

The origin and characterisation of new nuclear genes originating from a cytoplasmic organellar genome

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Table of Contents

Abstract	vi
Declaration	vii
Acknowledgements	viii
List of abbreviations	ix
Chapter 1: Introduction	1
1.1 Introduction	1
1.2 Origin of the chloroplast	1
1.3 Organelle genome reduction	1
1.3.1 <i>Evolutionary gene transfer to the nucleus</i>	1
1.3.2 <i>Recent gene transfer events</i>	3
1.3.3 <i>Why relocate?</i>	4
1.3.4 <i>Why retain an organellar genome?</i>	5
1.4 Ongoing organelle DNA transfer to the nucleus	6
1.4.1 <i>Organelle sequences in nuclear genomes</i>	6
1.4.2 <i>Experimental transfer to the nucleus</i>	7
1.4.3 <i>Mutational fate of norgs</i>	8
1.5 Mechanisms of gene transfer to the nucleus	9
1.5.1 <i>Escape of genetic material from the organelle</i>	9
1.5.2 <i>Is there an RNA or DNA intermediate?</i>	10
1.5.3 <i>Integration into nuclear chromosomes</i>	11
1.5.4 <i>Proteins involved</i>	12
1.6 Activation of newly transferred organelle genes	13
1.6.1 <i>Examples of organellar gene activation in the nucleus</i>	13
1.6.2 <i>Experimental activation of a chloroplast gene transferred to the nucleus</i>	14
1.7 Summary	14
1.8 Project Aims	15

Chapter 2: Functional transfer of a Chloroplast Transgene to the Nucleus in Tobacco

16

2.1	Introduction	16
2.2	Results	18
2.2.1	<i>Screen for aadA activation</i>	18
2.2.2	<i>Multiple copy insertion of aadA leads immediately to aminoglycoside resistance</i>	18
2.2.3	<i>Frequency of multiple copy aadA insertion</i>	19
2.2.4	<i>Mutational activation of aadA</i>	19
2.2.5	<i>Activation of aadA in sr1 occurred by acquisition of the 35S nuclear promoter</i>	20
2.2.6	<i>Activation of aadA in sr2 occurred by nuclear activation of the native chloroplast promoter</i>	20
2.2.7	<i>Maturation of aadA transcripts</i>	21
2.2.8	<i>Frequency of gene activation</i>	22
2.3	Discussion	22
2.3.1	<i>Multiple copy insertion of aadA leads to nuclear expression</i>	22
2.3.2	<i>Multi-copy insertion must lead to very large chloroplast DNA inserts</i>	24
2.3.3	<i>Secondary rearrangements lead to aadA activation</i>	24
2.3.4	<i>Maturation of aadA transcripts</i>	26
2.4	Conclusion	26
2.5	Materials and methods	27
2.5.1	<i>Plant growth conditions</i>	27
2.5.2	<i>Starting material</i>	27
2.5.3	<i>Selection for spectinomycin resistance</i>	27
2.5.4	<i>Analysis of antibiotic resistance in seedlings</i>	27
2.5.5	<i>Nucleic acid isolation</i>	27
2.5.6	<i>PCR and sequencing</i>	28
2.5.7	<i>RT-PCR</i>	29
2.5.8	<i>Real Time Quantitative PCR</i>	29
2.5.9	<i>TAIL-PCR</i>	29
2.5.10	<i>Genome walking</i>	29
2.5.11	<i>RACE</i>	29
2.5.12	<i>Cell counts</i>	29
2.5.13	<i>Construct design of transient expression vectors</i>	30
2.5.14	<i>Transient Expression Analysis</i>	30
2.5.15	<i>DNA Blot Analysis</i>	31

Chapter 3: Characterisation of a *de novo* nuclear insertion of chloroplast DNA

32

3.1	Introduction	32
3.2	Results	35
3.2.1	<i>Cloning and confirmation of the kr2.2 integrant and pre-insertion site</i>	35
3.2.2	<i>The kr2.2 integrant and pre-insertion site sequence</i>	35
3.3	Discussion	37
3.4	Conclusions	39
3.5	Materials and methods	39
3.5.1	<i>Plant growth</i>	39
3.5.2	<i>DNA extraction</i>	39
3.5.3	<i>Inverse PCR</i>	39
3.5.4	<i>Genome walking</i>	39
3.5.5	<i>PCR</i>	40
3.5.6	<i>Sequencing</i>	40
3.5.7	<i>Sequence analysis</i>	40

Chapter 4: Design and evaluation of an experimental system for the detection of organelle sequence insertion at sites of DNA double strand break repair **41**

4.1	Introduction	41
4.2	Outline of the experimental system	43
4.3	Results	45
4.3.1	<i>Transformation with vectors pdao1 and pAlcR:ISceI</i>	45
4.3.2	<i>Transformation with vector pGU.D.US</i>	45
4.3.3	<i>Evaluation of the use of dao1 as a selectable marker gene in tobacco</i>	46
4.3.4	<i>Evaluation of I-SceI ethanol induction</i>	47
4.3.5	<i>Generation of experimental lines</i>	47
4.3.6	<i>Induction of DSBs and selection for dao1 excision</i>	48
4.4	Discussion	49
4.4.1	<i>Evaluation of dao1 as a selectable marker gene in tobacco</i>	49
4.4.2	<i>Seedling screen for dao1 excision</i>	50
4.5	Conclusion	51
4.6	Methods	51
4.6.1	<i>Plant Growth</i>	51
4.6.2	<i>Nucleic Acid Extraction</i>	51
4.6.3	<i>PCR and Sequencing</i>	52
4.6.4	<i>Construct Design</i>	52

4.6.5	<i>Transformation</i>	53
4.6.6	<i>Analysis of Antibiotic Resistance in Seedlings</i>	53
4.6.7	<i>dao1 seedling selection</i>	53
4.6.8	<i>dao1 tissue culture selection</i>	53
4.6.9	<i>Ethanol induction of I-SceI for RT-PCR</i>	53
4.6.10	<i>RT-PCR</i>	54
4.6.11	<i>Experimental induction of DSBs</i>	54
4.6.12	<i>Seedling screen for dao1 excision</i>	54
Chapter 5: Investigating DSB repair by single molecule PCR		55
<hr/>		
5.1	Introduction	55
5.2	Results	57
5.2.1	<i>PCR detection of DSB repair events</i>	57
5.2.2	<i>Single molecule PCR</i>	57
5.3	Discussion	60
5.3.1	<i>Single molecule PCR</i>	60
5.4	Conclusion	61
5.5	Methods	61
5.5.1	<i>Experimental induction of DSBs</i>	61
5.5.2	<i>DSB PCR</i>	62
5.5.3	<i>smPCR</i>	62
5.5.4	<i>Statistical analysis</i>	62
5.5.5	<i>Sequence analysis</i>	62
Chapter 6: Discussion and Conclusions		63
<hr/>		
6.1	Introduction	63
6.2	Insertion	64
6.3	Activation	65
6.4	The role of double strand break repair	66
6.5	A role for the male germline?	67
6.6	Conclusion	68
Appendix 1		69
Appendix 2		70
Appendix 3		72
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Appendix 4	73
Appendix 5	74
References	75

Abstract

Endosymbiotic transfer of DNA and functional genes from the cytoplasmic organelles (mitochondria and chloroplasts) to the nucleus has been a major factor driving the origin of new nuclear genes, a process central to eukaryote evolution. Recent developments have allowed the experimental reconstruction of DNA transfer and functional gene transfer, enabling investigation of the molecular mechanisms involved in these important evolutionary processes.

To simulate the process of functional endosymbiotic gene transfer, a chloroplast reporter gene *aadA*, which had been transferred from the chloroplast to the nucleus, was monitored for nuclear activation. In total 16 plant lines were screened, each line representing an independent nuclear insertion of the *aadA* gene. For each line ~50 million cells were screened resulting in three plants being recovered in which *aadA* showed strong nuclear activation. Activation occurred by acquisition of the CaMV 35S nuclear promoter or by nuclear activation of the native chloroplast promoter. Two fortuitous sites resident within the 3' UTR of *aadA* mRNA both promoted polyadenylation without any sequence change. In addition, cryptic nuclear activity of the chloroplast promoter was revealed which became conspicuous when present in multiple nuclear copies.

To determine the method of chloroplast DNA insertion into the nucleus the insertion site was sequenced in line kr2.2. Complete characterisation of the nuclear sequence before and after gene transfer demonstrated simultaneous insertion of multiple chloroplast DNA fragments *via* synthesis dependent non-homologous end joining, probably at a site of double strand break (DSB) repair.

To further investigate the role of DSB repair in the nuclear insertion of organelle DNA, DSBs were induced at a specific nuclear location using the rare-cutting endonuclease I-SceI and the resulting repair events were observed. Analysis of ~300 DSB repair events indicated that most involved the loss of nucleotides from one or both ends being joined. Insertions were observed in five repair events. None of the inserted sequences were of organelle origin. Notably, the amount of nuclear sequence deleted was significantly larger in repair events involving insertion than in those without insertion, indicating that the two types of repair may be mediated by distinct pathways.

Declaration

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List of abbreviations

4-MU	4-Methylumbelliferone
A	adenine
ATP	adenosine triphosphate
BAC	bacterial artificial chromosome
bp, kb	base pairs, kilobase pairs
C	cytosine
CaMV	cauliflower mosaic virus
°C	degrees Celsius
cDNA	complementary deoxyribonucleic acid
cv	cultivar
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
DSB	double strand break
EDTA	ethylenediaminetetraacetic acid disodium salt
FISH	fluorescence <i>in situ</i> hybridisation
g	force of gravity
g, mg, µg, ng, pg	gram, milligram, microgram, nanogram, picogram
G	guanine
hr	hour
iPCR	inverse PCR
kr	kanamycin resistant
L, mL, µL	litre, millilitre, microlitre
M, µM	moles per litre, micromoles per litre
min	minute
mRNA	messenger RNA
cm, mm	centimetre, millimetre
MUG	4-methylumbelliferyl-beta-D-glucuronide
NHEJ	non-homologous end joining
<i>numt</i>	nuclear integrant of mitochondrial DNA
<i>nupt</i>	nuclear integrant of plastid DNA
<i>norg</i>	nuclear integrant of organellar DNA
nt	nucleotide
PCR	polymerase chain reaction
pmol	picomole
PVPP	polyvinylpolypyrrolidone
RACE	rapid amplification of cDNA ends
RLM-RACE	RNA ligase-mediated

RNA	ribonucleic acid
RNase A	ribonuclease A
rRNA	ribosomal RNA
RT-PCR	reverse transcription PCR
RT-QPCR	real-time quantitative PCR
sec	second
smPCR	single molecule PCR
sr	spectinomycin/streptomycin resistant
T	thymine
T-DNA	transfer DNA
TAE	Tris-acetate-EDTA
TAIL-PCR	thermal asymmetric interlaced PCR
<i>Taq</i>	<i>Thermus aquaticus</i>
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer RNA
U	uracil
U	Unit(s) of enzyme
UTR	untranscribed region
v/v	volume per volume
w/ v	weight per volume