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Similarities and differences between colicin and filamentous phage uptake by bacterial cells.

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Abstract

Gram-negative bacteria have evolved a complex envelope to adapt and survive in a broad range of ecological niches. This physical barrier is the first line of defense against noxious compounds and viral particles called bacteriophages. Colicins are a family of bactericidal proteins produced by and toxic to *Escherichia coli* and closely related bacteria. Filamentous phages have a complex structure, composed of at least five capsid proteins assembled in a long thread-shaped particle that protect the viral DNA. Despite their difference in size and complexity, group A colicins and filamentous phages both parasitize multiprotein complexes of their sensitive host for entry. They first bind to a receptor located at the surface of the target bacteria before specifically recruiting components of the Tol system to cross the outer membrane and find their

way through the periplasm. The Tol system is thought to use the proton-motive force of the inner membrane to maintain outer membrane integrity during the life cycle of the cell. This review will describe the sequential docking mechanisms of group A colicins and filamentous phages during their uptake by their bacterial host, with a specific focus on the translocation step, promoted by interactions with the Tol system.

Introduction

The cell envelope of Gram-negative bacteria, such as *Escherichia coli*, is characterized by the presence of two membranes, the inner (IM) and outer (OM) membranes, separated by the periplasm and a thin layer of peptidoglycan (PG). This envelope is a formidable barrier against a myriad of harmful compounds, while simultaneously allowing the entry of nutrients necessary for cell survival. However, this barrier, like the "Maginot Line" in France during the Second World War, is not completely impenetrable and exogenous particles, including some toxins and viruses, can pierce it.

Colicins are plasmid-encoded toxins (40 to 80 kDa), produced by *E. coli* under conditions of stress, that allow the efficient killing of related bacteria competing for space and resources. The toxicity of colicins relies on various modes of action, including IM depolarization, the inhibition of PG synthesis, and the degradation of DNA or RNA of the target cell. Colicins can be classified into two groups, A and B, depending on whether they use the Tol or Ton protein complexes to cross the OM and translocate through the periplasm of their host (for a review, see 1)

Filamentous bacteriophages or *Inoviridae* are elongated viruses (~7 by ~800-2000 nm) that must pierce the whole envelope to deliver their nucleic acid into the cytoplasm of the target cell to complete their life cycle. The virion particle consists of an assembly of the major coat

protein pVIII, capped with the adsorption protein pIII and minor coat proteins (pVI, pVII and pIX), encapsulating the circular single-stranded viral DNA. Among inoviruses, Ff coliphages have garnered particular scientific attention because of their extensive application in genetic engineering and phage display technology (reviewed in 2), along with the vibriophage CTX, which converts *Vibrio cholerae* to pathogenicity (3).

While colicins and phages are very different particles, their mode of uptake across the bacterial envelope shares both structural and functional similarities. They both use a multistep process that relies on the parasitism of structures produced by sensitive bacteria. Group A colicins and filamentous phages are first specifically recruited via an interaction with a bacterial surface receptor (reception step), followed by crossing of the OM and transport through the periplasm (translocation step). This step requires interactions with one or several proteins of the Tol system, a conserved macromolecular motor of the cell (4). This review describes the similarities and differences observed between the group A colicin and filamentous phage models of adsorption and translocation into their host and the role of cellular energy in these processes.

Colicins and the Filamentous Phage Adsorption Protein pIII Show Similar Structural Organization.

As for many toxins, colicins are organized into three structural domains that perform specific functions (**Fig. 1A**); the central domain (receptor domain, R) binds a specific bacterial OM protein, the N-terminal domain (translocation, T domain) is required for colicin translocation across the OM and interacts with the Tol system, and the C-terminal domain encodes the toxic activity (5-10). The crystallographic structures of some colicins have highlighted two important features: long antiparallel coiled-coil α -helices within the R-domain that separate this domain from the T and C-domains and a disordered region within the T

domain (11-14). Importantly, nuclease colicins are produced in a complex with an immunity protein, preventing their lethal action in the producing cell (**Fig. 1B**).

Filamentous phage infection is driven by three to five copies of the minor coat protein pIII (also called G3P), located at the tip of the particle (2, 15). As for colicins, phage pIII protein is organized into three functional domains, separated by two flexible regions (16, 17) (**Fig. 1A**). The central (N2) domain is responsible for adsorption to a pilus, whereas the N-terminal (N1) domain interacts with the Tol system. The pIII C-terminal domain anchors pIII to the phage particle and is required for DNA injection into the host. The structure of pIII-N1 and pIII-N1-N2 has been solved for the Ff and CTX phages by crystallography and NMR studies (**Fig. 1B**) (16, 18-22)

Binding to the Primary Receptor and Crossing of the OM.

The initial cellular reception of most group A colicins requires a high affinity OM protein receptor, such as BtuB for colicins A and E1 to E9 (**Table1**) (23-25). The structure of the colicin E3 and E2 R domain (135 residues) bound to BtuB has been solved (26, 27). In the current model of colicin uptake, the elongated helical coiled-coil R-domain binds BtuB with the T and C domains extending at a 45° angle out over the OM. Then, the intrinsically disordered region of the T-domain, adjacent to the OM, searches for a more abundant second receptor (called “the translocator”), in this case OmpF, and crosses the OM through one of the OmpF pores, which are wide enough to accommodate unfolded peptides. This model is based on numerous experiments, such as occlusion of the OmpF channel in planar lipid bilayers by colicin T domains and isolation of an intact complex of colicin with BtuB and OmpF, confirmed by crystallization of colicin T-domain peptides within the OmpF pore (28-32). The resulting Colicin-BtuB-OmpF complex is called the “colicin OM translocon” (**Fig. 2A**).

Filamentous phages have been reported to use host-specific Type IV pili or fimbriae that extend from the cell surface as extracellular receptors (**Table1**). Adsorption of the phage to the pilus is dependent on pIII-N2, the central domain of the capsid protein pIII (17, 33). For Ff phages, N2 binding to the *E. coli* F pilus leads to conformational changes in pIII that unmask the TolA binding site on N1, allowing phage infection to proceed (34) (**Fig. 2B**). How these phages cross the OM to reach the periplasmic space is unknown, but they may follow pilus retraction through the pilus secretin that spans the OM (35-37). Interestingly, the reception step is not strictly required for phage infection, as phages can bypass the pilus to infect cells at low frequencies (17, 38, 39). In the absence of the F pilus receptor, the addition of CaCl₂ to the medium has been reported to enhance phage infection, possibly by neutralizing negative charges at the cell surface (38).

After the binding step, which allows specific targeting of the bacterial host, both group A colicins and filamentous phages parasitize a trans-envelope protein complex, the Tol system, to cross the periplasm. The Tol-system components required for this step can vary depending on the group A colicin or phage involved (**Table1**).

The Tol Macrocomplex: A Conserved Molecular Motor of the Bacterial Envelope

The Tol system is highly conserved among Gram-negative bacteria (40). It consists of five proteins, TolQ, TolR, TolA, TolB, and Pal, which form a complex in the cell envelope. TolQRA are IM proteins that interact via their trans-membrane (TM) segments to form an IM complex (41-44). TolQ is a polytopic protein with three TM segments, whereas TolA and TolR are bitopic proteins with a large periplasmic domain (43, 45-48). TolB is a periplasmic protein composed of two subdomains, called D1 and D2, in which D2 forms a six-bladed β -propeller (49, 50). Finally, Pal is a lipoprotein tethered to the OM via its N-terminal acylated residue, and its C-terminal domain is free to interact with the peptidoglycan (PG) layer and TolB (51-55).

Thus, TolB-Pal forms the OM complex of the Tol system and TolB competes with the PG to bind Pal (56). The two Tol subcomplexes are transiently connected via the C-terminal domain of TolA (called TolAIII), which interacts with both TolB-D1 and Pal (57-60). The TolQ-TolR complex uses the proton motive force (PMF) to induce conformational changes in the periplasmic domain of TolA, promoting the TolA-Pal interaction (4, 41, 61). Thus, the Tol system works as a molecular motor, using the PMF to form a link between PG and the inner and outer membranes.

The Tol system has been reported to be essential in many bacterial species, whereas it is dispensable in the *E. coli* K12 strain (62-66). In *E. coli*, deletion of any of the *tol* genes causes a pleiotropic phenotype. Indeed, *tol* mutants are highly sensitive to detergents and some antibiotics, they release periplasmic proteins into the extracellular medium and they form numerous OM vesicles (67-71). These phenotypes all suggest a potential role of the Tol system in the maintenance of OM integrity. This role can be partially explained by the involvement of the Tol complex in OM lipid homeostasis (72, 73). Finally, the Tol system has been described to be involved in the late stage of the cell-division process, more precisely in OM invagination during cell division (62, 74).

The Tol System Serves as a Versatile Import Machinery for Parasites and Toxins.

Various *tol* mutants have been reported to show resistance both to group A colicins and filamentous phages. Indeed, direct or indirect interactions of these particles with components of the Tol complex have been described. When they reach the periplasmic space, most group A colicins first recruit the β -propeller domain of TolB through their N-terminal T domain (50, 58, 75-77). Conversely, TolB is not required for phage uptake (39, 78). The affinity of ColE9 to TolB has been shown to be strong enough to competitively displace TolB from Pal (77, 79). Structural data has shown that, in the presence of Pal, the N-terminal 12 residues of TolB,

encompassing the TolA binding domain, are ordered and sequestered to the TolB surface, whereas these residues are disordered and accessible to TolA binding in the presence of the ColE9 T-domain (79, 80). Thus, in the presence of ColE9, TolB binds to TolAIII and the toxin can continue its translocation. Although this model can be applied to the other nuclease colicins, some results suggest that the mechanism for the recruitment of TolB by the pore-forming colicin A may be different. As ColA is unable to competitively displace TolB in the TolB-Pal complex, the model suggests that the toxin binds to free periplasmic TolB and then to TolAIII, for which it has a higher affinity (81) (**Fig. 2A**).

All group A colicins and filamentous bacteriophages studied to date require TolA for their transit into the periplasm (**Table 1**). Direct interaction between the TolAIII domain and the colicin A, E1, N, and K T-domain has been shown by numerous *in vivo* and *in vitro* experiments (82-85). Similarly, the pIII protein at the tip of the phage particle is responsible for TolA binding (19, 22, 86, 87). The phage pIII-N1 and pIII-N2 domains have been reported to bind *E. coli* TolAIII and TolAII domains, respectively (88). No direct interaction has yet been observed between TolA and enzymatic colicins, even though *tolA* mutants are tolerant to these toxins.

Several studies indicate that TolA has diverse binding sites, which enable multiple interactions with other partners. First, point mutations or deletions in *tolA* differentially affect colicin import; some remain active, whereas others lose their ability to kill mutated strains (59, 89). Second, structural data have shown that the colicin A T-domain interacts with the convex site of TolAIII, whereas the phage Ff and IF1 pIII-N1 domain and colicin N T-domain bind to the concave side of TolAIII (18, 21, 90-93) (**Fig. 1B**). In contrast to coliphage Ff, the vibriophage CTX pIII-N1 domain binds to the convex site of *V. cholerae* TolAIII (22).

The role of the TolQ and TolR proteins in both phage and colicin uptake is less clear. Although TolQ and TolR are necessary but not essential for phage infection (39, 94), both are

absolutely required for the translocation of group A colicins (excepted ColE1). However, only a few studies have reported a direct interaction between colicins and either TolQ or TolR. The ColA and ColE3 T-domain has been shown to interact with TolR by cross-linking experiments (95) and the interaction between the ColK T-domain with TolQ or TolR has been revealed by coimmunoprecipitation (85). The final steps concerning the toxic effect of colicins and phage DNA injection are not discussed here but are briefly summarized in **Figure 2**.

Importance of Protein Structural Flexibility in Uptake Pathways.

An interesting aspect of the current models of colicin uptake is that their T-domains are disordered and that they fold into an ordered structure upon binding to a Tol protein. This disorder-to-order transition could explain the ability of colicins to bind several target partners and progress through the periplasm.

Another important aspect in both colicin and phage translocation is the large-scale structural modification of the molecules during the process. First, studies have shown that colicins A and E2 remain bound to their receptor and the Tol machinery, whereas their C and T domains are translocated (96, 97). Second, *in vivo* and *in vitro* experiments have shown that colicin A unfolds during its import into the cells (98, 99). This unfolding is triggered either by binding to the first ColE1 receptor, which is then propagated to the distal ends of the T and C domains of the toxin (26, 100), or it occurs after recruitment of the second receptor and formation of the “colicin OM translocon” for ColE9 (101). Similarly, the extensive structural modifications of coliphage pIII protein during initial binding to the F-pilus, followed by interaction with TolA in the periplasm, have been proposed to lead to the insertion of pIII-C into the IM of the host and the formation of a multimeric channel that allows phage DNA injection into the bacterial cytoplasm, concomitant with disassembly of the capsid by diffusion of the major coat protein pVIII in the membrane (102, 103).

Energetics of Colicin and Filamentous Phage Uptake.

Despite the large number of publications on colicin and phage import, only a few studies have focused on the energetic aspect of this process. As discussed above, the Tol system use the IM PMF, by means of the TolQR motor, to power mechanisms involved in the maintenance of OM integrity and cell constriction. However, initial studies suggested that the Tol-dependent import of two pore-forming colicins, ColA and ColE1, is energy-independent (104, 105). These results were later confirmed by point-mutations introduced into the TolQR motor that affect the physiological function of the Tol system without preventing the killing action of pore-forming colicins. These mutations were called “discriminative mutations” (106, 107). Energy requirements for the import of nuclease colicins appear to be different. It is clear that release of the immunity protein from nuclease colicins in the external medium requires functional import machinery (108, 109). Intriguingly, the release of the ColE9 immunity protein was shown to be energy dependent (109), whereas strains harboring discriminative TolQR mutations remain susceptible to ColE2 (107). Based on these divergent results, the question of the energy requirement for the translocation of group A colicins is still open. For coliphages, experiments using arsenate or protonophores has led to the hypothesis that phage uptake may be dependent on both ATP and PMF, possibly for pilus retraction and phage particle transit through the periplasm, respectively (110, 111).

In summary, it is fascinating to observe that small toxins, such as colicins, and complex viral structures, such as filamentous phages, are shaped so as to use similar molecular mechanisms to pierce the envelope of their target cell. These include a sequential docking mechanism, the strict requirement for the hub protein TolA, and large-scale structural modifications of the proteins during their transit. Although some key steps in these processes

225 have been well characterized, numerous questions are still unanswered concerning the
226 dynamics, sequence of events, and role of host-cell energy in the uptake of these
227 nanostructures.

228

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Table1. Host proteins required for reception and translocation of filamentous phages and group A colicins.

Name	Receptor	Translocator	Uptake Proteins	Cytotoxicity/Host	Ref.
Group A colicins					
ColA	BtuB	OmpF	TolA, B, Q, R	Pore-forming	
ColE2, E7, E8, E9	BtuB	OmpF	TolA, B, Q, R	Dnase	
ColE3, E4, E6	BtuB	OmpF	TolA, B, Q, R	rRNase	
ColE1	BtuB	TolC	TolA, Q	Pore-forming	
ColN	LPS	OmpF	TolA, Q, R	Pore-forming	(112)
ColE5	BtuB	OmpF	TolA, B, Q, R	tRNase	
ColK	Tsx	OmpF/OmpA	TolA, B, Q, R	Pore-forming	(113)
ColU	OmpA	OmpF	TolA, B, Q, R	Pore-forming	(114)
Filamentous phages					
Ff (Fd, f1, M13)	F-pilus		TolA, Q, R	<i>E. coli</i>	
IKE	N-pilus		TolA, Q, R	<i>E. coli</i>	(115)
IF1	I-pilus		n.d.	<i>E. coli</i>	
CTX	TCP pilus		TolA, Q, R	<i>V. cholerae</i>	
VGJ	MshA pilus		n.d.	<i>V. cholerae</i>	(116)
Pf1	type IV PAK pilus		n.d.	<i>P. aeruginosa</i>	(117)
Pf3	RP4 pilus		n.d.	<i>P. aeruginosa</i>	(117)

Only references that do not appear in the text are cited in this table. n.d.: not determined.

Figure legends

Figure 1. Colicin and phage minor coat protein organization and crystal structures. (A)

Schematic representation highlighting the similar general organization of colicin and phage pIII proteins for Translocation (T or N1 domains), Reception (R or N2 domains), and Activity or Anchoring (A or C domain), respectively. **(B)** Structures of: full length colicin E3 (top left, PDB: 1JCH) bound to its immunity protein (in green); full length colicin N (top right, PDB: 1A87); M13 phage protein pIII N1-N2 domains (bottom left, PDB: 1G3P); and superposition (bottom right) of *E. coli* TolAIII domain (grey) interacting with the colicin A T-domain on its convex side (co-crystal PDB: 3QDR) and *E. coli* TolAIII domain (grey) interacting with G3P-N1 on its concave side (co-crystal PDB: 1TOL). The color code used for each protein domain is the same for panels A and B.

Figure 2. Models of group A colicin (A) and Tol-dependent filamentous phage import (B)

into sensitive *E. coli* cells. (A) In stage 1, colicin binds to the OM receptor by its central domain (26, 27). In stage 2(a), the disordered N-terminal segment of the T-domain translocates through the OM β -barrel and interacts with a free periplasmic TolB or dissociates TolB from Pal (28-31, 77, 79, 81). In stage 2(b), the N-terminal segment interacts with other Tol proteins (82-85, 95). At this stage, the immunity protein of nuclease colicins is released (108, 109). Then, the unfolded C-terminal domain is thought to cross the OM through the interface between OmpF and the lipid bilayers (118) or directly through the OmpF porin (28). In stage 3, for pore-forming colicins (i) the C-terminal domain inserts spontaneously into the IM and form voltage-gated channels that depolarize and kill the target bacteria (for a review see, 119). For nuclease colicins (ii), the C-terminal domain is cleaved by FtsH (120, 121), an essential ATP-dependent IM protease, and spontaneously crosses the IM (122) or uses FtsH for its transfer (121). **(B)** In stage

1, the phage minor coat protein pIII-N2 domain binds to the tip of a F-pilus protruding from the cell surface (33). In stage 2, pilus retraction pulls the phage into the cell periplasm, possibly through the pilus secretin pore. Once there, the phage pIII-N1 domain interacts with the globular domain of TolA (TolAIII) (86, 87). In *E. coli*, a direct interaction between TolAII and phage pIII-N2 has been reported (dash arrow) (88). The PMF-dependent TolQR motor may trigger conformational changes of TolA that brings the phage particle in close contact with the IM. The phage uncapping process during the uptake stage (3) is speculative. In the model, pIII oligomerizes to form a channel in the IM of the host through its C-ter domain (pIII-C). Then, diffusion of the phage pVIII major coat protein in the IM leads to disassembly of the capsid, releasing the internal pressure of the structure. This force is thought to drive phage DNA injection through the IM pIII-C channel (102, 103).

OM, outer membrane; IM, inner membrane; PG, peptidoglycan; peri, periplasm; cyto, cytoplasm; rec, receptor; trans, translocator. The phage is composed of three to five copies of pIII, but only one copy has been represented, and other minor virion coat proteins have been omitted for simplicity.

A

B

Figure: 2