

The Texas Cytoplasm of Maize: Cytoplasmic Male Sterility and Disease Susceptibility

CHARLES S. LEVINGS, III

The Texas cytoplasm of maize carries two cytoplasmically inherited traits, male sterility and disease susceptibility, which have been of great interest both for basic research and plant breeding. The two traits are inseparable and are associated with an unusual mitochondrial gene, *T-urf13*, which encodes a 13-kilodalton polypeptide (URF13). An interaction between fungal toxins and URF13, which results in permeabilization of the inner mitochondrial membrane, accounts for the specific susceptibility to the fungal pathogens.

THE TEXAS, OR T, CYTOPLASM (*cms-T*) OF MAIZE WAS FIRST described in Texas in the Golden June line of maize (1). Maize geneticists and breeders were immediately interested in the Texas cytoplasm because it carried a trait for cytoplasmically inherited male sterility, which they wished to use in maize hybrid production in order to exploit hybrid vigor. This impetus led to many applied and basic studies dealing with cytoplasmic male sterility and disease susceptibility of the Texas cytoplasm and its relation to mitochondrial and nuclear genes. In this review, I have attempted to provide a contemporary treatment of the molecular basis of cytoplasmic male sterility and disease susceptibility of the Texas cytoplasm of maize.

Cytoplasmic Male Sterility

When plants fail to produce functional pollen grains, they are called male-sterile. Male sterility may be conditioned by either nuclear or cytoplasmic genes. If the sterility trait is inherited in a non-Mendelian fashion, it is designated as cytoplasmic male sterility (CMS). Cytoplasmic genes are most often maternally transmitted in plants. CMS is common among plant species; it has been reported in 140 species from 47 genera and 20 families (2). Among these CMS types, more than one-half arose naturally, about 20% were discovered in intraspecific crosses, and the remainder in interspecific crosses (3). Although CMS causes abortion of the male gametophyte, it typically does not affect female gametophytic development.

Three major CMS types are recognized in maize—*cms-C*, *cms-S*, and *cms-T* (4). Male-sterile cytoplasm is distinguished by specific nuclear genes that restore pollen fertility. These genes, called restorers of fertility (*Rf*), suppress the male-sterile effect of the

cytoplasm, allowing the production of viable pollen. Restorer genes do not cause heritable changes in maize cytoplasm, although in bean the restorer gene is implicated in causing alterations in mitochondrial DNA (mtDNA) (5). Other characteristics also differentiate the three male-sterile cytoplasm and normal maize. They include mtDNA restriction fragment length polymorphisms, variations in mitochondrial RNAs (mtRNAs) revealed by Northern blot analyses, and differences in mitochondrial translational products (6–8). Particular cytoplasm can also be distinguished by differences in disease resistance, toxin and insecticide resistance, histological variations, reversion to fertility, and mtDNA and mtRNA plasmids. Together, the differences in restorer genes and other characteristics suggest that distinctive cytoplasmic factors are responsible for the various CMS types.

In *cms-T* maize, male sterility is characterized by failure of anther protrusion and by pollen abortion, the failure of pollen to develop. The *cms-T* of maize has been extensively studied, primarily because of its widespread use in hybrid seed production and its unique disease susceptibility. For nearly two decades, the 1950s and 1960s, *cms-T* was used in hybrid production in order to avoid hand or mechanical emasculation. It was popular among seedsmen because it provided a reliable and stable source of CMS for seed production. In 1969 and 1970, a fungal disease known as Southern corn leaf blight, which is caused by *Bipolaris maydis* race T (formerly known as *Helminthosporium maydis* race T), occurred in epidemic proportions in the South and Corn Belt regions of the United States (9, 10). This fungal pathogen severely blighted maize carrying *cms-T*, which at that time constituted more than 85% of the U.S. corn acreage. The pathogen has only mild effects on maize plants carrying normal cytoplasm. It soon became apparent that *cms-T* was directly related to the blight, and the large-scale utilization of *cms-T* for hybrid seed production was discontinued. The specific susceptibility of *cms-T* maize to the blight once again demonstrated the dangers of genetic uniformity, although in this instance vulnerability was attributable to cytoplasmic, rather than nuclear, factors. Another fungal pathogen, *Phyllosticta maydis*, is also specifically virulent to maize carrying *cms-T*. The disease outbreak caused by this pathogen, however, was less serious than the Southern corn leaf blight because it was generally limited to the cooler northern regions of the United States.

Bipolaris maydis race T and *P. maydis* produce the pathotoxins BmT and Pm, respectively, which are host-specific to *cms-T* maize (11). Daly and co-workers purified and characterized these toxins and showed that they had specific activity toward *cms-T* maize (12). These pathotoxins adversely affect mitochondria from *cms-T* maize but not mitochondria from other maize cytoplasm or other plant species. The effects of these toxins on *cms-T* mitochondria include the inhibition of malate-supported state 3 respiration, the stimula-

The author is in the Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614.

tion of reduced nicotinamide adenine dinucleotide (NADH)-mediated state 4 respiration, the induction of organelle swelling and the leakage of small molecules [Ca^{2+} and nicotinamide adenine dinucleotide (NAD^+)], and the uncoupling of oxidative phosphorylation (13, 14). Pm and BmT toxins have similar structures, which contain repeated linear β -oxydioxo or oxy-oxo polyketol groups, respectively, on a methylene backbone. BmT toxins are longer, ranging from 35 to 45 carbon atoms in length, whereas Pm toxins range from 16 to 25 carbons (15). Although the relation between toxin structure and toxicity is unclear, several observations may be relevant. In order to show toxicity equivalent to native Pm toxins, synthetic versions of Pm toxins that are 24 and 16 carbons in length must be present at concentrations 10 and 1000 times greater than those of the native toxins, respectively (16). Moreover, Pm and BmT toxins retain toxicity toward *cms-T* mitochondria even when their ketone groups are reduced with sodium borohydride (17). The carbamate insecticide methomyl {S-methyl-N-[(methylcarbamoyl)-oxy]thioacetimidate}, the active ingredient in Du Pont's systemic insecticide Lannate, causes equivalent toxic effects on *cms-T* mitochondria (18). This is unexpected because Pm and BmT toxins are structurally unrelated to methomyl.

Normal maize and maize with *cms-S* and *cms-C* support only limited colonization by *B. maydis* race T. Colonization is normally restricted to boat-shaped lesions on the leaves that do not coalesce but remain small and isolated. Consequently, *B. maydis* race T is generally not a serious pathogen on maize with these cytoplasms. In marked contrast, *B. maydis* race T can rapidly and completely colonize *cms-T* maize. Lesions enlarge rapidly, coalesce, and spread throughout the plant, causing profound damage and, sometimes, death. Evidence indicates that the specific susceptibility of *cms-T* maize to *B. maydis* race T is due to the unique sensitivity of its mitochondria to BmT toxin; mitochondria of disease-resistant maize types are insensitive to BmT toxin. Specific virulence of *P. maydis* has a common basis with that of *B. maydis* race T in that this pathogen produces a structurally similar toxin to which *cms-T* mitochondria are also sensitive.

The Mitochondrial Gene *T-urf13*

The *cms-T* of maize carries a mitochondrial gene that is designated *T-urf13* (19). This gene encodes a 13-kD polypeptide (URF13) that is a component of the inner mitochondrial membrane. Early studies indicated that URF13 might be associated with Complex IV of the electron transfer complex (20) or the F_0F_1 -ATPase (adenosine triphosphatase) (21). More recent results, however, show that URF13 can associate with several different complexes, and it is doubtful that URF13 is specifically bound to a particular inner mitochondrial membrane component (22).

T-urf13 is located in an unusual 3547-nucleotide mtDNA sequence (19) that contains two open reading frames: one coding for *T-urf13* and the other for *orf221*. The latter, which is located 77 nucleotides downstream from *T-urf13*, is transcribed and would encode a polypeptide of about 25 kD (221 amino acids) if translation starts at the first methionine codon in the open reading frame. The most unusual aspect of the 3547-nucleotide sequence is its composition; it contains sequences with significant similarity both to two other mitochondrial genes and to a chloroplast gene. Specifically, the sequence contains stretches homologous with the 5' flanking region of the *atp6* gene, the 3' flanking region of the *rm26* gene (which encodes 26S rRNA), a part of the coding region of *rm26*, and a chloroplast gene encoding a tRNA for arginine. At least seven recombination sites are easily identified in the chimeric sequence, which appears to have originated by rearrangements

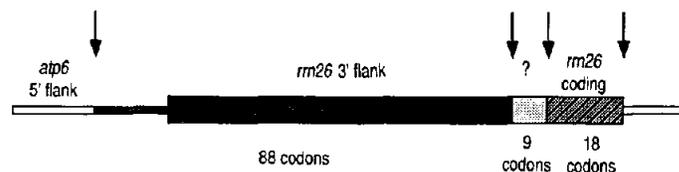


Fig. 1. The *T-urf13* mitochondrial gene of *cms-T* maize. Narrow horizontal lines indicate 5' and 3' noncoding regions; broad horizontal lines in different patterns indicate coding regions. Regions of homology with other mitochondrial genes are identified above the horizontal line; ? indicates sequence of unknown origin. Arrows indicate sites of recombinations. Adapted from (26) with permission of publisher.

involving both intramolecular and intermolecular recombinational events.

The specific composition of *T-urf13* is shown in Fig. 1. The coding region consists of 115 codons; the first 88 codons are similar to the 3' flanking region of *rm26*, the next nine codons are of unknown origin, and the last 18 codons are similar to the coding region of *rm26*. A large 5' flanking region (5 kb) of *T-urf13*, which is presumed to contain the promoter region, is similar to the 5' flanking region of *atp6*. Thus, *T-urf13* and *atp6* appear to have similar promoters. In addition to the recombinant fragment, the *cms-T* mitochondrial genome has complete, functional copies of *atp6* and *rm26* located elsewhere.

It is interesting that *T-urf13*, a protein-encoding gene, could have arisen from these complex rearrangements. The *T-urf13* coding region is composed largely of sequences from the coding and flanking regions of *rm26*, a gene coding for a structural RNA. Besides creating a 345-nucleotide open reading frame, rearrangements have also positioned *T-urf13* downstream from a sequence with mitochondrial promoter activity. *T-urf13* is not found in other male-sterile or male-fertile maize cytoplasms, or in other plant species that have been examined. Because *T-urf13* is not necessary for normal mitochondrial function and because of its complex origin, it is not surprising that it appears to be unique to *cms-T* maize.

Rearrangements of mtDNA are a significant force in causing gene mutations and in altering the organization of the genome. The plant mitochondrial literature contains many examples documenting the widespread occurrence of mtDNA rearrangements (6-8, 19, 23, 24). Investigations of plant mitochondrial genes have revealed mutations that feature rearrangements containing DNA sequences from other mitochondrial genes or from unknown sources. Recombinations between chloroplast and mitochondrial genomes have also resulted in mitochondrial genes that contain sequences homologous with chloroplast DNA. It is evident, therefore, that both intramolecular and intermolecular recombination can play a significant role in the origin and alteration of plant mitochondrial genes. The organization of the *cms-T* mitochondrial genome has been described (25); it differs in organization and size from the mitochondrial genome of normal maize (normal, 570 kb; *cms-T*, 540 kb). These differences reflect the effects of rearrangements on the genomic organization.

Substantial correlative evidence suggests that *T-urf13* is responsible for CMS in *cms-T* maize. The gene is apparently constitutively expressed in *cms-T* mitochondria and its transcripts are present in coleoptiles, ear shoots, leaves, roots, and tassels. A chemically synthesized oligopeptide based on the deduced amino acid sequence encoded by *T-urf13* was used to prepare an antiserum that detected URF13 (21, 26). The 13-kD polypeptide was readily identified by protein immunoblot analyses in *cms-T* mitochondria from all organs examined. Before the identification of *T-urf13*, Leaver and co-workers (27) used an in organello protein-synthesizing system to show that a 13-kD protein is synthesized specifically in *cms-T* mitochondria. They also demonstrated that the 13-kD protein is not

detected in mitochondria from normal or other male-sterile maize. Moreover, a labeled 13-kD protein was immunoprecipitated by the URF13-specific antiserum from the in organello translation products of *cms-T* mitochondria (26). These results show that the T-*urf13*-encoded polypeptide is associated only with the *cms-T*-type of CMS.

The dominant nuclear alleles of the *Rf1* and *Rf2* loci act jointly to suppress pollen abortion in *cms-T* maize (4). *Rf1* alters the transcriptional profile of T-*urf13* and decreases the abundance of URF13 by approximately 80% (26, 28). The homozygous recessive genotype *rf1 rf1*, in contrast, does not alter the transcriptional profile of T-*urf13* or affect the abundance of URF13. The means by which *Rf1* affects the expression of T-*urf13* is not understood but effects on RNA processing may be involved.

Rf1 may affect the expression of other mitochondrial genes in addition to T-*urf13*; however, studies to date have not detected additional activity. We do not know if the *Rf1* gene originated before or after T-*urf13* arose. In contrast to *Rf1*, *Rf2* does not seem to influence the expression of T-*urf13*, and it is uncertain how it contributes to the restoration of male fertility. However, because it is difficult to study restorer gene activities in particular anther cell layers, tissue-specific effects could easily be overlooked. Most maize inbred lines carry the *Rf2* restorer allele but lack the *Rf1* allele. The fact that *Rf1* affects the expression of T-*urf13* strengthens the association of T-*urf13* with CMS. Finally, although the gene product of T-*urf13* appears responsible for CMS, the possibility that CMS is due to a position effect cannot be ruled out as yet.

A strong association between T-*urf13* and the two traits, CMS and disease susceptibility, has been suggested from the analysis of *cms-T* revertants. Although spontaneous mutations that restore male fertility to *cms-T* plants have not been confirmed, reversion to male fertility is observed in plants derived from cell culture experiments. When calli initiated from immature embryos of *cms-T* maize are cultured on medium containing BmT toxin, some toxin-resistant calli are obtained. Usually, *cms-T* calli are sensitive to the toxin and grow poorly, if at all. Whole plants regenerated from resistant calli are often male-fertile, toxin-insensitive, and resistant to *B. maydis* race T (29). Some plants regenerated from resistant calli, however, remain male-sterile and disease susceptible. When similar experiments are conducted without toxin selection, male-fertile and toxin- and disease-resistant plants are also obtained (30). These latter experiments suggest that somaclonal variation may contribute to the reversion phenomenon. Studies of the revertants indicate that CMS and disease susceptibility are inseparable. Among true-breeding revertants, only male-fertile and disease-resistant phenotypes are found; the alternative combinations, male-sterile and disease-resistant and male-fertile and disease-susceptible, are not encountered. Genetic analyses show that reversion is due to changes in cytoplasmic genes rather than nuclear genes. These results show that nuclear mutations that give rise to new suppressor genes are not responsible for reversion. Several revertants have been characterized by restriction enzyme mapping and nucleotide sequencing. These investigations reveal that T-*urf13* is missing from several of the revertants and that homologous recombination is probably responsible for the deletion events (31). One revertant, designated T4, contains a mutation of T-*urf13* that results in a premature stop codon and a truncated URF13 polypeptide (32). Collectively, these results indicate that T-*urf13* is required for both the CMS and disease susceptibility phenotypes of *cms-T*.

Many mitochondrial genes that feature rearrangements are suspected of causing CMS. The best documented is *pcf*, a distinctly chimeric sequence that is associated with CMS in petunia. The *pcf* gene is composed of 5' flanking and coding sequences acquired from *atp9*, coding sequences from *coxII*, and sequences from unknown sources that are designated S-*urf* (33). Complete, functional *atp9* and

coxII genes are located elsewhere in the mitochondrial genome. Immediately downstream of *pcf* are two essential mitochondrial genes, *nad3* and *rps12* (34), which are cotranscribed with *pcf*. The *pcf* gene has been associated with CMS by its cosegregation with male sterility in somatic hybrids and by the demonstration that expression of the *pcf*-encoded polypeptide is affected by the nuclear restorer gene conferring fertility (35).

The origin, chimeric nature, activity, and organization of *pcf* and T-*urf13* are strikingly similar. CMS has also been correlated with chimeric gene sequences in *Brassica* (36), possibly in sorghum (37), in the *cms-C* of maize (23), and in other species. It is evident that mtDNA rearrangements have played a significant role in the origin of CMS. Finally, although *pcf* and T-*urf13* are newly created, nonessential genes, it is likely that mutations of essential mitochondrial genes can also cause CMS.

Other maternally inherited abnormalities are attributable to mitochondrial gene mutations. Nonchromosomal stripe (NCS) mutants of maize, in which overall growth is profoundly affected, contain sectors of reduced and normal growth in the leaves (8, 38). Sectoring is explained by heteroplasmy, in which affected plants carry a mixture of defective and functional organelles, resulting in both aberrant and normal growth. NCS mutations seem to be extremely deleterious to all cell types and seem to survive only in a heteroplasmic condition. Investigation of one such mutant, designated NCS5, has revealed that a partial deletion of the cytochrome oxidase subunit II gene (*coxII*) is correlated with the abnormal phenotype. Moreover, it appears that the NCS5 mutant arose by amplification of a homologous recombinational product. NCS mutants differ from CMS plants in that the deleterious effects are expressed in all cell types, whereas the effects of CMS mutations are expressed only in specific cells. This distinction probably accounts for the greater frequency of CMS mutations relative to other types of mutations in higher plants.

Susceptibility to Fungal Pathogens

There is little doubt that T-*urf13* is responsible for the specific virulence of *B. maydis* race T toward *cms-T* maize. Supportive evidence has come from pathotoxin studies conducted in *Escherichia coli* expressing the T-*urf13* mitochondrial gene. The coding region of T-*urf13* has been cloned into inducible expression vectors and transformed into *E. coli*. When induced, *E. coli* express the 13-kD polypeptide encoded by T-*urf13*, as shown by an antiserum prepared to the protein (39). The URF13 polypeptide is located in the plasma membrane of *E. coli*, analogous to its location in the inner mitochondrial membrane of *cms-T* maize. *Escherichia coli* not expressing URF13 are insensitive to the BmT and Pm toxins and methomyl; however, *E. coli* expressing URF13 are sensitive to these compounds. These compounds inhibit glucose-driven respiration and growth and cause spheroplast swelling and massive ion leakage in *E. coli* expressing URF13 (39, 40). Thus, toxic effects in *E. coli* correspond to those exhibited by *cms-T* mitochondria. These studies demonstrate that T-*urf13* is responsible for pathotoxin sensitivity.

Recently, RNA editing has been described for mitochondrial genes of wheat and *Oenothera* (41). This editing process modifies mRNAs so that they do not perfectly reflect the sequence of the corresponding DNA template. In plant mitochondrial genes, the C to U conversion is observed most frequently. Because it has not been determined whether the T-*urf13* transcript undergoes RNA editing, it is uncertain whether the amino acid sequence of URF13 differs from the sequence deduced from the nucleotide sequence. Because of RNA editing, the mitochondrial form of URF13 may not be identical to that synthesized by the T-*urf13*-transformed *E. coli*.

Nevertheless, bacterial and mitochondrial forms of URF13 respond in a similar fashion to the toxins.

T-urf13 has also been shown to confer toxin sensitivity to yeast mitochondria (42). A chimeric gene comprising *T-urf13* fused to the mitochondrial targeting sequence from the gene for the adenosine triphosphate (ATP) synthase subunit 9 precursor of *Neurospora crassa* was constructed to facilitate mitochondrial import. When expressed in yeast, this gene construct produced a polypeptide that was translocated into the mitochondrial membrane fraction and was processed to a protein of a size similar to that of maize URF13. BmT and Pm toxins and methomyl each inhibit the growth on glycerol medium of yeast cells expressing the fused gene and also stimulated NADH-driven respiration by isolated mitochondria from these cells. In contrast, these compounds do not affect either growth or respiration of yeast cells expressing *T-urf13* without a transit peptide. These findings indicate that *T-urf13* is sufficient to confer toxin sensitivity in a heterologous eukaryotic system and that mitochondrial localization of URF13 is essential for these functions. The yeast and bacterial studies jointly furnish compelling evidence that URF13 is responsible for toxin sensitivity.

Ion uptake experiments in *E. coli* show that a pathotoxin-URF13 interaction results in rapid and massive leakage of small molecules through the plasma membrane (40). The addition of Pm or BmT toxins or methomyl to nontransformed *E. coli* cells does not affect the uptake of ^{86}Rb ; however, the addition of these compounds to *E. coli* expressing URF13 causes a rapid permeabilization of the plasma membrane and ^{86}Rb leakage from the cells. These results indicate that URF13 is a channel-forming protein in the presence of toxin. A parallel response to pathotoxin and URF13 occurs in mitochondria from *cms-T* maize—incubation of toxin with mitochondria produces rapid leakage of Ca^{2+} and NAD^+ (14). The toxin-URF13 interaction permeabilizes the inner mitochondrial membrane, resulting in the dissipation of the membrane potential and loss of mitochondrial function. Methomyl causes the same effects in *E. coli* cells and *cms-T* maize mitochondria as those produced by the fungal toxins. The loss of mitochondrial function caused by the toxin-URF13 interaction probably accounts for the specific virulence of *B. maydis* race T and *P. maydis* to *cms-T* maize.

An association between URF13 and the pathotoxins is indicated by the specificity of BmT and Pm toxins for *cms-T* mitochondria. In fact, we have shown that toxin specifically binds to URF13 in *E. coli* and in *cms-T* mitochondria in binding experiments with ^3H -labeled Pm toxin (43). URF13 binds toxin in a positive cooperative fashion in *E. coli* (Hill coefficient = 1.5), with an apparent dissociation constant of 50 to 70 nM and a maximal binding of 350 pmol of toxin per milligram of *E. coli* protein. Much less toxin is bound in *cms-T* maize mitochondria—approximately 15 pmol of toxin per milligram of mitochondrial protein—and cooperativity is not detected. Greater toxin binding in *E. coli* than in mitochondria is probably due to the greater abundance of URF13 in *E. coli* than in mitochondria. Mitochondria from restored *cms-T* maize bind slightly less toxin than mitochondria from nonrestored *cms-T* maize. This may reflect the lower abundance of URF13 in the former than in the latter (26, 27). In this connection, it has been reported that restorer genes diminish the sensitivity of *cms-T* mitochondria to the pathotoxins (44). Displacement and competitive toxin binding experiments show that toxin binding is reversible and that BmT and Pm toxins and methomyl all bind to either the same site or to overlapping sites on URF13.

Mutational analysis is being applied in order to determine how URF13 causes toxin sensitivity. More than 100 different *T-urf13* mutations have been made by random and site-directed mutational techniques and the corresponding mutants have been screened for toxin insensitivity in *E. coli*. A few of the resulting toxin-insensitive

mutants have been characterized to determine why they are unable to mediate toxin sensitivity (43). Toxin binding experiments were performed to determine if toxin insensitivity is caused by changes in the capacity of URF13 to bind toxin. Two insensitive mutants, one containing valine instead of aspartic acid at residue 39 and one containing a COOH-terminal deletion of 33 amino acids that results in a truncated molecule of 82 amino acids, each exhibit a drastic decline in toxin binding. Even though these two mutants do bind a small amount of toxin, it is apparently insufficient to induce membrane permeabilization. In contrast, a third toxin-insensitive mutant, which contains an internal deletion of amino acid residues 2 to 11, binds about 300 pmol of toxin per milligram of *E. coli* protein (versus a value of 350 for the nonmutated URF13). This finding shows that toxin insensitivity can arise from causes other than a defect in toxin binding. Amino acids 2 to 11, thus, apparently make a significant contribution to membrane permeabilization. Together our results indicate that URF13 is a receptor for the toxin and that toxin-URF13 binding is essential for membrane permeabilization.

The structure of URF13 and its topology in biological membranes are relevant to its role as a channel-forming protein. Because URF13 binds toxin and permeabilizes membranes in both *cms-T* maize mitochondria and *E. coli*, these membranes must have common characteristics relevant to toxin sensitivity and URF13 function during channel formation. Experiments with *E. coli* have suggested a preliminary model for the orientation of URF13 in the plasma membrane (45). The NH_2 -terminus of URF13 is located on the cytoplasmic side of the plasma membrane, whereas the COOH-terminus is situated on the periplasmic side (Fig. 2); these locations were shown by experiments with right-side-out and inside-out vesicles and specific immunological markers for the identification of the termini of the protein. The locations of the NH_2 - and COOH-termini indicate that URF13 has an odd number of membrane-spanning regions, with the protein crossing the membrane three times. There are no experimental data precisely defining the three

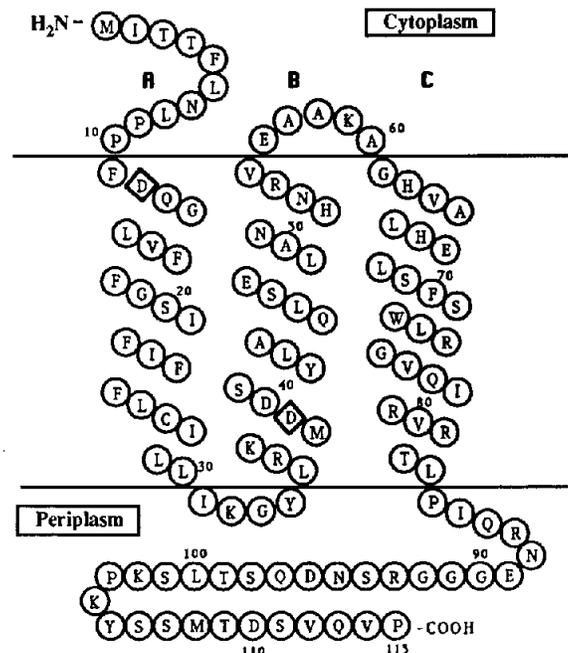


Fig. 2. Proposed orientation of URF13 within the plasma membrane of *E. coli*. Diamonds denote DCCD-binding residues. Horizontal lines indicate boundaries of plasma membrane. Three α helices are designated A, B, and C. Amino acid residues are designated by the single-letter code (53); numbers indicate position of amino acid residues. Adapted from (45) with permission of publisher.

membrane-spanning regions; they may be longer, shorter, or begin and end at different residues than shown. The aspartic acid residues at positions 12 and 39 covalently bind dicyclohexylcarbodiimide (DCCD). These two residues are predicted to be within the membrane, because DCCD forms stable adducts only with acidic residues located in a hydrophobic, nonaqueous environment. Residues 12 and 39 are located within the membrane-spanning regions of helices A and B, respectively, and are expected to reside in regions of hydrophobicity.

The cooperativity associated with the binding of toxin to URF13 suggests that URF13 exists in an oligomeric state within the membrane (45). Indeed, cross-linking experiments indicate that a fraction of URF13 in the membrane is present as a dimer or larger species. Cross-linking in the presence of toxin shows the same pattern, perhaps because channel formation involves changes in URF13 structure too subtle for the cross-linking reaction to resolve. The current model for the formation of hydrophobic channels by proteins within membranes indicates an association of several amphipathic α helices in the membrane, in which the polar faces of the helices orient inward to form the lining of the water-filled channel and the hydrophobic faces point outward, interacting with the hydrocarbon phase of the lipid bilayer (46). We thus propose that an oligomeric structure composed of two to four individual URF13 molecules produces a channel within the membrane in the presence of toxin.

Mechanisms of CMS

Efforts to understand CMS have also focused on contrasting anther development in normal and *cms-T* maize. Light and electron microscopic studies have revealed important differences between fertile and sterile anthers (47). The maize anther wall is four cell layers thick. The innermost cell layer is the tapetum, which surrounds the developing pollen grains, and tapetal cells serve to nourish developing pollen by exporting nutrients and other molecules needed for pollen formation. Mitochondria in the tapetum and the adjacent middle cell layer of sterile anthers start to degenerate soon after meiosis, and by the intermediate microspore stage they have become saclike and swollen. At a comparable developmental stage in fertile anthers, mitochondria are condensed with dark-staining matrices and angular cristae. Plastids and other organelles from sterile and fertile anthers do not differ structurally until late in anther development. From these observations Warmke and Lee (47) suggested that mitochondrial degeneration in the tapetum of sterile anthers is the first sign of abnormality and that it initiates events leading to pollen abortion. These workers have also shown that rapid division of mitochondria occurs in tapetal and sporogenous cells of both fertile and sterile anthers during early development. A 20- to 40-fold increase in the number of mitochondria per cell precedes the time of tapetal breakdown that occurs in sterile anthers; plastid numbers do not increase. Because the period of rapid mitochondrial division takes place before tapetal mitochondria of sterile anthers become internally disorganized, Warmke and Lee have proposed that fertile and sterile anthers differ in their capacity to cope with the demands associated with rapid mitochondrial replication. Moreover, they observed that female development is normal in *cms-T* maize and that rapid mitochondrial replication does not occur in the female reproductive structures.

Colhoun and Steer (48) have studied sterile and fertile maize anthers by microscopy and have concluded that mitochondrial degeneration is not necessarily the earliest detectable event in pollen abortion of *cms-T* maize. Furthermore, although Warmke and Lee (47) indicate that pollen abortion occurs between the tetrad and

vacuolate pollen stages, Colhoun and Steer have observed sporocyte abortion at these stages and also at or before the dyad-tetrad stage of meiosis. Clearly, there are disagreements as to when the various events take place.

Although *T-urf13* is rather convincingly associated with CMS by molecular analysis of revertants and its interaction with a restorer gene, there are no conclusive experimental data indicating the mechanism by which the *T-urf13* gene product causes pollen abortion. It is perplexing that a mitochondrial gene mutation specifically interferes with pollen formation and does not noticeably affect other plant developmental processes. As evidenced by their widespread agricultural usage before the blight epidemic, *cms-T* plants are vigorous and productive. In other seed crops—for example, sorghum—CMS is also not harmful to productivity. This observation suggests that pollen development has a special dependence on mitochondrial function that is not characteristic of other plant developmental processes.

If pollen development requires higher levels of mitochondrial activity, even a slight amount of mitochondrial dysfunction could result in pollen abortion. The 20- to 40-fold increase in mitochondrial numbers, which occurs during microsporogenesis in the tapetal cell layer of the anther and in developing microspores of maize, certainly indicates an increased requirement for mitochondria in pollen formation. Under these conditions, a mitochondrial gene mutation, such as *T-urf13*, could seriously impair mitochondrial biogenesis; whereas in other less demanding developmental processes, the mutation may not be limiting. The onset of pollen abortion in *cms-T* plants is correlated with the precocious degeneration of tapetal cells. URF13 may interfere with rapid mitochondrial biogenesis in these cells and cause cell degeneration and subsequently pollen abortion. In other species, different mitochondrial mutations may interrupt mitochondrial biogenesis in anther cells and account for CMS.

For the most part, plant mitochondrial genes encode polypeptides that are components of the electron transport chain or the F_0F_1 -ATPase (6), which are essential for mitochondrial respiration. Mutant forms of these polypeptides can cause respiration deficiencies. Mitochondrial gene mutations in humans can show their harmful effects in specific tissues. Leber's hereditary optic neuropathy, which causes optic nerve degeneration and cardiac dysrhythmia, has been traced to a mitochondrial gene mutation affecting a subunit of Complex I of the electron transport chain (49). This mutant gene product exhibits its deleterious effects on the optic nerve and heart function. This shows that mitochondrial gene mutations may not produce effects in all tissues, although the altered gene product is expressed in all cell types. Similarly, a CMS gene mutation may express its adverse effect only in tissues involved in pollen development, whereas other tissues are unaffected.

Lower alternative oxidase activity has been correlated with CMS in petunia (50). CMS lines carrying *pcf* exhibit less alternative oxidase activity than fertile lines when immature anthers and suspension cultures are assayed. Moreover, restored lines (that is, CMS lines restored to fertility by the nuclear restorer gene *Rf*) have normal alternative oxidase activity. These results suggest that lowered alternative oxidase activity and pollen sterility are induced by *pcf*. Although there are other explanations for these data, the relation between CMS and alternative oxidase activity provides an interesting correlation. In maize, no differences in alternative oxidase activity are found among mitochondria isolated from normal, *cms-T*, and restored *cms-T* plants (51). Thus, different biochemical mechanisms appear to be operative in CMS in petunia and *cms-T* maize. Indeed, there may be many different mechanisms that account for pollen abortion among the various types of CMS.

There is another explanation for CMS that is unique to *cms-T* and

depends on the properties of URF13. Flavell (52) proposed, long before the discovery of *T-urf13*, that an anther-specific substance exists that affects mitochondria in a fashion similar to the BmT toxin of *B. maydis* race T. In accordance with our present understanding, the anther-specific substance might interact with URF13 to inhibit mitochondrial activity in a manner analogous to the toxin-URF13 interaction. Because the resultant loss of mitochondrial activity occurs in anther cells involved in pollen formation, viable pollen production would not take place. The anther-specific substance would not adversely affect pollen development in other maize cytoplasm, because URF13 is expressed only in *cms-T* mitochondria. As yet, an anther-specific substance with these specific properties has not been found; nevertheless, it is an interesting possibility because it provides a mechanism that accounts for the dual role of *T-urf13* in causing CMS and disease susceptibility.

REFERENCES AND NOTES

- J. S. Rogers and J. R. Edwardson, *Agron. J.* **44**, 8 (1952); P. H. Harvey, C. S. Levings, III, E. A. Wernsman, *Adv. Agron.* **24**, 1 (1972).
- K. D. Laser and N. R. Lersten, *Bot. Rev.* **38**, 425 (1972); M. R. Hanson and M. F. Conde, *Int. Rev. Cytol.* **94**, 213 (1985).
- S. A. Frank, *Am. Nat.* **133**, 345 (1989).
- J. R. Laughnan and S. Gabay-Laughnan, *Annu. Rev. Genet.* **17**, 27 (1983).
- S. A. Mackenzic, D. R. Pring, M. J. Bassett, C. D. Chase, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2714 (1988).
- V. K. Eckenrode and C. S. Levings, III, in *Tailoring Genes for Crop Improvement*, J. Bruening, J. Harada, T. Kosuge, A. Hollaender, Eds. (Plenum, New York, 1987), pp. 69-84.
- C. J. Leaver and M. W. Gray, *Annu. Rev. Plant Physiol.* **33**, 373 (1982).
- K. J. Newton, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 503 (1988).
- A. J. Ullstrup, *Annu. Rev. Phytopathol.* **10**, 37 (1972).
- D. R. Pring and D. M. Lonsdale, *ibid.* **27**, 483 (1989).
- S. M. Lim and A. L. Hooker, *Phytopathology* **62**, 968 (1972); J. R. Laughnan and S. J. Gabay, *Theor. Appl. Genet.* **43**, 109 (1973).
- Y. Kono and J. M. Daly, *Bioorg. Chem.* **8**, 391 (1979); Y. Kono, S. Takeuchi, A. Kawarada, J. M. Daly, H. W. Knoche, *Tetrahedron Lett.* **21**, 1537 (1980); *Bioorg. Chem.* **10**, 206 (1981); Y. Kono, S. J. Danko, Y. Suzuki, S. Takeuchi, J. M. Daly, *Tetrahedron Lett.* **24**, 3803 (1983).
- R. J. Miller and D. E. Koeppe, *Science* **173**, 67 (1971); B. G. Gengenbach, R. J. Miller, D. E. Koeppe, C. J. Arntzen, *Can. J. Bot.* **51**, 2119 (1973); D. E. Matthews, P. Gregory, V. E. Gracen, *Plant Physiol.* **63**, 1149 (1979); A. Bervillé, A. Ghazi, M. Charbonnier, J.-F. Bonavent, *ibid.* **76**, 508 (1984); R. R. Klein and D. E. Koeppe, *ibid.* **77**, 912 (1985).
- M. J. Holden and H. Szc, *Plant Physiol.* **84**, 670 (1987).
- S. J. Danko *et al.*, *Biochemistry* **23**, 759 (1984); Y. Kono, S. Suzuki, S. Takeuchi, H. W. Knoche, J. M. Daly, *Agric. Biol. Chem.* **49**, 559 (1985).
- Y. Suzuki *et al.*, *Agric. Biol. Chem.* **49**, 149 (1985).
- K. A. Frantzen, J. M. Daly, H. W. Knoche, *Plant Physiol.* **83**, 863 (1987).
- D. E. Koeppe, J. K. Cox, C. P. Malone, *Science* **201**, 1227 (1978).
- R. E. Dewey, C. S. Levings, III, D. H. Timothy, *Cell* **44**, 439 (1986).
- C. J. Leaver, personal communication.
- R. P. Wise, A. E. Fliss, D. R. Pring, B. G. Gengenbach, *Plant Mol. Biol.* **9**, 121 (1987).
- S. Ferguson-Miller, W. Peiffer, K. Korth, personal communication.
- L. L. Fragosso, S. E. Nichols, C. S. Levings, III, *Genome* **31**, 160 (1989).
- D. M. Lonsdale, *Plant Mol. Biol.* **3**, 201 (1984); D. B. Stern and J. D. Palmer, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 1946 (1984); D. R. Pring and D. M. Lonsdale, *Int. Rev. Cytol.* **97**, 1 (1985); C. S. Levings, III, and G. G. Brown, *Cell* **56**, 171 (1989); D. M. Lonsdale, in *The Biochemistry of Plants, A Comprehensive Treatise: Molecular Biology*, A. Marcus, Ed. (Academic Press, New York, 1989), vol. 15, pp. 229-295; I. Small, R. Suffolk, C. J. Leaver, *Cell* **58**, 69 (1989).
- C. Fauron and M. Havlik, *Curr. Genet.* **15**, 149 (1989); C. Fauron, M. Havlik, D. Lonsdale, L. Nichols, *Mol. Gen. Genet.* **216**, 395 (1989).
- R. E. Dewey, D. H. Timothy, C. S. Levings, III, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5374 (1987).
- B. G. Forde, R. J. C. Oliver, C. J. Leaver, *ibid.* **75**, 3841 (1978); B. G. Forde and C. J. Leaver, *ibid.* **77**, 418 (1980).
- J. C. Kennell, R. P. Wise, D. R. Pring, *Mol. Gen. Genet.* **210**, 399 (1987); J. C. Kennell and D. R. Pring, *ibid.* **216**, 16 (1989).
- B. G. Gengenbach, C. E. Green, C. D. Donovan, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5113 (1977); P. F. Umbeck and B. G. Gengenbach, *Crop. Sci.* **23**, 584 (1983).
- R. I. S. Brettell, B. V. D. Goddard, D. S. Ingram, *Maydisa* **24**, 203 (1979); R. I. S. Brettell, E. Thomas, D. S. Ingram, *Theor. Appl. Genet.* **58**, 55 (1980).
- W. H. Rottmann, T. Brears, T. P. Hodge, D. M. Lonsdale, *EMBO J.* **6**, 1541 (1987); C. M.-R. Fauron, M. Havlik, R. I. S. Brettell, *Genetics* **124**, 423 (1990).
- R. P. Wise, D. R. Pring, B. G. Gengenbach, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2858 (1987).
- E. G. Young and M. R. Hanson, *Cell* **50**, 41 (1987).
- J. Rasmussen and M. R. Hanson, *Mol. Gen. Genet.* **215**, 332 (1989).
- M. L. Boeshorc, M. R. Hanson, S. Izhar, *Plant Mol. Biol.* **4**, 125 (1985); H. T. Nivison and M. R. Hanson, *Plant Cell* **1**, 1121 (1989).
- C. A. Makaroff and J. D. Palmer, *Mol. Cell. Biol.* **8**, 1474 (1988).
- C. J. Leaver *et al.*, *Philos. Trans. R. Soc. London Ser. B* **319**, 165 (1988); D. R. Pring, B. G. Gengenbach, R. P. Wise, *ibid.*, p. 187.
- K. J. Newton, C. Knudsen, S. Gabay-Laughnan, J. R. Laughnan, *Plant Cell* **2**, 107 (1990).
- R. E. Dewey, J. N. Siedow, D. H. Timothy, C. S. Levings, III, *Science* **239**, 293 (1988).
- C. J. Braun, J. N. Siedow, M. E. Williams, C. S. Levings, III, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4435 (1989).
- P. S. Covello and M. W. Gray, *Nature* **341**, 662 (1989); J. M. Gualberto, L. Lamattina, G. Bonnard, J. H. Weil, J. M. Grienenberger, *ibid.*, p. 660; R. Hiesler, B. Wissinger, W. Schuster, A. Brennicke, *Science* **246**, 1632 (1989).
- J. Huang *et al.*, *EMBO J.* **9**, 339 (1990).
- C. J. Braun, J. N. Siedow, C. S. Levings, III, *Plant Cell* **2**, 153 (1990).
- D. H. P. Barratt and R. B. Flavell, *Theor. Appl. Genet.* **45**, 315 (1975); L. S. Watrud, J. R. Laughnan, S. J. Gabay, D. E. Koeppe, *Can. J. Bot.* **54**, 2718 (1976).
- K. L. Korth, F. Struck, C. I. Kaspi, J. N. Siedow, C. S. Levings, III, in *Plant Molecular Biology*, R. G. Herrmann and B. A. Larkins, Eds. (Plenum, London, in press).
- R. O. Fox and F. M. Richards, *Nature* **300**, 325 (1982).
- H. E. Warmke and S.-L. J. Lee, *J. Hered.* **68**, 213 (1977); S.-L. J. Lee and H. E. Warmke, *Am. J. Bot.* **66**, 141 (1979).
- C. W. Colhoun and M. W. Steer, *Ann. Bot.* **48**, 417 (1981).
- D. C. Wallace *et al.*, *Science* **242**, 1427 (1989).
- M. B. Connert and M. R. Hanson, *Plant Physiol.* **93**, 1634 (1990).
- J. N. Siedow, personal communication.
- R. Flavell, *Plant Sci. Lett.* **3**, 259 (1974).
- Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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