Bacteria produce under stress conditions bacteriocins and microcins that display antibacterial activity against closely related species for survival. Bacteriocins and microcins exert their antibacterial activity by either disrupting the membrane or inhibiting essential intracellular processes of the bacterial target. To this end, they can lyse bacterial membranes and cause subsequent loss of their integrity or nutrients, or hijack membrane receptors for internalisation. Both bacteriocins and microcins are ribosomally synthesised and several are posttranslationally modified, whereas others are not. Such peptides are also toxic to the producing bacteria, which utilise immunity proteins or dedicated ATP-binding cassette (ABC) transporters to achieve self-immunity and peptide export. In this review, we discuss the structure and mechanism of self-protection that is conferred by these ABC transporters.

**Keywords:** ABC transporters; antibacterial peptides; bacteriocins; lantibiotics; mechanism; microcin; multidrug resistance; self-immunity; structure
Bacteriocins from Gram-positive bacteria

The AMP class of lanthipeptides is defined by two particular amino acid modifications being the bis-amino-bis-acids lanthionine (Lan) or 3-methyllanthionine (McLan) [5,6]. This PTM is catalysed by one or two specific enzymes in two steps. First, serine and threonine residues are dehydrated resulting in the unnatural α, β unsaturated amino acids 2,3-didehydroalanine (Dha) and 2,3-didehydrobutyrine (Dhb) [7,8], followed by a Michael-type condensation of these amino acids with cysteine residues yielding a thioether crosslink, so-called lanthionine rings (Lan or McLan rings). In general, the lanthipeptide is transcribed as a core peptide fused to an N-terminally located leader peptide. The modifications occur only in the core peptide and not in the N-terminal leader peptide, which is recognised by the modification enzymes and by the ATP-binding cassette (ABC) transporter which is used for secretion. After secretion, the leader peptide is cleaved off by either specific or nonspecific peptidases, as in the case of subtilin [9]. This cleavage represents the activation step of lanthipeptides.

Lanthipeptides are produced by specific biosynthetic clusters mainly in Gram-positive bacteria, but their occurrence is not restricted to this group; they are also found in Gram-negative bacteria [10] and cyanobacteria [11,12]. Lanthipeptides have been classified by their properties (e.g. their antimicrobial activity) or by their structural diversity in the past [13–16]. In 2013, a new and still commonly accepted classification was introduced based on the characteristics of the modification enzymes [1]. Recently, a new class was identified, which brings the number of different classes to five [17,18]. In class I, the enzymes needed for the modification are two single monofunctional enzymes where the dehydratase (LanB) and the cyclase (LanC) are forming a complex in the cell. In class II, these two activities are performed by a single, bifunctional large protein termed LanM containing both the dehydratase domain and a LanC-like cyclase domain. Class III and class IV contain trifunctional synthetases, including lyase, kinase and cyclase domains, LanKC and LanL, respectively, but with different cyclase domains [1]. Class V contains a triprotein synthetase (LanK, LanX and LanY) [17,18]. The different structures and mechanisms of the enzymes from class I-IV have been reviewed in detail in references [19,20].

Lanthipeptide operons are organised as biosynthetic gene clusters, which encode a precursor peptide (LanA) that is located with the enzymes for modification (class I: LanB and LanC or class II: LanM/LanKC), transport (LanT), processing (LanP), regulation (LanR and LanK), immunity for class I and class II (LanI, LanFEG) and in some cases accessory proteins found in class II (e.g. LanH; Fig. 1).

Some gene clusters are located on chromosomes (e.g. subtilin [21]), on mobile gene elements such as conjugative transposons (e.g. nisin [22,23]) or plasmids (e.g. epidermin [24] or Pep5 [25]). The mobile gene elements point to the possibility of horizontal gene transfer during evolution in Gram-positive bacteria [26,27].

In general, the biosynthesis of lanthipeptides starts at the ribosome, where the mRNA is translated into the corresponding amino acid sequence of the unmodified precursor peptide [28]. This is the target of the different modification enzymes (Fig. 1). The maturation starts by dehydration of Ser and Thr residues resulting in Dha or Dhb, which is catalysed by LanB, in the case of class I lanthipeptides, by a bifunctional enzyme termed LanM, for class II, the trifunctional LanKC, or LanL in class III and class IV or triprotein synthetases (LanK, LanX and LanY) in class V, respectively (Fig. 1) [20,29–32]. As a second step, the lanthionine rings are formed by a Michael-type addition with a cysteine side chain.

The modified lanthipeptide precursor peptides are set for either direct secretion or cytoplasmic maturation prior to the translocation process, which is mediated by specific ABC transporters (see section Lanthipeptide export by ABC transporters – structure and mechanism). After the final steps of lanthipeptide secretion and maturation, the lanthipeptide is released. The active and mature form of the lanthipeptide would not only target other Gram-positive bacteria but also the cellular membrane or specific receptors of the producer strain itself. This especially holds true for lanthipeptides, which display antimicrobial activity in the nanomolar range (termed lantibiotics). The simultaneous expression of specific immunity proteins confers immunity to the producing strain against its own lantibiotic and is therefore a prerequisite. The genes encoding these proteins are located adjacent on the same gene cluster as the genes coding for maturation, transport and activation proteins (Fig. 1).

Antimicrobial activity has been reported for class I and class II lanthipeptides, which are termed lantibiotics. Many of these are highly active against Gram-positive bacteria from the genera of Bacillus, Clostridium, Lactococcus, Micrococcus, Streptococcus and Staphylococcus [33–36]. Lantibiotics appear to have only a very limited effect on Gram-negative bacteria or yeasts [37,38]. Very likely, the additional outer membrane of Gram-negative bacteria or the cell wall of yeasts acts as a barrier and is generally not permeable to these peptides. Lantibiotics possess antimicrobial activity.
through several mechanisms including pore formation, inhibition of peptidoglycan synthesis or rather specific mode of action (e.g. targeting a certain receptor in the cell wall). Nisin, subtilin and mersacidin inhibit the peptidoglycan biosynthesis [39-41]. The mechanism of inhibition by nisin has been investigated in detail on the molecular level. Nisin interacts with the pyrophosphate of lipid II via its N terminus to form a complex at the membrane with a ratio of 1 : 1 [42]. As a consequence, the complex dissociates from the septum and the cell wall precursor lipid II is no longer available for cell wall synthesis [43]. Mersacidin [44], nukacin ISK-1 [45], plantaricin C [46], lacticin 3147 [47] and lichenicidin [48] also bind to lipid II or other peptidoglycan precursors. Additionally, nisin has a pore-forming mode of action, where the aforementioned membrane complex of nisin and lipid II subsequently forms a membrane pore of four nisin and eight lipid II molecules with a diameter of 2-2.5 nm [49-51]. These pore-forming complexes are responsible for cell lysis [49,52-56].

Although class III and class IV lanthipeptides are not associated with antimicrobial activity, some other bioactivities have been described for a small number of class III lanthipeptides. The lanthipeptide NAI-112 has low antimicrobial activity against staphylococci and streptococci, but more interestingly a highly antinociceptive activity has been determined [57]. The antiviral activity against HIV and HSV has been described for labyrinthopeptin A1 [58], where the lanthipeptides SapT and SapB have a morphogenic activity on the hyphae of streptomycetes [59,60].

Microcins from Gram-negative bacteria

Microcins are antibacterial peptides of low molecular weight, < 10 kDa, that are produced by Enterobacteriaceae. Like other AMPs, they are secreted under
conditions of nutrient exhaustion and exert potent antibacterial activity against closely related species. Microcins are ribosomally synthesised, and some of them are posttranslationally modified. Their biosynthetic cluster encodes a linear precursor peptide, the maturation enzymes that modify the peptide and immunity proteins that are either degrading enzymes, binding proteins or transporters (for an in-depth review on microcin biosynthesis see reference [61]; Fig. 2). The linear precursors have a leader sequence that is important for recognition by the synthetases and in a few cases by their ABC transporters, which display peptidase activity to remove it (see section Class II microcin export by ABC transporters – structure and mechanism). Microcins can be classified into class I and class II [1,61]. Class I microcins are usually plasmid-encoded and posttranslationally modified. Microcins that belong to the class I include MccB17, containing thiazole and oxazole rings [62], MccJ25, possessing a lasso topology [4], or MccC7/C51, containing a nucleotide [63]. Class II microcins can be subdivided into IIa and IIb; microcins of class IIa are plasmid-encoded but not modified (MccL, MccV, Mcc24), whereas microcins of class IIb are chromosomally encoded linear peptides with a C-terminal siderophore modification (MccE492, MccM, MccH47 and MccI47).

Microcins are targeting and inhibiting essential enzymes located in the cytoplasm such as DNA gyrase (MccB17 [64,65]) or RNA polymerase (MccJ25 [66]). Some microcins disrupt the bacterial inner membrane by pore formation like MccE492 [67]; its pore formation activity is dependent on the mannose transporter ManYZ, but the exact mechanism is unknown [68]. In order to reach their targets, they need to cross both, the outer and inner membrane of Gram-negative bacteria. Although these microcins are structurally very different, they all cross the outer membrane by hijacking outer membrane proteins [69], including siderophore receptors or porins (Fig. 2). Upon crossing the outer membrane, microcins that do not disrupt the inner membrane utilise secondary transporters such as SbmA [70] or ABC transporter (importer) YejABC [71] for reaching their targets in the cytoplasm.

Microcin J25 (MccJ25) is one of the best characterised microcins in terms of biosynthesis, export and mode of action. MccJ25 is a plasmid-encoded, ribosomally synthesised and posttranslationally modified 21-amino acid AMP that displays a unique lasso topology as a result of its C-terminal tail threading through an N-terminal eight-residue macrolactam ring. The macrolactam ring is formed by linkage of the N-terminal amino group of the Gly1 residue and the Glu8 side-chain carboxylate, and the loop stability is provided by locking it by steric hindrance from two bulky amino acid side chains, Phe19 and Tyr20, on either side of the macrolactam ring [4,72]. The biosynthetic

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**Fig. 2.** Biosynthetic gene clusters of class I and IIa/b microcins. Representative examples for each class are shown. Genes encoding self-immunity proteins are included in the biosynthetic clusters and ensure efficient protection against the produced peptides. Colour code: precursor peptide (yellow); posttranslational modification enzymes (green); self-immunity by transporters (blue and purple); self-immunity proteins (excluding export) (red); unknown function (grey). The white arrows indicate known promoters. Protein names are written under the genes.
The role of ABC transporters in lantibiotic and microcin export

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cluster consists of four genes [73], mcjABCD, where mcjA encodes the 58-amino acid peptide precursor McjA that is posttranslationally modified by the maturation enzyme McjBC [74] (Fig. 2); McjB is an ATP-dependent cysteine protease, and McjC is an ATP-dependent asparagine synthetase [75]. Self-immunity is provided by the ABC transporter McjD [76] (see section Self-immunity to microcins conferred by ABC transporters).

In *Escherichia coli*, MccJ25 enters the target bacteria by hijacking the outer membrane siderophore receptor FhuA-TonB-dependent pathway [77,78] and inner membrane protein SbmA [70,79,80] (Fig. 3). Once inside the cell, MccJ25 inhibits the bacterial RNA polymerase [66,81] by binding to the secondary channel [82,83] (Fig. 3). Unlike other microcins, MccJ25 self-immunity does not require other immunity proteins.

**General introduction on ABC transporters**

Export of lantibiotics and microcins out of the producing cells is usually mediated by ABC transporters (Figs 1 and 2), which ensure that the toxic peptides are secreted out of the cells for both, self-immunity and release, into the medium to exert their killing activity against closely related species for survival. ABC transporters are one of the largest superfamilies and can be found in bacteria, archaea and eukaryotes [84]. They have multiple roles including nutrient uptake, exporting signalling molecules or toxic compounds such as xenobiotics and toxic metabolites, as well as conferring cells with multidrug resistance (MDR). Nutrient uptake is usually mediated by ABC importers (type I and type II; not discussed here) [85], whereas ABC exporters (type IV) mediate the export of toxic molecules out of the cells [86]. ATP binding and hydrolysis energise the ABC transporters for substrate export. The core architecture of ABC transporters consists of a transmembrane domain (TMD), homodimeric or heterodimeric, of twelve transmembrane (TM) helices and a dimeric nucleotide-binding domain (NBD) [85]. The TMD forms the translocation pathway across the membrane bilayer and contains the substrate recognition site, but it does not display any significant sequence conservation amongst different species, where the NBD contains several conserved motifs for ATP binding and hydrolysis (see ref [85] for a comprehensive review).

![Fig. 3. Mode of action of microcins. The lasso peptide MccJ25 hijacks the siderophore receptor FhuA at the outer membrane and triggers a transport event via the ExbBD-TonB-dependent pathway that results in its internalisation. The MccB17 is internalised by crossing the outer membrane through the OmpF porin. Inside the periplasm, both the MccJ25 and MccB17 hijack the SbmA transporter for transport across the inner membrane and subsequently inhibit the RNA polymerase and DNA gyrase, respectively.](image-url)
In this review, we have tried to capture the ‘diverse’ export systems associated with self-immunity against AMPs in both Gram-positive and Gram-negative bacteria. Although most of the export systems belong to the ABC transporter superfamily, in many cases these transporters also play an important role in the maturation process, which is distinct from multidrug transporters that just export structurally distinct molecules [87]. The self-immunity ABC transporters appear specific to their substrates.

**Lantibiotics and ABC transporters**

**Lantibiotic secretion by ABC transporters**

Class I lantipeptides are mainly secreted by a LanT-type (or NisT-type) ABC transporter; prominent members are NisT [88], SpaT [89] or PepT [90], and they export the precursor peptide directly into the extracellular space (Fig. 4 and Table 1). In the operons of these examples, genes encoding specific peptidases are also present. These leader peptide peptidases (LanP) remove the leader peptide in the final maturation step and thereby activate the lantipeptides. LanPs are either located in the cytoplasm (e.g. PepP [90], EciP [91], ElxP [92]) or anchored to the extracellular membrane (e.g. EpiP [93] and NisP [94]). In the latter, cleavage of the leader peptide takes place after secretion ensuring that the lantipeptide remains inactive in the cytosol and cannot exert its antibacterial activity.

In contrast to class I, many class II lantipeptides are secreted by ABC transporters, which combine the final maturation and secretion step (Table 1). The final maturation step is performed by an additional C39 peptidase domain (Fig. 1). The prototype of this family is SunT [20,95]. They are bifunctional, homodimeric transporters, whose secretion and proteolytic activity are
tightly coupled [96,97] (see also section Class II microcin export by ABC transporters – structure and mechanism). Examples include the exporter LctT [98], MrsT [99], BovT [100], LicT [101] and NukT [102]. Noteworthy, in the operon of LicT, there is an additional gene encoding a peptidase, LicP, which further trims the leader peptide of the precursor peptide, yielding the mature peptide after translocation [103].

Class III lanthipeptides are secreted by LanT-type ABC transporters (as in class I) after their final maturation step. It was first suggested that the proteolytic cleavage of the leader peptide is performed by prolyl oligopeptidases (POPs) [104]. They are, however, not always present in the biosynthetic gene clusters of class III lanthipeptides and are therefore not included in the general biosynthetic gene cluster scheme. The biosynthesis of NAI-112 requires a Zn$^{2+}$-dependent protease, AplP, that catalyses the cleavage of the leader peptide [105]. In class IV lanthipeptides, little is known about the transporter and no protease has been identified [10,106]. In these biosynthetic gene clusters, lanT and lanH encode two proteins, LanT and LanH, responsible for the translocation process. It is assumed that the heterodimeric transporter LanTH is bifunctional and can act as a peptide exporter and an immunity transporter [10,107].

### Lanthipeptide export by ABC transporters – structure and mechanism

In the gene clusters of class I and class II lanthipeptides, at least one or two genes are encoding an immunity system (Fig. 1). These are termed LanI protein and LanFEG ABC transporter (Fig. 4). LanI is a lipoprotein located extracellularly, and it is anchored to the membrane by a fatty acid chain attachment to an N-terminal cysteine residue [108]. Deletion of lanI increases the sensitivity of the producer strain to its own lanthipeptide as shown for the nisin immunity protein NisI [109]. LanI proteins are also found in the biosynthetic gene clusters of Pep5 [91], epicidin 280 [91] and subtilin [111] and for the two-component lanthipeptides lacticin 3147a and b [112]. For NisI, which binds nisin, and SpaI, which binds subtilisin, the specific interaction between LanI and the lanthipeptide has been demonstrated [108,111,113,114]. The NMR structures of SpaI [115] and NisI [114], as well as the crystal structure of NisI [116], revealed a central six/seven-stranded $\beta$-sheet with a hydrophobic patch located in the C-terminal region (Fig. 4). NisI contains an N-terminal and a C-terminal domain, whereas SpaI possesses only one domain (Fig. 4). Although the sequence identity is low, both domains of NisI can be separately aligned to the SpaI structure. NMR titration experiments of NisI with nisin revealed that the main binding site is located in the C-terminal domain of NisI [116]. This finding supported former in vivo studies, in which the deletion of the C terminus of NisI decreased the immunity of Lactococcus lactis against nisin [117,118]. The LanI protein PepI also highlighted the importance of the C-terminal domain to confer full immunity [119]. The interaction of LanI with the lanthipeptides prevents

| Lanthipeptide ABC transporters | LanT | SunT (fused NBD and TMD) + C39 | LanFEG | BceAB |
|-------------------------------|------|-------------------------------|--------|-------|
| Sequence length (amino acids) | 602  | 710 | 225 + 242 + 214 | 253 + 648 |
| Predicted transmembrane helices | 6 | 5 or 6 | LanE: 6 | BceE: 10 |
| Stoichiometry | Homodimer | Homodimer | Heterodimer | BceAB: unknown (dimeric BceA) |
| Special sequence motif | – | N-terminal C39 peptidase domain | LanF: E-loop | BceA: Q-loop |
| Inactive transport mutant | H-loop | H-loop | H-loop | H-loop |
| Observed mechanism | Secretion of produced and or processing of lantibiotic | Leader processing and secretion | Expelling AMP | Expelling AMP/ shielding of membrane target |
| Substrate specificity | AMP (recognised by leader sequence) | AMP (recognised by leader sequence) | Immunity against produced AMP | Resistance against AMP and bacitracin |

Nisin: recognition of C terminus Nisin: recognition of N terminus

Table 1. Characteristics and substrate specificity of ABC transporters involved in lanthipeptide immunity or resistance.
membrane insertion and oligomerisation, being the prerequisite for pore formation [117] by inhibiting the interaction of receptor/LanA, for example membrane/lipid II for nisin [114,116,120].

The second set of immunity proteins are the LanFEG proteins, which belong to LanFEG-type ABC transporters [95]. They confer immunity against their own, specific lanthipeptide by extrusion from the cytoplasmic membrane into the extracellular medium [113,121,122]. The LanFEG-type ABC transporters consist of the TMDs LanE and LanG, and the NBD LanF (Fig. 4 and Table 1). A fully functional LanFEG-type ABC transporter consists of a heterodimer of LanE and LanG together with a homodimer of LanF. Interestingly, immunity systems have been described, in which only LanI or LanFEG is expressed, which seems to correlate with the mode of action of the lanthipeptide [123].

For lantibiotics that display a dual antimicrobial activity (e.g. inhibition of cell wall biosynthesis and pore formation within in the membrane), LanI and LanFEG work in a cooperative manner to confer full immunity [111,113]. Both genes encoding for the LanFEG and LanI proteins are present in the biosynthetic cluster of nisin [125], epidermin [121], subtilin [126] and streptin [127]. However, if the lantibiotics only form pores, only lanI, but not lanFEG, is present in the cluster (e.g. Pep5 [110,128] epicidin 280 [91] and lactocin S [129]). The LanFEG-type ABC transporter is encoded in gene clusters of lantibiotics, whose mode of action is to (a) bind to a specific receptor (epidermin [121]), or (b) interfere with cell wall biosynthesis (mersacidin [99]) or (c) modify the lipid composition of the membranes (cinnamycin [130]).

The biosynthetic cluster of gallidermin and epidermin encodes an accessory protein termed LanH (or GslH and EpiH, respectively; Fig. 4), instead of a lipoprotein, which contributes to higher immunity towards its cognate lanthipeptide [131]. Similarly, in Staphylococcus warneri ISK-1 the membrane protein NukH is present, which acts together with the LanFEG-type ABC transporter NukFEG to confer full immunity against nukacin ISK-1 [132,133]. Another example of a biosynthetic cluster encoding a lipoprotein, an ABC transporter and an accessory protein is found in the NAI-107 producer Microbispora ATCC PTA-5042; MlbQ is the lipoprotein that confers immunity to NAI-107-like lanthipeptides analogously to LanI proteins [124]. Additionally, the ABC transporter MlbYZ and the accessory protein MlbJ (functioning as a LanH protein) also contribute to the immunity. Class II lanthipeptides contain two-component lanthipeptides (α- and β-mLanA), in which one variant has a pore-forming mode of action and the other inhibits peptidoglycan biosynthesis [47,134]. Consequently, in these operons genes encoding a LanI lipoprotein and a LanFEG-type ABC transporter are present, which when expressed can confer immunity against both lanthipeptide variants (e.g. lichenicidin BGC [135,136]). In these operons, however, an additional accessory protein, LanH, can be found. This appears to be an exception since in the biosynthetic gene cluster of lactacin 3147, only ltnI and ltnEF were identified and not ltnH [112]. In many cases, the components of the immunity system seem to have co-evolved to the activity of the lanthipeptide resulting in different strategies for immunity mechanisms.

In the biosynthetic gene cluster of class III and class IV lanthipeptides, lanI and lanFEG are absent (Fig. 1), which correlates with the lack of antimicrobial activity of these lanthipeptides. In class IV lanthipeptides, the ABC transporter LanTH appears to be important for immunity [10,107] (Fig. 1). The detailed mechanism of action by class IV lanthipeptides is not well-understood, and the exact nature of the immunity mediated by these ABC transporters also remains enigmatic. In the new class V, only a gene encoding a LanI protein is present, which function is, however, not resolved so far [17].

In many biosynthetic clusters of class I and class II lanthipeptides, the genes encoding LanFEG-type transporters can be found as secondary export systems besides the genes for LanT/LanC39PT-type transporters [121,126,131,137–140]. Throughout the lanthipeptide biosynthetic clusters, the encoded LanFEG-type transporters are homologous to each other and share the same domain organisation (Fig. 5).

In contrast to LanT-type ABC transporters, the subunits of the LanFEG-type ABC transporters are encoded on separate genes with a fully assembled transporter in a proposed stoichiometry of 2 : 1 : 1 (LanF2EG) [137] based on bioinformatic analysis (Fig. 5 and Table 1). The LanE and LanG are the TMDs, and they all contain six predicted TM helices [111]. The TMDs are functional heterodimers as shown for NisFEG and SpaFEG, in which deletion of one of the proteins resulted in loss of immunity, suggesting that they are important for substrate binding and translocation [111,113,141]. The LanF proteins are the NBDs and share the common conserved ABC motifs of the ABC protein superfamily. Within the conserved ATPase motifs, an E-loop instead of the Q-loop can be found, that is highly conserved in LanFEG-type ABC transporters and is apparently involved in the communication of the NBDs with the TMDs that is important for lantibiotic immunity [142].
The LanFEG-type ABC transporters belong to the ABC-2 subfamily of MDR proteins, which are involved in the efflux of macrolides, antibiotics, toxins or other compounds [143,144]. The LanFEG-type transporters are specific for their dedicated lantibiotic and confer immunity without any cross-reactivity [122]. Although the exact mechanism is still unknown, LanFEG-type transporters may function like other ABC transporters. It has been proposed that the exporter extrudes the hydrophobic substrate from the inner/outer leaflet of the membrane to the trans-side of the membrane and/or extracellular space, respectively (Fig. 5). This efflux mechanism was proposed and shown for the lantibiotics nisin, subtilin, epidermin and nukacin ISK-1 [111,113,122,133]. The fast extrusion from the membrane appears to prevent pore formation as shown for nisin [141]; pore formation was monitored via a SYTOX green assay, and it clearly demonstrated that the expression of NisFEG prevented pore formation. Important to note is the cooperative function of the LanFEG-type transporter with the associate membrane-bound immunity proteins (LanI or LanH) to confer full immunity against the lantibiotic [108,118,133,145–147]. An independent action of only LanFEG-type transporter has also been observed for NisFEG and SpaFEG [111,113]; however, full immunity could only be observed if both proteins were expressed.

**Resistance to lantibiotics by ABC transporters expressed in human pathogens**

Although resistance against lantibiotics is rarely observed, still some inherent resistance mechanisms have been reported, mainly in human pathogenic bacteria. This resistance can arise from modifications of the cell wall or membrane and the formation of biofilms or endospores (reviewed in [148]). In all cases, the presence of lantibiotics is sensed by specific protein detection systems (e.g. two-component systems; TCS), which alter the gene regulation and thereby the expression of enzymes involved in peptidoglycan modification, lipid biosynthesis or resistance proteins (e.g. ABC transporters in Firmicutes). The encoded ABC transporter either belongs to the group of CprABC-type transporter (Cpr: cationic AMP resistance) or belongs to the group of BceAB-type transporter (Bce: bacitracin efflux) [87,95,149] (Table 1).

The first group are CprABC-type transporters, which are similar to the LanFEG-type transporters [150,151]. All proteins are encoded by the same operon but expressed separately under the control of the
corresponding TCS, which is responsible for lantibiotic sensing. The functional transporter consists of two NBDs and a heterodimeric TMD. Unusually for LanFEG-type transporters, the ABC transporter CprA2BC from the Gram-positive bacterium Clostridioides difficile confers resistance against multiple lantibiotics such as nisin and gallidermin [151]. In contrast to LanFEG-type transporters of lantibiotic-producing bacteria, these ABC transporters have a wide substrate spectrum and are not specific for a single lantibiotic. Another CprABC-type transporter is LetFEG from Streptococcus mutans, which confers resistance against nukacin ISK-1 and lactacin 481 under the control of the TCS LcrRS [152]. Interestingly, on a different gene locus, the TCS NsrRS regulates the expression of the ABC transporter NsrFE1E2G, which confers resistance against nisin but not to other lantibiotics such as nukacin ISK-1. In the Streptococcus pyogenes, SF370 bacterium, the TCS SrtRK and the ABC transporter SrtFEG only confer resistance to nisin [153].

The second group is composed of BceAB-type transporters, which are part of broad stress response systems [154]. These exporters display substrate promiscuity and export lantibiotics, bacteriocins or other AMPs, glycopeptides and even antibiotics (e.g. bacitracin or β-lactam antibiotics) suggesting a different mechanism [95]. The domain organisation of these ABC transporters is completely different in comparison with the LanT-type or LanFEG-type transporters [155]. The BceB protein (TMD) is predicted to have 10 TM helices and an additional large extracellular sub-domain (ECD) between TM helix 7 and TM helix 8 of roughly 200 amino acids in size, where the BceA protein is the NBD [156] (Table 1). Interestingly, the topology of the C-terminal four TM helices and the ECD is similar to MacB [157]. The ECD domain is believed to be important for sensing the putative substrate. It is proposed that these transporters have co-evolved with their TCS, as the histidine kinase (HK) lacks the sensor domain normally present in other putative HKs [158,159]. The presumed functions of BceAB-type transporters are either the translocation of the substrate (bacitracin or AMP) and/or the flipping of the membrane target, which is sensed by the associated TCS [160–162]. The suggested stoichiometry of the BceAB-type transporter is 2 : 1, as shown for the ABC transporter BceAB from Bacillus subtilis, where the HK BceS interacts with BceB [162]. This transporter mainly expels bacitracin and forms a complex with the bacitracin sensing TCS BceRS [159,163]. Additionally, it confers resistance against plectasin, actagardine and mersacidin [164]. Similarly, the BceAB paralog PsdAB from B. subtilis confers resistance against the lantibiotics mersacidin, actagardine, gallidermin, nisin and the peptide antibiotic bacitracin [164]. Further BceAB-type transporter examples include MbrAB from Staphylococcus mutans [165], VraDE from Staphylococcus aureus [166], YsaBC from L. lactis IL1403Nis’ [167] and AnrAB from Listeria monocytogenes, which confer resistance against nisin, bacitracin and some β-lactam antibiotics (e.g. oxacillin and penicillin G) [168]. It has been proposed that the BceAB transporter form B. subtilis confers resistance by transiently ‘free’ lipid II cycle intermediates from the inhibitory AMP complexes; however, the exact molecular mechanism is unknown [169].

A special variation of the standard operon of BceAB-type transporter can be mainly found in pathogenic strains such as S. aureus, Streptococcus agalactiae, Enterococcus faecium and Streptococcus sanguinis [170]. These operons include genes that encode a TCS, a BceAB-type transporter and an additional lipoprotein. Interestingly, strains containing such an operon do not produce AMPs, but are resistant against some lantibiotics. For example, in the human pathogen S. agalactiae COH1 an operon for nisin resistance can be found, which encodes the lipoprotein SaNSR, a serine protease belonging to the S41 protease family, a BceAB-type transporter SaNsrFP and the TCS SaNsrRK [120,170–172].

The CprABC and BceAB transporters appear to have different number of transmembrane helices and structural arrangements compared with the NisFEG transporter from L. lactis and the NsrFP from S. agalactiae. This is reflected by their substrate specificity; NisFEG recognises the C terminus of nisin, suggesting that mainly the pore-forming mode of action is targeted [141], whereas the SaNsrFP transporter recognises the N terminus, suggesting that it interferes with the binding of nisin towards the membrane or lipid II [173].

**Self-immunity to microcins conferred by ABC transporters**

The biosynthetic gene cluster of microcins, which belong to class I and class II, usually encodes the precursor peptide, enzymes involved in PTMs and at least one efflux system for export and subsequent self-immunity (Fig. 2). These efflux systems are usually ABC transporters, but an involvement of transporters that belong to the major facilitator superfamily (MFS) has also been reported [174]; immunity to the MccC7/C51 microcin requires the MFS transporter MccC [174] and immunity proteins MccE (also involved in the posttranslational modification of the MccC7/C51; an
acetyl-CoA-dependent acetyltransferase acetylates the primary amino group of the aminoacyl moiety of the processed microcin that cannot bind to aspartyl-tRNA-synthase [175] and MccF (a serine carboxypeptidase that degrades only mature MccC7) [175, 176]. The precursor peptides in both classes have a leader sequence that is cleaved prior to export. Class I microcin ABC transporters display a typical ABC transporter fold, a TMD and an NBD, to export the mature microcins, whereas the class II microcin ABC transporters contain an additional accessory N-terminal peptidase domain that is involved in the final maturation step of the microcin by cleaving its leader peptide prior to export (Table 2). In both systems, they ensure efficient export out of the producing bacteria to alleviate the microcin toxic effect.

### Class I microcin export by ABC transporters – structure and mechanism

Bacteria that synthesise class I microcins can confer self-immunity by either heterodimeric or homodimeric ABC transporters. In some cases, full self-immunity is conferred by additional proteins. The ABC transporter genes are found at the end of the biosynthetic clusters ensuring efficient protection against the accumulation of endogenous peptides during biosynthesis, whereas the immunity one is found near the microcin structural gene (Fig. 2). Biosynthesis of the microcin MccB17, which contains thiazole and oxazole rings, requires seven genes, 

| Microcin ABC transporters | MccD | MccFE |
|--------------------------|------|-------|
| Sequence length (amino acids) | 580 | 241 + 247 |
| Predicted transmembrane helices | 6 | MceE: 6 |
| Stoichiometry | Homodimer | Heterodimer |
| Special sequence motif | – | – |
| Inactive transport mutant | Walker B | Walker B |
| Observed mechanism | Secretion of produced microcin | Secretion of produced microcin |
| Substrate specificity | Lasso peptide MccJ25 | MccB17 |

The export of the microcin MccJ25 is one of the better understood systems amongst microcins belonging to the class I. As mentioned earlier, the biosynthetic cluster consists of four genes [73], mejABC, where mejA encodes the 58-amino acid peptide precursor MejA that is posttranslationally modified by MejB, an ATP-dependent cysteine protease, and MejC, a lactam synthetase [61]. Self-immunity is provided by the ABC transporter McjD [73, 76]. Unlike other class I microcins, self-immunity to MccJ25 is only conferred by the ABC transporter McjD (Fig. 2 and Table 2) and not additional immunity proteins. The mechanism of export has been extensively studied and has provided novel insights on the self-immunity process of class I microcins. The crystal structure of McjD has been determined in the presence and absence of nucleotides and it displays a characteristic homodimeric ABC transporter architecture with a TMD, which consists of 12 TM helices, and a dimeric NBD [76, 178] (Fig. 6). Its overall architecture is similar to Sav1866 from S. aureus [179] and MsbA from E. coli [180], but it does not display an accessible open cavity for substrate translocation regardless of the presence or absence of nucleotides [178]. In the absence of nucleotides, MsbA adopts an inward-closed conformation, whereas in the presence of nucleotides both Sav1866 and MsbA have been shown to adopt outward-open conformations; in both the inward-closed and outward-open conformations, the TMD is open to the inside or the outside of the membrane for ligand binding and release, respectively. The TMD of McjD is occluded at both sides of the membrane as a result of the movement of TM 1 and TM 2 towards the TMs 1′ and 2′ of the opposite monomer. The occluded TMD defines a large cavity of around 5900 Å³ that can accommodate MccJ25. In addition to the occluded TMD, McjD does not display TM helices intertwining unlike MsbA and Sav1866, as a result of the TM 1 and TM 2 movement. No structure
of McjD has been reported in the presence of MccJ25, but mutagenesis studies within the cavity have identified key residues for the binding of MccJ25 including charged and hydrophobic residues that could coordinate its binding [76]. Further evidence of MccJ25 binding within the cavity was provided by accessibility assays in inside-out vesicles using modification of cysteines by maleimide-PEG10K, which showed that the absence of an McjD-MccJ25 crystal structure, NMR studies revealed that only three residues from MccJ25 interact with McjD (shown in red sticks) (right panel). In the absence of an McjD-MccJ25 complex structure, NMR studies revealed that only three residues from MccJ25 interact with McjD (shown in red sticks) (right panel).

The role of ABC transporters in lantibiotic and microcin export

**Burkholderia** species with self-immunity against the lasso peptide capistruin [182,183]. Capistruin is a lasso peptide that displays structural similarities to MccJ25 as well as a similar mode of inhibition of RNA polymerase [83]. Interestingly, CapD did not provide *E. coli* cells with resistance against MccJ25 suggesting that ABC transporters associated with microcin biosynthetic clusters have a dedicated role in the self-immunity against the synthesised microcin. Considering the structural similarities between MccJ25 and capistruin, it would have been predicted that these two transporters should have substrate overlap, although the TMDs do not display any significant sequence similarity, but they display a single substrate specificity. As toxic levels of these microcins are building up in the cytoplasmic side of the inner membrane, it would be counterintuitive for these transporters to export other compounds. It cannot be excluded that multidrug ABC transporters have ‘evolved’ from such dedicated systems by altering their cavities for selectivity.

ABC transporters use the alternating access mechanism for substrate export based on the structures of Sav1866 from *S. aureus* [179] and MsbA from *E. coli* [180]. Although this model is applicable to many ABC transporters, there are several other models that are emerging (see Ford and Beis [184] for a comprehensive review). In the alternating access mechanism model, the transporter switches from an inward-facing to an outward-facing conformation that is driven by ATP binding. The role of ATP hydrolysis is to revert the transporter in an inward-facing conformation for another transport cycle. Several biophysical techniques have provided additional insights on the transport cycle. A consensus mechanism for ABC exporters has been proposed, where the apo inward-facing TMD can bind its substrate. In the inward-facing apo conformation, the NBDs are disengaged, but substrate binding induces small conformational changes along the TMD that subsequently brings the NBDs closer. ATP binding causes the NBDs to dimerise, which in turn causes major conformational changes resulting in the outward-open conformation and release of the substrate. ATP hydrolysis resets the transporter back to the inward-facing conformation.

In the light of the structural work on McjD, it is apparent that its overall structure and mechanism are distinguishable from the multidrug transporters MsbA and Sav1866. As mentioned earlier, McjD adopts an occluded TMD in the presence and absence of nucleotides as verified by crystallography, pulsed electron-electron double resonance (PELDOR) and cross-linking assays in inside-out vesicles, suggesting that its
mechanism of export is different from the alternative access model [178]. Although these studies uniformly showed that McjD is occluded in the membrane, they have been unable to provide evidence of TMD opening for substrate release. The first evidence of TMD opening came from single molecule Förster resonance energy transfer (sm-FRET) experiments with McjD reconstituted in liposomes. Donor and acceptor FRET labels were placed at the TM1, and they showed that in the absence or presence of nucleotides, the TMD remained occluded as it was previously reported [178], but the TMD displayed an opening in the presence of both ATP and MccJ25 [185]. This is a strikingly different regulation of an ABC transporter that could be unique to microcin ABC transporters. This tight regulation of both ATP and substrate binding to trigger the opening of the TMD and substrate release could possibly provide these self-immunity ABC transporters with substrate selectivity as well.

Two mechanisms have been proposed for McjD, one that can describe the futile ATP hydrolysis and one for the ligand-induced ATPase activity [178,185]. In the absence of its substrate MccJ25 and ATP, the TMD of McjD remains occluded but it samples an inward-open conformation as shown by the maleimide-PEG10K experiments [178], although the inward-occluded conformation is more readily sampled. Binding of ATP triggers dimerisation of the NBDs without adopting an outward-open conformation as seen in Sav1866 and MsbA [179]. ATP hydrolysis results in an inward-occluded conformation with NBD disengagement and reset of the transport cycle. In the presence of MccJ25, the peptide can bind to the large cavity and subsequent ATP binding triggers NBD dimerisation and conformational changes along the TMD for adopting a ‘transient’ or short-lived nucleotide (ATP)-bound outward-open conformation for releasing MccJ25. Upon release of MccJ25, McjD adopts a stable nucleotide (ATP)-bound outward-open conformation. ATP hydrolysis results in McjD adopting an inward-occluded conformation with NBD disengagement.

Resistance to MccJ25 has also been reported in target bacteria to overcome its toxic effects. In E. coli, resistance to MccJ25 is conferred by the ABC transporter YojI and outer membrane protein TolC [186]. YojI is a partially characterised ABC transporter whose function or substrates are not known, but it has been shown that it cannot confer resistance to antibiotics, suggesting that it is not an MDR ABC transporter but likely a promiscuous peptide exporter. It cannot be excluded that other ABC transporters can confer resistance to other microcins as well.

Class II microcin export by ABC transporters – structure and mechanism

Microcins that belong to class II result from a similar gene organisation as the class I ones (Fig. 2). Self-immunity is also conferred by ABC transporters and immunity proteins. Unlike class I microcins, the class II microcin ABC transporters participate in the maturation process of the precursor microcins by removing the leader sequence of the microcin prior to export (Table 2); they contain an additional N-terminal domain that poses peptidase activity that acts on the C-terminal side of a double-Gly type motif of the leader peptide [187,188]. The consensus sequence of cleavage of the leader peptide is L(−12)XXXE(−8)L(−7)XXXXG(−2)G(−1), where X could be any amino acids. Protein sequence analyses have shown similarity to the C39 peptidase superfamily, which has the catalytic triad Cys-His-Asp [189,190]. They are termed bifunctional ABC transporters and belong to the ABC transporter maturation secretion (AMS) superfamily, or peptidase-containing ATP-binding transporters (PCAT) [191].

Maturation of the class II microcins, MccL, Mcc24, MccE492, MccH47 and MccM, requires such a bifunctional peptidase/export system (Table 2). Since the final maturation step is associated with the ABC transporter, it can provide producing cells with a very efficient self-immunity since the cleavage and export are tightly regulated. At the time of this review, there were no structures of either isolated class II microcin C39 peptidase domains or full transporters. Details on the function of the C39 peptidase domain come from the functional characterisation of microcin V (MccV) from E. coli [189,190] (Table 2). MccV is a class IIa microcin, and self-immunity requires a tripartite efflux system CvaA-CvaB-TolC in E. coli [192]. CvaB is the ABC transporter and belongs to the AMS/PCAT family. Mutagenesis studies on the isolated peptidase domain have identified a catalytic triad consisting of Cys32, His105 and Asp121. Activity of the protease also requires Ca2+ [189,190]. The first structural insights on an isolated C39 peptidase domain were for the ComA ABC transporter from S. mutans [193]. ComA is responsible for the maturation and export of the quorum-sensing peptide ComC. The overall fold of the C39 peptidase domain is similar to the papain-like cysteine proteases, including three helices in the N terminus and β-sheets and a helix (α4) in the C terminus [193] that contain the catalytic triad Cys17, His96 and Asp112. The overall structure displays a shallow hydrophobic concave surface that can bind the leader sequence of ComC.
The crystal structure of PCAT1 from *Clostridium thermocellum* [194] provided the first structural information on the interaction between the C39 peptidase domain and the ABC transporter (Fig. 7). In the absence of a Gram-negative ABC transporter structure that belongs to the AMS/PCAT family, the PCAT1 structure from *C. thermocellum* is a good representative to gain insights on the domain architecture and function of this class of ABC transporters. PCAT1 removes the leader peptide and secretes a small protein of 66 residues, Cthe_0535 (CtA). The structure of PCAT1 has been determined in the presence and absence of substrate and nucleotides and has provided insights on both the leader sequence recognition/processing by the C39 peptidase and export mechanism [194,195] (Fig. 7). The overall transporter architecture is very similar to other type IV ABC transporters including McjD and MsbA. In the absence of nucleotides or the presence of CtA only, PCAT1 adopts an inward-open conformation. In the apo PCAT1, the C39 peptidase domain is interacting with the open TMD and the catalytic triad is facing the open TMD. In the presence of CtA, only part of the leader peptide sequence could be resolved in the cryo-EM maps; it displays an L-shaped structure that wraps around the C39 peptidase domain with the N-terminal end facing the cytosol and the C-terminal one towards the open TMD. Binding of the leader peptide to the C39 peptidase is coordinated by van der Waals interactions with Ala55 and Ile59. The double glycine motif, G23-G24, faces the catalytic triad, Cys21Ala (inactive mutant), His99 and Asp115. Some weak density corresponding to the rest of the CtA sequence could be observed inside the TMD. Although PCAT1 is a homodimer, and densities for two leader sequences could be observed at the C39 peptidase domains, and only density for one CtA molecule was found inside the TMD, suggesting that PCAT1 can only transport one molecule, whereas the second CtA molecule is outside the TMD [195]. Binding of CtA results in a more open cytoplasmic gate as a result of movement of TMs 3 and 4. In addition, the NBDs show larger separation relative to the apo structure. In the nucleotide-bound structure, PCAT1 adopts an outward-occluded conformation, similar to McjD, and no density for the C39 peptidase could be resolved, suggesting that it dissociates from the TMD upon nucleotide binding and the conformational changes associated with it.

Based on the available PCAT1 structures, a processing and export mechanism has been proposed: in the absence of ATP, PCAT1 adopts an inward-facing conformation with its TMD open to the cytoplasm so that it can recruit the C39 peptidase domain. The C39 peptidase domain can bind two substrates, but only one is processed and facing the TMD for export. Upon cleavage of the leader sequence and binding of ATP at the NBDs, the peptidase domain is displaced as a result of the conformational changes and the transporter adopts a transient nucleotide-bound outward-open conformation for substrate release. Upon exit of the substrate from the cavity, the TMD adopts a nucleotide-bound outward-occluded conformation. ATP hydrolysis resets the transporter to an inward-facing conformation with the C39 peptidase domain.
re-associating with the TMD. PCAT1 is a very good model to understand the mechanism of class II microcin export by bifunctional ABC transporters, but it is unclear whether the class II microcin transporters can process and transport one or two microcins since their size is smaller than the PCAT1 substrate; CtA has a molecular weight of around 10 kDa, whereas class II microcins range between 4.8 and 8.9 kDa. We cannot exclude that a variation of the processed and transported substrates can occur by their respective ABC transporters depending on their relative size.

Conclusions and perspectives

Unlike the NBDs that display several conserved motifs, the TMD of ABC transporters does not have conserved motifs or significant sequence conservation that could possibly explain substrate overlap between multidrug transporters, which often transport common drugs. In an attempt to investigate if certain motifs or conserved regions exist in the TMDs of these self-immunity proteins, we performed sequence alignments between the lantibiotic and microcin transporters discussed in this review. Such analyses did not reveal any significant motifs or homology between the different ABC transporters that could explain substrate specificity. Interestingly, specificity cannot be predicted based on the sequence of the lantibiotics and microcins either, because many of them have a similar scaffold and yet can only be transported by their respective self-immunity transporter. This does not exclude that a more in-depth analysis, with the inclusion of more sequences, may reveal such conserved motifs.

In conclusion, ABC transporters are fascinating and complex machines and their role in protecting cells against intrinsic (e.g. AMPs) or extrinsic (e.g. antibiotics) toxic molecules is remarkable. Although our understanding of self-immunity to AMPs conferred by ABC transporters has increased in recent years, a lot of unanswered questions remain. To date, we have no structures available for full-length lanthipeptide ABC transporters that could explain their organisation and mechanism. Additionally, we require more structural and functional studies of ABC transporters from different classes of microcins. Do all self-immunity ABC transporters from Gram-positive and Gram-negative bacteria show a single substrate selectivity or are there exceptions? Is it possible that such systems may allow us to decipher why certain ABC transporters are MDR? We believe that the AMP field is gaining a very good understanding on the biosynthesis of these complex peptides, but the self-immunity transporters are usually overlooked and they play a pivotal role in the survival of bacteria. We hope that the cryo-EM era will allow for more structures to be determined, which in turn will stimulate more research on the function of these ABC transporters.

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