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Loss of the *rpl32* gene from the chloroplast genome and subsequent acquisition of a preexisting transit peptide within the nuclear gene in *Populus* ☆

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Abstract

Gene transfer events from organelle genomes (mitochondria and chloroplasts in plants) to the nuclear genome are important processes in the evolution of the eukaryotic cell. It is highly likely that the gene transfer event is still an ongoing process in higher plant mitochondria and chloroplasts. The number and order of genes encoded in the chloroplast genome of higher plants are highly conserved. Recently, several exceptional cases of gene loss from the chloroplast genome have been discovered as the number of complete chloroplast genome sequences has increased. The *Populus* chloroplast genome has lost the *rpl32* gene, while the corresponding the chloroplast *rpl32* (cp *rpl32*) gene has been identified in the nuclear genome. Nuclear genes transferred from the chloroplast genome need to gain a sequence that encodes a transit peptide. Here, we revealed that the nuclear cp *rpl32* gene has acquired the exon sequence, which is highly homologous to a transit peptide derived from the chloroplast Cu–Zn superoxide dismutase (cp *sod-1*) gene. The cp *rpl32* gene has acquired the sequence that encodes not only for the transit peptide, but also for the conserved N-terminal portion of the mature SOD protein from the cp *sod-1* gene, suggesting the occurrence of DNA sequence duplication. Unlike cp SOD-1, cp RPL32 did not show biased localization in the chloroplasts. This difference may be caused by mutations accumulated in the sequence of the SOD domain on the cp *rpl32* gene. We provide new insight into the fate of the inherent sequence derived from a transit peptide.
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Keywords: Gene transfer; Chloroplast; Ribosomal protein; Exon-shuffling

1. Introduction

It is generally accepted that mitochondria and chloroplasts are descendants of α-proteobacteria and cyanobacteria, respectively. Most of the genes in the ancestral endosymbiont have either been translocated to the nuclear genome of the host cell or have been lost during evolution after the initial endosymbiotic event (Gray, 1992; Martin, 2003). Although such gene transfers are important genetic events in evolution, little is known about the process.

Compared with plant mitochondrial genomes, the number and order of genes encoded in chloroplast genomes are well conserved among species in higher plants (Timmis et al., 2004). Therefore, it is assumed that gene transfer events from the chloroplast genome are less active than those from the mitochondrial genome. Furthermore,

Abbreviations: kb; kilobase; cDNA; DNA complementary to RNA; *sod-1*; Cu–Zn superoxide disumtase; *rpl22*; ribosomal protein L22; *rpl32*; ribosomal protein L32; *rps10*; ribosomal protein S10; *rps9*; ribosomal protein S9; *rps11*; ribosomal protein S11; *rps14*; ribosomal protein S14; *rps16*; ribosomal protein S16; *sod*; superoxide dismutase; *infA*; translational initiation factor A; GFP; green fluorescence protein; EST; expressed sequence tag; RT; reverse transcriptase; cp; chloroplast; nt; nucleotide; bp; base pair; ORF; open reading frame; PCR; polymerase chain reaction; CaMV; cauliflower mosaic virus; TIGR; The Institute for Genomic Research; RI; replication intermediate.

☆ Sequences obtained in this work have been deposited in DDBJ/EMBL/GenBank with accession numbers: AB302216 and AB302218–AB302220.

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the mutation rate in the mitochondrial genome is generally lower than that in the nuclear and chloroplast genome in higher plants (Wolfe et al., 1987). Results indicate that the protein-coding sequences in the mitochondrial genome are highly conserved among higher plants. This enables comparative analyses to be conducted by Southern blotting to determine the presence or absence of genes in higher plant mitochondrial genomes. It is also possible to trace a transferred gene from the mitochondria to the nuclear genome. Vestiges of the evolutionary gene transfer event from the mitochondria to the nucleus still remain for recently transferred genes. The activation process of transferred genes, such as the acquisition of a sequence that encodes a mitochondrial targeting signal (called the presequence), the promoter, and RNA-mediated gene transfer (Adams and Palmer, 2003) have been gradually revealed. On the other hand, the number and order of the genes encoded in the chloroplast genome of higher plants are highly conserved. Therefore, examples of recent gene transfers during higher plant evolution are rarely identified.

A transferred gene from the chloroplasts must gain a sequence that encodes a transit peptide since translated protein in the cytosol is sorted by the chloroplasts. However, evidence of chloroplast targeting signal (called the transit peptide) acquisition is rarely reported, compared with the several examples in mitochondrial proteins. These act via duplication and subsequent recombination (exon shuffling) as in *Oryza sativa rps11* (Kadowaki et al., 1996), alternative splicing as in *O. sativa rps14* (Kubo et al., 1999), accumulation of point mutations as in *O. sativa rps10* (Kubo et al., 2000), and so on (see review, Adams and Palmer, 2003). To the best of our knowledge, transit peptide acquisition by the exon shuffling of an existing transit peptide has been reported (McFadden, 1999; Timmis et al., 2004). Examples are in the genes for *O. sativa rps9* (Arimura et al., 1999) and maize (*Zea mays*) glyceraldehyde-3-phosphate dehydrogenase (Quigley et al., 1988), which were transferred from the chloroplast to the nucleus before the divergence of the angiosperms. In the above gene cases, the origin of a transit peptide has not been determined but has been proved to share a common origin. Recent evolutionarily transferred genes include *rpl22* (Gantt et al., 1991) and *infA* (Millen et al., 2001). However, the origin of a transit peptide remains unknown in each gene.

As mentioned above, the mechanism of acquisition for a sequence that encodes a transit peptide has rarely been reported. Several cases of recent gene loss from the chloroplast genome have been found thanks to the increased number of chloroplast genomes that have been completely sequenced. These include all *ndh* genes in *Pinus* (Wakasugi et al., 1994), the *rps16* gene in *Medicago* (Saski et al., 2005), *Pinus* (Wakasugi et al., 1994), and *Populus* (Steane, 2005; Okumura et al., 2006), and the *rpl32* gene in *Populus* (Steane, 2005; Okumura et al., 2006), which are generally encoded in higher plant chloroplast genomes. It is still unknown as to whether these genes were successfully transferred to the nuclear genome or were lost from the cell entirely.

In *Escherichia coli*, L32 is a late assembly protein incorporated at the replication intermediate (RI)*$_{50}$ stage *in vitro* and is mapped near L6, L13, L17, L20, and L21 on the stalk side of the 50S subunit. No *E. coli* mutants lacking L32 have been isolated, indicating a probable essential role for L32 in ribosomal function (Subramanian, 1993). Recently, the transferred cp *rpl32* gene has been successfully obtained in the nuclear genome. It is also strongly suggested that the cp *rpl32* gene has acquired the sequence that encodes the transit peptide from the chloroplast Cu–Zn superoxide dismutase (cp *sod-1*) gene (Cusack and Wolfe, 2007). In *P. alba*, we have demonstrated that the cp *rpl32* and cp *sod-1* genes are actually located in the chloroplasts, and have discussed the fate of the inherent sequence derived from the cp *sod-1* gene in the cp *rpl32* gene after the acquisition of a sequence that encodes a transit peptide.

2. Materials and methods

2.1. Plant material and growth conditions

P. alba, *Idesia polycarpa*, *Salix gracilistyla*, *O. sativa* (Nipponbare), and *Arabidopsis thaliana* (Columbia) were used as plant material. *A. thaliana* was grown in a growth chamber at 22 °C with a short day photoperiod (10-h/14-h light/dark cycle). *O. sativa* was grown in a growth chamber at 28 °C under continuous dark. *P. alba* was grown in a chamber at 25 °C with a long day photoperiod (16-h/8-h light/dark cycle). *I. polycarpa* and *S. gracilistyla* were grown in the Koishikawa Botanical Gardens (Tokyo, Japan).

2.2. Database analysis

We used the available *Populus* EST database provided by The Institute for Genomic Research (TIGR). The BLAST search was conducted using TIGR Unique Gene Indices (http://tigrblast.tigr.org/tgi/). Subcellular localization of the protein was predicted by TargetP (Emanuelsson et al., 2000) and Predotar (Small et al., 2004) version 0.5 (http://www.inra.fr/predotar/). Each program was conducted using default parameters. ClustalX (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) was used for the multiple alignment of protein sequences.

2.3. DNA cloning of RT-PCR and the genomic PCR products

Total DNA and RNA were isolated from the green leaves of plants using the DNeasy Plant Mini Kit and the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA), respectively. Isolated RNA in *P. alba* was further purified by incubation with RNase-free DNase I (TaKaRa, Kyoto, Japan), according to the manufacturer's instructions. First-strand cDNA synthesis was performed using 1 μg of purified RNA, 0.5 U of SuperScript III reverse transcriptase, and 20 pmol of random hexamer primer (Invitrogen, Carlsbad, CA, USA). The resultant cDNAs were used as templates to amplify the cp *rpl32* and cp *sod-1* cDNA. Primer pairs for the amplification of each gene are as follows. Cp *rpl32* cDNA: primers P1 (5′-GAAATGCAATAGCAGTAGCC-3′) and P2 (5′-TGTAGAATCTGTTGGATAGC-3′). Cp *sod-1* cDNA: primers P3 (5′-GCACATTATCATTTTATCTAAAA-3′) and P4 (5′-CTGAATTTGAACAGCAGCTG-3′). These primers are designed from ESTs in the NCBI database

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