Endosymbiotic Evolution: Transfer of Plastid DNA to the Nucleus and its Stability Following Integration

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Contents

Amendments to Thesis

Page 18, last line should read:

synthesised using random hexamer primers followed by PCR amplification of the *neo* cDNA

Page 19, 3rd paragraph should read:

For technical reasons, the two editing regions were amplified separately from cDNA samples synthesised using different *neo* primers. In contrast to the results obtained using random hexamer cDNA, some editing was observed at all three editing sites, but the majority of transcript remained unedited (Figure 5). The proportion of edited transcript was slightly higher in *tp*neoACG8-2 compared to *tp*neoACG5-3, which may or may not have a biological basis. To confirm that the presence of detectable edited transcript was the result of using a different primer for reverse transcription, rather than different PCR primers, the experiment using random hexamer cDNA was repeated with the same PCR primers as those used for the *neo*-specific cDNA experiment. Again this revealed no detectable editing (Figure 4B).

Chapter 3, Figure 4 legend should read:

Arrows indicate primers that were used for PCR prior to sequencing. In **A**, PCR was performed with primers spanning the entire *neo* gene and in **B**, PCRs were performed separately for the two regions containing editing sites. Primers indicated are (left to right) psbF+Xho1 and neoR2(AL) in **A** and psbF+Xho1, TneoR1, NPTF2 and neoR2(AL) in **B**. See Table 2 for primer sequences.

Abstract

In eukaryotes, cytoplasmic organellar genomes have contributed to a wide variety of nuclear genes. Therefore DNA transfer from plastids and mitochondria to the nucleus has been an important driving force in eukaryotic evolution. Recent advances have enabled this transfer process to be demonstrated experimentally for the plastid in *Nicotiana tabacum*. Investigation of the frequencies, mechanisms and consequences of plastid-to-nucleus DNA transfer will shed further light on the complexities of endosymbiotic evolution.

To determine the frequencies of plastid-to-nucleus DNA transfer in the male and female germlines of *N. tabacum*, reciprocal crosses were performed using a transplastomic line (*tp*7) as either the male or the female parent. In its plastid genome *tp*7 contained a kanamycin resistance gene (*neo*) under the control of a nuclear promoter, so any resistant progeny were expected to be the result of *neo* transfer to the nucleus. In the cross where *tp*7 was the male parent, 13 resistant plants were obtained from a screen of 146,000 progeny indicating a transfer frequency in the male germline of approximately 1 event for every 11,000 pollen grains. Screening of 273,000 progeny from the cross where *tp*7 was the female parent revealed only 1 resistant plant, demonstrating a much lower transfer frequency in the female germline. The programmed degradation of plastids during pollen development may release DNA fragments that integrate into the nucleus, thus explaining the high transfer frequency in the male germline.

To investigate plastid-to-nucleus DNA transfer in somatic cells, a transplastomic line (*tp*GUS) was generated containing *gus* under the control of a nuclear promoter in the plastid genome. Staining of various somatic tissues revealed discrete foci of *gus* expression. The frequency of *gus* transfer to the nucleus was estimated to be 1 event for every 200,000 mature leaf cells. Self-fertilised progeny of *tp*GUS were used to detect stable transfer of *gus* to the nucleus, revealing a similar germline transfer frequency to that obtained with *tp*7.

To determine whether an RNA intermediate is involved in sequence transfer from the plastid to the nucleus, a transplastomic line (*tp*neoACG) was generated containing, in its plastid genome, a nuclear promoter-driven *neo* gene with a start codon that required plastid RNA editing. Screening revealed a number of kanamycin-resistant progeny plants. Surprisingly, *neo* was unedited in these plants, indicating that *neo* was active in the absence of RNA editing. Analysis of *tp*neoACG revealed that only a low proportion of *neo* transcripts were edited, thus precluding unequivocal conclusions regarding the importance of RNA in plastid-to-nucleus transfer.

The stability of newly integrated plastid DNA sequences in the nucleus was analysed using kanamycin-resistant progeny plants of *tp*7. Around half of the plants showed non-Mendelian

segregation, with fewer resistant progeny than expected. Loss of the *neo* gene was shown to be responsible for this instability. Therefore, plastid DNA integration into the nuclear genome and its subsequent deletion both occur, with the latter process varying from very frequent at some nuclear loci to undetectable at others. These may be important evolutionary processes in the generation of novel nuclear genes.

Declaration

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List of Publications

Sheppard A.E., Ayliffe M.A., Blatch L., Day A., Delaney S.K., Khairul-Fahmy N., Li Y., Madesis P., Pryor A.J. & Timmis J.N. (2008) Transfer of Plastid DNA to the Nucleus Is Elevated during Male Gametogenesis in Tobacco. *Plant Physiology* **148**, 328-36.

Sheppard A.E. & Timmis J.N. (2009) Instability of Plastid DNA in the Nuclear Genome. *PLoS Genetics* **5**(1), e1000323. doi:10.1371/journal.pgen.1000323

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