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Mini Review

Plant mitochondrial genome: “A sweet and safe home’’ for transgene

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Transfer of transgene through pollens to related plant species is a big environmental concern. Mitochondrion is also a superb and putative aspirant for transgene containment like plastids. Having its own transcription and translation machinery, and maternal inheritance gives assurance of transgene containment with high expression level. Regulation of mtDNA is under nuclear control, mediated by nuclear encoded proteins and protein complexes. These features offer a novel way, to engineer plant mtDNA and reduce the risk of transgene escape.

Key words: mtDNA, genome, transgene, organism.

INTRODUCTION

Plant cells contain three types of genomes, namely: nuclear, mitochondrial and chloroplast. Among these, nuclear genes are biparently inherited and organelle genes are inherited maternally, that is why chloroplast and mitochondrial genomes may provide transgene containment which is a crucial concern in genetically modified crops, particularly in crops having out crossing wild relatives. Mitochondria originated from incorporated K-purple bacteria (Grey et al., 1992) and transferred many of its essential genes to the nucleus, but still carry hallmarks of its bacterial ancestor. Mitochondria use an N-formylmethionyl-tRNA (fMet-tRNA) as initiator of protein synthesis (Galper and Darnell, 1969; Epler et al., 1970). Similarities in different features to bacteriophages, that are acquired by plant mitochondria revealed endosymbiotic relationships. Mitochondrial function is a prerequisite for the survival of higher plants. Proper mitochondrial DNA (mtDNA) maintenance and expressions have profound effects on mitochondrial function. The mtDNA of plants is moderately large at the structural level, and continuously reorganized by recombination between repeated sequences and in most species.

In higher plants, mitochondrial genomes are larger and more complex as compared to other organisms (Palmer and Schields, 1984). Based on their whole genome size, plant mitochondria have less DNA than expected (Satoh et al., 1993), therefore, mitochondrial fusion compensates for this shortage of DNA in higher plants. The mitochondrial genomes consist of multiple molecules with many repeated sequences that are characterized by active homologous recombination (Palmer and Schields, 1984). Within the plant kingdom, mitochondrial genome possesses a highly variable structure and size than most other eukaryotes (Wolstenholme and Fauron, 1995). One significant advance came with the sequencing of the entire mitochondrial genomes of Arabidopsis (Unseld et al., 1997) and liverwort (Oda et al., 1992), these give details clearly that, variation in the size may be brought about by high levels of recombination and extraneous DNA integration and can be accounted for by coding redundancy and changes in genome structure.

Due to changes in mitochondrial genome, induction of cytoplasmic male sterility is the common mark of the presence of second genomes under evolutionary forces, different from the ones acting on nuclear genes (Hanson, 1991). There is a bi-directional flow of information between the nucleus and mitochondrion and that mitochondria exert some control over nuclear genes (Parikh et al., 1987). Retrograde regulation (signaling from the mitochondrion to the nucleus) usually involves metabolites as

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Abbreviations: mtDNA, mitochondrial deoxyribonucleic acid; **RNA,** ribonucleic acid.

signals and is likely associated with multiple signal transduction pathways (Epstein et al., 2001). In plant mitochondria, transcription is mediated by at least one nuclear encoded RNA polymerase, having the RNA polymerases of bacteriophages T7, T3 and SP6 (Hedtke et al., 1997) and this nuclear controlled transcription in plant mitochondria, shows an effective and strong gene regulation (Gray, 1992).

SPECIALIZED GENETIC AND BIOCHEMICAL FEATURES UNIQUE TO PLANT MITOCHONDRIA

Like other eukaryotes, plant functions are also under stringent nuclear supervision. However, several fundamental metabolic and physiological processes are also controlled by plastids and mitochondrial genomes. A little bit genetic information for their functions and biogenesis are encoded by mitochondrial genomes and the remaining is derived a nuclear way. Within the plant kingdom, nuclear–mitochondrial interactions are highly specialized, unusual and exclusive genetic, and biochemical features displayed in plant mitochondria, arose in the milieu of a nuclear–mitochondrial co-evolution. Plant mitochondria have exhibited distinctive strategies for organelle segregation, genome maintenance, decoding, regulation, and with evolved physiological and biochemical functions, to meet the specific demands of photosynthetic organisms “rooted” in place (Mackenzie and Macintosh, 1999).

The makeup of several mitochondrial complexes reveals that, mitochondrial function is mediated by the coordinated expression of genes encoded in the nucleus and mitochondrion, including the translational machinery that involves nuclear-encoded polypeptides and mitochondrially-encoded rRNAs and tRNAs, as well as the large respiratory complexes that have subunits encoded in each of the genomes. In plant mitochondria complex, the pattern of transcripts arising from a given mitochondrial gene-coding region is an inquisitive feature of gene expression, and multiple transcription initiation and termination sites, as well as post-transcriptional cleavage and splicing may yield transcripts with variable sizes (Gray et al., 1992). DNA binding proteins associated with transcription initiation have been characterized (Hatzack et al., 1998).

In plants, to direct proteins from cytosol to the mitochondria, over 80 pre-sequences are required (Whelan and Glaser, 1997) and mitochondrial processing peptidase (MPP) is responsible for removing the pre-sequences upon import via proteolytic cleavage. In mitochondrial genomes of most plant species, recombinationally active repeated sequences exist and they subdivide the genome into a number of different, highly redundant, subgenomic molecules and in direct orientation. Plant mitochondrial genomes replicate by a rolling circle mechanism (Backert et al., 1997) and exist as linear and branched molecules (Oldenburg and Bendich, 1998). Evolution in plant mitochondrial genome is extremely slow and involves nucleotide substitution. That is why; gene sequences in plant mitochondria are outstandingly unvarying and reveal the presence of a strong DNA repair system.

Conversely, plant mitochondrial gene expression is strongly regulated at transcriptional, post transcriptional, translational and post translational levels, and having complex processes of 5 and 3´ RNA trimming and editing by C to U conversions, splicing of introns, polyadenylation and formation of secondary structures for transcript stability (Binder et al., 1996; Gagliardi and Leaver, 1999). For plant mitochondria, different genetic codes were proposed because, at conserved tryptophan positions, the CGG codon was found instead of UGG (Fox and Leaver, 1981). RNA editing was first discovered in plant mitochondria (Covello and Gray, 1989; Heisel et al., 1989) and revealed that translation of plant mitochondrial transcripts follows the standard genetic code.

NUCLEAR–MITOCHONDRIAL COMMUNICATION

Organelles communicate by means of essential polypeptides and this bidirectional information flow is imperative for organogenesis and responses to the environment. For nuclear–mitochondrial interaction, yeast is an excellent model system (Poyton and McEwen, 1996). Inter-organellar DNA exchanges appear to be common and identification of molecules mediating inter-organellar communication is one of the chief emerging areas of mitochondrial research. Different cytological proofs addresses that, physical contacts may exist between organelles, including mitochondrion–endoplasmic reticulum (Staehelin, 1997), mitochondrion–chloroplast (Kohler et al., 1997) and mitochondrion–nucleus (Southworth et al., 1997) interactions which may provide a means for the transfer and exchange of genetic information, to and from the mitochondrial genome (Unseld et al., 1997). It also involves the exchange of membrane components and the inter-organellar signals delivery.

Spontaneous genomic rearrangements *in vitro* (Kanazawa et al., 1994) and *in vivo* (Janska et al., 1998) endorse the ability of these molecules to undergo sudden changes in copy number; suppression of copy number may lead to the silencing of encoded genes. Substoichiometric retention of plant mtDNA molecules is extensively reported (Janska et al., 1998). For horizontal transfer of a single group 1 intron sequence, the genome of plant mitochondria has been targeted (Adams et al., 1998). Such a gene transfer actually occurs via RNA intermediates, seemingly a vestige of earlier endosymbiotic processes.

ENGINEERING OF PLANT MITOCHONDRIA

Attempts have been made to engineer mitochondria, but adapted to a limited number of organisms. Engineering of mitochondria in yeast by transferring both foreign and mitochondrial genes have been successfully done by using a biolistic delivery system (Johnston et al., 1988; Fox et al., 1988). Incorporation of genes in mitochondrial genome of yeast by homologous recombination, using biolistic transformation method has been reported. Sequencing of mitochondrial genomes in most of the plant species has been done. Maternal inheritance, own transcription translation machinery, communication with other organelles and high expression levels, present plant mitochondria as a putative organelle for transformation purposes. Due to these specialized and extraordinary features, plant mitochondria is deserving of the title “A Sweet and Safe Home” for transgene.

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