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URF13, a Ligand-Gated, Pore-Forming Receptor for T-toxin in the Inner Membrane of *cms-T* Mitochondria

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URF13 is the product of a mitochondrial-encoded gene (T-*urf13*) found only in maize plants containing the Texas male-sterile cytoplasm (*cms-T*), and it is thought to be responsible for both cytoplasmic male sterility and the susceptibility of *cms*-T maize to the fungal pathogens *Bipolaris maydis* race T and *Phyllosticta maydis*. Mitochondria isolated from *cms*-T maize are uniquely sensitive to pathotoxins (T-toxin) produced by these fungi and to methomyl (a commercial insecticide). URF13 acts as a receptor that specifically binds T-toxin to produce hydrophilic pores in the inner mitochondrial membrane. When expressed in *Escherichia coli* cells, URF13 also forms hydrophilic pores in the plasma membrane if exposed to T-toxin or methomyl. Topological studies established that URF13 contains three membrane-spanning α-helices, two of which are amphipathic and can contribute to pore formation. Chemical cross-linking of URF13 was used to demonstrate the existence of URF13 oligomers in *cms-T* mitochondria and *E. coli* cells. The ability of the carboxylate-specific reagent, *N,N'*-dicyclohexycarbodiimide, to cross-link URF13 was used in conjunction with site-directed mutagenesis to establish that the URF13 tetramer has a central core consisting of a four-α-helical bundle which undergoes a conformational change after interaction with T-toxin or methomyl. Overall, the experimental evidence indicates that URF13 functions as a ligand-gated, pore-forming T-toxin receptor in *cms-T* mitochondria.

KEY WORDS: Maize; mitochondria; cytoplasmic male sterility; URF13; T-*urf13;* T-toxin; pathogenesis; pore-forming receptor.

CHARACTERISTICS OF THE TEXAS MALE-STERILE CYTOPLASM

The Texas, or T, cytoplasm (*cms-T*) of maize was discovered in the variety Golden June in Texas (Rogers and Edwardson, 1952). Maize geneticists immediately showed an interest in this new cytoplasm because it carried a cytoplasmically inherited male sterility—a trait they wished to exploit in hybrid corn production. In plants carrying the Texas male-sterile cytoplasm, anthers fail to exert, and pollen does not develop (pollen abortion). Female flowers of *cms-T* plants set seed in a normal fashion when provided with pollen. *cms-T* plants also exhibit a few minor effects on plant development besides male sterility (Duvick, 1965). These plants have fewer leaves, tend to be shorter, and most interestingly, have reduced yields. Although *cms-T* reduces yield about 2%, pollen sterility raises yield so that only restored genotypes, as compared with their sterile counterparts, show a true yield decrease. In spite of these minor effects, the Texas male-sterile cytoplasm achieved widespread usage in the production of hybrid maize during the 1950s and 1960s.

Light and electron microscopic studies have shown significant developmental differences between fertile (normal) and sterile (*cms-T*) anthers (Warmke and Lee, 1977; Lee and Warmke, 1979). The maize anther consists of four cell layers. The innermost cell

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layer of the anther, called the tapetum, surrounds the developing pollen grains and serves to nourish developing pollen by exporting nutrients and other essential molecules. Mitochondria of the tapetum and the adjacent middle cell layer of *cms-T* anthers begin to degenerate soon after meiosis and become saclike and swollen by the intermediate microspore stage. In contrast, mitochondria observed in fertile anthers feature dark-staining matrices and angular cristae at a comparable developmental stage. Until late in anther development, plastids and other organelles from sterile (*cms-T*) and fertile anthers do not differ structurally. Based upon these observations, Warmke and Lee (1977) proposed that the first sign associated with pollen abortion occurs when the mitochondria of the tapetal cell layer begin to degenerate. They also observed an unusually rapid division of mitochondria in the tapetal and sporogenous cells of both fertile and sterile anthers during early development (Warmke and Lee, 1977; Lee and Warmke, 1979). Because rapid mitochondrial divisions occur in sterile anthers before tapetal mitochondria become internally disorganized, Warmke and Lee (1977) speculated that fertile and sterile anthers differ in their capacity to withstand the demands associated with the high mitochondrial replication rate.

Not all investigators agree with the conclusions of Warmke and Lee (1977). Colhoun and Steer (1981) contend that mitochondrial degeneration in tapetal cells is not necessarily the first detectable event in pollen abortion in *cms-T* maize. They also differ regarding the time of pollen abortion. Warmke and Lee (1977) say that pollen abortion takes place between the tetrad and vacuolated pollen stages, whereas Colhoun and Steer (1981) described sporocytic abortion at these stages as well as at or before the dyad-tetrad stage of meiosis.

Several studies suggest that microsporogenesis has higher energy requirements than most plant processes. The 20- to 40-fold increase in mitochondria numbers, which takes place during microsporogenesis in the tapetal and sporogenous cells of the anther, indicates a greater demand for mitochondria during pollen formation. Because developing microspores and internal anther cells have only a limited amount of photosynthetic capacity and contain mostly undifferentiated plastids and amyloplasts, mitochondria must supply the energy for pollen development. Mitochondria could produce more energy by increasing their numbers per cell so that more ATP is synthesized per cell. Alternatively, they could increase their metabolic activity so that more ATP is generated per mitochondrion. Some studies suggest that increases in mitochondria number occur in reproductive cells to meet the higher energy demands of pollen formation. Warmke and Lee (1977) observed an increase in the number of mitochondria in developing maize anthers. In tobacco, *Nicotiana sylvestris*, gametophytic cells contain many more mitochondria than other cell types (De Paepe *et al.*, 1993). Further, tobacco flower and leaf mitochondria contain similar amounts of the Rieske iron-sulfur protein, although the transcript level is much higher in flowers (Huang *et al.*, 1994). A large increase in the number of mitochondria per cell accounts for the higher levels of Rieske iron-sulfur protein mRNA in flowers. Collectively, these results from maize and tobacco support the notion that a large increase in mitochondria number occurs during microsporogenesis to meet the greater demand for energy during pollen development.

SOUTHERN CORN LEAF BLIGHT

In the 1950s and 1960s, *cms-T* provided a stable and reliable source of cytoplasmic male sterility (CMS) for use in hybrid seed production to avoid the more costly process of hand or mechanical emasculation (detasseling). This ended when in 1969 and 1970 an epidemic of Southern corn leaf blight, which is caused by the fungal pathogen *Bipolaris maydis* race T (formerly *Helminthosporium maydis* race T), swept the vast corn-growing regions of the South and Corn Belt (Ullstrup, 1972; Pring and Lonsdale, 1989). It was immediately evident that maize carrying the Texas cytoplasm was specifically susceptible to *B. maydis* race T, and the large-scale use of *cms-T* in seed production was terminated. A second fungal pathogen, *Phyllosticta maydis*, also showed a specific virulence to maize carrying *cms-T;* however, it was less serious than the Southern corn leaf blight because *P. maydis* is limited to the cooler northern regions of the United States.

Both pathogens, *B. maydis* race T and *P. maydis*, produce pathotoxins, BmT and Pm, respectively (collectively referred to as T-toxin), that are host specific to *cms-T* maize (Lim and Hooker, 1972; Laughnan and Gabay, 1973). These toxins were purified and characterized by Daly and co-workers who showed they had specific activity toward *cms-T* maize (Kono and Daly, 1979; Kono *et al.*, 1980, 1981, 1983). Chemically, T-toxins represent a family of compounds composed of alkanes with 35 to 45 carbon atoms in a linear

chain and regularly repeating polyketol groups having either a 1,5-dioxo-3-hydroxy or a 1-oxo-3,5-dihydroxy structure, in the case of BmT toxin (Fig. 1A), or 33 to 35 carbon atoms long and having repeating 1-oxo-3-hydroxy polyketol structures, in the case of the Pm toxin (Fig. 1B).

Maize carrying normal and other sterile cytoplasms such as *cms-S* and *cms-C* supports only a limited colonization by *B. maydis* race T, which is normally restricted to boat-shaped lesions on the leaves that remain isolated and small. *B. maydis* race T can, however, quickly and completely colonize *cms-T* maize, causing severe damage and even plant death. In this case, lesions grow rapidly, coalesce, and spread over the whole plant. The specific susceptibility of *cms-T* to *B. maydis* race T is caused by the unique sensitivity of its mitochondria to BmT toxin; in contrast, the mitochondria of disease-resistant maize types are insensitive to BmT toxin (Levings, 1990). Miller and Koeppe (1971) first demonstrated the specific inhibitory effects of T-toxin on isolated *cms*-T mitochondria. These effects include inhibition of electron transfer with malate as the electron donor, but not with substrates such as succinate or external NADH (Matthews *et al.*, 1979; Bervillé *et al.*, 1984), stimulation of state 4 (minus ATP) electron transfer when external NADH is the substrate (Bednarski *et al.*, 1977; Matthews *et al.*, 1979), induction of mitochondrial swelling (Klein and Koeppe, 1985), dissipation of the membrane potential across the inner mitochondrial membrane (Bervillé *et al.*, 1984; Holden and Sze, 1987), and rapid leakage of small molecules, such as

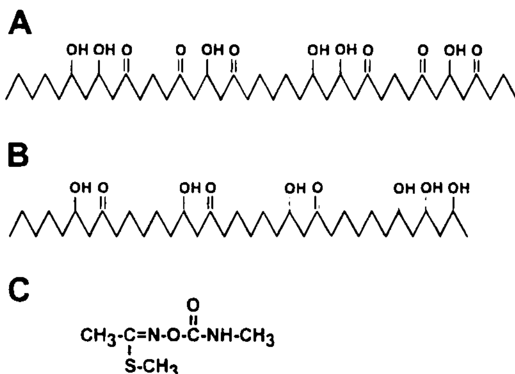

Fig. 1. Structure of representative fungal pathotoxins and methomyl. (A) Structure of band 1′ component of BmT toxin. (B) Structure of component C of Pm-toxin. The jagged lines represent methylene backbones and the hydrogens bound to the backbone are omitted for clarity. (C) Structure of *S*-methyl-*N*-[(methylcarbamoyl)oxy] thioacetimidate (methomyl). Redrawn from Braun *et al.* (1990).

Ca^{2+} (Holden and Sze, 1984) and NAD^+ (Bervillé *et al.*, 1984; Bednarski *et al.*, 1977) out of the mitochondrial matrix. These effects are all observed within the same low concentration range of T-toxin, 20 to 100 nM (Levings and Siedow, 1992).

Taken together, the results outlined above suggest that the interaction of T-toxins with *cms*-T mitochondria results in a rapid permeabilization of the inner mitochondrial membrane. For example, the inhibition of malate-stimulated electron transfer by T-toxin is associated with the loss of NAD^+ from the mitochondrial matrix, effectively inhibiting malate dehydrogenase activity. Substantial restoration of malate oxidation after the addition of T-toxin can be obtained by the subsequent addition of high concentrations of NAD^+ to the reaction medium (Matthews *et al.*, 1979). The T-toxin-induced permeabilization of the inner membrane also accounts for the loss of membrane potential as protons can readily cross the permeabilized membrane dissipating the protonmotive force and stimulating the rate of electron transfer under state 4 conditions. The permeability of the inner membrane to ions such as Ca^{2+} and NAD^+ suggests that the permeabilizing effect of T-toxin is not specific to protons, and the fact that NAD^+ (M_w = 663) can cross the membrane would suggest that the resulting hydrophilic pores are as large as 8–15 Å in diameter (Levings and Siedow, 1992). No loss of soluble enzymes from the matrix has been observed after the addition of T-toxin to *cms*-T mitochondria, which indicates that the resulting membrane permeabilization, while associated with the formation of a relatively large hydrophilic pore, does not result in wholesale disruption of the inner membrane.

The inhibitory effects of T-toxin have not been reported with mitochondria isolated from any other type of maize or from any other plant species, a correlation that also applies to the specific susceptibility of *cms*-T maize to *B. maydis* race T and *P. maydis*. This suggests that the site of specificity of these two fungi for *cms*-T maize lies at the level of the unique sensitivity of *cms*-T mitochondria to T-toxin. The subsequent loss of mitochondrial function must result in the death of the infected cells, which, in turn, promotes the further proliferation of the fungus on the plant.

Methomyl {*S*-methyl-*N*-[(methylcarbamoyl)oxy] thioacetimidate} (Fig. 1C), the active ingredient in the systemic insecticide Lannate, shows the same inhibitory effects on *cms*-T mitochondria as T-toxins but at concentrations of 1 to 10 mM, four to five orders of magnitude higher than that required of T-toxins. The

reduced effectiveness of methomyl is apparently related to its low lipid solubility. Analogues in which the methyl side chain on the carbamate group of methomyl is replaced with side chains 12 to 14 carbon atoms in length are much more lipid soluble and are as effective as T-toxin on a concentration basis (Aranda *et al.*, 1987). In spite of the ability of T-toxin and methomyl to promote the same biological effects in *cms-T* mitochondria, there are no obvious structural similarities between T-toxins and methomyl (Fig. 1).

CMS AND THE T-*urf13* GENE

An early indication that mitochondria might be responsible for *cms-T* male sterility came from *in organello* protein synthesis studies in which a 13-kDa protein was uniquely synthesized by *cms-T* mitochondria but not by mitochondria of other sterile types or normal maize (Forde *et al.*, 1978; Forde and Leaver, 1980). When restored to pollen fertility, *cms-T* mitochondria contain substantially less 13-kDa protein. Subsequently, a 345-nucleotide open reading frame was discovered in a DNA fragment that hybridized more intensely to *cms-T* mitochondrial RNA than to mitochondrial RNA from normal maize (Dewey *et al.*, 1986). Designated T-*urf13*, this novel open reading frame originated by a series of recombinational events involving other mitochondrial genes. The coding region of T-*urf13* contains 115 codons of which the first 88 codons have nucleotide similarity with the 3′ flanking region of a 26S ribosomal RNA gene, *rrn26*, the next nine codons have no known similarity, and the final 18 codons have similarity to the coding region of *rrn26*. Moreover, the large 5′ flanking region (5 kb) of T-*urf13* is similar to the 5′ flanking region of the *atp6* gene. Because this region probably contains the promoter region of T-*urf13*, both *atp6* and T-*urf13* must have promoters consisting of similar nucleotide sequences. The 13-kDa protein encoded by the T-*urf13* gene that is unique to *cms-T* maize is called URF13. Because no other plant mitochondrial genome carries this gene, we assume the gene is not required for mitochondrial function. Following the characterization of T-*urf13*, an antibody was raised against an oligopeptide that corresponds to a 15-amino acid sequence located in the carboxyl-terminal region of URF13. This antibody was subsequently used to demonstrate that the URF13 protein is present in *cms*-T mitochondria but not in any other type of maize mitochondria, and that URF13 is an integral protein associated with the inner mitochondrial membrane (Dewey *et al.*, 1987). Although novel when discovered, it is now apparent that many other CMS genes have arisen by recombinational events involving other mitochondrial-encoded genes. In fact, mitochondrial DNA rearrangements are a significant force in changing the genome organization and in causing mutations in the mitochondrial genomes of higher plants (Mackenzie *et al.*, 1994).

Strong correlative evidence supports a causal relationship between CMS and the T-*urf13* gene. The gene is found only in *cms-T* mitochondria where it is constitutively expressed. Specific nuclear genes, called restorers of fertility (*Rf*), suppress the male-sterile effect of the *cms-T* cytoplasm, resulting in the production of viable pollen. Acting jointly, the dominant alleles of *Rf1* and *Rf2* restore pollen fertility to the Texas male-sterile cytoplasm. The dominant allele of *Rf1* modifies the expression of T-*urf13* by altering the transcription profile of T-*urf13* and reducing the abundance of URF13 by approximately 80% (Dewey *et al.*, 1987; Kennell *et al.*, 1987; Kennell and Pring, 1989). The fact that the restorer gene *Rf1* affects the expression of T-*urf13* provided early evidence that T-*urf13* may cause CMS. In contrast, the recessive genotype, *rf1rf1*, has no influence on the transcription of T-*urf13* or the abundance of the URF13 protein. We do not understand the mechanism by which *Rf1* modifies the expression of T-*urf13*; however, changes in the processing of T-*urf13* mRNAs may account for the response.

Analysis of *cms-T* revertants has provided the strongest evidence of an association between T-*urf13* and CMS. Although spontaneous mutations that restore pollen fertility to *cms-T* plants do not occur in whole plants, reversion to pollen fertility has been found among plants regenerated from cell culture. When callus tissue derived from immature embryos of *cms-T* maize is grown on medium containing BmT toxin, toxin-resistant calli can be selected. Normally, *cms-T* calli are toxin sensitive and soon die on a toxin-containing medium. Toxin-resistant calli, when regenerated, often give rise to revertant plants that are male fertile, toxin insensitive, and resistant to *B. maydis* race T (Gengenbach *et al.*, 1977; Umbeck and Gengenbach, 1983). Surprisingly, when these same experiments are carried out without toxin selection, male-fertile, toxin-insensitive, and disease-resistant plants are also found (Bretell *et al.*, 1979, 1980). These results suggest that somaclonal variation is responsible for the reversional events. Only male-fertile, toxin-insensitive, disease-resistant phenotypes are detected among the true-

breeding revertants, whereas neither male-sterile, disease-resistant nor male-fertile, disease-susceptible plants are obtained. This outcome suggests that CMS and disease susceptibility are inseparable. Many revertants have been studied by restriction enzyme mapping and nucleotide sequencing. These studies have shown that T-*urf13* is absent in most revertants and that homologous recombination is responsible for the deletional events (Rottmann *et al.*, 1987; Fauron *et al.*, 1990). An unusual revertant, called T4, carries a mutation of T-*urf13*, resulting in a premature stop codon and a truncated URF13 polypeptide. Taken together, these findings indicate that T-*urf13* is necessary for the CMS and disease susceptibility traits in *cms-T* maize.

INTERACTIONS BETWEEN URF13 AND T-TOXIN

When T-*urf13* was expressed in the bacterium *Escherichia coli*, the URF13 protein was localized exclusively to the plasma membrane, and the resulting bacterial cells attained a sensitivity to both T-toxin and methomyl that was not observed in cells not expressing URF13. The inhibitory effects included an immediate inhibition of oxygen uptake associated with cellular respiration and cell death, indicated by the cessation of growth in culture after T-toxin addition or the failure of the toxin-treated cells to grow when plated on media that lacked T-toxin (Dewey *et al.*, 1988). The observation that addition of T-toxin or methomyl to *E. coli* expressing URF13 led to the immediate and complete loss of the potassium analogue ^{86}Rb from the cells supported the concept that the interaction of T-toxin or methomyl with URF13 resulted in the formation of a hydrophilic pore in the plasma membrane (Braun *et al.*, 1989) and that URF13 expressed in bacterial cells was responding to T-toxin in a manner identical to its response in *cms*-T mitochondria. These studies provided definitive experimental proof of the relationship between T-toxin sensitivity and the URF13 protein (Dewey *et al.*, 1988). Additional support for this relationship has been shown with the expression of URF13 in the yeast *Saccharomyces cerevisiae* (Huang *et al.*, 1990; Glab *et al.*, 1990), in insect cells using a baculovirus-mediated expression system (Korth and Levings, 1993), and in transgenic tobacco (Chaumont *et al.*, 1995).

The effects of T-toxin on biological membranes containing URF13 suggested an interaction between the two species, with URF13 acting as a ligand-gated, pore-forming T-toxin receptor. To test this directly, binding studies using tritiated, borohydride-reduced Pm toxin were undertaken (Braun *et al.*, 1990; Kaspi and Siedow, 1993). Reduced Pm toxin binds to URF13 expressed in *E. coli* and the binding is specific and saturable (Braun *et al.*, 1990). The apparent K_d for the interaction is in the range of 50–70 nM, the same concentration required to observe inhibition of respiration in intact *E. coli* cells by added T-toxin (Dewey *et al.*, 1988). The maximum amount of T-toxin bound varied somewhat, but consistently fell between 200 and 450 pmol of toxin bound per mg *E. coli* protein. The binding of T-toxin to URF13 expressed in *E. coli* also displays a distinct cooperativity, with Hill coefficients ranging between 1.4 and 2.0. Competition studies using unlabeled methomyl indicated that Pm toxin and methomyl bind to URF13 in a mutually exclusive fashion, indicative of their acting at either identical or, at the least, overlapping binding sites on URF13 (Braun *et al.*, 1990).

URF13 SECONDARY STRUCTURE AND ORIENTATION IN THE MEMBRANE

Based on analyses of the primary amino acid sequence, URF13 is predicted to have three transmembrane α-helices (Fig. 2). Helix I consists of residues 10–30 and is predicted, by hydropathy analysis (Braun *et al.*, 1989), to be hydrophobic. Helices II and III consist of residues 35–55 and 61–83, respectively, and are predicted to be amphipathic as determined by hydrophobic moment calculations and helical wheel projections (Levings and Siedow, 1992). The three-helix model of URF13 predicts that the amino and carboxyl termini of URF13 are on opposite sides of the membrane. This prediction is supported by protease accessibility studies (Korth *et al.*, 1991). These experiments employed mitoplasts, which expose the outer surface of the inner mitochondrial membrane, and submitochondrial particles, with the matrix side of the membrane exposed, to show that URF13 exists in the inner mitochondrial membrane of *cms-T* maize in a single orientation, with the C-terminus facing the matrix. When expressed in *E. coli* cells, URF13 is inserted into the plasma membrane predominantly with the N-terminus in the periplasmic space and the C-terminus on the cytoplasmic side of the membrane (Korth *et al.*, 1991), electrochemically identical to its orientation in *cms-T* mitochondria. The N- and C-

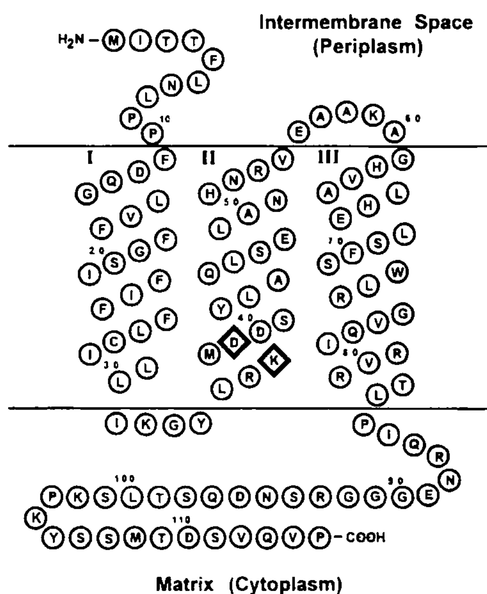

Fig. 2. Proposed topology of an URF13 monomer in the membrane. Amino acids are designated by the standard single-letter code, and Arabic numbers indicate position of amino acid residues. The three transmembrane α-helices are designated by roman numerals I, II, and III starting with the helix closest to the N-terminus. Lys-37 and Asp-39 are outlined in bold diamonds. Horizontal lines indicate boundaries of the membrane. Redrawn from Korth *et al.* (1991).

termini on opposite sides of the membrane is consistent with an odd number of membrane-spanning regions and the large size of the resulting proteolytic products suggests the presence of three membrane-spanning regions rather than one.

N,N'-Dicyclohexylcarbodiimide (DCCD) is a protein-modifying reagent that specifically reacts with carboxylate groups and either cross-links the side chains on Glu or Asp to the epsilon amino group of Lys residues or forms a stable covalent adduct with the acidic amino acids (Nalecz, *et al.*, 1986). In order to form a stable adduct, the reactive carboxylate residue must be localized in a hydrophobic environment. [^{14}C]DCCD covalently labels URF13 at Asp-39 and, to a lesser extent, Asp-12 (Braun *et al.*, 1989). This provides additional support for the three-transmembrane α-helix model because it indicates that Asp-12 and Asp-39 are in hydrophobic environments, as predicted by the model (Fig. 2). Further, treatment of either *cms-T* mitochondria (Bouthyette *et al.*, 1985; Holden and Sze, 1989) or *E. coli* cells expressing URF13 (Braun *et al.*, 1989) with DCCD results in a loss of URF13 sensitivity to T-toxin or methomyl.

URF13 TERTIARY AND QUATERNARY STRUCTURE

Studies of the interaction of *cms-T* mitochondria with T-toxin demonstrated a leakage of ions from the mitochondrial matrix (Matthews *et al.*, 1979), consistent with the formation of hydrophilic pores across the inner mitochondrial membrane. A standard model for the formation of hydrophilic pores by membrane proteins postulates the cylindrical association of several amphipathic membrane-spanning α-helices, with the hydrophilic faces of the helices lining the pore, and the hydrophobic faces exposed to the hydrocarbon phase of the bilayer (Ojcius and Young, 1991). In the three-helix model of URF13 (Fig. 2), helices II and III are amphipathic and could be envisioned to line the hydrophilic pore that results after interaction with T-toxin or methomyl. A pore having a diameter sufficient to allow NAD$^+$ to cross the membrane (8–10 Å) would require at least six, and more likely eight, amphipathic α-helices lining the cavity (Levings and Siedow, 1992). Monomeric URF13 would therefore not be capable of forming the pore produced after interaction with T-toxin. However, it was not known if URF13 was forming the pores by itself or was interacting with another protein(s) to form pores.

The cooperative nature of the binding of reduced, tritiated T-toxin to URF13 first suggested the oligomeric nature of URF13 (Braun *et al.*, 1990). The presence of URF13 oligomers was demonstrated directly by Korth *et al.* (1991) using the cross-linking reagent ethylene glycolbis(succinimidylsuccinate) (EGS). URF13 oligomers are at least tetrameric and are also cross-linked by DCCD (Korth *et al.*, 1991; Kaspi and Siedow, 1993). Susceptibility of *E. coli* cells to T-toxin or methomyl is strongly correlated with the presence of URF13 oligomers (Kaspi and Siedow, 1993).

We used a combination of the site-direct mutagenesis and chemical cross-linking to examine the disposition of the URF13 helices in the oligomeric structure (Rhoads *et al.*, 1994). These experiments took advantage of the observation that the carboxylate side chain of Asp-39 of URF13 is the primary carboxylate group that reacts with DCCD and is involved in DCCD cross-linking (Braun *et al.*, 1989; Kaspi and Siedow, 1993). DCCD cross-linking results in the formation of an amide bond between the carboxyl group of one amino acid and the amino group of the cross-linked partner, which is referred to as "zero length cross-linking." The three-helix model of URF13 (Fig. 2) predicts that either

Lys-32 or Lys-37 are positioned to be the likely cross-linking partners to Asp-39. We used site-directed mutagenesis to make amino acid substitutions for Lys-32 and Lys-37 individually and together. The results of these experiments showed that Lys-37 is the residue cross-linked by DCCD to Asp-39 (Rhoads *et al.*, 1994). The "zero-length" nature of DCCD cross-linking establishes some restraints on the relative positioning of Asp-39 and Lys-37. Specifically, these amino acids must be situated very close together in the URF13 oligomer, and this suggests that there must be a helix II central core in the URF13 oligomer (Fig. 3). Because DCCD (and other chemical cross-linkers) cross-links URF13 tetrameric species and tetramers are inherently symmetrical, it is attractive to postulate that the URF13 tetramer is the species capable of forming pores after interaction with T-toxin or methomyl. A schematic, three-dimensional model and helical wheel diagram of an URF13 tetramer is depicted in Fig. 3. These models present Asp-39 and Lys-37 positioned within the helix II central core so that they are readily cross-linked with DCCD and have the hydrophilic face of each helix II and each helix III facing into the region predicted to form the hydrophilic pore.

We propose that after interaction between T-toxin or methomyl and URF13, the URF13 oligomer undergoes a conformational change that allows the amphipathic helices III to participate in pore formation. This hypothesis is supported by two observations. DCCD cross-linking of URF13 prevents pore formation (Bouthyette *et al.*, 1985; Holden and Sze, 1989; Braun *et al.*, 1989), which is consistent with the covalent cross-linking locking URF13 into a closed state and preventing the conformational change that causes helices II to separate. In addition, the presence of methomyl or T-toxin prevents DCCD cross-linking (Rhoads *et al.*, 1994), which is consistent with the separation of helices II or a dramatic conformational change after binding. The T-toxin-induced conformational change moves Asp-39 and Lys-37 sufficiently far apart to eliminate DCCD cross-linking. Furthermore, the inhibitory effect of treatment with methomyl on DCCD cross-linking can be reversed by washing out the methomyl (Rhoads *et al.*, 1994). The restoration of cross-linking after washing is consistent with the reversible closing of the pore once the methomyl has been removed. These observations indicate that URF13 is a ligand-gated, pore-forming protein; binding of the

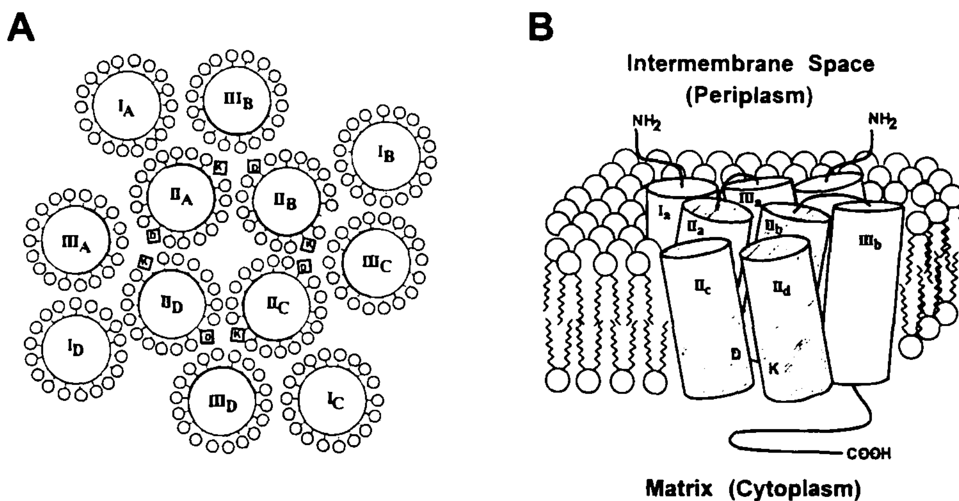

Fig. 3. Representations of URF13 tetramer. (A) Helical wheel representation of URF13 tetramer in which the amino acids of each helix are plotted as a two-dimensional projection of the helix parallel to the plane of the membrane. The hydrophilic face of each helix II and each helix III is represented by a bold arc and the hydrophobic face by a thin arc. The hydrophilic face of each helix III is positioned such that it could line the hydrophilic pore. Lys-37 and Asp-39 are outlined in bold squares. (B) Three-dimensional illustration of the postulated disposition of helices II of an URF13 tetramer in the membrane. Each shaded cylinder represents helix II of one URF13 molecule. Helices I_c, III_c, I_d, and III_d have been omitted for clarity. The positioning of helices I and III of molecules *a* and *b* is not known and should be viewed as arbitrary.

ligand (methomyl or T-toxin) to its receptor (URF13) is required for pore formation, and removal of the ligand results in closing of the pore.

CONCLUSIONS AND FUTURE DIRECTIONS

The presence of URF13 is strongly correlated with Texas-type cytoplasmic male sterility and URF13 is the receptor for the T-toxins produced by the pathogenic fungi *B. maydis* race T and *P. maydis*. The interaction between URF13 and T-toxins is the cause of the specific susceptibility of maize plants carrying the *cms*-T cytoplasm to *B. maydis* race T and *P. maydis*. The interaction between URF13 and T-toxin results in the formation of a hydrophilic pore across the inner membrane of *cms*-T mitochondria, severely disrupting mitochondrial bioenergetics and leading to the death of infected cells. Expression of URF13 in *E. coli* cells results in sensitivity to T-toxin and methomyl. The heterologous *E. coli*/URF13 system is a useful model system for studying URF13 structure and the interaction between T-toxin and URF13. These studies have led to the development of a model of the disposition of URF13 in the membrane that postulates the presence of three membrane-spanning α-helices (Fig. 2), two of which are amphipathic and line the hydrophilic pore that results after the binding of T-toxin to URF13 (Fig. 3). The oligomeric nature of URF13 has also been established and the utilization of techniques such as site-directed mutagenesis combined with chemical cross-linking have yielded significant insights into the tertiary structure of URF13 and hold promise for future studies directed at establishing the relative positions of helices I and III around the central core of helices II.

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