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Mitochondria and cytoplasmic male sterility in plants

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ABSTRACT

Mitochondria are essential organelles in cells not only because they supply over 90% of the cell's energy but also because their dysfunction is associated with disease. Owing to the importance of mitochondria, there are many questions about mitochondria that must be answered. Cytoplasmic male sterility (CMS) is a mysterious natural phenomenon, and the mechanism of the origin of CMS is unknown. Despite successful utilization of CMS and restoration of fertility (*Rf*) in practice, the underlying mechanisms of these processes remain elusive. This review summarizes the genes involved in CMS and *Rf*, with a special focus on recent studies reporting the mechanisms of the CMS and *Rf* pathways, and concludes with potential working models.

1. Introduction

In the plant kingdom, most flowers are bisexual, a feature that greatly contributes to plant evolution via crossing. The phenomenon of cytoplasmic male sterility (CMS) is found worldwide in nearly 200 species (Bentolila et al., 2002; Chase, 2007; Hu et al., 2012). Despite wide application to crop breeding and heterosis, the mechanisms controlling sterility and fertility are still unclear. Many studies have reported that CMS-associated genes are located in the mitochondrial genome, and restorer of fertility (*Rf*) genes that are responsible for fertility restoration in F_1 hybrids are derived from the nuclear genome (Budar et al., 2003). Crosstalk between the mitochondria and nuclei is an interesting field, and the CMS and *Rf* pathways are well suited to study mitochondrial–nuclear interactions (Chase, 2007).

Research into CMS revealed that genes or open reading frames (*orfs*) found in the mitochondria govern this complicated trait. Several CMS-associated genes have been identified and characterized in various species, and emerging evidence supports roles for programmed cell death (PCD) and reactive oxygen species (ROS) in the CMS pathway (Balk and Leaver, 2001; Ji et al., 2013; Wan et al., 2007). Even biogenesis of jasmonic acid was impaired in the CMS line (Liu et al., 2012). Furthermore, CMS products were established as being cytotoxic proteins to *Escherichia coli* and yeast, and these proteins interrupted either the assembly or functions of complexes in the electron transport chain (Liu et al., 2012; Peng et al., 2009; Wang et al., 2006, 2013).

Cloning and characterization of *Rf* genes for CMS was expanded in recent decades, including *Rf2* in T-CMS maize, *Rf17* in CW-CMS rice, *Rf1a* and *Rf1b* in BT-CMS rice, *Rf5* and *Rf6* in HL-CMS rice, *Rf3* and *Rf4* in WA-CMS rice, *Rf2* in LD-CMS rice, *Rfo* in Ogura-CMS, *PPR592* in petunia, and *PPR13* in sorghum (Bentolila et al., 2002; Brown et al., 2003; Cui et al., 1996; Desloire et al., 2003; Fujii and Toriyama, 2009; Hu et al., 2012; Itabashi et al., 2011; Klein et al., 2005; Koizuka et al., 2003; Wang et al., 2006). RFs derived from the nuclear genome can eliminate the impairments caused by CMS products. Two opinions regarding the action of RFs have been proposed. Some evidence has shown that RFs directly process CMS transcripts, and some evidence has shown that RFs function with co-factors for processing, and RF complexes were observed (Gillman et al., 2007; Hu et al., 2012; Kazama et al., 2008).

In this review, we focus on recent studies of the CMS and *Rf* pathways, which have greatly broadened our understanding of these processes. We believe that the exact mechanism of CMS among different species depends on the type of CMS. Consequently, we begin this review by describing the types of CMS, listing CMS and *Rf* genes, and ultimately discussing the mechanisms governing sterility and fertility in various species. We give more attention to rice, as many varieties and types of CMS have been studied.

2. Cytoplasmic male sterility and restoration

2.1. Types of CMS

Several types of male sterility have been uncovered and clarified in the last two decades. In this review, we focus on the recent studies of CMS and *Rf* that greatly expanded our understanding of such pathways.

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As shown in Fig. 1, CMS can be grouped into two classes according to the development of male gametes: sporophytic CMS (Sp-CMS) and gametophytic CMS (Ga-CMS). In the Sp-CMS system, the sterility of male gametes is dependent on the sporophyte genotype, whereas in the Ga-CMS system, the sterility of male gametes is dependent on the genotype of the gametophyte. Therefore, in F_1 individuals of the genotype *Rf*/*rf*, the male gametes were all functional in the Sp-CMS system, while in the Ga-CMS system, 50% of the male gametes carrying the *rf* allele were dysfunctional. As a consequence, the typical distinguishing feature between the two systems is the segregation ratio of the F_2 population. In Sp-CMS systems, a quarter of F_2 progeny are sterile, whereas in Ga-CMS systems all F_2 progeny are fertile (Fig. 2). Diverse CMS processes can be observed in nature, such as the Sp-CMS and Ga-CMS pathways in rice, suggesting the presence of complicated mechanisms.

2.2. Characteristics of CMS genes

The plant mitochondrial genome contains 33 coding genes and ranges in size from 200 kb to 2400 kb depending on the species (Knoop, 2012). Unlike in humans, the plant mitochondrial genome contains many *orf*s. Many reports have shown that CMS-associated genes were these *orf*s, which linked with electron transport chain (ETC) genes in the mitochondrial genome. To date, many CMS genes have been described; however, few have been characterized (Table 1). Based on this information, most researchers have investigated the *orf*s associated with functional genes and have identified the differences between the CMS and F_1 lines. Since the development of sequencing technology, the mitochondrial genomes of several species have been sequenced, including wild and domesticated species (Wang et al., 2012). Whole genome analysis has revealed some novel CMS genes and has confirmed the stability of the mitochondrial genome organization, and has shown that the mitochondrial genome is variable across species (Igarashi et al., 2013; Okazaki et al., 2013). With regards to the origin of CMS genes, these chimeric *orf*s are generally considered to result from mitochondrial genome rearrangements (Tuteja et al., 2013).

Because hybrid rice contributes greatly to food security, abundant germplasms have been discovered and CMS types have been investigated. As listed in Table 1, WA-CMS belongs to the Sp-CMS group, and the CMS gene WA352 was cloned and consists of 4 different segments (Luo et al., 2013). For the Ga-CMS group, a BT-CMS associated gene, *orf79*, is located downstream of *atp6* in the mitochondrial genome. The CMS transcript *atp6*–*orf79* and CMS protein ORF79 were observed by northern and western blot, respectively. For HL-CMS, the CMS gene *orfH79* was characterized as a gene homologous to *orf79*, with a 5-nucleotide substitution resulting in the alteration of 5 amino acids. In addition to *atp6*–*orfH79*, another novel CMS transcript, *orfH79*(*s*), has been observed, suggesting that there are different mechanisms for these transcripts at a transcriptional level. For LD-CMS, *orf79* was also found downstream of *atp6*, and consequently a CMS transcript, *atp6*–*orf79*, was observed; however, there was no ORF79 accumulation in the CMS line (Itabashi et al., 2009). Using whole genome sequencing, *orf307* was identified as a candidate CMS-associated gene for CW-CMS rice (Fujii et al., 2010a); however, there was no transcript pattern difference between the CMS line and the F_1 hybrid, implying that post-translational regulation was involved in the restoration. Furthermore, *orf113* and *orf352* were reported as candidate CMS-associated genes for RT98-CMS rice and RT102-CMS rice, respectively (Igarashi et al., 2013; Okazaki et al., 2013).

As in rice, several types of CMS were investigated in *Brassica*. In the Polima CMS line, *orf224*, located upstream of *atp6*, was identified as a CMS associated gene. Additionally, *orf222* was detected in the Napus CMS, and *orf138* was observed in both Ogura CMS and Tour CMS. The *orf125* gene, located upstream of *atp8*, was characterized in the Kosena radish. In PET1-CMS sunflowers, *orf552* is considered to be a CMS associated gene. In the petunia, the *s-pcf* was previously shown to consist of sequences from *atp9*, *cox II* and an unknown sequence (Hanson and Bentolila, 2004). An increasing number of CMS-associated *orf*s in the mitochondrial genome have been identified in recent decades, while the origins of these *orf*s are still unknown.

2.3. Characteristics of restorer of fertility genes

Cui et al. (1996), reported the first *Rf* gene in T-CMS maize in 1996 and proposed that RF2 eliminated the harm caused by CMS products. In the petunia, PPR592, which belongs to the PPR gene family, was characterized in 2002 (Bentolila et al., 2002). After these studies, additional *Rfs* were cloned and characterized as PPR genes (listed in Table 1).

PPR proteins form a large family in plants (approximately 450 genes in rice and 470 genes in *Arabidopsis*), have been shown to play important roles in post-transcriptional processes, and are considered to be RNA-binding proteins (O'Toole et al., 2008). To date, several *Rfs* have been cloned and characterized as PPR proteins, including PPR592 in petunia, *Rf1a* (same as *PPR791*) and *Rf1b* in BT-CMS rice, *Rf5* (same as *Rf1a*) and *Rf6* (unpublished) in HL-CMS rice, *Rfo* (same as *PPR687*) in Ogura-CMS, *Rfk* in Kosena-CMS, and *PPR13* in sorghum. The relationship between *Rfs* and PPRs is still unknown. In addition to PPR proteins, other types of *Rfs* have been cloned, implying the presence of complicated restoration pathways. LD-CMS rice *Rf2* encodes a glycine-rich protein (GRP) and CW-CMS rice *Rf17* encodes an unknown protein. In various species, other *Rfs* have been mapped and reported, but still require future detailed characterization. In any case, the common feature is that RFs should translocate into the target compartment to eliminate CMS products that were produced in mitochondria.

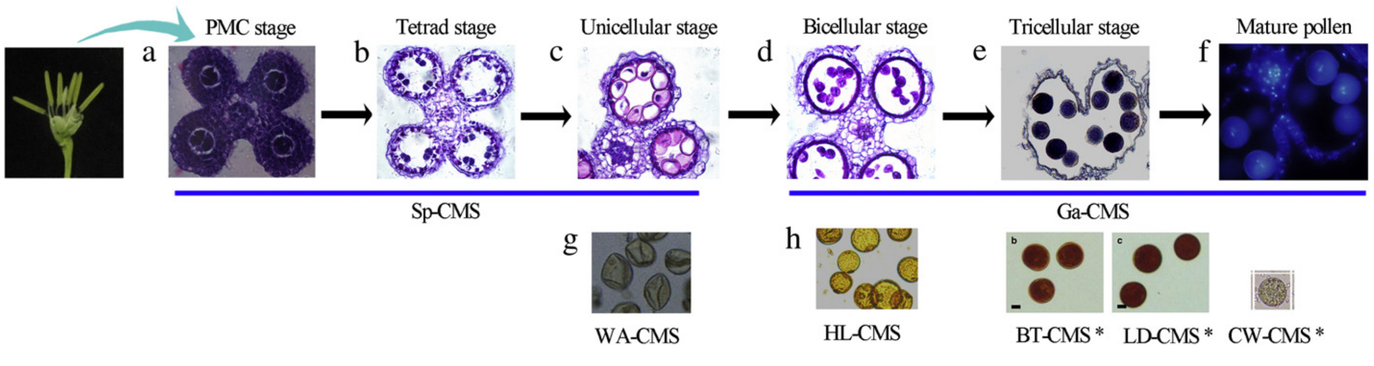

Fig. 1. Biogenesis of microspores and types of CMS in rice. Rice microspores develop through a pollen mother cell (PMC) (a), tetrad (b), unicellular (c), bicellular (d), and tricellular stages (e) to become mature pollens (f). a–e: Semi-thin sections, f: DAPI staining. Types of CMS can be grouped into sporophytic CMS (Sp-CMS) and gametophytic CMS (Ga-CMS), based on the development of microspores. WA-CMS belongs to Sp-CMS, while HL-CMS, BT-CMS, LD-CMS and CW-CMS belong to Ga-CMS. Pollen from the CMS lines was detected with 1% I_2-KI (g, h), and * (Fujii et al., 2010b; Itabashi et al., 2009).