Biochimica et Biophysica Acta 1843 (2014) 1717–1731

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Colicin import into *E. coli* cells: A model system for insights into the import mechanisms of bacteriocins

Young Chan Kim, Alexander W. Tarr, Christopher N. Penfold *

School of Life Sciences, University of Nottingham, Queens Medical Centre, Nottingham, NG7 2UH, UK

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 4 April 2014
Accepted 6 April 2014
Available online 16 April 2014

Keywords:
Colicin
Pyocin
Bacteriocin
Protein–protein interactions
Translocation
TolA
TonB

ABSTRACT

Bacteriocins are a diverse group of ribosomally synthesized protein antibiotics produced by most bacteria. They range from small lanthipeptides produced by lactic acid bacteria to much larger multi domain proteins of Gram negative bacteria such as the colicins from *Escherichia coli*. For activity bacteriocins must be released from the producing cell and then bind to the surface of a sensitive cell to instigate the import process leading to cell death. For over 50 years, colicins have provided a working platform for elucidating the structure/function studies of bacteriocin import and modes of action. An understanding of the processes that contribute to the delivery of a colicin molecule across two lipid membranes of the cell envelope has advanced our knowledge of protein–protein interactions (PPI), protein–lipid interactions and the role of order–disorder transitions of protein domains pertinent to protein transport. In this review, we provide an overview of the arrangement of genes that controls the synthesis and release of the mature protein. We examine the uptake processes of colicins from initial binding and sequestration of binding partners to crossing of the outer membrane, and then discuss the translocation of colicins through the cell periplasm and across the inner membrane to their cytotoxic site of action. This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

1. Introduction

Within the ecological niche of highly diverse communities, there is often fierce competition between various micro-organisms such as bacteria, fungi and Archaea for resources and space. Many species have evolved to produce a huge diversity of antimicrobial molecules that can either inhibit (bacteriostatic) or kill (bacteriocidal) competitor species including broad-spectrum antibiotics, proteinaceous exotoxins, lysozymes, and bacteriocins [1]. Since the discovery of the first bacteriocin by André Gratia nearly 90 years ago from *Escherichia coli* [2], bacteriocins have been found in nearly all bacterial lineages and some Archaea and are now regarded as the most diverse and naturally abundant classes of antimicrobial molecules [1,3,4]. Gratia's bacteriocin was termed a 'colicin' after the species name of the producing organism. A similar system of nomenclature was adopted for most bacteriocins discovered thereafter such as pyocins from *Pseudomonas aeruginosa* (formerly *Pseudomonas pyocyanea*), klebicin from *Klebsiella pneumoniae*, diffocins from *Clostridium difficile*, epidermin and gallidermin from *Staphylococcus epidermidis* and *S. gallinarum* respectively, and the newly discovered thuricin 17 from *Bacillus thuringiensis*. Despite huge differences in chemical structures and post-translational processing of bacteriocins between the small (un)modified lanthipeptides produced by lactic acid bacteria and the much larger multi domain polypeptides produced by Gram negative bacteria such as the colicins, all bacteriocins are ribosomally synthesized proteinaceous toxins that share a common biosynthetic pathway. Bacteriocins are generally narrow spectrum antibacterial agents with biological activity against closely related species and their genes are localized on transposable elements, plasmids or on the chromosome of the producer's genome. Modes of action range from depolarization of the lipid bilayer membranes [5,6], disruption of cell wall synthesis [7,8], inhibition of protein synthesis or degradation of host nucleic acids [9]. Mechanisms of cellular import are dependent on the target organism; enzymatic colicins that cross two lipid bilayer membranes and the peptidoglycan layer of the periplasmic space have a very different navigation pathway than lantibiotics such as mersacidin, which disrupt cell wall synthesis due to interactions with lipid II on the outside of the cell wall. The scope of this review is to investigate aspects of bacteriocin import. In particular, focus will be on the import processes adopted by colicins (especially enzymatic colicins), which have recently shown some exciting and novel mechanisms associated with cellular uptake [10–13].

Abbreviations: BRP, bacteriocin release protein; C domain, Cytotoxic domain; Col, colicin; IM, inner membrane; IUTD, intrinsically unstructured translocation domain; OM, outer membrane; OMP, outer membrane protein; PMF, proton motive force; R domain, Receptor binding domain; T domain, Translocation domain

☆ This article is part of a Special Issue entitled: Protein trafficking and secretion in bacteria. Guest Editors: Anastassios Economou and Ross Dalbey.

* Corresponding author. Tel.: +44 115 8467957; fax: +44 115 8230759.
E-mail address: chris.penfold@nottingham.ac.uk (C.N. Penfold).

http://dx.doi.org/10.1016/j.bbamcr.2014.04.010
0167-4889/

2. Colicin architecture

2.1. Domain organization and role in uptake

Colicins are modular proteins with three functional domains that act collectively to bind to the outside of a sensitive cell, cross the OM and translocate to a cytotoxic site of action (Fig. 1). Approximately 30–50% of the polypeptide is made up of a central receptor binding domain which interacts with a β-barrel shaped protein in the OM that is normally involved with the import of nutrients and metal ions such as vitamins, sugars and Fe^{3+}. An N-terminal translocation domain crosses the OM, in most cases through interactions with a second outer membrane protein that acts as a translocator and helps to establish a translocon through interactions with proteins termed Tol (mutations in which render the cell tolerant of the colicin) or Ton in the host periplasm. The translocator provides access for the colicin T domain to an energized translocation system in the periplasm. The T domain is divided into two parts; an intrinsically unstructured T domain (IUTD) and a larger structured T domain (STD) that is distal to the IUTD (Fig. 1). The final destination of the colicin molecule reflects the mode of action of the colicin which is either degradation of host nucleases in the cytoplasm (enzymatic colicins), depolarization of the cytoplasmic membrane (pore-forming colicins) or inhibition of peptidoglycan synthesis in the periplasm (colicin M) [14]. Based on this information, colicins are classified according to (i) the outer membrane receptor to which they bind and (ii) by the mechanism of translocation through the host periplasm according to Table 1. Import of Group A colicins occurs through the Tol-dependent translocation system which consists of the proteins TolA, TolB, TolQ, TolR and the peptidoglycan associated lipoprotein (Pal) whereas import of Group B colicins occurs through the TonB-dependent translocation system consisting of proteins TonB, ExbB and ExbD. Translocons of Tol-dependent colicins generally consist of interactions of the colicin with BtuB, OmpF and the Tol–Pal system, whereas translocons of the Ton-dependent system involve interactions of colicin with Ton and either FepA, FhuA or Cir. The primary receptor Tsx transcends the boundaries between Tol and Ton as it acts as primary receptor for the Tol-dependent colicin K and the Ton-dependent colicins 5 and 10 (Table 1). Interestingly, uptake of vitamin B12 which is TonB-dependent occurs from an initial interaction at the cell surface with BtuB whereas the BtuB-mediated uptake of colicins is exclusively associated with the Tol-dependent translocation of group A colicins rather than TonB (Table 1) [15].

2.2. Genetic arrangement of ORFs

Colicins are encoded on colicinogenic plasmids which are either low molecular weight, multi-copy plasmids of approximately 6 kb containing few other genetic determinants or high molecular weight, single copy plasmids in excess of 40 kb that carry many additional genes and are self-transmissible by conjugation [16]. The genetic organization of colicins varies depending on the type of colicin. The operon of enzymatic colicins consists of three genes; the first open reading frame in the operon specifies the structural gene of the colicin (named *cxa* for *c*olicin *x a*ctivity). *cxa* is immediately followed by a gene for immunity (*cxi*, named *c*olicin *x i*mmunity) which constitutively expresses a smaller polypeptide from its own promoter within *cxa* to neutralise the cytotoxic nuclease domain and prevent cell killing of the producing cell on synthesis of *cxa*. The third gene in the operon is termed *cxl* (named *c*olicin *x l*ysis), which is located distal to *cxi* and encodes a lysis protein that is involved in the extracellular release of colicins through lysis of the producer cell.

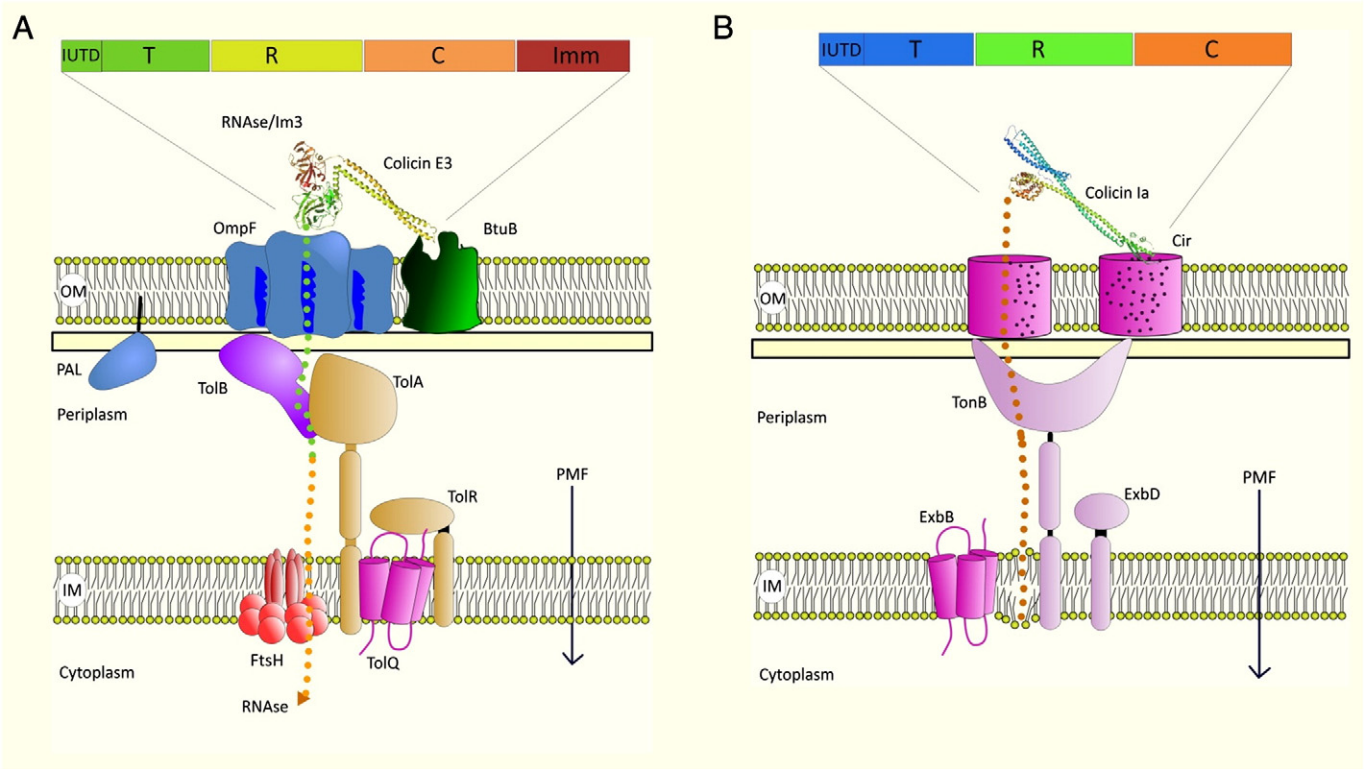

Fig. 1. Model of the colicin import mechanism. A. Enzymatic colicins such as colicin E3 bind to the outer membrane receptor, BtuB via their central receptor binding (R) domain at an angle of 45° from the cell surface. This permits the passage of the IUTD (green dots) through the lumen of an ompF monomer and across the OM to capture TolB. The resultant colicin translocon is energized by TolA, immunity is released from the colicin-immunity complex and the nuclease domain crosses the cell envelope (orange dots) by an as yet unknown mechanism. FtsH dependent processing of the colicin at the IM cleaves the nuclease domain from the colicin in the cytoplasm. In the absence of colicin, TolB forms an interaction with Pal at the membrane surface. B. Colicin Ia uses two copies of the outer membrane receptor, Cir; one for receptor binding and the 2nd one as a translocator. The Colla IUTD penetrates the cavity of the translocator (blue dots) following distortion of the Cir plug (black dots) to contact TonB at the boundary between OM and periplasm. Energy generated by the TonB–ExbB–ExbD complex promotes transport of the pore-forming domain into the IM (orange dashes).

Table 1
Colicin characteristics pertinent to cellular import. Colicins are grouped into two groups, A and B based on the mechanism of translocation across the periplasm.

Colicin	Outer membrane receptor "primary receptor"	"Translocator" or "secondary receptor"	Mechanism of translocation	Cytoxicity
Group A				
A	BtuB	OmpF	Tol A,B,Q,R	Pore-forming
E2, E7, E8, E9	BtuB	Omp F	Tol A,B,Q,R	DNase
E3, E4, E6	BtuB	OmpF	Tol A,B,Q,R	rRNase
E5	BtuB	OmpF	Tol A,B,Q,R	tRNase
E1	BtuB	TolC	Tol A,R	Pore-forming
N	LPS	OmpF	Tol A,Q,R	Pore-forming
K	Tsx	OmpF	Tol A,B,Q,R	Pore-forming
S4	OmpW	Omp F	Tol A,B,Q,R	Pore-forming
U	Omp A	Omp F, LPS	Tol A,B,Q,R	Pore-forming
28b	Omp A	Omp F, LPS	Tol A,B,Q,R	Pore-forming
Cloacin DF13	IutA	Unknown	Tol A,Q,R	RNase
Group B				
5, 10	Tsx	TolC	TonB, Exb B,D	Pore-forming
Ia, Ib	Cir	Cir	TonB, Exb B,D	Pore-forming
B	FepA	Unknown	TonB, Exb B,D	Pore-forming
D	Fep A	Unknown	TonB, Exb B,D	tRNase
M	Fhu A	Unknown	TonB, Exb B,D	Peptidoglycan synthesis inhibitor

An open reading frame for immunity against pore forming colicins is located in the intergenic gap between the colicin and lysis genes and is constitutively transcribed from the opposite DNA strand from its own promoter ensuring a constant supply of immunity protein for complete protection against the colicin [17–19]. Redundancies in the structure and organization of colicin operons occurs in E group colicins which often contain additional immunity genes to another nuclease colicin from a different incompatibility group; for example, the colicin E9 operon on the ColE9-J plasmid contains immunity genes to both ColE9 (DNase) and ColE5 (RNase; [20]). In addition, the ColE9 operon contains two lysis genes, a complete ColE5 lysis gene and a ColE9 lysis gene that is truncated and resembles a pseudogene [21] whereas no lysis gene has been described for the TonB dependent colicins, ColB and ColM [22].

2.3. Extracellular release

Colicins are released by *Escherichia coli* to kill neighbouring strains of *E. coli* and sometimes other enterobacteriacaea such as *Citrobacter and Shigella* [23,24]. Colicin expression is tightly regulated by the LexA and RecA proteins of the SOS response [19,25]. LexA binds to the SOS promoter and represses colicin gene expression which is reversed by the RecA dependent inactivation of LexA following a period of DNA damage. As colicin release kills the producing cell (reviewed below), colicin production is further regulated by IscR, a global transcriptional regulator that co-operatively stabilizes LexA at the SOS promoter and temporarily prevents the RecA-mediated inactivation of LexA ensuring colicins are only produced in response to persistent DNA damage and after all DNA repair mechanisms have been exhausted [26]. Populations of colicin producing *E. coli* produce colicin by autoinduction [27–29] when the cell enters the stationary growth phase, presumably as an offensive strategy to move into unoccupied environments offering more favourable conditions when nutrients become depleted and the cell enters a period of environmental stress [30,31]. The majority of studies on colicin release were conducted over twenty years ago on group A colicins; colicins A and N, E type colicins such as colicin E2 and E3, and cloacin DF13, a colicin orthologue produced by *Enterobacter cloacae* which all contain a lysis gene downstream of immunity on the colicin operon [32–37]. Colicins do not possess their own N-terminal signal sequence [38] and colicin release is independent of any inner membrane secretion pathway or defined excretion pathway [37]. Release of colicins, along with other intracellular proteins such as Elongation Factor-Tu and alkaline phosphatase, occurs by leakage through a permeabilized OM following lysis or quasi-lysis (dependent on the level of colicin induction) after expression of the lysis gene, which encodes a protein called the bacteriocin release protein (BRP), lysis or Kil protein from the same SOS promoter that regulates colicin production [34]. This ensures timely release of colicin following concomitant synthesis of both colicin and BRP, and controlled repression of BRP synthesis in the absence of colicin expression as the BRP protein can cause host cell lysis in the absence of colicin production [38,39]. The BRP is a lipoprotein with a stable signal sequence that is synthesized as a precursor and contains a conserved sequence of residues, Leu-X-Y-Cys, called the lipobox, around the cleavage site and where X and Y are neutral amino acids [40,41]. The signal sequence inserts into the IM and maturation of the lysis protein occurs following proteolytic cleavage of the signal sequence and modification of the BRP by acylation of the Cys residue [40]. Insertion of the mature BRP into the IM causes localized disruption and movement of the colicin across the IM [42] by an unknown process that requires co-operative behaviour between the BRP and the cleaved BRP signal sequence [42–44]. Mutations to key residues in the lipobox of the BRP prevented any accumulation of the colicin in the periplasm presumably by disruption to the transport processes of colicin across the IM resulting from a näive BRP [42,45]. The BRP of colicins and cloacin DF13 traverse the IM and associate with the OM [44]. Disruption of the local symmetry of the OM activates phospholipase A resulting in increased permeability of the lipid bilayer most probably through phospholipase A mediated changes to the lipid composition of the bilayer [47]. The role of phospholipase A in colicin export has been supported by the observation that *pldA* mutants defective in lipase activity do not appear to release colicin following induction of the colicin operon [37]. However, BRP-mediated perturbations of the OM are enough to cause colicin release, albeit at a reduced rate, in the absence of any phospholipase activity [47].

Colicins do not kill cells immediately after synthesis [48]; a delay of several hours for a colicin to be exported following a period of induction has been reported [23,49]. Colicin E2 has been found in both the cytoplasm and periplasm following induction [50] whereas colicin A and cloacin DF13 were found principally in the cytoplasm [44,51]. A one step model for colicin export was proposed in which colicin is released directly into the extracellular environment through imperfections in the lipid bilayer at points of close association between the IM and OM and thus bypassing the periplasm [44]. However, colicin A was subsequently shown to accumulate in the periplasm at various time points following induction with mitomycin C suggesting a two-step model of release after the Cal-mediated translocation across the IM [45]. No BRP has been reported for colicin B [18,52] and the mechanism of release of

colicin B is currently unknown but it may escape from cells by leakage [53].

3. Crossing the outer membrane

Colicin import is a unique event in the transport of proteins across membranes interfaces. The colicin is faced with a number of obstacles in its navigation across the OM, through the periplasmic space and peptidoglycan layer and, in the example of the enzymatic colicins, across the IM. The first step in colicin transport of all colicins, except colicin N, is the recognition by the R domain of a high affinity outer-membrane protein receptor on the *E. coli* cell surface such as BtuB, Cir, FepA, FhuA, Tsx or porins OmpA and OmpW (see Table 1). In contrast, the R domain of ColN targets the sugar moieties of LPS as its initial binding step [54] in a process that is more analogous to type RF pyocins and lectin-like bacteriocins from *Pseudomonas* spp. than other group A colicins [55–57]. High affinity outer membrane receptors are 22-stranded β-barrels that contain an N-terminal globular domain that inserts into the cavity of the barrel and acts as an internal plug. The plug domain contains a short stretch of ~5 residues at the N-terminus called the TonB box because it interacts with the C-terminus of TonB at the periplasmic side of the OM [58]. It has been suggested that movement of the plug domain into and out of the β-barrel facilitates the uptake of small metabolites and ion-chelators through the channel of the barrel [59].

Colicins also have similar short consensus sequences in their N-terminal translocation domains termed a TonB box (for TonB dependent colicins) or TolB box (for Tol-dependent colicins; colicins N and E1 are exceptions as they are TolB independent and do not have a TolB box; Table 1). Based on the positions of the TonB boxes in the crystal structures of ColIa and ColB, it was suggested that colicins also penetrate the outer membrane through the cavity of the barrel in an unfolded state following competitive displacement of the plug from its interaction with TonB [60,61]. Later studies investigating the effect of ColB on the exposure of cysteine residues engineered into the FepA plug domain to externally added fluorophores either supported [59] or challenged [62] the validity of this classical model of colicin translocation [63]. Similar studies using cysteine mutations of residues in constricting loops of the channels of the OmpF porins on the activity of colicins A and N demonstrated that neither colicins A nor N crossed the OM through the lumen of the OmpF pore [64,65]. Instead, colicin N feeds down the side of OmpF following displacement of tightly bound lipid on the exterior of its translocator [66]. The recently discovered phenomenon that ColN binds to LPS as its primary receptor and uses OmpF as a translocator has prompted an alternative model of ColN import [46]. Since the LPS is further from the surface of the membrane than OMPs, binding of the R domain to LPS sugars positions the IUTD above the exterior of ompF and may promote threading of the IUTD through the lumen of an adjacent OmpF barrel using the OBS as a pulling mechanism once inside the pore [46]. Recent studies by Jakes and Finklestein [11] have demonstrated that two copies of Cir are required for the activity of ColIa; one as the primary high affinity receptor and the second copy as a translocator for insertion of the ColIa IUTD [11]. By swapping the R domain of ColIa with that from ColE3 which binds BtuB as its primary OM receptor they created a chimeric colicin called IaE3R that became dependent on the E3 receptor, BtuB for its killing activity. However, cells devoid of Cir were equally resistant to IaE3R as well as ColIa suggesting that Cir might also be responsible for translocation processes of colicin import after receptor binding. Interestingly, both the wild type ColIa, IaE3R and even a deletion mutant of ColIa with no R domain still retained marginal cell killing activity against a *BtuB* mutant while the isolated pore-forming C-domain on its own showed no in vivo toxicity suggesting that the ColIa T domain may indeed bind to Cir in the absence of the R domain to initiate cell killing [11]. The isolated IUTD of ColN has recently been found to confer residual activity against *E. coli* by a mechanism that remains dependent on OmpF and TolA but independent of membrane insertion [67].

The crystal structure of colicins in complex with their primary receptors [68–70] have been instrumental in proposing novel models of colicin uptake across the OM [71,72]. Alternative mechanisms for colicin translocation across the OM have been proposed that involves the formation of a colicin translocon [10,73–75].

3.1. Formation of a colicin translocon

Despite high affinity binding ($kD < 10^{-9}$ M) between the long coiled-coiled R domains of ColE3 or ColE2 and the primary receptor BtuB, the central plug containing the TonB box of BtuB did not move significantly to expose the pore wide enough for the colicin to enter through nor was there a significant change in conformation of the colicin or BtuB to reconcile transport of the colicin through the BtuB channel [68,69]. Moreover, the R domain was found to be angled at 45° with respect to the OM surface which positioned the other two domains (C and T domains) further away from the primary receptor and towards other more abundant Omps (Fig. 2) [68,69]. The extended length and trajectory of the R domain of ColE2 and ColE3 acts as a 'fishing pole' for the T domain to locate its translocator, OmpF in this case, for transport across the OM (Fig. 1). OmpF is one of the most abundant proteins in the OM and exists as a trimer of identical 16 stranded β-barrels [76]. It is essential for the translocation of TolQR-dependent E colicins and colicins A and N [77,78]. Occlusion of the OmpF channels in planar lipid bilayer experiments by the T domain of ColE3 implied that the T domain may insert into the porin, which was later confirmed by the crystal structure of the IUTD of ColE3 in complex with OmpF [79]. Evidence for formation of a colicin translocon came from the extraction of an intact complex at the cell surface of BtuB-ColE9-Im9-OmpF that was dependent on the presence of the 83 residues of the intrinsically unstructured T domain (IUTD) for quaternary complex formation [75]. Deletion mutagenesis of the IUTD of ColE9 and interactions of the mutants with OmpF revealed two non-contiguous regions, or OmpF binding sites (OBS1 and OBS2) of the ColE9 IUTD that interacted with OmpF with binding affinities of 2 μM and 24 μM, respectively [71]. Site-directed mutagenesis of individual residues across OBS1 did not abolish the interaction of the IUTD peptide with OmpF but demonstrated the importance of flexibility of the whole molecule to threading of the peptide through the lumen of OmpF [80]. Although neither OBS is essential for the cytotoxicity of the enzymatic colicin, the presence of two OBS domains make killing more efficient by acting sequentially to deliver the TolB box (sandwiched between both OBS regions) through the OM and to its binding site on TolB in the periplasm. Recent data by Colin Kleanthous and colleagues has advanced our understanding of the colicin translocon model showing that TolB is tethered to the colicin translocon via both OmpF binding sites, which occupy two of the three subunits of OmpF in an antiparallel configuration as OBS1 is able to insert into OmpF in either orientation (Fig. 3; [10]). It remains to be seen whether a similar model of translocator threading occurs for other Tol-dependent colicins such as ColA which have different biological activities or structural characteristics.

3.2. Immunity release

All *E. coli* cells producing colicins protect themselves by co-synthesizing a plasmid-encoded immunity protein (Im) that prevents the host cells committing suicide prior to colicin secretion. This is imperative for enzymatic colicins as the nuclease domains are active on synthesis and would degrade cellular DNA/RNA without the neutralization by constitutively expressed immunity protein [28]. Enzymatic immunity proteins protect host cells against cognate colicins with binding affinities in the femtomolar range but are unable to offer protection against non-cognate colicins at physiological levels despite showing micromolar binding affinities in vitro [81,82]. Indeed, dual

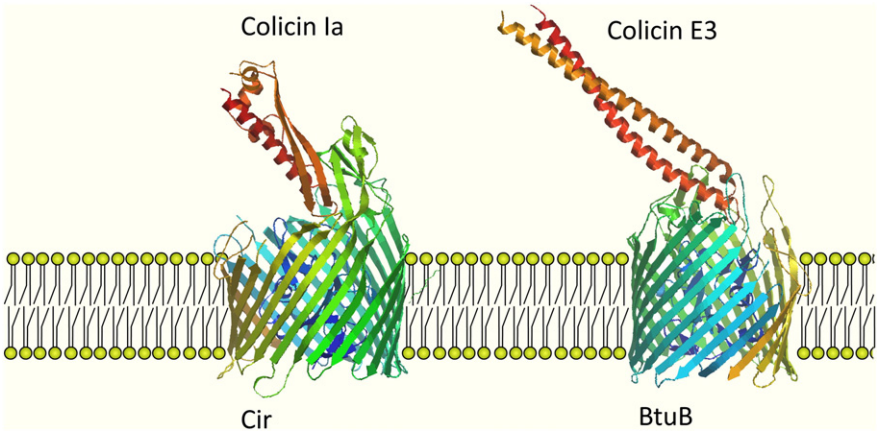

Fig. 2. Crystal structures of colicins in complex with their primary receptor. Complexes of the R domains of ColIa with Cir (pdb, 2HDI) [93] and ColE3 with BtuB (pdb, 1UJW) [95] demonstrating binding of the colicin R domain to the receptor at an oblique binding angle of ~45° to point the colicin away from the primary receptor and towards other Omps during the formation of a translocon.

immunity genes from different incompatibility groups are often present on the same colicinogenic plasmids expressing enzymatic colicins presumably to offer protection against attack by the cognate nuclease [83]. For producers of pore-forming colicins such as ColA that exhibit single hit kinetics (one colicin molecule kills one cell), the immunity protein forms part of the integral IM proteins that either prevent or block the depolarizing channel formation by the invading colicin pore-forming domain at the cytoplasmic membrane [84]. ColM differs from other colicins because it kills cells by inhibiting peptidoglycan biosynthesis [85]. The ColM immunity gene, *cmi* is found adjacent to the ColM activity gene, *cma* on the pColBM plasmid but both genes are transcribed in opposite directions and ColM does not form a complex with

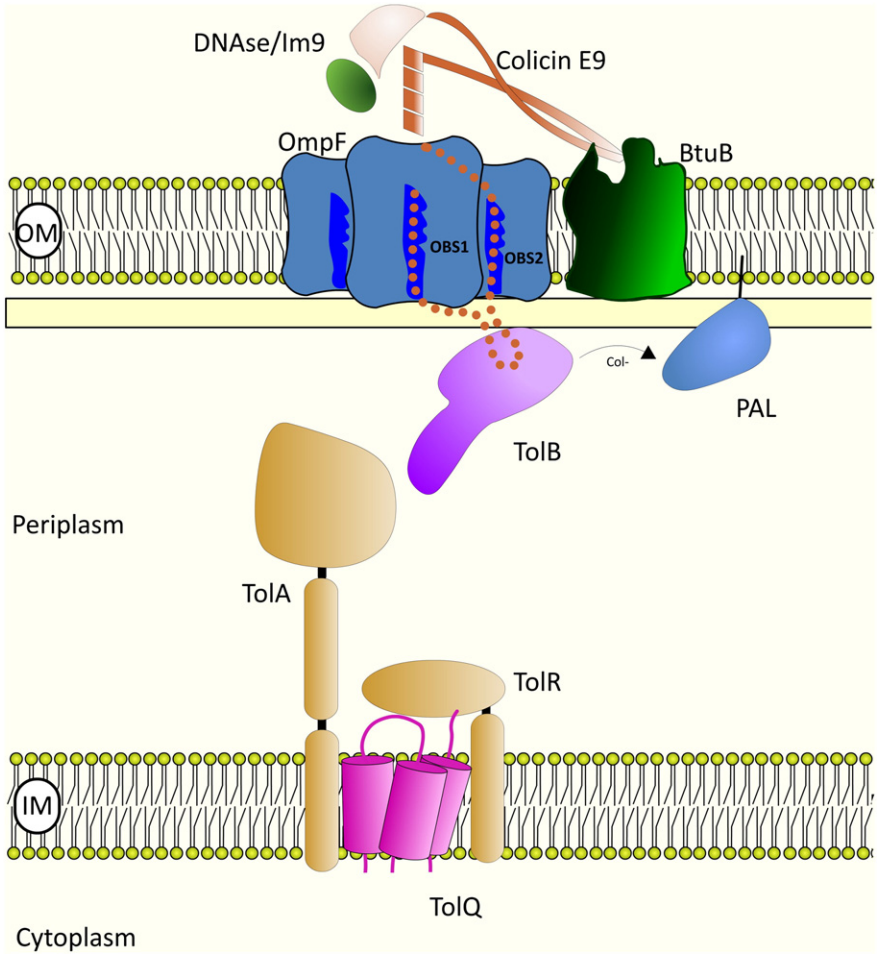

Fig. 3. Model of the colicin translocon. ColE9-Im9 complex binds to its high-affinity primary receptor BtuB recruiting the translocator protein OmpF via its IUTD (red dots). Both OmpF binding sites (OBS1 and OBS2) within the IUTD of ColE9 occupy two of the three subunits of OmpF in an antiparallel configuration to tether TolB in periplasm to the colicin translocon in a fixed orientation. Allosteric signalling of TolB forces immunity release at the cell surface.

its immunity protein before it is released from the cell. Instead, the immunity protein of ColM is anchored to the outer leaflet of the IM by an N-terminal hydrophobic α-helix that positions a globular α/β folded C-terminal domain into the periplasm to inactivate the imported ColM at the membrane interface by an unknown mechanism [86,87].

Nuclease immunity proteins are approximately 10 kDa and released from cells as a heterodimer of ~70 kDa with the colicin [88,89]. They inactivate enzymatic colicins either by binding directly to the nuclease active site and mimic their substrate RNA (Eg. tRNase-specific immunity proteins, Im5 and ImD) [90,91] or by binding at an exosite away from but adjacent to the active site that blocks access to their substrates, DNA or rRNA (e.g., DNase colicins E2, E7, E9 or rRNase colicins E3, E4, E6 [92–94]).

Enzymatic colicins bind to outer membrane receptors as an inactive complex with their cognate immunity proteins. At some point after binding, immunity is jettisoned from the colicin complex and the nuclease is transported to the cytoplasm to kill the receptive cell. Studies with recombinant colicin/Im complexes and/or uncomplexed colicin have shown that DNase colicins retain full activity in the absence of any complexed immunity whereas the activity of RNase colicins such as ColE3 or Cloacin DF13 is dependent on the presence of complexed immunity that is then lost after receptor binding [95–98]. The crystal structure of the ColE3-Im3 complex revealed that in addition to its interaction with the C domain (RNase), up to 38% of the Im3 protein also interacts with T domain [99,100] and that the bipartite binding of immunity to both domains in RNase colicins stabilizes the colicin in a favourable confirmation for biological activity prior to colicin import [95].

It is believed that the immunity protein is released from its heterodimer at the cell surface shortly after binding to its high affinity outer membrane receptor [95] but prior to the transport of the nuclease domain across the outer membrane [71,101]. Immunity protein of cloacin DF13 was found in culture supernatants of *E. coli* cells 10–20 mins after treatment with cloacin DF13 [95] and alexafluor labelled Im9 has been detected in culture supernatants of cells following reduction and resumption of activity at the cell surface of an inactive disulphide locked ColE9 [101]. Recent evidence has suggested that ColE3-Im3 complex dissociation requires the translocation of the IUTD of ColE3 through OmpF which weakens the interactions of Im3 with the T domain encouraging the release of Im3 at the cell surface [96,102,103]. However, the T domain of ColE9 is known to have limited interactions with wild-type ColE9 or the isolated DNase domain suggesting that the sandwiching of immunity proteins between the C and T domains may not be a general feature of all nuclease colicins [81]. It was demonstrated that receptor binding and recruitment of OmpF by the T domain of ColE9 during the formation of the BtuB-OmpF-ColE9 translocon was not sufficient to release Im9 from the ColE9-Im9 complex [75] but that unfolding must occur after binding to BtuB and prior to immunity release [75,101]. Furthermore, Duché et al. [104] have shown that release of Im2 from the ColE2-Im2 complex required an outer membrane translocon including BtuB, OmpF or OmpC as well as functional TolA and TolB proteins, and the interaction of a TolA binding region of TolB (termed the TolA box) with TolAIII was necessary for Im9 release from ColE9-Im9 and import of the ColE9 DNase domain [105]. In addition to a functional import machinery, the energy supply dissipated by the IM PMF via the Tol system was also found to be essential for Im9 release from the ColE9-Im9 complex [106].

The crystal structures of the complexes of the BtuB receptor and the R domains of colicins E2 and E3 not only provided evidence for the formation of the translocon with OmpF and Tol (detailed in Section 3.1) but implicated the importance of flexibility and unfolding of the extended helical arms of the R domain for immunity release and entry of the nuclease domains across the OM [68,69]. Rigidity engineered across both helical arms of the R domain by the insertion of an inactivating disulfide bond at the neck of the R domain prevented activity that was only reversed by DTT treatment [107], and global conformational rearrangement across all three domains of ColE9 at the cell surface has recently been demonstrated for ColE9 activity and Im9 release [108].

One of the biggest conundrums associated with immunity release over the previous 20 years has been the discrepancy that exists between the high binding affinities associated with the slow dissociation rate constants for the colicin-immunity interaction and the energy source needed to accomplish rapid dissociation of immunity from colicin at the cell surface [109]. The minimum amount of energy required for removal of immunity protein with a Kd = ~10^{-14} was estimated to be between −20 Kcal mol^{-1} and −24 Kcal mol^{-1} [72] whereas all known energy transducing systems available such as the PMF are thought to be able to supply up to 3.5 Kcal mol^{-1} which assumes a huge shortfall in the energy requirements needed [13]. However, recent work by Farrance et al. [12] using atomic force microscopy and disulfide bonds engineered into the DNase domain have shown that Im9 is released from the colE9-Im9 complex following a conformational rearrangement of the DNAse domain in response to low forces (<20 pN) triggered by a conformational remodelling of the T Domain on binding TolB. This results in an enormous increase (10^6-fold) in the dissociation rate of the ColE9-Im9 complex and reduces the lifespan of the complex from days to milliseconds commensurate with the speed of intoxication, supporting a role for the establishment of the outer membrane translocon and PMF in immunity protein release [75,101,106].

4. Translocation

4.1. Tol-dependent translocation

Conservation of the sequence of genes of the *tol–pal* operon across a variety of bacterial orders suggests important functional significance of the Tol proteins [110]. Though their normal cellular function in *E. coli* is still uncertain [110], the Tol–Pal complex appears to play a role in maintaining the integrity of the cell envelope, transducing energy from the cytoplasmic membrane, and may form a dynamic sub-complex at constriction sites to promote the energy dependent septal wall formation across invaginating peptidoglycan and inner membrane layers during cell division [111–114]. In addition, the Tol-Pal complex has become a surrogate platform for entry of external agents such as bacteriocins and filamentous bacteriophage. The Tol–Pal system consists of five proteins, TolA, TolB, TolR, TolQ and Pal. TolA is a 44 kDa periplasmic protein organized into three domains which are separated by clusters of glycine residues [115]. It is anchored to the cytoplasmic membrane via its N-terminus [116], spans the periplasm via its extended central domain (TolAII), and binds to both TolB [117,118] and Pal [119] via its C-terminal domain (TolAIII). All group A colicins are dependent on TolA which is integral to the Tol–Pal complex and has been likened to a promiscuous hub protein that forms interactions with intrinsic and extrinsic proteins at several different interaction sites [120,121]. TolQ and TolR are transmembrane proteins that are involved in the PMF-dependent activation of TolA [111], which shuttles energy from the IM to OM because of its association with Pal anchored to the outer membrane [122,123]. TolB is a periplasmic protein, that is associated with the outer membrane via an interaction of its C-terminal β-propeller domain with the peptidoglycan-associated lipoprotein (Pal) [124], and with TolA via its N-terminal domain [117]. Apart from the translocation of ColN, ColE1 and cloacin DF13, recruitment of TolB appears essential for the import of Group A colicins (Table 1). Comparison of the three-dimensional structures of TolB on its own [125,126] and in complex with the T domains of ColA and ColE9 carrying linear TolB binding epitopes, named the TolB box, have revealed important functional differences between the enzymatic colicins and ColA for the common TolB binding interface [127,128]. The affinity of TolB for the TolB box of ColE9 is approximately 10 fold higher than that of the TolB box of ColA with TolB [129]. Consequently the TolB box of ColE9, but not of ColA, is able to competitively recruit TolB from its physiological interaction with Pal producing a conformational change in TolB that encourages a low affinity interaction of the N-terminus of TolB with TolAIII, which is important for immunity protein release of the ColE9/Im9

complex [105,106]. Although competitive recruitment of TolB by ColA was established after favorable substitutions were engineered into the ColA TolB box [127], it is clear that recruitment of TolB by ColA is different to that of the enzymatic colicins. TolB binds to other outer membrane receptors such as Lpp and OmpA [130,131] and maybe present in the periplasm as a smaller protein that shuttles between membranes [132,133]. It is therefore possible that ColA interacts with TolB directly in the periplasm or from a lower affinity complex at the outer membrane. The lower affinity interaction of the TolB box of ColA with TolB, as compared to that of ColE9, may be important for the subsequent higher affinity interaction of ColA with TolA [120,129,134], which is a component of the Brownian ratcheting process that has been proposed to drive the unidirectional translocation of group A colicins [135]. ColA is unique in colicin biology in possessing different binding sites for two Tol proteins and as such resembles the infection of *E. coli* by filamentous phages [136]. Filamentous phages infect *E. coli* in a two-step process that involves the interaction of two N terminal domains of the g3p protein of phage fd (D2 and D1) that bind to cell pili and TolAIII respectively in a sequential manner [137]. The g3p interaction site with TolAIII contains a tetrapeptide motif (CYGT) that is also conserved in the TolA binding domains of colicins A, K and N [14]. There is currently no published evidence to suggest that the TolA binding region (extending from residues 52-97) and TolB box domains of ColA interact with each other in a similar way to the D1 and D2 domains of g3p. Comparison of the crystal structures of TolA in complex with bacteriophage fd g3p protein and the TolA binding domain of ColA [135,138] shows both complexes have opposing binding interfaces [120,136]. Nevertheless, the establishment of a trimeric complex of TolA:TolB:ColA in vitro suggests that ColA might interact with TolA and TolB simultaneously following a two-step process [139].

TolA is also required for the entry of Colicins N and E1 [140,141]. NMR experiments have revealed that the IUTD of ColN binds to TolAIII in an interaction that is strikingly similar to the g3p–TolAIII complex [136,142,143]. The lack of any sequence or structural homology between the TolAIII binding sites of ColN and g3p implies that they have evolved by convergent evolution to fulfil the same biological task [143]. Colicin E1 interacts with TolA differently than other group A colicins; deletion of domain II of TolA, whilst being catastrophic to the activity of other groups A colicins, only has a slight effect on ColE1 activity that reflects its unusual dependence on TolC for transport through the OM [144]. ColA is also dependent on TolC for biological activity [145] but a role for TolC in the import of ColA, unlike that of ColE1, has not been established (Table 1). TolC is present in the cell envelope at adhesion zones [146] and may therefore provide a direct route for ColE1 to the TolQRA complex through constrictions of the cell membranes.

Despite the identification of a putative TolR box in the N-terminus of colicin E3 a direct interaction of TolQ or TolR with any colicin has not been demonstrated [147]. The IM transmembrane regions of TolQRA stabilize TolA in a suitable confirmation for dissipation of the IM PMF that ensures the maintenance of OM stability [116]. TolR interacts with TolA by interactions with its N-terminal transmembrane domain and surface exposed C-terminal domain and with TolQ through an interaction of its C-terminal domain which becomes associated with the IM by its interaction with TolQ [148,149]. TolQ has three transmembrane helical domains and interacts with TolA through an interaction with the first helix and with TolR by an interaction with its second and third helices [149,150]. Disruption of IM interactions by site directed mutations to discriminative residues of TolQR that reside in a putative aqueous ion channel resulted in destabilization of the OM but did not affect colicin activity [112].

4.2. Ton-dependent translocation

The Ton system consists of an OM receptor and three proteins anchored in the IM; TonB, ExbB and ExbD that share functional and structural similarities to the Tol system proteins TolA, TolQ and TolR respectively [151,152]. The Ton system has been parasitized by Group B colicins and bacteriophages T1 and phi80 for import into sensitive cells [153]. ExbB and ExbD are analogous to TolQ and TolR respectively, and harvest the energy from the IM PMF to energize TonB [154]. Mutational and cross-linking studies show that partners may be interacting as oligomers [157] with the ratios of TonB:ExbB:ExbD per cell equivalent to 1:7:2 respectively [158]. ExbB is thought to be a primary scaffolding protein since both TonB and ExbD are unstable in its absence [155], and is an integral component of the energy coupling system for the transport of vitamin B12 [156]. ExbD interacts with TonB in the periplasm and has a role in regulating the energy-dependent conformation of TonB for specific interactions with transporters [159,160].

Cross complementation between the Tol and TonB systems has been demonstrated such that TolQ and TolR can replace ExbB and ExbD respectively, albeit at low levels (10–20%) for TonB-dependent translocation of colicins [151,152] and the Ton system can functionally replace TolB in the uptake of colicin U by converting the ColU TolB box to a TonB box of its T domain [161]. Braun showed that colicins B and D, two group B colicins are still active on ExbBD mutants. Activities of group B colicins and cobalamin transport were completely abolished in a TolQR-ExbBD mutant [156,152] while the over expression of TolQ and TolR in an ExbBD mutant background rendered cells more sensitive to those two colicins [152]. In contrast, no cross-complementation between TolA and TonB has been observed [152] despite a common evolutionary relationship between their C terminal domains [162].

TonB is a ~26 kDa protein that is mechanistically analogous to TolA [163]. It is divided up into three domains: Domain I contains the amino terminus from residues 1–65 that has a standard hydrophobic signal sequence necessary for the Sec-dependent export of TonB to the IM. TonB is exported without proteolytic cleavage which serves to anchor TonB to the IM allowing the predominantly hydrophilic domain II (residues 66–102) to extend into the periplasm. Domain II is unusually rich in proline residues which are not necessary for energy dissipation to the OM but encourage a conformational plasticity that extends the protein across the periplasm towards the inner leaflet of the OM [164]. The third domain consists of residues 103–239 and has a hydrophobic region at its C-terminus that may localise TonB to the OM. The C terminal domain interacts with the TonB box of the OM receptors such as BtuB, FepA and FhuA [165,166] to facilitate energy transduction across both membranes for TonB-dependent coupling of IM and OM processes. Group B colicins have a sequence of 5 residues in the N-terminus that has been termed the TonB Box from its conservation with the TonB box of the plug domain of OM receptors [77]. Mutations to the TonB box of colicins B and M produced mutants that were inactive due to a perturbation in translocation of the colicin that were partially suppressed by mutations in TonB, suggesting that uptake is TonB dependent [167,168]. It was originally envisaged that colicins use the TonB box to interact with TonB following displacement of the TonB box of the plug of the OM receptor [85,86]. However, the crystal structure of colicin Ia bound to its OM receptor, Cir showed that the tip of the R domain of ColIa penetrated the outer surfaces of the ligand-binding pocket of Cir to expose but not dislodge the plug from its central position [95]. Nevertheless, individual missense mutations to residues Arginine 158 and Proline 161 of TonB, which lie at the binding interface of the TonB:TonB box of the FhuA and BtuB plug domains [83,169], rendered cells resistant to ColD but sensitive to Colicins B, M, Ia and bacteriophage phi80 [170]. Sensitivity to ColD was restored in suppressor mutations of the TonB box of ColD [170]. Given that the TonB box of ColB can functionally replace the equivalent pentapeptide of ColD without affecting the activity of ColD, it is likely that translocation of colicins B and D is functionally dissimilar despite sharing 96% identity across the T and R domains [170]. Interestingly, microcins require TonB for cellular import but do not possess an apparent TonB box [171] and given their small size probably enter the cell in a process that is similar to the uptake of iron siderophore complexes [172] or antibiotics that bind the OM plug independently of the TonB: TonB box interaction [173].

Bacteriophage T1 and phi80 require TonB and FhuA for transport across the cell but the precise mechanism of uptake is currently unclear [174,175]. Meanwhile, colicins 5 and 10 have a TonB box in their T domain and are Ton dependent but independent of the Ton system with regard to the OM receptor, Tsx [176] (Table 1).

The recent discovery that colicin Ia uses a second copy of Cir as a translocator raises the question of whether two copies of TonB are necessary for translocation of the Colla pore-forming domain. The current model of Colla import assumes that the formation of the Colla-Cir(R-domain)-Cir (translocator) complex mimics binding of its natural ligand to the receptor which facilitates binding of the TonB box of Cir to the C-terminal domain of TonB following an order–disorder transition of the Cir TonB box [177]. The Cir plug becomes distorted or displaced out of the barrel to allow movement of the IUTD through the pore into the periplasm and promote binding of the TonB box of Colla to either a second copy of TonB or the same copy of TonB following displacement from its interaction with the TonB box of Cir (Fig. 1) [11]. TonB is able to dimerize in vivo [178] and forms a 2:1 (TonB:FhuA) stoichiometry with FhuA [179] that is dependent on the presence of the TonB box [180] but recent evidence using double electron–electron resonance has shown that when bound to either FhuA, FepA or BtuB, the TonB dimer is converted back to its monomeric form [181].

5. Crossing the inner membrane

5.1. Integrations with the cytoplasmic membrane

Colicin DNases and rRNases are characterized by sequences rich in basic residues within their C-domains despite being structurally very different, suggesting that their import across the IM is electrostatically driven and may be structurally independent [93,182]. Studies looking at the interactions in vitro, between the membrane and the nuclease domains of ColE9 and ColE3 showed significant structural destabilization as result of their interaction with anionic phospholipids [183,184]. Colicin DNases such as ColE9 are able to form short lived, non-voltage gated channels with planar lipid bilayer in contrast to pore forming colicins that do not cross the IM but are inserted into the IM to form voltage dependent channels upon helix reorganization with longer half-lives and with the ability to cause IM depolarization [185]. This DNAse-induced channel activity was prevented by an internal disulfide bond within the nuclease domain that also abolished Col toxicity and implied that structural flexibility indicative of destabilization of the nuclease domain was essential for interactions with the IM [185]. In other studies, it was shown that there is a direct correlation between the net positive charge on the DNase and the cell killing efficiency such that colE7 > colE2 > colE8 > colE9 implying that DNase colicins with a higher net positive charge have more desirable pharmacokinetic properties [186]. However, a correlation between net positive nuclease charge and association kinetics of the nuclease with artificial large unilamellar vesicles (LUVs) in vivo was not substantiated, suggesting that in the presence of sufficient negative charge at the membrane surface, membrane affinities of nuclease domains are not affected by increases in net positive charge of the nuclease [187]. Nuclease domains bind anionic membranes only with micro-molar affinity and via a co-operative binding mechanism such that the initial non-specific electrostatic membrane association with basic nuclease domains may allow a conformational rearrangement to facilitate further specific hydrophobic interactions that drive insertion depth and destabilization at the membrane interface [187]. Interestingly, for all colicins tested, insertion depth correlated well with increased lipid mixing and vesicle aggregation and was a reflection of relative hydrophobicity such that the greatest insertion depth was observed for ColE3 followed by the DNases E7 > E9 > E8 [187,188]. Colicin E3 inserts into the top region of the hydrophobic core and the DNases traverse the interfacial region of the bilayer suggesting a direct correlation between individual cleavage mechanisms and depth of lipid bilayer insertion (Fig. 4).

Evidence for a role of the IM-associated translocator, FtsH in the import of nuclease domains through the IM by a process of retro-translocation analogous to that used by bacterial toxins to move unabated through the lipid membranes of eukaryotic cells has been established [186]. FtsH is an essential ATP dependent protease in the *E. coli* that spans the IM with two transmembrane regions and extends into the cytoplasm through a large cytosolic domain that contains the protease activity [189,190]. It functions to dislocate mis-folded membrane proteins from the IM to the cytoplasm and then degrades them [191]. Retro-translocation of colicins across the IM is one of the least known processes in colicin import. IM-associated destabilization of colicin nuclease domains may provide a conduit to FtsH directly or alternatively membrane embedded chaperone proteins such as HflC and HflK may facilitate access to FtsH through their interaction with FtsH as a large multimeric complex of proteins [192] (Fig. 3). FtsH is required for the biological activity of both group A and group B nuclease colicins (DNase, tRNAse or rRNase) and has been implicated in the IM translocation of ColD but not ColE3 through its interaction with LepB [193] but is not required for the cytotoxicity of pore-forming colicins of either translocation dependent system [186]. How the unfolded colicin evades FtsH mediated degradation at the interface between IM and cytoplasm is unknown but it maybe a combination of the rapid re-folding characteristics of colicin nuclease domains in an aqueous environment and/or the inability of FtsH to degrade sequestered proteins that contain periplasmically exposed domains with tightly folded regions [194].

5.2. Proteolytic processing

In the third and final step of translocation, pore-forming colicins generate a pore in the inner membrane causing a localized depolarization whilst nuclease colicins require further translocation through the inner-membrane (IM) and into the cytoplasm to access their target material such as DNA or RNA. As most research on colicin transport over the last decade has focused on OM translocation, progress on an understanding of translocation through the inner membrane remains relatively sketchy. Recent studies have revealed that IM translocation of enzymatic colicins is likely to involve some proteolytic processing of the colicin with some degree of interaction between the cytotoxic domain of colicin and the IM, retro-translocation that involves a localized destabilization of the nuclease domain and refolding into holoprotein once reaching the cytoplasm [183,187]. The role of endo-proteolytic cleavage of the active cytotoxic domain into the cytoplasm remains a topic of conjecture. It has been reported through studies on colicins E2 and E3 that the colicin remains in tight binding with their primary OM receptor and import machinery during the IM translocation of nuclease domains into the cytoplasm suggesting that cleavage of the colicin shortly after receptor binding or transport through the OM to release the cytotoxic domain at an early stage of translocation does not occur [195]. However, many studies have suggested that proteolytic cleavage of colicin nuclease domains does occur, but its link to the import mechanism is often less clear. Bacterial OM protease OmpT has been an attractive candidate for proteolytic cleavage of colicins at the cell surface; colicins E1-E3 and A were shown to be cleaved by OmpT releasing a fragment containing cytotoxic domain [196,197]. ColE7 also undergoes cleavage at the residue arginine 447 of the colicin molecule in an OmpT dependent manner [198] but the gene product responsible for such a proteolytic event could not be identified from a genome-wide screen of the Keio collection [145], and the corresponding residue in the DNase domain of ColE9 was shown to be an essential residue for DNAse activity of colicin E9 [199]. In addition, the BtuB bound ColE2-Im2 complex protected OmpT proteolytic processing of ColE2 at the cell surface [196]. However, the involvement of OmpT for the import and toxicity of nuclease colicins has recently been refuted as an OmpT deletion mutant of *E. coli* retained full-sensitivity to the ColD and ColE3 RNases [193], and it has been suggested that OmpT plays a part of the general bacterial defence system against exogenous toxins such

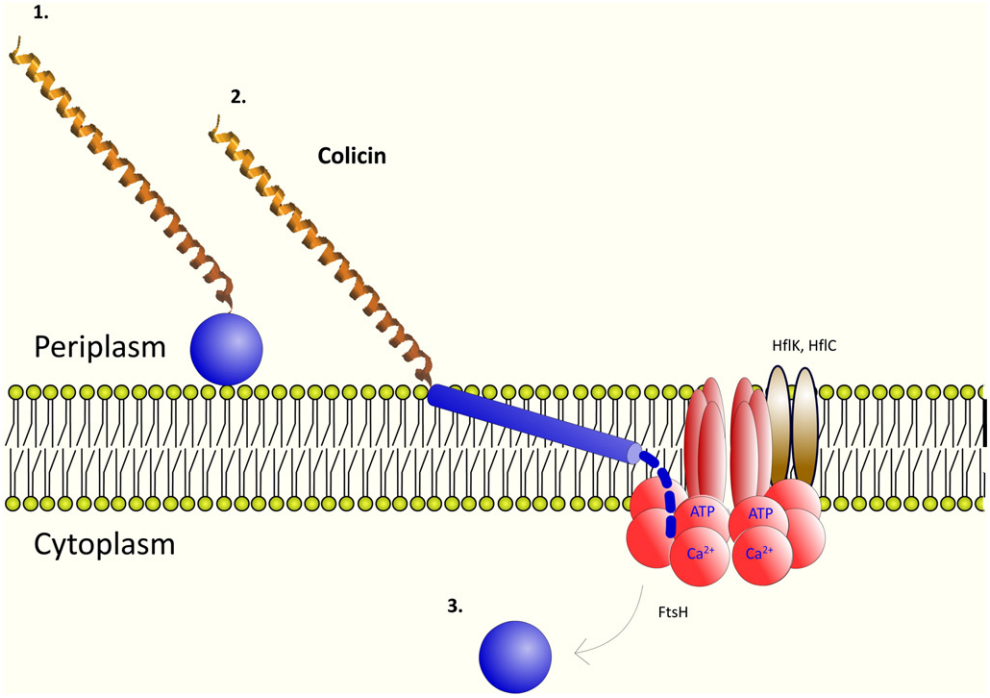

Fig. 4. Transport of the nuclease domain through the IM. The nuclease domain of colicins is selectively transported from the periplasm to cytoplasm across the inner membrane through an interaction with FtsH. 1. The nuclease domain interacts with the inner membrane. 2. Nuclease unfolding occurs within the membrane. RNase colicins such as ColE3 penetrate into the hydrophobic core whereas DNAse colicins enter the IM at the interfacial region of the lipid bilayer. 3. Following interaction with a complex of FtsH, Hflk and HflC, the nuclease is re-folded into its active form in the cytoplasm.

as colicins by hydrolysing them at the cell surface [197,200,201]. The IM leader protease LepB was implicated in the processing of ColD to release the RNase fragment [202,203]. LepB interacts with ColD in vitro, and mutated forms of LepB carrying the mutations Arg274Lys and Lys145Ala as individual substitutions prevented binding and catalysis of ColD, respectively [202,204]. The same group recently showed that LepB in isolation failed to cleave ColD implying that it is necessary, but not sufficient, for endoproteolytic processing of ColD on its own [193]. Moreover, LepB protein was not found to be necessary for cytotoxicity of other colicins (ColB and ColE1-E3) indicating that this is a very specific effect for ColD.

6. Import of colicin-like bacteriocins from other Gammaproteobacteria

Bacteriocins with similarities in their genetic organization and domain structure to colicins have been identified in several other γ-proteobacteria such as pyocins from *Pseudomonas aeruginosa*, pesticins from *Yersinia pestis*, klebicins (Klebocins) from *Klebsiella pneumoniae*, cloacins from *Enterobacter cloacae*, lumicins from *Photorhabdus luminescens* and several colicin-like bacteriocins active against plant pathogenic bacteria from *Ps. syringae* and *Pectobacterium* spp. Partial DNA sequence homologies with colicin domains, X-ray structure determination and site-directed mutagenesis studies have advanced our understanding of the mechanisms by which these colicin–like bacteriocins are able to bind cells and to kill them but very few studies have provided compelling evidence for any specific import processes across the cell envelope. In the absence of any direct binding studies that show an interaction of the T domain of a colicin-like bacteriocin with a host cell protein or other macromolecule, for example, TolA/B or TonB, most evidence for any import mechanisms of colicin-like bacteriocins relies on indirect comparisons of the translocation processes adopted by colicins.

6.1. Pyocins

Pyocins are chromosomally encoded bacteriocins classified into three different types (Type R, F and S) which are structurally and functionally distinct. They are produced by more than 90% of all *Pseudomonas aeruginosa* strains with any one strain being capable of producing more than one pyocin type [205].

Pyocin production is induced by DNA damaging agents such as ultraviolet (UV) irradiation and mitomycin C via the RecA dependent pathway. Mutagenic agents trigger the expression of RecA which causes the cleavage and release of the pyocin repressor, PrtR from the promoter of *prtN* allowing synthesis of PrtN and subsequent expression of the pyocin genes. They are usually produced against related strains but activity has been reported against other distantly related Gram negative organisms [206].

The majority of studies on pyocin import so far have concentrated on the identity of the primary cell surface receptors used by pyocins for receptor binding. The ferripyoverdine or ferripyochelin receptors normally involved in the import of iron-siderophore complexes are involved in the uptake of soluble pyocins via the N-terminal R domain whereas the lipopolysaccharides (LPS) are implicated in the entry of the phage-like RF type pyocins via interactions of the tail fibre region of RF type pyocins with sugar residues of the outer core of LPS [56,207–210]. The newly discovered pyocin L1 from *P. aeruginosa* has similarity to the lectin-like family of bacteriocins from *Pseudomonas spp.* and binds the monosaccharide D-rhamnose of the common polysaccharide antigen (CPA) of *P. aeruginosa* LPS [55].

Very little is known about the mechanism of cell entry following receptor binding, translocation to the active site and the cascade of events that culminate in the death of the non-immune cell. Based on similarities of the domain organization of soluble pyocins to the colicins, it is likely that the translocation of the soluble pyocins occurs through protein–protein interactions of the translocation domain with either the Tol-dependent or the TonB-dependent translocation systems. The Tol-

dependent system has been suggested to be the system used for the translocation of soluble pyocin AP41 following restoration of activity of pyocin AP41 to a *tol*-like mutation in *P. aeruginosa* strain PAO1652 by complementation with *tolQRA* [211]. The crystal structure of TolAIII of *P. aeruginosa* is similar to that of *E. coli* either alone [120] or in complex with bacteriophage g3p [136] despite there being only marginal homology of about 20% in the participating residues [162]. This might imply that the recruitment of TolAIII by the translocation domain of the soluble pyocins, for example pyocin AP41 from *Pseudomonas aeruginosa* is actually very similar to that of TolAIII from *E. coli* by group A colicins or phage g3p protein despite the interactions occurring across a different binding interface [120,136,139]. However, the role of TolA in the infection process of pyocins has not been conclusively determined because mutations of TolA, as controls for cell killing, have proved problematic [211].

The role of TonB in the uptake of soluble pyocins has also been inferred as TonB has a close association with the ferripyoverdine receptor, FpvA and participates in pyoverdine mediated signalling [212]. The crystal structure of FpvA is a transmembrane 22-stranded β-barrel, with an extended N-terminal TonB box that forms intermolecular interactions with TonB, and shares structural similarity to BtuB [213]. In addition, it has been suggested that uptake of the S type pyocins, S1, S2 and S3 occurs via the TonB-dependent uptake system because these pyocins show increased activity under iron limiting conditions and bind to a ferripyoverdine receptor despite the absence of an obvious TonB box in their translocation domains [214].

6.2. Pesticin

Pesticin is produced by *Yersinia pestis* to kill non-immune *Y. pestis* strains, other *Yersinia* spp. and *Escherichia coli* strains that express the primary receptor, FyuA used by pesticin to bind to sensitive cells [215]. The pesticin structural gene, *pst* is synthesized in the opposite polarity to an adjacent immunity gene, *pim* on a small ~10 kb plasmid that does not possess a lysis gene for lysis mediated pesticin release [216].

The crystal structure of pesticin has revealed a domain organization that is very similar to the three domain organization of colicins: an N-terminal translocation domain, a central receptor binding domain and a C-terminal cytotoxic domain [217]. The first 40 residues of the N-terminus are intrinsically unstructured and are not visible in the crystal structure; a characteristic similar to those colicins of which the X-ray structure has also been determined. The cytotoxic domain has structural homology to phage T4 lysozymes and kills cells by the hydrolysis of peptidoglycan similar to ColM although ColM interferes with peptidoglycan synthesis rather than degradation of the murein layer [218]. The pesticin OM receptor, FyuA has two domains: a β-barrel domain and an internal plug with a TonB box that is required for the uptake of the iron siderophore, yersiniabactin [219]. Uptake of pesticin occurs through the cavity of FyuA in an energy dependent process that is driven by TonB, ExbB and ExbD [219]. Pesticin contains a TonB box (DTMVV) at residues 3–7 of the N-terminal translocation domain that likely interacts with TonB following displacement of the plug of FyuA from its interaction with TonB [217]. Complete unfurling of the IUTD appears critical for full activity of pesticin as any reduction in the length of the imported IUTD has been predicted to significantly diminish its activity [217].

6.3. Colicin-like bacteriocins from phytopathogens (plant pathogenic bacteria)

Recent advances in our understanding of the import processes and biological activities of colicin-like bacteriocins from phytopathogens justify their inclusion in this review. The majority of studies on colicin-like bacteriocins active against plant pathogenic bacteria have been conducted on carotovoricin, carocin, pectocin and syringicin. Carotovoricin and carocin S1, S2 and D, are produced by *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotorova*), which causes soft-rot disease in plants. Carotovoricin Er is a high molecular weight bacteriocin with high sequence homology to bacteriophage proteins from *Salmonella typhi* [220]. The genetic locus most closely resembles the locus of RF-type pyocins and controls the expression of at least 10 proteins that assemble into a phage structure with an antenna-like tail, base plate and contractile sheath [220,221]. The mechanism of import is currently unknown but presumably occurs by contraction of the sheath and delivery of the core protein across the OM following adsorption of carotovoricin to the cell surface. Carocins S1 and S2 are low molecular weight bacteriocins with DNase and RNase activities respectively, and are produced as a complex with their cognate immunity proteins similar to the enzymatic colicins [222–224]. Carocin S1 gene has been shown to have high homology to the pyocin S3 and pyocin AP41 genes of *P. aeruginosa,* and gene expression is induced by the presence of energy sources such glucose or lactose. The type III secretion system, integral to the secretion of flagella in *P. carotovorum* is involved in the extracellular secretion of carocin S1 [223]. Unsurprisingly, domain III (cytotoxic domain) of carocin S2 has greater identity with RNase domains of colicin D and klebicin D than with carocin S1 [224].

Carocin D has DNase activity and is produced as a complex with a cognate immunity protein from adjacent genes, *caro*DK and *caro*DI, respectively. Carocin D is a multi-domain protein with homology near the N-terminus to the translocation domains of colicin E3 and cloacin DF13 and at the C-terminus to both the translocation and cytotoxic domains of S-type pyocins suggesting that the carocin D has two separate translocation domains similar to colicin D and klebicin D [225]. An N-terminal TonB box, DTMTV at residues 13–17 suggests that import of carocin D across the periplasm is via the TonB-dependent translocation system but a TonB-dependent high affinity receptor or a receptor binding domain has not, as yet, been elucidated [225].

Two novel bacteriocins, Pectocin M1 and M2, have recently been identified from *Pectobacterium spp*. with sequence homology at the C-terminus to colicin M and at the N-terminus to iron-sulfur containing plant ferredoxins [226]. Competitive inhibition of the biological activity of pectocin M by spinach ferredoxin I or by an inactive mutant of pectocin M defective in the catalytic domain showed that receptor binding of pectocin M and plant ferredoxin were mutually exclusive and bind to an as yet unidentified common receptor [227]. In the absence of a structure for pectocin M, it is probable that the ferredoxin domain of pectocin provides the receptor binding function and another region of the protein provides a mechanism for translocation through the cell envelope to the cytotoxic site of action [227].

Syringacin 4A and M are other examples of colicin-like bacteriocins produced by the plant-pathogenic bacterium, *Pseudomonas syringae*. Syringacin 4A is induced by ultraviolet irradiation or mitomycin C [228] and therefore is most likely regulated by the SOS response. Syringacin M is a colicin M-like bacteriocin that kills susceptible cells by disrupting peptidoglycan synthesis analogous to the mechanism of peptidoglycan inhibition described for colicin M [7,229,230]. Despite limited identity of the primary sequence of syringacin M with the corresponding sequence of colicin M, the recent crystal structure of syringacin M showed striking structural similarities between syringacin M and colicin M, not only of the expected cytotoxic C-domains but also the receptor binding and translocation domains [231]. Weak electron density at the N-terminus of syringacin indicated that the T domain is unstructured and analogous to the IUTD of colicin M. Although an obvious TonB box has not been identified in the T domain of syringacin M, the marked similarity with ColM suggests that import of syringacin M across the cell envelope of *P. syringae* is TonB-dependent [231]. Indeed, a mutant of syringacin M devoid of the likely TonB box-containing region at the N-terminus is inactive. This unexpected evolutionary relationship between colicin M-like bacteriocins such as syringacin M and colicins suggests the evolution of novel bacteriocins from a common ancestor occurs through a process of diversifying selection rather than a recombination event.

7. Conclusions and future perspectives

Colicins offer excellent opportunities for a detailed understanding of the processes that facilitate the import of bacteriocins and other protein metabolites across the cell envelope of bacteria and/or membranes of eukaryotic organelles. Over the last ten years, the determination of crystal structures of colicins and the structures of colicins in complex with binding ligands has resulted in rapid progress in our knowledge and understanding of the mechanisms that control outer membrane penetration of colicin domains through the formation of colicin translocons at the cell surface. Similarly, the role of the Tol and Ton translocation systems in the cell periplasm and the interactions of Tol and Ton with intrinsic and extrinsic proteins have provided answers to questions on uptake of colicins across the OM and the energy dependent release of immunity protein from the nuclease domains of enzymatic colicins. Indeed, the age-old paradox that existed between rapid immunity protein release at the cell surface and the perceived astronomical forces needed to achieve this feat has now been resolved [12], and the exciting new discovery that not all colicins bind high affinity OM protein receptors as the primary receptor binding step has widened our knowledge of colicin import processes [54]. In contrast, much less is known about the transport of the cytotoxic domain through either the OM or IM, and the mechanisms of proteolytic cleavage and transport of nuclease domains across the IM which need further investigations.

With the impending demise of current antibiotic regimens against many multi-drug resistant bacteria especially against Gram negative organisms producing extended spectrum β-lactamases (ESBLs), the application of bacteriocins as alternatives to antibiotics is gaining increased popularity [232,233]. Nisin has been introduced as a preservative in the food industry and as a prophylactic in veterinary healthcare. Probiotics often produce bacteriocins that contribute to the establishment of beneficial bacterial communities in host micro-environments. Several lanthipeptides are currently in clinical trials at different phases for investigations as potential chemotherapeutic agents [232]. Colicins have recently been shown to have good activity against pathogenic *E. coli* existing within biofilm communities [234,235], colicin M has been suggested as a candidate for a novel antimicrobial agent because its mode of action is common to all bacteria [236], and a hybrid phage lysin based on the structure of pesticin has promising narrow spectrum activity against members of the enterobactericeae [219]. However, the clinical application of colicins is unlikely to depend on their mode of action per se but may focus on an understanding of the import processes and ensuing interactions to disrupt essential cellular processes with colicin domains or novel synthetic peptide mimics.

Acknowledgements

We thank Jeremy Lakey for sharing unpublished data during the writing of this review, and we are grateful to Mireille Vankemmelbeke for helpful comments on the manuscript.

References

[1] M.A. Riley, J.E. Wertz, Bacteriocins: evolution, ecology, and application, Annu. Rev. Microbiol. 56 (2002) 117–137.
[2] J.-P. Gratia, André Gratia: a forerunner in microbial and viral genetics, Genetics 156 (2000) 471–476.
[3] M.A. Riley, J.E. Wertz, Bacteriocin diversity: ecological and evolutionary perspectives, Biochimie 84 (2002) 357–364.
[4] E. O'Connor, R. Shand, Halocins and sulfolobicins: the emerging story of archaeal protein and peptide antibiotics, J. Ind. Microbiol. Biotechnol. 28 (2002) 23–31.
[5] J. Nissen-Meyer, C. Oppegård, P. Rogne, H. Haugen, P. Kristiansen, Structure and mode-of-action of the two-peptide (class-IIb) bacteriocins probiotics, Antimicrob. Proteins 2 (2010) 52–60.
[6] C.J. Lazdunski, D. Baty, V. Géli, D. Cavard, J. Morlon, R. Lloubès, S.P. Howard, M. Knibiehler, M. Chartier, S. Varenne, et al., The membrane channel-forming colicin A: synthesis, secretion, structure, action and immunity, Biochim. Biophys. Acta 947 (1988) 445–464.
[7] M. El Ghachi, A. Bouhss, H. Barreteau, T. Touzé, G. Auger, D. Blanot, D. Mengin-Lecreulx, Colicin M exerts its bacteriolytic effect via enzymatic degradation of undecaprenyl phosphate-linked peptidoglycan precursors, J. Biol. Chem. (2006) M602834200.
[8] H. Brötz, G. Bierbaum, K. Leopold, P.E. Reynolds, H.-G. Sahl, The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II, Antimicrob. Agents Chemother. 42 (1998) 154–160.
[9] R. James, C.N. Penfold, G.R. Moore, C. Kleanthous, Killing of *E. coli* cells by E group nuclease colicins, Biochimie 84 (2002) 381–389.
[10] N.G. Housden, J.T.S. Hopper, N. Lukoyanova, D. Rodriguez-Larrea, J.A. Wojdyla, A. Klein, R. Kaminska, H. Bayley, H.R. Saibil, C.V. Robinson, C. Kleanthous, Intrinsically disordered protein threads through the bacterial outer-membrane porin OmpF, Science 340 (2013) 1570–1574.
[11] K.S. Jakes, A. Finkelstein, The colicin Ia receptor, Cir, is also the translocator for colicin Ia, Mol. Microbiol. 75 (2010) 567–578.
[12] O.E. Farrance, E. Hann, R. Kaminska, N.G. Housden, S.R. Derrington, C. Kleanthous, S. E. Radford, D.J. Brockwell, A force-activated trip switch triggers rapid dissociation of a colicin from its immunity protein, PLoS Biol. 11 (2013) e1001489.
[13] K.S. Jakes, W.A. Cramer, Border crossings: colicins and transporters, Annu. Rev. Genet. 46 (2012) 209–231.
[14] E. Cascales, S.K. Buchanan, D. Duché, C. Kleanthous, R. Lloubès, K. Postle, M. Riley, S. Slatin, D. Cavard, Colicin biology, Microbiol. Mol. Biol. Rev. 71 (2007) 158–229.
[15] L.C. DeVeaux, R.J. Kadner, Transport of vitamin B12 in *Escherichia coli*: cloning of the btuCD region, J. Bacteriol. 162 (1985) 888–896.
[16] K.G. Hardy, Colicinogeny and related phenomena, Bacteriol. Rev. 39 (1975) 464–515.
[17] H. Pilsl, V. Braun, Strong function-related homology between the pore-forming colicins K and 5, J. Bacteriol. 177 (1995) 6973–6977.
[18] E. Schramm, T. Olschläger, W. Troger, V. Braun, Sequence, expression and localization of the immunity protein for colicin B, Mol. Gen. Genet. 211 (1988) 176–182.
[19] R. Lloubès, D. Baty, C. Lazdunski, The promoters of the genes for colicin production, release and immunity in the ColA plasmid: effects of convergent transcription and Lex A protein, Nucleic Acids Res. 14 (1986) 2621–2636.
[20] K.-F. Chak, R. James, Characterization of the ColE9-J plasmid and analysis of its genetic organization, J. Gen. Microbiol. 132 (1986) 61–70.
[21] R. James, M. Jarvis, D.F. Barker, Nucleotide sequence of the immunity and lysis region of the ColE9-J plasmid, Microbiology 133 (2014) 1553–1562.
[22] V. Braun, S. Gaisser, C. Glaser, R. Harkness, T. Ölschläger, J. Mende, Import and export of Colicin M, in: R. James, C. Lazdunski, F. Pattus (Eds.), Bacteriocins, Microcins and Lantibiotics., vol. H65, Springer-Verlag, Berlin Heidelberg, 1992, pp. 225–242.
[23] S. Varenne, D. Cavard, C. Lazdunski, Biosynthesis and export of colicin A in *Citrobacter freundii* CA31, Eur. J. Biochem. 116 (1981) 615–620.
[24] D. Smajs, H. Pilsl, V. Braun, Colicin U, a novel colicin produced by *Shigella boydii*, J. Bacteriol. 179 (1997) 4919–4928.
[25] O. Gillor, J.A.C. Vriezen, M.A. Riley, The role of SOS boxes in enteric bacteriocin regulation, Microbiology 154 (2008) 1783–1792.
[26] M. Butala, S. Sonjak, S. Kamenšek, M. Hodošček, D.F. Browning, D. Žgur-Bertok, S.J.W. Busby, Double locking of an *Escherichia coli* promoter by two repressors prevents premature colicin expression and cell lysis, Mol. Microbiol. 86 (2012) 129–139.
[27] J. Mulec, Z. Podlesek, P. Mrak, A. Kopitar, A. Ihan, D. Žgur-Bertok, A cka-gfp transcriptional fusion reveals that the colicin K activity gene is induced in only 3 percent of the population, J. Bacteriol. 185 (2003) 654–659.
[28] A.P. Pugsley, Autoinduced synthesis of colicin E2, Mol. Gen. Genet. 190 (1983) 379–383.
[29] L. Ghazaryan, M.I.M. Soares, O. Gillor, Auto-regulation of DNA degrading bacteriocins: molecular and ecological aspects, Antonie Van Leeuwenhoek (2014) 1–12.
[30] I. Kuhar, D. Žgur-Bertok, Transcription regulation of the colicin K *cka* gene reveals induction of colicin synthesis by differential responses to environmental signals, J. Bacteriol. 181 (1999) 7373–7380.
[31] M.A. Riley, D.M. Gordon, The ecological role of bacteriocins in bacterial competition, Trends Microbiol. 7 (1999) 129–133.
[32] J. Luirink, D.M. Clark, J. Ras, E.J. Verschoor, F. Stegehuis, F.K. de Graaf, B. Oudega, pCloDF13-encoded bacteriocin release proteins with shortened carboxyl-terminal segments are lipid modified and processed and function in release of cloacin DF13 and apparent host cell lysis, J. Bacteriol. 171 (1989) 2673–2679.
[33] J. Luirink, J. de Jong, A.J. van Putten, F.K. de Graaf, B. Oudega, Functioning of a hybrid BRP-beta-lactamase protein in the release of cloacin DF13 and lysis of *Escherichia coli* cells, FEMS Microbiol. Lett. 49 (1989) 25–31.
[34] K.S. Jakes, P. Model, Mechanism of export of colicin E1 and colicin E3, J. Bacteriol. 138 (1979) 770–778.
[35] K.S. Jakes, N.D. Zinder, Plasmid ColE3 specifies a lysis protein, J. Bacteriol. 157 (1984) 582–590.
[36] A.P. Pugsley, Genetic analysis of ColN plasmid determinants for colicin production, release, and immunity, J. Bacteriol. 158 (1984) 523–529.
[37] A.P. Pugsley, M. Schwartz, Colicin E2 release: lysis, leakage or secretion? Possible role of a phospholipase, EMBO J. 3 (1984) 2393–2397.
[38] D. Baty, R. Lloubès, V. Géli, C. Lazdunski, S.P. Howard, Extracellular release of colicin A is non-specific, EMBO J. 6 (1987) 2463–2468.
[39] A.P. Pugsley, M. Schwartz, Expression of a gene in a 400-base-pair fragment of colicin plasmid ColE2-P9 is sufficient to cause host cell lysis, J. Bacteriol. 156 (1983) 109–114.
[40] J. Luirink, S. Hayashi, H.C. Wu, M.M. Kater, F.K. de Graaf, B. Oudega, Effect of a mutation preventing lipid modification on localization of the pCloDF13-encoded bacteriocin release protein and on release of cloacin DF13, J. Bacteriol. 170 (1988) 4153–4160.
[41] D. Cavard, D. Baty, S.P. Howard, H.M. Verheij, C. Lazdunski, Lipoprotein nature of the colicin A lysis protein: effect of amino acid substitutions at the site of modification and processing, J. Bacteriol. 169 (1987) 2187–2194.

[42] Y.-R. Chen, T.-Y. Yang, G.-S. Lei, L.-J.R. Lin, K.-F. Chak, Delineation of the translocation of colicin E7 across the inner membrane of *Escherichia coli*, Arch. Microbiol. 193 (2011) 419–428.
[43] J. Luirink, B. Duim, J.W. de Gier, B. Oudega, Functioning of the stable signal peptide of the pCloDF13-encoded bacteriocin release protein, Mol. Microbiol. 5 (1991) 393–399.
[44] F.J. van der Wal, B. Oudega, M.M. Kater, C.M. ten Hagen-Jongman, F.K. de Graaf, J. Luirink, The stable BRP signal peptide causes lethality but is unable to provoke the translocation of cloacin DF13 across the cytoplasmic membrane of *Escherichia coli*, Mol. Microbiol. 6 (1992) 2309–2318.
[45] D. Cavard, Role of Cal, the colicin A lysis protein, in two steps of colicin A release and in the interaction with colicin A-porin complexes, Microbiology 150 (2004) 3867–3875.
[46] K.S. Jakes, Daring to be different: colicin N finds another way, Mol. Microbiol. (2014), http://dx.doi.org/10.1111/mmi.12569.
[47] N. Dekker, J. Tommassen, H.M. Verheij, Bacteriocin release protein triggers dimerization of outer membrane phospholipase A *in vivo*, J. Bacteriol. 181 (1999) 3281–3283.
[48] H.R. Herschman, D.R. Helinski, Comparative study of the events associated with colicin induction, J. Bacteriol. 94 (1967) 691–699.
[49] D. Cavard, A. Bernadac, C. Lazdunski, Exclusive localization of colicin A in cell cytoplasm of producing bacteria, Eur. J. Biochem. 119 (1981) 125–131.
[50] A.P. Pugsley, J.P. Rosenbusch, Release of colicin E2 from *Escherichia coli*, J. Bacteriol. 147 (1981) 186–192.
[51] D. Cavard, A. Bernadac, J.M. Pages, C. Lazdunski, Colicins are not transiently accumulated in the periplasmic space before release from colicinogenic cells, Biol. Cell. 51 (1984) 79–86.
[52] E. Schramm, J. Mende, V. Braun, R.M. Kamp, Nucleotide sequence of the colicin B activity gene *cba*: consensus pentapeptide among TonB-dependent colicins and receptors, J. Bacteriol. 169 (1987) 3350–3357.
[53] V. Braun, S.I. Patzer, Intercellular communication by related bacterial protein toxins: colicins, contact-dependent inhibitors, and proteins exported by the type VI secretion system, FEMS Microbiol. Lett. 345 (2013) 13–21.
[54] C.L. Johnson, H. Ridley, R. Marchetti, A. Silipo, D.C. Griffin, L. Crawford, B. Bonev, A. Molinaro, J.H. Lakey, The antibacterial toxin Colicin N binds to the inner core of lipopolysaccharide and close to its translocator protein, Mol. Microbiol. (2014), http://dx.doi.org/10.1111/mmi.12568.
[55] L.C. McCaughey, R. Grinter, I. Josts, A.W. Roszak, K.I. Waløen, R.J. Cogdell, J. Milner, T. Evans, S. Kelly, N.P. Tucker, O. Byron, B. Smith, D. Walker, Lectin-like bacteriocins from *Pseudomonas spp.* utilise D-rhamnose containing lipopolysaccharide as a cellular receptor, PLoS Pathog. 10 (2014) e1003898.
[56] T. Köhler, V. Donner, C. van Delden, Lipopolysaccharide as shield and receptor for R-pyocin-mediated killing in *Pseudomonas aeruginosa*, J. Bacteriol. 192 (2010) 1921–1928.
[57] D. Kocíncová, J.S. Lam, A deletion in the wapB promoter in many serotypes of *Pseudomonas aeruginosa* accounts for the lack of a terminal glucose residue in the core oligosaccharide and resistance to killing by R3-pyocin, Mol. Microbiol. 89 (2013) 464–478.
[58] P.D. Pawelek, N. Croteau, C. Ng-Thow-Hing, C.M. Khursigara, N. Moiseeva, M. Allaire, J.W. Coulton, Structure of TonB in complex with FhuA, *E. coli* Outer membrane receptor, Science 312 (2006) 1399–1402.
[59] S. Devanathan, K. Postle, Studies on colicin B translocation: FepA is gated by TonB, Mol. Microbiol. 65 (2007) 441–453.
[60] M. Wiener, D. Freymann, P. Ghosh, R.M. Stroud, Crystal structure of colicin Ia, Nature 385 (1997) 461–464.
[61] J.L. Hilsenbeck, H. Park, G. Chen, B. Youn, K. Postle, C. Kang, Crystal structure of the cytotoxic bacterial protein colicin B at 2.5 Å resolution, Mol. Microbiol. 51 (2004) 711–720.
[62] C.R. Smallwood, A.G. Marco, Q. Xiao, V. Trinh, S.M.C. Newton, P.E. Klebba, Fluoresceination of FepA during colicin B killing: effects of temperature, toxin and TonB, Mol. Microbiol. 72 (2009) 1171–1180.
[63] Z. Cao, P.E. Klebba, Mechanisms of colicin binding and transport through outer membrane porins, Biochimie 84 (2002) 399–412.
[64] G. Bainbridge, G.A. Armstrong, L.G. Dover, K.F. Whelan, J.H. Lakey, Displacement of OmpF loop 3 is not required for the membrane translocation of colicins N and A *in vivo*, FEBS Lett. 432 (1998) 117–122.
[65] G. Bainbridge, H. Mobasheri, G.A. Armstrong, E.J. Lea, J.H. Lakey, Voltage-gating of *Escherichia coli* porin: a cystine-scanning mutagenesis study of loop 3, J. Mol. Biol. 275 (1998) 171–176.
[66] T.G. Baboolal, M.J. Conroy, K. Gill, H. Ridley, V. Visudtiphole, P.A. Bullough, J.H. Lakey, Colicin N binds to the periphery of Its receptor and translocator, outer membrane protein F, Structure 16 (2008) 371–379.
[67] C.L. Johnson, H. Ridley, R.J. Pengelly, M.Z. Salleh, J.H. Lakey, The unstructured domain of colicin N kills *Escherichia coli*, Mol. Microbiol. 89 (2013) 84–95.
[68] G. Kurisu, S.D. Zakharov, M.V. Zhalnina, S. Bano, V.Y. Eroukova, T.I. Rokitskaya, Y.N. Antonenko, M.C. Wiener, W.A. Cramer, The structure of BtuB with bound colicin E3 R-domain implies a translocon, Nat. Struct. Biol. 10 (2003) 948–954.
[69] O. Sharma, E. Yamashita, M.V. Zhalnina, S.D. Zakharov, K.A. Datsenko, B.L. Wanner, W.A. Cramer, Structure of the complex of the colicin E2 R-domain and its BtuB receptor: the outer membrane colicin translocon, J. Biol. Chem. 282 (2007) 23163–23170.
[70] S.K. Buchanan, P. Lukacik, S. Grizot, R. Ghirlando, M. Ali, T.J. Barnard, K.S. Jakes, P.K. Kienker, L. Esser, Structure of colicin I receptor bound to the R-domain of colicin Ia: implications for protein import, EMBO J. 26 (2007) 2594–2604.
[71] N.G. Housden, J.A. Wojdyla, J. Korczynska, I. Grishkovskaya, N. Kirkpatrick, A.M. Brzozowski, C. Kleanthous, Directed epitope delivery across the *Escherichia coli* outer membrane through the porin OmpF, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 21412–21417.
[72] N.G. Housden, C. Kleanthous, J.M.H. Michael, L. Johnson, K.A. Gary, Thermodynamic Dissection of Colicin Interactions, Methods in Enzymology, vol. 488, Academic Press, 2011. 123–145.
[73] S.D. Zakharov, O. Sharma, M.V. Zhalnina, E. Yamashita, W.A. Cramer, Pathways of colicin import: utilization of BtuB, OmpF porin and the TolC drug-export protein, Biochem. Soc. Trans. 40 (2012) 1463–1468.
[74] J. Spector, S. Zakharov, Y. Lill, O. Sharma, W.A. Cramer, K. Ritchie, Mobility of BtuB and OmpF in the *Escherichia coli* outer membrane: implications for dynamic formation of a translocon complex, Biophys. J. 99 (2010) 3880–3886.
[75] N.G. Housden, S.R. Loftus, G.R. Moore, R. James, C. Kleanthous, Cell entry mechanism of enzymatic bacterial colicins: porin recruitment and the thermodynamics of receptor binding, PNAS 102 (2005) 13849–13854.
[76] J.P. Bourdineaud, H.P. Fierobe, C. Lazdunski, J.M. Pages, Involvement of OmpF during reception and translocation steps of colicin N entry, Mol. Microbiol. 4 (1990) 1737–1743.
[77] H. Bénédetti, M. Frenette, D. Baty, R. Lloubès, V. Géli, C. Lazdunski, Comparison of the uptake systems for the entry of various BtuB group colicins into *Escherichia coli*, J. Gen. Microbiol. 135 (1989) 3413–3420.
[78] R. El Kouhen, J.M. Pages, Dynamic aspects of colicin N translocation through the *Escherichia coli* outer membrane, J. Bacteriol. 178 (1996) 5316–5319.
[79] E. Yamashita, M.V. Zhalnina, S.D. Zakharov, O. Sharma, W.A. Cramer, Crystal structures of the OmpF porin: function in a colicin translocon, EMBO J. 27 (2008) 2171–2180.
[80] N.G. Housden, C. Kleanthous, Colicin translocation across the *Escherichia coli* outer membrane, Biochem. Soc. Trans. 40 (2012) 1475–1479.
[81] R. Wallis, K.Y. Leung, A.J. Pommer, H. Videler, G.R. Moore, R. James, C. Kleanthous, Protein–protein interactions in colicin E9 DNase-immunity protein complexes. 2. Cognate and noncognate interactions that span the millimolar to femtomolar affinity range, Biochemistry 34 (1995) 13751–13759.
[82] W. Li, A.H. Keeble, C. Giffard, R. James, G.R. Moore, C. Kleanthous, Highly discriminating protein–protein interaction specificities in the context of a conserved binding energy hotspot, J. Mol. Biol. 337 (2004) 743–759.
[83] P.C. Cooper, F.K. Hawkins, R. James, Incompatibility between E colicin plasmids, J. Gen. Microbiol. 132 (1986) 1859–1862.
[84] D. Espesset, D. Duché, D. Baty, V. Géli, The channel domain of colicin A is inhibited by its immunity protein through direct interaction in the *Escherichia coli* inner membrane, EMBO J. 15 (1996) 2356–2364.
[85] K. Schaller, J.V. Höltje, V. Braun, Colicin M is an inhibitor of murein biosynthesis, J. Bacteriol. 152 (1982) 994–1000.
[86] T. Olschläger, V. Braun, Sequence, expression, and localization of the immunity protein for colicin M, J. Bacteriol. 169 (1987) 4765–4769.
[87] T. Olschläger, A. Turba, V. Braun, Binding of the immunity protein inactivates colicin M, Mol. Microbiol. 5 (1991) 1105–1111.
[88] K. Jakes, N.D. Zinder, T. Boon, Purification and properties of colicin E3 immunity protein, J. Biol. Chem. 249 (1974) 438–444.
[89] J. Sidikaro, M. Nomura, E3 immunity substance. A protein from E3-colicinogenic cells that accounts for their immunity to colicin E3, J. Biol. Chem. 249 (1974) 445–453.
[90] M. Graille, L. Mora, R.H. Buckingham, H. Van Tilbeurgh, M. de Zamaroczy, Structural inhibition of the colicin D tRNase by the tRNA-mimicking immunity protein, EMBO J. 23 (2004) 1474–1482.
[91] C. Luna-Chavez, Y.-L. Lin, R.H. Huang, Molecular basis of inhibition of the ribonuclease activity in colicin E5 by its cognate immunity protein, J. Mol. Biol. 358 (2014) 571–579.
[92] C. Kleanthous, D. Walker, Immunity proteins: enzyme inhibitors that avoid the active site, Trends Biochem. Sci. 26 (2001) 624–631.
[93] C. Kleanthous, U.C. Kühlmann, A.J. Pommer, N. Ferguson, S.E. Radford, G.R. Moore, R. James, A.M. Hemmings, Structural and mechanistic basis of immunity towards endonuclease colicins, Nat. Struct. Biol. 6 (1999) 243–252.
[94] Y.-S. Cheng, K.-C. Hsia, L.G. Doudeva, K.-F. Chak, H.S. Yuan, The crystal structure of the nuclease domain of colicin E7 suggests a mechanism for binding to double-stranded DNA by the H-N-H endonucleases, J. Mol. Biol. 324 (2002) 227–236.
[95] W.J. Krone, P. de Vries, G. Koningstein, A.J. de Jonge, F.K. de Graaf, B. Oudega, Uptake of cloacin DF13 by susceptible cells: removal of immunity protein and fragmentation of cloacin molecules, J. Bacteriol. 166 (1986) 260–268.
[96] D. Walker, G.R. Moore, R. James, C. Kleanthous, Thermodynamic consequences of bipartite immunity protein binding to the ribosomal ribonuclease colicin E3, Biochemistry 42 (2003) 4161–4171.
[97] R. Wallis, A. Reilly, A. Rowe, G.R. Moore, R. James, C. Kleanthous, *In vivo* and *in vitro* characterization of overproduced colicin E9 immunity protein, Eur. J. Biochem. 207 (1992) 687–695.
[98] M. Vankemmelbeke, B. Healy, G.R. Moore, C. Kleanthous, C.N. Penfold, R. James, Rapid detection of colicin E9 induced DNA damage using *Escherichia coli* cells carrying SOS promoter::*lux* fusions, J. Bacteriol. 187 (2005) 4900–4907.
[99] S. Soelaiman, K. Jakes, N. Wu, C. Li, M. Shoham, Crystal structure of colicin E3: implications for cell entry and ribosome inactivation, Mol. Cell 8 (2001) 1053–1062.
[100] D. Walker, L. Lancaster, R. James, C. Kleanthous, Identification of the catalytic motif of the microbial ribosome inactivating cytotoxin colicin E3, Protein Sci. 13 (2004) 1603–1611.
[101] Y. Zhang, M.N. Vankemmelbeke, L.E. Holland, D.C. Walker, R. James, C.N. Penfold, Investigating early events in receptor binding and translocation of colicin E9 using synchronized cell killing and proteolytic cleavage, J. Bacteriol. 190 (2008) 4342–4350.

[102] S.D. Zakharov, O. Sharma, M.V. Zhalnina, W.A. Cramer, Primary events in the colicin translocon: FRET analysis of colicin unfolding initiated by binding to BtuB and OmpF, Biochemistry 47 (2008) 12802–12809.
[103] S.D. Zakharov, M.V. Zhalnina, O. Sharma, W.A. Cramer, The colicin E3 outer membrane translocon: immunity protein release allows interaction of the cytotoxic domain with OmpF porin, Biochemistry 45 (2006) 10199–10207.
[104] D. Duché, A. Frenkian, V. Prima, R. Lloubès, Release of immunity protein requires functional endonuclease colicin import Machinery, J. Bacteriol. 188 (2006) 8593–8600.
[105] D.A. Bonsor, O. Hecht, M. Vankemmelbeke, A. Sharma, A.M. Krachler, N.G. Housden, K.J. Lilly, R. James, G.R. Moore, C. Kleanthous, Allosteric [beta]-propeller signalling in TolB and its manipulation by translocating colicins, EMBO J. 28 (2009) 2846–2857.
[106] M. Vankemmelbeke, Y. Zhang, G.R. Moore, C. Kleanthous, C.N. Penfold, R. James, Energy-dependent immunity protein release during tol-dependent nuclease colicin translocation, J. Biol. Chem. 284 (2009) 18932–18941.
[107] C.N. Penfold, B. Healy, N.G. Housden, R. Boetzel, M. Vankemmelbeke, G.R. Moore, C. Kleanthous, R. James, Flexibility in the receptor-binding domain of the enzymatic colicin E9 is required for toxicity against *Escherichia coli* cells, J. Bacteriol. 186 (2004) 4520–4527.
[108] M. Vankemmelbeke, N.G. Housden, R. James, C. Kleanthous, C.N. Penfold, Immunity protein release from a cell-bound nuclease colicin complex requires global conformational rearrangement, Microbiol. Open 2 (2013) 853–861.
[109] G. Papadakos, J.A. Wojdyla, C. Kleanthous, Nuclease colicins and their immunity proteins, Q. Rev. Biophys. 45 (2012) 57–103.
[110] J. Sturgis, Organisation and evolution of the tol-pal gene cluster, J. Mol. Microbiol. Biotechnol. 3 (2001) 113–122.
[111] E. Cascales, R. Lloubès, J.N. Sturgis, The TolQ-TolR proteins energize TolA and share homologies with the flagellar motor proteins MotA-MotB, Mol. Microbiol. 42 (2001) 795–807.
[112] E.L. Goemaere, E. Cascales, R. Lloubès, Mutational analyses define helix organization and key residues of a bacterial membrane energy-transducing complex, J. Mol. Biol. 366 (2007) 1424–1436.
[113] M.A. Gerding, Y. Ogata, N.D. Pecora, H. Niki, P.A.J. de Boer, The trans-envelope Tol–Pal complex is part of the cell division machinery and required for proper outer-membrane invagination during cell constriction in *E. coli*. Mol. Microbiol. 63 (2007) 1008–1025.
[114] M.A. Teleha, A.C. Miller, R.A. Larsen, Overexpression of the *Escherichia coli* TolQ protein leads to a null-FtsN-like division phenotype, Microbiol. Open 2 (2013) 618–632.
[115] S.K. Levengood, W.F. Beyer Jr., R.E. Webster, TolA: a membrane protein involved in colicin uptake contains an extended helical region, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 5939–5943.
[116] P. Germon, M.-C. Ray, A. Vianney, J.C. Lazzaroni, Energy-dependent conformational change in the TolA protein of *Escherichia coli* involves its N-terminal domain, TolQ, and TolR, J. Bacteriol. 183 (2001) 4110–4114.
[117] J.F. Dubuisson, A. Vianney, J.C. Lazzaroni, Mutational analysis of the TolA C-terminal domain of *Escherichia coli* and genetic evidence for an interaction between TolA and TolB, J. Bacteriol. 184 (2002) 4620–4625.
[118] A. Walburger, C. Lazdunski, Y. Corda, The Tol/Pal system function requires an interaction between the C-terminal domain of TolA and the N-terminal domain of TolB, Mol. Microbiol. 44 (2002) 695–708.
[119] E. Cascales, M. Gavioli, J.N. Sturgis, R. Lloubès, Proton motive force drives the interaction of the inner membrane TolA and outer membrane Pal proteins in *Escherichia coli*, Mol. Microbiol. 38 (2000) 904–915.
[120] C. Li, Y. Zhang, M. Vankemmelbeke, O. Hecht, F.S. Aleanizy, C. Macdonald, G.R. Moore, R. James, C.N. Penfold, Structural evidence that colicin A protein binds to a novel binding site of TolA protein in *Escherichia coli* Periplasm, J. Biol. Chem. 287 (2012) 19048–19057.
[121] C.N. Penfold, C. Li, Y. Zhang, M. Vankemmelbeke, R. James, Colicin A binds to a novel binding site of TolA in the *Escherichia coli* periplasm, Biochem. Soc. Trans. 40 (2012) 1469–1474.
[122] R. Lloubès, E. Cascales, A. Walburger, E. Bouveret, C. Lazdunski, A. Bernadac, L. Journet, The Tol–Pal proteins of the *Escherichia coli* cell envelope: an energized system required for outer membrane integrity? Res. Microbiol. 152 (2001) 523–529.
[123] E. Cascales, A. Bernadac, M. Gavioli, J.C. Lazzaroni, R. Lloubès, Pal lipoprotein of *Escherichia coli* plays a major role in outer membrane integrity, J. Bacteriol. 184 (2002) 754–759.
[124] E. Bouveret, R. Derouiche, A. Rigal, R. Lloubès, C. Lazdunski, H. Bénédetti, Peptidoglycan-associated lipoprotein-TolB interaction. A possible key to explaining the formation of contact sites between the inner and outer membranes of *Escherichia coli*, J. Biol. Chem. 270 (1995) 11071–11077.
[125] S. Carr, C.N. Penfold, V. Bamford, R. James, A.M. Hemmings, The structure of TolB, an essential component of the *tol*-dependent translocation system, and its protein–protein interaction with the translocation domain of colicin E9, Structure 8 (2000) 57–66.
[126] C. Abergel, E. Bouveret, J.-M. Claverie, K. Brown, A. Rigal, C. Lazdunski, H. Bénedétti, Structure of the *Escherichia coli* TolB protein determined by MAD methods at 1.95 Å resolution, Structure 7 (1999) 1291–1300.
[127] Y. Zhang, C. Li, M.N. Vankemmelbeke, P. Bardelang, M. Paoli, C.N. Penfold, R. James, The crystal structure of the TolB box of colicin A in complex with TolB reveals important differences in the recruitment of the common TolB translocation portal used by group A colicins, Mol. Microbiol. 75 (2010) 623–636.
[128] S.R. Loftus, D. Walker, M.J. Mate, D.A. Bonsor, R. James, G.R. Moore, C. Kleanthous, Competitive recruitment of the periplasmic translocation portal TolB by a natively disordered domain of colicin E9, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12353–12358.
[129] S.L. Hands, L.E. Holland, M. Vankemmelbeke, L. Fraser, C.J. Macdonald, G.R. Moore, R. James, C.N. Penfold, Interactions of TolB with the translocation domain of colicin E9 require an extended TolB Box, J. Bacteriol. 187 (2005) 6733–6741.
[130] A. Rigal, E. Bouveret, R. Lloubès, C. Lazdunski, H. Bénédetti, The TolB protein interacts with the porins of *Escherichia coli*, J. Bacteriol. 179 (1997) 7274–7279.
[131] T. Clavel, P. Germon, A. Vianney, R. Portalier, J.C. Lazzaroni, TolB protein of *Escherichia coli* K-12 interacts with the outer membrane peptidoglycan-associated proteins Pal, Lpp and OmpA, Mol. Microbiol. 29 (1998) 359–367.
[132] M. Isnard, A. Rigal, J.C. Lazzaroni, C. Lazdunski, R. Lloubès, Maturation and localization of the TolB protein required for colicin import, J. Bacteriol. 176 (1994) 6392–6396.
[133] S.K. Levengood, R.E. Webster, Nucleotide sequences of the *tolA* and *tolB* genes and localization of their products, components of a multistep translocation system in *Escherichia coli*, J. Bacteriol. 171 (1989) 6600–6609.
[134] R. Derouiche, G. Zeder-Lutz, H. Bénédetti, M. Gavioli, A. Rigal, C. Lazdunski, R. Lloubès, Binding of colicins A and E1 to purified TolA domains, Microbiology 143 (1997) 3185–3192.
[135] L. Journet, E. Bouveret, A. Rigal, R. Lloubès, C. Lazdunski, H. Bénédetti, Import of colicins across the outer membrane of *Escherichia coli* involves multiple protein interactions in the periplasm, Mol. Microbiol. 42 (2001) 331–344.
[136] J. Lubkowski, F. Hennecke, A. Pluckthun, A. Wlodawer, Filamentous phage infection: crystal structure of g3p in complex with its coreceptor, the C-terminal domain of TolA, Structure 7 (1999) 711–722.
[137] L. Riechmann, P. Holliger, The C-terminal domain of TolA is the coreceptor for filamentous phage infection of *E. coli*, Cell 90 (1997) 351–360.
[138] E. Bouveret, A. Rigal, C. Lazdunski, H. Bénédetti, Distinct regions of the colicin A translocation domain are involved in the interaction with TolA and TolB proteins upon import into *Escherichia coli*, Mol. Microbiol. 27 (1998) 143–157.
[139] O. Hecht, Y. Zhang, C. Li, C.N. Penfold, R. James, G.R. Moore, Characterisation of the interaction of colicin A with its co-receptor TolA, FEBS Lett. 584 (2010) 2249–2252.
[140] E.M. Raggett, G. Bainbridge, L.J. Evans, A. Cooper, J.H. Lakey, Discovery of critical TolA-binding residues in the bactericidal toxin colicin N: a biophysical approach, Mol. Microbiol. 28 (1998) 1335–1343.
[141] H. Bénédetti, C. Lazdunski, R. Lloubès, Protein import into *Escherichia coli*: colicins A and E1 interact with a component of their translocation system, EMBO J. 10 (1991) 1989–1995.
[142] G. Anderluh, I. Gökçe, J.H. Lakey, A natively unfolded toxin domain uses its receptor as a folding template, J. Biol. Chem. 279 (2004) 22002–22009.
[143] O. Hecht, H. Ridley, R. Boetzel, A. Lewin, N. Cull, D.A. Chalton, J.H. Lakey, G.R. Moore, Self-recognition by an intrinsically disordered protein, FEBS Lett. 388 (2009) 880–893.
[144] S.L. Schendel, E.M. Click, R.E. Webster, W.A. Cramer, The TolA protein interacts with colicin E1 differently than with other group A colicins, J. Bacteriol. 179 (1997) 3683–3690.
[145] O. Sharma, K.A. Datsenko, S.C. Ess, M.V. Zhalnina, B.L. Wanner, W.A. Cramer, Genome-wide screens: novel mechanisms in colicin import and cytotoxicity, Mol. Microbiol. 73 (2009) 571–585.
[146] M.E. Bayer, Areas of adhesion between wall and membrane of *Escherichia coli*, J. Gen. Microbiol. 53 (1968) 395–404.
[147] E. Bouveret, L. Journet, A. Walburger, E. Cascales, H. Bénédetti, R. Lloubès, Analysis of the *Escherichia coli* Tol-Pal and TonB systems by periplasmic production of Tol, TonB, colicin, or phage capsid soluble domains, Biochimie 84 (2002) 413–421.
[148] R. Derouiche, H. Bénédetti, J.C. Lazzaroni, C. Lazdunski, R. Lloubès, Protein complex within *Escherichia coli* inner membrane. TolA N-terminal domain interacts with TolQ and TolR proteins, J. Biol. Chem. 270 (1995) 11078–11084.
[149] L. Journet, A. Rigal, C. Lazdunski, H. Bénédetti, Role of TolR N-terminal, central, and C-terminal domains in dimerization and interaction with TolA and TolQ, J. Bacteriol. 181 (1999) 4476–4484.
[150] E.L. Goemaere, A. Devert, R. Lloubès, E. Cascales, Movements of the TolR C-terminal domain depend on TolQR ionizable key residues and regulate activity of the Tol complex, J. Biol. Chem. 366 (2007) 1424–1436.
[151] K. Eick-Helmerich, V. Braun, Import of biopolymers into *Escherichia coli*: nucleotide sequences of the *exbB* and *exbD* genes are homologous to those of the *tolQ* and *tolR* genes, respectively, J. Bacteriol. 171 (1989) 5117–5126.
[152] V. Braun, C. Herrmann, Evolutionary relationship of uptake systems for biopolymers in Escherichia coli: cross-complementation between the TonB-ExbB-ExbD and the TolA-TolQ-TolR proteins, Mol. Microbiol. 8 (1993) 261–268.
[153] H. Killmann, G. Videnov, G. Jung, H. Schwarz, V. Braun, Identification of receptor binding sites by competitive peptide mapping: phages T1, T5, and phi 80 and colicin M bind to the gating loop of FhuA, J. Bacteriol. 177 (1995) 694–698.
[154] V. Braun, Energy-coupled transport and signal transduction through the gram-negative outer membrane via TonB-ExbB-ExbD-dependent receptor proteins, FEMS Microbiol. Rev. 16 (1995) 295–307.
[155] E. Fischer, K. Gunter, V. Braun, Involvement of ExbB and TonB in transport across the outer membrane of *Escherichia coli*: phenotypic complementation of *exb* mutants by overexpressed *tonB* and physical stabilization of TonB by ExbB, J. Bacteriol. 171 (1989) 5127–5134.
[156] C. Bradbeer, The proton motive force drives the outer membrane transport of cobalamin in Escherichia coli, J. Bacteriol. 175 (1993) 3146–3150.
[157] P.I. Higgs, P.S. Myers, K. Postle, Interactions in the TonB-dependent energy transduction complex: ExbB and ExbD form homomultimers, J. Bacteriol. 180 (1998) 6031–6038.
[158] P.I. Higgs, R.A. Larsen, K. Postle, Quantificaiton of known components of the Escherichia coli TonB energy transduction system:TonB, ExbB, ExbD and FepA, Mol. Microbiol. 44 (2002) 271–281.
[159] A.A. Ollis, K. Postle, ExbD mutants define initial stages in TonB energization, J. Mol. Biol. 415 (2012) 237–247.

[160] A.A. Ollis, K. Postle, Identification of functionally important TonB-ExbD periplasmic domain interactions in vivo, J. Bacteriol. 194 (2012) 3078–3087.
[161] H. Pilsl, V. Braun, The Ton system can functionally replace the TolB protein in the uptake of mutated colicin U, FEMS Microbiol. Lett. 164 (1998) 363–367.
[162] M. Witty, C. Sanz, A. Shah, J.G. Grossmann, K. Mizuguchi, R.N. Perham, B. Luisi, Structure of the periplasmic domain of *Pseudomonas aeruginosa* TolA: evidence for an evolutionary relationship with the TonB transporter protein, EMBO J. 21 (2002) 4207–4218.
[163] R. Koebnik, The molecular interaction between components of the TonB-ExbBD-dependent and of the TolQRA-dependent bacterial uptake systems, Mol. Microbiol. 9 (1993) 219.
[164] R.A. Larsen, G.E. Wood, K. Postle, The conserved proline-rich motif is not essential for energy transduction by *Escherichia coli* TonB protein [published erratum appears in Mol Microbiol 1994 Jun;12(5):857], Mol. Microbiol. 10 (1993) 943–953.
[165] N. Cadieux, R.J. Kadner, Site-directed disulfide bonding reveals an interaction site between energy-coupling protein TonB and BtuB, the outer membrane cobalamin transporter, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10673–10678.
[166] M. Ogierman, V. Braun, Interactions between the outer membrane ferric citrate transporter FecA and TonB: studies of the FecA TonB box, J. Bacteriol. 185 (2003) 1870–1885.
[167] J. Mende, V. Braun, Import-defective colicin B derivatives mutated in the TonB box, Mol. Microbiol. 4 (1990) 1523–1533.
[168] H. Pilsl, C. Glaser, P. Gross, H. Killmann, T. Olschläger, V. Braun, Domains of colicin M involved in uptake and activity, Mol. Gen. Genet. 240 (1993) 103–112.
[169] D.D. Shultis, M.D. Purdy, C.N. Banchs, M.C. Wiener, Outer membrane active transport: structure of the BtuB:TonB complex, Science 312 (2006) 1396–1399.
[170] L. Mora, N. Diaz, R.H. Buckingham, M. de Zamaroczy, Import of the transfer RNase Colicin D requires site-specific interaction with the energy-transducing protein TonB, J. Bacteriol. 187 (2005) 2693–2697.
[171] V. Braun, S.I. Patzer, K. Hantke, Ton-dependent colicins and microcins: modular design and evolution, Biochimie 84 (2002) 365–380.
[172] A.D. Ferguson, E. Hofmann, J.W. Coulton, K. Diederichs, W. Welte, Siderophore-mediated iron transport: crystal structure of FhuA with bound lipopolysaccharide, Science 282 (1998) 2215–2220.
[173] I. Mathavan, K. Beis, The role of bacterial membrane proteins in the internalization of microcin MccJ25 and MccB17, Biochem. Soc. Trans. 40 (2012) 1539–1543.
[174] F. Endriß, V. Braun, Loop deletions indicate regions important for FhuA transport and receptor functions in *Escherichia coli*, J. Bacteriol. 186 (2004) 4818–4823.
[175] J. Langenscheid, H. Killmann, V. Braun, A FhuA mutant of *Escherichia coli* is infected by phage T1-independent of TonB, FEMS Microbiol. Lett. 234 (2004) 133–137.
[176] H. Pilsl, V. Braun, Novel colicin 10: assignment of four domains to TonB- and TolC-dependent uptake via the Tsx receptor and to pore formation, Mol. Microbiol. 16 (1995) 57–67.
[177] C. Kleanthous, Translocator hunt comes full Cir-Col, Mol. Microbiol. 75 (2010) 529–533.
[178] A. Sauter, S.P. Howard, V. Braun, In vivo evidence for TonB dimerization, J. Bacteriol. 185 (2003) 5747–5754.
[179] C.M. Khursigara, G. De Crescenzo, P.D. Pawelek, J.W. Coulton, Kinetic analyses reveal multiple steps in forming TonB-FhuA complexes from *Escherichia coli*, Biochemistry 44 (2005) 3441–3453.
[180] C.M. Khursigara, G. De Crescenzo, P.D. Pawelek, J.W. Coulton, Enhanced binding of TonB to a ligand-loaded outer membrane receptor: role of the oligomeric state of TonB in formation of a functional FhuA.TonB complex, J. Biol. Chem. 279 (2004) 7405–7412.
[181] D.M. Freed, S.M. Lukasik, A. Sikora, A. Mokdad, D.S. Cafiso, Monomeric TonB and the Ton Box are required for the formation of a high-affinity transporter-TonB complex, Biochemistry 52 (2013) 2638–2648.
[182] S. Carr, D. Walker, R. James, C. Kleanthous, A.M. Hemmings, Inhibition of a ribosome-inactivating ribonuclease: the crystal structure of the cytotoxic domain of colicin E3 in complex with its immunity protein, Struct. Fold. Des. 8 (2000) 949–960.
[183] K. Mosbahi, D. Walker, E. Lea, G.R. Moore, R. James, C. Kleanthous, Destabilization of the colicin E9 endonuclease domain by interaction with negatively charged phospholipids: implications for colicin translocation into bacteria, J. Biol. Chem. 279 (2004) 22145–22151.
[184] K. Mosbahi, D. Walker, R. James, G.R. Moore, C. Kleanthous, Global structural rearrangement of the cell penetrating ribonuclease colicin E3 on interaction with phospholipid membranes, Protein Sci. 15 (2006) 620–627.
[185] K. Mosbahi, C. Lemaître, A.H. Keeble, H. Mobasheri, B. Morel, R. James, G.R. Moore, E.J.A. Lea, C. Kleanthous, The cytotoxic domain of Colicin E9 is a channel-forming endonuclease, Nat. Struct. Biol. 9 (2002) 476–484.
[186] D. Walker, K. Mosbahi, M. Vankemmelbeke, R. James, C. Kleanthous, The role of electrostatics in colicin nuclease domain translocation into bacterial cells, J. Biol. Chem. 282 (2007) 31389–31397.
[187] M. Vankemmelbeke, P. O'Shea, R. James, C.N. Penfold, Interaction of nuclease colicins with membranes: insertion depth correlates with bilayer perturbation, PLoS ONE 7 (2012) e46656.
[188] M. Vankemmelbeke, R. James, C.N. Penfold, Membrane activities of colicin nuclease domains: analogies with antimicrobial peptides, Biochem. Soc. Trans. 40 (2012) 1517–1521.
[189] S. Krzywda, A.M. Brzozowski, C. Verma, K. Karata, T. Ogura, A.J. Wilkinson, The Crystal Structure of the AAA Domain of the ATP-Dependent Protease FtsH of *Escherichia coli* at 1.5 Å Resolution, Structure 10 (2002) 1073–1083.
[190] R. Suno, H. Niwa, D. Tsuchiya, X. Zhang, M. Yoshida, K. Morikawa, Structure of the whole cytosolic region of ATP-dependent protease FtsH, Mol. Cell 22 (2006) 575–585.
[191] K. Ito, Y. Akiyama, Cellular functions, mechanism of action, and regulation of FtsH protease, Annu. Rev. Microbiol. 59 (2005) 211–231.
[192] N. Saikawa, Y. Akiyama, K. Ito, FtsH exists as an exceptionally large complex containing HflKC in the plasma membrane of *Escherichia coli*, J. Struct. Biol. 146 (2004) 123–129.
[193] M. Chauleau, L. Mora, J. Serba, M. de Zamaroczy, FtsH-dependent processing of RNase colicins D and E3 means that only the cytotoxic domains are imported into the cytoplasm, J. Biol. Chem. 286 (2011) 29397–29407.
[194] Y. Akiyama, Quality control of cytoplasmic membrane proteins in *Escherichia coli*, J. Biochem. 146 (2009) 449–454.
[195] D. Duché, Colicin E2 is still in contact with its receptor and import machinery when its nuclease domain enters the cytoplasm, J. Bacteriol. 189 (2007) 4217–4222.
[196] D. Duché, M. Issouf, R. Lloubès, Immunity protein protects colicin E2 from OmpT protease, J. Biochem. 145 (2009) 95–101.
[197] M. Masi, P. Vuong, M. Humbard, K. Malone, R. Misra, Initial steps of colicin E1 import across the outer membrane of *Escherichia coli*, J. Bacteriol. 189 (2007) 2667–2676.
[198] Z. Shi, K.-F. Chak, H.S. Yuan, Identification of an essential cleavage site in ColE7 required for import and killing of cells, J. Biol. Chem. 280 (2005) 24663–24668.
[199] D.C. Walker, T. Georgiou, A.J. Pommer, D. Walker, G.R. Moore, C. Kleanthous, R. James, Mutagenic scan of the H-N-H motif of colicin E9: implications for the mechanistic enzymology of colicins, homing enzymes and apoptotic endonucleases, Nucleic Acids Res. 30 (2002) 3225–3234.
[200] S. Stumpe, R. Schmid, D.L. Stephens, G. Georgiou, E.P. Bakker, Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*, J. Bacteriol. 180 (1998) 4002–4006.
[201] L. Vandeputte-Rutten, R.A. Kramer, J. Kroon, N. Dekker, M.R. Egmond, P. Gros, Crystal structure of the outer membrane protease OmpT from *Escherichia coli* suggests a novel catalytic site, EMBO J 20 (2001) 5033–5039.
[202] M. de Zamaroczy, L. Mora, A. Lecuyer, V. Géli, R.H. Buckingham, Cleavage of colicin D is necessary for cell killing and requires the inner membrane peptidase LepB, Mol. Cell 8 (2001) 159–168.
[203] M. de Zamaroczy, R.H. Buckingham, Importation of nuclease colicins into *E. coli* cells: endoproteolytic cleavage and its prevention by the immunity protein, Biochimie 84 (2002) 423–432.
[204] M. de Zamaroczy, L. Mora, Hijacking cellular functions for processing and delivery of colicins E3 and D into the cytoplasm, Biochem. Soc. Trans. 40 (2012) 1486–1491.
[205] Y. Michel-Briand, C. Baysse, The pyocins of *Pseudomonas aeruginosa*, Biochimie 84 (2002) 499–510.
[206] S.A. Morse, B.V. Jones, P.G. Lysko, Pyocin inhibition of *Neisseria gonorrhoeae*: mechanism of action, Antimicrob. Agents Chemother. 18 (1980) 416–423.
[207] C. Baysse, J.-M. Meyer, P. Plesiat, V.R. Geoffroy, Y. Michel-Briand, P. Cornelis, Uptake of pyocin S3 occurs through the outer membrane ferripyoverdine type II receptor of *Pseudomonas aeruginosa*, J. Bacteriol. 181 (1999) 3849–3851.
[208] S. Denayer, S. Matthijs, P. Cornelis, Pyocin S2 (Sa) kills *Pseudomonas aeruginosa* strains via the FpvA type I ferripyoverdine receptor, J. Bacteriol. 189 (2007) 7663–7668.
[209] A. Elfarash, Q. Wei, P. Cornelis, The soluble pyocins S2 and S4 from *Pseudomonas aeruginosa* bind to the same FpvAI receptor, Microbiol. Open 1 (2012) 268–275.
[210] A. Elfarash, J. Dingemans, L. Ye, A.A. Hassan, M. Craggs, C. Reimmann, M.S. Thomas, P. Cornelis, Pore-forming pyocin S5 utilizes the FptA ferripyochelin receptor to kill *Pseudomonas aeruginosa*, Microbiology 160 (2014) 261–269.
[211] J.J. Dennis, E.R. Lafontaine, P.A. Sokol, Identification and characterization of the tolQRA genes of *Pseudomonas aeruginosa*, J. Bacteriol. 178 (1996) 7059–7068.
[212] M. Shirley, I.L. Lamont, Role of TonB1 in pyoverdine-mediated signaling in *Pseudomonas aeruginosa*, J. Bacteriol. 191 (2009) 5634–5640.
[213] D. Cobessi, H. Celia, N. Folschweiller, I.J. Schalk, M.A. Abdallah, F. Pattus, The crystal structure of the pyoverdine outer membrane receptor FpvA from *Pseudomonas aeruginosa* at 3.6 Angstroms resolution, J. Mol. Biol. 347 (2005) 121–134.
[214] I. Ohkawa, S. Shiga, M. Kageyama, Effect of iron concentration in the growth medium on the sensitivity of *Pseudomonas aeruginosa* to pyocin S2, J. Biochem. 87 (1980) 323–331.
[215] V. Braun, S. Helbig, S.I. Patzer, Import of periplasmic bacteriocins targeting the murein, Biochem. Soc. Trans. 40 (2012) 1449–1455.
[216] A. Rakin, E. Boolgakowa, J. Heesemann, Structural and functional organization of the *Yersinia pestis* bacteriocin pesticin gene cluster, Microbiology 142 (1996) 3415–3424.
[217] S.I. Patzer, R. Albrecht, V. Braun, K. Zeth, Structural and mechanistic studies of pesticin, a bacterial homolog of phage lysozymes, J. Biol. Chem. 287 (2012) 23381–23396.
[218] W. Vollmer, H. Pilsl, K. Hantke, J.V. Höltje, V. Braun, Pesticin displays muramidase activity, J. Bacteriol. 179 (1997) 1580–1583.
[219] P. Lukacik, T.J. Barnard, P.W. Keller, K.S. Chaturvedi, N. Seddiki, J.W. Fairman, N. Noinaj, T.L. Kirby, J.P. Henderson, A.C. Steven, B.J. Hinnebusch, S.K. Buchanan, Structural engineering of a phage lysin that targets Gram-negative pathogens, Proc. Natl. Acad. Sci. 109 (2012) 9857–9862.
[220] K. Yamada, M. Hirota, Y. Niimi, H.A. Nguyen, Y. Takahara, Y. Kamio, J. Kaneko, Nucleotide sequences and organization of the genes for carotovoricin (Ctv) from *Erwinia carotovora* indicate that Ctv evolved from the same ancestor as *Salmonella typhi* prophage, Biosci. Biotechnol. Biochem. 70 (2006) 2236–2247.
[221] A.H. Nguyen, T. Tomita, M. Hirota, T. Sato, Y. Kamio, A simple purification method and morphology and component analyses for carotovoricin Er, a phage-tail-like bacteriocin from the plant pathogen *Erwinia carotovora* Er, Biosci. Biotechnol. Biochem. 63 (1999) 1360–1369.
[222] D.-Y. Chuang, Y.-C. Chien, H.-P. Wu, Cloning and expression of the *Erwinia carotovora* subsp. *carotovora* gene encoding the low-molecular-weight bacteriocin carocin S1, J. Bacteriol. 189 (2007) 620–626.

[223] Y.-C. Chan, H.-P. Wu, D.-Y. Chuang, Extracellular secretion of Carocin S1 in *Pectobacterium carotovorum* subsp. *carotovorum* occurs via the type III secretion system integral to the bacterial flagellum, BMC Microbiol. 9 (2009) 181.
[224] Y.-C. Chan, J.-L. Wu, H.-P. Wu, K.-C. Tzeng, D.-Y. Chuang, Cloning, purification, and functional characterization of Carocin S2, a ribonuclease bacteriocin produced by *Pectobacterium carotovorum*, BMC Microbiol. 11 (2011) 99.
[225] E. Roh, T.-H. Park, M.-I. Kim, S. Lee, S. Ryu, C.-S. Oh, S. Rhee, D.-H. Kim, B.-S. Park, S. Heu, Characterization of a new bacteriocin, carocin D, from *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21, Appl. Environ. Microbiol. 76 (2010) 7541–7549.
[226] R. Grinter, J. Milner, D. Walker, Ferredoxin containing bacteriocins suggest a novel mechanism of iron uptake in *Pectobacterium* spp, PLoS ONE 7 (2012) e33033.
[227] R. Grinter, J. Milner, D. Walker, Beware of proteins bearing gifts: protein antibiotics that use iron as a Trojan horse, FEMS Microbiol. Lett. 338 (2013) 1–9.
[228] W.L. Haag, A.K. Vidaver, Purification and characterization of syringacin 4-A, a bacteriocin from *Pseudomonas syringae* 4-A, Antimicrob. Agents Chemother. 6 (1974) 76–83.
[229] R.E. Harkness, V. Braun, Inhibition of lipopolysaccharide O-antigen synthesis by colicin M, J. Biol. Chem. 264 (1989) 14716–14722.
[230] R.E. Harkness, V. Braun, Colicin M inhibits peptidoglycan biosynthesis by interfering with lipid carrier recycling, J. Biol. Chem. 264 (1989) 6177–6182.
[231] R. Grinter, A.W. Roszak, R.J. Cogdell, J.J. Milner, D. Walker, The crystal structure of the lipid II-degrading bacteriocin syringacin M suggests unexpected evolutionary relationships between colicin M-like bacteriocins, J. Biol. Chem. 287 (2012) 38876–38888.
[232] J. Dischinger, S. Basi Chipalu, G. Bierbaum, Lantibiotics: promising candidates for future applications in health care, Int. J. Med. Microbiol. 304 (2014) 51–62.
[233] P.D. Cotter, R.P. Ross, C. Hill, Bacteriocins — a viable alternative to antibiotics? Nat. Rev. Microbiol. 11 (2013) 95–105.
[234] C.L. Brown, K. Smith, L. McCaughey, D. Walker, Colicin-like bacteriocins as novel therapeutic agents for the treatment of chronic biofilm-mediated infection, Biochem. Soc. Trans. 40 (2012) 1549–1552.
[235] O. Rendueles, C. Beloin, P. Latour-Lambert, J.M. Ghigo, A new biofilm-associated colicin with increased efficiency against biofilm bacteria, ISME J. (2014), http://dx.doi.org/10.1038/ismej.2013.238.
[236] S. Kamenšek, D. Žgur-Bertok, Global transcriptional responses to the bacteriocin colicin M in *Escherichia coli*, BMC Microbiol. 13 (2013) 42.