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Review

Organellar maturases: A window into the evolution of the spliceosome☆

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ABSTRACT

During the evolution of eukaryotic genomes, many genes have been interrupted by intervening sequences (introns) that must be removed post-transcriptionally from RNA precursors to form mRNAs ready for translation. The origin of nuclear introns is still under debate, but one hypothesis is that the spliceosome and the intron–exon structure of genes have evolved from bacterial-type group II introns that invaded the eukaryotic genomes. The group II introns were most likely introduced into the eukaryotic genome from an α-proteobacterial predecessor of mitochondria early during the endosymbiosis event. These self-splicing and mobile introns spread through the eukaryotic genome and later degenerated. Pieces of introns became part of the general splicing machinery we know today as the spliceosome. In addition, group II introns likely brought intron maturases with them to the nucleus. Maturases are found in most bacterial introns, where they act as highly specific splicing factors for group II introns. In the spliceosome, the core protein Prp8 shows homology to group II intron-encoded maturases. While maturases are entirely intron specific, their descendant of the spliceosomal machinery, the Prp8 protein, is an extremely versatile splicing factor with multiple interacting proteins and RNAs. How could such a general player in spliceosomal splicing evolve from the monospecific bacterial maturases? Analysis of the organellar splicing machinery in plants may give clues on the evolution of nuclear splicing.
Plants encode various proteins which are closely related to bacterial maturases. The organellar genomes contain one maturase each, named MatK in chloroplasts and MatR in mitochondria. In addition, several maturase genes have been found in the nucleus as well, which are acting on mitochondrial pre-RNAs. All plant maturases show sequence deviation from their progenitor bacterial maturases, and interestingly are all acting on multiple organellar group II intron targets. Moreover, they seem to function in the splicing of group II introns together with a number of additional nuclear-encoded splicing factors, possibly acting as an organellar protospliceosome. Together, this makes them interesting models for the early evolution of nuclear spliceosomal splicing. In this review, we summarize recent advances in our understanding of the role of plant maturases and their accessory factors in plants. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. A short introduction to group II introns

Nuclear genomes of eukaryotes are replete with introns. Their importance for gene expression is obvious, but their evolutionary origin was and still is a contested field of research. A widely accepted scenario posits that introns invaded the eukaryotic genome early, likely upon emergence of the eukaryotic cell itself [65]. Key players in this invasion were group II introns, which have been suggested to have entered the nucleus from mitochondria: the "mitochondrial seed" hypothesis (Fig. 1; [76]). According to this hypothesis, the α-proteobacterial endosymbiont that evolved into present-day mitochondria brought group II introns into the eukaryotic host cell. These were transferred via DNA-intermediates into the nuclear genome and spread as mobile genetic elements throughout nuclear chromosomes. Later, group II introns evolved into spliceosomal introns as we know them today. We do not wish to gloss over the fact that this hypothesis has been criticized, not the least since it is difficult to test experimentally, and that there are alternative explanations for the origin of spliceosomal introns [18]. However, before we consider evidence in favor of the "mitochondrial seed" hypothesis and group II introns as ancestors of spliceosomal introns, we briefly introduce a few salient features of group II introns.

Almost 30 years ago group II introns were demonstrated to be autocatalytic RNAs, *i.e.* that they could splice in the absence of any protein cofactors [96,131]. The secondary structure of typical group II introns is characterized by six double-helical domains (DI-DVI) arising from a central hub (Fig. 2A; [84]). These domains form a complex, globular structure that was recently solved by X-ray crystallography [105,128]. Most important domains for catalysis are V and VI, which are at the catalytic core of group II introns and co-ordinate bivalent metal ions necessary for splicing chemistry [128]. Under physiological conditions, the

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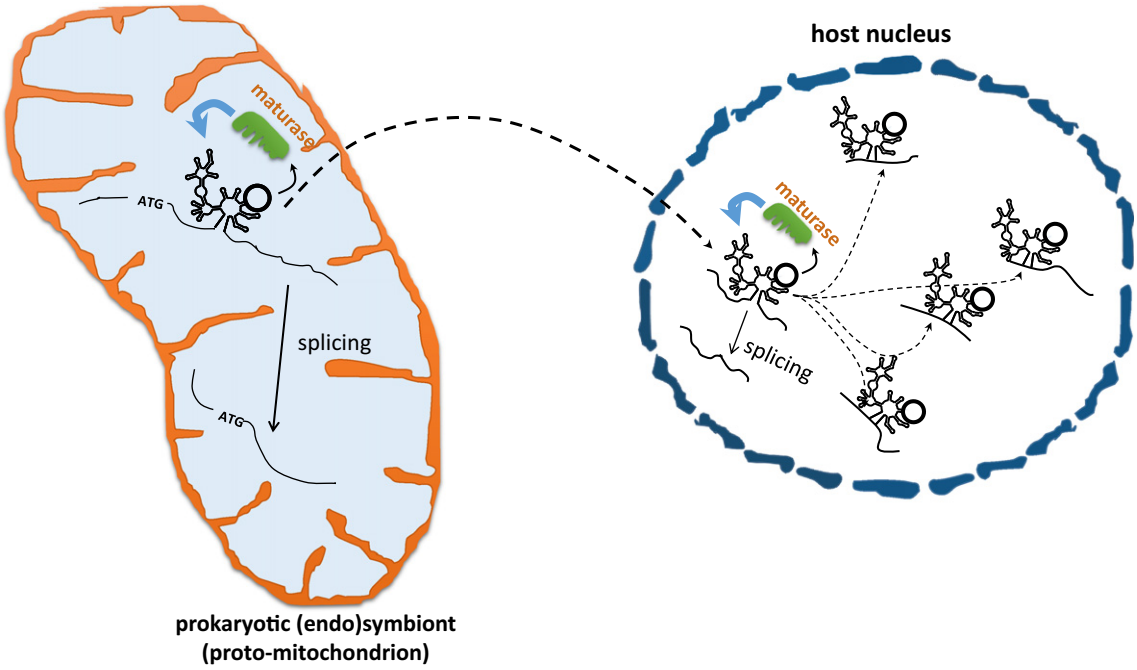

Fig. 1. Maturases and the mitochondrial seed hypothesis. A prokaryotic endosymbiont, progenitor of present-day mitochondria (orange), contained group II introns within some of its mRNAs (solid lines with ATG start codon). The intron codes for a maturase (green) that is required for splicing of its home intron (light blue arrow). DNA fragments containing a group II intron were transferred to the host nucleus (blue). If expressed, the intron together with its cognate maturase could spread through the host genome (dashed arrows).

splicing process is aided *in vivo* by a multidomain intron-encoded protein, termed a maturase. The maturase facilitates splicing, usually with high specificity towards the intron in which it is encoded. The maturase also has reverse transcriptase activity, which is required in an RNA-mediated mobility process called 'retrohoming'. Retrohoming is a reverse splicing of the excised intron RNA into a DNA site [71]. Usually, these sites represent intronless alleles of the same gene the intron resides in. Remarkably, some group II introns are also capable of colonizing ectopic sites, which essentially makes them mobile genetic elements [71]. Group II introns are found in all three domains of life and are, in particular, prevalent in organelles of land plants [71]. Their role as ancestors of spliceosomal introns is widely accepted.

2. Group II introns as ancestors of spliceosomal introns

The mobility of group II introns is one asset that makes them appealing candidates as progenitors of spliceosomal introns. In prokaryotes, group II's are found in many bacteria and some archaea [14] but are often found outside genes or near *rho* elements [30]. Importantly, group II introns are not only mobile within a given organism, but they have been shown to be capable of transgressing boundaries between species and even boundaries between kingdoms. For example, sequencing analyses indicate that group II introns moved from bacteria into the archaea *Methanosarcina acetivorans* and *Methanosarcina mazei* [103] and a mitochondrial group II intron was transferred into the nucleus in the monocot *Washingtonia robusta* and morphed into a spliceosomal intron (this counts only as a trans-kingdom movement if we consider mitochondria as part of the eubacterial division [69]). The latter event is obviously significant for the mitochondrial seed hypothesis. Transfer of mitochondrial DNA into the nucleus has indeed been reported in many species, including humans [104], yeast [124] and tobacco [136]. Similarly, transfer of plastid DNA into the nucleus has been demonstrated as well, and proceeds at extraordinarily high frequencies in both the germline [55] as well as somatically [121]. Endosymbiotic gene transfer into the host genome has been and still is a normality, leading to multiple chromosomal integrants of organellar genomes into nuclear chromosomes [127]. Genes are transferred as functional units and it can be speculated that group II introns could remain intact after transfer as well, if their maturases are expressed or on their own if they are capable of autocatalytic splicing. Indeed, the bacterial group II intron LtrB introduced in parallel with its cognate maturase LtrA into the yeast nucleus was spliced efficiently both after export of the pre-mRNA to the cytosol [20] or when forced to remain within the nucleus [98]. In sum, the transfer event itself as proposed by the mitochondrial seed hypothesis is a likely event. Moreover, group II introns together with their maturase can be active and functional in a eukaryotic genome.

Further support for the hypothesis that group II introns were the progenitors of spliceosomal introns comes from analyses of their splicing chemistry. The splicing of group II introns is mediated via two transesterification reactions and the excision of the intron as a lariat RNA [148]. In fact, it was noted early on after the discovery of autocatalytic introns that these processes are identical to the splicing of spliceosomal introns in eukaryotic pre-RNAs [19,112]. This similarity prompted more detailed mechanistic analyses that confirmed the indistinguishable catalytic mechanism between the two intron types. Both splicing mechanisms use an adenosine residue as a 2′ hydroxyl nucleophile in a single-nucleotide-bulge from a helical region for the first chemical step of the splicing reaction, leading to the release of the 5′-exon. In the second step, the 3′ OH of the cleaved 5′ exon is the nucleophile and attacks the 3′-splice site, resulting in exon ligation and the excision of the intron RNA as a lariat [94,109]. Reactants and products of each reaction step are the same as is the stereochemical course of the reactions [87,93,120]. Importantly, even the divalent metal ion interactions with selected oxygens in the phosphodiester bonds are identical.

Given the chemical and structural similarities between these two ribonucleoprotein machineries, it was argued that group II intron elements may be able to complement spliceosomal RNAs and that components of the spliceosome, in particular the snRNAs, may be able to complement group II intron mutants. Indeed, a conserved region of the spliceosomal U5 snRNA was used to successfully replace an exon binding site within a group II intron *in vitro* [51]. Similarly, DV in group II introns is functionally equivalent to an U6 snRNA stem-loop in the spliceosome [1,56,64,77,95,115,142]. Both DV and U6 stem-

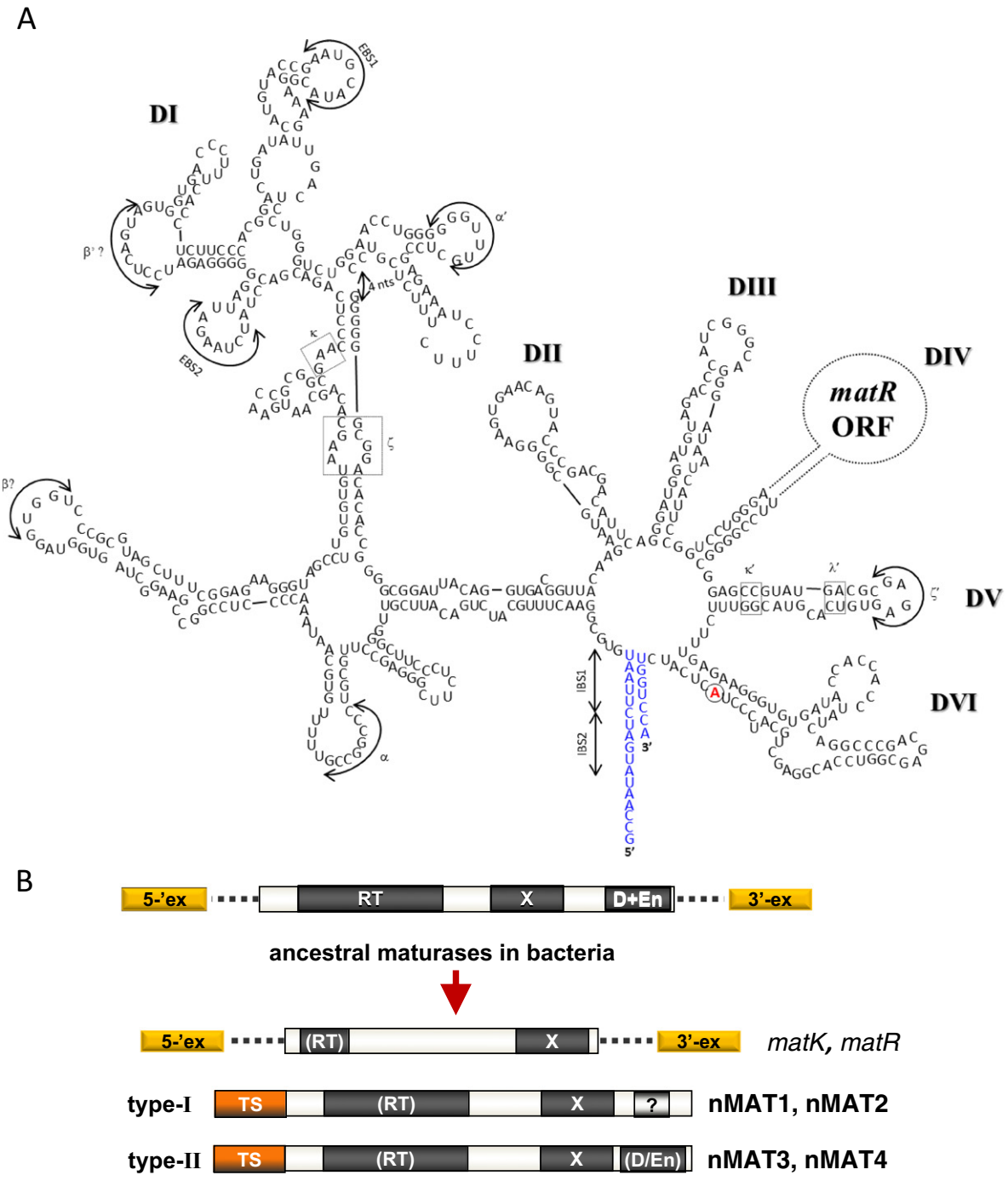

Fig. 2. Group II intron secondary structure and schematic representation of maturase protein structures. (A) As an example for the secondary structure of a plant mitochondrial group II intron, the *Arabidopsis thaliana nad1* intron 4 is illustrated schematically within this figure. Each subdomain of DI and DII, DIII, DIV, DV and DVI is outlined within the structure. The conserved bulged-A residue in DVI, the exon–intron binding sites (*i.e.* EBS1/IBS1 and EBS2/IBS2), and tertiary interactions between different intron regions (indicated by roman letters) are shown in the model structure. The ORF encoding the *MatR* protein in *nad1* intron 4 is encoded in intron domain IV. The structure of *nad1* intron 4 was modeled based on the proposed conserved structural features of different group II intron types in bacteria, yeast and plant organelles [16]. Like other mitochondrial introns in angiosperms, *nad1* intron 4 belongs to the subgroup IIA [13]. The putative secondary structure of the introns was generated by the prediction algorithms MFOLD [146] and alifold [54], based on the known *nad1* intron 4 sequences found in various plant species for which complete mitochondria sequences are available (NCBI Organelle Genome Resources). (B) Plant genomes encode six proteins that are closely related to group II intron maturases. These include *matK* in *trnK* in the plastids, the mitochondrial *matR* ORF encoded in *nad1* intron 4, and four nuclear encoded maturases (nMAT 1-to-4). The typical reverse-transcriptase (RT), RNA binding and splicing domain (X) and the DNA binding/endonuclease domain (D/En) are illustrated schematically in each protein. The mitochondrial targeting signals (TSs) are shown in the nuclear encoded proteins.

loops co-ordinate catalytically important metal ion interactions in the active site of the spliceosome [40,64,116,141]. The recent progress in structural analyses of group II introns has now established the functional equivalence of D5 and U6 as well as that of other conserved sequence elements [105,128].

In sum, the striking similarities between the two intron systems is consistent with the hypothesis that an ancestral group II intron invaded the nuclear genome and broke up into small RNAs that evolved to act *in trans* on other introns to facilitate their splicing. These sRNAs were presumably the progenitors of present-day snRNAs such as U6.

[35,83,106,112,117,119]. The role of the maturase in this evolutionary process has rarely been addressed. We shall come back to this point after an introduction to bacterial and organellar maturases.

3. The ancestors of plant intron maturases: bacterial maturases

In eubacteria and archaea, many of the group II sequences encode maturases in domain IV [149], whereas in higher plant organelles only one plastidial intron (*trnK*) and a single mitochondrial intron (the forth intron in *nad1*) have preserved ORFs similar to prokaryotic-type maturases. The canonical maturase proteins, like the well-characterized LtrA protein encoded by the *Lactococcus lactis* Ll.LtrB intron, have three distinct domains (Fig. 2B): a reverse transcriptase (RT), an RNA binding and splicing domain (X), and a DNA binding/endonuclease domain (D/En). The RT and D/En motifs are required for intron mobility [70,71], while domain X was mainly associated with intron recognition and splicing activities [86,88]. Together with the reverse transcriptase domain, domain X confers specificity to RNA recognition and promotes formation of the active ribozyme structure, either by stabilizing or nucleating secondary and long-range inter-domain RNA tertiary contacts [27,107,137]. Loss of the reverse transcriptase or endonuclease sequences seem common in bacteria, which probably affects retrohoming but leaves splicing capability untouched [72]. Mutagenesis screens and footprinting analyses of LtrA have identified regions required for splicing both in the maturase as well as in the *LtrB* target intron. These data suggest that an extensive protein surface comprising the reverse transcriptase, domain X, and maybe additional areas of the protein make contacts with a stem-loop in intron domain IV, as well as with regions in domains I, II and VI [12,27,29,43,79,137]. Further biochemical data support a model in which LtrA binds the intron as a dimer [100,107]. Together, current models suggest that LtrA's role in promoting splicing depends on its ability to stabilize both short- and long-distance interactions between various intron regions and within intron domains, in particular in the large domain I of the intron [72]. Although very limited experimental data exist, similar scenarios are expected to apply to other group II intron-encoded maturases as well.

4. Land plant organellar maturases: the last survivors of the bacterial RNA processing machinery in organellar genomes

Plant organellar RNA metabolism is characterized by a multitude of independent RNA processing events. Plant organellar RNAs are trimmed and cut by a variety of nucleases and are at the same time stabilized against decay by a host of protective RNA binding proteins. In addition, many RNAs are spliced and individual nucleotides are edited [9,48]. This complexity is rather surprising given that both chloroplasts and mitochondria are derived from bacterial ancestors (cyanobacteria and α-proteobacteria, respectively) via endosymbiosis, and prokaryotes are known for their rather simple RNA metabolism. Many components of RNA metabolism found in bacteria persisted in extant plant organelles, such as exoribonucleolytic PNPase [122], endoribonucleolytic RNase E [135], 70S ribosomes and, important for the discussion here, group II introns and corresponding intron maturases. Of course, there is a variety of add-ons that were acquired during chloroplast and mitochondria evolution, including additional subunits of ribosomes [126], novel RNA polymerases [139], and a collection of RNA binding proteins with diverse functions [144]. A hallmark of these novel factors, as well as of the prokaryotic contributions to plant organellar RNA metabolism, is that the underlying genes are found in the nucleus. In fact, all factors required for organellar RNA processing in land plants are nuclear-encoded, with one notable exception: both mitochondrion and chloroplast genomes harbor a single gene each for an intron maturase. In chloroplasts, this maturase is called MatK, since the gene is positioned within an intron of the *trnK*-UUU gene. In mitochondria, the maturase gene is found within intron 4 of the *nad1* gene and is called *matR* (or mat-r; for maturase-related protein; [134]). Below, we summarize the current state of knowledge for these two plant organellar maturases.

5. Chloroplast maturase MatK

5.1. Phylogeny of MatK

The chloroplast maturase MatK is one of the fastest evolving plastid genes and has therefore been used widely for phylogenetic studies at the species-level [11]. Despite this apparent evolutionary volatility, the gene has been surprisingly persistent in plants, where it is found in all autotrophic land plant chloroplast genomes that contain group II introns. The gene can be traced back to the origin of land plants within the basal streptophyte algae. Here, it is found already within the *trnK* intron, suggesting that MatK, together with its intron, invaded plants early in the streptophyte lineage [108,130]. Interestingly, phylogenetic analyses indicated that MatK clusters with maturases found in the mitochondria and nuclear genomes of different plants, suggesting the possible transfer of a mitochondrial intron to the chloroplast in an ancestor of modern land plants and charophyte algae [15,150]. However, as no indications exist for a gene transfer from mitochondria to chloroplasts, and since the branch support for the position of the *matK* clade was considered weak [45], such observations should be interpreted with care.

5.2. Evidence for MatK as a general chloroplast splicing factor

In bacteria, most group II maturases are highly specific for splicing only of their host intron RNAs. But there are exceptions that can serve as models for how maturase-based intron-specific splicing may have traversed the boundary towards splicing of multiple introns by one machinery, *i.e.* spliceosomal intron splicing. In a few cases, proliferation of an intron in bacteria led to a number of closely related introns that are now served by only a single maturase [28,82]. More striking, recent data indicate that MatK has expanded its target range to more distantly related introns. Evidence for the function of MatK as a general splicing factor is still only indirect and is summarized below.

5.2.1. matK is an essential gene

The MatK protein was identified early on as a homologue of prokaryotic intron maturases [90]. A role in splicing is therefore logical. Still, up to now there is no direct genetic evidence for a role of MatK in splicing activity of plastidial introns. Most mutational approaches failed so far. None of the attempts to use chloroplast transformation to disrupt the reading frame [34] or to introduce missense mutations [145] were successful. These attempts had led to heteroplastomic plants, *i.e.* a state in which the introduced mutant chromosome and the wild-type chromosome exist side-by-side in the highly polyploidy plastid genome. Complete removal of wild-type chromosomes was not achieved so far, suggesting that the gene is essential for chloroplast and plant cell survival [34,145]. Recently, a *matK* frame-shift mutation was reported in the cypress *Cryptomeria japonica,* but whether introns remain unspliced in this material could not be ascertained [52]. Thus, while there have been no genetic inroads into MatK function so far, biochemical and phylogenetic evidence strongly supports a direct role in splicing.

5.2.2. Loss of plastid translation entails loss of a subset of splicing events likely based on MatK activity

MatK is one of the most degenerated maturases found to date and has lost the protein domains required for intron mobility [10,86], but has retained a recognizable splicing domain X (Fig. 2B; [71]). Despite its rapid evolution, which in fact prompted speculation that MatK is on the way to becoming a pseudogene, its expression has been validated in various land plants on the RNA and protein levels [10]. In mutants of the plastid translational apparatus, which no longer can express MatK,

the *trnK* precursor RNA is not spliced [133]. In addition, an entire subgroup of chloroplast introns, the group IIA introns, failed to splice in such translation-incompetent tissue as well [50,132]. The only conceivable factor that would require functioning chloroplast translation for splicing is MatK, which led to the proposition that MatK serves splicing of all group IIA introns [132].

5.2.3. Phylogenetic evidence for MatK as a general splicing factor

In the streptophyte algae *Zygnema*, in the fern *Adiantum capillus-veneris* and also in the parasitic land plants *Epifagus virginiana*, *Cuscuta exaltata*, and *Cuscuta reflexa, matK* is present as a stand-alone reading frame whereas the *trnK* gene has been lost [38,80,129,140]. This suggests a function of *matK in trans*, most likely for splicing of other introns in the plastid genome. This hypothesis is further supported by the few species that have completely lost *matK*. Among all embryophytes analyzed, only species from the parasitic angiosperm subgenus *Cuscuta* grammica and from the orchid *Rhizantella gardneri* have lost *matK* [38, 81]. These non-autotrophic plants have also lost group IIA introns with the exception of the structurally derived and evolutionarily younger *clpP*-2 intron [38,81,130]. Obviously, the presence of *matK* is evolutionarily linked to the presence of a cluster of group IIA introns in strephtophytes [67]. Together, these phylogenetic analyses further support the notion that MatK is required for more than just splicing of its cognate *trnK* intron.

5.2.4. Biochemical evidence for MatK as a general splicing factor

In addition to these indirect observations, more direct support for a role of MatK in splicing comes from biochemical data on MatK RNA ligands. A direct association of MatK with intron RNA was first demonstrated by *in vitro* binding assays [73]. Later, a tagged version of *matK* was introduced into the tobacco chloroplast genome and the gene products were co-precipitated with seven group IIA introns, including its own host RNA, the *trnK* intron [145]. These are the same introns that fail to splice in translation-incompetent chloroplasts (see above). Together with the genetic and phylogenetic data, the biochemical approaches strongly support that MatK is involved in the splicing of multiple chloroplast introns, all belonging to the subgroup IIA (introns listed in Fig. 3A). Further attempts at expressing aberrant versions of MatK or induced knock-down approaches are needed to directly prove that this protein has indeed traversed the boundary to become a general splicing factor.

6. The mitochondrial maturase MatR

Similarly to MatK, MatR is the only maturase-related ORF that has retained in the mtDNAs in angiosperms. MatR is highly conserved and is represented within all angiosperm lineages [2] and is therefore expected to be essential for mitochondrial group II introns splicing. Moreover, sequencing analyses indicate various RNA-editing events, which restore highly conserved amino acids in different plant species [123], further indicating that *matR* encodes to a functional mitochondrial protein. Despite being transcribed, there is no direct evidence that *matR* is translated into a protein that functions in splicing, mainly because transformation methods are currently not available for mitochondria. Using high-throughput sequencing techniques, the mtDNA and transcriptome of cauliflower (*Brassica oleracea*) mitochondria (NCBI accession no. KJ820683.1) was sequenced [42]. Based on these analyses, three synthetic peptides corresponding to amino acids 36–55, 145–161 and 512–530 (*i.e.* IKEEWGISRWFLEFDIRKCF, RRIDDQENPGEEASFNA and SSEFPIKIEAPIKKILRRL, respectively) were used to generate MatR-specific antisera. To identify the RNA ligands of MatR, a modified RIP-chip analysis was designed [110], based on the specific antibodies raised against Bo-MatR. The preliminary data indicate that MatR is associated with various pre-RNAs *in vivo*, all belong to the group II intron class (Sultan et al; Manuscript in preparation). If true, these results would make MatR a polyvalent splice factor similar to MatK.

7. Nuclear descendants of organellar maturases—the nMats

In addition to the organellar encoded maturases (*i.e.* MatK and MatR), the genomes of plants also harbor several genes that are closely related to group II intron maturases. These are encoded in the nucleus as standalone genes, outside the context of their evolutionary related group II intron hosts [15,45,60,85]. In angiosperms, these include four proteins (nMAT 1–4; Fig. 2B) that are highly conserved between monocot and dicot species and are thus expected to retained similar functions in all angiosperms [15,85]. GFP-assays indicated that the four nMATs in Arabidopsis are all localized to mitochondria *in vivo*, while nMAT4 may reside in the plastids as well [59,60]. The four nuclear maturases in angiosperms are divided into subtypes I and II, which differ in their C-terminal domains (Fig. 2B): nMAT1 and nMAT2 belong to type I maturases that lack the D/EN domain and instead contain degenerated long C-termini domains, while the type II maturases, nMAT3 and nMAT4, have both retained the three domains characteristic for model group II intron-encoded maturases (*i.e.* RT, domain X and the D/EN domain). The precise biochemical functions of the plant nMATs in the splicing process still need to be elucidated, but genetic studies clearly demonstrated that these are all required for the splicing of multiple mitochondrial-encoded group II introns in land-plants [15].

7.1. nMAT1, 2 and 4 support splicing of multiple mitochondrial mRNAs

Analyses of the RNA profiles associated with wild-type (Col-0) and various *nmat* mutant plants established the roles of nMAT1, nMAT2 and nMAT4 in the splicing of different subsets of group II introns in Arabidopsis mitochondria (reviewed in [15]). nMAT1 was found to be required for the *trans*-splicing of *nad1* intron 1, *nad2* intron 1 and *nad4* intron 2 [59], while mutations in the *nMat2* gene resulted in reduced splicing efficiencies of many organellar introns, but particularly affected the splicing of *nad1* intron 2, *nad7* intron 1 and the single intron in *cox2* [60]. Recently, it was shown that nMAT4 is involved in the splicing of three out of the four introns in *nad1* (*i.e. nad1* introns 1, 3 and 4), and its activities are essential to the maturation of *nad1* mRNA [22].

The role(s) of nMAT3 in the metabolism of organellar transcripts in plants remains unknown, although it is anticipated that this protein will also function in the splicing of group II introns. GFP-assays indicate that the *nMat3* gene in Arabidopsis (At5g04050) encodes to a mitochondrial protein. The established activities of its three paralogs in Arabidopsis (*i.e.* nMAT1, nMAT2 and nMAT4) strongly support that nMAT3 acts in the splicing of mitochondrial introns as well. Our initial analysis of a T-DNA insertional line in Arabidopsis (SALK-line 144082) indicates a role for nMAT3 in the splicing or processing of several mitochondrial group II introns (O. Ostersetzer and G. Brown, unpublished data). The effects of nMAT3 knockout and knockdown on the RNA metabolism and the biogenesis of the respiratory system are currently being analyzed.

7.2. The activities of the mitochondrial MAT proteins are critical to NAD1 maturation and complex I biogenesis

The *nmat* mutant lines in Arabidopsis show altered growth and developmental defect phenotypes, modified respiration and altered stress responses, which are all tightly correlated to complex I defects [22,59, 60,89]. A particularly important subunit of complex I is encoded by the *nad1* gene. It has been suggested that processing of *nad1* pre-mRNAs in angiosperms mitochondria may serve as a key step in regulating complex I biogenesis [151].

Analyses of *nad1* mutants in animals and algae, and recently in angiosperms, indicate that NAD1 is most likely incorporated at the very earliest stage in the complex I assembly [5,17,22,36,42,59,102]. In plants, at least six different splicing factors are required for the excision of the four introns in *nad1*, and in each case these involve at least one maturase protein. OTP43 [36], nMAT1 [59] and nMAT4 [143] are required for the

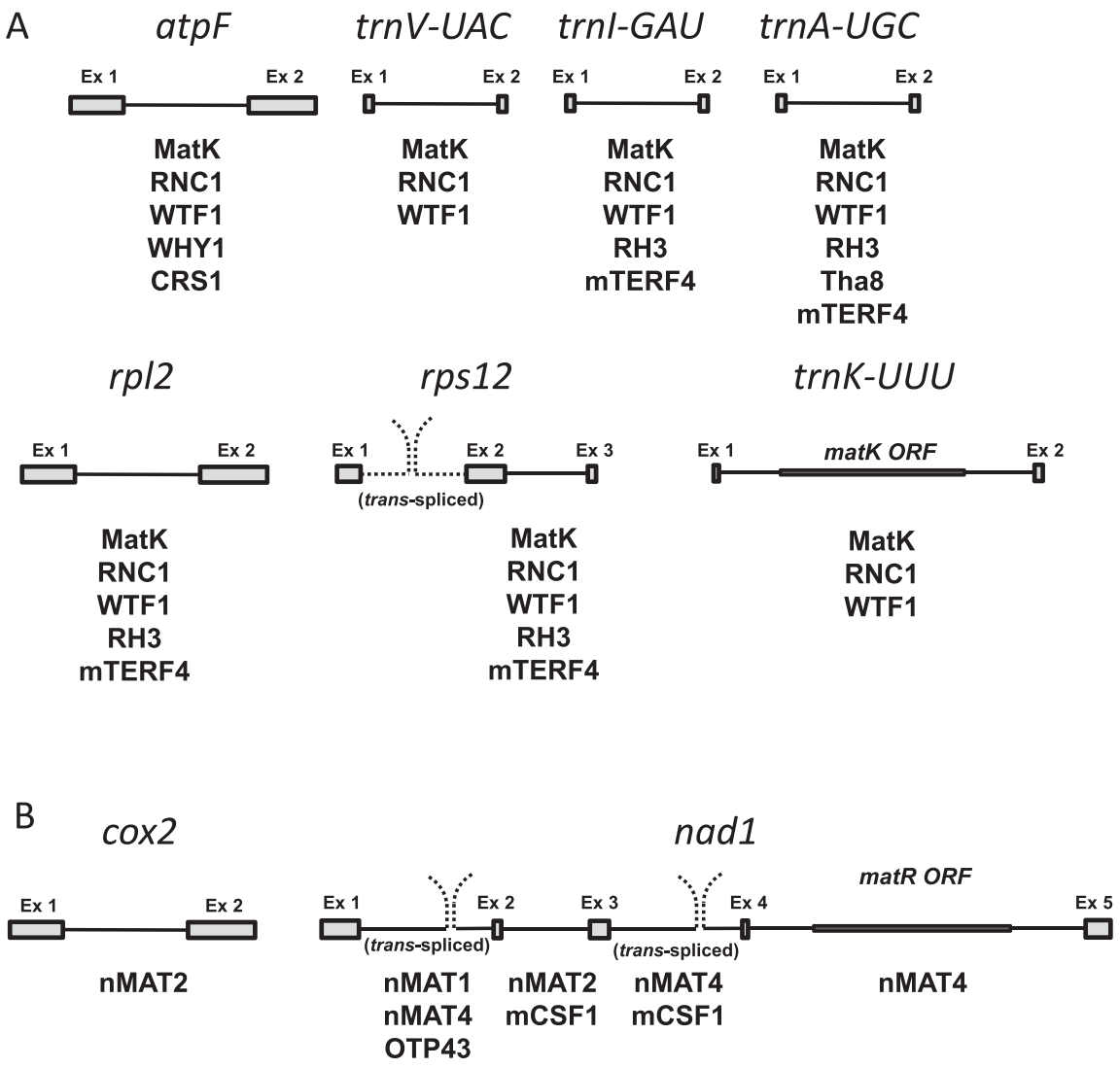

Fig. 3. Target introns of MatK, nMATs together with putative nuclear-encoded co-factors. (A) Schematic representation of MatK target RNAs with exons (ex) depicted as boxes, introns as lines. The trans-spliced first intron of *rps12* is indicated as an interrupted, dashed line. The *matK* reading frame in the *trnK* intron is shown as a bold line. Note that while for MatK, no direct confirmation of a function in splicing has been demonstrated for the introns shown here, all other factors have been demonstrated genetically to be involved for splicing of these introns. This was done for CRS1, RNC1, THA8, WHY1, WTF1, mTERF4 in maize and for CRS1, THA8, RH3 in Arabidopsis [138,68,147,57,125,47], (Khrouchtchova, Monde et al. 2012). (B) Schematic representation of nMAT target RNAs. The *matR* reading frame in the fourth intron of *nad1*is shown as a bold line. Other symbols as in (A).

trans-splicing of *nad1* intron 1, whereas the splicing of *nad1* intron 2 involves both nMAT2 [60] and a CRM related protein, mCSF1 [143]. Similarly, the trans-splicing of *nad1* intron 3 requires mCSF1 and nMAT4 [22], while the removal of *nad1* intron 4 involves at least nMAT4 [22]. Our preliminary results also indicate a role for both MatR and nMAT3 in the processing of several *nad1* introns (unpublished). Intriguingly, this situation may represent an evolutionary step in the gradual transition from the monospecific maturase-facilitated splicing, as observed in bacteria, towards the more general and complex spliceosomal machinery found in the genomes of eukaryotes ([72,78]). Together, the expression profiles of mitochondrial maturases and their established roles in the splicing of complex I subunits strongly suggest that these factors have key roles for the biogenesis of the mitochondrial complex I in plants.

7.3. The mechanistic role of nMATs in splicing

Bioinformatic analyses failed to reveal the presence of a common sequence motif or conserved structural features that could explain the specificities of the different nMATs to their genetically defined pre-RNAs [22,60]. Still, it was noticed that the three intron targets of nMAT1 all lack the canonical bulged A residue [59], which is essential for the first trans-esterification step leading to the release of the 5′ exon. [59]. An intriguing possibility is that nMAT1 may facilitate the hydrolysis of the phosphodiester bond at the 5′ splice site, an activity that may relate to its degenerated C-terminus domain. In addition, nMATs may also recruit other proteins required for the folding or the processing (*e.g.* endonucleolytic activity) of the degenerated mitochondrial introns in plants [13,15]. *In silico* analyses of the model tertiary structures of nMAT1 and nMAT4, two representatives of types I and II maturases (respectively) in Arabidopsis indicate the presence of highly positively charged surfaces that are postulated to serve as RNA-binding modules, while uncharged or negatively charged regions may be required for specific protein–protein interactions (Fig. 4). Attempts to establish the 'co-crystal structures' of mitochondrial group II introns with their genetically identified RNA ligands are underway. Similarly, co-immunoprecipitations or two hybrid screens may lead to the identification of proteins interacting with the nMATs and their introns RNAs *in vivo* (see for example [60]).

7.4. nMATs with additional roles in mitochondrial DNA-metabolism?

In addition to splicing, the nuclear-encoded nMATs may also carry out other essential functions in the biogenesis of plant organelles.

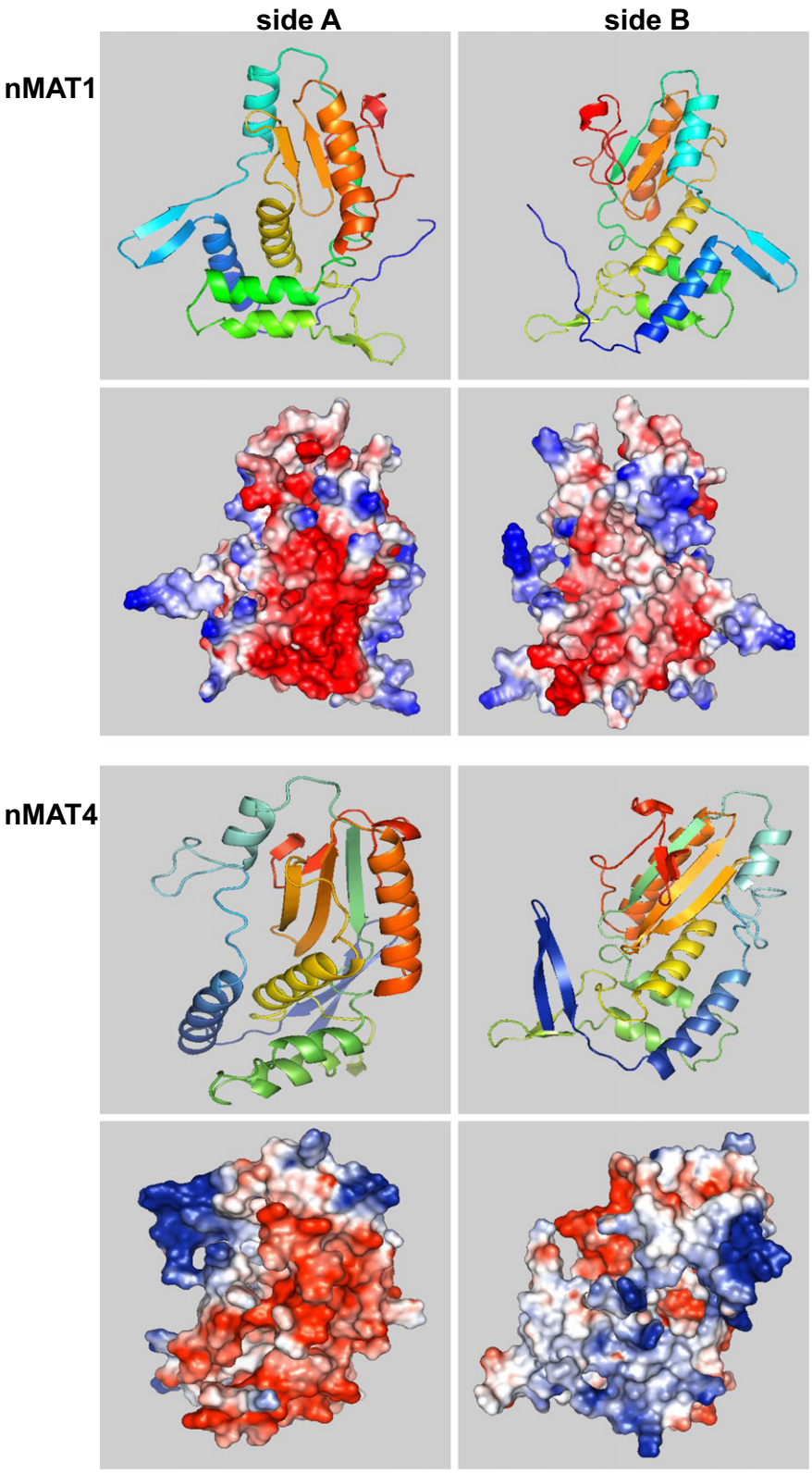

Fig. 4. Schematic representation of the putative 3D structures of plant nuclear encoded maturases (nMATs). The nuclear genomes in plants encode several proteins, which are closely related to maturases encoded within group II introns in bacteria and in fungal mitochondria. nMAT1 belongs to type I maturases, which lack the D/En (DNA binding and Endonuclease) region, while nMAT4 represents plant type II maturases, which harbor the three domains characteristic to model group II intron-encoded maturases. To get more insights into their putative mode of action, in particular of RNA recognition, we performed an atomic model of nMAT1 and nMAT4 proteins using the 'Protein Homology/Analogy Recognition Engine' (Phyre) [58]. The model structures of nMAT1 and nMAT4 were generated by the PyMol software suite [32]. Similarly to the bacterial LtrA maturase [12], the predicted 3D structures of both nMAT 1 and 4 share high similarity (confidence > 98.5) with the HIV-1 reverse transcriptase (PDB c1mu2A). The color code is red for negative values, white for near zero values, and blue for positive values. While positively charged surfaces are expected to be critical for RNA recognition and binding, uncharged or positively charged regions may function in protein–protein interactions.

Sequencing analyses of mitochondrial DNAs obtained from various plants and several CMS (cytoplasmic male sterile) lines indicated the presence of both circular and linear DNA fragments and also revealed an unexpected high frequency of both intra- and inter-molecular recombination events (reviewed by [44]). These processes are characteristic of plant mitochondrial genetics and appear to be the basis for the evolution of the mitochondrial DNA and the generation of new mitotypes in plants. Yet, the identity of the factors involved in these processes is still largely unknown. Among the important components of the mitochondrial recombination machinery is the MSH1 protein, which encodes a MutS-like protein [6,113,114]. In bacteria, MutS recruits an endonuclease, the MutH protein, for DNA mismatch repair and the suppression of ectopic recombination [111]. Reductions in the levels of MutS or MutH result in increased recombination events. However, there is no known MutH-like gene in Arabidopsis. Both MatK and MatR lack the D/En (*i.e.* DNA binding and endonuclease) domains and retained only remnants of the maturase related RT (Reverse Transcriptase) motif, and are thus expected to have lost the maturase-associated 'retrohoming' activities [26]. Yet, the high similarity between nMATs proteins and bacterial group II intron-encoded ORFs [15,85] may indicate that in addition to splicing, nMAT proteins have also retained the homing endonucleases functions, *i.e.* promote RNA splicing and intron mobility reactions through their ability to act not only on RNA but also on DNA substrates. It would be therefore interesting to test whether the nMAT proteins have also been pivotal in the evolution of mitochondrial genome structure in plants.

8. Host-derived cofactors in splicing

In addition to the nMATs, other RNA-binding proteins were also recruited to function in the splicing of organellar group II introns in angiosperms. Here, we list only factors that serve introns that are targeted by maturases. For a description of the large number of additional nuclear encoded splicing factors for non-maturase dependent introns, the reader is referred to recent reviews of chloroplast [31] and mitochondrial [15] splicing.

Genetic screens generated a large amount of data regarding the roles of nuclear-encoded factors in the splicing of plastidial introns in maize (reviewed in [9]). Seven of these factors are required for introns that are also associated with MatK (Fig. 3). Among these are two proteins, RNC1 and WTF1, that co-precipitate with all the group IIA introns that are also found as ligands of MatK [68,138]. Both proteins support splicing of their intron ligands in maize [68,138]. It is tempting to speculate that MatK, RNC1 and WTF1 form a functional complex required for recognizing the group IIA intron structure. Another two proteins, RH3 and mTERF4 are associated with and required for splicing of a common set of four group IIA introns [7,47]. Again, formation of a subcomplex together with MatK and also RNC1 and WTF1 seems possible. In addition to these five more general splicing factors, further RNA binding proteins are necessary for splicing of specific MatK-associated introns. First, the CRM-domain protein CRS1 is essential for splicing the *atpF* intron [57, 92,110,125] and second, the short PPR protein THA8 is specific for the *trnA*-UGC intron [61]. Whether direct contacts exist between this set of factors or whether the proteins are attached at different positions within their target introns without making protein–protein contacts still needs to be determined. Also, contacts to RNA could occur consecutively during the splicing process thus precluding a direct interaction between the different proteins listed here. This is amenable to experimental testing. Yeast-two hybrid experiments together with a more detailed analysis of the exact intron target sequences of these proteins by RIP-Seq or related techniques could clarify this point. In any case, maturase-based splicing clearly requires additional nuclear-encoded factors—it appears as if the chloroplast splicing machinery is on its way towards a more complex machinery, a chloroplast spliceosome.

A similar situation is now starting to emerge for the mitochondrial introns in land-plants ([15,23]). As in plastids, the mitochondrial splicing factors also belong to a diverse set of protein families [23,15, 48]. Some, as the maturase nMAT2 [60], the RNA-helicase PMH2 [63] and a CRM-related protein, mCSF1 [143], are required for optimal processing of a larger set of organellar introns. Others, such as PPR (pentatricopeptide repeat) proteins [25,36,46,66,75], mTERF (human mitochondrial transcription termination factor) proteins [47] and PORR (plant organellar RNA recognition) proteins [24] appear to be more specific, influencing the splicing of a single or only a few group II introns.

At the moment, far fewer nuclear-encoded splicing factors were isolated for mitochondria than for chloroplasts. Only two of these serve introns also requiring nMATs (Fig. 3B). *nad1* intron 1 requires the PPR protein OTP43 in addition to nMAT1 and nMAT4 [36]. *nad1* intron 2 requires nMAT2 and mCSF1 for splicing and *nad1* intron 3 requires nMAT4 and also mCSF1 [143]. Future experiments need to elucidate whether these factors act together with the maturases or rather act in a consecutive fashion to remove their particular intron targets.

9. Why are maturase genes stuck within organellar genomes?

Unlike *matR* and *matK*, many other chloroplast and also mitochondrial genes have been transferred multiple times in different land plant lineages to the nucleus [62]. As outlined above, this includes the nMATs, which can serve organellar splicing of several mitochondrial group II introns [22,59,60,89]. This demonstrates that maturases can in principle be transferred to the nucleus and still function in splicing. An important question therefore is why *matR* and *matK* are evolutionary maintained on the organellar chromosomes? As discussed above, *matK* was lost only in plants that lost photosynthesis (*matR* has never been lost in plant evolution). As a consequence of the loss of the ability to photosynthesize, these species could dispense with genes coding for components of photosynthetic machinery [67]. Together with these genes, introns requiring MatK were lost as well. Consequently there was no need to maintain *matK*, which also indicates that *matK* does not serve additional functions outside of splicing. While this is evidence that *matK* is essential for the expression of the photosynthetic apparatus, it is unclear why it could not fulfill this function as a nuclear gene—just as the nMATs do (see, Section 2).

Several arguments have been put forward to explain the retention of plastid and mitochondrial genes within organelles (summarized in [3,8]). One of these arguments is that essential organelle-internal regulatory circuits would prevent gene transfer. For example, chloroplast-borne signals, like changes of the redox balance of the electron transport chain, have an impact on chloroplast gene expression. If such regulatory influence is essential for adapting gene expression to photosynthesis, the gene will be "stuck" within the organelle, a hypothesis called CORR (co-location for redox regulation of gene expression; [4]). For example, transcription of the *psaA/B* operon is under the control of the rapidly changing chloroplast redox state [97]. It is presumably faster and certainly more direct to signal the redox state within the chloroplast than to bounce it first to the nucleus.

For organellar maturases, there is no direct evidence for a connection to such regulatory circuits. MatK is required for the expression of the AtpF subunit of the ATP synthase. This enzyme complex has been proposed to be under redox control [3,4] and thus MatK might participate here in signal transduction. However, it remains to be determined whether MatK is indeed linked to a signaling pathway and would be for example under control of a plastid kinase.

Apart from such metabolic considerations, genes would also be evolutionarily stuck within the organelle if their gene product interacted with other chloroplast genes for regulatory reasons. For example, in bacteria most of the operons for ribosomal proteins encode a regulatory r-protein that serves as an operon-specific translational repressor. These proteins bind to their own mRNA and thus prevent further translation (reviewed in [91]). Similar internal connections within the chloroplast have been demonstrated in *Chlamydomonas* chloroplasts,

where unassembled subunits of thylakoid membrane complexes repress translation of their own or related complex subunits (a phenomenon called control of epistasy of synthesis = CES; [21]). In addition, a recent study in *Chlamydomonas* demonstrated feedback loops that affect chloroplast transcription and translation [101]. For example, a negative impact of the ClpP protein on its own mRNA accumulation has been demonstrated [101]. How prevalent such regulatory loops are in embryophytes is not yet known.

MatK does bind to its own mRNA, specifically to the 5′-UTR and might act as a translational repressor [49]. Similarly, the bacterial maturase LtrA also targets the 5′-UTR and in fact thus down-regulates its own translation [27,118]. Expression data of the MatK protein and *matK* mRNA support autoregulation. In young tissue, the lowest *matK* mRNA amounts are found in parallel with the highest MatK protein levels [49]. Later in development, mRNA levels rise but protein levels fall. Such a reciprocal relationship might well be explained by negative translational auto-regulation and was indeed further supported by mathematical modeling of the MatK gene expression network [49]. If essential for MatK function, such regulatory loops would keep the *matK* gene within the organelle. A proof for auto-regulation of MatK could be attempted in an *in vitro* chloroplast translation system [53], but is currently lacking.

10. MatK, MatR and nMATs as missing links in the evolution of the spliceosome

The mitochondrial seed hypothesis has gained support from the striking similarities between group II introns and spliceosomal introns, in particular spliceosomal snRNAs. In contrast, a similarly well-supported hypothesis for the origin of the spliceosomal proteins was never provided. This has changed recently with the observation that the core nuclear spliceosomal factor, Prp8 displays homologies to group II intron maturases [33]. Prp8 is, with its 280 kDa, the largest and also most conserved protein within the spliceosome [41]. It makes contacts with all three snRNPs involved in splicing catalysis, *i.e.* U2, U5 and U6 [39]. Among its various domains, a reverse transcriptase, an endonuclease domain and a domain X bear similarities to maturase proteins. Mutational analyses indicate that Prp8 is essential for forming the catalytic center of the spliceosome [74], although it does not itself contribute residues to splicing—a job recently shown to be purely RNA-based [37]. In sum, Prp8 is a key player in spliceosomal splicing that likely evolved from an ancestral maturase.

One interesting open question is how a bacterial maturase was turned into a core spliceosomal factor? After gene transfer and spread of introns through the genome, maturase genes were present in many introns. These were free to degenerate as long as there was one or a few maturases capable of taking care of splicing of all introns. Concomitantly, some introns degenerated to evolve into the snRNPs. snRNPs then provided the general tools for a maturase to take care of multiple introns *in trans*. Such a maturase possibly co-evolved with snRNPs to become Prp8. Likely, domains present in extant Prp8 but unknown from bacterial maturases (like the Jab1/MPN domain) were gained later during evolution by gene fusion or other processes [99]. Such a scenario posits that during the evolution towards Prp8, a progenitor maturase was likely to have expanded its intron target range. Possibly, it already had a relaxed target specificity before transfer into the nucleus, if it had evolved inside a proto-mitochondrion similar as MatR. Or, it gained this ability to recognize and splice multiple introns later, within the nuclear genome. In any case, understanding how MatR and MatK manage to serve multiple group II introns will help us to formulate a hypothesis on the early events during Prp8 and thus spliceosomal evolution. Structural analyses with MatK or MatR bound to intron ligands and detailed analyses of MatK/MatR binding sites on introns will likely help solve this issue in the near future.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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