



HAL
open science

Similarities and Differences between Colicin and Filamentous Phage Uptake by Bacterial Cells

Denis Duché, Laetitia Houot

► **To cite this version:**

Denis Duché, Laetitia Houot. Similarities and Differences between Colicin and Filamentous Phage Uptake by Bacterial Cells. *EcoSal Plus*, 2019, 8 (2), 10.1128/ecosalplus.ESP-0030-2018 . hal-02395622

HAL Id: hal-02395622

<https://hal.science/hal-02395622v1>

Submitted on 31 Jan 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3

4 Denis Duché* and Laetitia Houot*

5

6 From the Laboratoire d'Ingénierie des Systèmes Macromoléculaires, UMR7255, Institut de
7 Microbiologie de la Méditerranée, Aix-Marseille Univ - CNRS, 31 Chemin Joseph Aiguier,
8 13402 Marseille Cedex 20, France

9

10 *To whom correspondence should be addressed: Laboratoire d'Ingénierie des Systèmes
11 Macromoléculaires, UMR7255, Institut de Microbiologie de la Méditerranée, Aix-Marseille
12 Univ - CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France; Tel: +33 491-
13 164-663; Fax: +33 491 712 124; E-mail: duche@imm.cnrs.fr and lhouot@imm.cnrs.fr

14

15

16 **Abstract**

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

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

31

32

33 **Introduction**

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

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

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

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

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

66

67 **Colicins and the Filamentous Phage Adsorption Protein pIII Show Similar Structural** 68 **Organization.**

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

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

78 Filamentous phage infection is driven by three to five copies of the minor coat protein
79 pIII (also called G3P), located at the tip of the particle (2, 15). As for colicins, phage pIII protein
80 is organized into three functional domains, separated by two flexible regions (16, 17) (**Fig. 1A**).
81 The central (N2) domain is responsible for adsorption to a pilus, whereas the N-terminal (N1)
82 domain interacts with the Tol system. The pIII C-terminal domain anchors pIII to the phage
83 particle and is required for DNA injection into the host. The structure of pIII-N1 and pIII-N1-
84 N2 has been solved for the Ff and CTX phages by crystallography and NMR studies (**Fig. 1B**)
85 (16, 18-22)

86

87 **Binding to the Primary Receptor and Crossing of the OM.**

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

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

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

115

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

117 The Tol system is highly conserved among Gram-negative bacteria (40). It consists of
118 five proteins, TolQ, TolR, TolA, TolB, and Pal, which form a complex in the cell envelope.
119 TolQRA are IM proteins that interact via their trans-membrane (TM) segments to form an IM
120 complex (41-44). TolQ is a polytopic protein with three TM segments, whereas TolA and TolR
121 are bitopic proteins with a large periplasmic domain (43, 45-48). TolB is a periplasmic protein
122 composed of two subdomains, called D1 and D2, in which D2 forms a six-bladed β -propeller
123 (49, 50). Finally, Pal is a lipoprotein tethered to the OM via its N-terminal acylated residue, and
124 its C-terminal domain is free to interact with the peptidoglycan (PG) layer and TolB (51-55).

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

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

141

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

143 Various *tol* mutants have been reported to show resistance both to group A colicins and
144 filamentous phages. Indeed, direct or indirect interactions of these particles with components
145 of the Tol complex have been described. When they reach the periplasmic space, most group
146 A colicins first recruit the β -propeller domain of TolB through their N-terminal T domain (50,
147 58, 75-77). Conversely, TolB is not required for phage uptake (39, 78). The affinity of ColE9
148 to TolB has been shown to be strong enough to competitively displace TolB from Pal (77, 79).
149 Structural data has shown that, in the presence of Pal, the N-terminal 12 residues of TolB,

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

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

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

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

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

181

182 **Importance of Protein Structural Flexibility in Uptake Pathways.**

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

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

200

201 **Energetics of Colicin and Filamentous Phage Uptake.**

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

219

220 In summary, it is fascinating to observe that small toxins, such as colicins, and complex
221 viral structures, such as filamentous phages, are shaped so as to use similar molecular
222 mechanisms to pierce the envelope of their target cell. These include a sequential docking
223 mechanism, the strict requirement for the hub protein TolA, and large-scale structural
224 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

229 **Acknowledgements**

230 We thank Roland Lloubes, Laure Journet, and Jean Peuplu for careful reading of the
231 manuscript. Work in the laboratory is supported by the Centre National de la Recherche
232 Scientifique (CNRS), the Aix-Marseille Univ., and funded by the Agence Nationale de la
233 Recherche (MEMOX, ANR-18-CE11-0027)

234

235 **References**

236

237 1. **Cascales E, Buchanan SK, Duché D, Kleanthous C, Llobès R, Postle K, Riley**
238 **M, Slatin S, Cavard D.** 2007. Colicin biology. *Microbiol Mol Biol Rev.* **71**:158-229.

239

240 2. **Rakonjac J, Bennett NJ, Spagnuolo J, Gagic D, Russel M.** 2011. Filamentous
241 bacteriophage: biology, phage display and nanotechnology applications. *Curr Issues*
242 *Mol Biol.* **13**:51-76.

243

244 3. **Waldor MK, Mekalanos JJ.** 1996. Lysogenic conversion by a filamentous phage
245 encoding cholera toxin. *Science.* **272**:1910-4.

246

247 4. **Cascales E, Llobès R, Sturgis JN.** 2001. The TolQ-TolR proteins energize TolA
248 and share homologies with the flagellar motor proteins MotA-MotB. *Mol Microbiol.*
249 **42**:795-807.

250

251 5. **Baty D, Frenette M, Llobès R, Géli V, Howard SP, Pattus F, and Lazdunski C.**
252 1988. Functional domain of Colicin A. *Mol Microbiol.* **2**: 807-11.

253

254 6. **Dankert, JR, Uratani Y, Grabau C, Cramer WA, Hermodson M.** 1982. On a
255 domain structure of colicin E1. A COOH-terminal peptide fragment active in
256 membrane depolarization. *J Biol Chem.* **257**: 3857-63.

257

258 7. **Escuyer V, Mock M.** 1987. DNA sequence analysis of three missense mutations
259 affecting colicin E3 bactericidal activity. *Mol Microbiol.* **13**: 82-5.

260

261 8. **Martinez C, Lazdunski C, Pattus F.** 1983. Isolation, molecular and functional
262 properties of the C-terminal domain of colicin A. *EMBO J.* **2**: 1501-7

263

264 9. **Ohno-Iwashita Y, Imahori K.** 1980. Assignment of the functional loci in colicin E2
265 and E3 molecules by characterization of their proteolytic fragments. *Biochemistry.*
266 **19**: 652-9

267

268 10. **Ohno-Iwashita Y, Imahori K.** 1982. Assignment of the functional loci in the
269 colicin E1 molecule by characterization of its proteolytic fragments. *J Biol Chem.*
270 **257**: 6446-51

271

272 11. **Wiener M, Freymann D, Ghosh P, Stroud RM.** 1997. Crystal structure of colicin
273 Ia. *Nature.* **385**: 461-4.

274

275 12. **Vetter IR, Parker MW, Tucker AD, Lakey JH, Pattus F, Tsernoglou D.** 1988.
276 Crystal structure of a colicin N fragment suggests a model of toxicity. *Structure.* **6**:
277 863-74.

278

279 13. **Soelaiman S, Jakes K, Wu N, Li C, Shoham M.** 2001. Crystal structure of colicin
280 E3: implications for cell entry and ribosome inactivation. *Mol Cell.* **8**: 1053-62.

281

282 14. **Deprez C, Blanchard L, Guerlesquin F, Gavioli M, Simorre JP, Lazdunski C,**
283 **Marion D, Lloubès R.** 2002. Macromolecular import into *Escherichia coli*: the
284 TolA C-terminal domain changes conformation when interacting with the colicin A
285 toxin. *Biochemistry.* **41**:2589-98.

286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310

15. **Grant RA, Lin TC, Konigsberg W, Webster RE.** 1981. Structure of the filamentous bacteriophage fl. Location of the A, C, and D minor coat proteins. *J Biol Chem.* **256**:539-46.
16. **Holliger P, Riechmann L.** 1997. A conserved infection pathway for filamentous bacteriophages is suggested by the structure of the membrane penetration domain of the minor coat protein g3p from phage fd. *Structure.* **5**:265–75.
17. **Heilpern AJ, Waldor MK.** 2003. pIIICTX, a predicted CTXphi minor coat protein, can expand the host range of coliphage fd to include *Vibrio cholerae*. *J Bacteriol.* **185**:1037-44.
18. **Lubkowski J, Hennecke F, Plückthun A, and Wlodawer A.** 1999. Filamentous phage infection: crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA. *Structure.* **7**:711-22.
19. **Lubkowski J, Hennecke F, Plückthun A, Wlodawer A.** 1998. The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. *Nature Struct Biol.* **5**:140 –7.
20. **Holliger P, Riechmann L, Williams RL.** 1999. Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 Å: evidence for conformational lability. *J Mol Biol.* **288**:649-57.

- 311 21. **Lorenz SH, Jakob RP, Weininger U, Balbach J, Dobbek H, Schmid FX.** 2011.
312 The filamentous phages fd and IF1 use different mechanisms to infect *Escherichia*
313 *coli*. *J Mol Biol.* **405**:989-1003.
314
- 315 22. **Ford CG, Kolappan S, Phan HT, Waldor MK, Winther-Larsen HC, Craig L.**
316 2012. Crystal structures of a CTXphi pIII domain unbound and in complex with a
317 *Vibrio cholerae* TolA domain reveal novel interaction interfaces. *J Biol Chem.*
318 **287**:36258-72.
319
- 320 23. **Di Masi, DR, White JC, Schnaitman CA, Bradbeer C.** 1973. Transport of vitamin
321 B12 in *Escherichia coli*: common receptor sites for vitamin B12 and the E colicins
322 on the outer membrane of the cell envelope. *J Bacteriol.* **115**: 506-13.
323
- 324 24. **Cavard, D, Lazdunski C.** 1981. Involvement of BtuB and OmpF proteins in binding
325 and uptake of colicin A. *FEMS Microbiol Lett.* **12**: 311-6.
326
- 327 25. **Chai T, Wu V, Foulds J.** 1982. Colicin A receptor: role of two *Escherichia coli*
328 outer membrane proteins (OmpF protein and *btuB* gene product) and
329 lipopolysaccharide. *J Bacteriol.* **151**: 983-8.
330
- 331 26. **Kurisu G, Zakharov SD, Zhalnina MV, Bano S, Eroukova VY, Rokitskaya TI,**
332 **Antonenko YN, Wiener MC, Cramer WA.** 2003. The structure of BtuB with
333 bound colicin E3 R-domain implies a translocon. *Nat Struct Biol.* **10**:948-54.
334

- 335 27. **Sharma O, Yamashita E, Zhalnina MV, Zakharov SD, Datsenko KA, Wanner**
336 **BL, Cramer WA.** 2007. Structure of the complex of the colicin E2 R-domain and its
337 BtuB receptor. The outer membrane colicin translocon. *J Biol Chem.* **282**:23163-70.
338
- 339 28. **Zakharov, SD, Eroukova VY, Rokitskaya TI, Zhalnina MV, Sharma O, Loll PJ,**
340 **Zgurskaya HI, Antonenko YN, Cramer WA.** 2004. Colicin occlusion of OmpF
341 and TolC channels: outer membrane translocons for colicin import. *Biophys J.* **87**:
342 3901-11.
343
- 344 29. **Yamashita E, Zhalnina MV, Zakharov SD, Sharma O, Cramer WA.** 2008.
345 Crystal structures of the OmpF porin: function in a colicin translocon. *EMBO J.* **27**:
346 2171-80.
347
- 348 30. **Housden, NG, Wojdyla JA, Korczynska J, Grishkovskaya I, Kirkpatrick N,**
349 **Brzozowski AM, Kleanthous C.** 2010. Directed epitope delivery across the
350 *Escherichia coli* outer membrane through the porin OmpF. *Proc Natl Acad Sci U S*
351 *A.* **107**: 21412-7.
352
- 353 31. **Housden NG, Hopper JT, Lukyanova N, Rodriguez-Larrea D, Wojdyla JA,**
354 **Klein A, Kaminska R, Bayley H, Saibil HR, Robinson CV, Kleanthous C.** 2013.
355 Intrinsically disordered protein threads through the bacterial outer-membrane porin
356 OmpF. *Science.* **340**: 1570-4.
357
- 358 32. **Housden NG, Rassam P, Lee S, Samsudin F, Kaminska R, Sharp C, Goult JD,**
359 **Francis ML, Khalid S, Bayley H, Kleanthous C.** 2018. Directional Porin Binding

- 360 of Intrinsically Disordered Protein Sequences Promotes Colicin Epitope Display in
361 the Bacterial Periplasm. *Biochemistry*. **57**:4374-81.
- 362
- 363 33. **Deng LW, Perham RN**. 2002. Delineating the site of interaction on the pIII protein
364 of filamentous bacteriophage fd with the F-pilus of *Escherichia coli*. *J Mol Biol*.
365 **319**:603-14.
- 366
- 367
- 368 34. **Martin A, Schmid FX**. 2003. A proline switch controls folding and domain
369 interactions in the gene-3-protein of the filamentous phage fd. *J Mol Biol*. **331**:1131–
370 40
- 371
- 372 35. **Jacobson A**. 1972. Role of F pili in the penetration of bacteriophage fl. *J Virol*.
373 **10**:835–43.
- 374
- 375 36. **Gao Y, Hauke CA, Marles JM, Taylor RK**. 2016. Effects of tcpB mutations on
376 biogenesis and function of TCP, the Type IVb pilus of *Vibrio cholerae*. *J Bacteriol*.
377 **198**:2818-28.
- 378
- 379 37. **Ng D, Harn T, Altindal T, Kolappan S, Marles JM, Lala R, Spielman I, Gao Y,**
380 **Hauke CA, Kovacikova G, Verjee Z, Taylor RK, Biais N, Craig L**. 2016. The
381 *Vibrio cholerae* Minor Pilin TcpB Initiates Assembly and Retraction of the Toxin-
382 Coregulated Pilus. *PLoS Pathog*. **12**:e1006109.
- 383

- 384 38. **Russel M, Whirlow H, Sun TP, Webster RE.** 1988. Low-frequency infection of F-
385 bacteria by transducing particles of filamentous bacteriophages. *J Bacteriol.*
386 **170**:5312-6.
387
- 388 39. **Heilpern AJ, Waldor MK.** 2000. CTXphi infection of *Vibrio cholerae* requires the
389 tolQRA gene products. *J Bacteriol.* **182**:1739-47.
390
- 391 40. **Sturgis JN.** Organisation and evolution of the *tol-pal* gene cluster. 2001. *J Mol*
392 *Microbiol Biotechnol.* **3**:113-22.
393
- 394 41. **Derouiche R, Bénédetti H, Lazzaroni JC, Lazdunski C, Llobès R.** 1995. Protein
395 complex within *Escherichia coli* inner membrane. TolA N-terminal domain interacts
396 with TolQ and TolR proteins. *J Biol Chem.* **270**: 11078-84.
397
- 398 42. **Germon, P, Clavel T, Vianney A, Portalier R, Lazzaroni JC.** 1998. Mutational
399 analysis of the *Escherichia coli* K-12 TolA N-terminal region and characterization of
400 its TolQ-interacting domain by genetic suppression. *J Bacteriol.* **180**: 6433-9.
401
- 402 43. **Journet L, Rigal A, Lazdunski C, Bénédetti H.** 1999. Role of TolR N-terminal,
403 central, and C-terminal domains in dimerization and interaction with TolA and TolQ.
404 *J Bacteriol.* **181**:4476-84.
405
- 406 44. **Lazzaroni JC, Vianney A, Popot JL, Bénédetti H, Samatey F, Lazdunski C,**
407 **Portalier R, and Géli V.** 1995. Transmembrane alpha-helix interactions are required

408 for the functional assembly of the *Escherichia coli* Tol complex. *J Mol Biol.* **246**: 1-
409 7.
410
411 45. **Bourdineaud JP, Howard SP, Lazdunski C.** 1989. Localization and assembly into
412 the *Escherichia coli* envelope of a protein required for entry of colicin A. *J Bacteriol.*
413 **171**: 2458-65.
414
415 46. **Kampfenkel K, Braun V.** 1993. Membrane topologies of the TolQ and TolR
416 proteins of *Escherichia coli*: inactivation of TolQ by a missense mutation in the
417 proposed first transmembrane segment. *J Bacteriol.* **175**: 4485-91.
418
419 47. **Vianney A, Lewin TM, Beyer WF Jr, Lazzaroni JC, Portalier R, Webster RE.**
420 1994. Membrane topology and mutational analysis of the TolQ protein of
421 *Escherichia coli* required for the uptake of macromolecules and cell envelope
422 integrity. *J Bacteriol.* **176**: 822-9.
423
424 48. **Levengood SK, Beyer WF Jr., Webster RE.** 1991. TolA: a membrane protein
425 involved in colicin uptake contains an extended helical region. *Proc Natl Acad Sci U*
426 *S A.* **88**: 5939-43.
427
428 49. **Abergel C, Bouveret E, Claverie JM, Brown K, Rigal A, Lazdunski C, and**
429 **Bénédicti H.** 1999. Structure of the *Escherichia coli* TolB protein determined by
430 MAD methods at 1.95 Å resolution. *Structure.* **7**: 1291-300.
431

- 432 50. **Carr S, Penfold CN, Bamford V, James R, Hemmings AM.** 2000. The structure
433 of TolB, an essential component of the tol-dependent translocation system, and its
434 protein-protein interaction with the translocation domain of colicin E9. *Structure*.
435 **8:57-66.**
- 436
- 437 51. **Lazzaroni JC, Portalier R.** 1992. The *excC* gene of *Escherichia coli* K-12 required
438 for cell envelope integrity encodes the peptidoglycan-associated lipoprotein (PAL).
439 *Mol Microbiol.* **6:** 735-42.
- 440
- 441 52. **Bouveret E, Bénédicti H, Rigal A, Loret E, Lazdunski C.** 1999. In vitro
442 characterization of peptidoglycan-associated lipoprotein (PAL)-peptidoglycan and
443 PAL-TolB interactions. *J Bacteriol.* **181:** 6306-11.
- 444
- 445 53. **Ray MC, Germon P, Vianney A, Portalier R, Lazzaroni JC.** 2000. Identification
446 by genetic suppression of *Escherichia coli* TolB residues important for TolB-Pal
447 interaction. *J Bacteriol.* **182:** 821-4.
- 448
- 449 54. **Cascales E, Bernadac A, Gavioli M, Lazzaroni JC, Lloubes R.** 2002. Pal
450 lipoprotein of *Escherichia coli* plays a major role in outer membrane integrity. *J*
451 *Bacteriol.* **184:**754-9.
- 452
- 453 55. **Parsons LM, Lin F, Orban J.** 2006. Peptidoglycan recognition by Pal, an outer
454 membrane lipoprotein. *Biochemistry.* **45:** 2122-8.
- 455

- 456 56. **Bouveret E, Derouiche R, Rigal A, Llobès R, Lazdunski C, Bénédicti H.** 1995.
457 Peptidoglycan-associated lipoprotein-TolB interaction. A possible key to explaining
458 the formation of contact sites between the inner and outer membranes of *Escherichia*
459 *coli*. *J Biol Chem.* **270**: 11071-7.
- 460
- 461 57. **Cascales E, Gavioli M, Sturgis JN, Llobès R.** 2000. Proton motive force drives
462 the interaction of the inner membrane TolA and outer membrane Pal proteins in
463 *Escherichia coli*. *Mol Microbiol.* **38**: 904-15.
- 464
- 465 58. **Walburger A, Lazdunski C, Corda Y.** 2002. The Tol/Pal system function requires
466 an interaction between the C-terminal domain of TolA and the N-terminal domain of
467 TolB. *Mol Microbiol.* **44**: 695-708.
- 468
- 469 59. **Dubuisson JF, Vianney A, Lazzaroni JC.** 2002. Mutational analysis of the TolA C-
470 terminal domain of *Escherichia coli* and genetic evidence for an interaction between
471 TolA and TolB. *J Bacteriol.* **184**: 4620-5.
- 472
- 473 60. **Cascales E, Llobès R.** 2004. Deletion analyses of the peptidoglycan-associated
474 lipoprotein Pal reveals three independent binding sequences including a TolA box.
475 *Mol Microbiol.* **51**:873-85.
- 476
- 477 61. **Germon P, Ray MC, Vianney A, Lazzaroni JC.** 2001. Energy-dependent
478 conformational change in the TolA protein of *Escherichia coli* involves its N-
479 terminal domain, TolQ, and TolR. *J Bacteriol.* **183**:4110-4.
- 480

- 481 62. **Yeh YC, Comolli LR, Downing KH, Shapiro L, McAdams HH.** 2010. The
482 caulobacter Tol-Pal complex is essential for outer membrane integrity and the
483 positioning of a polar localization factor. *J Bacteriol.* **192**: 4847-58.
484
- 485 63. **Houot L, Navarro R, Nouailler M, Duché D, Guerlesquin F, Lloubes R.** 2017.
486 Electrostatic interactions between the CTX phage minor coat protein and the
487 bacterial host receptor TolA drive the pathogenic conversion of *Vibrio cholerae*. *J*
488 *Biol Chem.* **292**:13584-98 Erratum in: *J Biol Chem.* 2018, **293**(19):7263
489
- 490 64. **Gaspar JA, Thomas JA, Marolda CL, Valvano MA.** 2000. Surface expression of
491 O-specific lipopolysaccharide in *Escherichia coli* requires the function of the TolA
492 protein. *Mol Microbiol.* **38**:262-75.
493
- 494 65. **Dennis JJ, Lafontaine ER, Sokol PA.** 1996. Identification and characterization of
495 the *tolQRA* genes of *Pseudomonas aeruginosa*. *J Bacteriol.* **178**:7059-68.
496
- 497 66. **Meury J, Devilliers G.** 1999. Impairment of cell division in *tolA* mutants of
498 *Escherichia coli* at low and high medium osmolarities. *Biol Cell.* **91**:67-75.
499
- 500 67. **Bernstein A, Rolfe B, Onodera K.** 1972. Pleiotropic properties and genetic
501 organization of the *tolA,B* locus of *Escherichia coli* K-12. *J Bacteriol.* **112**: 74-83.
502
- 503 68. **Bernadac A, Gavioli M, Lazzaroni JC, Raina S, and Lloubès R.** 1998.
504 *Escherichia coli tol-pal* mutants form outer membrane vesicles. *J Bacteriol.*
505 **180**:4872-8.

506

507 69. **Fognini-Lefebvre N, Lazzaroni JC, Portalier R.** 1987. *tolA*, *tolB* and *excC*, three
508 cistrons involved in the control of pleiotropic release of periplasmic proteins by
509 *Escherichia coli* K12. *Mol Gen Genet.* **209**: 391-5.

510

511 70. **Lazzaroni JC, Portalier RC.** 1981. Genetic and biochemical characterization of
512 periplasmic-leaky mutants of *Escherichia coli* K-12. *J Bacteriol.* **145**: 1351-8.

513

514 71. **Prouty AM, Van Velkinburgh JC, Gunn JS.** 2002. *Salmonella enterica* serovar
515 typhimurium resistance to bile: identification and characterization of the *tolQRA*
516 cluster. *J Bacteriol.* **184**: 1270-6.

517

518 72. **Shrivastava R, Jiang X, Chng SS.** 2017. Outer membrane lipid homeostasis via
519 retrograde phospholipid transport in *Escherichia coli*. *Mol Microbiol.* **106**: 395-408.

520

521 73. **Masilamani R, Cian MB, Dalebroux ZD.** 2018. Salmonella Tol-Pal Reduces Outer
522 Membrane Glycerophospholipid Levels for Envelope Homeostasis and Survival
523 during Bacteremia. *Infect Immun.* **86**:e00173-18.

524

525 74. **Gerding MA, Ogata Y, Pecora ND, Niki H, de Boer PA.** 2007. The trans-envelope
526 Tol-Pal complex is part of the cell division machinery and required for proper outer-
527 membrane invagination during cell constriction in *E. coli*. *Mol Microbiol.* **63**: 1008-
528 25.

529

- 530 75. **Bouveret E, Rigal A, Lazdunski C, Bénédicti H.** 1997. The N-terminal domain of
531 colicin E3 interacts with TolB which is involved in the colicin translocation step.
532 *Mol Microbiol.* **23**:909-20.
533
- 534 76. **Bouveret E, Rigal A, Lazdunski C, Bénédicti H.** 1998. Distinct regions of the
535 colicin A translocation domain are involved in the interaction with TolA and TolB
536 proteins upon import into *Escherichia coli*. *Mol Microbiol.* **27**:143-57.
537
- 538 77. **Loftus SR, Walker D, Maté MJ, Bonsor DA, James R, Moore GR, Kleanthous**
539 **C.** 2006. Competitive recruitment of the periplasmic translocation portal TolB by a
540 natively disordered domain of colicin E9. *Proc Natl Acad Sci U S A.* **103**:12353-8.
541
- 542 78. **Sun TP, Webster RE.** 1986. fii, a bacterial locus required for filamentous phage
543 infection and its relation to colicin-tolerant tolA and tolB. *J Bacteriol.* **165**:107-15.
544
- 545 79. **Bonsor DA, Grishkovskaya I, Dodson EJ, Kleanthous C.** 2007. Molecular
546 mimicry enables competitive recruitment by a natively disordered protein. *J Am*
547 *Chem Soc.* **129** :4800-7.
548
- 549 80. **Bonsor DA, Hecht O, Vankemmelbeke M, Sharma A, Krachler AM, Housden**
550 **NG, Lilly KJ, James R, Moore GR, Kleanthous C.** 2009. Allosteric beta-propeller
551 signalling in TolB and its manipulation by translocating colicins. *EMBO J.* **28**:2846-
552 57. Erratum in: *EMBO J.* **28**: 2858.
553

- 554 81. **Zhang Y, Li C, Vankemmelbeke MN, Bardelang P, Paoli M, Penfold CN, James**
555 **R.** 2010. The crystal structure of the TolB box of colicin A in complex with TolB
556 reveals important differences in the recruitment of the common TolB translocation
557 portal used by group A colicins. *Mol Microbiol.* **75**:623-36.
- 558
- 559 82. **Bénédicti H, Lazdunski C, and Llobès R.** 1991. Protein import into *Escherichia*
560 *coli*: colicins A and E1 interact with a component of their translocation system.
561 *EMBO J.* **10**:1989-95.
- 562
- 563 83. **Derouiche R, Zeder-Lutz G, Bénédicti H, Gavioli M, Rigal A, Lazdunski C,**
564 **Llobès R.** 1997. Binding of colicins A and E1 to purified TolA domains.
565 *Microbiology.* **143**:3185-92.
- 566
- 567 84. **Raggett EM, Bainbridge G, Evans LJ, Cooper A, Lakey JH.** 1998. Discovery of
568 critical Tol A-binding residues in the bactericidal toxin colicin N: a biophysical
569 approach. *Mol Microbiol.* **28**:1335-43.
- 570
- 571 85. **Barnéoud-Arnoulet A, Gavioli M, Llobès R, and Cascales E.** 2010. Interaction
572 of the colicin K bactericidal toxin with components of its import machinery in the
573 periplasm of *Escherichia coli*. *J Bacteriol.* **192**:5934-42.
- 574
- 575 86. **Click EM, Webster RE.** 1997. Filamentous phage infection: required interactions
576 with the TolA protein. *J Bacteriol.* **179**:6464-71.
- 577
- 578 87. **Riechmann L, Holliger P.** 1997. The C-terminal domain of TolA is the coreceptor

- 579 for filamentous phage infection of *E. coli*. *Cell*. **90**:351–60.
- 580
- 581 88. **Karlsson F, Borrebaeck CA, Nilsson N, Malmberg-Hager AC**. 2003. The
582 mechanism of bacterial infection by filamentous phages involves molecular
583 interactions between TolA and phage protein 3 domains. *J Bacteriol*. **185**:2628-34.
- 584
- 585 89. **Schendel SL, Click EM, Webster RE, Cramer WA**. 1997. The TolA protein
586 interacts with colicin E1 differently than with other group A colicins. *J Bacteriol*.
587 **179**:3683-90.
- 588
- 589 90. **Anderluh G, Gökçe I, and Lakey JH**. 2004. A natively unfolded toxin domain uses
590 its receptor as a folding template. *J Biol Chem*. **279**:22002-9.
- 591
- 592 91. **Li C, Zhang Y, Vankemmelbeke M, Hecht O, Aleanizy FS, Macdonald C,**
593 **Moore G.R, James R, Penfold CN**. 2012. Structural evidence that colicin A protein
594 binds to a novel binding site of TolA protein in Escherichia coli periplasm. *J Biol*
595 *Chem*. **287**:19048-57.
- 596
- 597 92. **Hecht O, Ridley H, Lakey JH, Moore GR**. 2009. A common interaction for the
598 entry of colicin N and filamentous phage into Escherichia coli. *J Mol Biol*. **388**: 880-
599 93.
- 600
- 601 93. **Ridley H, Lakey JH**. 2015. Antibacterial toxin colicin N and phage protein G3p
602 compete with TolB for a binding site on TolA. *Microbiology*. 161: 503-15.

- 603 94. **Sun TP, Webster RE.** 1987. Nucleotide sequence of a gene cluster involved in entry
604 of colicins and single-stranded DNA of infecting filamentous bacteriophages into
605 *Escherichia coli*. *J. Bacteriol.* **169**: 2667–74.
606
- 607 95. **Journet L, Bouveret E, Rigal A, Lloubes R, Lazdunski C, Bénédicti H.** 2001.
608 Import of colicins across the outer membrane of *Escherichia coli* involves multiple
609 protein interactions in the periplasm. *Mol Microbiol.* **42**:331-44.
610
- 611 96. **Duché D, Letellier L, Géli V, Bénédicti H, Baty D.** 1995. Quantification of group
612 A colicin import sites. *J Bacteriol.* **177**:4935-9.
613
- 614 97. **Duché D.** 2007. Colicin E2 is still in contact with its receptor and import machinery
615 when its nuclease domain enters the cytoplasm. *J Bacteriol.* **189**:4217-22.
616
- 617 98. **Bénédicti H, Lloubès R, Lazdunski C, Letellier L.** 1992. Colicin A unfolds during
618 its translocation in *Escherichia coli* cells and spans the whole cell envelope when its
619 pore has formed. *EMBO J.* **11**:441-7.
620
- 621 99. **Duché D, Baty D, Chartier M, Letellier L.** 1994. Unfolding of colicin A during its
622 translocation through the *Escherichia coli* envelope as demonstrated by disulfide
623 bond engineering. *J Biol Chem.* **269**:24820-5.
624
- 625 100. **Griko YV, Zakharov SD, Cramer WA.** 2000. Structural stability and domain
626 organization of colicin E1. *J Mol Biol.* **302**:941-53.
627

- 628 101. **Housden NG, Loftus SR, Moore GR, James R, Kleanthous C.** 2005. Cell entry
629 mechanism of enzymatic bacterial colicins: porin recruitment and the
630 thermodynamics of receptor binding. *Proc Natl Acad Sci U S A.* **102**:13849-54.
631
- 632 102. **Bennett NJ, Rakonjac J.** 2006. Unlocking of the filamentous bacteriophage virion
633 during infection is mediated by the C domain of pIII. *J Mol Biol.* **356**:266–73.
634
- 635 103. **Glaser-Wuttke, G, Keppner J, Rasched I.** 1989. Pore-forming properties of the
636 adsorption protein of filamentous phage fd. *Biochim Biophys. Acta* **985**:239–47
637
- 638 104. **Braun V, Frenz J, Hantke K, Schaller K.** 1980. Penetration of colicin M into cells
639 of *Escherichia coli*. *J Bacteriol.* **142**:162-8.
640
- 641 105. **Bourdineaud JP, Boulanger P, Lazdunski C, Letellier L.** 1990. In vivo properties
642 of colicin A: channel activity is voltage dependent but translocation may be voltage
643 independent. *Proc Natl Acad Sci U S A.* **87**:1037-41.
644
- 645 106. **Goemaere EL, Cascales E, Lloubès R.** 2007. Mutational analyses define helix
646 organization and key residues of a bacterial membrane energy-transducing complex.
647 *J Mol Biol.* **366**:1424-36.
648
- 649 107. **Lloubès R, Goemaere E, Zhang X, Cascales E, Duché D.** 2012. Energetics of
650 colicin import revealed by genetic cross-complementation between the Tol and Ton
651 systems. *Biochem Soc Trans.* **40**:1480-5.
652

- 653 108. **Duché D, Frenkian A, Prima V, Lloubès R.** 2006. Release of immunity protein
654 requires functional endonuclease colicin import machinery. *J Bacteriol.* **188**:8593-
655 600.
- 656
- 657 109. **Vankemmelbeke M, Zhang Y, Moore GR, Kleanthous C, Penfold CN, James R.**
658 2009. Energy-dependent immunity protein release during tol-dependent nuclease
659 colicin translocation. *J Biol Chem.* **284**:18932-41.
- 660
- 661 110. **Yamamoto M, Kanegasaki S, Yoshikawa M.** 1981. Role of membrane potential
662 and ATP in complex formation between Escherichia coli male cells and filamentous
663 phage fd. *J Gen Microbiol.* **123**:343-9.
- 664
- 665 111. **Häse CC.** 2003. Ion motive force dependence of protease secretion and phage
666 transduction in *Vibrio cholerae* and *Pseudomonas aeruginosa*. *FEMS Microbiol Lett.*
667 **227**:65-71
- 668
- 669 112. **Johnson CL, Ridley H, Marchetti R, Silipo A, Griffin DC, Crawford L, Bonev**
670 **B, Molinaro A, Lakey JH.** 2014. The antibacterial toxin colicin N binds to the
671 inner core of lipopolysaccharide and close to its translocator protein. *Mol Microbiol.*
672 **92**: 440-52.
- 673
- 674 113. **Bradley DE, Howard SP.** 1992. A new colicin that adsorbs to outer-membrane
675 protein Tsx but is dependent on the tonB instead of the tolQ membrane transport
676 system. *J Gen Microbiol.* **138**: 2721-4.
- 677

- 678 114. **Smajs D, Pils H, Braun V.** 1997. Colicin U, a novel colicin produced by *Shigella*
679 *boydii*. *J Bacteriol.* **179**: 4919-28.
- 680
- 681 115. **Jakob RP, Geitner AJ, Weininger U, Balbach J, Dobbek H, Schmid FX.** 2012.
682 Structural and energetic basis of infection by the filamentous bacteriophage IKE. *Mol*
683 *Microbiol.* **84**:1124-38.
- 684
- 685 116. **Campos J, Martínez E, Suzarte E, Rodríguez BL, Marrero K, Silva Y, Ledón T,**
686 **del Sol R, Fando R.** 2003. VGJ phi, a novel filamentous phage of *Vibrio cholerae*,
687 integrates into the same chromosomal site as CTX phi. *J Bacteriol.* **185**:5685-96.
- 688
- 689 117. **Holland SJ, Sanz C, Perham RN.** 2006. Identification and specificity of pilus
690 adsorption proteins of filamentous bacteriophages infecting *Pseudomonas*
691 *aeruginosa*. *Virology.* **345**:540-8
- 692
- 693 118. **Baboolal TG, Conroy MJ, Gill K, Ridley H, Visudtiphole V, Bullough PA,**
694 **Lakey JH.** 2008. Colicin N binds to the periphery of its receptor and translocator,
695 outer membrane protein F. *Structure.* 2008 **16**: 371-9.
- 696
- 697 119. **Lakey JH, Slatin SL.** 2001. Pore-forming colicins and their relatives. *Curr Top*
698 *Microbiol Immunol.* **257**: 131-61.
- 699
- 700 120. **Walker D, Mosbahi K, Vankemmelbeke M, James R, Kleanthous C.** 2007. The
701 role of electrostatics in colicin nuclease domain translocation into bacterial cells. *J*
702 *Biol Chem.* **282**:31389-97

703

704 121. **Chauleau M, Mora L, Serba J, de Zamaroczy M.** 2011. FtsH-dependent
705 processing of RNase colicins D and E3 means that only the cytotoxic domains are
706 imported into the cytoplasm. *J Biol Chem.* **286**: 29397-407.

707

708 122. **Mosbahi K, Lemaître C, Keeble AH, Mobasher H, Morel B, James R, Moore**
709 **GR, Lea EJ, Kleanthous C.** 2002. The cytotoxic domain of colicin E9 is a channel-
710 forming endonuclease. *Nat Struct Biol.* **9**:476-84.

711

712

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

714

715	Name	Receptor	Translocator	Uptake Proteins	Cytotoxicity/Host	Ref.
-----	------	----------	--------------	-----------------	-------------------	------

717

718 **Group A colicins**

719

720	ColA	BtuB	OmpF	TolA, B, Q, R	Pore-forming	
721	ColE2, E7, E8, E9	BtuB	OmpF	TolA, B, Q, R	Dnase	
722	ColE3, E4, E6	BtuB	OmpF	TolA, B, Q, R	rRNase	
723	ColE1	BtuB	TolC	TolA, Q	Pore-forming	
724	ColN	LPS	OmpF	TolA, Q, R	Pore-forming	(112)
725	ColE5	BtuB	OmpF	TolA, B, Q, R	tRNase	
726	ColK	Tsx	OmpF/OmpA	TolA, B, Q, R	Pore-forming	(113)
727	ColU	OmpA	OmpF	TolA, B, Q, R	Pore-forming	(114)

728

729 **Filamentous phages**

730	Ff (Fd, f1, M13)	F-pilus		TolA, Q, R	<i>E. coli</i>	
731	IKE	N-pilus		TolA, Q, R	<i>E. coli</i>	(115)
732	IF1	I-pilus		n.d.	<i>E. coli</i>	
733	CTX	TCP pilus		TolA, Q, R	<i>V. cholerae</i>	
734	VGJ	MshA pilus		n.d.	<i>V. cholerae</i>	(116)
735	Pf1	type IV PAK pilus		n.d.	<i>P. aeruginosa</i>	(117)
736	Pf3	RP4 pilus		n.d.	<i>P. aeruginosa</i>	(117)

737

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

739

740 **Figure legends**

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

742 Schematic representation highlighting the similar general organization of colicin and phage pIII
743 proteins for Translocation (T or N1 domains), Reception (R or N2 domains), and Activity or
744 Anchoring (A or C domain), respectively. **(B)** Structures of: full length colicin E3 (top left,
745 PDB: 1JCH) bound to its immunity protein (in green); full length colicin N (top right, PDB:
746 1A87); M13 phage protein pIII N1-N2 domains (bottom left, PDB: 1G3P); and superposition
747 (bottom right) of *E. coli* TolAIII domain (grey) interacting with the colicin A T-domain on its
748 convex side (co-crystal PDB: 3QDR) and *E. coli* TolAIII domain (grey) interacting with G3P-
749 N1 on its concave side (co-crystal PDB: 1TOL). The color code used for each protein domain
750 is the same for panels A and B.

751

752

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

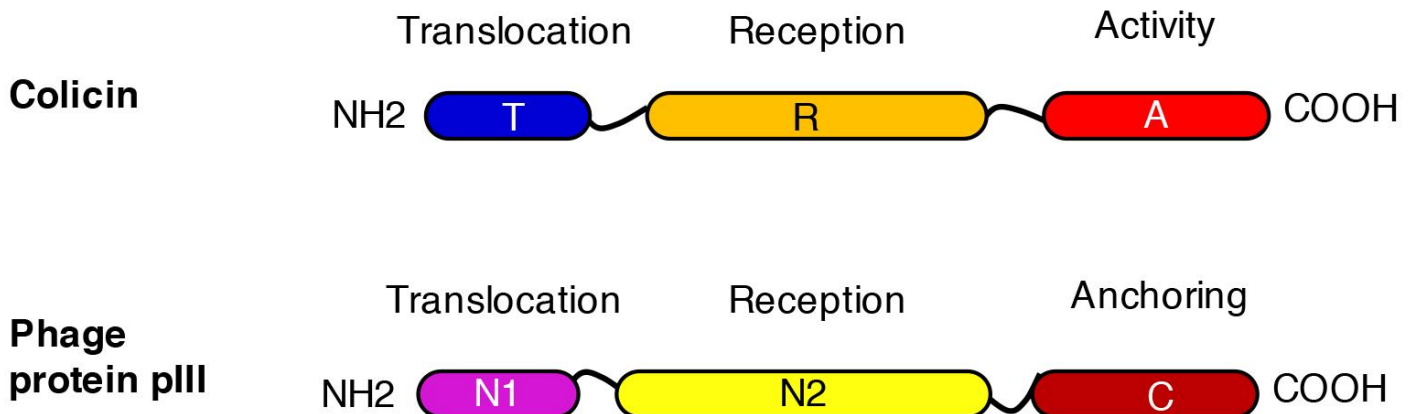
754 **into sensitive *E. coli* cells. (A)** In stage 1, colicin binds to the OM receptor by its central domain
755 (26, 27). In stage 2(a), the disordered N-terminal segment of the T-domain translocates through
756 the OM β -barrel and interacts with a free periplasmic TolB or dissociates TolB from Pal (28-
757 31, 77, 79, 81). In stage 2(b), the N-terminal segment interacts with other Tol proteins (82-85,
758 95). At this stage, the immunity protein of nuclease colicins is released (108, 109). Then, the
759 unfolded C-terminal domain is thought to cross the OM through the interface between OmpF
760 and the lipid bilayers (118) or directly through the OmpF porin (28). In stage 3, for pore-forming
761 colicins (i) the C-terminal domain inserts spontaneously into the IM and form voltage-gated
762 channels that depolarize and kill the target bacteria (for a review see, 119). For nuclease colicins
763 (ii), the C-terminal domain is cleaved by FtsH (120, 121), an essential ATP-dependent IM
764 protease, and spontaneously crosses the IM (122) or uses FtsH for its transfer (121). **(B)** In stage

765 1, the phage minor coat protein pIII-N2 domain binds to the tip of a F-pilus protruding from the
766 cell surface (33). In stage 2, pilus retraction pulls the phage into the cell periplasm, possibly
767 through the pilus secretin pore. Once there, the phage pIII-N1 domain interacts with the
768 globular domain of TolA (TolAIII) (86, 87). In *E. coli*, a direct interaction between TolAII and
769 phage pIII-N2 has been reported (dash arrow) (88). The PMF-dependent TolQR motor may
770 trigger conformational changes of TolA that brings the phage particle in close contact with the
771 IM. The phage uncapping process during the uptake stage (3) is speculative. In the model, pIII
772 oligomerizes to form a channel in the IM of the host through its C-ter domain (pIII-C). Then,
773 diffusion of the phage pVIII major coat protein in the IM leads to disassembly of the capsid,
774 releasing the internal pressure of the structure. This force is thought to drive phage DNA
775 injection through the IM pIII-C channel (102, 103).

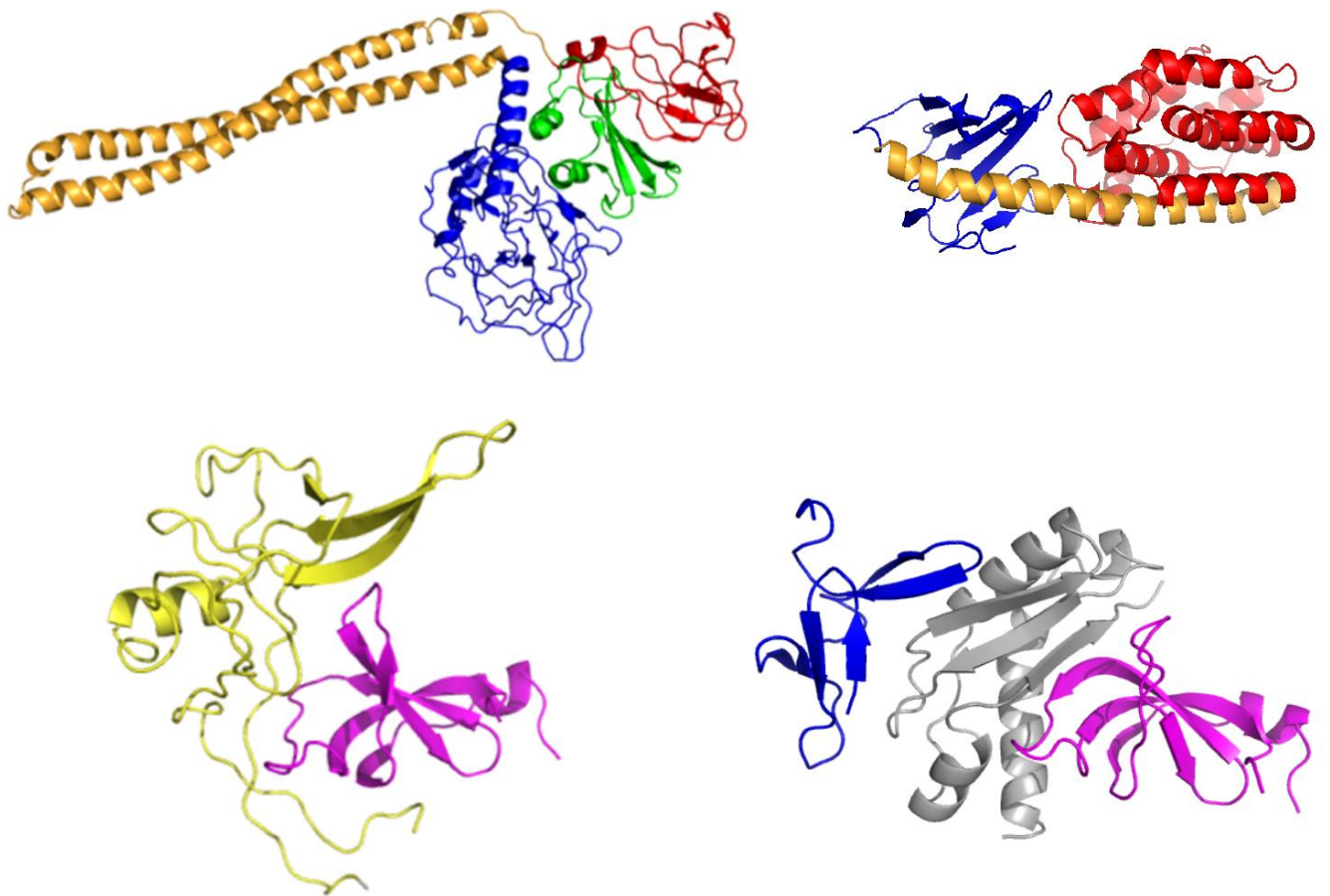
776 OM, outer membrane; IM, inner membrane; PG, peptidoglycan; peri, periplasm; cyto,
777 cytoplasm; rec, receptor; trans, translocator. The phage is composed of three to five copies of
778 pIII, but only one copy has been represented, and other minor virion coat proteins have been
779 omitted for simplicity.

780

A



B



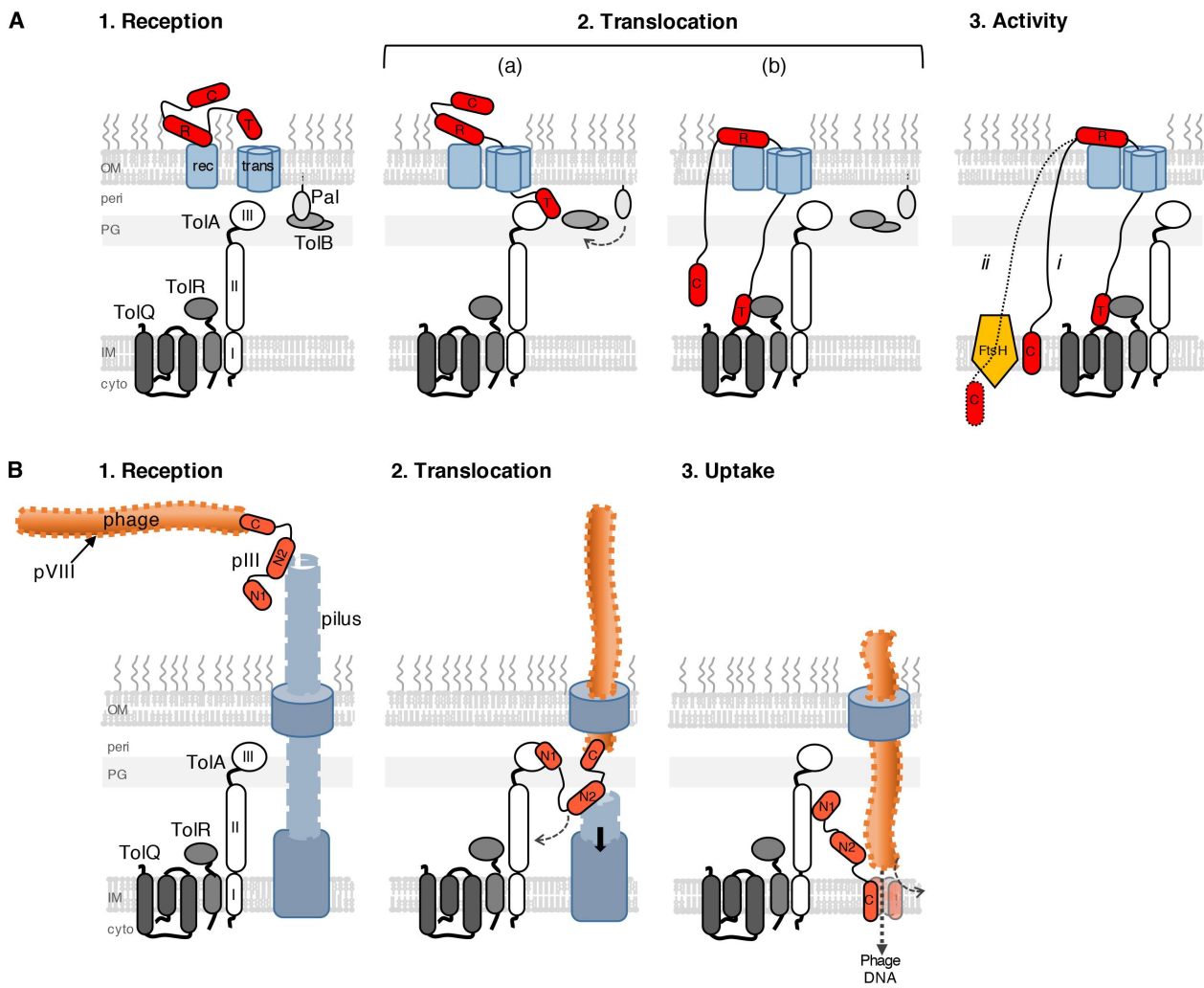


Figure: 2