N,N'-Dicyclohexylcarbodiimide cross-linking suggests a central core of helices II in oligomers of URF13, the pore-forming T-toxin receptor of cms-T maize mitochondria

(Texas cytoplasm/cytoplasmic male sterility/protein structure/α-helices)

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ABSTRACT URF13 is a mitochondrially encoded, integral membrane protein found only in maize carrying the cms-T cytoplasm. URF13 is associated with cytoplasmic male sterility, Texas type, and causes susceptibility to the fungal pathogens Bipolaris maydis race T and Phylosticta maydis. URF13 is predicted to contain three transmembrane α-helices and is a receptor for the pathotoxins (T-toxins) produced by B. maydis race T and P. maydis. Binding of T-toxin to URF13 leads to membrane permeability. Cross-linking of URF13 oligomers with N,N'-dicyclohexylcarbodiimide (DCCD) protects Escherichia coli cells expressing URF13 and cms-T mitochondria from the permeability caused by T-toxin or methomyl. Using mutated forms of URF13 expressed in E. coli cells, we determined the molecular mechanism of DCCD protection. We separately changed Lys-37 in helix II to isoleucine (K37I-URF13) and Lys-32 in the helix I/helix II loop region to alanine (K32A-URF13). DCCD treatment of K37I-URF13-expressing cells did not protect the cells from permeability caused by T-toxin or methomyl. DCCD cross-linking was greatly reduced in K37I-URF13 and in D39V-URF13-expressing cells, but it was unaffected in K32A-URF13-expressing cells. Binding of methomyl or T-toxin decreases DCCD cross-linking of URF13 oligomers expressed in either E. coli or cms-T mitochondria. We conclude that Asp-39 in helix II is cross-linked by DCCD to Lys-37 in helix II of an adjacent URF13 molecule and that this cross-linking protects against toxin-mediated permeabilization. Our results also indicate that helices II form a central core in URF13 oligomers.

Maize plants (Zea mays L.) that exhibit Texas cytoplasmic male sterility (cms-T) are unable to produce pollen and are specifically susceptible to the fungal pathogens Bipolaris maydis race T and Phylosticta maydis (1, 2). Early experiments established that mitochondria isolated from cms-T maize plants and exposed to the host-specific toxins (T-toxins) produced by B. maydis race T or P. maydis (BmT-toxin and Pm-toxin, respectively) exhibit rapid swelling, uncoupling of oxidative phosphorylation, inhibition of malate-driven respiration, and leakage of NAD⁺ and other ions (1–5).

A 13-kDa protein, URF13, is the product of the mitochondrial gene T-urfl3, which is specific to cms-T maize (6). URF13 is located in the inner mitochondrial membrane of cms-T maize plants (7, 8) and is predicted to contain three transmembrane α-helices (Fig. 1), two of which (helices II and III) are amphipathic (1, 2). Protease accessibility studies support this postulated topology of URF13 in the membrane (9). Escherichia coli cells that express URF13 are also sensitive to T-toxin, and the effects of the toxin are similar to those observed when T-toxin interacts with isolated cms-T mitochondria (10–12). When methomyl, a compound structurally unrelated to T-toxin, is added to cms-T mitochondria or E. coli cells expressing URF13, the effects are the same as when T-toxin is added (12). Radiolabeled Pm-toxin binds specifically to cms-T mitochondria and to E. coli cells expressing URF13 (11). The binding to URF13 in E. coli cells is cooperative, suggesting that URF13 exists as oligomers in E. coli membranes (11). These results show that URF13 is the T-toxin receptor responsible for fungal susceptibility in cms-T maize and suggest that URF13 and T-toxin (or methomyl) interact to produce hydrophilic pores in cms-T maize mitochondrial and E. coli membranes (1, 2).

Because NAD⁺ (Mᵣ = 662) can pass through the pores formed by URF13 (4), a pore diameter of 0.8–1.5 nm is indicated (13). Even with a pore diameter of 0.8 nm, six amphipathic helices would be needed to form the channel

Fig. 1. Proposed topology of a URF13 monomer in the membrane. Amino acids are designated by the standard single-letter code, and arabic numbers indicate positions 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. Horizontal lines indicate the boundaries of the membrane. Asp-39, Lys-32, and Lys-37 are designated with boldface circles.

Abbreviations: DCCD, N,N'-dicyclohexylcarbodiimide; EGS, ethylene glycol bis(succinimidyl succinate).

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lining (13, 14). Based on the three-helix model of URF13 and the observation that only helices II and III are amphiphatic and likely to be involved in pore formation, a trimeric URF13 quaternary structure is minimally required to produce the observed pores. Direct evidence for the existence of URF13 oligomers (dimers, trimers, tetramers, and perhaps higher-order oligomers) was provided by cross-linking of URF13 using ethylene glycol bis(succinimidyl succinate) (EGS; ref. 9) and N,N'-dicyclohexylcarbodiimide (DCCD; ref. 15). Cross-linking with DCCD protects cms-T mitochondria (16, 17) and E. coli cells expressing URF13 (10, 15) from the effects of added T-toxin or methomyl.

In proteins, DCCD reacts with the carboxyl group of glutamate and aspartate residues to form an activated intermediate (18). Site-directed mutagenesis showed that Asp-39 is the primary amino acid with which DCCD reacts (10). If a nucleophile such as the ε-amino group of a lysine residue is suitably positioned, it can displace DCCD from the activated intermediate and form a covalent amide bond, resulting in intermolecular cross-linking if the residues are on distinct proteins. The DCCD cross-linking of the URF13 oligomers is a result of intermolecular cross-linking of those URF13 monomers that comprise the oligomers (15).

Based on the three-helix model for URF13 (Fig. 1), we hypothesized that DCCD cross-links Asp-39 of one URF13 monomer to Lys-37 of an adjacent monomer because of the potential for these two amino acids to be close together when helices II of adjacent monomers are aligned. In the studies presented here, we used site-directed mutagenesis to determine that Asp-39 and Lys-37 are the residues in URF13 that are cross-linked by DCCD and have developed a structural model of URF13 oligomers to explain the results.

**MATERIALS AND METHODS**

Oligonucleotide Synthesis, Site-Directed Mutagenesis, and DNA Sequence Analysis. The expression vector pKK223-3 (Pharmacia) containing T-urf13 downstream of the isopropyl β-d-thiogalactoside-inducible tac promoter and the M13 phage gene 17, designated pKK13T1 (19), was used to make changes in specific codons of T-urf13, according to the method of Kunkel (20). The primer used to change the codon for lysine to a codon for alanine at position 32 (designated a K32A mutation) had the sequence 5'-GATATCCCGTATCA-3'. The plasmid containing this change was designated pKK-K32A, and the protein expressed from this plasmid was designated K32A-URF13. The primer used to change the codon for lysine to a codon for isoleucine at position 37 (K37I) had the sequence 5'-CATCCTATTACGGAGA-3'. The plasmid containing this change was designated pKK-K37I. A plasmid containing both mutations, designated as pKK-K32A/K37I, was constructed by replacing the EcoRV–Sac I fragment of pKK-K32A with the EcoRV–Sac I fragment of pKK-K37I. DNA sequence analysis was done according to Sanger et al. (21) using a primer complementary to nt 1593–1609 of T-urf13 (6).

Expression of URF13 in E. coli. Spheroplast Preparation, and Mitochondria Isolation. URF13 and mutated URF13 were expressed from the pKK13T1 constructs in XL1-Blue E. coli cells (Stratagene) using isopropyl β-d-thiogalactoside according to the procedure suggested by the manufacturer or from the pET5b13T1 construct in BL21(DE3)pLysS E. coli cells (Novagen). The pET5b13T1 vector was created by ligation of the EcoRV–Sac I fragment (containing the fl intergenic region) from pKK13T1 into EcoRV–Sac I-digested pET5.13T (9). This creates a chimeric protein with 11 amino acids of a portion of the fl7 gene 10 protein (s10) fused to the N terminus of URF13. Stationary-phase cultures were diluted 1:50 into Luria–Bertani broth (22) containing the appropriate antibiotics and grown at 37°C for 3 hr. Cells were harvested by centrifugation at 5000 × g for 5 min at 4°C, washed in either M9 salts (42 mM Na2HPO4/22 mM KH2PO4/8.6 mM NaCl/19 mM NH4Cl) for respiration assays or phosphate-buffered saline (PBS; 140 mM NaCl/2 mM KCl/10 mM Na2HPO4/2 mM KH2PO4, pH 7.2) for cross-linking experiments. E. coli spheroplasts were prepared as described by Witholt et al. (23). Washed mitochondria were isolated from 6- or 7-day-old seedlings of B37 cms-T maize, as described in Stegink and Siedow (24). Protein concentrations were determined as described in Larson et al. (25).

DCCD and EGS Cross-Linking. DCCD and EGS cross-linking were done as described (9, 15). For the methomyl and toxin treatment experiments, 0.5 ml of E. coli spheroplasts or mitochondria at a concentration of 1.0 mg of protein per ml in a volume of 0.5 ml were incubated with 8.0 mM methomyl or 1.0 μM Pm-toxin at room temperature for 15 min before addition of DCCD. For the reversibility experiment, spheroplasts incubated with methomyl for 15 min were subsequently diluted by addition of 10 ml of PBS, incubated at room temperature for 10 min, and centrifuged at 14,460 × g for 10 min. The pellets were resuspended in 200 μl of PBS, and the protein concentration was determined. The concentration was adjusted to 1.0 mg of protein per ml with PBS, and DCCD cross-linking was done as described (9).

Immunoblot Analysis. Spheroplasts or mitochondrial proteins at 1.0 mg of protein per ml were solubilized in SDS/PAGE buffer, boiled for 5 min, and separated by SDS/PAGE on gels consisting of 18% acrylamide as described in Braun et al. (10). Immunoblotting was done by using the monoclonal antibody MAb-C that recognizes the C-terminal domain of URF13 and anti-s10 antibodies (Novagen) that recognize the N-terminal amino acids corresponding to the portion of the s10 protein encoded by the pET5b vector (9). The pET5b constructs and anti-s10 antibodies were used for the EGS cross-linking experiments because EGS treatment greatly decreases the recognition of the URF13 epitope by MAb-C.

Assays of Methomyl Sensitivity. The sensitivity of glucose-driven respiration to methomyl in intact E. coli cells was measured polarographically using a Clark-type oxygen electrode as described (12).

**RESULTS**

Respiration Assays Using DCCD-Treated and Untreated Cells. When methomyl was added to E. coli cells expressing URF13, the cells rapidly lost the ability to sustain glucose-driven respiration (Fig. 2A); however, treatment with 0.5 mM DCCD before the respiration assays made them insensitive to methomyl (Fig. 2B), as reported (10, 12, 15). Cells expressing URF13 with the K32A mutation (K32A-URF13) were insensitive to methomyl after DCCD treatment (Fig. 2D) and were sensitive if untreated (Fig. 2C), suggesting that Lys-32 is not involved in the DCCD cross-linking of URF13. Cells expressing URF13 with the D39V mutation (D39V-URF13) were insensitive to methomyl when either untreated (Fig. 2G), as reported (10), or when DCCD-treated (Fig. 2H). However, cells expressing URF13 containing the K37I mutation (K37I-URF13) were sensitive to methomyl whether they were treated with DCCD (Fig. 2F) or not (Fig. 2E), suggesting that the K37I-URF13 molecules were not significantly cross-linked by DCCD.

DCCD and EGS Cross-Linking. DCCD cross-links URF13 in the plasma membrane of E. coli cells (Fig. 3A, lane 2, and B, lane 2). Asp-39 is involved in DCCD cross-linking (15); a change at residue 39 from aspartate to valine (D39V-URF13) results in a dramatic decrease in DCCD cross-linking of URF13 oligomers (Fig. 3A, lane 6). When Lys-37 was changed to isoleucine (K37I-URF13), a dramatic reduction in cross-linking after DCCD treatment was observed (Fig. 3A, lane 4, and B, lane 5). However, s10:K37I-URF13 molecules
could be cross-linked by EGS (Fig. 3B, lane 6) to the same extent as s10:URF13 (Fig. 3B, lane 3), and D39V-URF13 molecules could be cross-linked with EGS to the same extent as URF13 (data not shown), indicating that these mutations do not significantly disrupt the secondary or tertiary structure of the K371-URF13 molecules or the quaternary structure of URF13 oligomers. The 11 amino acids of the s10 protein at the N terminus do not alter EGS cross-linking of either s10:URF13 (Fig. 3B, lane 2) or s10:K371-URF13 (Fig. 3B, lane 6) and do not alter the DCCD cross-linking of s10:URF13 (Fig. 3B, lane 2). When Lys-32 was changed to alanine (K32A-URF13), the K32A-URF13 molecules were cross-linked after DCCD treatment (Fig. 3A, lane 3) to the same extent as URF13 (Fig. 3A, lane 2).

**Mathemyl and T-Toxin Inhibition of DCCD Cross-Linking.**

In the presence of 8.0 mM methomyl, the cross-linking of URF13 in *E. coli* spheroplasts was greatly reduced (Fig. 4A, lane 3) relative to the cross-linking observed in the absence of methomyl (Fig. 4A, lane 2). The effect of methomyl could be reversed by washing the spheroplasts after incubation with methomyl (Fig. 4A, lane 5). In the presence of 1.0 μM T-toxin, the cross-linking of URF13 molecules by DCCD was also greatly reduced (Fig. 4A, lane 4) relative to the cross-linking in the absence of T-toxin (Fig. 4A, lane 2). Analogous effects of methomyl and T-toxin on DCCD cross-linking of URF13 were observed in isolated *cms-7* mitochondria (Fig. 4B). Because the chemical structures of methomyl and T-toxin differ greatly, it is unlikely that either compound interacts directly with DCCD to prevent cross-linking. A URF13 mutant containing a Ser-41 → Pro change binds T-toxin at very low levels relative to URF13 (G. C. Ward and C.S.L., unpublished work). DCCD cross-links S41P-URF13 oligomers to the same degree in the presence or absence of methomyl or T-toxin, suggesting that neither methomyl nor T-toxin reacts chemically with DCCD to prevent cross-linking of URF13 (data not shown).

**Fig. 2.** Affects of methomyl on respiration in *E. coli* cells expressing URF13 or mutated URF13. *E. coli* cells expressing URF13 (A and B), K32A-URF13 (C and D), K371-URF13 (E and F), or D39V-URF13 (G and H) were either untreated (A, C, E, and G) or treated with 500 μM DCCD (B, D, F, and H). Respiration of 500 μg of *E. coli* protein was measured as described (12). Methomyl (8 mM) was added at the time indicated by the arrows. Respiration rates are given below each trace as nmol of oxygen per min per mg of protein.

**Fig. 3.** SDS/PAGE immunobots of URF13 and mutated URF13 proteins in *E. coli* spheroplasts treated with DCCD or EGS. Samples were untreated (A and B, lanes 1, B, lane 4) or treated with either 250 μM DCCD (A, lanes 2–6, B, lanes 2 and 5) or 5 mM EGS (B, lanes 3 and 6). The proteins expressed are given above each lane. Each lane contained 15 μg of total protein, and the immunoblots were probed with either the MAb-C monoclonal antibody (A) or a monoclonal antibody to the s10 protein fused to URF13 (B). Relative molecular masses, in kDa, of protein standards (Bethesda Research Laboratories) are indicated at left. URF13 oligomeric species are indicated at right and designated as monomer (M), dimer (D), trimer (Tr), and tetramer (T) in A and dashes in the same order from the bottom in B.

**Fig. 4.** SDS/PAGE immunoblots showing the effects of methomyl and T-toxin on DCCD cross-linking of URF13 monomers in *E. coli* spheroplasts (A) and mitochondria (B). (A) *E. coli* spheroplast samples were untreated (lane 1) or treated with 500 μM DCCD (lanes 2–5) either after preincubation in 8.0 μM methomyl (lane 3) or 1.0 μM T-toxin (lane 4) for 15 min or after preincubation in methomyl followed by a wash (w) (lane 5). (B) Mitochondrial samples were untreated (lane 1) or treated (lanes 2–4) with 250 μM DCCD after preincubation in 8.0 μM methomyl (lane 3) or 1.0 μM T-toxin (lane 4) for 15 min. Each lane contained 15 μg of total protein, and the immunoblot was probed with the MAB-C monoclonal antibody. Relative molecular masses, in kDa, of protein standards (Bethesda Research Laboratories) are indicated at left, and oligomeric species of URF13 are indicated at right and designated as for Fig. 3.
DISCUSSION

The derived amino acid sequence of URF13 was used to predict that this protein contains three transmembrane α-helices linked by two short turns (Fig. 1) (1, 2). This model is supported by proteolysis studies (9). DCCD protects E. coli cells expressing URF13 from the permeabilizing effects of T-toxin or methomyl (10). This protection was first thought to be caused by a covalent modification of Asp-39 (10). Later, it was determined that DCCD cross-links the URF13 oligomers and that cross-linking results in the protection (15). Based on the current model for the disposition of URF13 in membranes (Fig. 1), we postulated that DCCD cross-links Asp-39 to either Lys-37 or Lys-32. The involvement of Asp-39 and Lys-37 seemed more likely because the proposed alignment of helices II (Figs. 1 and 5) positions these residues close together. Close positioning of an aspartate and a lysine is required for DCCD cross-linking because the nucleophilic ε-amino group of the attacking lysine residue must be in position to react with the carbonyl of the activated carboxylate to displace DCCD (18).

We expressed URF13 mutants in E. coli cells to test the hypothesis that Asp-39 and Lys-37 are the amino acids involved in DCCD cross-linking of URF13 oligomers. Cells expressing forms of URF13 that can be DCCD cross-linked should be T-toxin and methomyl-insensitive after DCCD treatment, whereas cells expressing forms of URF13 that cannot be cross-linked should remain T-toxin- and methomyl-sensitive after DCCD treatment. An exception would be URF13 mutants that are insensitive to methomyl. The Asp-39 mutation is an example of this category of mutant. As expected, cells expressing D39V-URF13 were insensitive to methomyl whether they were treated with DCCD (Fig. 2H) or not (Fig. 2G), and cross-linking of D39V-URF13 (Fig. 3A, lane 6) by DCCD was greatly reduced relative to the amount of cross-linking of URF13. The failure of DCCD cross-linking in D39V-URF13 does not result from the failure to form oligomers because D39V-URF13 oligomers are cross-linked by EGS (G. C. Ward and C. S. L., unpublished work). When cells expressing K37I-URF13 were treated with DCCD, these cells remained sensitive to methomyl (Fig. 2F), suggesting that K37I-URF13 molecules were not cross-linked. Immunoblot analysis confirmed that cross-linking of K37I-URF13 by DCCD (Fig. 3A, lane 4, and B, lane 5) was greatly reduced relative to DCCD cross-linking of URF13 (Fig. 3A, lane 2, and B, lane 2). The failure of DCCD cross-linking in K37I-URF13 does not result from a failure to form oligomers because the s10:K37I-URF13 protein (Fig. 3B, lane 6) was cross-linked by EGS to the same extent as s10:URF13 (Fig. 3B, lane 3). In contrast, when cells expressing K32A-URF13 were treated with DCCD, the cells were no longer sensitive to methomyl (Fig. 2D), and immunoblot analysis confirmed that K32A-URF13 oligomers (Fig. 3A, lane 3) were cross-linked to the same extent as URF13 (Fig. 3A, lane 2). These results strongly suggest that Asp-39 and Lys-37 are the residues in URF13 oligomers that are cross-linked by DCCD.

Even though higher-order oligomers are not cross-linked, a small amount of dimer is observed when E. coli spheroplasts expressing K37I-URF13, K32A/K37I-URF13, or D39V-URF13 are treated with DCCD (Fig. 3A, lanes 4, 5, and 6, and B, lane 5). Small amounts of dimers are often observed on immunoblots of URF13 samples (mutant or nonmutant), even without cross-linking treatment (Fig. 3A and B, lanes 1; refs. 8 and 9). Presumably these dimers are held together by interactions between monomers that cannot be overcome by SDS.

The water-soluble 1-ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC) cross-links proteins by the same mechanism as DCCD (18). There is one report in which serine acts as the nucleophile to displace EDC from the Glu-EDC intermediate (26). The resulting ester linkage is susceptible to hydrolysis by NaOH and NH4OH (26). Neither the linkages formed by DCCD cross-linking of URF13 oligomers nor the small amount of K37I-URF13 dimer cross-linked by DCCD is susceptible to base hydrolysis (data not shown). Also, S41P-URF13 oligomers are cross-linked by DCCD (data not shown). This result suggests that serine residues (e.g., Ser-41) are not significantly involved in DCCD cross-linking of URF13 oligomers.

Our results are consistent with the three-membrane-spanning α-helical model of the structure of URF13 (Fig. 1) (1, 2) and provide insights into the quaternary structure of URF13 oligomers. For the carboxyl group of Asp-39 in one URF13 monomer to be intermolecularly cross-linked by DCCD to the α-amino group of Lys-37 on an adjacent URF13 molecule, helix II of one URF13 must be closely aligned with helix II of an adjacent URF13. To obtain URF13 higher-order oligomers (i.e., trimer, tetramer, and larger) that can be cross-linked by DCCD, each URF13 oligomer must have a central core of helices II. A helical wheel representation and a schematic illustration of a four-helix core that we propose for URF13 tetramers are displayed in Fig. 5 A and B, respectively. As implied by the DCCD cross-linking data, the Asp-39 and Lys-37 residues are close together in the helices II of the tetramers. Two sets of helix–helix interactions are required for the formation of this four-helix core: one set of interactions between helices I, II, and III, and another second set of interactions between helices II, III, and IV. Because helix–helix interactions between adjacent monomers in a URF13 trimer would differ intrinsically from the interactions between URF13 monomers in a tetramer, we suggest that trimers on immunoblots represent incompletely cross-linked tetramers. For example, if DCCD first cross-linked only three monomers and then imbibed the sample, the trimer cross-linked by DCCD could be detected, but the tetramer cross-linked by DCCD would be undetected.

![Figure 5](https://example.com/figure5.png) Representation of the putative central core of helices II within a URF13 tetramer. (A) Helical wheel representation 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 is represented by a bold arc, and the hydrophobic face is represented by a thin arc. Circled amino acids are hydrophilic. Lys-37 and Asp-39 are circled with boldface lines. (B) Three-dimensional illustration of the postulated disposition of helices II of a URF13 tetramer in the membrane. The shaded cylinder represents helix II of one URF13 molecule. Helices I1, I2, I3, and I4 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.
links helix I_D to I_E (Fig. 5) and helix I_E to I_D, then tetramers would result from cross-linking either helix I_D to I_F or helix I_E to I_D. However, if DCCD first cross-links helix I_E to I_D and helix I_D to I_E, and there is a subsequent conformational change that prevents further DCCD cross-linking, then only trimers would be formed.

We have demonstrated that methomyl or T-toxin greatly reduces DCCD cross-linking of URF13 oligomers in both E. coli spheroplasts and cms-T mitochondria and that the effects of methomyl on DCCD cross-linking of URF13 in E. coli spheroplasts can be eliminated by removing the methomyl. This reversibility demonstrates that the methomyl does not cause a permanent change in the structure of URF13 that affects DCCD cross-linking. The DCCD cross-linking that occurs in the presence of methomyl or T-toxin probably occurs, in part, because DCCD cross-linking is irreversible, but the binding of T-toxin and methomyl is reversible. We have not determined whether higher amounts of either methomyl or T-toxin would completely inhibit DCCD cross-linking. Methomyl or T-toxin may bind at or near the helix-helix interface where Lys-37 of one URF13 monomer is adjacent to Asp-39 of a second URF13 monomer and prevent DCCD cross-linking by simple steric hindrance. Alternatively, binding of methomyl or T-toxin may cause a conformational change in URF13 that moves the helices II sufficiently far apart to prevent DCCD cross-linking. It is intuitively attractive to postulate that the binding of methomyl or T-toxin causes a conformational change that results in the insertion of helix III between adjacent helices II such that the central core of helices II is forced apart allowing the amphiphilic faces of helices III to line the pore along with the amphiphilic faces of helices II. This separation of helices II may prevent DCCD cross-linking because Lys-37 is no longer sufficiently close for its ε-amino group to displace DCCD from the Asp-39–DCCD intermediate. Insensitivity to methomyl or T-toxin of DCCD cross-linked cells expressing URF13 may occur because cross-linking locks the URF13 oligomers into a rigid structure that cannot change conformation. This prevents insertion of helices III between helices II, thereby preventing pore formation and sensitivity to methomyl or T-toxin.

Channel- and pore-forming proteins are prevalent and important components of the membranes of both prokaryotes and eukaryotes, in which they have roles in many physiological processes. Protein complexes that likely form pores or channels include (i) bacterial toxins, including anthrax (27), botulinum (28), cholera (29), diphtheria (28), and tetanus toxins (28); (ii) colicins (13); (iii) cercopins and magainins (30); and (iv) thionins (31). Structural data on membrane proteins, in general, and channel- and pore-forming proteins, in particular, are limited. Antiparallel four α-helix bundles have been identified from the crystal structures of several soluble proteins (32). For membrane proteins, however, little direct structural information exists, and the presence of α-helical cores that line hydrophilic pores has not been directly demonstrated, although they are likely components of several pore- and channel-forming protein complexes (33). This study provides evidence that the putative helices II of URF13 oligomers form an α-helix core that probably lines the URF3 hydrophilic pore. Continued study of URF3 should provide insight into the structural biochemistry of pore-forming, integral membrane proteins in general.

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