24 Grandmougin, A. *et al.* (1989) Cyclopropyl sterols and phospholipid composition of membrane fractions from maize roots treated with fenpropimorph, *Plant Physiol.* 90, 591–597
25 Schuler, I. *et al.* (1990) Soybean phosphatidylcholine vesicles containing plant sterols: a fluorescence anisotropy study, *Biochim. Biophys. Acta* 1028, 82–88
26 Schuler, I. *et al.* (1991) Differential effects of plant sterols on water permeability and on acyl chain ordering of soybean phosphatidylcholine bilayers, *Proc. Natl. Acad. Sci. U. S. A.* 88, 6926–6930
27 Krajewsky-Bertrand, M-A., Milon, A. and Hartmann, M-A. (1992) Deuterium-NMR investigation of plant sterol effects on soybean phosphatidylcholine acyl chain ordering, *Chem. Phys. Lipids* 63, 235–241
28 Grandmougin-Ferjani, A., Schuler-Muller, I. and Hartmann, M-A. (1997) Sterol modulation of the plasma membrane H^+-ATPase activity from corn roots reconstituted into soybean lipids, *Plant Physiol.* 113, 163–174
29 Bloch, K.E. (1983) Sterol structure and membrane function, *Crit. Rev. Biochem.* 14, 47–82
30 Nes, W.D. *et al.* (1993) The structural requirement of sterols for membrane function in *Saccharomyces cerevisiae*, *Arch. Biochem. Biophys.* 300, 724–733
31 Rodriguez, R.J. *et al.* (1985) Multiple functions for sterols in *Saccharomyces cerevisiae*, *Biochim. Biophys. Acta* 837, 336–343
32 Lees, N.D. *et al.* (1995) Cloning of the late genes in the ergosterol biosynthetic pathway of *Saccharomyces cerevisiae* – a review, *Lipids* 30, 221–226
33 Bach, T.J. and Benveniste, P. (1997) Cloning of cDNAs or genes encoding enzymes of sterol biosynthesis from plants and other eukaryotes: heterologous expression and complementation analysis of mutations for functional characterization, *Prog. Lipid Res.*, 36, 197–226
34 Gachotte, D., Meens, R. and Benveniste, P. (1995) An *Arabodopsis* mutant deficient in sterol biosynthesis: heterologous complementation by *ERG3* encoding a Δ^7-sterol-C5-desaturase from yeast, *Plant J.* 8, 407–416
35 Schaller, H. *et al.* (1995) Expression of the *Hevea brasiliensis* (H.B.K.) Müll. Arg. 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 in tobacco results in sterol overproduction, *Plant Physiol.* 109, 761–770
36 Marsan, M.P., Muller, I. and Milon, A. (1996) Ability of clionasterol and poriferasterol (24-epimers of sitosterol and stigmasterol) to regulate membrane lipid dynamics, *Chem. Phys. Lipids* 84, 117–121
37 Goad, L.J. (1990) Application of sterol synthesis inhibitors to investigate the sterol requirements of protozoa and plants, *Biochem. Soc. Trans.* 18, 63–65
38 Cerana, R. *et al.* (1984) Regulating effects of brassinosteroids and of sterols on growth and H^+ secretion in maize roots, *Z. Pflanzenphysiol.* 114, 221–225
39 Cornelius, F. (1995) Cholesterol modulation of molecular activity of reconstituted shark Na^+,K^+-ATPase, *Biochim. Biophys. Acta* 1235, 205–212

Note added in proof

M. Havaux has recently discussed the absence of sterols in thylakoid membranes (*Trends Plant Sci.* 3, 147–151). In such membranes the function played by sterols might be fulfilled by other isoprenoids, such as xanthophyll carotenoids (e.g. zeaxanthin) and α-tocopherol.

Marie-Andrée Hartmann is at the Institut de Biologie Moléculaire des Plantes du Centre National de la Recherche Scientifique (CNRS), 28 rue Goethe, 67083 Strasbourg, France (tel +33 3 88 35 83 70; fax +33 3 88 35 84 84; e-mail ma.hartmann@ibmp-ulp.u-strasbg.fr).

The molecular basis of cytoplasmic male sterility and fertility restoration

Patrick S. Schnable and Roger P. Wise

Cytoplasmic male sterility (CMS) is a maternally inherited condition in which a plant is unable to produce functional pollen. It occurs in many plant species and is often associated with chimeric mitochondrial open reading frames. In a number of cases, transcripts originating from these altered open reading frames are translated into unique proteins that appear to interfere with mitochondrial function and pollen development. Nuclear restorer (*Rf* or *Fr*) genes function to suppress the deleterious effects of CMS-associated mitochondrial abnormalities by diverse mechanisms. There are now several well-characterized CMS systems, for which the mitochondrial sequences thought to be responsible have been described. Possible mechanisms by which nuclear restoration occurs in these systems can now be postulated.

Cytoplasmic male sterility (CMS) has been observed in over 150 plant species[1–3]. CMS systems make excellent models in which to study the interaction between nuclear and cytoplasmic factors, because fertility restoration relies on nuclear genes that suppress cytoplasmic dysfunction (Fig. 1). Analyses of the molecular mechanisms by which fertility loss and restoration occur can also help elucidate pollen development and normal mitochondrial function.

Nuclear restoration allows the commercial exploitation of CMS systems in the production of hybrid seed. This is because, in combination with CMS, it eliminates the need for hand emasculation and yet ensures the production of male-fertile, first-generation (F_1) progeny. For example, prior to the epidemic of southern corn leaf blight in 1970, male-sterile T (Texas)-cytoplasm maize system was used to produce approximately 85% of hybrid seed in the USA. Breeders produce hybrid seed using a CMS system by developing female lines that carry CMS cytoplasm but lack restorer genes and by developing male lines that carry the appropriate restorer genes. F_1 hybrid seed produced by the female lines carry the CMS cytoplasm but yield fertile plants because of the action of the paternally contributed nuclear restorers.

Origin of cytoplasmic male sterility

Because of their value in hybrid seed production, CMS systems have been identified and characterized in many crop species, including *Phaseolus vulgaris*, beet, carrot, maize, onion, petunia,

 1360 - 1385/98/$19.00 PII: S1360-1385(98)01235-7

Fig. 1. Tassels from sibling maize plants that carry the T (Texas) cytoplasm. T-cytoplasm confers male sterility on plants that do not carry both of the nuclear restorer genes, *Rf1* and *Rf2* (right). In contrast, T-cytoplasm plants that carry both of these nuclear restorers are completely male fertile (left).

Brassica napus, rice, rye, sorghum, sunflower and wheat[4]. CMS can arise spontaneously in breeding lines, following mutagenesis, as a result of wide crosses, or the interspecific exchange of nuclear and cytoplasmic genomes. For example, several CMS cytoplasms have been recovered from breeding lines without intentional intervention: the maize T-cytoplasm; the pol cytoplasm of *B. napus* (which arose in the cultivar Polima); the male-sterile cytoplasm of *Phaseolus*; and the S-cytoplasm of onion. Because plants that carry a CMS cytoplasm plus the appropriate restorers are often indistinguishable from normal plants, CMS cytoplasms can inadvertently be maintained in breeding lines until a mutation or segregation removes a restorer gene[5,6]. Such an event might explain the appearance of CMS following mutagenesis of sunflowers[7]. In rice, the male-sterile, Bo cytoplasm arose when the *Indica* cultivar Chinsurah Boro II was recurrently backcrossed with the *Japonica* cultivar Taichung 65. Interspecific hybridizations can be achieved between otherwise incompatible species via sexual crosses followed by embryo rescue or via protoplast fusion and the production of cybrids. These approaches have resulted in the identification of CMS-inducing cytoplasms in a number of genera. For example, the best characterized CMS-inducing cytoplasm of sunflower, PET1, was derived from an interspecific cross between *Helianthus petiolaris* and *H. annuus*[7]. Similarly, the ogu cytoplasm, which confers male sterility upon *B. napus*, was derived from radish. Wide crosses have also been used extensively to generate CMS systems in the family Triticeae[8,9].

Morphological changes associated with male sterility

The morphological changes that precede CMS in the various systems occur at different developmental stages and in different tissues. Nevertheless, in a number of systems, one of the first visible signs of CMS is the premature degeneration of the tapetum layer of the anther. Although the molecular basis for this tissue specificity is not known, it seems logical that tapetal degeneration would cause CMS because this structure plays a significant role in microspore development. In T-cytoplasm maize, the first obvious morphological lesion occurs soon after meiosis, when the mitochondria of the tapetum and middle layer of the anther begin to degenerate[10]. PET1-mediated male sterility in sunflower is also associated with a degeneration of the tapetum and tetrads after meiosis II. However, in this system differences between fertile and sterile plants first become visible as early as the beginning of meiosis. Similarly, in petunia with CMS, cells of the tapetal layer become vacuolated during meiosis[11].

Identification and structure of sterility-associated genes

By virtue of its maternal inheritance, it was initially assumed that CMS is the result of lesions in either the mitochondrial or the chloroplast genomes. Indeed, in all cases where a specific CMS-associated gene has been identified and shown via correlative or direct means to be responsible for CMS, the lesion has been in the mitochondrial genome (Fig. 2). However, because plant mitochondrial genomes are large (200–2400 kb), it is often difficult to identify the sequences responsible for CMS. Several approaches have been used to narrow the search, including comparative physical mapping and the identification of differences in mitochondrial gene-expression patterns between normal fertile, male-sterile, restored fertile, and fertile revertant plants. The key test, a functional assay for a candidate sequence, has been reported in only a few cases.

Detailed RFLP analyses revealed that the PET1 cytoplasm of sunflower differs from fertile cytoplasms (including that of *H. petiolaris*) within a 17-kb region of the mitochondrial genome that includes a 12-kb inversion and a 5-kb insertion flanked by 261-bp inverted repeats[7]. These polymorphisms relative to the cytoplasm of *H. petiolaris* are particularly interesting given that the PET1 cytoplasm is derived from an interspecific hybridization of *H. petiolaris* and *H. annuus*. The 5-kb insertion created a 522-bp open reading frame (ORF) downstream of the *atpA* gene[12] (Fig. 2). This ORF encodes a 16-kDa protein that accumulates in both sterile and restored PET1 seedlings[13]. Accumulation of the *orfH522* transcript and its encoded protein exhibit tissue-specific[14] and cell-specific[15] reductions following restoration. These results therefore provide strong support for the role of *orfH522* in CMS.

In *B. napus*, there are several CMS-associated ORFs associated with male sterility (Fig. 2). Physical mapping has revealed differences between the male-sterile pol and male-fertile cam mitochondrial genomes that are confined to a rearranged region around the *atp6* gene[16]. This result led to the discovery of the CMS-associated *orf224*/*atp6* locus[17], which is present only in the pol cytoplasm. Additional evidence for the involvement of this locus in the CMS phenotype is that *orf224*/*atp6* transcripts are differentially processed in plants that carry the *Rfp1* or *Rfp2* restorers[18,19]. Interestingly, the predicted ORF224 protein exhibits 79% sequence similarity to the predicted protein of the *orf222* portion of the CMS-associated *orf222*/*nad5c*/*orf139* region in the male-sterile nap cytoplasm[20]. The basis for calling this region CMS-associated is that *orf222*/*nad5c*/*orf139* transcripts are less abundant and are qualitatively different in restored and non-restored (i.e. sterile) plants. Again, based on physical mapping studies, *orf138* is the best candidate as the CMS-associated gene in the male-sterile ogu cytoplasm[21]. Unlike the CMS-associated genes in the pol and nap

cytoplasms, however, the accumulation of *orf138*-specific transcripts is not correlated with the presence or absence of the *Rfo* restorer. Nevertheless, restoration does appear to be associated with a reduced accumulation of the 19-kDa ORF138 mitochondrial-membrane protein[22].

In maize, there are numerous RFLPs between the mitochondrial genomes of the male-fertile (N-) and male-sterile T-cytoplasms. This greatly complicated the search for the gene responsible for sterility in the mitochondrial genome. The T-*urf13* gene was ultimately identified via two complementary approaches[23,24]. One approach identified a mitochondrial DNA fragment that hybridized to a family of T-specific transcripts. Sequence analysis of this T-specific region revealed two ORFs, T-*urf13* and the co-transcribed *orf221* (Ref. 25) (Fig. 2). Alterations in T-*urf13*-specific transcript accumulation occur in plants restored to fertility by the *Rf1* gene and this alteration is absent in plants carrying mutant *rf1-m* alleles[26,27]. Recently, it has been revealed that the *Rf8* and *Rf** partial restorers also have specific effects (distinct from *Rf1*) on T-*urf13* transcript accumulation[28]. The second approach took advantage of tissue culture-induced mitochondrial mutations that restored fertility to T-cytoplasm. Physical mapping experiments revealed a common RFLP in 19 out of 20 such mutants. Subsequent analyses revealed that the T-*urf13* reading frame had been lost by homologous recombination in each of these mutants[23,24]. The remaining fertile mutant, T-4, which did not exhibit the RFLP, contained a 5-bp insertion in the T-*urf13* coding region that truncated this reading frame[29]. In all of these mutants, the co-transcribed *orf221* reading frame is unaltered. T-*urf13* encodes a 13-kDa mitochondrial pore-forming protein (URF13) that assembles as an oligomer in the inner mitochondrial membrane. The accumulation of this protein is reduced in plants restored to fertility by *Rf1* (Ref. 26) or *Rf8* (Ref. 28). Hence, in combination, these approaches provide strong evidence that the T-*urf13* gene is responsible for CMS in T-cytoplasm maize.

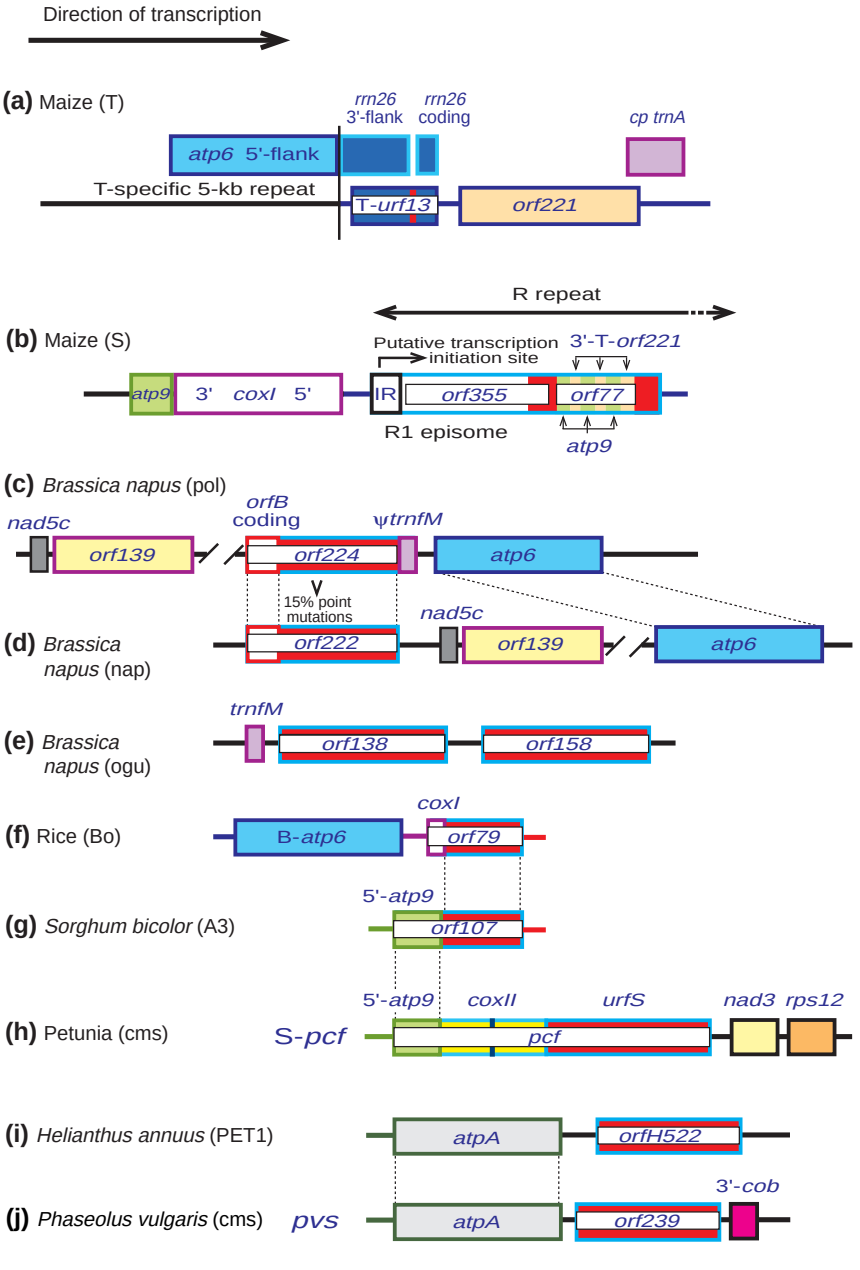

Fig. 2. Chimeric cytoplasm male sterility (CMS)-associated regions in the mitochondrial genomes of various species. CMS-associated open reading frames (ORFs) (open boxes) often include novel sequences of unknown origin (red). Other colors indicate known mitochondrial genes and are designated by standard mitochondrial gene nomenclature. Parallel dashed lines indicate sequence similarities. The direction of transcription (from the 5′- to the 3′-end) is as indicated at the top of the figure, except in the case of *coxI* within the chimeric R region of S-cytoplasm maize, which is oriented as illustrated.

The mitochondrial genome of the male-sterile S-cytoplasm of maize contains the repeated DNA region 'R', which contains two chimeric ORFs[30] (Fig. 2). This region is rearranged in fertile revertants of S-cytoplasm plants. In addition, the nuclear restorer *Rf3* alters the abundance of the major R transcripts. More specifically, an R-derived 1.6-kb transcript that accumulates in sterile plants is absent in fertile revertants and reduced in plants restored to fertility by *Rf3* (Ref. 30).

To identify the CMS-associated gene of petunia, somatic hybrids of fertile and CMS lines were constructed via protoplast fusion[11]. Within these hybrids, the mitochondrial genomes of the two parents were able to recombine. The resulting recombinant genomes then segregated during subsequent cell divisions. Comparative mtRFLP analyses of male-sterile and fertile progeny revealed a chimeric ORF consisting of the 5′-portion of the *atp9* gene, parts of the first and second exons of the *coxII* gene, and an unidentified sequence (termed *urfS*) that co-segregated with male sterility. This chimeric 1.2-kb ORF, *pcf* ('petunia CMS-associated fused'), is co-transcribed with *nad3* and *rps12* (Ref. 31) (Fig. 2). In the presence of the *Rf* gene, the accumulation of *pcf*-derived transcripts is altered. In addition, the *Rf* gene reduces the accumulation of the 25-kDa PCF protein.

Unlike the mitochondrial genome of normal fertile (*Japonica*) rice, the mitochondrial genome in the Bo cytoplasm contains two copies of the *atp6* gene[32,33]. Downstream of (and co-transcribed with) the second copy of the *atp6* gene (B-*atp6*) is the unique CMS-associated *orf79* (Ref. 34) (Fig. 2). As in petunia, comparisons between mtDNAs in male-fertile and male-sterile somatic hybrids were used to correlate the presence of the *orf79* gene with male sterility[32,34]. Additional evidence for the role of this gene in CMS is that the *Rf1* restorer influences the processing and editing of the 2.0-kb B-*atp6/orf79* transcript[32].

During investigations of the *atp9* gene from the A3 cytoplasm of sorghum with CMS, cosmid clones were identified that contained an unusual chimeric ORF, designated *orf107* (Ref. 35) (Fig. 2). *orf107* is comprised of sequences similar to the 5′-end of *atp9* and the 3′-end of rice *orf79*. The processing of *orf107* transcripts cosegregates with *Rf3*-conditioned fertility restoration.

The mitochondrial genome of CMS wheat that arose via an interspecific cross between *Triticum timopheevi* (female parent) and *T. aestivum* carries a chimeric gene, *orf256*. Although present in the mitochondrial genome of *T. timopheevi*, *orf256* is absent from *T. aestivum*. A 7-kDa protein encoded by *orf256* is present in the inner mitochondrial membrane of wheat with CMS, but is not in either parent or wheat restored to fertility by nuclear genes derived from *T. timopheevi*[9].

An interesting type of mitochondrial DNA polymorphism is observed in male-sterile, male-fertile and restored lines of *Phaseolus*[36]. Detailed analyses have revealed that a portion of the mitochondrial genome [designated *pvs* (‘*Phaseolus vulgaris* sterility sequence’)] is physically lost upon restoration by the *Fr* gene[37,38]. The loss of *pvs* also occurs coincidentally with spontaneous reversions to fertility that occur at high rates in this system. The *pvs* region contains two ORFs, *orf239* and *orf98* (Ref. 39) (Fig. 2), and transcripts from it are modified during restoration by *Fr2*, another restorer of this cytoplasm. The definitive evidence that *orf239* is responsible for CMS was provided by the finding that transgenic tobacco plants that express this ORF often exhibit a semisterile or male-sterile phenotype[40].

CMS-associated genes are often chimeric, having been derived from fusions of portions of known genes with previously unknown sequences. In most instances the sequences of CMS-associated ORFs are unrelated. However, many of these CMS-associated genes do share the common feature that they encode large hydrophobic domains[3,23]. There are only two examples of CMS-associated genes that exhibit sequence similarity (Fig. 2). In *B. napus*, *orf222* from the nap cytoplasm is 79% similar at the amino acid level to *orf224* from the pol cytoplasm[20]. *orf107* of the A3 cytoplasm of sorghum and the 3′-region of *orf79* from rice comprise the only pair of interspecific CMS-associated ORFs known to exhibit a high level of sequence similarity[35].

Function of genes associated with cytoplasmic male sterility

Clues as to how CMS-associated genes cause male sterility have arisen in several systems. For example, in *Phaseolus* the ORF239 protein is associated with the cell walls of developing microspores[39]. A similar association is observed in transgenic tobacco expressing nuclear copies of *orf239* (Ref. 40). These transgenic plants are also male sterile when the transgene lacks a mitochondrial targeting sequence. Thus, it can be concluded that the ORF239 protein is toxic to developing microspores regardless of where it is encoded or translated. These results suggest that CMS in *Phaseolus* is not directly related to mitochondrial function.

In addition to being male sterile, maize that carries T-cytoplasm is highly sensitive to the host-selective toxin (T-toxin) produced by race T of *Cochliobolus heterostrophus* Drechsler (asexual stage *Bipolaris maydis* Nisikado and Miyake), the causal organism of southern corn leaf blight. By contrast, tissue culture-derived male-fertile mutants of T-cytoplasm that have lost the T-*urf13* gene are toxin insensitive. This finding suggests that T-*urf13* is responsible for both the male sterility and toxin sensitivity[23]. Indeed, the URF13 protein confers toxin sensitivity when expressed in *E. coli*, yeast or tobacco[41–45]. However, none of the toxin-sensitive, transgenic tobacco plants produced to date has been male sterile even when URF13 has been targeted to the mitochondria. Similarly, expression of the PCF protein (which is associated with CMS in petunia) has not caused male sterility in transgenic tobacco or petunia produced to date[46]. The failure to obtain male-sterile plants from these two experiments suggests that correct tissue-specific expression and subcellular localization are required for some CMS-associated proteins to cause male sterility.

Restoration of fertility

Genetics of restoration

There is a great deal of diversity in the genetics of restoration both among and within species. Restoration systems are classified as being either sporophytic or gametophytic: sporophytic restorers act prior to meiosis or in sporophytic tissues; gametophytic restorers act after meiosis in microspores or pollen grains. These differences lead to very different transmission patterns. A diploid plant that carries a male-sterile cytoplasm and is heterozygous for a restorer will produce two classes of pollen grains: those that carry the restorer and those that do not. In the case of a sporophytic restorer, both genotypic classes of gametes will be functional. By contrast, in the case of a plant heterozygous for a gametophytic restorer, only those gametes that carry the restorer will be functional. S-cytoplasm maize is an example of a well-characterized CMS system that is restored gametophytically[47].

The diversity in restoration systems extends to the number of restorer genes. In some systems, one or two major restorer loci confer complete restoration. In others, full restoration requires the concerted action of a number of genes, many of which provide only small incremental effects. For practical reasons most research has been conducted on systems in which restoration is conditioned by relatively few genes. In most such systems, a single restorer is sufficient. By contrast, in T-cytoplasm maize, PET-cytoplasm sunflower and T-cytoplasm onion, two unlinked restorers are required for full restoration.

Duplicate restorer loci exist in a number of systems. For example, in maize, *Rf8* can at least partially substitute for *Rf1* to restore T-cytoplasm maize[28]. Similar cases exist in, for example, the PET1 cytoplasm of sunflower, the T-cytoplasm of onion and *Phaseolus* with CMS. Such overlapping functions could be a consequence of duplication of gene functions or an indication that multiple mechanisms can induce restoration.

Population genetics of restorers

Information regarding the allelic frequencies of restorers can prove useful in trying to understand their evolutionary origins. For example, although the *Rf1* restorer of T-cytoplasm maize is quite rare among maize lines, the *Rf2* restorer of this cytoplasm is present in almost all maize lines, even though most of these lines have never been exposed to the T-cytoplasm. This suggests that the *Rf2* gene has been maintained during evolution by selection and must therefore have a significant function independent of restoration[48]. The ogu, pol and nap cytoplasms of *B. napus* induce sterility in all, some, and only a few cultivars, respectively[19]. Hence, one might deduce that the ogu restorer (*Rfo*) is absent from *B. napus* germplasm, pol restorers are rare, and nap restorers are more common.

Mechanisms of restoration

The mechanisms by which restoration occurs are probably as diverse as the mechanisms by which mitochondrial mutations cause CMS. The physical loss of a CMS-associated gene from the mitochondrial genome is one means by which this can occur. Such a case has been observed in *Phaseolus*, where, in the presence of the nuclear gene *Fr*, the mitochondrial sequence responsible for CMS (*pvs*) is lost. Although several interesting hypotheses that could explain this process have been proposed (and in some cases tested), the actual mechanism by which it occurs has not yet been established[38].

Nuclear restorers often alter the expression of CMS-associated genes, and thereby presumably lessen or eliminate the deleterious effects associated with their gene products. For example, restoration of fertility is associated with processing of CMS-associated transcripts in a number of systems. In most cases, alterations are observed in the accumulation of specific transcripts via northern blot analyses. Hence, it is often impossible to distinguish between transcriptional and post-transcriptional mechanisms. However, there are numerous examples in which the accumulation patterns of specific transcripts have been found to be altered. For example, in T-cytoplasm maize, in the presence of the *Rf1* restorer, the accumulation of 1.6-kb and 0.6-kb T-*urf13* transcripts is greatly enhanced and the accumulation of the 13-kDa URF13 protein is reduced[25–27]. Capping experiments have revealed that these transcripts probably do not arise via novel initiation sites[49]. Instead, they probably arise via processing from a larger transcript. However, even in the presence of *Rf1*, the steady-state accumulation of the larger T-*urf13* transcripts is not measurably reduced. Similarly, a measurable decrease of the steady state accumulation of the larger T-*urf13* transcripts has not been observed in plants carrying *Rf8* or *Rf** (Ref. 28). By contrast, the *Rf3* restorer of sorghum conditions the accumulation of a novel 0.38-kb *orf107* transcript and a marked corresponding decrease in the 1.11-, 0.87- and 0.81-kb unprocessed transcripts[35].

It is possible that post-transcriptional RNA editing plays a role in fertility restoration in some systems. For example, editing might change the length of predicted CMS-associated ORFs by creating new start (AUG) and/or stop (i.e. UAA, UAG, or UGA) codons, because the most prevalent example of editing in plant mitochondrial sequences is C-to-U (Ref. 31). It is also possible that tissue-specific editing might allow a CMS-associated sequence to become deleterious only at microsporogenesis or microgametogenesis. For example, editing of the mitochondrial *atp6* gene of CMS sorghum is strongly reduced relative to normal fertile sorghum in anthers, but not in seedlings[50]. RNA editing of this gene increases following fertility restoration. Because the role of *atp6* in sorghum with CMS is unclear, further experiments are needed in order to establish the association of this intriguing tissue-specific editing with fertility restoration.

Although the analysis of diploid pollen suggests that the *Rf3* restorer of S-cytoplasm maize is a functional allele[47], it has been suggested that the other novel restorers of S-cytoplasm that arise at a high rate result from loss-of-function nuclear mutations that affect mitochondrial gene expression. This hypothesis is consistent with the observation that such restorers generally condition pleiotropic deleterious phenotypes when homozygous. If this hypothesis is correct, the analysis of such restorer loci should prove particularly informative.

In general, sequence analysis of restorer genes should provide significant clues as to their functions. However, precisely because little is known regarding the biochemical nature of the gene products of restorer genes, these genes must be cloned via genetic methods. To compound the difficulty, in many plant species genetic-based systems for cloning genes are not well developed. Hence, to date, only one restorer gene has been cloned. The *rf2* gene, required for fertility restoration in T-cytoplasm maize, encodes a predicted protein with a high degree of sequence similarity to mammalian mitochondrial aldehyde dehydrogenases[51]. Based on this finding, several general hypotheses have been put forward to explain the mechanism by which this gene functions. According to the 'metabolic hypothesis', URF13 alters mitochondrial function such that additional aldehydes are produced. In this scenario, the role of the RF2 protein would be either to detoxify these aldehydes or to catalyze their oxidation into compounds that are essential in plants harboring T-cytoplasm mitochondria. The 'interaction hypothesis' suggests that the RF2 protein interacts either directly or indirectly with URF13 and thereby diminishes or eliminates its deleterious effects. For example, the RF2 protein could catalyze the oxidation of an aldehyde component of the inner mitochondrial membrane, thereby altering the interaction between URF13 and the membrane where it normally accumulates.

Future perspectives

Efforts are under way in a number of labs to clone additional nuclear restorer genes. Hence, within the next few years we should have a better understanding of the diverse mechanisms by which CMS and restoration occur. However, as has been demonstrated for T-cytoplasm maize following the cloning of the *rf2* gene, the identification of the biochemical function of a restorer gene might yield more questions than answers.

Acknowledgements

We thank the many colleagues who sent reprints and in particular Greg Brown, Sally Mackenzie, Chris Makaroff, Maureen Hanson and Daryl Pring for important clarifications. We also thank John Imsande and Thomas Baum for helpful comments on the manuscript. Our research is supported by competitive grants from the US Dept of Agriculture (USDA) National Research Initiative (NRI) (96-00804) and Pioneer Hi-Bred. This is Journal Paper No. J-17676 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, USA, Project Nos. 3390 and 3368, supported by Hatch Act and State of Iowa funds.

References

1 Laser, K.D. and Lersten, N.R. (1972) Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms, *Bot. Rev.* 38, 425–454

2 Mackenzie, S., Shichuan, H. and Lyznik, A. (1994) The elusive plant mitochondrion as a genetic system, *Plant Physiol.* 105, 775–780

3 Levings, C.S., III and Vasil, I.K., eds (1995) *The Molecular Biology of Plant Mitochondria*, Kluwer

4 Kück, U. and Wricke, G., eds (1995) *Advances in Plant Breeding 18, Genetic Mechanisms for Hybrid Breeding*, Blackwell Wissenschafts-Verlag

5 Havey, M.J. and Bark, O.H. (1994) Molecular confirmation that sterile cytoplasm has been introduced into open-pollinated grano onion cultivars, *J. Am. Soc. Hort.* 119, 90–93

6 Mackenzie, S.A. (1991) Identification of a sterility-inducing cytoplasm in a fertile accession line of *Phaseolus vulgaris* L., *Genetics* 127, 411–416

7 Horn, R. *et al.* (1995) Development and molecular analysis of alloplasmatic male sterility in sunflower, in *Advances in Plant Breeding 18, Genetic Mechanisms for Hybrid Breeding*, (Kück, U. and Wricke, G., eds), pp. 89–110, Blackwell Wissenschafts-Verlag

8 Kück, U. (1995) CMS in wheat and triticale, in *Advances in Plant Breeding 18, Genetic Mechanisms for Hybrid Breeding*, (Kück, U. and Wricke, G. eds), pp. 19–36, Blackwell Wissenschafts-Verlag

9 Song, J. and Hedgcoth, C. (1994) A chimeric gene (*orf256*) is expressed as protein only in cytoplasmic male-sterile lines of wheat, *Plant Mol. Biol.* 26, 535–539

10 Warmke, H.E. and Lee, S-L.J. (1977) Mitochondrial degeneration in Texas cytoplasmic male-sterile corn anthers, *J. Hered.* 68, 213–222
11 Hanson, M.R. (1991) Plant mitochondrial mutations and male sterility, *Annu. Rev. Genet.* 25, 461–486
12 Köhler, R.H. *et al*. (1991) Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene, *Mol. Gen. Genet.* 227, 369–376
13 Horn, R., Höhler, R.H. and Zetsche, K. (1991) A mitochondrial 16 kD protein is associated with cytoplasmic male sterility in sunflower, *Plant Mol. Biol.* 17, 29–36
14 Monéger, F., Smart, C.J. and Leaver, C.J. (1994) Nuclear restoration of cytoplasmic male sterility in sunflower is associated with the tissue-specific regulation of a novel mitochondrial gene, *EMBO J.* 13, 8–17
15 Smart, C.J., Monéger, F. and Leaver, C.J. (1994) Cell-specific regulation of gene expression in mitochondria during anther development in sunflower, *Plant Cell* 6, 811–825
16 L'Homme, Y. and Brown, G.G. (1993) Organizational differences between cytoplasmic male sterile and male fertile *Brassica* mitochondrial genomes are confined to a single transposed locus, *Nucleic Acids Res.* 21, 1903–1909
17 Singh, M. and Brown, G.G. (1991) Suppression of cytoplasmic male sterility by nuclear genes alters expression of a novel mitochondiral gene region, *Plant Cell* 3, 1349–1362
18 Singh, M. *et al*. (1996) Nuclear genes associated with a single brassica CMS restorer locus influence transcripts of three different mitochondrial gene regions, *Genetics* 143, 505–516
19 Jean, M., Brown, G.G. and Landry, B.S. (1997) Genetic mapping of nuclear fertility restorer genes for the 'Polima' cytoplasmic male sterility in canola (*Brassica napus* L.) using DNA markers, *Theor. Appl. Genet.* 95, 321–328
20 L'Homme, Y. *et al*. (1997) *Brassica nap* cytoplasmic male sterility is associated with expression of a mtDNA region containing a chimeric gene similar to the *pol* CMS-associated *orf224* gene, *Curr. Genet.* 31, 325–335
21 Bonhomme, S. *et al*. (1992) Sequence and transcript analysis of the *Nco2.5* Ogura-specific fragment correlated with cytoplasmic male sterility in *Brassica* cybrids, *Mol. Gen. Genet.* 235, 340–348
22 Grelon, M. *et al*. (1994) Ogura cytoplasmic male-sterility (CMS)-associated *orf138* is translated into a mitochondrial membrane polypeptide in male-sterile *Brassica* cybrids, *Mol. Gen. Genet.* 243, 540–547
23 Levings, C.S., III (1993) Thoughts on cytoplasmic male sterility in *cms-T* maize, *Plant Cell* 5, 1285–1290
24 Ward, G.C. (1995) The Texas male-sterile cytoplasm of maize, in *The Molecular Biology of Plant Mitochondria*, (Levings, C.S., III and Vasil, I.K., eds), pp. 433–459, Kluwer
25 Dewey, R.E., Levings, C.S., III and Timothy, D.H. (1986) Novel recombinations in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm, *Cell* 44, 439–449
26 Dewey, R.E., Timothy, D.H. and Levings, C.S., III (1987) A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize, *Proc. Natl. Acad. Sci. U. S. A.* 84, 5374–5378
27 Wise, R.P., Dill, C.L. and Schnable, P.S. (1996) *Mutator*-induced mutations of the *rf1* nuclear fertility restorer of T-cytoplasm maize alter the accumulation of T-*urf13* mitochondrial transcripts, *Genetics* 143, 1383–1394
28 Dill, C.L., Wise, R.P. and Schnable, P.S. (1997) *Rf8* and *Rf** mediate unique T-*urf13*-transcript accumulation, revealing a conserved motif associated with RNA processing and restoration of pollen fertility in T-cytoplasm maize, *Genetics* 147, 1367–1379
29 Wise, R.P., Pring, D.R. and Gengenbach, B.G. (1987) Mutation to male fertility and toxin insensitivity in Texas (T)-cytoplasm maize is associated with a frame shift in a mitochondrial open reading frame, *Proc. Natl. Acad. Sci. U. S. A.* 84, 2858–2862
30 Zabala, G., Gabay-Laughnan, S. and Laughnan, J.R. (1997) The nuclear gene *Rf3* affects the expression of the mitochondrial chimeric sequence R implicated in S-type male sterility in maize, *Genetics* 147, 847–860
31 Hanson, M.R., Sutton, C.A. and Lu, B. (1996) Plant organelle gene expression: altered by RNA editing, *Trends Plant Sci.* 1, 57–64
32 Iwabuchi, M., Kyozuka, J. and Shimamoto, K. (1993) Processing followed by complete editing of an altered mitochondrial *atp6* RNA restores fertility of cytoplasmic male sterile rice, *EMBO J.* 12, 1437–1446
33 Kadowaki, K., Suzuki, T. and Kazama, S. (1990) A chimeric gene containing the 5′ portion of *atp6* is associated with cytoplasmic male-sterility of rice, *Mol. Gen. Genet.* 224, 10–16
34 Akagi, H. *et al*. (1995) Genetic diagnosis of cytoplasmic male sterile cybrid plants of rice, *Theor. Appl. Genet.* 90, 948–951
35 Tang, H.V. *et al*. (1996) Transcript processing internal to a mitochondrial open reading frame is correlated with fertility restoration in male-sterile sorghum, *Plant J.* 10, 123–133
36 Johns, C. *et al*. (1992) A mitochondrial DNA sequence is associated with abnormal pollen development in cytoplasmic male sterile bean plants, *Plant Cell* 4, 435–449
37 Mackenzie, S.A. and Chase, C.D. (1990) Fertility restoration is associated with loss of a portion of the mitochondrial genome in cytoplasmic male-sterile common bean, *Plant Cell* 2, 905–912
38 He, S., Lyznik, A. and Mackenzie, S. (1995) Pollen fertility restoration by nuclear gene *Fr* in CMS bean: nuclear-directed alteration of a mitochondrial population, *Genetics* 139, 955–962
39 Abad, A.R., Mehrtens, B.J. and Mackenzie, S. (1995) Specific expression in reproductive tissues and fate of a mitochondrial sterility-associated protein in cytoplasmic male-sterile bean, *Plant Cell* 7, 271–285
40 He, S.C. *et al*. (1996) A cytoplasmic male sterility-associated mitochondrial protein causes pollen disruption in transgeneic tobacco, *Proc. Natl. Acad. Sci. U. S. A.* 11763–11768
41 Glab, N. *et al*. (1990) Expression in *S. cerevisiae* of a gene associated with cytoplasmic male sterility from maize: respiratory dysfunction and uncoupling of yeast mitochondria, *Mol. Gen. Genet.* 223, 24–32
42 Huang, J. *et al*. (1990) Expression in yeast of the T-URF13 protein from Texas male-sterile maize mitochondria confers sensitivity to methomyl and to Texas-cytoplasm-specific fungal toxins, *EMBO J.* 9, 339–347
43 Von Allmen, J-M. *et al*. (1991) Transfer of methomyl and *HmT*-toxin sensitivity from T-cytoplasm maize to tobacco, *Mol. Gen. Genet.* 229, 405–412
44 Dewey, R.E. *et al*. (1988) A 13-kilodalton maize mitochondrial protein in *E. coli* confers sensitivity to *Bipolaris maydis* toxin, *Science* 239, 292–295
45 Chaumont, F. *et al*. (1995) Targeting the maize T-*urf13* product into tobacco mitochondria confers methomyl sensitivity to mitochondrial respiration, *Proc. Natl. Acad. Sci. U. S. A.* 92, 1167–1171
46 Wintz, H. *et al*. (1995) Expression of the CMS-associated urfS sequence in transgenic petunia and tobacco, *Plant Mol. Biol.* 28, 83–92
47 Kamps, T.L., McCarty, D.R. and Chase, C.D. (1996) Gametophyte genetics in *Zea mays* L.: dominance of a restoration-of-fertility allele (*Rf3*) in diploid pollen, *Genetics* 142, 1001–1007
48 Schnable, P.S. and Wise, R.P. (1994) Recovery of heritable, transposon-induced, mutant alleles of the *rf2* nuclear restorer of T-cytoplasm maize, *Genetics* 136, 1171–1185
49 Kennell, J.C. and Pring, D.R. (1989) Initiation and processing of *atp6*, T-*urf13* and *ORF221* transcripts from mitochondria of T-cytoplasm maize, *Mol. Gen. Genet.* 216, 16–24
50 Howad, W. and Kempken, F. (1997) Cell type-specific loss of *atp6* RNA editing in cytoplasmic male sterile *Sorghum bicolor*, *Proc. Natl. Acad. Sci. U. S. A.* 94, 11090–11095
51 Cui, X.Q., Wise, R.P. and Schnable, P.S. (1996) The *rf2* nuclear restorer gene of male-sterile, T-cytoplasm maize, *Science* 272, 1334–1336

Patrick S. Schnable is at the Dept of Agronomy, Iowa State University, Ames, IA 50011, USA (tel +1 515 294 0975; fax +1 515 294 2299; e-mail schnable@iastate.edu);
Roger P. Wise is at the Corn Insects and Crop Genetics Research Unit of the US Dept of Agriculture (USDA)-Agricultural Research Service and the Dept of Plant Pathology, Iowa State University, Ames, IA 50011, USA (tel +1 515 294 9756; fax +1 515 294 9420; e-mail rpwise@iastate.edu).