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Immunity to lantibiotics

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Abstract

Bacteria producing bacteriocins have to be protected from being killed by themselves. This mechanism of self-protection or immunity is especially important if the bacteriocin does not need a specific receptor for its action, as is the case for the type A lantibiotics forming pores in the cytoplasmic membrane. At least two different systems of immunity have evolved in this group of bacteriocins containing modified amino acids as a result of posttranslational modification. The immunity mechanism of Pep5 in *Staphylococcus epidermidis* is based on inhibition of pore formation by a small 69-amino acid protein weakly associated with the outer surface of the cytoplasmic membrane. In *Lactococcus lactis* and *Bacillus subtilis* the putative immunity lipoproteins NisI and SpaI, respectively, are also located at the outer surface of the cytoplasmic membrane, suggesting that a similar mechanism might be utilized by the producers of nisin and subtilin. In addition an ABC-transport system consisting of two membrane proteins, (NisEG, SpaG and the hydrophobic domain of SpaF, and EpiEG) and a cytoplasmic protein (NisF, the cytoplasmic domain of SpaF, and EpiF) play a role in immunity of nisin, subtilin and epidermin by import, export or inhibition of pore formation by the membrane components of the transport systems. Almost nothing is known of the immunity determinants of newly described and other type of lantibiotics.

Introduction

Production of bacteriocins is typically coupled to the expression of immunity peptides protecting the producer strains from the lethal action of their own product. In *Escherichia coli* the killing action of colicins E2 and E3 is inhibited by stoichiometric complex formation with the immunity protein in the cytoplasmic membrane (Bowman et al. 1971). The transport system of channel forming colicins transporting the bacteriocin across the outer membrane to the periplasmic space has been proposed to be involved in immunity by a specific interaction with the immunity protein (Song & Cramer 1991). Until recently, almost nothing was known about the immunity systems of producers of lantibiotics, a group of bacteriocins containing lanthionine and other modified amino acids due to post-translational events. These bacteriocins are first translated to a precursor with a leader sequence, then modified, secreted and finally activated by a protease that cleaves the leader from the modified precursor. Processing of the N-terminus of some lantibiotics might also occur inside the cell as suggested by the lack of a *sec*-dependent secretion signal of the LasP and PepP proteases involved in the biosynthesis of lactocin S (Skaugen 1994) and Pep5 (Meyer et al. 1995), respectively. The bactericidal effect of linear lantibiotics is based on depolarization of energized bacterial cytoplasmic membranes by pore formation. Several immunity proteins have been identified from the producers of the elongated (type A) lantibiotics (nisin, subtilin, epidermin and Pep5) but the immunity proteins from producers of other lantibiotics still remain to be characterized. In spite of the knowledge of the components needed for immunity, the mechanism of immunity is still poorly understood.

Nisin immunity

Nisin, produced by some strains of *Lactococcus lactis* subsp. *lactis*, is the most extensively studied lantibiotic and of significant importance because it is used as a preservative in the food industry. Nisin production and immunity can be transferred to other *L. lactis* strains by conjugation of a 70 kb conjugative transposon (Gasson 1984; Horn et al 1991; Rauch & de Vos 1992). The structural and biosynthetic genes of nisin A (*nisABTCIPRK* and *nisFEG*, Buchman et al. 1988; Dodd et al. 1990; Steen et al. 1991; Kuipers et al. 1993; van der Meer et al. 1993; Engelke et al. 1994; Siegers & Entian 1995) and the structural variant nisin Z (*nisZBTCIPRK* and *nisFEG*, Graeffe et al. 1991; Mulders et al. 1991; Immonen et al. 1995; Immonen & Saris unpubl.) have been cloned and sequenced. The precursor of nisin is encoded by either *nisA* or *nisZ*; NisB and NisC are likely to be involved in dehydration and lanthionine formation; NisT is an ABC transport protein likely to transport the modified precursor; NisP is the protease cleaving the leader from the modified precursor, NisR and NisK form a two-component regulatory pair, NisI and NisFEG are suggested to be involved in immunity.

Is NisI an immunity protein?

NisI (245 aa) is a predominantly hydrophilic protein that has a typical lipoprotein signal sequence with a Cys at position + 1 of the cleavage site for signal peptidase II. This protein becomes lipid-modified and membrane-anchored on the extracellular side of the cytoplasmic membrane after signal peptide cleavage as shown by labelling with ^{3}H-palmitic acid (Immonen & Saris unpubl.). No sequence homology to proteins in data banks has been found (Kuipers et al. 1993; Engelke et al. 1994; Immonen et al. 1995) except for the signal sequence with the very conserved consensus sequence for the signal peptidase II.

Kuipers et al. (1993) showed that expression of NisI and the N-terminal part of NisP using the T7 promoter in *E. coli* could partially protect EDTA-treated cells from being killed by nisin. However, this protection could not be confirmed by expressing NisI without the truncated NisP protein in *E. coli* with the same promoter (Qiao & Saris unpubl.). The level of NisI production was significantly higher using the construct without than with the *nisP* part in the expression vector. The produced NisI was secreted and modified, indicating a correct location, as it could be labelled with ^{3}H-palmitic acid.

Expression of the *nisI* gene in *L. lactis* using a plasmid expression vector resulted in cells with 1–4% of wild type immunity assayed in liquid cultures (Kuipers et al. 1993) or in cells with increased immunity assayed by the plate diffusion assay (Engelke et al. 1994). No data of the production level of the NisI protein were presented in these reports. Therefore, the low level of nisin immunity of the cells producing NisI could have been due to a lower level of NisI production compared to the level of a nisin producer. When NisI was produced in *L. lactis* with the P45 promoter (Sibakov et al. 1991) the production level, analyzed using NisI-antibodies, was slightly higher than the level in nisin producers, but still the immunity was only 1% of the wild type immunity assayed from liquid cultures (Qiao & Saris unpubl.). Assayed by the plate diffusion bioassay, these cells were slightly more immune (5% of the wild type level), but still far less immune than cells of nisin producers. This clearly showed that having the NisI lipoprotein at a wild type level on the outer surface of the membrane is not enough for self-protection and that additional immunity proteins are needed for high level of nisin immunity.

What can be learned from strains with a mutation in the nisin biosynthetic operon?

Several mutant strains with mutations in the biosynthetic operon have recently been constructed. Inactivation of the *nisA* gene has been made by an insertion of a selection marker (Dodd et al. 1992), a 4 bp internal deletion (Kuipers et al. 1993) and a plasmid producing antisense-*nisZ*-RNA (Hakola & Saris unpubl.). The *nisBTI* (successive in frame deletions), *nisCK* (plasmid insertions) (Kuipers et al. unpubl.) and the *nisI* (insertion of a selection marker) (Siegers & Entian 1995) genes of nisin A producers have been knocked out. The structural gene (by a gene replacement) of a nisin Z producer and the *nisBTP* genes (plasmid insertion) have also been inactivated (Qiao & Saris unpubl.). Knocking out the structural gene or the *nisBTCK* genes resulted in loss of nisin production and a reduced level of immunity due to lack of transcription from the promoter in front of the structural gene (Kuipers et al. 1993; Kuipers et al. unpubl.; Qiao & Saris unpubl.). Inactivation of the *nisP* gene reduced the transcription and immunity level to approximately 10% of the wild-type. Transcription of the nisin oper-

on and the lost nisin immunity of the mutant strains could be partially restored (to maximally 80% of the wild-type immunity) by an external addition of nisin to the cells (Kuipers et al. unpubl.; Ra & Saris unpubl.), except in the case of the NisK mutant strain (Kuipers et al. unpubl.), which showed that the nisin promoter is autoregulated and that NisK is probably important for the signal transduction.

The two NisI mutant strains still produced nisin. The nisin immunity and production by the NisI mutant strain (in frame deletion) was reduced to a level of approximately 25% of the wild-type strain (Kuipers et al. unpubl.). The other NisI mutant strain had also an increased sensitivity to externally added nisin (Siegers & Entian 1995) and produced a significant amount of nisin (Siegers & Entian unpubl.). The cells of the NisI mutant strains were immune to the relatively high amount of nisin they produced, arguing for that other proteins are responsible for the remaining immunity. A more drastic decrease in immunity would have been likely if NisI would play a crucial role in immunity. However, knocking out an immunity protein immediately selects for secondary mutations leading to reduced sensitivity, which might have contributed to the immunity level of the NisI mutant strains.

The studies of the nisin mutant strains suggested that of the proteins encoded by the *nisBTCIPRK* genes, NisK and NisR are necessary for the induction of immunity and NisI for high level of nisin immunity and production. The NisK mutant strain was completely nisin sensitive, whereas the immunity of the other mutant strains varied from 1–25% of wild-type immunity (Kuipers et al. unpubl.; Qiao & Saris unpubl.). This indicated that NisK is also regulating some other genes involved in immunity.

The nisFEG encoding an ABC transport system is involved in immunity

Recently, genes downstream of the *nisABTCIPRK* and the *nisZBTCIPRK* operon have been cloned and sequenced (Siegers & Entian 1995; Immonen & Saris unpubl.). Three open reading frames *nisFEG* were identified with sequence homology to ABC transport systems. The homology of NisF was highest with SpaF (44% identical residues, Immonen & Saris unpubl.; 45.5% identical residues, Siegers & Entian 1995). The *nisEG* genes encode proteins that are hydrophobic. These proteins contain six putative transmembrane domains, which is a typical amount found in channel forming proteins of both the exporters and importers of the ABC-family (Saier 1994). There is a weak similarity between NisF and NisE and the McbF-McbE transporter of *E. coli* (Siegers & Entian 1995). The McbF-McbE transport system is suggested to be involved in the transport and immunity of microcin B17 (Garrido et al. 1988), suggesting that the NisF, NisE and NisG may be involved in similar functions.

Insertion of a resistance marker into the *nisFEG* genes affected nisin production and immunity (Siegers & Entian 1995). The interruption of the *nisF* gene resulted in a minor decrease of nisin production and immunity. Inactivation of the *nisE* gene resulted in a clearly decreased nisin production and immunity. The NisG mutant strain produced slightly less nisin than the parental strain, but the increase of sensitivity to exogenously added nisin was not obvious. The cells of the mutant strains survived the relatively high amounts of nisin they produced, which means that the cells were by definition immune. However, two of the mutant strains were more sensitive than the wild type strain to exogenously added nisin indicating that the immunity system of the mutant strains were adjusted to the level of nisin the cells produced. A clear correlation is seen between the level of immunity and the amount of nisin produced, which is likely to be an effect of the autoregulation in the system. Therefore, cells with any mutation leading to a lower level of nisin production, have less transcription of all of the nisin genes, resulting in higher sensitivity to exogenously added nisin, when the immunity levels are compared with cells producing higher amounts of nisin like cells of wild-type nisin producers. This makes it difficult to make reliable conclusions of what protein is an immunity protein from studies of cells producing a lowered amount of nisin, because the amount of all the other proteins involved in nisin biosynthesis and immunity is also likely to be lowered. This holds true especially if the immunity assay used detects instant killing, which gives no time for the mutant cells to increase the level of immunity proteins induced by nisin used in the immunity assay.

Inhibition of translation of *nisF*-mRNA, *nisEG*-mRNA and *nisG*-mRNA by antisense-RNA produced from an expression plasmid did decrease the level of nisin produced (25–50% of the wild type) in the early logarithmic growth phase (Immonen & Saris unpubl.) but the cells were more resistant to exogenously added nisin (40–80%) than cells of the parent strain, except the *nisG*-antisense strain which had only 20% of wild type immunity. However, cells of the *nisG*-antisense

strain showed wild type level of nisin immunity in the stationary growth phase. Northern analysis with *nisF, nisE* and *nisG* probes of RNA isolated from cells of the *NisEG*-antisense strain (40% more immune than the wild type strain) in the early logarithmic growth phase revealed that it contained almost no *nisF*-mRNA and significantly less *nisE*- or *nisG*-mRNA than the wild type strain (Immonen & Saris unpubl.). In the stationary growth phase the same strain contained barely detectable *nisFEG*-mRNA, whereas the level of immunity was still higher than in the wild type strain. These results indicated, in contradiction with the results reported by Siegers & Entian (1995), that the products of the *nisFEG* genes are not needed for the development of high nisin immunity. Rather the NisF/NisE/NisG-ABC transporter has a role in regulation indicated by the changes in the level of nisin production and immunity in the antisense strains compared to the wild type strain. When these strains were grown over night they produced as much nisin as the wild-type nisin producer, indicating that the cells were immune and that the actual mechanism of self-protection is dependent on other proteins.

A putative transcription stop loop was found downstream of the *nisK* gene (Engelke et al. 1994; Immonen et al. 1995). Northern analysis of RNA from a nisin producer with a probe recognizing sequences of the *nisF* gene showed that the three genes downstream of the *nisK* gene form a separate operon (Ra & Saris unpubl.). From the nisin biosynthetic mutant strains of the nisin Z producer, having no detectable *nisZBTCIPRK*-mRNA, *nisFEG*-mRNA could not be detected (Ra & Saris unpubl.). After nisin induction the level of the *nisFEG*-mRNA in these strains was approximately as high as in the wild-type nisin producer (Ra & Saris unpubl.). However, the immunity level of the nisin induced mutant strains varied (10–80% of wild type), which suggested that the level of nisin immunity does not completely correlate with the expression level of the *nisFEG* genes. This is further supported by phenotype of the *nisA* mutant strain FI7332 (Dodd et al. 1992) which does not produce nisin and has wild type level of immunity, probably due to transcription of the *nisBTCIPRK* genes from a promoter of IS*905*. According to the requirement of nisin for the transcription of the *nisFEG* operon (Ra & Saris unpubl.) this strain probably lacks transcription of the *nisFEG* operon. However, the strain was still fully nisin immune (Dodd et al. 1992). In conclusion, results both support the involvement of the NisF/NisE/NisG transport system (Siegers & Entian 1995) in immunity or suggest that it is not involved in immunity (Immonen & Saris unpubl.; Dodd et al. 1992). Clearly, more experiments have to be done before the function of the NisF/NisE/NisG proteins can be assigned.

Subtilin immunity

Subtilin produced by one *Bacillus subtilis* strain and epidermin by *Staphylococcus epidermidis* are structurally similar to nisin. Therefore it is likely that the mechanism of immunity is similar. Several genes involved in biosynthesis, regulation and immunity have been cloned and sequenced. The subtilin operons consist of *spaBTCA* and *spaIFGRK* (Banerjee & Hansen 1988; Klein et al. 1992; Klein et al. 1993). All the genes showed high homology with genes in the nisin operons, with the exception of the *spaI* and *spaG* genes. The genes have been disrupted with a resistance marker. No subtilin was produced by the SpaB, SpaC, SpaA, SpaR and SpaK mutant strains. Of these strains, the SpaB and SpaC mutants were still immune to subtilin (Klein & Entian 1994). This showed that active subtilin is neither needed for subtilin immunity nor for stimulation of other immunity factors from outside of the cell. However, the interruption of the *spaA* gene, the last gene in the *spaBTCA* operon, resulted in subtilin sensitive cells. Therefore, the unmodified precursor might be directly involved in immunity or indirectly by stimulating the transcription of immunity genes. The strains with the nonfunctional regulatory SpaR and SpaK proteins were also sensitive to subtilin, indicating that they are needed in the signal transduction system to turn on immunity genes. The SpaI, SpaF and SpaG mutant strains still produced subtilin, but less than the parental strain. This indicated that also the expression level of the structural gene was lowered and because this gene product seems to be needed for the development of immunity, the SpaI, SpaF and SpaG mutant strains are likely to be sensitive to higher subtilin amounts than they produce themselves as was reported (Klein & Entian 1994). The SpaI, SpaF and SpaG mutant strains were immune to the remarkable amounts of subtilin they produced, indicating that other components are more important for immunity to subtilin. The difference between nisin and subtilin immunity seems to be that subtilin immunity is not stimulated by autoregulation of the end product from outside of the cell, but rather from inside of the cell by unmodified subtilin precursor. The experimental results do not exclude a direct role of subtilin precur-

sor in immunity, as some results of nisin immunity of nisin mutant strains indicate (Dodd et al. 1992). Another difference between these lantibiotics is that the SpaI lipoprotein is encoded in the same operon as the ABC transport system consisting of the SpaF-SpaG proteins. Lipoproteins encoded in the same operon as ABC transporters (Gilson et al. 1988; Russell et al. 1992; Sutcliffe et al 1993; Tynkkynen et al. 1993) are in gram-positive bacteria counterparts of periplasmic binding proteins of gram-negative import systems. By analogue one might speculate that the transport system encoded by the *spaFG* genes is an importer. However, it has been suggested (Klein & Entian 1994) that the subtilin leader might be cleaved inside the cell or that some of the active subtilin would penetrate the cytoplasmic membrane from the outside of the cell and that the SpaF-SpaG proteins would be needed as an additional transport system to transport active subtilin out of the cell.

Epidermin immunity

The genes needed for epidermin biosynthesis are organized in several operons, *epiABCD, epiPQ, epiT* and *epiFEG* (Schnell et al. 1992; Peschel & Götz unpubl.). Between the *epiT* gene and the *epiFEG* genes there still is a partly uncharacterized region, but this region encodes a membrane protein and not a lipoprotein (Peschel & Götz unpubl.). Inactivation of the *epiA* or *epiB* genes had no effect on epidermin immunity (Peschel & Götz unpubl.). Active epidermin has been produced in *S. carnosus* (Schnell et al. 1992) by transformation of a plasmid harbouring the *epiABCD-PQ* genes. The epidermin production was very low, below the minimal inhibitory concentration of the *S. carnosus* strain. Transformation of the *epiEFG* operon into *S. carnosus* increased the resistance to epidermin approximately 8 fold. All the genes were needed for this effect (Peschel & Götz unpubl.). The level of immunity mediated by this transport system was still clearly lower compared to the level of epidermin immunity expressed by the natural epidermin producer. A *S. carnosus* strain having both the biosynthetic machinery of epidermin and the EpiFEG transport system produced more epidermin but epidermin immunity was reduced (Peschel & Götz unpubl.). These results indicated that other immunity factors might be needed for wild type level of epidermin immunity.

The most remarkable difference between the epidermin immunity compared to nisin and subtilin immunity is that epidermin production itself is not needed for immunity or regulation of immunity. Furthermore, the biosynthetic operons of epidermin do not contain genes encoding a lipoprotein or a functional EpiT protein. A common feature is the presence of the LanFEG transport system in the producers of these lantibiotics. The conservation of this transport system in the evolution of lantibiotics argues for the importance of this system in the biosynthesis or immunity and potentially also in regulation, because strains having mutations in these genes produce less lantibiotic than the wild-type strains.

Pep5 immunity

The molecular mechanism of immunity against Pep5 clearly differs from those described for nisin, subtilin and epidermin. This could be a reflection of the fact that the proteolytic activation of Pep5 takes place inside the cells (Meyer et al. 1995). The structural organization of the immunity gene *PepI* in the Pep5 gene cluster as well as the characteristics of the PepI peptide rather point to a close relationship of the Pep5 immunity system to those of the unmodified bacteriocins, e.g. lactococcin A produced by *Lactococcus lactis* subsp. *cremoris* (Nissen-Mayer et al. 1993) or the recently characterized immunity protein of Carnobacteriocin B2 produced by *Carnobacterium piscicola* (Quadri et al. 1995).

The immunity gene *pepI*, coding for the 69 amino acid PepI peptide was localized upstream of the structural gene *pepA* on the 20 kb plasmid pED503 of *S. epidermidis* 5 (Reis & Sahl 1991). This plasmid was shown to contain the genetic information for Pep5 production and immunity (Ersfeld-Dreßen et al. 1984) organized in the Pep5 biosynthetic gene cluster *pepTIAPBC* (Meyer et al. 1995; Bierbaum et al. this volume).

In contrast to the lantibiotics nisin and subtilin no graduated level of immunity could be seen in the case of Pep5. Wild-type strain level of insensitivity towards Pep5 could be restored in *S. epidermis* 5 $Pep5^-$ mutants (devoid of plasmid pED503 with a $Pep5^-$ Imm^- phenotype) by transformation with a DNA fragment containing the intact structural gene *pepA* and the immunity gene *pepI*. Deletion clones harbouring either *pepI* without *pepA* or *pepI* with incomplete *pepA* were not immune, which clearly demonstrates the involvement of the structural gene in immunity (Reis & Sahl 1991). The question is how *pepA* is involved in the expres-

sion of the immunity phenotype. Obviously some yet unidentified regulatory mechanism prevents PepI synthesis when *pepA* is absent.

The immunity peptide PepI is characterized by a striking charge distribution. It consists of a N-terminal 20 amino acid hydrophobic region, followed by a hydrophilic C-terminal domain with a net positive charge. The immunity peptide could be detected by immunoblotting with anti-PepI-antiserum in soluble and membrane fractions of the wild-type Pep5 producer strain *S. epidermidis* 5 and of all the variants expressing the immunity phenotype. Membrane washing experiments further indicated that PepI should be weakly associated with the cytoplasmic membrane. The association of PepI with the cytoplasmic membrane is thought to be mediated by the hydrophobic N-terminus of the peptide; it is supposed to be located on the outer surface of the cell membrane because the level of PepI was significantly reduced by protease digests performed with osmotically stabilized protoplasts (Reis et al. 1994).

In contrast to the potential immunity peptides NisI and SpaI, which have typical lipoprotein consensus sequences (Kuipers et al. 1993; Klein et al. 1994; Immonen et al. 1995), PepI contains no characteristic signal sequence which would direct secretion of the peptide in a *sec*-dependent process. Therefore a specific transporter similar to those found in other lantibiotic gene clusters, e.g. the gene products of *nisT* (Engelke et al. 1992; Steen et al. 1991) and *spaT* (Klein et al. 1992; Chung et al. 1992), could be necessary for translocation. This function cannot be ascribed to the transporter encoded by *pepT* (Meyer et al. 1995) but rather must be mediated by a hypothetic transporter protein encoded on the chromosome of *S. epidermidis* 5: this was concluded from results with the recombinant plasmid pMR11 (containing only *pepA* and *pepI*) which conferred full immunity to Pep5 when transferred into *S. epidermidis* 5 Pep5$^-$ (Reis & Sahl 1991).

So far there is no information on the molecular mechanism of the Pep5 immunity system. The antagonizing function of PepI could be demonstrated by ^{14}C-labeled L-proline uptake and efflux experiments performed with the Pep5 sensitive mutant *S. epidermidis* 5 Pep5$^-$ and the immune mutant harbouring *pepA* and *pepI* (pMR2). In contrast to the sensitive mutant, no Pep5 induced inhibition of proline accumulation could be seen with the cells of the immune variant (pMR2). Moreover, strain pMR2 did not show any significant efflux of accumulated amino acids after Pep5 addition (Reis et al. 1994). This clearly demonstrated that PepI prevents the pore formation by Pep5 and that the site of its action is the cytoplasmic membrane.

To study the functional role of PepI, site-directed mutagenesis was used to introduce charged residues into the hydrophobic N-terminal part of PepI and to shorten the C-terminal domain (Pag et al. unpubl.). All mutants showed reduced levels of immunity as compared to the wild-type strain, but were less sensitive against Pep5 than the pED503-cured variant *S. epidermidis* 5 Pep5$^-$. However, immunoblots of cell extracts of these clones indicated degradation of the mutated PepI molecules (Pag et al. unpubl.) thus making interpretations as to structural requirements for PepI activity very difficult. This could be due to instability of the mutated peptides probably subjected to an accelerated degradation. The reduced level of functional PepI would explain the decrease of immunity in these mutants.

Immunity to other lantibiotics

Several other lantibiotics of type A have been described, like gallidermin (Kellner et al. 1988), salivaricin (Ross et al. 1993), lactococcin DR (Rince et al. 1994), lacticin 481 (Piard et al. 1993), carnocin U149 (Stoffels et al. 1992), SA-FF22 (Hynes et al. 1993), mutacin (Novak et al. 1994), cytolysin (Gilmore et al. 1990) and lactocin S (Mørtvedt et al. 1991). The immunity mechanism of gallidermin and epidermin producers is likely to be similar, because the lantibiotics are structurally identical except for one amino acid. Nearly nothing is known about the genes involved in immunity of the other lantibiotics. The protease processing cytolysin seems to be involved in the protection of the producer via a potential additional cleavage of the bacteriocin at the cell surface. Downstream of the *lasA* gene eight genes (*lasM, orf239, lasT, orf93, orf125, lasP, orf57* and *orf414*) involved in biosynthesis of lactocin S have been cloned and sequenced (Skaugen 1994). Insertion of IS*1163*, with a potential polar effect, into the first (*lasM*) and second gene (*lasT*) abolished the lactocin S production but did not affect immunity. This showed that the potential transport protein and LasM are not needed for lactocin S immunity. Any involvement of the other gene products of the *las* operon in lactocin S immunity is still unknown. However, no lipoprotein or ABC transporter similar to SpaFG, EpiFEG and NisFEG is encoded by the *las* operon indicating a different mechanism of immunity if the *las* encodes an immunity factor.

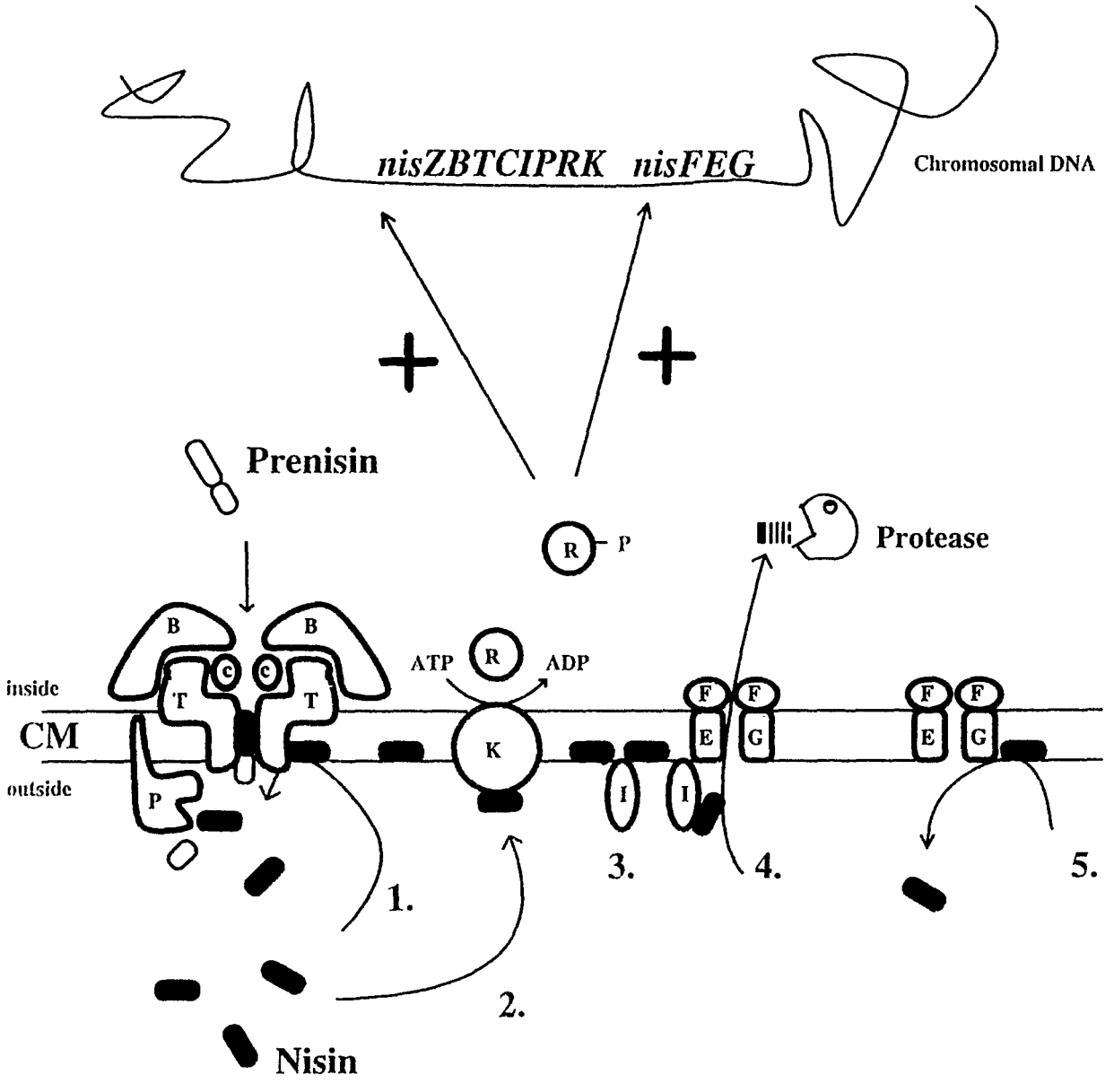

Fig. 1. Hypothetical mechanisms of nisin immunity. Prenisin is modified, secreted and processed (van der Meer et al. 1993) by the biosynthetic machinery. 1. Active nisin adsorbed to the membrane is recognized by NisT and translocated from the membrane. 2. Nisin activates NisK which autophosphorylates NisR. In this form NisR activates the transcription of the nisin operons resulting in immunity (suggested by induction results; Kuipers unpubl.; Ra & Saris unpubl.). 3. The lipoprotein NisI inhibits pore formation or (4) binds nisin associated with the cell surface and facilitates nisin import via the NisFEG transport system. Import is followed by degradation inside the cell. 5. The NisFEG transport system recognizes membrane bound nisin and translocates it or destabilizes pore formation. The translocation process could be analogous to the function of multidrug resistance pumps (Lewis 1994). NisI could also be involved in all of the described processes. Immunity would result either from an active translocation (NisT and NisF are ATP binding proteins) of nisin from the membrane or from protein interactions inhibiting pore formation or activity.

Mechanism of immunity

Inhibition of the bactericidal effect of the pore-forming lantibiotics might be achieved by several strategies. Adsorption of the lantibiotic to the membrane could be inhibited, membrane adsorbed bacteriocins could be translocated or taken into the cytoplasm for degradation or transport. Several molecules of a lantibiotic are required to form a pore. This assembly process could be inhibited by specific interactions of membrane associated immunity proteins. The assembled pore could be destabilized or the pore could be plugged by a peptide. There is no cross immunity between producers of lantibiotics. Nisin producers are sensitive to subtilin and *vice versa*, which shows that the interactions resulting in immunity are very specific. The modification machinery is not as specific, because a subtilin producer can be modified to produce active nisin (Rintala et al. 1993). Cross immunity has been observed between strains producing natural variants, such as nisin A and nisin Z (de Vos et al. 1993) or epidermin and gallidermin (Peschel & Götz unpubl).

At least two different mechanisms of immunity have evolved. The mechanism of Pep5 immunity seems to involve an interaction of the PepI protein with Pep5 at the outer surface of the cytoplasmic membrane. The structure of PepI does not indicate that any transport events would be involved in Pep5 immunity. It is rather thought that the mechanism involves inhibition of pore formation by interactions at the outer surface of the cytoplasmic membrane.

Inhibition of pore formation by protein interactions at the surface of the cytoplasmic membrane seems also to be a potential immunity mechanism of subtilin and nisin producers, suggested by the location of the lipoproteins SpaI and NisI (Fig. 1). The potential immunity activity of NisI requires the assistance of other proteins. Utilization of transport either into or out of the cell could also be an additional mechanism of immunity of nisin, subtilin and epidermin resulting in a low concentration of the lantibiotic in the membrane low enough to inhibit pore formation. Other mechanisms of immunity involving inhibition of membrane adsorption or pore assembly might also contribute to the total level of immunity. Potentially all of the proteins in the biosynthetic machinery have affinity to the lantibiotic and could therefore have direct interactions with the lantibiotic. The biosynthetic proteins form most likely a complex located in the membrane. Wild type level of immunity might require a functional biosynthetic complex. This could be the situation in nisin immunity, because wild type level of nisin immunity has been achieved only when all of the components of the biosynthetic machinery are present. Additional proteins involved in immunity might also yet be found.

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