THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Tissue Specific Expression Studies of Nuclear Maturases in *A. thaliana*

Sunita Malik
Amity Institute of Biotechnology, Amity University, Haryana, India

Abstract:
Post-transcriptional processes play a crucial role in the regulation of gene expression. In general, such regulatory processes are regulated by binding of specific proteins to structures present on some mRNA or pre-mRNAs but not others. Post transcriptional regulatory processes are particularly prevalent in plant mitochondria. The removal of group II introns from plant mtDNA is essential for normal function of mitochondria, and thus respiration. Intron encoded maturases are proteins which facilitate self splicing of group II introns in bacteria and organellar genomes of several lower eukaryotes. Plant organelles also contain group II introns, but have lost maturase ORF. Nuclear maturases (nMATs) are the proteins encoded by nuclear genes of angiosperms. The four nMAT proteins are classified as nMAT1, nMAT2, nMAT3, and nMAT4. nMATs are transported to mitochondria for specific and efficient splicing of group II introns of mitochondria encoded genes. The roles of three of these paralogs in Arabidopsis (nMAT1, nMAT2, and nMAT4) in the splicing of mitochondrial introns have been established. Deficiency of these proteins in nmat mutants causes embryo-defective and developmentally delayed phenotypes. Present study shows that all four NMATs are transcribed in flower, silique, root, cotyledon, hypocotyl, rosette leaf tissue at various degrees for different genes, with highest in flower and silique tissue for all nMATs.

Key words: *Maturases, Group II introns, splicing, gene expression, flower*

1. Introduction

Mitochondria are organelles responsible for aerobic energy production in eukaryotic cells (Knoop, 2012; Michel *et al.*, 1989). Although most of the proteins of mitochondria are coded by nuclear DNA, a number of essential proteins are coded by mitochondrial DNA (mtDNA) that resides within the mitochondria itself (Knoop, 2012). mtDNA encodes essential subunits of inner mitochondrial membrane respiratory chain complexes, the main function of which is to produce energy, in the form of ATP, from the oxidation of glucose and fatty acids through the process of oxidative phosphorylation (Michel *et al*., 1989). According to the traditional endosymbiont theory, present day mitochondria evolved from an aerobic bacterium that invaded a primordial eukaryotic cell and became a symbiotic partner that provided metabolic machinery for the complete oxidation of fermentation end products. Over evolutionary time, genes were transferred from the genome of the aerobic partner into what became the eukaryotic nucleus, leading, eventually, to the reduced genomes found within the mitochondrion in present day eukaryotes.

The mitochondrial genome has several unique features relative to the nuclear genome. The organization of mitochondrial DNA (mtDNA) is different from that of nuclear (nDNA), in some organisms its genetic code is different, and its replication is independent of the cell cycle. Furthermore, there is widespread diversity among modern mitochondrial genomes with respect to both their structure and their organization. Most mtDNAs encode a similar set of proteins which can be categorized into two groups. Information processing genes, such as mitochondrial ribosomal RNA (rRNA) genes and transfer RNA (tRNA) genes encompass the first group. The second assortment consists of protein-coding genes that are components of the mitochondrial respiratory chain. Specifically, genes for complex I (*nad*), complex III (*cob*), complex IV (*cox*) and complex V (*atp*) are typically encoded by mtDNAs (Gray *et al*, 1996).

In contrast to the vertebrate mt-genomes that are compact and small, the mt-genomes of higher plants exhibit extraordinarily large sizes, extending from 222 Kbp of the *Brassica napus* (*B. napus*) mt-genome (Handa 2003) up to and over 2000 Kbp for the mt-genomes of some species within the Cucurbitaceae (Ward *et al*., 1981). Due to large mtDNA sizes, gene density is relatively low in plant mtDNAs. In *Arabidopsis thaliana (A. thaliana)* and *B. napus,* identified genes account for only 10% of the genome. Introns, duplications, integrations of DNA of nuclear and plastid origin and large unidentified open reading frames (ORFs) account for another 30% (Handa 2003). In the dicot sugarbeet, 55.6% of the mt-genome has no clear function (Kubo *et al*., 2000). In the case of two grasses, maize and rice, the scenario repeats itself as non-coding sequences represent 83% and 76% of their mt-genomes, respectively (Clifton *et al*., 2004) . Gene content is also somewhat variable among plant mtDNAs: some individual genes have been transferred to the nucleus in some species whereas in others they remain in the mitochondrial genome. Despite this, a population of genes that encode highly hydrophobic proteins for the complexes of the oxidative respiratory chain and the ATP synthase such as *nad*1, *nad*4, *nad*5, *atp*8, and *atp*6, are consistently maintained within the higher plant's mt-genomes (Handa, 2003).

In addition to, or perhaps as a result of, the complex mt-genomes in plants, regulation of gene expression of this organelle has distinctive features. Plant mitochondrial RNA polymerases and promoters, as well as post-transcriptional and translational

controls, including 5' and 3' stability, *cis*- and *trans*-splicing, and RNA editing (Hofmann *et al*., 2001; Binder *et al*., 1996) are also important in plant mitochondria. Introns of organellar genomes, including those of plant mitochondria, can be divided into two main classes: group I and groupII that differ by a number of criteria, such as differences in their predicted secondary structures and the mechanism by which some introns of both groups can undergo self-splicing *in vitro (*Bonen, 2008; Bonen & Vogel, 2001; Lambowitz & Zimmerly, 2004; Michel, 1989; Zimmerly *et al*., 2001). In land plant mitochondria, protein-coding genes frequently contain group I and group II introns (Lehmann & Schmidt, 2003). In *Marchantia* mtDNA, 32 introns, including both group I and group II introns, are present (Oda *et al*., 1992), whereas most of the 25 introns identified to date in flowering plant mitochondria (Kubo *et al*., 2000; Handa, 2003) are group II introns.

Group II introns are large, natural ribozymes found in prokaryotes and eukaryotic organelles (mitochondria, chloroplast); they are identified by characteristic primary, secondary and tertiary RNA structural elements (Michel & Ferat, 1995). *In vitro*, some group II introns can splice autocatalytically without requiring any additional protein but *in vivo* all are thought to require accessory proteins for efficient splicing. The best known of these accessory proteins are the maturases, which in most cases, are encoded within the intron itself. Group II introns are further divided into groups IIA and IIB according to differences in the structure and the mechanism of splicing (Lehmann & Schmidt, 2003). Most group II intron maturases are encoded by the intron themselves, but some are encoded by other genes of the host organism (in the case of bacteria and higher plant mitochondria) or organelle (chloroplasts, plant and fungal mitochondria). Most maturases contain conserved domains involved in reverse transcription of the intron (RT), intron-RNA binding (X), DNA binding (D) and DNA endonuclease activity (En). Among these, the RT, D, and En domains are involved in intron mobility and the RT and X domains are involved in splicing (Carignani *et al*., 1983; Matsuura *et al*., 1997). Generally, maturases mediate the splicing and retrotransposition of only the introns that encode them, or of closely related introns.

Lately, four maturase-like proteins were identified in the *A. thaliana* nuclear genome (Mohr & Lambowitz, 2003). The proteins encoded by these genes are termed nuclear maturases (nMAT) and were predicted to splice group II introns in mitochondria. The four *nMat* genes are classified as *nMat*1 (At1g30010), *nMat*2 (At5g46920), *nMat*3 (At5g04050), and *nMat*4 (At1g74350) (Mohr & Lambowitz, 2003; Keren *et al*., 2009; Keren *et al*., 2012). These proteins are transported to mitochondria for splicing group II introns present in mtDNA. In Arabidopsis, maturases seem particularly important for the maturation of primary *nad* transcripts. Homozygote *nmat* mutants show altered growth and developmental phenotypes, modified respiration and altered stress responses, which are tightly correlated with mitochondrial complex I defects (Nakagawa & Sakurai, 2006; Keren *et al*., 2009). While nMAT1 is required in trans-splicing of *nad*1 intron 1, *nad*2 intron 1 and *nad*4 intron 2 (Keren *et al*., 2009), nMAT2 functions in the efficient splicing of *nad*1 intron 2, *nad*7 intron 1 and the single intron in the cytochrome oxidase subunit 2 gene (*cox*2 intron 1) (Keren *et al*., 2009), nMAT4 functions in efficient processing of *nad*1 intron 1, 3 and 4 (Cohen *et al*., 2014). The accurate biochemical functions of nuclear-encoded maturases in the splicing process have not yet been established. Present study focuses on study of difference in level of expression of nMATs in different tissues of Arabidopsis.

2. Material and Methods

Seeds for Wild type (WT) *A. thaliana* (Col-0) were placed on 1% AT sucrose plates (Haughn *et al*., 1986) and plates were kept at a temperature of 4°C for 4 days. Plates were then transferred to the growth chamber for 14 days. The seedlings obtained were transferred to 5 inch pots with Sunshine Mix #5 (Sun Gro Horticulture Inc.) and 20:20:20 fertilizer (Plant-Prod) was diluted 80:1000 with distilled water and 50 ml was sprinkled evenly on the top of the soil in each pot. The pots were then covered with plastic wrap for three days. Following this the plants were maintained for one month with 24 hour light photoperiod.

To obtain tissue for roots, cotyledons and hypocotyls, seeds (Col-0) were grown in liquid culture as follows: 4.4 g MS salts, 0.5 g MES, 10 g sucrose were added to 1 Liter (L) water and the pH was set to 5.7 with 1M KOH (about 1 ml). This solution was divided into 250 ml aliquots in 1 L flasks and autoclaved 15 minutes. 1 ml Gamborg's B-5 vitamin mix from 1000 times stock was added to the cooled autoclaved media. About 200 seeds were sterilized (Axelos *et al*., 1992) and added to the flasks. The flasks were shaken on orbital shaker at 80-100 rpm for two weeks with continuous light. The seedlings were blot dried, divided into roots, hypocotyls, and cotyledons and immediately frozen in liquid nitrogen.

RNA isolation for leaf, flower, root, silique (10 days after flowering), cotyledon and hypocotyl was done using the Qiagen RNeasy mini kit (QIAGEN Sciences). Isolated RNA was treated with DNaseI (Invitrogen-amplification grade, 100 units-1U/µL). PCR amplification without added reverse transcriptase was performed on DNaseI-treated RNA samples with actin gene primers to confirm the absence of DNA.

RT-PCR was performed using the Qiagen Omniscript RT kit. 1µg total RNA from the respective plant tissue was reverse transcribed for 1 hour at 42°C to obtain complementary DNA (cDNA). PCR was performed on 1 µl cDNA with initial denaturation at 94°C for 4 minutes, denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 45 seconds and final elongation at 72°C for 10 minutes, as specified in the kit. Different numbers of PCR cycles were used to determine expression in different tissues. The number of cycles that best displayed expression was 32 for *nMat1, nMat2, nMat3 and 30 for nMat4.* 10 µl of PCR products were analyzed on 1% agarose gels loaded. The primers used to analyze tissue specific expression of the four *nMats* in 5` to 3` direction were cattagagggcctagagaggatgcag and gggcaaggttagaccctcgcg for *nMat1;* gttccacgcaactcaagctcatacg and catgcgtgcaatgcgaagcccatc for *nMat2;* gggagaggaatcgtaagacactggggc and ggtgcccatgccaggaaccc for *nMat3;* gaatatggatggaggacgtgctaagaacc and acacagccctattctccttccatttaagc for *nMat4.*

3. Results and Discussion

nMat expression in various tissues was characterized in WT *A. thaliana*. Transcripts of all the *nMAT* genes were detected in all the tissues analyzed (leaf, flower, silique, root, hypocotyl, and cotyledon). The *nMat1* and *nMat2* genes were highly expressed in all the tissues. In nMAT1, the *nMat3* and *nMat4* genes were also expressed moderately in all the tissues (Figure 1).

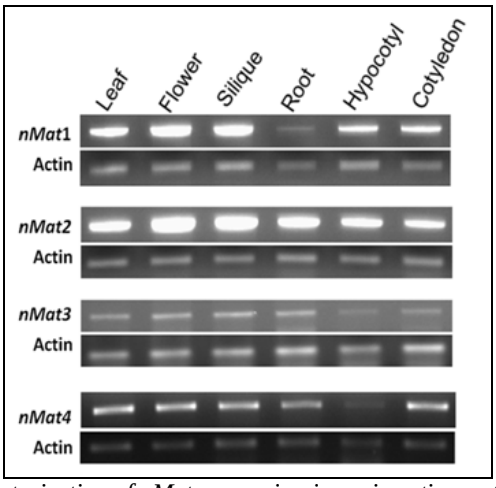

Figure 1: Characterization of nMat expression in various tissues through RT-PCR

Till date, several developmentally delayed phenotypes have been observed for different *nmats (*Keren *et al*., 2009; Keren *et al*., 2012; Nakagawa & Sakurai, 2006). Genetic studies indicate that nMATs are involved in the splicing of mitochondrial introns in angiosperms (Keren *et al*., 2009; Keren *et al*., 2012; Nakagawa & Sakurai, 2006). *nMat* gene expression for all four nMATs was conducted to determine if *nMat* expression was tissue or organ specific. The results show that all the tissues expressed all the *nMats* comparably. The expression of the *nMat*s in all tissues analyzed is consistent with their role in seed germination, and growth and development (Keren *et al*., 2009; Keren *et al*., 2012; Nakagawa & Sakurai, 2006).

4. References

1. Axelos M, Curie C, Mazzolini L, Bardet C, Lescure B, (1992). A protocol for transient expression in Arabidopsis thaliana protoplasts isolated from cell suspension cultures, Plant Physiology and Biochemistry, 30 (1), 123-128.
2. Binder S, Marchfelder A & Brennicke A, (1996). Regulation of gene expression in plant mitochondria, Plant Molecular Biology, 32, 303-314.
3. Bonen, (2008). L, Cis- and trans-splicing of group II introns in plant mitochondria. Mitochondrion, 8, 26-34.
4. Bonen L & Vogel J, (2001). The ins and outs of group II introns, Trends in Genetics, 17, 322-331.
5. Carignani G, Groudinsky O, Frezza D, Schiavon E, Bergantino E, Slonimski P P, (1983). An mRNA Maturase is encoded by the first intron of the mitochondrial gene for the subunit I of cytochrome oxidase in S.servisiae, Cell, 35, 733-742.
6. Clifton S W, Minx P, Fauron C M R, Gibson M, Allen O J et al, (2004). Sequence and comparative analysis of the Maize NB mitochondrial genome, Plant Physiology, 136, 3486-3503.
7. Cohen S, Zmudjak M, Small C C D F, Malik S, Shaya F et al, (2014). nMAT4, a maturase factor required for nad1 pre-mRNA processing and maturation, is essential for holocomplex I biogenesis in Arabidopsis mitochondria, The Plant Journal (Accepted).
8. Gray M W, Spencer D F, (1996). Organellar evolution in evolution of microbial life, Cambridge University Press, 109-126.
9. Handa H, (2003). The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (Brassica napus L.): comparative analysis of the mitochondrial genomes of rapeseed and A. thaliana, Nucleic Acids Research, 31, 5907-5916.
10. Haughn G W, Somerville C R, Sulfonylurea-resistant mutants of Arabidopsis thaliana, Mol and Genetics and Genomics, 204 (1986) 430-434.
11. Hofmann M, Kuhn J, Daschner K, Binder S, (2001). The RNA world of plant mitochondria, Progress in Nucleic Acids Research and Molecular Biology, 70, 119-154.
12. Keren I, Bezawork-Geleta A, Kolton M, Maayan I, Belausov E et al, (2009). AtnMat2, a nuclear-encoded maturase required for splicing of group-II introns in Arabidopsis mitochondria, RNA, 5, 2299-2311.
13. Keren I, Tal L, des Francs-Small C C, Araújo W L, Shevtsov S et al, (2012). nMAT1, a Nuclear-encoded Maturase involved in the trans-splicing of nad1 intron 1, is essential for mitochondrial complex I assembly and function, The Plant Journal, 71, 413-426.
14. Knoop V, (2012). Seed Plant Mitochondrial Genomes: Complexity Evolving in Genomics of Chloroplasts and Mitochondria (Springer, eds. Bock & Knoop), 175-195.
15. Kubo T, Nishizawa S, Sugawara A, Itchoda N, Estiati A et al, (2000). The complete nucleotide sequence of the mitochondrial genome of sugar beet (Beta vulgaris L.) reveals a novel gene for tRNACys (GCA), Nucleic Acids Research, 28, 2571-2576.
16. Lambowitz A M & Zimmerly S, (2004). Mobile group II introns, Annual Reviews of Genetics, 38, 1-35.
17. Lehmann K & Schmidt U, (2003). Group II Introns: Structure and catalytic versatility of large natural ribozymes, Critical Reviews Biochemistry and Molecular Biology, 38, 249-303.

18. Matsuura M, Saldanha R, Wank H, Yang J, Mohr G et al, (1997). A bacterial group II intron encoding reverse transcriptase, maturase, and DNA endonuclease activities: biochemical demonstration of maturase activity and insertion of new genetic information within the intron, Genes and Development, 11, 2910-2924.
19. Michel F & Ferat J L, (1995). Structure and activities of group II introns, Annual Reviews of Biochemistry, 64 435-461.
20. Michel F, Umesono K & Ozeki H, (1989). Comparative and functional anatomy of group II catalytic introns - a review, Gene, 82, 5-30.
21. Mohr G & Lambowitz A, (2003). Putative proteins related to group II intron reverse transcriptase/maturases are encoded by nuclear genes in higher plants, Nucleic Acids Research, 31, 647-652.
22. Moore M J, (2005). From Birth to death: The Complex Lives of Eukaryotic mRNAs, Science, 309, 1514-1518.
23. Nakagawa N & Sakurai N, (2006). A Mutation in At-nMat1a, which encodes a nuclear gene having high similarity to group II intron maturase, causes impaired splicing of mitochondrial nad4 transcript and altered carbon metabolism in A. thaliana, Plant Cell Physiology, 47, 772-783.
24. Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M et al, (1992). Gene organization deduced from the complete sequence of liverwort Marchantia polymorpha mitochondrial DNA, A primitive form of plant mitochondrial genome, Journal of Molecular Biology, 223, 1-7.
25. Ward B L, Anderson R S, Bendich A J, (1981). The mitochondrial genome is large and variable in a family of plants (cucurbitaceae), Cell, 25, 793-803.
26. Zimmerly S, Hausner G, Wu X, (2001). Phylogenetic relationships among group II intron ORFs, Nucleic Acids Research, 29, 1238-1250