○ T(aCg)	M(aUg)
rpl20	1	103	S(ucu)	103	L(uua)	---	103	S(uCa)	L(uUa)
atpA	1	264	L(cuc)	264	L(cuc)	---	264	L(cuc)	---
atpA	2	265	S(ucc)	265	S(ucc)	---	265	S(ucc)	---
atpA	3	383	○ S(uca)	383	○ S(uCa)	L(uUa)	383	○ S(uCa)	L(uUa)
atpF	1	31	L(cua)	31	L(cua)	---	31	L(cua)	---
psbE	1	72	S(ucu)	72	S(ucu)	---	72	S(ucu)	---
psbL	1	1	M(aug)	1	M(aug)	---	1	M(aug)	---
petB	1	204	P(cca)	204	L(cua)	---	223	P(cCa)	L(cUa)
ndhA	1	17	L(uug)	17	L(uug)	---	17	○ S(uCg)	L(uUg)
ndhA	2	113	L(uua)	113	L(uua)	---	113	L(uua)	---
ndhA	3	158	○ S(uca)	158	○ S(uCa)	L(uUa)	158	○ S(uCa)	L(uUa)
ndhA	4	188	○ S(uca)	188	○ S(uca)	nd	188	○ S(uCa)	L(uUa)
ndhA	5	357	S(ucu)	357	S(uCc)	F(uUc)	357	S(uCc)	F(uUc)
ndhB	1	50	* S(uca)	50	L(uua)	---	50	L(uua)	---
ndhB	2	156	P(ccu)	156	P(cCa)	L(cUa)	156	P(cCa)	L(cUa)
ndhB	3	196	H(cau)	196	H(Cau)	Y(Uau)	196	H(Cau)	Y(Uau)
ndhB	4	204	S(uca)	204	S(uCa)	L(uUa)	204	S(uCa)	L(uUa)
ndhB	5	235	○ S(ucc)	235	○ S(uCc)	F(uUc)	235	F(uuc)	---
ndhB	6	246	P(ccu)	246	P(cCa)	L(cUa)	246	P(cCa)	L(cUa)
ndhB	7	249	F(uuu)	249	F(uuu)	---	249	F(uuu)	---
ndhB	8	277	S(uca)	277	S(uCa)	L(uUa)	277	S(uCa)	L(uUa)
ndhB	9	279	S(uca)	279	S(uCa)	L(uUa)	279	L(uua)	---
ndhB	10	494	P(cca)	494	P(cCa)	L(cUa)	494	P(cCa)	L(cUa)
ndhD	1	1	M(aug)	1	M(aug)	---	1	M(aug)	---
ndhD	2	128	L(uua)	128	L(uua)	---	128	L(uua)	---
ndhD	3	293	○ S(uca)	293	○ S(uCa)	L(uUa)	293	○ S(uCa)	L(uUa)
ndhF	1	21	○ S(uCa)	21	○ S(uCa)	L(uUa)	21	○ S(uCa)	L(uUa)
ndhF	2	97	L(uua)	97	L(uua)	---	97	L(uua)	---
matK	1	451	○ H(cac)	451	○ H(cac)	nd	451	○ H(Cau)	Y(Uau)
ycf3	1	15	○ S(ucc)	15	F(uuc)	---	15	○ S(uCc)	F(uUc)
ycf3	2	62	○ T(acg)	62	○ T(aCg)	M(aUg)	62	○ T(aCg)	M(aUg)
5'UTR of *ndhG*	1	-10bp	(u)	-10bp	○ (c)	(U)	-10bp	○ (C)	(U)

Fig. 1. Continued.

transcript, leading to the formation of a new protein-coding gene at the mRNA level, the *petL* gene in black pine chloroplasts being thus produced (Wakasugi et al. 1996). Therefore, protein-coding regions or protein sequences cannot always be predicted from their genome sequences in chloroplasts. An exception to the above cases is that an RNA editing event was observed at the third position of a serine codon (UCC to UCU) in the tobacco *atpA* transcript, thus not leading to amino acid change (silent editing) (Hirose et al. 1996), and that it was also detected in the 5′ untranslated regions of maize and rice *ndhG* mRNAs (Bock et al. 1997a; Corneille et

al. 2000), and of Ginkgo *psbJ* transcripts (Kudla and Bock 1999).

RNA editing has been found in chloroplast transcripts from all major lineages of land plants (Freyer et al. 1997) but has not been reported in those from algae and cell transcripts from cyanobacteria. Most editing events found so far in vascular plants are C to U conversions, but U to C inverse editing, in addition to C to U changes, has been reported in the *rbcL* and *atpB* transcripts from a bryophyte, the hornwort *Anthoceros formosae* (Yoshinaga et al. 1996, 1997). However, no editing was found in a bryophyte, the liverwort *Marchantia polymorpha*, whereas another liverwort *Bazzania trilobata* exhibits RNA editing at least in *ndhB* and *rbcL* transcripts (Freyer et al. 1997).

Comparison of RNA Editing Patterns in Angiosperms

Occurrence of RNA editing in specific chloroplast transcripts from a wide range of plant species was examined, and it was shown that neither editing frequencies nor editing patterns correlate with the phylogenetic tree of the plant kingdom (Freyer et al. 1997). Here we compared editing sites in transcripts from completely sequenced chloroplast genomes of angiosperms. The complete chloroplast DNA sequence has so far been determined from tobacco (*Nicotiana tabacum* var. Bright Yellow 4, 155.939 bp, Shinozaki et al. 1986, accession no. Z00044), spinach (*Spinacia oleracea*, 150.725 bp, accession no. AJ400848), *Arabidopsis thaliana* ecotype Columbia (154.478 bp, Sato et al. 1999, accession no. AP000423), evening primrose (*Oenothera elata* ssp. hookeri, 159.443 bp, Hupfer et al. 2000, accession no. AJ271079), rice (*Oryza sativa* var. Nipponbare, 134.525 bp, Hiratsuka et al. 1989, accession no. X15901), maize (*Zea mays*, 140.387 bp, Maier et al. 1995, accession no. X86563), and wheat (*Triticum aestivum* cv. Chinese spring, 134.540 bp, Ogihara et al. 2000, accession no. AB042240) among angiosperms. The number of editing sites was systematically analyzed experimentally in several species; so far 27 have been found in maize (Maier et al. 1995; Bock et al. 1997a), 26 in black pine (Wakasugi et al. 1996), 31 in tobacco (Hirose et al. 1999), and 21 in rice (Corneille et al. 2000).

Figure 1 summarizes editing sites identified and predicted so far in the transcripts from the seven angiosperm chloroplasts. All those editing events are C-to-U conversions, and no U-to-C inverse editing was reported. On comparing editing sites among tobacco (31 sites, Fig. 2), maize (27 sites), and rice (21 sites), 12 sites are common between the dicot (tobacco) and the monocot (maize or rice), and 20 sites between the two monocots (maize and rice). Therefore, at least 12 common sites may have been present before divergence to monocots and dicots. When compared with the 26 editing sites found in the chloroplast transcripts from the gymnosperm black pine (Wakasugi et al. 1996), only five sites [*rpoB* (position 159), *rpoC2* (position 1284), *rps8* (position 61), *rps14* (position 49), and *psbE* (position 72)] are conserved among the total of 47 different sites identified in the three angiosperm species. This observation raises a possibility that many editing sites were acquired in the evolution of angiosperms.

Based on edited sites identified in tobacco, maize, and rice chloroplast transcripts, possible sites were predicted in the transcripts from spinach, *Arabidopsis*, *Oenothera*, and wheat chloroplasts; 22 sites in spinach, 19 in *Arabidopsis*, 23 in *Oenothera*, and 28 in wheat (see Fig. 1). Lack of several editing sites in the four species with respect to those found in tobacco and maize is due to the presence of T residues in relevant positions, suggesting that the back mutation from C to T at the DNA level may have occurred after divergence to individual species.

RNA editing occurs in 21 mRNAs out of 80 different protein-coding genes present in tobacco, rice, and maize chloroplasts; six mRNAs from 36 photosynthetic genes, ten mRNAs from 26 genetic system genes, and five mRNAs from 11 NADH dehydrogenase genes (*ndhs*). Namely, RNA editing is found more frequently in *ndh* gene transcripts than the other transcripts; 20 sites in *ndh* mRNAs out of the total of 47 identified sites. Accumulation of editing sites in *ndh* mRNAs may be due to non-essential nature of *ndh* genes (Corneille et al. 2000). The *ndhB* transcript has the highest number of editing sites (nine sites in tobacco), and these sites are highly conserved among species examined. The *ndhB* gene is located in the inverted repeat sequence, suggesting that this situation may prevent the back mutation from C to T.

Creation of Translational Initiation Codons

RNA editing produces AUG from ACG in some transcripts from *rpl2*, *psbL*, and *ndhD*, and the resulting AUG triplet was proved to be a functional initiation codon (Hirose and Sugiura 1997 and unpublished observations). Occurrence of these editing events shows some correlation with the phylogenetic position of angiosperm species (Fig. 3). No corresponding sites have been found in chloroplast transcripts from the gymnosperm black pine (Wakasugi et al. 1996). The ACG to AUG editing site in *rpl2* transcripts is seen only in monocots, whereas that in *ndhD* and *psbL* transcripts is present only in dicots. These observations may be explained if respective editing sites were acquired during divergence to monocots and dicots. *Arabidopsis*, *Oenothera*, and monocots lost such editing site in *psbL* transcripts thereafter, which may be due to the back mutation from C to T.

Problems and Prospects

The list of identified editing sites shown in Fig. 1 is by no means complete because search does not take account of

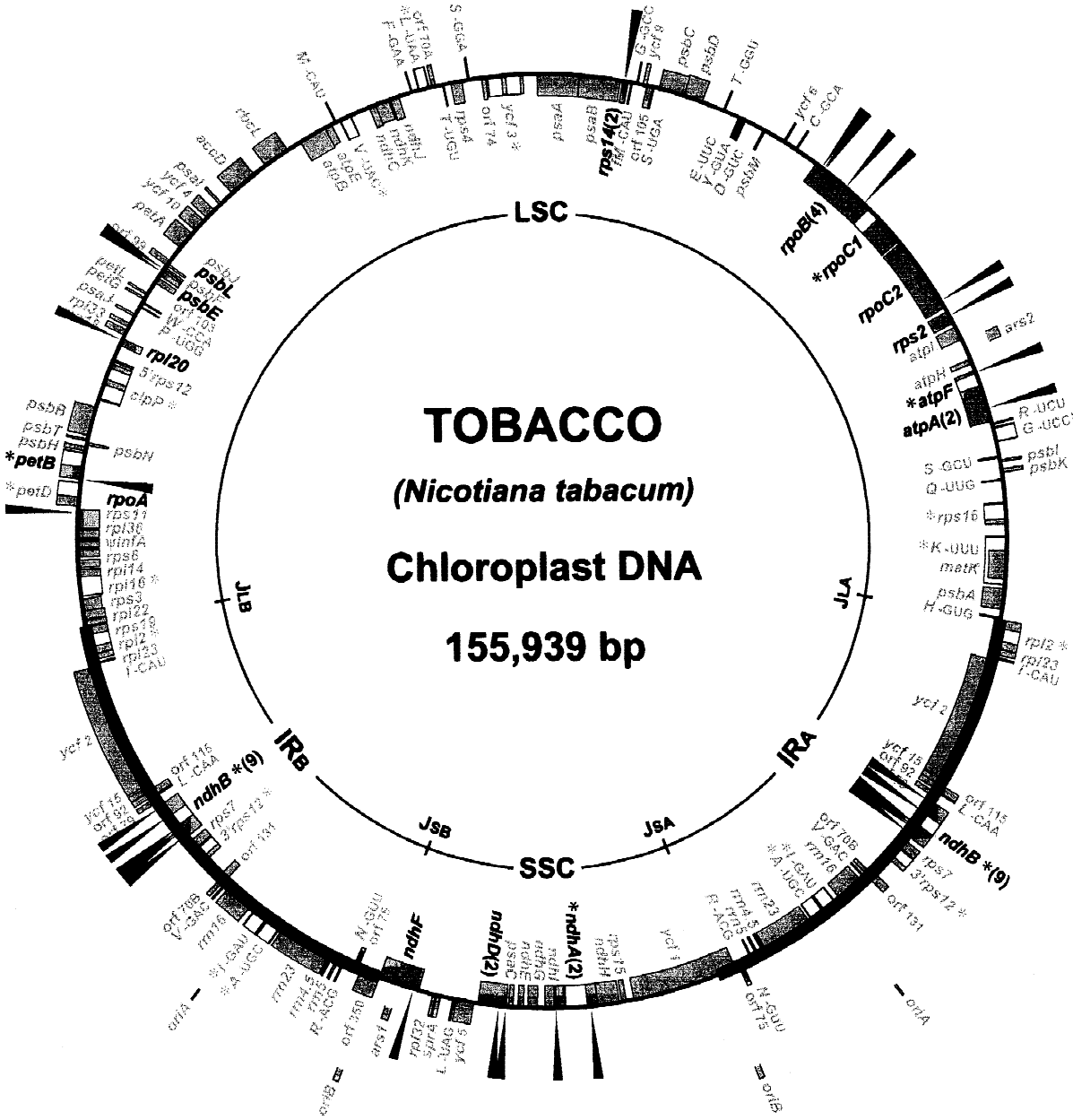

Fig. 2. Distribution of RNA editing sites on the gene map of tobacco chloroplast DNA. Identified RNA editing sites in the protein-coding regions of transcripts are indicated by sharp triangles with bold gene names. Numerals in parentheses are numbers of editing sites, and no parenthesis indicates single sites.

editing sites outside the protein-coding regions, the third positions of codons, or tRNA/rRNA transcripts. Efforts have to be continued to identify additional editing sites, especially in these regions of chloroplast transcripts. The mechanism of RNA editing in chloroplast is being studied by using tobacco (Bock et al. 1994, 1996, 1997b; Bock and Maliga 1995; Chaudhuri et al. 1995; Bock and Koop 1997; Chaudhuri and Maliga 1997; Karcher and Bock 1998; Hermann and Bock 1999; Hirose and Sugiura 2001). The chloroplast DNA from tobacco has frequently served as a reference for chloroplast genomes, and both chloroplast transformation technique (Svab and Maliga 1993) and chloroplast in vitro RNA editing system (Hirose and Sugiura 2001) are available only for tobacco so that at a moment the tobacco chloroplast is the best model for molecular analyses of RNA editing. Based on limited data, site-specific *trans*-acting factors (probably proteins) are required to recognize editing sites in chloroplasts. Therefore, to gain an editing site during evolution, both T-to-C mutation in a genome and acquisition of a cognate *trans*-acting factor seem to be required. If each editing site is recognized by a unique *trans*-acting factor, a chloroplast has to possess dozens of these factors. It should be prerequisite to elucidate the structure of these *trans*-factors in order to understand how diverse *trans*-factors have emerged.

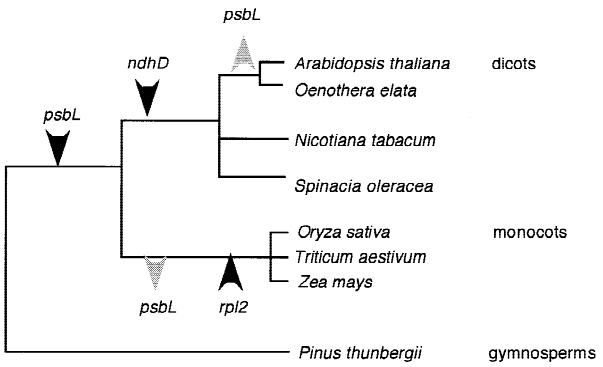

Fig. 3. Phylogeny and RNA editing at translation initiation sites. The phylogenetic tree was constructed according to Soltis et al. (2000). The length of branches does not reflect the phylogenetic distance. Acquisition and loss of RNA editing sites are indicated by filled arrowheads and an open arrowhead, respectively.

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