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Nuclear genome diversity in somatic cells is accelerated by environmental stress

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DNA transfer to the nucleus from prokaryotic ancestors of the cytoplasmic organelles (mitochondria and plastids) has occurred during endosymbiotic evolution in eukaryotes. In most eukaryotes, organelle DNA transfer to nucleus is a continuing process. The frequency of DNA transposition from plastid (chloroplast) to nucleus has been measured in tobacco plants (*Nicotiana tabacum*) experimentally. We have monitored the effects of environmental stress on the rate of DNA transfer from plastid to nucleus by exploiting nucleus-specific reporter genes in two transplastomic tobacco lines. DNA migration from plastids to the nucleus is markedly increased by mild heat stress. In addition, insertions of mitochondrial DNA into induced double-strand breaks are observed after heat treatment. These results show that movement of organelle DNA to the nucleus is remarkably increased by heat stress.

Keywords: environmental stress, DNA transfer, chloroplast, mitochondria, endosymbiotic evolution, cell division

Abbreviations: nupt, nuclear integrant of plastid DNA; numt, nuclear integrant of mitochondrial DNA; norg, nuclear integrant organelle DNA; cp, chloroplast; ptDNA, plastid DNA; DSB, double-strand break; orgDNA, organelle DNA; orgV, organellar value; EGT, endosymbiotic gene transfer; ORF, open reading frame

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Mitochondria and chloroplasts originated through endosymbiosis from α-proteobacteria and cyanobacteria, respectively.[1,2] After the engulfment of the two prokaryotic endosymbionts, there followed large-scale translocation of genes and DNA fragments from the endosymbionts' genome to the nuclear genome of the host cell.[3,4] Nuclear integrants of mitochondrial DNA or plastid DNA are described as *numts*[5] or *nupts*,[6] respectively. Collectively, they are called nuclear organelle DNAs (*norgs*),[7] and *norg* sequences not only create new genes,[8] but also contribute autonomously replicating sequence elements, promoters, introns and novel exons to functional genes in the nucleus.[4,9,10]

Experimental Detection of Plastid DNA Transfer to the Nucleus in Tobacco

To determine the frequency of DNA transfer from chloroplast (plastid) to nucleus in a higher plant, a nucleus-specific neomycin phosphotransferase gene (*neoSTLS2*) was experimentally integrated into the tobacco chloroplast (cp) genome and the progeny of transplastomic plants were screened for kanamycin resistance.[11] In 16 out of approximately 250,000 progeny produced by fertilization of wild-type females with pollen from transplastomic plants containing cp-*neoSTLS2*, the *neoSTLS2* maker had been integrated into a nuclear chromosome, where it conferred kanamycin resistance in a genetic screen. This equated to a chloroplast-to-nucleus DNA transfer frequency of one in 16,000 male gametes tested.[11] In contrast, the frequency of transplastomic *neoSTLS2* transfer in the female nuclear germline of tobacco was remarkably low: only one transposition was identified in a screen of 273,000 transplastomic ovules.[12] The same strategy was also used to detect chloroplast-to-nucleus transposition in somatic cells.[13] A transposition event was estimated to occur in about 5 million somatic cells by this procedure.[13] Therefore, taken at face value, the frequency of plastid-to-nucleus transposition in pollen is much higher than that in ovules or somatic cells. There is ample evidence that both loss of plastids and degradation of plastid DNA (ptDNA) occur during

pollen grain development, particularly in species which show maternal inheritance of plastid genes (such as tobacco).[14] This process may release fragmented organelle DNA to the cytoplasm, enabling it to transfect the nucleus. It is probable therefore, that the programmed degeneration of plastids occurring during pollen development explains the higher rate of plastid to nucleus transfer through the male germline.[6]

Environmental Stress Increases DNA Transfer from Organelle-to-Nucleus in Plants

Previous works measuring DNA transposition from plastid to nucleus were performed under ideal plant growth conditions.[11-13] To understand the impact of environmental factors on the frequency of DNA transfer from plastid-to-nucleus, two transplastomic tobacco lines (one plastome carries the nuclear specific antibiotic resistance gene *neo*,[11] the other has a nuclear specific GUS reporter gene[12]) were used.[15] The transposition of DNA from plastid to nucleus is significantly increased in the leaves of seedlings treated at 45°C. Comparison between untreated and treated plants demonstrated that ptDNA transfer frequency is increased approximately 3.5-fold by 5 h heat stress. To investigate the reason for the large rise in plastid-to-nucleus DNA transfer induced by heat stress, the integrity of cp membrane was examined. Laser scanning confocal microscopy showed that cp membranes of heat-treated leaves were disrupted, implying that abundant cpDNA was released into the cytosol and intercellular spaces. In addition, the presence of a considerable amount of freely available mitochondrial DNA after heat treatment was suggested by investigation of induced double-strand break (DSB) repair. Using a recently-established system that included single molecule PCR,[16] we investigated the repair of DSBs and observed mitochondrial DNA insertions at sites of nuclear DSB repair in heat-treated seedlings but no such integrants were seen in untreated plants. Thus, it appears that a large amount of orgDNA is available for entry to the nucleus after heat stress, resulting in a remarkable increase in the frequency of transfer. Our results in the absence of heat stress are similar to experiments performed in tobacco in the absence of stress[17,18] which also detected no cytoplasmic orgDNA insertions at sites of nuclear DSBs. As the numbers of DSB repair junctions studied were similar in all series, a minimum concentration of orgDNA may be necessary before norgs are sufficiently frequent to be detected in genetic screens of this size. This minimal value of orgDNA concentration is defined as organellar value (*orgV*) here. Increased free orgDNA produced by heat stress satisfies the *orgV* threshold and results in experimentally detectable integration at DSBs.

In the period after the engulfment of the ancestral prokaryotes, the climate of ancient earth was highly variable and included extremes that may explain why the bulk of endosymbiotic gene transfer (EGT) occurred early in eukaryote evolution. It is possible that severe abiotic stress environments created very disruptive environments for the endosymbionts while cell viability was maintained because they were far more tolerable to the archaebacterial ancestor of the nucleated cell. This may have caused disruption of endosymbiont membranes, resulting in an abundant supply of endosymbiont DNA fragments, strongly promoting DNA transfer to the experimental arena of the nucleus. Then followed the generation of novel nuclear genes were as well as replacement genes for those initially located in the endosymbionts.

As an extension of this argument to extant cells: the greater than two orders of magnitude higher migration frequency in the male gametophyte[11,12] compared with somatic cells[13,15] is also explained by differences in the amount of free DNA fragments. Compared with somatic cells, pollen easily approaches *orgV* because of the programmed degradation of plastids associated with uniparental inheritance resulting in the observed higher gene transfer frequency. Similarly de novo nupt formation is almost undetectable in female gametes where greater stability of the plastome is essential for maternal inheritance of plastid genes.

Such high levels of orgDNA insertion into the nuclear genome suggest that eukaryotes must accumulate genome heterogeneity in their somatic cells. It is well recognized that a sequenced reference genome gives only a snapshot and a general picture upon which many individual organisms' variations are superimposed. We can now add the multiple somatic variations that must occur in many organisms because of nuclear invasion of the nuclei by DNA from mitochondria and chloroplasts. Because the rate of DNA transfer from organelle to nucleus can be accelerated by environmental factors,[15] we expect orgDNA to contribute extensive polymorphism to the nuclear genome, for example in different ecotypes, of *Arabidopsis thaliana* derived from populations accessed from different environments.[19,20] In addition, the nuclear influx of organellar DNA is potentially a major source of mutation by insertional inactivation for nuclear genes. The novel "transplanted" prokaryotic-type genes may also acclimatize to their new nuclear environment and become activated. It is known that adaptation of orgDNA sequences after integration to nuclear genome can produce new protein-coding sequences by several pathways: maintaining partial conservation of the original open reading frame (ORF) of the orgDNA; replacement of a former ORF of a protein-coding orgDNA by a novel reading frame; drastic sequence diversification and a high rate of non-synonymous substitutions independent of the coding capacity of the original orgDNA.[10] We conclude that increased frequencies of orgDNA transposition caused by environmental factors will significantly diversify the nuclear genetic system. The potentially large range of stress factors that stimulate organelle DNA transfer to the nucleus remains to be seen. Plants are clearly prime targets for experimentation because of their close dependence on fluctuating soil and ambient climatic conditions, coupled with their inability to move position in order to avoid adverse conditions.

The Role of Cell Division in orgDNA Transfer to Nucleus

Compared with the high frequency of DNA transfer from plastid to nucleus found in leaves of heat-treated seedlings,

no significant increase was observed in cotyledons.[15] It is clear that cell division of tobacco cotyledon cells is finished 6–7 d after sowing. However, the heat treatment was imposed when seedlings were 2 weeks old,[15] which is after the end of cell division in cotyledons.[21] This suggests that there is a mechanistic correlation between orgDNA transfer and nuclear division. Because the nuclear membrane disappears during mitosis, cell division may provide easier access of orgDNA fragments to nuclear chromosomes.

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