Published in final edited form as:

Mol Microbiol. 2014 May ; 92(3): 435–439. doi:10.1111/mmi.12569.

DARING TO BE DIFFERENT: COLICIN N FINDS ANOTHER WAY

Karen S. Jakes

Albert Einstein College of Medicine, Department of Physiology and Biophysics, 1300 Morris Park Avenue, Bronx, NY 10461, USA

Summary

The mechanisms by which colicins, protein toxins produced by *Escherichia coli*, kill other *E. coli*, have become much better understood in recent years. Most colicins initially bind to an outer membrane protein receptor, and then search for a separate nearby outer membrane protein translocator that serves as a pathway into target cells. Many colicins use the outer membrane porin, OmpF, as that translocator, while using a different primary receptor. Colicin N is unique among known colicins in that only OmpF had been identified as being required for uptake of the colicin and it was presumed to somehow serve as both receptor and translocator. Genetic screens also identified a number of genes required for lipopolysaccharide (LPS) synthesis as uniquely required for killing by colicin N, but not by other colicins. In this issue of *Molecular Microbiology*, Johnson *et al.* show that the receptor-binding domain of colicin N binds to LPS, and does not require OmpF for that binding. LPS of a minimal length is required for binding, explaining the requirement for specific elements of the LPS biosynthetic pathway. For colicin N, the receptor-binding domain does not recognize a protein, but rather the most abundant component of the outer membrane itself, LPS.

Keywords

colicin; lipopolysaccharide; membrane translocation; receptors; porin

Escherichia coli bacteria live in an environment of constant competition for nutrients with other closely related bacteria. In order to eliminate some of that competition, they make plasmid-encoded protein toxins called colicins, which kill related bacteria by disrupting some basic function—degrading their DNA or RNA, collapsing their inner membrane potential by making voltage-dependent channels, or degrading peptidoglycan in the membrane (Cascales *et al.*, 2007). The dozens of known colicins are remarkably similar in most respects and share a common domain structure, exemplified in Figure 1A by colicin Ia. Binding to an outer membrane receptor is carried out by the central part of the molecule (receptor-binding or R domain). The outer membrane receptors to which colicin R domains bind are, for the most part, plugged β -barrel transporters, such as FepA, FhuA, Cir, or BtuB, whose normal role in the life of *E. coli* is to bind essential nutrients such as siderophore-bound iron or vitamins. Translocation across the outer membrane is then mediated by the N-terminal portion of the molecule (T domain); for all of the complete colicin molecules

whose 3-D structures have been solved, a significant part of the T domain is flexible and unstructured and therefore not seen in the crystal structure (Wiener *et al.*, 1997; Vetter *et al.*, 1998; Soelaiman *et al.*, 2001; Hilsenbeck *et al.*, 2004; Zeth *et al.*, 2008). The killing activity—enzymatic or pore-forming (P) domain—is at the C-terminal end of the molecule. In addition to cannibalizing nutrient receptors to attach to sensitive bacteria, the colicins also use either of two groups of inner and periplasmic proteins, TolA, B, Q, R or TonB, ExbB, ExbD, to provide the necessary energy to move the colicin across the outer membrane (Jakes & Cramer, 2012). There is an essential step between the initial binding of the colicin to the outer membrane and its engagement, via its T domain, with either the Tol or Ton machinery in the periplasm that involves a second, usually different, outer membrane protein, whose role is that of a translocator, to act as a passageway across the outer membrane. For most of the Tol-dependent colicins, such as the enzymatic E colicins E2–E9 and the channel-forming colicin A, the translocator is the trimeric porin, OmpF (Cavard & Lazdunski, 1981; Bénédicti *et al.*, 1989). A model for how the colicin accesses its target inside the *E. coli* cell was first proposed for colicin E3 (Kurusu *et al.*, 2003). The colicin first binds, via the tip of its extended (~100 Å) coiled-coil R domain, to its outer membrane receptor, BtuB, the plugged β -barrel vitamin B₁₂ receptor. The position of the bound colicin, extending like a fishing rod over the bacterial surface, allows the unstructured N-terminal T domain to search for a nearby copy of the ubiquitous [$\sim 10^5$ copies/cell (Rosenbusch, 1974)] OmpF trimer. Two binding sites for OmpF within the T domain, OBS1 and OBS2, have been elegantly characterized by Kleanthous and colleagues for the closely-related colicin E9 (Housden *et al.*, 2005; Housden *et al.*, 2010; Housden *et al.*, 2013), and colicin E3 or its T domain and the E9 OBS1 peptide have been shown to occlude OmpF channels *in vitro*, in planar lipid bilayer membranes (Zakharov *et al.*, 2004; Housden *et al.*, 2013). In fact, short segments of both the E3 and E9 unstructured T domains have been co-crystallized within the aqueous channel of OmpF (Yamashita *et al.*, 2008; Housden *et al.*, 2010). Although no heterologous translocator has been identified for any TonB-dependent colicin (*e.g.*, colicins Ia and Ib, B, or M), a similar binding and translocation mechanism has been proposed for colicin Ia, which uses one copy of its receptor, Cir, for initial binding via its R domain, and then recruits a second copy of the same protein to initiate translocation via its T domain (Jakes & Finkelstein, 2010).

Colicin N has always seemed to be different from other colicins: unlike the other members of the family of Tol-dependent colicins, only OmpF had been genetically identified as its receptor and translocator, instead of a pair such as BtuB and OmpF. It is significantly smaller than other pore-forming colicins (~40 kDa instead of ~60 kDa) (Pugsley, 1987), although its pore-forming domain is very similar to other pore-forming colicins. Instead, the difference from other colicins lies in the length and structure of its R and T domains (Vetter *et al.*, 1998). It lacks the long R domain that allows the E colicins and colicin Ia to search for a remote translocator protein (Fig 1). Another notable difference between colicin N and many other colicins was revealed by an exhaustive genetic screen by Sharma *et al.* (Sharma *et al.*, 2009). A genome-wide screen, using the Keio collection of *E. coli* single-gene knockout mutations, identified several gene products uniquely required for killing by colicin N, but not by the other colicins screened in the study. All of those gene products are

involved in the biosynthesis of lipopolysaccharide (LPS), specifically in the extension of the polysaccharide chain to form the inner and outer core LPS (Fig. 2).

Now Johnson *et al.* in this issue (2014) reconcile a number of the unusual aspects of colicin N in a way that is both satisfying and suggestive of a unique mechanism by which colicin N could bind to target *E. coli* and translocate across the outer membrane. Using a variety of biophysical methods, they show that the receptor-binding domain of colicin N binds LPS even in the absence of OmpF. Thus, unlike any other colicin, colicin N's R domain does not target a protein receptor, but rather, the principal component of the outer membrane itself, which is present at $\sim 10^6$ molecules/cell (Raetz & Whitfield, 2002). Using whole colicin N or purified R or TR domains bound to chips, they used surface plasmon resonance to demonstrate R domain-dependent binding to Rc LPS, but not to the slightly shorter Rd LPS (Fig. 2), implying a requirement for a minimum length of the sugar chain on LPS. They further showed that no major rearrangement of the R domain was required for the binding, since a disulphide-locked R domain bound virtually as well as the reduced version of the protein. In fact, although the binding was weaker, colicin N's R domain could even be shown, by isothermal calorimetry (ITC), to bind delipidated LPS Rc, but not Rd, so it is primarily the sugar moieties of LPS to which the colicin domain is binding. Delipidated Rc and Rd LPS were also used for saturation transfer difference (STD) NMR experiments that suggest that the terminal glucose and heptose (heptose III) (Fig. 2) are essential for binding of R domain. Rd LPS, which is missing those terminal residues, gave no STD signal. Phosphorylation and pyrophosphorylation sites on the terminal sugars in Rc may also be involved in the recognition of Rc LPS by R domain. Thus, sugar and sugar phosphate moieties extending away from the outer membrane surface are serving as the binding sites for the colicin to attach to target bacteria, a scenario far different from that known for other colicins.

The discovery that the R domain actually binds LPS could explain some previously confusing results. ITC experiments measuring binding of colicin N or its domains to OmpF, *in vitro*, had shown that while whole colicin had a dissociation constant of $\sim 2 \mu\text{M}$, the K_d for both R and R-P domains was ~ 50 -fold weaker (Evans *et al.*, 1996a; Evans *et al.*, 1996b). Thus there was biophysical evidence that much of the affinity of colicin N for OmpF resided in the N-terminal T domain, which was absent from those two constructs. The magnitude of that affinity is more comparable to that measured for other colicins' T domains to their translocators than the much tighter binding ($\sim 1 \text{ nM } K_d$) of their R domains to protein outer membrane receptors (Buchanan *et al.*, 2007; Housden *et al.*, 2010). However, previous experiments that also imply a direct interaction of R domain with OmpF, both *in vivo* and *in vitro* (Evans *et al.*, 1996a; Evans *et al.*, 1996b; Stora *et al.*, 1999), will require further work to understand that interaction. Some ambiguity may result from differences between the lengths of the domain constructs used in those earlier studies and this one, whether the constructs had histidine tags, and whether those were at the N- or C-termini of the expressed domains. Hopefully, future experiments will identify binding sites for LPS in the R domain and answer the question whether there are also R domain binding sites on OmpF.

Earlier work from the Lakey group seemed to show colicin N bound in the cleft between OmpF monomers of the trimeric protein, in 2D crystals (Baboolal *et al.*, 2008), rather than

on the external opening of its OmpF receptor, where other colicins have been shown to bind their specific receptors [reviewed in Jakes and Cramer (2012)]. There was evidence from that work and neutron-scattering experiments (Clifton *et al.*, 2012) that the colicin or a construct containing the receptor and pore-forming domains may displace LPS from OmpF trimers, since LPS was present in the purified OmpF used for those studies. Models for the binding and translocation of colicin N were proposed as a result of those experiments that would have the colicin bound in the outer membrane adjacent to OmpF and somehow sliding down the outside of the OmpF barrels. While such a model was consistent with the structural data from those experiments, it could not explain earlier experiments with mutants of OmpF. In particular, mutant OmpF G119N is resistant to colicin N (Fourel *et al.*, 1993). The x-ray crystal structure of the mutant protein revealed that the mutated residue protrudes into the porin channel, dividing it into compartments of only 3–4 Å diameter and therefore strongly implying that the colicin or some part of it must either bind within or actually travel through the wild-type channel for normal activity (Jeanteur *et al.*, 1994).

An intriguing model for how colicin N reaches its target at the inner membrane of *E. coli* is suggested by the new results of Johnson *et al.* (2014) (Fig. 3). The colicin's R domain inserts into the "field" of LPS on the surface of the outer membrane, perhaps attracted to specific sites (not yet identified) in LPS adjacent to OmpF, in space made in the membrane by that protein. Since the LPS sugar and phosphate residues that appear to mediate binding are further from the surface of the membrane, the rest of the colicin molecule—its T and P domains—stick up in this model, possibly protruding somewhat above the ends of the LPS. This arrangement would position the unstructured N-terminal T domain to search for a nearby opening in one of the $\sim 10^5$ copies of OmpF. As has been shown to occur for the T domains of colicin E3 and E9 and suggested by the colicin N resistance of OmpF mutant G119N (Fourel *et al.*, 1993; Housden *et al.*, 2005; Yamashita *et al.*, 2008; Housden *et al.*, 2010; Housden *et al.*, 2013), the T domain could then thread into one of the three pores of the trimeric OmpF, using the recently-identified OBS of colicin N to bind within the pore (Johnson *et al.*, 2013). Once the unfolded T domain has threaded through a pore of OmpF, presumably via some sort of pushing/pulling mechanism (Housden *et al.*, 2010; Thoren & Krantz, 2011), the TolA binding site on the T domain (Raggett *et al.*, 1998) would be exposed in the periplasm. It could then bind TolA to further energize the unfolding and import of the rest of the colicin molecule, although, as for other colicins, the source of that energy remains something of a mystery (Jakes & Cramer, 2012). An alternative pathway down the outside of the OmpF trimer is suggested by 2D crystallization and neutron scattering experiments that show displacement of LPS from OmpF by the pore domain and binding of that domain in the cleft adjacent to OmpF (Baboolal *et al.*, 2008; Clifton *et al.*, 2012). That model cannot be ruled out at this time and is also shown in Fig. 3. Resolving whether the colicin ultimately travels through the porin, as do other colicins, or down its outside remains to be answered. Whatever the ultimate OmpF pathway and whatever the engine to pull the pore-forming domain through the outer membrane, colicin N has proven to be unique in adapting to use the most ubiquitous component of the *E. coli* outer membrane as its primary binding site and may yet prove to use its OmpF translocator in a totally unique way, as well.

Acknowledgments

I thank Alan Finkelstein for helpful comments on this MicroCommentary, and Onkar Sharma and Peter Jakes for invaluable help preparing the figures. This work was supported by R01AI091633 from the National Institute of Allergy and Infectious Diseases.

References

- Baboolal TG, Conroy MJ, Gill K, Ridley H, Visudtiphole V, Bullough PA, Lakey JH. Colicin N binds to the periphery of its receptor and translocator, outer membrane protein F. *Structure*. 2008; 16:371–379. [PubMed: 18334212]
- Bénédicti H, Frenette M, Baty D, Llobès R, Géli V, Lazdunski C. Comparison of the uptake systems for the entry of various BtuB group colicins into *Escherichia coli*. *J Gen Microbiol*. 1989; 135:3413–3420. [PubMed: 2699893]
- Buchanan SK, Lukacik P, Grizot S, Ghirlando R, Ali MMU, Barnard TJ, Jakes KS, Kienker PK, Esser L. Structure of colicin I receptor bound to the R-domain of colicin Ia: implications for protein import. *EMBO J*. 2007; 26:2594–2604. [PubMed: 17464289]
- Cascales E, Buchanan SK, Duché D, Kleanthous C, Llobès R, Postle K, Riley M, Slatin S, Cavard D. Colicin biology. *Microbiol Mol Biol Rev*. 2007; 71:158–229. [PubMed: 17347522]
- Cavard D, Lazdunski C. Involvement of BtuB and OmpF proteins in binding and uptake of colicin A. *FEMS Microbiol Lett*. 1981; 12:311–316.
- Clifton LA, Johnson CL, Solovyova AS, Callow P, Weiss KL, Ridley H, Le Brun AP, Kinane CJ, Webster JR, Holt SA, Lakey JH. Low resolution structure and dynamics of a colicin-receptor complex determined by neutron scattering. *J Biol Chem*. 2012; 287:337–346. [PubMed: 22081604]
- Evans LJ, Cooper A, Lakey JH. Direct measurement of the association of a protein with a family of membrane receptors. *J Mol Biol*. 1996a; 255:559–563. [PubMed: 8568897]
- Evans LJ, Labeit S, Cooper A, Bond LH, Lakey JH. The central domain of colicin N possesses the receptor recognition site but not the binding affinity of the whole toxin. *Biochemistry*. 1996b; 35:15143–15148. [PubMed: 8952461]
- Fourel D, Mizushima S, Bernadac A, Pages JM. Specific regions of *Escherichia coli* OmpF protein involved in antigenic and colicin receptor sites and in stable trimerization. *J Bacteriol*. 1993; 175:2754–2757. [PubMed: 7683021]
- Hilsenbeck JL, Park H, Chen G, Youn B, Postle K, Kang C. Crystal structure of the cytotoxic bacterial protein colicin B at 2.5 Å resolution. *Mol Microbiol*. 2004; 51:711–720. [PubMed: 14731273]
- Housden NG, Hopper JT, Lukyanova N, Rodriguez-Larrea D, Wojdyla JA, Klein A, Kaminska R, Bayley H, Saibil HR, Robinson CV, Kleanthous C. Intrinsically disordered protein threads through the bacterial outer-membrane porin OmpF. *Science*. 2013; 340:1570–1574. [PubMed: 23812713]
- Housden NG, Loftus SR, Moore GR, James R, Kleanthous C, Housden NG, Loftus SR, Moore GR, James R, Kleanthous C. Cell entry mechanism of enzymatic bacterial colicins: porin recruitment and the thermodynamics of receptor binding. *Proc Natl Acad Sci U S A*. 2005; 102:13849–13854. [PubMed: 16166265]
- Housden NG, Wojdyla JA, Korczynska J, Grishkovskaya I, Kirkpatrick N, Brzozowski AM, Kleanthous C. Directed epitope delivery across the *Escherichia coli* outer membrane through the porin OmpF. *Proc Natl Acad Sci U S A*. 2010; 107:21412–21417. [PubMed: 21098297]
- Jakes KS, Cramer WA. Border crossings: colicins and transporters. *Annu Rev Genet*. 2012; 46:209–231. [PubMed: 22934645]
- Jakes KS, Finkelstein A. The colicin Ia receptor, Cir, is also the translocator for colicin Ia. *Mol Microbiol*. 2010; 75:567–578. [PubMed: 19919671]
- Jeanteur D, Schirmer T, Fourel D, Simonet V, Rummel G, Widmer C, Rosenbusch JP, Pattus F, Pages JM. Structural and functional alterations of a colicin-resistant mutant of OmpF porin from *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1994; 91:10675–10679. [PubMed: 7524100]
- Johnson CL, Ridley H, Pengelly RJ, Salleh MZ, Lakey JH. The unstructured domain of colicin N kills *Escherichia coli*. *Mol Microbiol*. 2013; 89:84–95. [PubMed: 23672584]

- Johnson CL, Ridley H, Marchetti R, Silipo A, Griffin DC, Crawford L, Bonev B, Molinaro A, Lakey JH. The antibacterial toxin colicin N binds to the inner core of lipopolysaccharide and close to its translocator protein. *Mol Micro.* 2014; 92:440–452.
- Kurisu G, Zakharov SD, Zhalnina MV, Bano S, Eroukova VY, Rokitskaya TI, Antonenko YN, Wiener MC, Cramer WA. The structure of BtuB with bound colicin E3 R-domain implies a translocon. *Nat Struct Biol.* 2003; 10:948–954. [PubMed: 14528295]
- Pugsley AP. Nucleotide sequencing of the structural gene for colicin N reveals homology between the catalytic, C-terminal domains of colicins A and N. *Mol Microbiol.* 1987; 1:317–325. [PubMed: 2834623]
- Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annu Rev Biochem.* 2002; 71:635–700. [PubMed: 12045108]
- Raggett EM, Bainbridge G, Evans LJ, Cooper A, Lakey JH. Discovery of critical Tol A-binding residues in the bactericidal toxin colicin N: a biophysical approach. *Mol Microbiol.* 1998; 28:1335–1343. [PubMed: 9680221]
- Rosenbusch JP. Characterization of the major envelope protein from *Escherichia coli*. Regular arrangement on the peptidoglycan and unusual dodecyl sulfate binding. *J Biol Chem.* 1974; 249:8019–8029. [PubMed: 4609976]
- Sharma O, Datsenko KA, Ess SC, Zhalnina MV, Wanner BL, Cramer WA. Genome-wide screens: novel mechanisms in colicin import and cytotoxicity. *Mol Microbiol.* 2009; 73:571–585. [PubMed: 19650773]
- Soelaiman S, Jakes K, Wu N, Li CM, Shoham M. Crystal structure of colicin E3: Implications for cell entry and ribosome inactivation. *Mol Cell.* 2001; 8:1053–1062. [PubMed: 11741540]
- Stora T, Lakey JH, Vogel H. Ion-channel gating in transmembrane receptor proteins: Functional activity in tethered lipid membranes. *Angew Chem Int Edit.* 1999; 38:389–392.
- Thoren KL, Krantz BA. The unfolding story of anthrax toxin translocation. *Mol Microbiol.* 2011; 80:588–595. [PubMed: 21443527]
- Vetter IR, Parker MW, Tucker AD, Lakey JH, Pattus F, Tsernoglou D. Crystal structure of a colicin N fragment suggests a model for toxicity. *Structure.* 1998; 6:863–874. [PubMed: 9687368]
- Wiener M, Freymann D, Ghosh P, Stroud RM. Crystal structure of colicin Ia. *Nature.* 1997; 385:461–464. [PubMed: 9009197]
- Yamashita E, Zhalnina MV, Zakharov SD, Sharma O, Cramer WA. Crystal structures of the OmpF porin: function in a colicin translocon. *EMBO J.* 2008; 27:2171–2180. [PubMed: 18636093]
- Zakharov SD V, Eroukova Y, Rokitskaya TI, Zhalnina MV, Sharma O, Loll PJ, Zgurskaya HI, Antonenko YN, Cramer WA. Colicin occlusion of OmpF and TolC channels: Outer membrane translocons for colicin import. *Biophys J.* 2004; 87:3901–3911. [PubMed: 15465872]
- Zeth K, Romer C, Patzer SI, Braun V. Crystal structure of colicin M, a novel phosphatase specifically imported by *Escherichia coli*. *J Biol Chem.* 2008; 283:25324–25331. [PubMed: 18640984]

Fig. 1.

(A.) Colicin Ia crystal structure (Wiener *et al.*, 1997), with T domain in blue, R domain in green and channel-forming domain in red. The same color scheme is used for colicin N (B.), but only a single residue of the 92-residue T domain is seen in this structure (Vetter *et al.*, 1998). The two molecules are not shown exactly to scale; their channel-forming domains, in red, are actually nearly identical in size. Note the very long coiled-coil that separates the tip of the colicin Ia receptor domain from the other two domains and the much less extended R domain of colicin N. Enzymatic E colicins have an extended structure similar to colicin Ia, bind via the tip of their long R domain to their BtuB receptor and search, via the unstructured N-terminal T domain, for a nearby OmpF translocator.

Fig. 2.

Schematic diagram of lipopolysaccharide (LPS), showing basic components of lipid A, the inner core (Rd) and outer core (Rc). KDO is 3-deoxy-D-manno-octulosonic acid; III designates heptose III, which is considered to be part of Rc, and was shown by Johnson *et al.* (in press) to be required for colicin N activity. Additional sugar residues beyond those required for colicin N activity and O-antigen repeats are not shown, as the O-antigen was not present in experiments described by Johnson *et al* (in press).

Fig. 3.

Cartoon model for colicin N binding and translocation. Colicin N is shown with its R domain (in green) binding to outer core glucose and heptose III (R_c) residues of LPS (symbols for sugars and phosphates are as in Fig. 2), adjacent to a molecule of trimeric OmpF, positioning its pore domain (red) away from the outer membrane (gray smear). This arrangement allows the unstructured N-terminal T domain (blue) to find an opening in OmpF and begin translocation of the colicin molecule either through an OmpF pore, or, alternatively, down the outside of an OmpF trimer in the membrane, after which the T domain can then be engaged by TolA in the periplasm (not shown) to initiate translocation of the rest of the colicin across the outer membrane. As discussed in the text, the pore domain may actually also insert in the membrane adjacent to OmpF (Baboolal *et al.*, 2008). O-antigens present on the surface of some *E. coli* strains are not shown in the figure.