Membrane Insertion of the Heptameric Staphylococcal α-Toxin Pore

A DOMINO-LIKE STRUCTURAL TRANSITION THAT IS ALLOSTERICALLY MODULATED BY THE TARGET CELL MEMBRANE*

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Staphylococcal α-toxin forms heptameric pores on eukaryotic cell membranes. After binding to the cell membrane in its monomeric form, the toxin first assembles into a heptameric pre-pore. Subsequently, the pre-pore transforms into the final pore by membrane insertion of an amphipathic β-barrel, which comprises the “central loop” domains of all heptamer subunits. The process of membrane insertion was analyzed here using a set of functionally altered toxin mutants. The results show that insertion may be initiated within an individual protomer when its NH₂ terminus activates its central loop. The activated state is then shared with the central loops of the residual heptamer subunits, which results in cooperative membrane penetration. This cooperation of the central loops commences while these are still remote from the lipid bilayer. Nevertheless, it is subject to modulation by the target membrane, which therefore acts across a distance much like an allosteric effector. However, while allosteric transitions usually are reversible, membrane insertion of α-toxin is an irreversible event, and we show here that it can proceed to completion in a domino-like fashion when triggered by as little as a single foreign atom within the entire heptamer.

Staphylococcus aureus α-toxin, a protein of 293 amino acid residues (M₀: 33,000), was the first pore-forming bacterial cytolysin to be identified (1) and to date is still one of the most studied members within this class of toxins. The molecule is secreted as a water-soluble monomer that binds to cytoplasmic membranes of eukaryotic target cells, where it subsequently forms transmembrane pores of heptameric stoichiometry (2). The crystal structure of the pore heptamer has been solved (3). The transmembrane part of the pore consists of an amphipathic β-barrel of 7-fold rotational symmetry, and it comprises the amino acid residues 118–140 of each subunit (4). Within this central loop, the odd-numbered residues face the lumen of the pore, while the even-numbered ones are in contact with the surrounding lipid bilayer (3–5).

The conformation of the monomer is likely to be similar to that of the homologous toxin leukocidin (6, 7), so that structural information is available for both the initial and the final stages of pore formation (Fig. 1A). In addition, two intermediate stages can be distinguished: the membrane-bound monomer and the heptameric pre-pore (8, 9). The pre-pore appears to be irreversibly associated, but the β-barrel is not yet inserted in the membrane (Fig. 1B). During insertion, the central loop requires assistance by NH₂-terminal segments of the polypeptide chain, which also undergoes a major conformational change (10–12).

A variety of both recombinant and chemical modification techniques have been applied to the α-toxin pore to explore its application as a biosensor and to extend its utility in cell biological research (13–15). The mutation characterized in the present study was initially designed for a cell biological experiment, too: the four amino acids (YTRF) that were inserted at the turn of the membrane-penetrating loop (following residue 129) represent a peptide signal for transferrin receptor endocytosis (16, 17). Accordingly, our intention was to observe endocytosis of 129YTRF heptamers on nucleated cells. This goal was not attained. However, the unique phenotype of 129YTRF could be exploited to reveal surprising features concerning the molecular mode of pore formation. In particular, when 129YTRF was co-oligomerized on fibroblast membranes with a series of point mutants of α-toxin, hybrid heptamers were detected that were either fully active or entirely inactive depending on only one amino acid within a single subunit. A single amino acid residue may thus trigger membrane insertion of the entire α-toxin heptamer, whereby the NH₂ terminus and the glycine-rich central loop cooperate both intra- and intermolecularly in an intricate fashion.

MATERIALS AND METHODS

Construction, Expression, Purification, and Chemical Modification of α-Toxin Mutants—The mutant 129YTRF was constructed by PCR mutagenesis according to Ref. 18 using two mutagenic primers (coding sequence, GGT GAT ACA TAC ACC ACG TTC GGA AAA ATT GCC GGC C; complementary, TTT TCC GAA CCT GCT GTA TCT ATC ACC AGT AAC; the four italicized codons represent the insertion) and verified by DNA sequencing. The mutant gene was cloned into the shuttle plasmid pDU1212 for expression in Staphylococcus aureus DU1090 (19) and purified from bacterial culture supernatants as described previously (20). The various single cysteine mutants used in this study have been described before (4, 5, 21). The double mutant I5C/129YTRF was constructed by cleaving the respective pDU1212-derived, single mutant plasmids with KpnI and recombining the appropriate restriction fragments. Analogously, the cysteine mutant I5C/G130C was subcloned from the I5C and G130C single mutants. To achieve the formation of intramolecular disulfide bonds with the latter mutant, the protein was transferred into phosphate-buffered saline (pH 7.5) and supplemented with an equimolar amount of dithiobisnitrobenzoic acid. After incubation at room temperature for 15 min, disulfide bond forma-

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the oligomers and fluorescein fluorescence quantified as above. Parallel samples were prepared with 10 μg of labeled K46C supplemented with 20 μg of either wild type hemolysin or 129YTRF, respectively.

**Functional Assays of α-Toxin Mutants on Rabbit Erythrocytes, Human Fibroblasts, and THP-1 Cells—** Hemolytic titration was carried out using rabbit erythrocytes (23) with visual reading after 120 min. For assays involving retention of marker molecules within human fibroblasts, these were grown to confluence in 6 (K+ or 96 (ATP)-well plates. The fibroblasts were washed with HBSS and then treated with the various toxin variants for 1 h at 37 °C; the wild type or mutant proteins used and their respective amounts are stated under “Results.” Following incubation, the supernatant was withdrawn, and the cells were washed once with HBSS. The cellular material was then recovered by lysis with Triton X-100 (Sigma; 1% in HBSS). Cellular ATP was assayed by luminometry, and cellular K+ was determined by flame photometry as described (24), whereby control cells incubated in parallel without toxins served as standards. THP1 cells were treated analogously, except that they were grown and treated with the toxin in suspension; washing and recovery were performed by centrifugation.

### RESULTS

**Construction and Pore-forming Properties of Mutant 129YTRF—** Four consecutive codons (YTRF: tyrosine, threonine, arginine, and phenylalanine) were inserted between residues Thr129 and Gly130 of α-toxin. The specific hemolytic activity of the ensuing mutant 129YTRF (as assayed with rabbit erythrocytes) corresponded to that of wild type toxin (data not shown). In contrast, when fibroblasts were treated with 129YTRF, no release of cellular K+ or ATP occurred, whereas either marker was efficiently depleted by wild type toxin (Fig. 2A). The 129YTRF mutant thus did not detectably permeabilize the fibroblasts, although it readily formed oligomers on these cells (Fig. 2B). Similar results were also obtained with the lymphoid cell line THP1 (data not shown). In sum, 129YTRF may form either lytic or nonlytic oligomers, depending on the target membrane.

**The 129YTRF Mutation Exerts a Dominant Negative Effect upon Membrane Insertion of Wild Type Toxin into Fibroblast Membranes—** To learn more about the functional properties of the 129YTRF mutant, we examined its interaction wild type toxin. A constant amount of wild type toxin was admixed with 129YTRF at various ratios, and the mixtures were applied to fibroblasts. Permeabilization of the cells was abolished when 129YTRF was employed in at least 2-fold excess over wild type toxin (Fig. 3A). This observed inhibition of wild type toxin by 129YTRF might conceivably be due to a competition of the two species for a limiting number of binding sites. The wild type toxin was therefore replaced with a fluorescein-labeled mutant (D108C) to facilitate the quantitation of the cell-bound active toxin. D108C indeed closely resembled wild type toxin in permeabilizing the fibroblasts in the absence but not in the presence of 129YTRF (data not shown). Nevertheless, the amount of labeled D108C that bound to the fibroblasts was unaffected by 129YTRF (Fig. 3B), indicating that 129YTRF had not dislodged D108C from its binding sites. We therefore considered the possibility that the inhibitory effect of 129YTRF was due to the formation of hybrid oligomers with the active toxin. The ability of 129YTRF to form hybrid with an active toxin species was confirmed using an experimental approach outlined previously (11). The fluorescein derivative of another active cysteine mutant (K46C) exhibits pronounced self-quenching of fluorescence upon oligomerization. When admixed to the labeled K46C, 129YTRF was indistinguishable from wild type toxin in its ability to prevent the self-quenching effect, indicating that both had formed hybrid oligomers with K46C to the same extent. This strongly suggests that oligomerization of all toxin species in question occurred entirely at random. Then, the stoichiometry of the hybrid oligomers ensuing from a binary mixture should be binomially distributed. With a 2-fold excess of 129YTRF over wild type toxin, the

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1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; HBSS, Hank’s balanced salts solution.
fraction of homogeneous wild type oligomers will then be virtually nil (0.05%), whereas 6% of the oligomers will consist of 129YTRF only. The majority of the oligomers will thus represent hybrids of the two proteins of varying composition. The observed lack of permeabilization therefore indicates that, within these hybrids, 129YTRF suppresses the lytic activity of wild type toxin. This finding tallies with the previous concept of cooperative membrane insertion of the heptamer subunits (4, 11).

Several "Breakthrough" Point Mutants Escape Inhibition by 129YTRF on Fibroblast Membranes—To characterize in more detail the cooperation among the subunits of the heptamer, we then tried to identify mutations that might complement the insertionally deficient phenotype of 129YTRF. To this end, we screened an existing collection of point mutants carrying unique cysteines substituted for various residues within the cysteine-less wild type a-toxin molecule (4, 5, 21, 25). All these mutants were hemolytically active, and although they varied to some extent in their permeabilizing activity toward fibroblasts, they all effected a reduction of cellular ATP by at least 60% when employed at 10 μg/ml (data not shown). Accordingly, 10 μg/ml of each cysteine mutant in question was admixed with a 3-fold excess of 129YTRF and applied to fibroblasts, which were then assayed for cellular ATP. In most experiments, the fibroblasts retained ATP, so that the mutant proteins resembled wild type toxin in being inhibited by 129YTRF (Fig. 4). However, the figure also shows that in several instances the cells had been permeabilized, indicating that these mutants had escaped inhibition by 129YTRF. Mutations of the latter type did not significantly compete for binding sites. C, fluorescence quenching assay of hybrid heptamer formation. The fluorescein-labeled mutant K46C (5 μg) was oligomerized on liposome membranes, and the fluorescence intensity was assayed. Admixing the labeled mutant with unlabeled toxin prior to oligomerization enhances fluorescence, i.e. reduces quenching. This reduction is the same with 129YTRF as with wild type hemolysin, indicating that either species is incorporated into hybrid oligomers with K46C to the same extent. (Bars represent means of three experiments).

Several "Breakthrough" Point Mutants Escape Inhibition by 129YTRF on Fibroblast Membranes—To characterize in more detail the cooperation among the subunits of the heptamer, we then tried to identify mutations that might complement the insertionally deficient phenotype of 129YTRF. To this end, we screened an existing collection of point mutants carrying unique cysteines substituted for various residues within the cysteine-less wild type a-toxin molecule (4, 5, 21, 25). All these mutants were hemolytically active, and although they varied to some extent in their permeabilizing activity toward fibroblasts, they all effected a reduction of cellular ATP by at least 60% when employed at 10 μg/ml (data not shown). Accordingly, 10 μg/ml of each cysteine mutant in question was admixed with a 3-fold excess of 129YTRF and applied to fibroblasts, which were then assayed for cellular ATP. In most experiments, the fibroblasts retained ATP, so that the mutant proteins resembled wild type toxin in being inhibited by 129YTRF (Fig. 4). However, the figure also shows that in several instances the cells had been permeabilized, indicating that these mutants had escaped inhibition by 129YTRF. Mutations of the latter type cluster in two regions: mutants S3C, I5C, I7C, and D13C are located at the far NH2 terminus, while substitutions D127C, D128C, T129C, G130C, and K131C are in immediate proximity to the insertional mutation of 129YTRF. They will collectively be referred to as breakthrough mutations in the following.

Breakthrough Mutants Effect Pore Formation when Co-assembled with 129YTRF into Hybrid Oligomers—The lacking inhibition of breakthrough mutants by 129YTRF might conceivably arise in two different ways: first, the mutants might sim-
with 5 m increasing amounts of 129YTRF. Surprisingly, in this experi-
ment, the permeabilization by hybrid heptamers is maintained at very high
"dilution" of 129YTRF with 123YTRF. (Error bars indicate S.D. from five
experiments).

Another possibility that might be considered is that, within the
hybrids, the breakthrough mutant molecules might insert on
their own, while the 129-YTRF subunits would not; the
partially inserted hybrid oligomer would then create a pore of
reduced lytic capability. However, this assumption is at odds
with the behavior of the same hybrid oligomers on a different
cell type. The myeloid cell line THP-1, while readily susceptible
to 129YTRF or I5C alone, resists attack by mixtures of either
mutant when admixed with 129YTRF (data not shown). Therefore,
much like wild type toxin on fibroblasts, G130C or I5C are
amenable to inhibition by 129YTRF, and they are thus not
insertionally uncoupled.

In sum, neither co-dominant control nor uncoupling of mem-
brane insertion may account for the experimental findings, so
that the observed reduction in activity of G130C/129YTRF hy-
drom heptamers as opposed to the homogeneous G130C ones
heptamers remains unexplained. This issue will be further
considered below (see “Discussion”).

The I5C Breakthrough Mutation Does Not Complement the
129YTRF Insertion When Both Are Located on the Same Mole-
cule—The existence of the NH₂-terminal cluster of break-
through mutations indicated that, in one way or the other, the
amino terminus of a given subunit of the heptamer promotes
membrane insertion of the central loop of a neighboring sub-
unit. This stimulatory effect of the NH₂ terminus might con-
ceivably be transmitted along different routes that are sche-
matically depicted in Fig. 6A. First, the two molecular regions
might be engaged in immediate interaction (as represented by the
full arrow). Alternatively, the stimulatory effect might first
be relayed to a corresponding element in cis (long broken ar-
row) or in trans (short broken arrow). To distinguish between
these possibilities, we combined the NH₂-terminal break-
through mutation I5C with 129YTRF to yield a double mutant,
which then again was functionally characterized within homo-
geneous and hybrid heptamers. The results are summarized in
Fig. 6, B–D. The double mutant I5C/129YTRF alone did not
exhibit any permeabilization of fibroblasts, which is inconsis-
tent with the hypothesis of an immediate effect of the NH₂
terminus upon the central loop in trans (Fig. 6B). The double
mutant also failed to activate the 129YTRF single mutant
within hybrid heptamers, which is at odds with the assumption
that the NH₂-terminal stimulus is first communicated in trans
to a neighboring NH₂ terminus and from there to the central

**Fig. 4.** Several single cysteine mutants of α-toxin escape inhibition by 129YTRF. Wild type (wt) α-toxin, which comprises 293 amino acids without cysteines, and a series of single cysteine mutants were admixed with 129YTRF in 3-fold excess before application to fibroblasts. The percentage of cellular ATP retained was quantified by comparison with control cells incubated without toxin. With several mutants (e.g. S3C, G130C), ATP is released, indicating that these proteins resist inhibition by 129YTRF. (Bars represent means of three experiments).

**Fig. 5.** The breakthrough mutant G130C forms lytic hybrids with 129YTRF. A, G130C (at the amounts indicated), alone or with a 3-fold excess of 129YTRF, was applied to fibroblasts, and the cells were assayed for ATP. Permeabilization by G130C is augmented by 129YTRF, indicating formation of lytic hybrids. B, a constant sublytic amount (2.5 µg/ml) of G130C was admixed with increasing amounts of 129YTRF. Permeabilization by the hybrid heptamers is maintained at very high "dilution" of G130C with 129YTRF. (Error bars indicate S.D. from five experiments).
loop in cis (Fig. 6C). However, much like the \textsuperscript{129}YTRF single mutant, I5C/\textsuperscript{129}YTRF was readily complemented when co-oligomerized with I5C (Fig. 5D, upper panel). Moreover, the double mutant also inhibited the activity of wild-type toxin within hybrid oligomers (Fig. 5D, lower panel). These two findings support the notion that the NH\textsubscript{2} terminus primarily acts upon the central loop in cis, which then shares its state of activation with the other central loops within the heptamer. As discussed below, this concept is also compatible with previous data obtained with an NH\textsubscript{2}-terminal deletion mutant (Fig. 6E).

The Amino Terminus and the Central Loop Region Are Close within the \textit{a}-Toxin Monomer—The amino terminus is located remotely from the central loop within the heptamer (3), but the two regions are likely to be in close proximity within the monomeric toxin molecule (cf. Fig. 1A). Their close cooperation during membrane insertion suggests that this proximity be maintained in the pre-pore stage. To address this question more directly, a double cysteine mutant (I5C/G130C) was constructed. Fig. 7A shows that, in its monomeric form, this protein was readily converted to an intramolecular disulfide, the yield being around 50% in the experiment depicted. Subsequently, the mutant protein was no longer hemolytically active, and it failed to form SDS-resistant heptamers on membranes of model liposomes (Fig. 7A). Interestingly, however, when the liposomes were solubilized and electrophoresed with the non-denaturing detergent deoxycholate, an oligomeric species was detected (Fig. 7B, lane 3). This oligomer migrated behind the one observed when the mutant protein was reduced prior to membrane binding (lane 4), but it could be reverted both to normal electrophoretic mobility and to SDS-resistance by post-treatment with dithiothreitol (Fig. 7B, A and B, lanes 5). These results suggest that the NH\textsubscript{2} terminus and the central loop indeed remain in close contact at an early stage of heptamer assembly. This contact probably is the structural basis of their close cooperation in the subsequent process of membrane insertion. The reduced electrophoretic mobility of the disulfide-bonded pre-pore heptamer might reflect its lack of a protruding \( \beta \)-barrel, which in case of the mature pore heptamer, confers additional electric charge by the binding of deoxycholate to its hydrophobic outer circumference (4). Our findings also provide another example of the previous notion that the pre-pore stage may be further subdivided into several forms that, among other features, are distinguished by their susceptibility to dissociation by SDS (11).

**DISCUSSION**

\( \alpha \)-Toxin is an important model system for the molecular study of pore-forming proteins. Crystallization of the heptamer has provided the first high-resolution structure of a bacterial toxin pore (3), which has recently been complemented by the crystal structure of the homologous toxin leukocidin (lukF) in its monomeric state (6). Comparison of the two structures highlights the crucial roles of both the amino-terminal latch and the...
The insertion of $^{129}$YTRF into fibroblast membranes triggered inhibitory activity toward all active toxin species tested on THP-1 cells, whereas it is inactive and also nonlytic on rabbit erythrocytes. In the heptamer, the central glycine-rich loop in the conformational transition associated with pore formation (Fig. 1). In the monomer, the two segments are packed against the core of the molecule, contacting each other at their tips. In the oligomer, both have undergone a large move and now make long excursions from the core of the protomer. At the same time, they are in close contact with their respective counterparts of the neighboring subunits, the central loops now forming the transmembrane $\beta$-barrel that is crucial to toxin function. In addition to these structurally defined end points, intermediate stages have been characterized by mutational analysis that have outlined the way leading from the monomer to the final membrane-inserted oligomer. It has thereby become clear that, in the process of pore formation, oligomerization precedes membrane insertion (8, 9). Insertion involves cooperation among neighboring protomers (4, 11).

Despite its various contacts within the monomeric and the oligomeric structures, respectively, the amino-terminal latch might conceivably contribute to membrane insertion through a variety of either intra- or intermolecular effects. The findings reported here now distinguish the essential role of the intramolecular effect the amino latch takes upon the central loop in cis. While this effect was observed here with individual (NH$_2$-terminal breakthrough mutant) subunits within hybrid heptamers, it must be assumed to occur with all the subunits of an active (wild type) heptamer. This leads us to the notion that, in membrane insertion, the central loop receives dual activation both from the NH$_2$ terminus in cis and from another central loop in trans. This model readily accommodates the second cluster of breakthrough mutations located within that loop, and it can also account for previous results obtained with an NH$_2$-terminal deletion mutant (12). The latter protein exhibits impaired hemolytic activity, which it also imparts on wild type toxin within hybrid heptamers (Fig. 6E).

Another important prerequisite for membrane insertion to be initiated is that the oligomer receives activation from the target lipid bilayer, a fact that is strikingly illustrated by the finding that polymorphonuclear granulocytes resist toxin attack by preventing membrane insertion of the pre-pore altogether (5). The $^{129}$YTRF phenotype now shows that this activation may be available to a different extent with various naturally susceptible membranes. The $^{129}$YTRF mutant is active on its own on rabbit erythrocytes, it is inactive and also inhibitory toward all active toxin species tested on THP-1 cells, and it is either inhibitory or subject to activation on fibroblasts. The insertion of $^{129}$YTRF into fibroblast membranes triggered by breakthrough mutants exemplifies that, within an oligomer, individual protomers may transmit their activated state to neighboring subunits. On the other hand, the inhibitory action of $^{129}$YTRF shows that, despite available promotion by the membrane, a functional subunit may still fail to insert when in contact with drowzy neighbors. Therefore, in deciding on whether or not to proceed with membrane insertion, each subunit of the oligomer integrates signals received both from adjacent subunits and from the membrane. At what stage, then, does the membrane signal affect insertion of the central loop region? Consider a hybrid heptamer comprised of one wild type molecule and six molecules of $^{129}$YTRF. This heptamer will readily insert into the rabbit erythrocyte membrane, but not so into the fibroblast membrane. However, by substituting one molecule of, e.g. ISC for the wild type subunit, insertion into the fibroblast membrane will be restored. The NH$_2$-terminal mutation therefore compensates for the insufficient membrane stimulus, and it does so by directly acting upon the central loop in cis. It thus turns out that the membrane signal takes effect, while the central loop is still in contact with the NH$_2$ terminus and, hence, remote from the target membrane.

Taken together, the above considerations unveil a striking resemblance between membrane insertion of the $\alpha$-toxin heptamer and the cooperative structural transition of an allosteric enzyme, which also senses signals from extraneous ligands, transmits them to the remotely located active site and integrates them among the subunits themselves. There remains, however, one important difference: while the reversibility of allosteric transitions is essential to their regulatory role in enzyme activity, membrane insertion of the $\alpha$-toxin pore is an irreversible process. This irreversibility is strikingly highlighted by the fact that a single mutant residue within the entire heptamer may trigger insertion. Amazingly, in case of the breakthrough mutant S3C, such lytic hybrids differ from the corresponding nonlytic wild type $^{129}$YTRF hybrids by as little as a single sulfur atom replacing an oxygen. In a reversible system, such a small change should not effect much more than a slight shift of equilibrium; but, as we can see here, it may suffice to trigger an irreversible, self-sustaining reaction that proceeds to completion much like a toppling cascade of dominoes.

Although the cellular acceptor sites for $\alpha$-toxin still await molecular characterization, the limited number of toxin molecules found on the surface of target cells indicates that there is selectivity in toxin binding, and it also suggests that the toxin remains associated with the binding sites after oligomerization. From this, it would follow that, with different cells, these binding sites deliver a different degree of activation to the pre-pore heptamer that is on the verge of inserting. The question then arises whether or not, on a given cell, all binding sites are homogeneous. If they were not, their heterogeneity could account for the yet unexplained finding that the hybrid oligomers of G130C (as well as the other breakthrough mutants) and $^{129}$YTRF are less efficient in permeabilizing fibroblasts than those consisting of G130C only. Let us consider a single hybrid heptamer that is sitting on a fibroblast; $n$ is the number of its G130C subunits. All subunits are randomly bound to either activating membrane sites or to nonactivating ones. One would then expect the membrane stimulus to take effect only at those activating sites that happen to carry a G130C subunit, while those sites carrying a $^{129}$YTRF subunit would be silenced. Let $A$ be the fraction of activating sites and $N$ the fraction of nonactivating ones; $A + N = 1$. Then, the probability of membrane insertion of the hybrid oligomer would equal $1 - N^n$, i.e. there would be a direct relationship between the fraction of G130C subunits and the readiness of an oligomer to achieve insertion. This would explain the reduced specific activity of G130C/$^{129}$YTRF hybrids as opposed to homogeneous G130C oligomers. On the other hand, when $n$ G130C subunits would be singly incorporated into $n$ hybrid oligomers with $^{129}$YTRF, the probability that at least one of them was inserted would again be $1 - N^n$, but with $n > 1$ there would be the additional chance that further oligomers would be inserted. This consideration would account for the enhancement of efficacy of G130C by its “titration” with increasing amounts of the nonlytic $^{129}$YTRF species. In sum, the experimental findings are in line with the assumption that single cells may afford binding sites that support membrane insertion to different degrees. We speculate that this heterogeneity might be related to the lateral segregation of lipids in cell membranes.

In conclusion, our study shows that membrane insertion of the $\alpha$-toxin heptamer occurs in an intricately concerted manner resembling the allosteric transition of an oligomeric enzyme. It is restricted or promoted to a varying extent by different natural target membranes, whereby the molecular nature of the...
incremental cell membrane constituents remains to be elucidated. At the same time, membrane insertion is exceedingly susceptible to minimal changes of protein structure, which reflects the irreversible nature of the event of insertion. Ultimately, therefore, we are left with the question why a pore-forming toxin that, after oligomerization on the target membrane, is committed to an irreversible, one-hit mode of action is activated in a fashion similar to allosteric enzymes that must reversibly adjust to the prevailing metabolic situation.

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