Accepted Manuscript

Surrogate mutants for studying mitochondrially encoded functions

Catherine Colas des Francs-Small, Ian Small

PII: S0300-9084(13)00291-5

DOI: [10.1016/j.biochi.2013.08.019](https://doi.org/10.1016/j.biochi.2013.08.019)

Reference: BIOCHI 4276

To appear in: *Biochimie*

Received Date: 5 July 2013

Accepted Date: 18 August 2013

Please cite this article as: C.C. des Francs-Small, I. Small, Surrogate mutants for studying mitochondrially encoded functions, *Biochimie* (2013), doi: 10.1016/j.biochi.2013.08.019.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Highlights

- Mitochondrial genome mutants are nearly impossible to obtain
- Mitochondrial gene expression is controlled by nuclear factors
- Loss or mutation of these factors can mimic mitochondrial genome mutants
- Such mutants are named indirect or “surrogate” mutants
- Surrogate mutants are valuable for studying the biogenesis of respiratory complexes

MINI-REVIEW

Surrogate mutants for studying mitochondrially encoded functions

Catherine Colas des Francs-Small¹ and Ian Small¹

¹Australian Research Council (ARC) Centre of Excellence in Plant Energy Biology, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

*Corresponding author
Ian Small

Corresponding author's e-mail address: ian.small@uwa.edu.au
Phone: + 61 8 64884499

Running head: Surrogate mutants for studying mitochondrially-encoded functions

Abstract

Although chloroplast transformation is possible in some plant species, it is extremely difficult to create or select mutations in plant mitochondrial genomes, explaining why few genetic studies of mitochondrially encoded functions exist. In recent years it has become clear that many nuclear genes encode factors with key functions in organelle gene expression, and that often their action is restricted to a single organelle gene or transcript. Mutations in one of these nuclear genes thus leads to a specific primary defect in expression of a single organelle gene, and the nuclear mutation can be used as a surrogate for a phenotypically equivalent mutation in the organelle genome. These surrogate mutations often result in defective assembly of respiratory complexes, and lead to severe phenotypes including reduced growth and fertility, or even embryo-lethality. A wide collection of such mutants is now available, and this review summarises the progress in basic knowledge of mitochondrial biogenesis they have contributed to and points out areas where this resource has not been exploited yet.

Highlights

- The phenotypes of plants carrying mutations in nuclear genes encoding factors that control mitochondrial gene expression can mimic mutations in mitochondrially encoded subunits of respiratory complexes;
- These mutants are named indirect or "surrogate" mutants;
- Surrogate mutants are a valuable resource for studying the biogenesis of respiratory complexes.

Keywords:

mitochondria; RNA-binding proteins; RNA processing; mutants; plants

1 INTRODUCTION

Plant cells have three distinct genomes, with expression of the much smaller mitochondrial and chloroplast genomes dependent on hundreds to thousands of nuclearly encoded gene products [1, 2]. Although the gene content of the organelles has drastically reduced over the last billion years or so, the genes that remain tend to be particularly important, coding for the major components of most of the respiratory and photosynthetic complexes of mitochondria and chloroplasts. Loss of expression of almost any of these genes gives rise to severe phenotypes, and total loss of gene expression of either organelle is lethal in almost all cases [3-6]. Despite their importance, the organellar genes have been little studied, as they are intractable to most of the standard methods of genetic analysis. Classically, gene function in plants has been investigated by forward genetics using mutagens such as ethyl methyl sulphonate (EMS) or by reverse genetics using collections of T-DNA or transposon insertion mutants. None of these approaches work effectively on the organelle genomes.

Spontaneous mutations in the organelle genomes are rare and reports are largely limited to highly visible phenotypes such as cytoplasmic male sterility (CMS) or leaf variegation. These mutations generally result from rearrangements or deletions in the organelle genomes (reviewed in [7]) and are difficult to provoke deliberately at high frequency [8-10]. CMS can also result from nucleo-cytoplasmic incompatibility generated by inter-specific protoplast fusion [11, 12].

Genetic manipulation of organelle genomes is not straightforward due to the

number of organelles per cell and the number of genome copies per organelle, which make selection difficult: for example, an *Arabidopsis* mesophyll cell contains about 120 plastids [13] and about 670 mitochondria [14], and numerous copies of each genome (1000 to 1700 copies of the chloroplast genome [13] and about 60 – 100 copies of the mitochondrial genome [15]). These technical difficulties have greatly hampered studies of organelle gene functions, although chloroplast transformation is possible, and has been extremely informative in tobacco and related species [16, 17]. In contrast, transformation has never been convincingly achieved for mitochondria of any land plant. Hence the creation of indirect or 'surrogate' mutants can be a helpful alternative way of generating specific alterations of organelle gene expression, and particularly that of the mitochondrial genome.

2. SURROGATE OR INDIRECT MUTANTS

2.1 What do we mean by 'surrogate' mutants?

The expression of organelle genes requires numerous stages, particularly during RNA processing, including protection of 3' and 5' termini, splicing and editing. These steps each require RNA-binding proteins that are encoded in the nucleus, translated in the cytosol and targeted to the organelle where they are needed. Several families of such RNA-binding proteins have been described, the largest being the pentatricopeptide repeat (PPR) family, which comprises around 450-500 members in angiosperms [18]. Smaller RNA-binding families are also known, such as the 'chloroplast RNA splicing and ribosome maturation' (CRM), and 'plant organellar RNA recognition' (PORR) [19, 20] and 'regulator of chromosome condensation' (RCC) [21] families,

which appear to be specialised in aiding intron splicing. Recently, a new type of RNA Editing Factors whose precise molecular function has not been elucidated, have been shown to be required for the editing of several chloroplast and mitochondrial genes, in conjunction with PPR proteins [22-24]. Importantly in the context of this review, the action of many of these RNA-binding proteins is highly specific, each protein only acting on a small number of transcripts, or even on a single transcript. Hence, loss of function of some of these RNA-binding proteins leads to a highly specific defect in expression of only a single mitochondrial gene. This opens the possibility of using mutants affected in these nuclear genes as surrogates for the unobtainable mitochondrial mutants. This approach is particularly powerful in model species such as *Arabidopsis* where large collections of nuclear mutants are widely available. Several such surrogate mutants have been described in the literature (if not generally described as such), and have proved useful for studies of the assembly and function of the main respiratory complexes. We believe, though, that their usefulness is under-appreciated by the researchers most able to take advantage of them. In this review, rather than cataloguing the molecular defects resulting from the loss of specific RNA-binding proteins that have been thoroughly reviewed elsewhere, we will focus on the physiological consequences of the defects in mitochondrial gene expression and give examples of interesting discoveries made during the study of such mutants.

2.2 What can be done with these mutants?

The primary interest of surrogate mutants does not lie in the identification of

the primary function of individual gene products. The functions of most mitochondrial gene products are already well understood, and unfortunately we lack surrogate mutants for the few genes whose function is not already known. Instead, we suggest these mutants be used to answer more complex and subtle questions.

Most of the mitochondrial genes encode subunits of very large and complicated protein complexes (Figure 1) whose biogenesis and activity needs to be tightly controlled. Indeed, there are strong arguments for thinking that it is precisely the necessity to control the assembly of these complexes that explains why genes are retained within the organellar genomes [25-27]. Nevertheless, we still only have a very vague understanding of how these complexes are assembled, and even less about how these processes are controlled. The difficulty of obtaining or constructing appropriate mutants partly explains why we know so little. We encourage researchers interested in these questions to consider the surrogate mutants we describe here as alternative and complementary approach to examine these important questions.

Numerous surrogate mitochondrial mutants have been described in plants, with various phenotypic defects. Table 1 lists these mutants, indicating the protein complex that is impaired and provide a brief description of the physiological and macroscopic phenotypes as well as the molecular defect(s) in the mutants described. In this review, we will cover findings that arose from the study of these surrogate mutants in the alga *Chlamydomonas*, the moss *Physcomitrella*, in monocots and dicots. The focus will be on mutants in which

expression of a single, or at most 2, organelle genes are directly affected. In mutants in which expression of more organelle genes are directly affected it is more difficult to relate the phenotype to a single organelle gene and thus interpret the results.

3 SURROGATE MITOCHONDRIAL MUTANTS

Plant mitochondrial genomes retain up to approximately 65 functional genes [28]. Commonly, these include 18 which encode mostly membrane proteins of the respiratory chain, i.e. *nad1-4*, *nad4L*, *nad5-7* and *nad9* genes (complex I), *cob* (complex III), *cox1, 2, 3* (complex IV), *atp1, 4, 6, 8, 9* (ATP synthase). In addition, the following genes are also usually present: *ccmB*, *ccmC*, *ccmF_C*, *ccmF_N* (cytochrome *c* maturation), *rps1, 2, 3, 4, 7, 12*, *rpl2, 5, 16* (encoding ribosomal subunits), *mttB* (protein translocator) and *matR* (splicing maturase). There is some variation between species as regards the genes retained [28].

ATP is the main source of cellular energy and is generally produced in mitochondria through the oxidative phosphorylation pathway (Figure 1). Reducing equivalents originating from catabolism of sugars, amino acids and fats are oxidised by the respiratory chain, which is composed of four main complexes, comprising subunits of nuclear and mitochondrial origins (with the exception of complex II, fully encoded by the nuclear genome in most land plants). Complex I is the biggest, composed of over 40 subunits in plants [29-31] and acts as a NADH-ubiquinone oxidoreductase. Complex III (ubiquinone-cytochrome *c* oxidoreductase and complex IV (cytochrome *c* oxidase) together transfer electrons from ubiquinone to molecular oxygen. Concomitant

with electron transfer, protons are pumped by these complexes to generate a proton motive force that is used by ATP synthase (complex V) to power the generation of ATP. Plant mitochondria also possess an alternative electron transport pathway, known as the non-phosphorylating pathway, which can bypass the cytochrome pathway but will not generate ATP. As none of the components of the alternative pathway are encoded in the mitochondrial genome, we will not consider this pathway in this review.

3.1 Complex I

Complex I is one of the most frequent targets of surrogate mutations, as it contains nine subunits encoded in the mitochondrial genome (*nad1* to *4*, *nad4L*, *nad5* to *7* and *nad9*), and the expression of all nine needs *cis*- and *trans*-splicing [32], thus requiring a plethora of RNA-binding proteins. The membrane arm of complex I is composed of seven mitochondrially encoded subunits (Nad1-6), and about 27 nuclearly encoded ones (18 are also found in the bovine complex I but the remainder are plant-specific, including the carbonic anhydrase-like subunits [31, 33, 34]). Apart from Nad7 and Nad9, the subunits composing the peripheral arm are all encoded in the nucleus [29, 30, 33].

Several mutants with reduced complex I activities have been described, including some with mutations in nuclear genes encoding complex I subunits such as *frol*, *ca2*, *ndufs4* in *Arabidopsis thaliana* [35-37], and natural mitochondrial mutants with rearrangements of their mitochondrial genomes in *Nicotiana sylvestris* [38, 39], or in maize [40, 41]. These studies have shown that the absence of complex I is not lethal in plants, and certainly not as

deleterious as it can be in humans [42]. The phenotypes are more or less severe, ranging from no macroscopic phenotype (*ca2* [36]), to slow growth and curled foliage (*ndufs4* [37]), male sterility (*cmsII* [39]) and variegated leaves (*ncs2* [41]), but share reduced amounts of assembled complex I. Additionally, several surrogate mutants have also been found, leading to interesting discoveries. The first surrogate mitochondrial mutant in plants was described in *Nicotiana sylvestris* and named *nms1* [43]. It was altered in a *nad4* splicing factor and displayed phenotypes similar to the *cmsII* and *cmsI* mutants, i.e. slow growth, reduced fertility and reduced accumulation of complex I [38, 43]. In *Arabidopsis*, the first and most severe surrogate complex I mutant to be described was *otp43* [44], a mutant altered in the expression of a PPR protein involved in the splicing of *nad1*. This mutant displayed a phenotype similar to that of complex I KO mutants such as *ndufs4*, i.e. reduced fertility, very slow growth and dark green curled rosette leaves. The defect was so severe that no fully assembled complex I could be detected by Blue Native PAGE. More surrogate mutants affected in the accumulation of complex I have been described since, with defects in different subunits: *nad2* [21, 45-47], *nad3* [48-50], *nad4* [43, 49, 51-54], *nad5* [48, 55-58], *nad7* [59, 60](see Table I). Rather interestingly, a huge range of developmental, physiological and biochemical phenotypes have been described for these different mutants, including sensitivity to sugar (*otp43*) or ABA (*abo5*, *ahg11*), tolerance to inhibitors of glutathione synthesis (*bir6*), defects in root growth [47, 50] and many others. The comprehensive studies of *cmsII* and *ndufs4* in *Nicotiana* and *Arabidopsis* suggest that all of these phenotypes result from the extremely pleiotropic effects of a loss of complex I

on central energy metabolism and signalling. The severity of the growth phenotype in these different mutants appears to correlate with the quantity of assembled complex I, rather than with which subunit is directly affected in the mutant (Table 1). Only a small quantity of fully assembled complex I is required for near normal growth, even if in such plants energy metabolism and signalling can still be profoundly perturbed.

Nevertheless, there remains a strong possibility that some differences in phenotype between surrogate mutants result from the specific effects of losing a particular subunit. Like most large protein complexes, complex I is assembled through association of multiple modules into various sub-complexes that can be viewed as assembly intermediates. Some of these sub-complexes show demonstrable oxidoreductase activity [41, 43, 54, 61, 62]. This is physiologically relevant as shown by the essential association of L-galactono-1,4-lactone dehydrogenase (GLDH) with complex I sub-complexes [63]. It is plausible, therefore, that some differences between mutants may reflect differential accumulation of specific sub-complexes that either provide useful functions (as in the case of GLDH) or on the contrary are deleterious through futile cycling of energy metabolites and/or generation of reactive oxygen species [41, 43]. There is a great opportunity to use the many surrogate mutants lacking different mitochondrially-encoded subunits to investigate the assembly pathway of complex I and the functions of the various sub-complexes. Meyer et al. [30] have made a useful start in this direction by using the sub-complexes present in different complex I nuclear mutants to generate a model of the assembly of complex I in *Arabidopsis thaliana*. The many surrogate mutants described above would be a great

addition to this study as they are mostly altered in subunits of the membrane arm, whose assembly is still not fully described in plants.

3.2 Complex III, complex IV and the c-type cytochrome maturation complex

The next three complexes will be treated together, as they are functionally linked by cytochrome *c*, generating similar phenotypes in the respective mutants. Complex III (also known as cytochrome *bc1* complex) is an integral membrane protein complex, which contains only one mitochondrially-encoded subunit, cytochrome *b* (Cob), whilst the other 9-10 subunits (including cyt *c1* and the Rieske protein) are nuclearly encoded [64]. Cytochrome *c* oxidase is a transmembrane protein complex composed of 10-12 subunits in plants [65]. It contains two haem molecules in cytochromes *a* and *a3*, and two copper centres. The three core subunits (Cox1, 2 and 3) are mitochondrially encoded. The assembly of cytochrome *c* oxidase requires the presence of holo-cytochrome *c* [66]. The biogenesis of cytochrome *c* itself is a complex process, which is still not fully understood in plants [67]. The cytochrome *c* apoprotein is synthesized in the cytosol and imported into the mitochondrial intermembrane space where its haem cofactor is covalently attached by the CCM (cytochrome *c* maturation) complex. This complex comprises four mitochondrially encoded subunits (CcmB, CcmC, CcmF_N and CcmF_C) or 5 in Brassicaceae (CcmF_N being split into CcmF_{N1} and CcmF_{N2}) and 3 nuclearly encoded subunits (CCMA, E and H) [67]. It is located in the inner membrane, orientated towards the inter-membrane space where cytochrome *c* will be assembled [67]. The CCM system in plant mitochondria is of the same type as

in bacteria (system I) and different from the system used in chloroplasts (system II) or in animal or yeast mitochondria (system III) [67]; nevertheless, many details about its composition and mode of action are still lacking. Very few direct mutants in complex III, complex IV and cytochrome *c* have been described because a total lack of either complex or cytochrome *c* is lethal. For example, maize NCS6 mutant embryos partially lacking *cox2* have to be rescued *in vitro* [68] and embryonic development of the *Arabidopsis* complex IV mutant *hcc1* [69] is arrested at the heart stage. HCC1 is the *Arabidopsis* homolog of the yeast copper chaperone SCO1 [69], which plays an essential role in the assembly of cytochrome *c* oxidase. Similarly, the *Chlamydomonas* mutant *dum-15*, carrying a deletion of the *cob* gene, is unable to grow under heterotrophic conditions [70]. Double mutants lacking expression of both nuclear *CYTC* genes in *Arabidopsis* are not viable, showing that cytochrome *c* is essential for embryo development [71]. Mutations disrupting the CCM complex (e.g. the *ccmh* mutant) lead to arrested embryonic development at the torpedo stage because of the lack of cytochrome *c* [5]. Clearly the cytochrome pathway of electron transport through complexes III and IV is essential in plants.

In this context, it is not surprising that the few surrogate mutants that have been described all appear to retain a minimal amount of functional complex III, IV and cytochrome *c* to allow development of the plant. The putative surrogate complex III mutants *ppr40* (unknown target, [72]) and *ppr2263* (*cob* and *nad3*, [56]) exhibit delayed germination and reduced growth phenotypes in *Arabidopsis* and maize respectively. The phenotypes are presumably due

to a severe ATP deficit and possibly over-reduction of ubiquinone in the mitochondrial membrane leading to ROS production, despite activation of the alternative oxidase pathway. Similar phenotypes are observed for surrogate complex IV mutants in *Physcomitrella* (disrupted in PpPPR_43 (*cox1*) and PpPPR_77 (*cox2* and *cox3*)[48, 73]).

Surrogate mutants affected in expression of the CCM machinery present similar phenotypes. For example the *Arabidopsis* mutant *wtf9*, almost completely lacking CcmF_C, has extremely stunted growth due to the almost complete lack of cytochromes *c* and *c1*, and consequently that of assembled complexes III and IV. The dwarf plants flower and produce short siliques containing one or two milky sterile seeds [74]. A similar growth phenotype was observed in the *Physcomitrella* editing mutant disrupted in PpPPR_71 and affected in the same CCM subunit [75]. Oddly, the processing mutants *rpf3* (*ccmC*) and *rpf2* (*cox3* and *nad9*) [76, 77] and the editing mutants *mef19* (*ccmF_{N2}*) and *mef21* (*cox3*)[49] do not display any growth phenotype. It is probable that, as we saw for complex I, even low levels of correctly assembled CCM complex are sufficient for apparently normal growth.

These surrogate mutants are precious additions to the limited collection of known mutants affected in the cytochrome pathway and will be valuable tools for the study of the composition and biogenesis of complexes III, IV and the CCM complex in plant mitochondria.

3.3 ATP synthase

F₁F_o ATP synthase is a rotary motor enzyme comprising 15-18 subunits. The F₁ extra-membranous catalytic core is formed by the α_3 , β_3 , γ , δ and ϵ

subunits and its structure is rather better conserved and documented than that of the F_0 membrane subcomplex [78]. Five subunits are encoded by the mitochondrial genes *atp1* (encoding the α subunit of F_1), *atp4*, 6, 8, and 9 (encoding subunits of F_0). Very few mutants in this complex have been described, most probably because the lack of mitochondrial ATP synthesis would be lethal, as explained in the previous section. In yeast, the few isolated mitochondrial ATP synthase mutants that have been generated display genetic instability and deletions in the mitochondrial DNA [79]. Using a gene replacement technique to avoid genetic instability, an *atp6* mutant was obtained, and resulted in a loss of ATPase activity with assembly of an incomplete ATP synthase, poor accumulation of cytochrome *c* oxidase and abnormal morphology of the mitochondrial cristae [79]. Similarly, a *Chlamydomonas* RNAi mutant with reduced expression of the nuclear *atp2* gene (encoding the F_1 β subunit) had no apparent assembled ATPase complex and a reduced respiration rate [80].

Two surrogate mutants affected in expression of mitochondrial *atp* genes have been described in *Arabidopsis* [81, 82]. The lack of editing of the *atp1* transcript in *otp87* generates an amino-acid substitution in the α subunit and results in a reduced growth phenotype [81]. The analysis of its mitochondrial membranes by blue native electrophoresis and SDS PAGE clearly indicates a specific problem in the assembly of ATP synthase. The second mutant, *mef3* (*atp4*), does not display an obvious macroscopic phenotype [82]. Studies of a mutant such as *otp87* should provide valuable information on the assembly of ATP synthase and feedbacks on mitochondrial biogenesis and metabolism.

3.4 Mitochondrial translation machinery

Mitochondria encode much of their own protein synthesis machinery (26S, 18S and 5S rRNAs; 11 native tRNAs as well as some chloroplast-derived tRNAs, and numerous ribosomal proteins) to produce the polypeptides encoded in the mitochondrial DNA [83]. Mutations in these genes would be expected to result in a reduction of mitochondrial translation and therefore be very deleterious or even lethal [84]. Unsurprisingly, therefore, few such mutants have been described as yet. The only two direct mutants that we know of are NCS3 and NCS4 from maize, which carry deletions in *rps3* leading to reduced mitochondrial translation and stunted growth [85] [86]. The plants only survive because they are heteroplasmic for the *rps3* deletion. In addition, four surrogate mutants are known (Table1). Two mitochondrial editing mutants altered in *rps4*, *mef1* [45] and *mef20* [49] do not show any macroscopic phenotype, like many editing mutants grown under normal conditions. The maize and *Arabidopsis ppr6* mutants [87] as well as a *Physcomitrella* mutant lacking PpPPR_78 and altered in expression of *rps14* [55] display slow growth. In *ppr6*, the *rps3* transcript is not processed correctly, resulting in very reduced mitochondrial translation, poor seed germination and slow growth. In *Arabidopsis*, *ppr596* is another mutant apparently impaired in *rps3*, but the editing defect itself is silent and cannot by itself explain the growth defect [88].

4 CONCLUSION AND PERSPECTIVES

4.1 What have we learnt and what can we still learn from the surrogate mutants?

The advantages offered by surrogate mutants are manifold. Firstly they simply provide numerous independent examples of mutants affected in specific complexes for which otherwise the choice might be limited. Secondly, they often display milder, partial or conditional phenotypes compared to knockout mutants of nuclearly encoded subunits because they only indirectly affect the target complex. These 'softer' phenotypes are invaluable for studying essential functions such as mitochondrial translation or ATP synthesis. Thirdly, they are in some cases exquisitely specific - RNA editing mutants generally differ from wild-type by only a single amino acid, and as these editing events are generally well conserved, it is a safe assumption that this single amino acid difference is likely to be physiologically important at least under some conditions at some stage of the life cycle. Last, but not least, the loss of a single organelle-encoded subunit of a large complex allows us to study the effect of blocking assembly of the complex at a specific stage. This has two important repercussions; it allows us to examine the order of assembly of the different modules of the complex, and the effect of the loss of assembly on the expression of the other subunits comprising the complex. It has frequently been noted that in the absence of assembled complex, some or all of its subunits fail to be synthesised, even those not directly affected by the mutation. This implies a feedback loop from assembly to translation, whereby unassembled subunits block translation of their own mRNA. In this way, translation of each organelle-encoded subunit is dependent on the presence of its assembly partner(s). In *Chlamydomonas* chloroplasts where this has been best studied, this phenomenon has been termed Control by Epistasy of Synthesis (CES) [89]. Biogenesis of PSI is an excellent example

of CES, where one subunit (PsaB) is dominant over another one (PsaA), which in turn will determine the synthesis of the next one (PsaC) [90]. It is worth emphasising that one of the first surrogate mitochondrial mutants described in yeast [91] suggested a similar dominant role for Cox1 over the other Cox subunits. Clues in favour of the occurrence of CES in higher plant chloroplasts have been reported, in particular the regulation of the translation of the large subunit of RuBisCo by the status of its assembly into the complex [92], and the “high chlorophyll fluorescence” mutant *lpa66* [93]; counter examples also have been given [94] [95], so the mechanism remains controversial in land plants [2]. Either way, the surrogate mutants described here will help confirm or contradict the hypothesis.

4.2 How can we get the “missing mutants”?

We lack surrogate mutants for several plant mitochondrial genes including *nad4L* and *nad9* encoding complex I subunits, and genes for subunits of complexes III and IV, the CCM complex and the translation apparatus (Table 2). For many of these genes, surrogate mutants are likely to exist, as they either contain introns (likely to require specific splicing factors) or editing sites (very likely to require specific editing factors). It is important to note that we now have the ability to predict the binding sites of editing factors at least [96] [97] [98], with the possibility that this could be extended to splicing factors in the near future [96]. Thus it is now possible to carry out targeted searches for surrogate mutants altered in the expression of specific mitochondrial genes by predicting the factor that binds to a desired target site and then analysing the relevant mutants [98] [97]. Some of these surrogate mutants will turn out to

have lethal phenotypes, but there are published methods to partially complement such mutants to allow their analysis [99].

Taking a step further, it should be possible in the near future to design RNA binding factors using the PPR 'code' [96] [97] to target sites in transcripts that are not normally subject to processing events for which the desired mutants can be obtained. This opens up the possibility of deliberately creating desired surrogate mutants by (for example) targeting an editing factor to alter a specific codon that is not naturally edited.

In conclusion, surrogate mitochondrial genome mutants form an under-used resource that has lots to offer researchers interested in the biogenesis and function of this organelle. We believe that most researchers interested in the physiological functions of mitochondria have not fully realised the opportunities offered by these mutants, whereas the researchers that are discovering and characterising these mutants are more interested in the molecular details of organelle gene expression than they are in broader, questions of physiological function. Our hope is that this review will stimulate the two groups to discuss how to work together for mutual benefit.

- [1] D. Gagliardi, S. Binder, Expression of the plant mitochondrial genome., in: D. Logan (Ed.), Plant Mitochondria, Blackwell Publishing, 2007, pp. 50-95.
- [2] A. Barkan, Expression of Plastid Genes: Organelle-Specific Elaborations on a Prokaryotic Scaffold, Plant Physiol. 155 (2011) 1520-1532.
- [3] I. Tzafrir, A. Dickerman, O. Brazhnik, Q. Nguyen, J. McElver, C. Frye, D. Patton, D. Meinke, The Arabidopsis SeedGenes Project, Nucleic Acids Res. 31 (2003) 90-93.

- [4] I. Tzafrir, R. Pena-Muralla, A. Dickerman, M. Berg, R. Rogers, S. Hutchens, T.C. Sweeney, J. McElver, G. Aux, D. Patton, D. Meinke, Identification of genes required for embryo development in Arabidopsis, *Plant Physiol.* 135 (2004) 1206-1220.
- [5] E.H. Meyer, P. Giegé, E. Gelhaye, N. Rayapuram, U. Ahuja, L. Thony-Meyer, J.M. Grienenberger, G. Bonnard, AtCCMH, an essential component of the c-type cytochrome maturation pathway in Arabidopsis mitochondria, interacts with apocytochrome c, *Proc. Natl Acad. Sci. USA* 102 (2005) 16113-16118.
- [6] N. Bryant, J. Lloyd, C. Sweeney, F. Myouga, D. Meinke, Identification of nuclear genes encoding chloroplast-localized proteins required for embryo development in Arabidopsis, *Plant Physiol.* 155 (2011) 1678-1689.
- [7] C.D. Chase, Cytoplasmic male sterility: a window to the world of plant mitochondrial-nuclear interactions, *Trends Genet.* 23 (2007) 81-90.
- [8] V. Zaegel, B. Guermann, M. Le Ret, C. Andres, D. Meyer, M. Erhardt, J. Canaday, J.M. Gualberto, P. Imbault, The plant-specific ssDNA binding protein OSB1 is involved in the stoichiometric transmission of mitochondrial DNA in Arabidopsis, *Plant Cell* 18 (2006) 3548-3563.
- [9] A.P.S. Sandhu, R.V. Abdelnoor, S.A. Mackenzie, Transgenic induction of mitochondrial rearrangements for cytoplasmic male sterility in crop plants, *Proc. Natl Acad. Sci. USA* 104 (2007) 1766-1770.
- [10] V. Shedge, M. Arrieta-Montiel, A.C. Christensen, S.A. Mackenzie, Plant mitochondrial recombination surveillance requires unusual RecA and MutS homologs, *Plant Cell* 19 (2007) 1251-1264.

- [11] P. Bergman, J. Edqvist, I. Farbos, K. Glimelius, Male-sterile tobacco displays abnormal mitochondrial atp1 transcript accumulation and reduced floral ATP/ADP ratio, *Plant Mol. Biol.* 42 (2000) 531-544.
- [12] S. Bonhomme, F. Budar, D. Lancelin, I. Small, M.C. Defrance, G. Pelletier, Sequence and transcript analysis of the Nco2.5 Ogura-specific fragment correlated with cytoplasmic male sterility in Brassica cybrids, *Mol. Gen. Genet.* 235 (1992) 340-348.
- [13] R. Zoschke, K. Liere, T. Börner, From seedling to mature plant: Arabidopsis plastidial genome copy number, RNA accumulation and transcription are differentially regulated during leaf development, *Plant J.* 50 (2007) 710-722.
- [14] M.B. Sheahan, D.W. McCurdy, R.J. Rose, Mitochondria as a connected population: ensuring continuity of the mitochondrial genome during plant cell dedifferentiation through massive mitochondrial fusion, *Plant J.* 44 (2005) 744-755.
- [15] A.J. Bendich, Why Do Chloroplasts and Mitochondria Contain So Many Copies of Their Genome, *Bioessays* 6 (1987) 279-282.
- [16] Z. Svab, P. Hajdukiewicz, P. Maliga, Stable Transformation of Plastids in Higher-Plants, *Proc. Natl Acad. Sci. USA* 87 (1990) 8526-8530.
- [17] P. Maliga, R. Bock, Plastid Biotechnology: Food, Fuel, and Medicine for the 21st Century, *Plant Physiol.* 155 (2011) 1501-1510.
- [18] C. Schmitz-Linneweber, I. Small, Pentatricopeptide repeat proteins: a socket set for organelle gene expression, *Trends Plant Sci.* 13 (2008) 663-670.

- [19] Y. Asakura, A. Barkan, Arabidopsis orthologs of maize chloroplast splicing factors promote splicing of orthologous and species-specific group II introns, *Plant Physiol.* 142 (2006) 1656-1663.
- [20] T.S. Kroeger, K.P. Watkins, G. Friso, K.J. van Wijk, A. Barkan, A plant-specific RNA-binding domain revealed through analysis of chloroplast group II intron splicing, *Proc. Natl Acad. Sci. USA* 106 (2009) 4537-4542.
- [21] K. Kühn, C. Carrie, E. Giraud, Y. Wang, E.H. Meyer, R. Narsai, C. Colas des Francs-Small, B.T. Zhang, M.W. Murcha, J. Whelan, The RCC1 family protein RUG3 is required for splicing of nad2 and complex I biogenesis in mitochondria of *Arabidopsis thaliana*, *Plant J.* 67 (2011) 1067-1080.
- [22] M. Takenaka, A. Zehrmann, D. Verbitskiy, M. Kugelman, B. Hartel, A. Brennicke, Multiple organellar RNA editing factor (MORF) family proteins are required for RNA editing in mitochondria and plastids of plants, *Proc. Natl Acad. Sci. USA* 109 (2012) 5104-5109.
- [23] S. Bentolila, W.P. Heller, T. Sun, A.M. Babina, G. Friso, K.J. van Wijk, M.R. Hanson, RIP1, a member of an Arabidopsis protein family, interacts with the protein RARE1 and broadly affects RNA editing, *Proc. Natl Acad. Sci. USA* 109 (2012) E1453-1461.
- [24] S. Bentolila, J. Oh, M.R. Hanson, R. Bukowski, Comprehensive High-Resolution Analysis of the Role of an Arabidopsis Gene Family in RNA Editing, *PLoS Genet.* 9 (2013) e1003584.
- [25] J.F. Allen, J.A. Raven, Free-radical-induced mutation vs redox regulation: costs and benefits of genes in organelles, *J. Mol. Evol.* 42 (1996) 482-492.

- [26] B.J. Pogson, N.S. Woo, B. Forster, I.D. Small, Plastid signalling to the nucleus and beyond, *Trends Plant Sci.* 13 (2008) 602-609.
- [27] E. Delannoy, S. Fujii, C. Colas des Francs-Small, M. Brundrett, I. Small, Rampant gene loss in the underground orchid *Rhizanthella gardneri* highlights evolutionary constraints on plastid genomes, *Mol. Biol. Evol.* 28 (2011) 2077-2086.
- [28] A.O. Richardson, D.W. Rice, G.J. Young, A.J. Alverson, J.D. Palmer, The "fossilized" mitochondrial genome of *Liriodendron tulipifera*: ancestral gene content and order, ancestral editing sites, and extraordinarily low mutation rate, *BMC Biol.* 11 (2013) 29.
- [29] J. Klodmann, H.P. Braun, Proteomic approach to characterize mitochondrial complex I from plants, *Phytochem.* 72 (2011) 1071-1080.
- [30] E.H. Meyer, C. Solheim, S.K. Tanz, G. Bonnard, A.H. Millar, Insights into the composition and assembly of the membrane arm of plant complex I through analysis of subcomplexes in *Arabidopsis* mutant lines, *J. Biol. Chem.* 286 (2011) 26081-26092.
- [31] K. Peters, K. Belt, H.P. Braun, 3D Gel Map of *Arabidopsis* Complex I, *Front. Plant Sci.* 4 (2013) 153.
- [32] L. Bonen, Cis- and trans-splicing of group II introns in plant mitochondria, *Mitochondrion* 8 (2008) 26-34.
- [33] E.H. Meyer, Proteomic investigations of complex I composition: how to define a subunit?, *Front. Plant Sci.* 3 (2012) 106.
- [34] J. Klodmann, S. Sunderhaus, M. Nimtz, L. Jansch, H.P. Braun, Internal architecture of mitochondrial complex I from *Arabidopsis thaliana*, *Plant Cell* 22 (2010) 797-810.

- [35] B.H. Lee, H.J. Lee, L.M. Xiong, J.K. Zhu, A mitochondrial complex I defect impairs cold-regulated nuclear gene expression, *Plant Cell* 14 (2002) 1235-1251.
- [36] M. Perales, H. Eubel, J. Heinemeyer, A. Colaneri, E. Zabaleta, H.P. Braun, Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces complex I and supercomplex I+III₂ levels and alters mitochondrial physiology in *Arabidopsis*, *J. Mol. Biol.* 350 (2005) 263-277.
- [37] E.H. Meyer, T. Tomaz, A.J. Carroll, G. Estavillo, E. Delannoy, S.K. Tanz, I.D. Small, B.J. Pogson, A.H. Millar, Remodeled Respiration in *ndufs4* with Low Phosphorylation Efficiency Suppresses *Arabidopsis* Germination and Growth and Alters Control of Metabolism at Night, *Plant Physiol.* 151 (2009) 603-619.
- [38] M. Pla, C. Mathieu, R. DePaepe, P. Chetrit, F. Vedel, Deletion of the Last 2 Exons of the Mitochondrial *Nad7* Gene Results in Lack of the *Nad7* Polypeptide in a *Nicotiana-Sylvestris* *Cms* Mutant, *Mol. Gen. Genet.* 248 (1995) 79-88.
- [39] S. Gutierrez, M. Sabar, C. Lelandais, P. Chetrit, P. Diolez, H. Degand, M. Boutry, F. Vedel, Y. de Kouchkovsky, R. DePaepe, Lack of mitochondrial and nuclear-encoded subunits of complex I and alteration of the respiratory chain in *Nicotiana sylvestris* mitochondrial deletion mutants, *Proc. Natl Acad. Sci. USA* 94 (1997) 3436-3441.
- [40] J.R. Marienfeld, K.J. Newton, The Maize *Ncs2* Abnormal Growth Mutant Has a Chimeric *Nad4-Nad7* Mitochondrial Gene and Is Associated with Reduced Complex-I Function, *Genetics* 138 (1994) 855-863.

- [41] O.V. Karpova, K.J. Newton, A partially assembled complex I in NAD4-deficient mitochondria of maize, *Plant J.* 17 (1999) 511-521.
- [42] D.C. Wallace, Mitochondrial diseases in man and mouse, *Science* 283 (1999) 1482-1488.
- [43] J. Brangeon, M. Sabar, S. Gutierrez, B. Combettes, J. Bove, C. Gendy, P. Chetrit, C. Colas Francis-Small, M. Pla, F. Vedel, R. De Paepe, Defective splicing of the first nad4 intron is associated with lack of several complex I subunits in the *Nicotiana sylvestris* NMS1 nuclear mutant, *Plant J.* 21 (2000) 269-280.
- [44] A. Falcon de Longevialle, E.H. Meyer, C. Andres, N.L. Taylor, C. Lurin, A.H. Millar, I.D. Small, The pentatricopeptide repeat gene OTP43 is required for trans-splicing of the mitochondrial nad1 Intron 1 in *Arabidopsis thaliana*, *Plant Cell* 19 (2007) 3256-3265.
- [45] A. Zehrmann, D. Verbitskiy, J.A. van der Merwe, A. Brennicke, M. Takenaka, A DYW Domain-Containing Pentatricopeptide Repeat Protein Is Required for RNA Editing at Multiple Sites in Mitochondria of *Arabidopsis thaliana*, *Plant Cell* 21 (2009) 558-567.
- [46] S. Bentolila, W. Knight, M. Hanson, Natural Variation in *Arabidopsis* Leads to the Identification of REME1, a Pentatricopeptide Repeat-DYW Protein Controlling the Editing of Mitochondrial Transcripts, *Plant Physiol.* 154 (2010) 1966-1982.
- [47] Y. Liu, J.N. He, Z.Z. Chen, X.Z. Ren, X.H. Hong, Z.Z. Gong, ABA overly-sensitive 5 (ABO5), encoding a pentatricopeptide repeat protein required for cis-splicing of mitochondrial nad2 intron 3, is involved in the abscisic acid response in *Arabidopsis*, *Plant J.* 63 (2010) 749-765.

- [48] S. Ohtani, M. Ichinose, E. Tasaki, Y. Aoki, Y. Komura, M. Sugita, Targeted gene disruption identifies three PPR-DYW proteins involved in RNA editing for five editing sites of the moss mitochondrial transcripts, *Plant Cell Physiol.* 51 (2010) 1942-1949.
- [49] M. Takenaka, D. Verbitskiy, A. Zehrmann, A. Brennicke, Reverse Genetic Screening Identifies Five E-class PPR Proteins Involved in RNA Editing in Mitochondria of *Arabidopsis thaliana*, *J. Biol. Chem.* 285 (2010) 27122-27129.
- [50] H. Yuan, D. Liu, Functional disruption of the pentatricopeptide protein SLG1 affects mitochondrial RNA editing, plant development, and responses to abiotic stresses in *Arabidopsis*, *Plant J.* 70 (2012) 432-444.
- [51] T.Y. Sung, C.C. Tseng, M.H. Hsieh, The SLO1 PPR protein is required for RNA editing at multiple sites with similar upstream sequences in *Arabidopsis* mitochondria, *Plant J.* 63 (2010) 499-511.
- [52] A. Holzle, C. Jonietz, O. Torjek, T. Altmann, S. Binder, J. Forner, A RESTORER OF FERTILITY-like PPR gene is required for 5'-end processing of the nad4 mRNA in mitochondria of *Arabidopsis thaliana*, *Plant J.* 65 (2011) 737-744.
- [53] M. Murayama, S. Hayashi, N. Nishimura, M. Ishide, K. Kobayashi, Y. Yagi, T. Asami, T. Nakamura, K. Shinozaki, T. Hirayama, Isolation of *Arabidopsis* ahg11, a weak ABA hypersensitive mutant defective in nad4 RNA editing, *J. Exp. Bot.* 63 (2012) 5301-5310.
- [54] N. Haïli, N. Arnal, M. Quadrado, S. Amiar, G. Tcherkez, J. Dahan, P. Briozzo, C. Colas des Francs-Small, V. N., H. Mireau, The pentatricopeptide

repeat MTSF1 protein stabilizes the nad4 mRNA in Arabidopsis mitochondria, Nucleic Acids Res. 41 (2013) 6650-6663.

[55] M. Uchida, S. Ohtani, M. Ichinose, C. Sugita, M. Sugita, The PPR-DYW proteins are required for RNA editing of rps14, cox1 and nad5 transcripts in Physcomitrella patens mitochondria, FEBS Lett. 585 (2011) 2367-2371.

[56] D. Sosso, S. Mbelo, V. Vernoud, G. Gendrot, A. Dedieu, P. Chambrier, M. Dauzat, L. Heurtevin, V. Guyon, M. Takenaka, P.M. Rogowsky, PPR2263, a DYW-Subgroup Pentatricopeptide repeat protein, is required for mitochondrial nad5 and cob transcript editing, mitochondrion biogenesis, and maize growth, Plant Cell 24 (2012) 676-691.

[57] T. Toda, S. Fujii, K. Noguchi, T. Kazama, K. Toriyama, Rice MPR25 encodes a pentatricopeptide repeat protein and is essential for RNA editing of nad5 transcripts in mitochondria, Plant J. 72 (2012) 450-460.

[58] D. Verbitskiy, A. Zehrmann, B. Haertel, A. Brennicke, M. Takenaka, Two related RNA editing proteins target the same sites in mitochondria of Arabidopsis thaliana, J. Biol. Chem. 287 (2012) 38064-38072.

[59] A. Koprivova, C. Colas des Francs-Small, G. Calder, S.T. Mugford, S. Tanz, B.R. Lee, B. Zechmann, I. Small, S. Kopriva, Identification of a pentatricopeptide repeat protein implicated in splicing of intron 1 of mitochondrial nad7 transcripts, J. Biol. Chem. 285 (2010) 32192-32199.

[60] M. Takenaka, MEF9, an E-Subclass Pentatricopeptide Repeat Protein, Is Required for an RNA Editing Event in the nad7 Transcript in Mitochondria of Arabidopsis, Plant Physiol. 152 (2010) 939-947.

- [61] B. Pineau, O. Layoune, A. Danon, R. De Paepe, L-galactono-1,4-lactone dehydrogenase is required for the accumulation of plant respiratory complex I, *J. Biol. Chem.* 283 (2008) 32500-32505.
- [62] P. Cardol, R.F. Matagne, C. Remacle, Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in *Chlamydomonas*. Implication for the structural organization of the enzyme, *J. Mol. Biol.* 319 (2002) 1211-1221.
- [63] P. Schertl, S. Sunderhaus, J. Klodmann, G.E. Grozeff, C.G. Bartoli, H.P. Braun, L-galactono-1,4-lactone dehydrogenase (GLDH) forms part of three subcomplexes of mitochondrial complex I in *Arabidopsis thaliana*, *J. Biol. Chem.* 287 (2012) 14412-14419.
- [64] H.P. Braun, U.K. Schmitz, The bifunctional cytochrome c reductase/processing peptidase complex from plant mitochondria, *J. Bioenerg. Biomembr.* 27 (1995) 423-436.
- [65] A.H. Millar, H. Eubel, L. Jansch, V. Kruff, J.L. Heazlewood, H.P. Braun, Mitochondrial cytochrome c oxidase and succinate dehydrogenase complexes contain plant specific subunits, *Plant Mol. Biol.* 56 (2004) 77-90.
- [66] A. Barrientos, D. Pierre, J. Lee, A. Tzagoloff, Cytochrome oxidase assembly does not require catalytically active cytochrome C, *J. Biol. Chem.* 278 (2003) 8881-8887.
- [67] P. Giegé, J.M. Grienberger, G. Bonnard, Cytochrome c biogenesis in mitochondria, *Mitochondrion* 8 (2008) 61-73.
- [68] J. Gu, S. Dempsey, K.J. Newton, Rescue of a maize mitochondrial cytochrome oxidase mutant by tissue culture, *Plant J.* 6 (1994) 787-794.

- [69] I. Steinebrunner, M. Landschreiber, U. Krause-Buchholz, J. Teichmann, G. Rodel, HCC1, the Arabidopsis homologue of the yeast mitochondrial copper chaperone SCO1, is essential for embryonic development, *J. Exp. Bot.* 62 (2011) 319-330.
- [70] M.P. Dorthu, S. Rémy, M.R. Michel-Wolwertz, L. Colleaux, D. Breyer, M.C. Beckers, S. Englebert, C. Duyckaerts, F.E. Sluse, R.F. Matagne, Biochemical, genetic and molecular characterization of new respiratory-deficient mutants in *Chlamydomonas reinhardtii*, *Plant Mol. Biol.* 18 (1992) 759-772.
- [71] E. Welchen, T.M. Hildebrandt, D. Lewejohann, D.H. Gonzalez, H.P. Braun, Lack of cytochrome c in Arabidopsis decreases stability of Complex IV and modifies redox metabolism without affecting Complexes I and III, *Biochim. Biophys. Acta* 1817 (2012) 990-1001.
- [72] L. Zsigmond, G. Rigo, A. Szarka, G. Szekely, K. Otvos, Z. Darula, K.F. Medzihradszky, C. Koncz, Z. Koncz, L. Szabados, Arabidopsis PPR40 connects abiotic stress responses to mitochondrial electron transport, *Plant Physiol.* 146 (2008) 1721-1737.
- [73] M. Ichinose, E. Tasaki, C. Sugita, M. Sugita, A PPR-DYW protein is required for splicing of a group II intron of *cox1* pre-mRNA in *Physcomitrella patens*, *Plant J.* 70 (2012) 271-278.
- [74] C. Colas des Francs-Small, T. Kroeger, M. Zmudjak, O. Ostersetzer-Biran, N. Rahimi, I. Small, A. Barkan, A PORR domain protein required for *rpl2* and *ccmF_C* intron splicing and for the biogenesis of c-type cytochromes in Arabidopsis mitochondria, *Plant J.* 69 (2012) 996-1005.

- [75] E. Tasaki, M. Sugita, The moss *Physcomitrella patens*, a model plant for the study of RNA editing in plant organelles, *Plant Signal Behav.* 5 (2010) 727-729.
- [76] C. Jonietz, J. Forner, T. Hildebrandt, S. Binder, RNA PROCESSING FACTOR3 Is Crucial for the Accumulation of Mature ccmC Transcripts in Mitochondria of *Arabidopsis* Accession Columbia, *Plant Physiol.* 157 (2011) 1430-1439.
- [77] C. Jonietz, J. Forner, A. Holzle, S. Thuss, S. Binder, RNA PROCESSING FACTOR2 Is Required for 5' End Processing of *nad9* and *cox3* mRNAs in Mitochondria of *Arabidopsis thaliana*, *Plant Cell* 22 (2010) 443-453.
- [78] N.V. Dudkina, J. Heinemeyer, W. Keegstra, E.J. Boekema, H.P. Braun, Structure of dimeric ATP synthase from mitochondria: an angular association of monomers induces the strong curvature of the inner membrane, *FEBS Lett.* 579 (2005) 5769-5772.
- [79] M. Rak, E. Tetaud, F. Godard, I. Sagot, B. Salin, S. Duvezin-Caubet, P.P. Slonimski, J. Rytka, J.P. di Rago, Yeast cells lacking the mitochondrial gene encoding the ATP synthase subunit 6 exhibit a selective loss of complex IV and unusual mitochondrial morphology, *J. Biol. Chem.* 282 (2007) 10853-10864.
- [80] M. Lapaille, M. Thiry, E. Perez, D. Gonzalez-Halphen, C. Remacle, P. Cardol, Loss of mitochondrial ATP synthase subunit beta (*Atp2*) alters mitochondrial and chloroplastic function and morphology in *Chlamydomonas*, *Biochim. Biophys. Acta* 1797 (2010) 1533-1539.

- [81] K. Hammani, C. Colas des Francs-Small, M. Takenaka, S.K. Tanz, K. Okuda, T. Shikanai, A. Brennicke, I. Small, The pentatricopeptide repeat protein OTP87 is essential for RNA editing of nad7 and atp1 transcripts in Arabidopsis mitochondria, *J. Biol. Chem.* (2011).
- [82] D. Verbitskiy, J.A. Merwe, A. Zehrmann, B. Hartel, M. Takenaka, The E-class PPR protein MEF3 of Arabidopsis thaliana can also function in mitochondrial RNA editing with an additional DYW domain, *Plant Cell Physiol.* 53 (2012) 358-367.
- [83] T. Kubo, K.J. Newton, Angiosperm mitochondrial genomes and mutations, *Mitochondrion* 8 (2008) 5-14.
- [84] S.Y. Gerdes, M.D. Scholle, J.W. Campbell, G. Balazsi, E. Ravasz, M.D. Daugherty, A.L. Somera, N.C. Kyrpides, I. Anderson, M.S. Gelfand, A. Bhattacharya, V. Kapatral, M. D'Souza, M.V. Baev, Y. Grechkin, F. Mseeh, M.Y. Fonstein, R. Overbeek, A.L. Barabasi, Z.N. Oltvai, A.L. Osterman, Experimental determination and system level analysis of essential genes in Escherichia coli MG1655, *J. Bacteriol.* 185 (2003) 5673-5684.
- [85] M.D. Hunt, K.J. Newton, The NCS3 mutation: genetic evidence for the expression of ribosomal protein genes in Zea mays mitochondria, *EMBO J.* 10 (1991) 1045-1052.
- [86] K.J. Newton, J.M. Mariano, C.M. Gibson, E. Kuzmin, S. Gabay-Laughnan, Involvement of S2 episomal sequences in the generation of NCS4 deletion mutation in maize mitochondria, *Dev. Genet.* 19 (1996) 277-286.
- [87] N. Manavski, V. Guyon, J. Meurer, U. Wienand, R. Brettschneider, An essential pentatricopeptide repeat protein facilitates 5' maturation and

translation initiation of rps3 mRNA in maize mitochondria, *Plant Cell* 24 (2012) 3087-3105.

- [88] Y. Doniwa, M. Ueda, M. Ueta, A. Wada, K. Kadowaki, N. Tsutsumi, The involvement of a PPR protein of the P subfamily in partial RNA editing of an *Arabidopsis* mitochondrial transcript, *Gene* 454 (2010) 39-46.
- [89] Y. Choquet, F.A. Wollman, Translational regulations as specific traits of chloroplast gene expression, *FEBS Lett.* 529 (2002) 39-42.
- [90] K. Wostrikoff, J. Girard-Bascou, F.A. Wollman, Y. Choquet, Biogenesis of PSI involves a cascade of translational autoregulation in the chloroplast of *Chlamydomonas*, *EMBO J.* 23 (2004) 2696-2705.
- [91] K.M. Calder, J.E. McEwen, Deletion of the COX7 gene in *Saccharomyces cerevisiae* reveals a role for cytochrome c oxidase subunit VII in assembly of remaining subunits, *Mol. Microbiol.* 5 (1991) 1769-1777.
- [92] K. Wostrikoff, D. Stern, Rubisco large-subunit translation is autoregulated in response to its assembly state in tobacco chloroplasts, *Proc. Natl Acad. Sci. USA* 104 (2007) 6466-6471.
- [93] W. Cai, D. Ji, L. Peng, J. Guo, J. Ma, M. Zou, C. Lu, L. Zhang, LPA66 is required for editing psbF chloroplast transcripts in *Arabidopsis*, *Plant Physiol.* 150 (2009) 1260-1271.
- [94] D.J. McCormac, A. Barkan, A nuclear gene in maize required for the translation of the chloroplast atpB/E mRNA, *Plant Cell* 11 (1999) 1709-1716.
- [95] R. Zoschke, K.P. Watkins, A. Barkan, A Rapid Ribosome Profiling Method Elucidates Chloroplast Ribosome Behavior in Vivo, *Plant Cell* (2013).

- [96] A. Barkan, M. Rojas, S. Fujii, A. Yap, Y.S. Chong, C.S. Bond, I. Small, A combinatorial amino acid code for RNA recognition by pentatricopeptide repeat proteins, *PLoS Genet.* 8 (2012) e1002910.
- [97] Y. Yagi, S. Hayashi, K. Kobayashi, T. Hirayama, T. Nakamura, Elucidation of the RNA recognition code for pentatricopeptide repeat proteins involved in organelle RNA editing in plants, *PLoS One* 8 (2013) e57286.
- [98] M. Takenaka, A. Zehrmann, A. Brennicke, K. Graichen, Improved computational target site prediction for pentatricopeptide repeat RNA editing factors, *PLoS One* 8 (2013) e65343.
- [99] B. Despres, M. Delseny, M. Devic, Partial complementation of embryo defective mutations: a general strategy to elucidate gene function, *Plant J.* 27 (2001) 149-159.
- [100] B. Hartel, A. Zehrmann, D. Verbitskiy, J.A. van der Merwe, A. Brennicke, M. Takenaka, MEF10 is required for RNA editing at nad2-842 in mitochondria of *Arabidopsis thaliana* and interacts with MORF8, *Plant Mol. Biol.* 81 (2013) 337-346.
- [101] A. Hauler, C. Jonietz, B. Stoll, K. Stoll, H.P. Braun, S. Binder, RNA PROCESSING FACTOR 5 is required for efficient 5' cleavage at a processing site conserved in RNAs of three different mitochondrial genes in *Arabidopsis thaliana*, *Plant J.* (2013).
- [102] L. Zsigmond, A. Szepesi, I. Tari, G. Rigo, A. Kiraly, L. Szabados, Overexpression of the mitochondrial PPR40 gene improves salt tolerance in *Arabidopsis*, *Plant Sci.* 182 (2012) 87-93.
- [103] A.L. Chateigner-Boutin, C. Colas des Francs-Small, S. Fujii, K. Okuda, S.K. Tanz, I. Small, The E domains of pentatricopeptide repeat proteins from

different organelles are not functionally equivalent for RNA editing, *Plant J.* 74 (2013) 935-945.

[104] D. Verbitskiy, B. Hartel, A. Zehrmann, A. Brennicke, M. Takenaka, The DYW-E-PPR protein MEF14 is required for RNA editing at site matR-1895 in mitochondria of *Arabidopsis thaliana*, *FEBS Lett.* 585 (2011) 700-704.

[105] K. Laluk, S. Abuqamar, T. Mengiste, The *Arabidopsis* mitochondria-localized pentatricopeptide repeat protein PGN functions in defense against necrotrophic fungi and abiotic stress tolerance, *Plant Physiol.* 156 (2011) 2053-2068.

[106] I. Keren, L. Tal, C.C. des Francs-Small, W.L. Araujo, S. Shevtsov, F. Shaya, A.R. Fernie, I. Small, O. Ostersetzer-Biran, nMAT1, a nuclear-encoded maturase involved in the trans-splicing of nad1 intron 1, is essential for mitochondrial complex I assembly and function, *Plant J.* 71 (2012) 413-426.

[107] I. Keren, A. Bezawork-Geleta, M. Kolton, I. Maayan, E. Belausov, M. Levy, A. Mett, D. Gidoni, F. Shaya, O. Ostersetzer-Biran, AtnMat2, a nuclear-encoded maturase required for splicing of group-II introns in *Arabidopsis* mitochondria, *RNA* 15 (2009) 2299-2311.

[108] D. Kohler, S. Schmidt-Gattung, S. Binder, The DEAD-box protein PMH2 is required for efficient group II intron splicing in mitochondria of *Arabidopsis thaliana*, *Plant Mol. Biol.* 72 (2010) 459-467.

[109] S.R. Kim, J.I. Yang, S. Moon, C.H. Ryu, K. An, K.M. Kim, J. Yim, G. An, Rice OGR1 encodes a pentatricopeptide repeat-DYW protein and is essential for RNA editing in mitochondria, *Plant J.* 59 (2009) 738-749.

- [110] K. Kobayashi, M. Suzuki, J.W. Tang, N. Nagata, K. Ohya, H. Seki, R. Kiuchi, Y. Kaneko, M. Nakazawa, M. Matsui, S. Matsumoto, S. Yoshida, T. Muranaka, LOVASTATIN INSENSITIVE 1, a novel pentatricopeptide repeat protein, is a potential regulatory factor of isoprenoid biosynthesis in *Arabidopsis*, *Plant Cell Physiol.* 48 (2007) 322-331.
- [111] D. Verbitskiy, A. Zehrmann, J.A. van der Merwe, A. Brennicke, M. Takenaka, The PPR protein encoded by the LOVASTATIN INSENSITIVE 1 gene is involved in RNA editing at three sites in mitochondria of *Arabidopsis thaliana*, *Plant J.* 61 (2010) 446-455.
- [112] Q. Zhu, J. Dugardeyn, C. Zhang, M. Takenaka, K. Kuhn, C. Craddock, J. Smalle, M. Karampelias, J. Denecke, J. Peters, T. Gerats, A. Brennicke, P. Eastmond, E.H. Meyer, D. Van Der Straeten, SLO2, a mitochondrial pentatricopeptide repeat protein affecting several RNA editing sites, is required for energy metabolism, *Plant J.* 71 (2012) 836-849.
- [113] Q. Zhu, H.M. E, D. Van Der Straeten, Functional analysis of SLO2 provides new insight into the role of plant PPR proteins, *Plant Signal Behav.* 7 (2012).
- [114] A. Zehrmann, J. van der Merwe, D. Verbitskiy, B. Hartel, A. Brennicke, M. Takenaka, The DYW-class PPR protein MEF7 is required for RNA editing at four sites in mitochondria of *Arabidopsis thaliana*, *RNA Biol.* 9 (2012) 155-161.
- [115] M. Rudinger, P. Kindgren, A. Zehrmann, I. Small, V. Knoop, A DYW-protein knockout in *Physcomitrella* affects two closely spaced mitochondrial editing sites and causes a severe developmental phenotype, *Plant J.* (2013).

- [116] B. Hartel, A. Zehrmann, D. Verbitskiy, M. Takenaka, The longest mitochondrial RNA editing PPR protein MEF12 in *Arabidopsis thaliana* requires the full-length E domain, *RNA Biol.* 10 (2013).
- [117] M.A. Arenas, M. Takenaka, S. Moreno, I. Gomez, X. Jordana, Contiguous RNA editing sites in the mitochondrial nad1 transcript of *Arabidopsis thaliana* are recognized by different proteins, *FEBS Lett.* 587 (2013) 887-891.
- [118] Y.J. Liu, Z.H. Xiu, R. Meeley, B.C. Tan, Empty pericarp5 encodes a pentatricopeptide repeat protein that is required for mitochondrial RNA editing and seed development in maize, *Plant Cell* 25 (2013) 868-883.
- [119] S. Bentolila, A.M. Babina, A. Germain, M.R. Hanson, Quantitative trait locus mapping identifies REME2, a PPR-DYW protein required for editing of specific C targets in *Arabidopsis* mitochondria, *RNA Biol.* 10 (2013).
- [120] M. Zmudjak, C. Colas des Francs-Small, I. Keren, F. Shaya, E. Belausov, I. Small, O. Ostersetzer-Biran, mCSF1, a nucleus-encoded CRM protein required for the processing of many mitochondrial introns, is involved in the biogenesis of respiratory complexes I and IV in *Arabidopsis*, *New Phytol.* 199 (2013) 379-394.

FIGURE LEGENDS

Figure 1. Mitochondrial electron transfer chain (ETC) and surrogate mutants

The diagram shows a simplified version of the ETC, with the main complexes and a list of the surrogate mutants affected in their assembly. CI: complex I (NADH-ubiquinone oxidoreductase); CII: complex II (FADH₂-ubiquinone oxidoreductase or succinate dehydrogenase); UQ: ubiquinone; CIII: complex III (ubiquinone-cytochrome c oxidoreductase); c: cytochrome c; CIV: complex IV (cytochrome c oxidase). The number of mitochondrially encoded subunits for each complex is indicated in brackets. The alternative dehydrogenases and AOX were omitted. M: matrix, IM: inner membrane, IMS: inner membrane space.

Table 1: A list of surrogate mutants with the complex and transcript affected, their species name or AGI number for Arabidopsis. Brief descriptions of the phenotypes as well as an indication of protein data when available are given. n/a means that no investigation on protein abundance or complex assembly has been performed. Each mutant is cited once per complex, even if altered in 2 subunits of the same complex. Silent editing sites are in brackets.

Complex I

<i>Transcript</i>	<i>AGI number /organism</i>	<i>mutant</i>	<i>phenotype</i>	<i>defect</i>	<i>complex accumulation</i>	<i>ref</i>
<i>nad1</i>	At1g74900	<i>otp43</i>	slow growth, curled leaves, low fertility	splicing	C I undetected by BN PAGE	[44]
	At3g25060	<i>mef25</i>	no macroscopic phenotype	editing	n/a	[117]
<i>nad2</i>	At1g51965	<i>abo5</i>	slow growth, ABA sensitive, low fertility	splicing	n/a	[47]
	At5g60870	<i>rug3</i>	slow growth, curled leaves	splicing	C I very reduced	[21]
	At3g11460	<i>mef10</i>	no macroscopic phenotype	editing	n/a	[100]
<i>(nad2)</i>	At2g03880	<i>reme1</i>	no macroscopic phenotype	editing	n/a	[46]
<i>nad2,(nad7)+rps4</i>	At5g52630	<i>mef1</i>	no macroscopic phenotype	editing	n/a	[45]
<i>nad3</i>	At3g12770	<i>mef22</i>	no macroscopic phenotype	editing	n/a	[49]
	At5g08490	<i>slg1</i>	slow growth, altered response to abiotic stresses	editing	C I reduced	[50]
<i>nad3+nad4</i>	<i>Physcomitrella</i>	PpPPR_56	slow growth	editing	n/a	[48]
<i>nad4</i>	<i>N. sylvestris</i>	<i>nms1</i>	slow growth, partially male sterile	splicing	C I reduced	[43]
	At2g44880	<i>ahg11</i>	ABA hypersensitive	editing	n/a	[53]
	At5g19020	<i>mef18</i>	no macroscopic phenotype	editing	n/a	[49]
	At1g12700	<i>rpf1</i>	no macroscopic phenotype	processing	n/a	[52]
	At1g06710	<i>mtsf1</i>	slow growth, curled leaves	splicing	C I undetected by BN PAGE	[54]
<i>nad4+nad9</i>	At2g22410	<i>slo1</i>	late germination, severe growth retardation	editing	n/a	[51]
<i>nad5</i>	<i>O. sativa</i>	<i>mpr25</i>	growth retardation, pale green leaves at early stages	editing	C I unchanged	[57]
	<i>Physcomitrella</i>	PpPPR_79	slow growth	editing	n/a	[55]
	<i>Physcomitrella</i>	PpPPR_91	very slow growth	editing	n/a	[48]
	At3g09040	<i>mef12</i>	no macroscopic phenotype	editing	n/a	[116]
<i>nad5+nad6</i>	At2g25580	<i>mef8</i>	no macroscopic phenotype	editing	n/a	[58]
	At4g32450	<i>mef8S</i>	no macroscopic phenotype	editing	n/a	[58]
<i>nad5+cob</i>	<i>Zea mays</i>	<i>ppr2263</i>	late germination, severe growth retardation	editing	C I unchanged, C III reduced	[56]
	At4g30700	<i>mef29</i>	not described	editing	n/a	[56]
<i>nad6</i>	At2g28010	<i>rpf5</i>	late germination, reduced germination capacity	processing	C I unchanged	[101]
<i>nad7</i>	At3g48250	<i>bir6</i>	slightly smaller than WT, glutathione accumulation	splicing	C I reduced	[59]

<i>nad9+cox3</i>	At1g62260	<i>mef9</i>	no macroscopic phenotype	editing	n/a	[60]
	At1g62670	<i>rpf2</i>	no macroscopic phenotype	processing	n/a	[77]
Complex III						
<i>Transcript</i>	<i>AGI number</i> <i>/organism</i>	<i>mutant</i>	<i>phenotype</i>	<i>defect</i>	<i>complex accumulation</i>	<i>ref</i>
?	At3g16890	<i>ppr40</i>	ABA hypersensitivity, growth retardation in ppr40-1	?	C I unchanged	[72, 102]
<i>nad5+cob</i>	<i>Zea mays</i>	<i>mppr2263</i>	late germination, severe growth retardation	editing	C I unchanged, C III reduced	[56]
	At4g30700	<i>mef29</i>	not described	editing	n/a	[56]
Cytochrome c and c1 maturation						
<i>ccmB</i>	At3g05240	<i>mef19</i>	no macroscopic phenotype	editing	n/a	[60]
<i>ccmFN2</i>	At1g64310	<i>otp71</i>	no macroscopic phenotype	editing	n/a	[103]
<i>ccmC</i>	At1g62930	<i>rpf3</i>	no macroscopic phenotype	processing	n/a	[76]
<i>ccmFc</i>	At2g39120	<i>wtf9</i>	very slow growth	splicing	no assembled C III and C IV	[74]
	<i>Physcomitrella</i>	PpPPR_71	very slow growth	editing	n/a	[75]
	<i>Physcomitrella</i>	DYW-PPR_65	very slow growth	editing	n/a	[115]
Complex IV						
<i>cox1</i>	<i>Physcomitrella</i>	PpPPR_43	very slow growth	splicing	n/a	[73]
<i>cox1+rps14</i>	<i>Physcomitrella</i>	PpPPR_78	slow growth	editing		[54]
<i>cox2+cox3</i>	<i>Physcomitrella</i>	PpPPR_77	very slow growth	editing	n/a	[48]
<i>cox3</i>	At2g20540	<i>mef21</i>	no macroscopic phenotype	editing	n/a	[49]
<i>cox3+nad9</i>	At1g62670	<i>rpf2</i>	no macroscopic phenotype	processing	Cox3 unchanged	[77]
ATP synthase						
<i>atp1+(nad7)</i>	At1g74600	<i>otp87</i>	late germination, slow growth	editing	reduced ATPase and F1 complex	[81]
<i>atp4</i>	At1g06140	<i>mef3</i>	no macroscopic phenotype	editing	n/a	[82]
Translation machinery						
<i>rps3</i>	<i>Zea mays</i>	<i>mppr6</i>	growth retardation, slow development	translation	reduced Rps3, low mito translation	[87]
	At1g77360	<i>appr6</i>	growth retardation, slow development	translation	reduced Rps3, low mito translation	[87]

<i>rps3+rps4</i>	At4g15720	<i>reme2</i>	lethal	editing	n/a	[119]
<i>rps4</i>	At3g18970	<i>mef20</i>	no macroscopic phenotype	editing	n/a	[49]
<i>rps4+nad2 (nad7)</i>	At5g52630	<i>mef1</i>	no macroscopic phenotype	editing	n/a	[45]
<i>rps14, cox1</i>	<i>Physcomitrella</i>	PpPPR_78	slow growth	editing		[55]
<i>rps3</i>	<i>Zea mays</i>	<i>mppr596</i>	very slow growth	editing?		[88]
<i>rpl16</i>	At3g13880	<i>otp72</i>	no macroscopic phenotype	editing	n/a	[103]
	<i>Zea mays</i>	<i>emp5</i>	slow growth, defective seeds	editing	n/a	[118]
	<i>Oryza sativa</i>	<i>emp5</i>	slow growth, defective seeds	editing	n/a	[118]

Others

<i>Transcript</i>	<i>AGI number</i> <i>/organism</i>	<i>mutant</i>	<i>phenotype</i>	<i>defect</i>	<i>complex accumulation</i>	<i>ref</i>
<i>matR</i>	At3g26780	<i>mef14</i>	no macroscopic phenotype	editing	n/a	[104]
<i>?</i>	At1g56570	<i>pgn</i>	hypersensitivity to biotic and abiotic stress	<i>?</i>	n/a	[105]

Multiple targets

At1g30010	<i>nMat1 (css1)</i>	sugar hypersensitivity, growth retardation (seedling)	splicing	C I very reduced	[106]
At5g46920	<i>nMat2</i>	low germination, slow growth, low fertility	splicing	Nad 9 reduced	[107]
At4g31010	<i>mcsf1</i>	embryo lethal. RNAi lines slow growth,	splicing	C I and C IV reduced in RNAi lines	[120]
At3g22330	<i>pmh2</i>	no macroscopic phenotype, possibly shorter roots?	splicing	n/a	[108]
<i>Oryza sativa</i>	<i>ogr1</i>	opaque endosperm, late germination, slow growth	editing	n/a	[109]
At4g14850	<i>mef11, loi1</i>	slow growth, slightly shorter roots (no lovastatin)	editing	n/a	[110, 111]
At2g13600	<i>slo2</i>	growth retardation, short roots, late flowering	editing	n/a	[112, 113]
At5g09950	<i>mef7</i>	no macroscopic phenotype	editing	n/a	[114]
multiple	<i>morfs</i>	no macroscopic phenotype	editing	n/a	[22]

Transcript	size (bp)	splicing	editing sites
<i>nad4L</i>	303	no	10
<i>nad9</i>	573	no	7
<i>cob</i>	1182	no	10
<i>ccmB</i>	621	no	39
<i>ccmFN1</i>	1149	no	22
<i>cox2</i>	783	yes	15
<i>atp6</i>	1321	no	1
<i>atp8</i>	477	no	0
<i>atp9</i>	256	no	4
<i>rpl2</i>	1050	yes	3
<i>rpl5</i>	558	no	10
<i>rps7</i>	369	no	1
<i>rps12</i>	378	no	8

Table 2: List of mitochondrial transcripts for which no specific RNA processing factor has been described yet. The size of each transcript as well as whether it has to undergo splicing and the number of known editing sites (according to the site REDIdb, http://biologia.unical.it/py_script/overview.html) are indicated.