Nuclear control and mitochondrial transcript processing with relevance to cytoplasmic male sterility in higher plants

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Recent advances in the molecular biology of plant mitochondria have yielded some newer insights. A common, basic set of genes is encoded in all mitochondrial genomes, although their sizes differ greatly, with plant mitochondrial DNAs being by far the most complex. Mitochondrial genome mutation encodes cytoplasmic male sterility (cms) which in turn leads to stamen sterility or pollen abortion in several higher plants. Cms is the resultant of an incompatibility between the nucleus and mitochondrial genomes such that male pollen is aborted or not properly formed. Cms system in agriculturally important crops has been used to produce high-yielding and heterotic hybrid seeds, because it eliminates the need for labour intensive and expensive hand emasculation. Hybrid seeds (or varieties) hold great potential for improving crop economic yields, even when the average yields are much higher in many of the traditional food and feed crops. An important feature of mutation(s) responsible for cms is the discovery of chimeric genes or chimeric locus and different open reading frames joined together, or placed in proximal locations for cotranscriptions with other standard mitochondrial genes. Twenty-nine mitochondrial cms related genes in over twelve so far studied higher plant species have been characterized together with their specific DNA coding sequences. The recent development of *in vitro* systems for transcription initiation and RNA processing has yielded intriguing details of transcriptional and post-transcriptional regulation of plant mitochondrial *cms* gene. The nuclear gene *Rf* or *Fr* (restorer of fertility) prevents the expression of male sterility gene in the cms system in rice (*B-atp* 6) and maize (mitochondrial aldehyde dehydrogenase enzyme) by influencing RNA processing and sequential post-transcriptional editing. The latest findings on the subject support the notion that 'intergenomic interaction', i.e. specific nuclear-mitochondrial gene(s) co-transcription is operational as the plausible molecular mechanism for the manifestation of maternally and cytoplasmically inherited cms genetical trait in crop plants.

THREE genetic systems, one nuclear and two cytoplasmic or extranuclear, mitochondria and chloroplasts are essential for expression and development of various quantitative and qualitative traits in higher plants. Plant mitochondria have been found to cover many facets of genetics and molecular biology[1]. The first evidence for the presence of genes outside the nucleus was provided by non-Mendelian inheritance in plants[2]. Non-Mendelian inheritance and somatic segregation are taken to indicate the existence of functional genes that reside outside the realm of the nucleus and do not undergo hereditary type conventional segregation and transmission on the meiotic and mitotic spindles to distribute genomic replicas to gametes or to daughter cells during microsporogenesis. The interpretation for the frequent occurrence of novel heritable genetic or even nonheritable epigenetic variations has been debated among geneticists and breeders for almost two decades. Exciting new findings have emerged in terms of plant organelle (mitochondria and chloroplast) gene expression[3,4] in relation to the manifestation of cytoplasmic male sterility (cms) and heterosis, after the author's earlier reviews on the related subject exhibited some kind of nuclear control of mitochondrial and chloroplast development and function in higher plants which now provide convincing evidence for a plausible DNA transfer (mobile genes) between the mitochondria, chloroplast and nucleus[5–7], i.e. intergenomic interaction. Cms provides a convenient and proprietary means to produce hybrid seeds. Srivastava[5] propounded a theory of 'intergenomic interaction' to explain genetical phenomenon of heterosis or hybrid vigour based on experimental evidences then available, that mitochondria may interact structurally, functionally and biosynthetically with other cellular-genetical components like nuclear and chloroplast genes so as to endow an overall superiority to heterotic hybrids in many measurable attributes, including crop grain yields. Much of the interest in cms plants stems from their use, or potential use, in producing heterotic hybrid seeds. Presently, hybrid seeds of some crops are produced with the use of cms inbred (predominantly homozygous and homogeneous) lines, while production of others depends upon tedious hand emasculation. The eukaryotic cell nucleur DNA content

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($\times 10^{-14}$ g) is immensely greater (4.0–7.0) compared to chloroplast DNA (0.3–0.9) and mitochondrial DNA (0.1–0.3)[5]. Mitochondria contain their own DNA (mt DNA) and the transcriptional and translational machinery necessary for protein synthesis. Mitochondria, however, are not fully autonomous, and their biogenesis and function depend on coordinated expression of both nuclear and organellar genomes and genes. mtDNA is particularly maternally or uniparentally inherited. Plant mitochondria also possess an alternative route for electron transport system or cyanide-resistant respiration[8]. Many independent mutations that disrupt pollen development or abnormal aborted pollen production have been observed in a wide variety of higher plant species. In tomato, for example, nuclear encoded male sterility mutations have been mapped to over 40 different loci. Many other male sterile mutations, however, do not exhibit Mendelian inheritance. Such cytoplasmic inherited mutations have been so far reported in over 140 higher plant species[9] and the possibility of discovery of novel plant cms gene(s) in other plant species exists through further intensive research. Despite identification of several mitochondrial genes that specify cms trait, its precise molecular basis or molecular mechanism is not understood in any plant species. An updated account of current knowledge concerning the nature of the nuclear–mitochondrial interaction resulting in plant mitochondrial gene mutation(s) encoding economically and commercially relevant cms trait in several important food and other crop species is provided in this article.

Plant mitochondrial genomes organization

The *in vivo* structure of mitochondrial genome organization has been adequately covered[1,10,11]. Earlier studies have elucidated the derivation of circular structures from physical mapping of overlapping restriction fragments; from these maps circular genome structures are predicted for the majority of mitochondrial genomes in eukaryotic cells[12]. A more comprehensive coverage of mitochondrial genome organization, evolution, cms, and analysis of mtDNA in somatic hybrids has been provided[4,13,14]. Several general features of higher plant mitochondrial genome organization have emerged: (i) The genomes are larger than those from animals and fungi. (ii) Restriction endonuclease mapping (using DNA markers such as RFLPs, RAPDs and AFLPs) indicates that plant mitochondrial genomes are usually organized as multiple circular molecules, with conversion of circle types mediated by recombination between repeated sequences. (iii) Mitochondrial genomes from closely related species are highly conserved in the primary sequence, but vary greatly in linear gene order. (iv) The plant mitochondrial genome, like that of other eukaryotes, encodes only a small percentage of proteins located within the mitochondria, indicating that over the course of evolution most of the genes that resided in the original endosymbiont (earlier as prokaryotes) have migrated to the nuclear genome. (v) In addition to high molecular weight mtDNA, plasmid-like molecules are present in the mitochondria. (vi) Chloroplast DNA and nuclear DNA sequences are often found inserted in the mtDNA.

Higher plants exhibit an extremely wide range of variation in mtDNA size with a minimum of ~ 100 kb. Higher plant mitochondrial genomes, which vary in size from 200 kb to 2500 kb are much larger. Analyses of Cucurbit mtDNAs demonstrated a seven-fold range in genome size within the family, from 330 kb in watermelon to 2500 kb in muskmelon. Despite the large size differences, there is no correlation of mitochondrial genome size with repeated DNA sequences, mitochondrial volume or the number of translational products. The function of additional DNA has not been precisely enumerated, but it seems likely that much of it either is noncoded or is subjected to some kind of shuffling mechanism between 'intron' and 'exon' sequences. The size of the genome makes it difficult to isolate the mtDNA intact, but restriction mapping (RFLPs, RAPDs and AFLPs) in several crop plants suggests that each has a single sequence organized as a circle. Within this circle there are short homologous sequences. Recombination between these elements generates smaller, subgenomic circular molecules that coexist with the complete 'master' genome or 'principal' genome explaining the apparent complexity of plant mtDNAs. The mixing of cytoplasms in higher plants promotes mitochondrial fusion and recombination between varying mitochondrial genomes[5,6].

Unlike animal and fungal mtDNAs, which are usually organized in a single circular molecule, plant mtDNA often consist of an assortment of genomic and subgenomic circular molecules of different sizes and frequencies[1]. The cause of this variability was first explained in *Brassica campestris*. Its mtDNA is primarily composed of a single large 218 kb circle called the 'master' chromosome plus smaller circles of 135 kb and 83 kb. Within the master chromosome are two 2 kb direct repeats separated by 83 kb on one side and 135 kb on the other. Recombination between the two repeats generates the smaller 135 kb and 83 kb circles. Moreover, recombination between the two smaller molecules can also regenerate the 'master' chromosome. The 570 kb maize genome has six major pairs of repeats, which may participate in recombination to produce a large array of circular molecules of various sizes and frequencies. The relative proportions of the different circles apparently depend on the recombinational frequencies among the different pairs of repeats and whether the various circles are capable of autonomous replication.

Moreover, genomic rearrangements of the *atpA* gene characteristic of cms-S (USDA) and cms-T (Texas) maize are found at low levels in N (normal) mitochon-

dria[15,16]. It is thought that these 'substoichiometric' *atpA* types are part of a larger circular or linear molecule present in low copy number relative to the rest of the mitochondrial genome in the cell. The substoichiometric molecules called 'sublimons' may have originated from infrequent recombinational events between very short regions of homology in the mitochondrial genome. Thus, the products of rare or unique recombinational events are retained in the genome at low levels and these sublimons may then be expected to exhibit rapid molecular evolution, because mutational events are more quickly fixed by chance in a small population of molecules. Occasional amplification of sublimons could cause sudden genomic organization, possibly leading to the evolution of cms[1,17]. It has been unequivocally demonstrated that mitochondrial genomes of many higher plants contain subprinciple genome DNAs and/or double-stranded RNAs which have been termed minilinear and minicircular[18] for convenience in distinguishing them from the higher molecular weight principal genome.

Tobacco tissue culture cells yield a large percentage of small circular DNAs, which are derived from the principal genome. Of the smaller circular DNAs, the smallest ones (10.1, 20.2 and 30.3 kb) merge to reach the size of some of the larger minicircular and minilinear DNAs, found in other species[19]. It is interesting that the coding sequences of cytochrome *c* oxidase subunit II have been shown to be located on the small circles of Oenothera[11,20,21]. The small, circular DNA molecules of animal cells of about 16 kb have been visualized by electron microscopy and can be isolated as supercoiled molecule populations by CsCl density gradient centrifugation[22]. The *in vivo* structure of the larger mitochondrial DNAs of yeast and plants, however, may be different from the structures predicted by frequent mapping. Pulse field gel electrophoresis exhibits one fraction of molecules to be locked into highly complex arrangements that migrate with an apparent size of more than one megabase[22]. Most plant mtDNAs behave like linear molecules ranging from 50 to 100 kb in a heterogeneous population visible as smear in the gels[10]. Linear DNA molecules have thus been proposed as the major *in vivo* form of mitochondrial genomes in higher plants[10]. Effects of DNA anchoring at membrane integral proteins and specific mechanisms of replication such as those leading to catenated network of trypanosome mtDNA[23,24] may be involved in determining the variation in linear and circular structures in the mitochondria.

Mitochondrial gene(s) and their transcription

Extranuclear genetic systems are a fundamental feature of higher plant cells. The cytoplasmic organelles possess their own DNA. The mitochondrial genomes contribute a limited (10%) but essential number of gene products to the biogenesis of mitochondria, that are competent to carry out oxidative phosphorylation and energetically important cellular respiratory functions. Mitochondria from all organisms provide ATP as the principal energy source for the cell and deliver numerous substrates via specific carriers for biosynthetic reactions in the cytoplasm. Many basic features of the mitochondrial structure and function, developed at an early stage of evolution, have been highly conserved between animals and plants despite a billion years of divergent evolution. The assembly and function of respiratory-competent mitochondrion in eukaryotes result from a collaboration and coordination between gene products derived from mitochondrial and nuclear genomes. The inventory of nuclear and mitochondrially coded proteins required to assemble a functional mitochondrion shows clearly that nuclear and mitochondrial genomes interact in at least two ways. First, both nuclear and mitochondrial genes contribute to mitochondrial protein function. Second, both nuclear and mitochondrial genomes interact to affect the synthesis and assembly of mitochondrial proteins[5,6]. Communication from the mitochondrion to the nucleus probably involves metabolic signals and one or more signal transduction pathways that function across the inner mitochondrial membrane.

Nuclear background influences extensive editing of plant mitochondrial transcription in particular genomic regions. The complete nucleotide sequences of several animal mitochondrial genomes have been published[25–27] and those of certain fungi are nearing completion[28]. These analyses have made possible the location of the coding regions (mitochondrial transcript) and construction of detailed mtDNA restriction maps[12,19,29–33]. The picture as regards the number of genes encoded in the higher plant mtDNA has become clearer. Isolated plant mitochondria synthesize some 20 to 50 polypeptides[34] which are presumed to be encoded by the mtDNA. It has been observed that higher plants also encode 26S rRNA (ref. 34) and in maize mitochondria *coxI*, *coxII*, *cob*, *atp* and 5S, 18S and 26S rRNA genes have been identified on the circular 570 kb restriction map[35]. The protein coding genes are obviously not clustered, whereas the 5S and 18S rRNA genes and the ATPase gene are transcribed from the same mtDNA strand, while the 26S rRNA, *coxI*, *coxII* and *cob* genes are transcribed from the opposite strand[36,37]. Approximately one-tenth of the mitochondrial proteins by weight are specified by the limited number of genes on the mitochondrial genome. Thus mitochondrial genomes contribute a limited but essential number of gene products to the biogenesis of mitochondria, that are fully competent to carry out respiratory functions such as electron transport and oxidative phosphorylation. It is obvious

then that a significant number of nuclear genes must be involved in the synthesis of mitochondrial components, expression of the mitochondrial genome, and control of mitochondrial biogenesis. Nuclear background alters transcription of *atpA* in the 'ogura' cytoplasm of radish[38] and the cms gene (*T-urf* 13) in the T cytoplasm of maize[39]. The *T-urf* 13 cms associated sequence is cotranscribed with the open reading frame orf 221. Specific inbred lines of maize revealed some marked influence of nuclear background on the pattern of transcription and mitochondrial function[40]. Rocheford *et al.*[41] demonstrated that the transcript changes were not only a function of the mitochondrial genomic environment encompassing the *T-urf* 13/orf 221 region but also of dominant nuclear gene action (following Mendelian hereditary transmission principles) influencing the pattern of post-transcriptional processing of the transcripts.

Mitochondrial transcription

The common functions of the mitochondria in plants, animals and fungi are reflected by similarities in their genomes[42]. The recent development of *in vitro* systems for transcription initiation and tRNA processing has yielded intriguing details of transcriptional and post-transcriptional regulation in plant mitochondria. Most of the coding regions are separated by several kilobases of non-coding regions[20,43–45]. This organization implies that most of the essential coding information is expressed as monocistronic transcripts from individual promoters, for example, *atp6*, *atp9*, *cytob*, *coxI*, *coxII*, and *coxIII* genes in most plants. When transcripts are analysed by Northern blot hybridization, the mRNAs are found to be generally much larger than the actual coding regions of the genes and to include extended non-coding 5′ and 3′ transcribed regions of several hundred nucleotides. The frequent genomic recombinations in plant mitochondrial genomes can place genes that are far apart in one genome into close proximity in another species.

Genes located close together almost invariably exhibit co-transcription[11,46,47], such as the *18S-5S*-RNA genes or *nad3-rps12* transcription units. The transcribed spacers between, for example, the *18S-5S* rRNA genes vary among plant species, ranging from just over 100 nt to more than 500 nt; sequences apparently can become integrated or lost by intra-or intermolecular recombination[48]. Larger trancription units would most likely be found upon further detailed investigation of higher plants. The stably transcribed fraction of higher plant mitochondrial genomes has been estimated to be about one-third of the genomic complexity, for example in Brassicaceae and in Cucurbitaceae[49]. This RNA population is equivalent to the entire sequence information common to all plant mitochondrial genomes. The remaining 70% of the plant mitochondrial genome appears to be non-essential sequence information derived partly from chloroplast DNA (ct DNA), partly from nuclear sequences and from the duplication of mitochondrial sequences degenerated to various degrees. The presence of numerous transcription units implies a corresponding multitude of individual promoters of transcription and the presence of several promoters per genome has been confirmed[4]. For some genes, multiple promoters have been identified which are actively engaged in transcription and may be used for differential regulation of the respective gene activity. The *coxII* gene in maize, for example, is transcribed from a closely stacked set of promoter sequences, and the *atp9* gene in this plant is preceded by at least six active promoters spaced over several hundred nucleotides upstream of the coding region[4,47,50]. The transcripts arising from such multiple promoters have different sizes and thus complicate transcription patterns of individual genes even in monocistronic units[4]. Transcription patterns of individual genes are further complicated by the presence of several gene copies in a given genome. In Northern blots, detectable transcripts of different sizes may arise from duplicated genes with different 5′ or 3′ regions adjacent to the respective open reading frame that leads to variable extensions at either end. Common to mitochondria in animals, fungi and plants appears to be the positioning of the conserved sequence block and the first transcribed nucleotide. The conserved sequence motif generally appears to include the first transcribed nucleotide of all mRNAs in yeast and the first two nucleotides in plant mitochondria[50]. Mitochondrial activity, although required in all plant tissues, is capable of adopting to specific requirements by regulated gene expression[4]. Investigation of the factors governing the quality and quantity of distinct RNAs will define the extent of interorganelle regulatory interface in mitochondrial gene expression.

In vitro transcription systems have been established and now permit the identification of sequences necessary and sufficient for efficient transcription initiation[4,51,52]. Post-translational control having some important role in the regulation of plant mitochondrial genome expression has been considered earlier[53,54]. Not much is known about the mechanisms that control gene expression at post-translational 3′ and 5′ ends of mRNAs[4]. Like chloroplast transcripts, plant mitochondrial mRNAs tend to contain inverted repeat sequences in their 3′-region that can fold into stem–loop structures[55–58]. The steady-state level of mRNA from the 'ogura' cms locus of *Brassica* cybrids being determined post-transcriptionally by its 3′ region has recently been demonstrated by Bellaoui *et al.*[12]. Their result strongly suggests that the steady-state level of mRNA from the orf 138 locus is determined post-transcriptionally, most likely by its 3′-region. In the presence of rapeseed mito-

chondrial lysate, synthetic RNAs corresponding to the 3′-region of the Nco 2.7/13 F transcript are, as expected, less stable than RNAs corresponding to the 3′-regions of the Nco 2.5/13S and Bam 4.8/18S transcripts (see Figure 1). Future research should focus on better understanding of the role of these 3′ regions and their post-transcriptional regulation in relation to mitochondrial gene expression in general and *cms* gene in particular.

Mitochondrial transcript processing

Transcript processing is a widespread phenomenon in plant mitochondria though not yet well understood mechanistically. It was first deduced by the observation that unusually complex transcripts in certain regions of the plant mitochondrial genome were produced not only by multiple transcription start and stop sites, but also by internal transcript processing[44]. Although the role of processing in gene regulation is not yet clear, nuclear regulation of transcript processing could be an important means of mitochondrial gene suppression[4]. This inference is based on the observation that three different nuclear *Rf* (restorer of fertility as dominant gene) loci directly influence transcript processing within the respective mitochondrial cms-associated regions. In cms-T maize, perhaps the most well-studied example of cms,

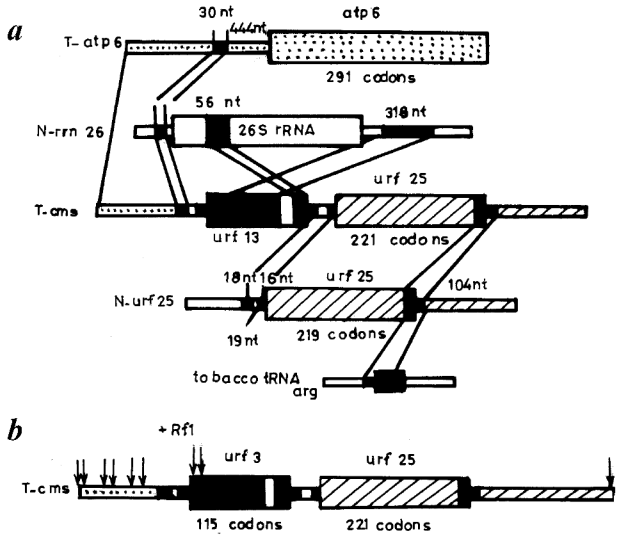

Figure 2. *a*, Structure of the maize (*Zea mays*) cms-associated chimeric gene. Portion of urf13 derived from a normal *atp* 6 gene and a normal 26S rRNA gene. *Atp6* and *26S* rRNA genes are present elsewhere in the maize cms-T mtDNA, but the *urf25* gene downstream of *urf13* is the only copy present in maize cms-T (refs 29, 31). Chloroplast DNA sequences derived from an arginine tRNA gene are found at the 3′ terminus of the *urf25* gene from both a normal fertile maize line and cms-T (ref. 32); *b*, Transcription termini in the maize cms associated chimeric gene. Arrows indicate the location of mapped termini. The arrow labelled *T–Rf*$_1$ indicates a transcript that is increased in abundance in lines containing *Rf*$_1$ allele[30]. (Figure adapted from Hanson[21]).

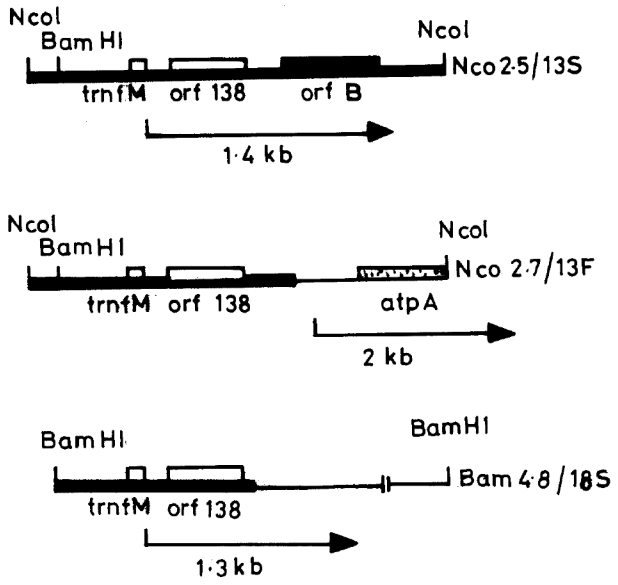

Figure 1. Restriction maps of the three configurations Nco 2.5/13S, Nco 2.7/13F and Bam 4.8/18S, specific to the 13S (sterile), 13F (fertile) and 18S (sterile) *Brassica cybrids*, respectively. The sequences of the Nco 2.7/13F and Bam 4.8/18S fragments which are common with the Nco 2.5/13S fragment are indicated by a thick black line. White box: *trn fM* gene coding for initiator methionine rRNA; light box with plain line; *orf138* gene; dark box: *orfB gene*; striped box: *atpA* gene. *In vivo* accumulated transcripts from each configuration are indicated by the bent arrows. (Adapted from Bellaoui *et al.*[12]).

fertility restoration (*Rf*) requires dominant alleles of two unlinked nuclear loci, *Rf*$_1$ and *Rf*$_2$ (ref. 59). Compelling evidence demonstrates that the product of *Rf*$_1$, essential though not sufficient to restore fertility, directly influences transcript processing of the *T-urf* 13 mitochondrial region (see Figure 2). Processing of *T-urf* 13 transcripts appears to be directly associated with a marked reduction in the expression of the encoded 13 kDa T-URF 13 polypeptide[20]. Although the function of *Rf*$_2$ gene in fertility restoration is not yet understood at the molecular genetic level, it has been speculated that the *Rf*$_2$ gene product may play a role in the detoxification of a pollen-specific product that interacts with the T-URF 13 protein to cause premature tapetal breakdown leading to premature or aborted pollen grains[60]. In *Sorghum* line IS 1112 C, cms is associated with expression of the open reading frame orf 107 (ref. 61). Again fertility restoration is associated with altered processing of orf 107 transcripts and the concomitant reduction in the accumulation of a 12 kDa polypeptide presumed to be the gene product[61]. Of particular interest is the observation that the transcript processing sites described in both cms-T maize and cms sorghum share sequence features[62], implying that sequence motifs exist within plant mitochondrial genes that may act as targets for nuclear-directed gene modulation.

Cms in the *B. napus* (oilseed rape) polima cytoplasm is associated with aberrant expression of a region encoding the *atp6* gene cotranscribed with a downstream chimeric sequence, orf 224 (ref. 63). Fertility restoration conditioned by either of two dominant single nuclear loci, results in a transcript processing event that generates predominantly monocistronic *atp6* transcripts[63]. Generation of monocistronic *atp6* transcripts cosegregates with a single dominant fertility restorer locus, *Rfp*. Most intriguing, however, is the observation that an alternate recessive allele at this locus (rfp_1) or a second locus tightly linked to rfp_1, influences transcript processing of two other mitochondrial genes not associated with male sterility, *nad4* and a cell-like gene that may be involved in mitochondrial cytochrome *c* biogenesis[63]. At all four processing sites under Rfp_1 or rfp_1 control, there is UUGUGG or UUGUUG sequence motif located very near the processing site.

RNA editing alters gene products

Although only 0.13% of the codons in the entire maize chloroplast genome is altered, plant mitochondrial protein coding genes typically have 3–15% of the codons affected by RNA editing[64]. The discovery of RNA editing has changed the way plant organelle genes must be analysed. Before 1991, sequencing of genomic DNA was thought to be sufficient to characterize the coding regions of most plant mitochondrial genes. Earlier literature did predict protein sequences from genomic DNA sequences[4]. However, some anomalies were noted; often plant mitochondrial proteins predicted from genomic sequences differed from mitochondrial proteins in other organisms. As a result, it was proposed that like yeast mitochondrial genes, the genetic code in plant mitochondria differed from the universal code. Because the CGG codon (which codes arginine) was present where homologous genes in other organisms exhibited 'tryptophan', codons such as TGG, CGG were at first proposed to encode tryptophan in the plant mitochondrial genetic code. Now it is known that the genetic code in plant mitochondria does not differ from the standard code but that some CGG codons actually specify UGG as a result of C to U RNA editing. When editing occurs, the encoded amino acid is likely to be altered because the codon position of the edited C is not random. In chloroplasts, the second codon position is the primary target of editing[65], in mitochondria, the first and second positions of codons are more often edited than the third position[66,67]. Plant organelle proteins are more similar to their homologous counterparts in other organisms than was originally realized[4]. Protein sequencing of two mitochondrial gene products (wheat ATP 9 and potato NAD 9) has confirmed the presence of the amino acid predicted from edited codons[68].

Editing of RNA may shorten the predicted open reading frame to the length expected from analysis of homologous genes. The stop codons UAA, UAG and UGA could be created by editing the C-containing codons. Mitochondrial *atp6*, *atp9* and *rsp10* genes from several plant species have 'edit encoded' stop codons, which slightly shorten the open reading frame[69,70]. A bizarre example of an edit-encoded stop codon occurs in the petunia *rpl16* gene. When 15 cDNAs were sequenced, all were found to carry an edit-encoded stop codon early in the open reading frame predicted from the genomic sequence[71]. Editing evidently has converted the mitochondrial *rpl16* gene (*mt-rpl16*) into a pseudogene. Presumably a functional *rpl16* gene may be present in the nucleus. Most plant chloroplast and plant mitochondrial genes carry genomically encoded AUG start codons, unlike human sleeping sickness blood stream protozoa trypanosome[72] mitochondrial genes, in which insertion of U often created the start codon. Nevertheless, ACG to AUG editing creates the start codons of transcripts of the wheat *mt-nad1* gene, the potato *mt-coxI* gene, the maize chloroplast *rpl12* gene (*cp-rpl12*) and the tobacco *cp-psbL* and *cp-ndhD* genes[73,74].

Transcripts of different genes vary in extent of editing

Mitochondrial genes can be divided into three classes with respect to their transcript editing. All transcripts of some mitochondrial genes appear to be fully edited; direct cDNA sequencing as well as sequencing of multiple, independently derived cDNA clones, indicate that all editing sites have Ts replacing Cs. Other genes exhibit partially edited precursor transcripts, but the mature transcript population is fully edited[75,76]. The third class of genes exhibits a low proportion of fully edited transcripts; most transcripts are partially edited[77,78]. When a gene exhibits partially edited transcripts, many individual cDNAs must be sequenced to make sure that every editing site is detected. The existence of partially edited transcipts, and the finding that spliced transcripts are more highly edited than unspliced transcripts, have led to the view that RNA editing in plant mitochondria is a post-transcriptional process[12,33]. Fewer data are available concerning the extent of editing of chloroplast transcripts, but partially edited transcripts of a few genes have been reported[4,12,33]. Editing can occur before splicing in both organelles[4,79]. Transcripts of almost all mitochondrial protein-coding genes contain at least one editing site (Table 1; also see Figures 1–4). Mitochondrial editing events have 5′ and 3′ non-translated regions, introns, ribosomal RNAs and transfer RNAs, but in general, coding regions are more highly edited than non-coding regions. Not only does the extent of editing of different mitochondrial genes' transcripts vary within

the same species, but the number of codons edited varies in transcripts of the same gene in different species[4,29–32] (Table 1).

In plant, there is no published evidence that transcripts present in comparable abundance in different tissues are differentially edited. Transcripts of *psbL* are present in similar amounts in chloroplasts and chromoplasts of ripening bell pepper (*Capsicum annum*), and editing occurs in both types of plastids[80]. When the degree of editing of spinach *cp-psbF* and *cp-psbL* transcripts was examined by direct sequencing of cDNAs,

Table 1. Altered codons in plant mitochondrial protein-coding genes*

Mitochondrial gene (cms related)	Number of codons altered (%)
atp6	6
atp9	13
CoxII	5
nad1	7
nad3	13
Pcf	3
rpl16	3
rps12	4
Rps19	5
mt-ATP 6 core region	Number of codons changed (%)
Rice	4
Sorghum	6
Radish	0.4
Petunia	6
Ocnothera	8

*Adapted from Hanson *et al.*[64] with modification.

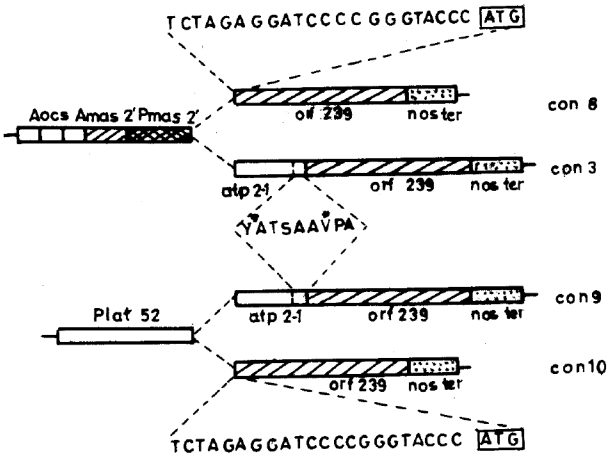

Figure 3. Construction of *orf239* chimeric gene in transgenic tobacco (*Nicotiana tabaccum*). Strategy for cloning each gene construct into the transferred DNA binary vector pB1101 is shown. Aocs, activator of the Octopine synthase gene (trimer); Amas 2′ gene, activator of mannopine synthase 2′ gene; Pmas 2′, promoter of the mannopine synthase 2′ gene; PLat 52, promoter of the tomato pollen specific gene Lat 52. The boxed ATG is the first codon of orf 239. ? , Site specific cleavage; *, the amino acid altered as a consequence of cloning (Adapted from He *et al.*[19]).

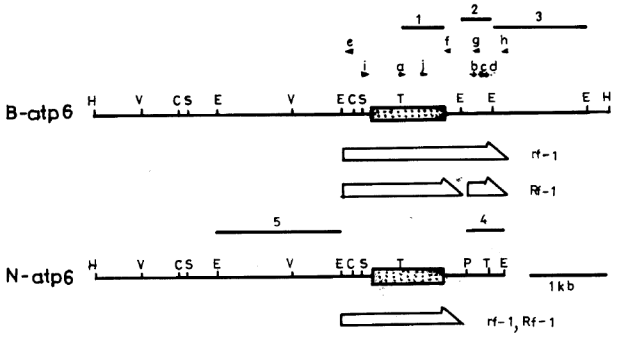

Figure 4. Schematic diagram of *atp6* region of cytoplasmic male sterile rice (*Oryza sativa*). The *N-atp6* and *B-atp6* genes isolated from N(r), B (R), and C. The coding region is shown on a dotted box. The probes used for nucleic acid hybridization are indicated by bars (1–5). The sizes of probes 1–5 are 0.55, 0.4, 1.3, 0.45 and 1.7 kb, respectively. The primers used for sequencing, PCR amplification and primer extension experiment are shown as arrow heads (a–j). The *atp6* RNAs are represented by arrows. The transcript of *B-atp6* is altered, whereas that of *N-atp6* is not changed by the presence or absence of the Rf_1 gene. The restriction sites are shown on E, *Eco*R1; H, *Hin*dIII; P, *Pst*I; T, *Eco*T221, S, *Sal*I and V, *Eco*RV. (Adapted from Iwabuchi *et al.*[33]).

Bock *et al.*[81] found that the extent of editing was lower in seeds and roots than in leaf etioplasts and chloroplasts. The reverse transcription-polymerase chain reaction (RT-PCR) assay was applied to search for differences in the extent of editing of petunia *nad3* transcripts in different tissues, no developmental regulation of the extent of editing was detected[21]. In the absence of a transformation system in which sequences can be deliberately altered, editing can be assessed in abnormal genes created by the rearrangement characteristic of plant mitochondrial genomes[82]. In genes created by such aberrant recombination events, pieces of the coding regions of mitochondrial genes are put into different contexts, but editing events in the translocated regions are usually still observed. Despite numerous recombination events placing portion of *atp9*, parts of the first and second exon of Cox II, and an unidentified reading frame (urf) together in the petunia *pcf* gene, almost all sites are edited[83]. A 193-nucleotide segment of wheat Cox II is edited when present as part of the *nad3/rps12* transcript[27]. In the *atp6* gene, found in maize T-cms, a segment containing 30 nucleotides of the coding region, plus some upstream sequence derived from the *atp9* coding region was still edited even when located next to an unidentified urf[84,85] (see Figure 2).

Nuclear-encoded genes required for normal mitochondrial gene expression have been described for several mitochondrial genes in yeast[86]. They regulate the expression of mitochondrial genes at both the transcriptional and post-transcriptional levels. In higher plants the expression of the nuclear and mitochondrial genomes must be coordinated to make a functional cell. Cms is a loss of functional pollen in plants that can be

attributed to unique mitochondrial genomes. Cms is the resultant of an incompatibility between nucleus and mitochondrial genomes such that pollen is not properly formed. However, there are nuclear genes designated *Rf* or *Fr* (restorer of fertility) that can correct this incompatibility and allow the development of functional pollen. Thus, cms offers a system with which to study the mechanism(s) by which nuclear genes directly regulate mitochondrial gene expression. Among the several *Rf* genes known to reduce or completely eliminate the cms phenotype, some specifically affect the transcription patterns of the *cms* gene. One of the restorers introduces new processing sites into the mutant *T-urf13* transcripts and leads to almost complete reduction of the corresponding polypeptide synthesis[17,86] (see Figure 2). The open reading frame (orf) of the mitochondrial complex IV gene *CoxI* is extended by a recombination event in sorghum cytoplasms and leads to a cms phenotype upon high expression[4]. As yet not known nuclear genes revert the cms phenotype and compensate for the expression of this aberrant protein[87]. In sunflower mitochondria, a recombined novel orf correlated to a cms phenotype is likewise influenced by specific nuclear gene expression[4].

Comparison of Northern blot analysis and run-on experiments has exhibited that the nuclear restorer genes act at the post-transcriptional level and alter transcript stability. Recent results suggest that RNA processing by Rf_1 in rice-cms system does influence the sequential post-transcriptional editing of the B-atp6 RNA[33] (see Figure 4). The *B-atp6* gene was identified as a cms gene by Southern analysis of the mitochondrial genome. The coding sequence of *B-atp6* was identical to the normal *N-atp6* gene but its 3′-flanking sequence was different starting at 49 bases downstream from the stop codon[33]. Northern analysis showed that *B-atp6*, transcribed into a 2.0 kb RNA, in the absence of the Rf_1 gene whereas two discontinuous RNAs, of 1.5 and 0.45 kb were detected in the presence of the Rf_1 gene. Iwabuchi *et al.*[33] interpreted these results by suggesting that the unprocessed RNAs of *B-atp6* are possibly translated into altered polypeptides and that interaction of RNA processing and editing plays a role in controlling cms expression and the restoration of fertility in rice. The recent identification of a nuclear restorer gene in maize (rf_2) coding from a mitochondrial aldehyde dehydrogenase suggests that metabolic enzymes influence directly through gain of function or indirectly by their metabolic activity[16].

Cms phenotype caused by mitochondrial mutation

The cms phenotype is now thought to be caused by mitochondrial mutations. Mitochondrial genomes of higher plants are too large (over 200–2400 kb) to permit identification of mutation by complete genomic sequencing. In plants, several strategies have been used to locate mtDNA regions of interest for sequencing. In maize, type cms-T, a genomic library was screened with cDNAs from mitochondria of fertile vs male sterile (cms) maize lines, to identify the gene region expressed differentially under the influence of the nucleus[4]. So far twenty-nine mitochondrial cms related region(s) or gene(s) (mostly chimeric in nature) in over twelve plant species have been completely or partially characterized (Table 2; also see Figures 1–4)). Mitochondrial genes have been reported to be associated with cms cytoplasm in maize, sunflower, petunia, sorghum and radish[88–96] (Table 2). In maize, petunia and sorghum a chimeric mitochondrial gene which produces a novel protein has been found in cms plant mitochondria[87,92,97]. In the mitochondria of cms sunflower a new open reading frame is co-transcribed with the *atp1* gene[98] and the protein supposed to be encoded by this orf is uniquely present[99]. Among these examples only the *T-urf13* (Figure 2) associated with maize cms-T cytoplasm has been strongly correlated with the cms phenotype based on the analysis of a number of mutants which are toxin resistant and fertile[100]. Although novel proteins uniquely present in the cms mitochondria, an altered gene expression caused by mitochondrial genome rearrangments is thought to be responsible for cms, the precise

Table 2. Mitochondrial region/gene(s) involved in cytoplasmic male sterile phenotype in several higher plant species

Plant species	Mitochondrial gene(s)/ chimeric mitochondrial gene	References
Rice (*Oryza sativa*)	*Atp* 6 genes (*N-atp* 6 and *B-atp* 6), *orf* 25, *cox* III	33, 46
Maize (*Zea mays*)	*T-urf* 13, *orf* 221, *atp* 6, *atp* 9	16, 21, 30, 34, 100, 116, 119–121
Sorghum (*Sorghum vulgare*)	orf 107, Cyto *c* oxidase	61, 87, 89
Sunflower (*Helianthus annuus*)	*atp* A, *atp* 9, *Cob*, *rrrn* 26, *orf* 552	98, 99, 122
Oilseed Rape (*Brassica napus*)	*atp* 6, *orf* 224, *nad* 4	63
Bean (*Phaseolus vulgaris*)	pvs sterility sequence, *orf* 98 and *orf* 239	13
Teosinte (Wild relative of maize)	*Cox* II	11, 47
Arabidopsis thaliana	*CHM*	123
Nicotiana tabacum	*orf* 25, *orf* 239	19, 107
Brasssica tournefortii	*orf* 3	108
Radish (*Raphanus sativum*)	*atp A*	96
Triticum timopheevi	*atp* 6, *orf* 25	110
Petunia (*Petunia hybrida*)	*pcf-a*	124
Brassica cybrids (Ogura cytoplasm)	*orf* 138, *orf B*	125, 126

molecular mechanism underlying cms expression still remains to be elucidated. Observation of altered electron transport in petunia and toxin-mediated membrane disruption in maize plants, bacteria and yeast expressing the maize *urf13* gene product provide clues to possible mechanism of disruption of pollen development or dysfunctional mitochondria in aborted pollen. Whether disruption in a particular mitochondrial function is at the root of cms in all higher plant species, or whether defects in numerous mitochondrial activities can produce pollen male sterility will only be unravelled by further probing of physiological and biochemical defects present in cms genotypes.

Nuclear gene influence

The nucleus somehow affects mitochondrial genome organization and function in higher plant systems. The plant mitochondrial genome, now fully sequenced in *Arabidopois*[101] and *Marcantia*[102], is organized in a much more complex and variable structure than is observed in other higher plants or eukaryotes[103]. In most higher plants, this organization is defined by the presence of recombinationally active repeated DNA sequences that allow for high- and low-frequency inter- and intramolecular recombination events to occur[104]. The physical organization of the mitochondrial genome in plants has proved to be rather difficult to define. Although most genomes map on circular molecules defined by overlapping clones, direct physical observation by pulsed field gel electrophoresis, electron microscopy and other procedures has revealed that the genome may consist of both linear and circular forms, with molecules much larger than the multiple circles constructed by clone analysis[105]. A multipartite genome organization exists in the mitochondria of most plant species, with each molecule containing only a portion of the genetic information. The relative copy number of the various mtDNA forms and their recombinational activity appears to be under nuclear control. One of the most pronounced examples is the observed loss of a mitochondrial genomic molecule in response to a single nuclear gene in common bean (*Phaseolus vulgaris*)[13,106]. The cms-associated mitochondrial mutation in common bean, pvs-orf 239, appears to be maintained on a single 210 kb molecule within a tripartite mitochondrial genome organization[13]. Introduction of a single dominant nuclear factor, *Fr*, (fertility restorer) results in a genomic shift of the pvs-orf 239-containing molecule to substoichiometric levels within the genome, thus restoring pollen fertility/male fertility. The development of alloplasmic lines, derived by recurrent backcrossing strategies or protoplast fusion to combine different mitochondrial and nuclear genotypes, routinely gives rise to changes in relative stoichiometries and mitochondrial genomic rearrangements in *Nicotiana*[107], *Brassica*[108,109] and *Triticum*[110–112]. In some cases, it has been possible to identify specific nuclear loci essential to establish compatibility in individual nuclear–cytoplasmic (intergenomic) combinations[113]. It may thus be possible to predict particular nuclear–cytoplasmic intergenomic interacting genetic combinations which would give rise to high frequency of specific mitochondrial mutations. Several nonchromosomal stripe mutations (ncs) affect distinct loci and have arisen by what appears to be different molecular events[11,47,114]. A characteristic of cms mutation in plants is the presence of chimeric genes or chimeric locus and different open reading frames are joined together or placed in proximal locations and co-transcribed with standard mitochondrial genes. Despite much progress, and the identification of several mitochondrial loci that specify cms in a number of crop plants (Table 2; also see Figures 1–4), the molecular basis of this nuclear–mitochondrial interactive gene-related genic defect is not well understood in any plant species. Observations of altered electron transport in wheat[115,116] and petunia and toxin-mediated membrane disruption in maize plants[15,16], bacteria, and yeast expressing the maize *urf13* gene product, provide clues to possible mechanisms for disruption of pollen development[117]. Whether disruption in a particular mitochondrial function[115,118,119] is at the root of cms in all species, or whether many nuclear–mitochondrial intergenomic interaction-related genetic defects in numerous key mitochondrial activities related to electron transport and oxidative phosphorylation (ATP energies) can produce male pollen sterility, will only be revealed by further probing of physiological and biochemical defects naturally or evolutionarily present in cms genotypes of higher plants. Cms mutations have proven particularly useful in defining regulation of mitochondrial respiratory functions. Data suggest that variation in promoter strength is likely a primary influence on relative transcription rates[4]. Nucleus genes regulating transcription rate or promoter selection are difficult to detect. Perhaps the most compelling demonstration of nucleus control on mitochondrial gene transcription is described in a *Zea mays/Zea perennis* alloplasmic line. A single nuclear gene, described *Mct*, influences promoter selection in *CoxII* gene in maize[11,47,114]. Transcriptional initiation at position 907 produces the predominant *CoxII* transcript of ~1900 nucleotides. The dominant nuclear *Mct* allele apparently directs transcriptional initiation at a second site (– 347) upstream to the *CoxII* locus. Interestingly, this alternate initiation site, detected as a shorter *CoxII* transcript, does not conform to the consensus promoter sequence described for maize[4,15]. This provided some circumstantial evidence that specific nuclear gene produced cofactors may influence promoter selection in cms-related genomic region of plant mitochondria. While nuclear genome is undoubtedly the

principal source of heredity and Mendelian gene transmission, the role played by cytoplasmic and maternally and non-Mendelian inherited mitochondrial genome through intergenomic interaction linked cotranscriptions in giving rise to evolutionarily and speciation wise important cms trait and resultant hybrid seeds with heterosis having high proprietary, economic, and commercial relevance in higher plant world needs further focusing and mechanistic unravelling[115–118].

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Genetic transformation of rice: Current status and future prospects

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Genetic transformation of rice has been an important area of research in the past few years. PEG, electroporation, microprojectile bombardment, and *Agrobacterium* have been used to mediate gene transfer. Genes for insect resistance, fungal resistance, virus resistance, herbicide resistance, bacterial resistance and nematode resistance have been utilized in rice transformation. The methods involved, the genes utilized and the promoters used in rice transformation along with integration and expression of transgenes are discussed in this review.

THE ever-increasing human population especially in the developing countries and various abiotic and biotic stresses have posed a challenge to boost the rice production in limited cultivable land[1,2]. Genetically engineered plants with genes of direct interest can be produced in a relatively short time and can be of direct value in the agri-food industry. Recently it has been recognized that marker-free transgenics may be more acceptable commercially[3,4]. Some good reviews are already available[5–8]. Initially rice was transformed with direct transformation methods such as particle bombardment. Recently it has been shown that *Agrobacterium tumefaciens* can efficiently transform rice also. In this review, we shall discuss the latest developments in the transformation of rice. We shall focus on advances in gene transfer techniques and we shall also mention specific genes that have recently been introduced into rice plants as well as various issues related to the integration and expression of foreign genes.

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Methods of gene delivery

PEG-mediated rice transformation

In 1986, Uchimiya *et al.*[9] first obtained transgenic calli after polyethylene glycol (PEG)-induced DNA uptake of the *nptII* gene into root-derived protoplasts, followed