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Divergent RNA editing frequencies in hornwort mitochondrial *nad*5 sequences

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Abstract

Hornwort mitochondrial genomes have some of the highest rates of RNA editing among plants. Comparison of eleven partial mitochondrial *nad*5 genomic and cDNA sequences from diverse taxa of hornworts reveal 125 edited sites in only 1107 nt. No single sample exhibits more than half of these sites. Ten of the 11 hornwort taxa have between 35 and 54 edited sties each; whereas, the eleventh taxon, *Leiosporoceros,* which represents a potential sister taxa to all other hornworts, has only eight sites. Comparison of multiple cDNA sequences from several individuals reveals the presence of many immature transcripts showing the heterogonous nature of the progression of editing. Phylogenetic analyses of hornwort genomic and cDNAs sequences reveal that 65 of the 94 phylogenetically informative sites within the hornwort clade are edited positions.

Keywords: *Leiosporoceros*; Phylogeny reconstruction; U to C editing

1. Introduction

RNA editing is a process in which the primary transcript is altered prior to translation. This process has been demonstrated across a diverse range of plant chloroplast and mitochondria genomes (Hiesel et al., 1994; Malek et al., 1996; Freyer et al., 1997) and usually takes the form of C to U conversions. While relatively uncommon in seed plants this process is more prevalent in both the chloroplast and mitochondrial transcripts of deeper land plant lineages. In particular, the highest rates of RNA editing have been observed in the non-vascular group, the hornworts. Kugita et al. (2003) characterized RNA editing across the entire chloroplast DNA genome of *Anthoceros* finding 942 total edited sites, more than twice that found in the fern chloroplast genome of *Adiantum* (Wolf et al., 2004). Nevertheless, partial sequences of the *nad*5 mitochondrial gene (Steinhauser et al., 1999) have shown that mitochondrial genes of hornworts are edited to even greater extent: two genera of hornworts exhibited a total of 71 total edited sites of approximately 1100 nucleotides. More recently, Groth-Malonek et al. (2005) reported high rates of editing in mitochondrial *nad*5 among several unrelated taxa, including the liverwort *Haplomitrium* and fern *Asplenium*. Aside from these studies, little is know about the frequency and pattern of RNA editing both across land plant lineages and across the entire mitochondrial genome. To further explore the distribution and frequency of editing among a single ancient land plant group, the hornworts, I extend the examination of RNA editing among *nad*5 transcripts to a range of taxonomically diverse hornworts and show extensive RNA editing of *nad*5 transcripts for all hornworts with the exception of the unusual taxon *Leiosporoceros*.

2. Materials and methods

2.1. Nucleic acid preparation and cDNA synthesis

DNA and RNA extractions were performed on plant tissues collected from the field as described previously (Duff and Moore, 2005). RNA was digested for one hour with DNAse-I (Qiagen) then subjected to first strand cDNA synthesis with the Sensuscript kit (Qiagen) using the reverse primer NAD5L (Steinhauser et al., 1999). Amplification of

Abbreviations: C, cytidine; U, uridine; cDNA, DNA complementary to RNA; PCR, polymerase chain reaction; nt, nucleotide(s); DNase, deoxyribonuclease.

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partial *nad*5 cDNAs and genomic DNAs were accomplished using the NAD5K and NAD5L primer pair (Steinhauser et al., 1999) with the DNAsed RNA extraction acting as a control reaction for the cDNA amplifications. Products were ligated into the TA TOPO vector (Invitrogen). Resultant colonies were screened via PCR using universal primers to evaluate insert size, and two plasmids for genomic sequences and up to ten positive colonies representing individual cDNAs were obtained. Sequencing of vector inserts was accomplished using universal primers as previously described (Duff et al., 2004). Multiple (2–10) cDNAs were sequenced for each hornwort sample. Genomic and cDNA sequences were aligned manually and compared to determine the presence of RNA editing sites.

2.2. Phylogenetic analyses

An alignment (1107 nt) including genomic and cDNA sequences for nine hornworts was combined with nine outgroup taxa including four liverworts, four mosses and the green algae *Chara*. Two data sets were used for phylogenetic analyses including either hornwort DNA or hornwort cDNA sequences along with outgroup genomic DNA sequences. Phylogeny reconstruction was by the maximum likelihood (ML) method using PAUP* version 4.0b10 (Swofford, 2003). For each analysis, we used an HKY85 model of DNA evolution (Hasegawa et al., 1985) for which base frequencies and the transition/transversion ratio was estimated from the data. To account for rate heterogeneity among sites we used a gamma distribution with the alpha shape parameter estimated from the data. Heuristic searches were performed on starting trees obtained by stepwise addition of randomly ordered taxa and subjected to tree bisection and reconnection (TBR) branch swapping. Reliability of inferred clades resulting from analyses was assessed using 100 bootstrap replications using the same conditions above.

3. Results and discussion

3.1. RNA editing distribution and frequency

Genomic and cDNA sequences were generated for nine hornworts. These new sequences combined with two sequences previously reported for *Anthoceros* (Steinhauser et al., 1999) demonstrate the presence of 125 total edited sites among the eleven total samples examined. Fig. 1 summarizes the positions, form of editing and numbers of edited sites for each taxa. Of the 125 sites 38 (31%) are U to C and 85 (68%) are C to U conversions with two sites (7 and 967) exhibiting editing in both directions. Fig. 1 includes total numbers of edited sites for each sample including predicted sites. For several taxa these sites are predicted to exist based on the lack of removal of stop codons and amino acid conservation (Duff and Moore, 2005) though they were not observed in cDNA-genomic DNA comparisons. Remarkably, the total number of edited positions constitute 11% of the entire nucleotide sequence examined. Furthermore, these edited sites are predicted to alter the expression of 95 amino acid residues constituting 26% of this portion of the *nad*5 peptide chain. Ten U to C conversions were identified which serve to repair internal stop codons with one sample, *Phaeoceros carolinianus*, possessing eight stop codons.

3.2. Immature transcripts and RNA editing prediction

One to five cDNA clones were sequenced for each sample. For most samples, each of the cDNAs were identical and appeared to represent fully edited transcripts. However, for three samples (*Megaceros fuegiensis*, *Megaceros flagellaris* and *P. carolinianus*) sites in the cDNA sequence necessary to repair stop codons were not corrected suggesting the transcripts sequenced were not completely mature. For two samples, *Phaeoceros pearsonii* and *Phaeoceros bulbiculosus*, an especially large diversity of edited sites among cloned cDNAs. For each, no two cDNA was observed sequences, among the 8 sequenced, were identical. Fig. 2 shows the variation among eight transcripts of *P. bulbiculosos*. Among these sequences, 53 edited sites were clearly identified with an additional site (Fig. 1, position 294) predicted to be likely edited but was not observed. In addition, 11 differences between the DNA sequence and a single cDNA clone were observed. Three of these were G to A or A to G transitions and the remaining eight were C to T or T to C transitions. Three of the latter were found in synonymous third sites and thus may represent additional edited sites. However the remaining sites are possibly, if not likely, the result of errors incurred either during transcription by RNA polymerase or from reverse transcription and subsequent cloning processes and thus are not treated as edited sites here. For example, the T to C change at position seven would result in the creation of a stop codon whereas in other hornwort taxa the same position is edited in the reverse direction to repair a stop codon. Similar to that observation of Lin et al. (2002) in dinoflagellates, four sites exhibited T to A differences in more than a single cDNA clone suggesting the possibility of an additional form of RNA editing in hornworts. Interestingly, one of these sites (Fig. 1, position 142) results in the predicted conversion of a stop codon to an arginine residue while cDNA copies 4 and 5 have a T to C change that also would convert the stop codon to the same amino acid.

For *P. bulbiculosus*, while a total of 49 of the total 53 observed edited sites were identified in a single transcript, as few as three were observed in another transcript captured from cDNAs synthesized from the same RNA extraction. More surprisingly, when one compares the transcripts exhibiting similar total numbers of edited sites, the position of observed edited sites were frequently very different. For example, cDNA-3 and cDNA-6 (Fig. 2) show 14 and 15 total edited sites, respectively, yet do not share a single edited site! Similarly, clones cDNA-6 and cDNA-1 have 15 and 18 edited sites yet only share 2 of them. Not surprisingly then, examination of the positions of edited sites reveals that while they are found non-randomly across the length of the gene the order in which each site is edited is highly variable.