Genome Barriers between Nuclei and Mitochondria Exemplified by Cytoplasmic Male Sterility

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Since plants retain genomes of an extremely large size in mitochondria (200–2,400 kb), and mitochondrial protein complexes are comprised of chimeric structures of nuclear- and mitochondrial-encoded subunits, coordination of gene expression between the nucleus and mitochondria is indispensable for sound plant development. It has been well documented that the nucleus regulates organelle gene expression. This regulation is called anterograde regulation. On the other hand, recent studies have demonstrated that signals emitted from organelles regulate nuclear gene expression. This process is known as retrograde signaling. Incompatibility caused by genome barriers between a nucleus and foreign mitochondria destines the fate of pollen to be dead in cytoplasmic male sterility (CMS), and studies of CMS confirm that pollen fertility is associated with anterograde/retrograde signaling. This review summarizes the current perspectives in CMS and fertility restoration, mainly from the viewpoint of anterograde/retrograde signaling.

Key words: Anterograde signaling • Cytoplasmic male sterility • Mitochondria • Retrograde signaling.

Abbreviations: AOX, alternative oxidase; CMS, cytoplasmic male sterility; MCAG, mitochondrial CMS-associated gene; PCD, programmed cell death; Rf, fertility restorer gene.

Introduction

Since its first observation in maize (Rhoades 1931), cytoplasmic male sterility (CMS) has been one of the best models for maternally inherited traits in plants. The phenomenon is largely utilized for hybrid breeding programs, taking advantage of its male sterility. CMS phenotypes are diverse even within species (Schnable and Wise 1998, Hanson and Bentolila 2004). For example, pollen-specific dysfunction occurs in S-type CMS maize and BT-type CMS rice, while anther development defective phenotypes are observed in T-type CMS maize and WA-type CMS rice. The former are restored gametophytically, and the latter are restored sporophytically by its corresponding nuclear-encoded fertility restorer genes (RfS). There is another class of CMS types, in which the plants exhibit conversion of stamens into other floral organs, often seen in alloplasmic type CMS strains which are derived from the cross of intergeneric crosses. Common to these CMS is non-mendelian but cytoplasmic inheritance of male organ dysfunction. CMS has been a great mystery to researchers for a number of years, and still presents the important question: do cytoplasmic genomes (mitochondria or plastid) contribute to male development? In 1970, a maize hybrid breeding system utilizing T-cytoplasm was hit by Southern Corn Leaf Blight caused by Helminthosporium maydis race T, which only infects T-cytoplasm but not the other CMS cytoplasts. It was shown later that the H. maydis toxin is only virulent on mitochondria of T-cytoplasm (Millar and Koepppe 1971). Since then, it was presumed that mitochondria are responsible for CMS. Abnormalities in the mitochondria structure during pollen development of T-cytoplasm-carrying maize were observed in an ultrastructural study (Warmke and Lee 1977), and mitochondrial restriction fragment length polymorphisms were observed in the same strain (Levings and Pring 1976). It should be noted here that CMS is distinguished from mitochondrial mutants, because most of the mitochondrial ‘loss-of-function’ mutants express apparent growth deficiency. For instance, maize mitochondrial gene deletional Non-Chromosome Stripe mutants exhibit abnormal leaf architecture and male sterility (Newton et al. 1990, Newton et al. 1996, Yamato and Newton 1999). It has been reported that tobacco CMS-I and CMS-II mutants lost the NAD7 subunit of mitochondrial electron chain complex I via protoplast culture (Cheruit et al. 1992, Pla et al. 1995, Gutierrez et al. 1997). These mutants directly lack genes encoded in mitochondria, and their growth behavior is poor under normal conditions. Arabidopsis css1 (Nakagawa and Sakurai 2006), otp43...
mutants (Falcon de Longevialle et al. 2007), and tobacco nms1 mutant (Brangeon et al. 2000) mimic these phenotypes, except that these mutants carry defects in the nuclear genome and they are indirectly impaired in the expression of mitochondrial nad transcripts. In contrast to these mutants, most of the known CMS strains from different species are remarkably normal in vegetative stages. They exhibit deficiency only in male development, and even female reproduction abilities are usually normal. This feature demonstrates that CMS is presumably caused by a ‘gain-of-function’ phenomenon rather than by a lack of mitochondrial activity. In another words, CMS-specific factors should be present in CMS mitochondria. At present, the key molecules involved in this ‘gain-of-function’ feature are identified from various plant species, and are referred to as mitochondrial CMS-associated gene (or protein) (MCAG) in this review. Since many CMS systems have been derived from intra- or inter-generic cytoplasmic substitutions, it can be assumed that genome barriers exist between nuclei and mitochondria that cause CMS, and MCAG accounts for one of these genome barriers. We will discuss CMS from the viewpoint of anterograde/retrograde signaling between nuclei and mitochondria.

**Unique gene structures found in mitochondrial genome of CMS lines**

The common rules of MCAGs at present are as follows: (i) is unique in CMS mitochondria, and (ii) receives post-transcriptional/translational regulation by nuclear-encoded fertility restorer gene(s). Details on (ii) are mentioned in the later sections. The first evidence of MCAG obtained is T-urf13, a gene encoding a 13-kDa protein found only in T mitochondrial genome in maize (Dewey et al. 1987, 1988). Later it was shown that T-urf13 gene products accumulate in the mitochondrial inner membrane (Hack et al. 1991), and T-URF13 was considered to be a pore-forming protein in the membrane, penetrating and possibly inducing voltage leakage. The genetic evidence of the involvement of T-urf13 in CMS was provided by taking advantage of fertility revertants obtained by cell cultures. Usually, maize T-CMS plants are unable to arise from suspension cells, but gene disruptions in T-urf13 allowed the regeneration of 19 developed plants (Rottman et al. 1987). One of the hallmark revertants contained a 5-bp deletion within the T-urf13 gene, resulting in a formation of premature stop codon (Wise et al. 1987).

A similar MCAG was also found in petunia. Male fertile and sterile lines with recombinant mitochondrial DNA caused by somatic hybrids were used to identify the pcf (petunia CMS-associated fused) locus (Boeshore et al. 1985). PCF protein was later shown to be expressed as 45-kDa protein and processed into the 19-kDa form, and proteins in both forms were present in soluble fractions or loosely attached to membrane (Nivison et al. 1994). A parallel situation was found in I-12 CMS derived from wild beet cytoplasm, in which a unique 12-kDa polypeptide was found by an in organello protein translation experiment (Yamamoto et al. 2008). As in the case of petunia PCF, 12-kDa polypeptide designated as ORF129 was loosely associated with mitochondrial membrane and was also found in the matrix fraction.

Unique MCAG features were found in the same sugar beet species but from a different strain, Owen CMS. The S’ leader sequence of an ATP synthase subunit encoding atp6 was found to encode a variant 35-kDa polypeptide only in Owen CMS mitochondria (Yamamoto et al. 2005). This 35-kDa polypeptide, designated as preSatp6, was found to be membrane localized, and BN-PAGE analysis concluded that it would form a pentamer or hexamer within the inner membrane (Yamamoto et al. 2005).

Genes unique in the CMS lines of radish, sunflower and rice have also been identified. In the case of Ogura CMS, the presence of a 19-kDa protein, ORF138, has been reported to be correlated with CMS (Bonhomme et al. 1992, Grelen et al. 1994). An orthologous sequence has been found from Kosena CMS radish, which strain carried 13 amino acid-truncated ORF125 (Iwabuchi et al. 1999). Sunflower PTT1-CMS line has been reported to carry an ORF comprised of 522 amino acids (orf522), and the 15-kD protein product of the ORF was only detected in the CMS lines (Meneger et al. 1994, Horn et al. 1996). Rice BT-type CMS plants have been known to contain an additional copy of atp6 (designated as B-atp6), and B-atp6 was co-transcribed with an ORF of 79 amino acids (Iwabuchi et al. 1993, Akagi et al. 1994).

Candidates for MCAG are found in variable species other than the ones stated in the above paragraphs (reviewed in Schnable and Wise 1998, Hanson and Bentolila 2004). However, it is often difficult to determine whether these candidate MCAGs are actually responsible for CMS because; (i) CMS mitochondrial genomic sequences frequently contain novel ORFs that are not present in normal mitochondria, and (ii) mitochondrial transformation is currently unavailable in higher plants, hence the direct assessment of MCAG involvement in male sterility is impossible. Recent genomic works have shown that the genomic organization of CMS mitochondria is largely reorganized compared to that of normal plant mitochondrial genomic sequences (Satoh et al. 2004, Allen et al. 2007). Several studies have attempted to introduce MCAG into the nuclear genome, fused with N-terminal mitochondrial targeting pre-sequence (Wintz et al. 1995, Chaumont et al. 1995, He et al. 1996, Duroc et al. 2006, Kim et al. 2007, Yamamoto et al. 2008). Three studies succeeded in producing tobacco or Arabidopsis transgenic lines disrupted in pollen development (He et al. 1996, Kim et al. 2007, Yamamoto et al. 2008), whereas three others failed to identify linkages between the introduced gene and male sterility (Wintz et al. 1995, Chaumont et al. 1995, Duroc et al. 2006).
Although Wintz et al. (1995) introduced pcf into its original species, they could not observe correlations between male defects and pcf expression. The results do not conclude that these candidate MCAG proteins were not causes of CMS, instead these works might suggest that accumulation of these proteins at proper spatial or temporal development stages, and correct sorting of these proteins within mitochondria are required to induce male sterility. It is even unclear how MCAG proteins produced in the cytoplasm get imported into mitochondria and distributed inside mitochondria. The establishment of a mitochondrial gene introduction system will be required to verify the toxicity of MCAG protein in male development.

Nuclear Restorer-of-fertility (Rf) genes revert male viability to a CMS plant

Molecular components in charge of the fertility restoration process are cloned from different species. The first Rf cloned, although still in argument (Touzet 2002, Schnable 2002), was Rf2a from maize encoding a protein with aldehyde dehydrogenase activity (Cui et al. 1996, Liu et al. 2001). Except for this Rf2a, all of the fertility restorer genes so far cloned have encoded proteins possessing tandem alignments of a 35 amino acid polypeptide, the so called PentatricoPeptide Repeat (PPR) motif (Bentolila et al. 2002, Desloire et al. 2003, Brown et al. 2003, Koizuka et al. 2004, Komori et al. 2004, Akagi et al. 2004, Wang et al. 2006). PPR is a degenerate repeat motif. This protein family is comprised of approximately 450 members in Arabidopsis and rice (reviewed in Small and Peeters 2000, Lurin et al. 2004, Andrés et al. 2007, O’Toole et al. 2008). It has been proposed that proteins encoded by the majority of this family are targeted to plastid or mitochondria (Lurin et al. 2004), and many genetic and biochemical studies conclude that PPRs directly bind to a specific RNA sequence and promote anterograde regulation such as post-transcriptional splicing, processing, editing or regulating mRNA stability (Fisk et al. 2000, Nakamura et al. 2003, Kotera et al. 2004, Schmitz-Linneweber et al. 2005, Okuda et al. 2006, Okuda et al. 2007, Hattori et al. 2007, Falcon de Longevialle et al. 2007). It is worth noting that only five of these family members are present in yeast and only six in humans. Surprisingly, only 12 were found from the green algae Chlamydomonas reinhardtii L., suggesting that plastid acquisition is not the only reason that higher plants are equipped with so many numbers of PPRs (Merchant et al. 2007). Considering that moss genome retains only 103 members, and there are up to 450 PPRs in angiosperms through retrotranspositioning (O’Toole et al. 2008), PPRs could be indispensable for complex development such as flowering in higher plants.

Rf was first cloned from petunia, RFPRESS2, which encoded a protein with 11 continuous PPR motifs (Bentolila et al. 2002). RFPRESS2 was targeted to mitochondria, and was also found to eliminate the CMS-specific protein PCF, which we described in the prior section. Later, Gillman et al. (2007) introduced RRPRESS592 fused with FLAG epitope tag at its C-terminus into a CMS plant, and RRPRESS92 was shown to reside within a mitochondrial membrane-associated, RNase-sensitive high-molecular-weight protein complex. RRPRESS92 immunoprecipitated with pcf RNA, suggesting that RRPRESS92 interacts with pcf directly or indirectly.

Rfs cloned from Kosena/Ogura radish also encoded a 687 amino acid PPR protein (Brown et al. 2003, Desloire et al. 2003, Koizuka et al. 2003). Transgenic CMS plants carrying Rf-orf687 exhibited reduction of the MCAG gene product, ORF138 (Koizuka et al. 2003). Rf1a cloned from rice also encoded a PPR protein (Kazama and Toriyama 2003, Komori et al. 2004, Akagi et al. 2004, Wang et al. 2006), and Rf1a was shown to promote processing of a MCAG, B-atp6-B-orf79, dicstronic transcript into a monocistronic B-atp6 and B-orf79 (Kazama and Toriyama 2003, Wang et al. 2006). Rf1b was also cloned as the fertility restorer for the same CMS type in rice, and Rf1b was highly identical to Rf1a in the amino acid level (Wang et al. 2006). Unlike Rf1a, Rf1b totally diminishes the B-orf79 part from the B-atp6-B-orf79 transcript instead of processing it. Interestingly, the biochemical function of Rf1a was epistatic to Rf1b, suggesting that Rf1b is unable to digest processed B-orf79 transcript (Wang et al. 2006). A recent work suggests that B-orf79 transcripts processed by the effect of Rf1a were not associated with polysomes, and would not be translated (Kazama et al. 2008).

There is an intriguing parallel with these Rfs cloned from independent plant species that should not be ignored, other than the fact that they all encode a PPR protein. These Rf loci all contain a cluster of similar PPRs within the region (Fig. 1). For example, petunia RFPRESS92 was flanked by a 93% identical RRPRESS91 (Bentolila et al. 2002) (Fig. 1). This locus included complex genome duplications and recombination in promoter regions and 3’UTR of RRPRESS91 and RRPRESS92. Radish Rf6 (PPR-B) was present between two similar genes, PPR-A and PPR-C, three of which are at least 72% identical with each other (Desloire et al. 2003, Brown et al. 2003) (Fig. 1). Rice Rf1a was followed by two or three (depending on the Rf-containing lines) other identical PPR-encoding genes, and Rf1b could be counted as one of them (Kazama and Toriyama 2003, Komori et al. 2004, Akagi et al. 2004, Wang et al. 2006) (Fig. 1). Including Rf1b, Wang et al. (2006) identified nine Rf1a homologous PPRs in Rf1 locus. Taking advantage of the completely sequenced rice genome (IRGSP 2005), we found that there are at least 15 Rf1a-like PPR genes (including pseudo-genes) near the Rf1a locus, even though the sequenced cultivar Nipponbare does not possess Rf1a (our unpublished data). It is also noteworthy that only one of these clustered PPRs is functional as Rf (referred to as ‘Rf-PPR’ in subsequent sentences) (Koizuka et al. 2003, Kazama and Toriyama 2003). This phenomenon could not be considered as a random coincidence. One fact it indicates
is that PPR functions are strictly dependent on their amino acid sequence, because even genes that are over 93.2% identical with Rf1a did not complement CMS phenotype to fertility restoration in rice (Kazama and Toriyama 2003). It is also worthwhile to mention that these PPRs located close to ‘Rf-PPRs’ may possess other housekeeping functions, and it is likely that CMS cytoplasm and the Rf locus co-evolved through many trial and errors to generate ‘Rf-PPRs’ that are functional against MCAGs. It seems that ‘The chicken or the egg’ dilemma exists between the rise of ‘Rf-PPRs’ and the presence of MCAGs, and deeper phylogenetic analysis would reveal their relationships.

These PPR-class Rfs are considered to function dominantly against CMS. However, since CMS is caused by the incompatibility between mitochondria and nucleus, it is also possible that recessive Rfs exist, namely, nuclear genes that should not be functional under CMS conditions. This loss-of-function type of fertility Rf locus was reported in maize S-CMS (Wen et al. 2003). The dominant Restorer-of-fertility lethal 1 (Rfl1) allele is positively related to the mitochondrial A-subunit of ATP synthase (ATPA) accumulation, and ATPA could interact with candidate MCAG orf355-orf77 gene product possibly inducing male sterility. When the Rfl1 is not present, plants restore fertility in haploid tissues. However, a plant homozygous for rfl1 is lethal because it lacks mitochondrial ATPA accumulation. Identification of rfl1 was possible because the fertility restoration of maize S-CMS occurs in haploid tissues (pollen), in which the dominant/recessive relationship is canceled. Since haploid type fertility restoration is often found in rice CMS, a factor like rfl1 could exist, and it is possible to mutagenize CMS plants and obtain fertility-reverted lines. Genes discovered from these analyses should help explain the uncovered gap between MCAG and CMS phenotype.

Retrograde signaling in CMS

It is widely accepted that mitochondrial genotype influences nuclear gene expression in eukaryotes. For example, mitochondrial DNA-depleted or respiratory-deficient yeast strains change the expression patterns of large numbers of nuclear genes (Parikh et al. 1987, Epstein et al. 2001, Traven et al. 2001). Or in Drosophila, mitochondria genotype is known to affect longevity, and not the ability of the mitochondria but it is the combination with proper nuclear background that is critical for aging (Rand 2005, Rand et al. 2006, Tang et al. 2007). These phenomena are believed to be caused by the effect of retrograde mitochondrial control of nuclear gene expression. In the case of Drosophila aging, it could be considered that correct mitochondrial–nuclear interaction plays a large role in longevity, and nuclear–mitochondrial imbalance caused by the cytoplasmic substitution may shorten the life expectancy of flies.

Involvement of retrograde signaling in CMS has been implicated from the studies of alloplasmic type CMS, a type of CMS that is derived from the inter-species or inter-genera crosses. Alloplasmic CMS plants often exhibit morphological changes in flower development, and in most cases the stamens are converted into other floral organs (reviewed in Zubko 2004, Linke and Börner 2005, Carlsson et al. 2008). These CMS phenotypes resemble those of mutants impaired in genes related in the ABC model (Coen and Myerowitz 1991), especially the class B and C mutants that do not produce male organs. As expected, a homeotic function B gene in tobacco alloplasmic CMS lines was down-regulated (Zubko et al. 2001). APETALA3 homolog was considerably down-regulated in alloplasmic wheat (Murai et al. 2002).
and GLOBOSA and DEFICIENS homologs were down-regulated in CMS carrots (Linke et al. 2003). APETALA3 gene was expressed ectopically in CMS B. napus (Geddy et al. 2004), and Teixeira et al. (2005) found that the expression levels of various homeotic genes are changed in the CMS cytoplasmic background. Although these studies still lack genetic evidences, misregulations of these genes could be directly involved in the induction of homeotic CMS phenotypes.

Recently, comprehensive transcript or protein expression studies on CMS have been performed by several groups to determine the factors regulated by the cytoplasmic genomes. Hochholdinger et al. (2004) compared the mitochondrial protein abundance status of plants carrying normal and T-CMS cytoplasm. They found that at least 27 proteins accumulate predominantly in the background of each cytoplasm, and these include such proteins as the F1 subunit of ATPase. Microarray-based transcriptomic studies have been performed on rice (Fujii et al. 2007) and B. napus (Carlsson et al. 2007). In the former study, 140 genes were differentially regulated between the CW-CMS line and its nuclear isogenic line Taichung 65. Rice CW-CMS plants exhibited a curious phenotype, in which the pollen developed normally to a mature state but failed to germinate tubes on stigma after anthesis (Fujii and Toriyama 2005). RNAi knockdown of Down-regulated in CW-CMS 11 (DCW11), one of the suppressed genes in the CW-CMS background, in wild-type Taichung 65, resulted in a pollen sterility phenotype that resembles that of CW-CMS (Fujii and Toriyama 2008). DCW11 is predicted to encode a mitochondrial protein phosphatase 2C protein, and this implies that the CW-CMS system could connote a phosphorylation process. Arabidopsis flower-specific cDNA microarray enabled identification of 244 differentially expressed genes in B. napus CMS line (Carlsson et al. 2007). They found at least 19 genes encoded a mitochondrial protein (of 244), and these included α-MPP and β-MPP, proteins involved in the cleavage of mitochondrial targeting signals (reviewed by Glaser and Dessi 1999), and mitochondrial outer membrane protein TOM40 (Lister et al. 2004, Lister et al. 2007). They concluded that reduction of these proteins may result in reduced ATP synthesis and lead to CMS induction. Based on a cDNA subtraction study on wheat pistillody CMS lines, AGC group protein kinase was shown to be up-regulated in the young spikes of the CMS line (Saraie et al. 2007). A lipid transfer protein gene was found to be strongly down-regulated in a sugar beet CMS line (Matsuhira et al. 2007). It is still difficult to determine whether these gene products are the direct cause of CMS, or the result of CMS, but we are sure that CMS signaling possesses the potential to enfold a complex protein–protein, and metabolite network.

Then what are the molecular components known in model cases of retrograde signaling? In yeast, RTG signaling has been elucidated extensively in mitochondrial DNA-depleted cells (reviewed in Butow and Avadhan 2004, Liu and Butow 2006) (Fig. 2A). Basic-helix-loop-helix transcription factors RTG1 and RTG3 have been shown to migrate to the nucleus to up-regulate citrate synthase 2 gene under mitochondrial stress conditions, whereas they are phosphorylated and remain in the cytoplasm in normal conditions (Liao and Butow 1993, Jia et al. 1997, Sekito et al. 2000). In the upstream of RTG1 and RTG3, ATP-binding motif-containing RTG2 positively regulates these transcription factors in RTG signaling (Liao and Butow 1993, Sekito et al. 2000). RTG2 was shown to bind to MKS1, an unknown protein which negatively regulates RTG signaling in the upstream of RTG1 and RTG3, and positively regulates RTG signaling by taking MKS1 out of the RTG pathway (Sekito et al. 2002, Liu et al. 2003). In parallel with RTG signaling, yeast when exposed to mitochondrial stress emits calcium ions to the cytoplasm. These calcium ions are sensed by factors such as calcium ion-dependent protein kinase C, and the signaling is passed downstream as a phospho-relay (Amuthan et al. 2001, Butow and Avadhan 2004). Proper retrograde signaling is known to be essential for progressing the cell cycle from the G1 phase to the S phase in the development of the compound eye in Drosophila melanogaster (Mandal et al. 2005, Owusu-Ansah et al. 2008) (Fig. 2B). In Drosophila lines without fully functional mitochondrial complex IV, ATP production is dropped and the AMP kinase-involving pathway is activated. AMP kinase positively regulates p53 and as a result, G1 to S phase cell cycle-promoting cyclinE expression is reduced (Mandal et al. 2005, Owusu-Ansah et al. 2008). On the other hand, Drosophila suffering from mitochondrial complex I deficiency accumulates reactive oxygen species (ROS) five times more than that of the wild-type, and ROS activates the p27 pathway which negatively regulates cyclinE-CDK2 complex (Owusu-Ansah et al. 2008).

Arabidopsis plastid-to-nucleus signaling is currently the model for plant retrograde signaling (reviewed by Woodson and Chory 2008) (Fig. 2C). The expression of plastid chlorophyll a/b binding protein encoding CAB gene is repressed under lincomycin- or norflurazon-treated conditions (Oelmuller and Mohr 1986, Susek et al. 1993). Mutants with impaired suppression of CAB gene under plastid-stressed conditions were designated as genomes uncoupled (gun) mutants (Susek et al. 1993). GUN2, GUN3, GUN4 and GUN5 encoded the enzymes involved in the different steps of chlorophyll biosynthesis (Mochizuki et al. 2001, Larkin et al. 2003), whereas GUN1 encoded a PPR protein which is likely to interact with DNA (Kousvevitzky et al. 2007). On the other hand, a large portion of mitochondrial retrograde signaling in Arabidopsis remains unknown. Although mutants unable to overdrive AOX gene under mitochondrial electron chain inhibitor antimycin A-treated conditions were isolated (Zarkovic et al. 2005), virtually nothing is understood. Currently, it is still impossible to imagine that the same pathways...
as yeast, *Drosophila* and plastid exist in CMS, however, considering that CMS is derived from a ‘gain-of-function’ effect, it is likely that unique retrograde signaling during CMS occurrence exists and influences nuclear gene expressions. Otherwise, it is possible that normal plant male development requires proper retrograde signaling, and when exposed to CMS cytoplasm, the signaling is impaired and male dysfunction occurs (Fig. 2D).

**Tissue specificity in CMS, why?**

Although the mitochondrion is a ubiquitous organelle, male-specific deficiency is observed in CMS. In addition, most MCAG products accumulate throughout plant development, and are not male specific (reviewed in Chase 2007).

The only MCAG found to accumulate specifically in anther is ORF239 in CMS common bean, and is degraded at vegetative tissues (Sarria et al. 1998). This characteristic poses a question. Why are defective phenotypes in CMS male specific? However, it also provides a hint which is that the function of MCAG is male specific. Furthermore, it should be considered that mitochondria have a special function in male development. For instance, mitochondria have been proposed to play a key role in programmed cell death (PCD) in mammalian cells (reviewed in Cai et al. 1998), PCD in anther tapetum cells is known to be essential during anther development in order to provide lipids that coat pollen exines (Piffanelli and Murphy 1998, Balk and Leaver 2001, Kawanabe et al. 2006). In sunflower PET1-CMS, in which the 15-kDa protein encoding MCAG orf522 is considered to be responsible for CMS, it is presumed to be suppressed by the presence of MCAG.

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**Fig. 2** Essence of retrograde signaling in model organisms. Proteins that positively regulate retrograde signaling are shown in white boxes, whereas genes or proteins that are negatively involved in the signaling are indicated with black boxes. (A) Yeast RTG-dependent signaling pathway. BMH1/2 are 14-3-3 proteins that bind to MKS1 when RTG signaling is inactive (Liu et al. 2003). The figure was modified from Butow and Avadhani (2004). (B) Retrograde signaling pathways involved in G1 phase to S phase cell-cycle transition of *Drosophila* compound eye development. ASK1 is a mitogen-activated protein kinase, JNK is a stress-responsive kinase and Foxo is a forkhead transcription factor involved in the positive regulation of p27. The figure was modified from Owusu-Ansah et al. (2008). (C) *Arabidopsis* plastid-signaling mediated by GUN1. ABI4 is an *Apetalla2* transcription factor that suppresses the mRNA expression of *Light Harvesting Chlorophyll a/b Binding* (*LHCB*) protein encoding gene (Koussevitzky et al. 2007). The figure was modified from Woodson and Chory (2008). (D) How about CMS? X could be a gene ectopically overdriven in CMS background like yeast CIT2, where MCAG may be the factor that starts the retrograde signaling. X may represent homeobox genes that changed expression in alloplasmic CMS plants, or *AOX* genes that showed abnormal up-regulation in rice CMS plant (Fujii et al. 2007). Y is possibly important for normal plant male development like *Drosophila* *CycE*, where it is presumed to be suppressed by the presence of MCAG.
As mentioned before, most mitochondrial ‘loss-of-functional’ mutants exhibit severe growth defect phenotypes. As far as we know, there are no mitochondrial mutants that show male-specific dysfunction. However, there are a few mutants that show defects in haploid development. A mutant with T-DNA inserted in the mitochondrial complex II succinate dehydrogenase (SDH) subunit suffers complete male sterility, namely no transmission of T-DNA-inserted allele from the male parent (Leon et al. 2007). When the SDH1-1/ hdh1-1 hemizygous line was used as the female parent, transmission efficiency of the T-DNA inserted allele decreased to 60%. Thus, sdh1-1 mutation caused complete loss of pollen viability, and partial loss of ability of female gametophyte. Recent analysis on mitochondrial Miro GTPase homologue in Arabidopsis also presents quite interesting data (Yamaoka and Leaver 2008). Miro1/miro1 exhibits normal growth and develops morphologically normal pollen; however, pollen germination was impaired and male transmission efficiency of the mutant allele was significantly reduced. Homozygous miro1 mutant aborts embryogenesis at the zygote stage. The inheritance trait of these two mitochondrial mutant alleles tells us that the function of mitochondria differs between male and female gametophyte development, and it seems that pollen development is more sensitive to mitochondrial dysfunction than female gametophyte development.

Above we mentioned the gametogenesis type mitochondrial mutants. There are also mitochondrial gene knockdown studies that report tapetum deficiency. The antisense suppression of mitochondrial Alternative Oxidase (AOX) under the control of tapetum-specific promoter resulted in reduced pollen viability in tobacco plants (Kitashiba et al. 1998). Tapetum-specific suppression of mitochondrial pyruvate dehydrogenase resulted in abnormal vacuolated cell progression in tobacco, and mimicked the sugar beet CMS phenotype (Yui et al. 2003). The key to these research findings is the specific suppression of mitochondrial genes in tapetum, strongly suggesting that mitochondrial functions in tapetum cells are special.

Although there have been great advancements in genetic tools to identify in planta gene functions in Arabidopsis, mysteries of organ specific mitochondrial gene products remain unsolved. The pollen-specific isoform of ATP synthase subunit b was found in diploid tobacco Nicotiana sylvestris L. (De Paepe et al. 1993), and later, a single gene corresponding to the subunit was isolated and confirmed to be bi-cellular pollen-specific (Lalanne et al. 1998). Mitochondrial Rieske proteins are preferentially increased in flowers compared to leaves along with the increase in the mitochondrial number during flower development (Huang et al. 1994). Unfortunately the destinies of plants that suffer impairments to these genes are still unknown, although functions of these proteins could provide a hint to solving male specificity of CMS.

Concluding remarks

A unique, male-specific dysfunction feature of CMS reveals two things; (i) male organ development is dependent on cytoplasmic genotypes, and (ii) there is more significance to mitochondria in plant development other than just energy production. An incongruity exists when considering that only the loss in ‘quantity’ of mitochondrial functions causes CMS. Rather, it seems more unassuming to consider that changes in ‘quality’ of mitochondrial functions cause CMS, and probably revealing factors involved in retrograde signaling from mitochondria to nucleus during CMS induction and fertility restoration could help shed light on the mysterious function of mitochondria and help tell the full story of genome barriers between mitochondria and the nucleus.

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