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MINIREVIEW

Regulation of VanA- and VanB-Type Glycopeptide Resistance in Enterococci

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Glycopeptide antibiotics vancomycin and teicoplanin inhibit cell wall synthesis by forming complexes with the peptidyl-D-alanyl–D-alanine (D-Ala–D-Ala) termini of peptidoglycan precursors at the cell surface (33). Acquired resistance to these antibiotics is mostly due to two types of gene clusters, designated *vanA* and *vanB*, that confer resistance by the same mechanism and encode related enzymes (Fig. 1) (10). In both cases, resistance is due to synthesis of peptidoglycan precursors ending in the depsipeptide D-alanyl–D-lactate (D-Ala–D-Lac) that bind to glycopeptides with reduced affinity (15, 17). The *vanA* and *vanB* clusters comprise three genes (*vanHAX* and $vanH_B$-BX_B) that are necessary and sufficient for resistance (9) and appear to originate from glycopeptide-producing bacteria (32). These genes encode a dehydrogenase (VanH or $VanH_B$) that reduces pyruvate into D-Lac, a ligase (VanA or VanB) that synthesizes D-Ala–D-Lac, and a D,D-dipeptidase (VanX or $VanX_B$) that hydrolyzes D-Ala–D-Ala. The combined action of these three enzymes results in incorporation of D-Ala–D-Lac instead of D-Ala–D-Ala into peptidoglycan precursors (8). In addition, the *vanA* and *vanB* clusters encode a D,D-carboxypeptidase (VanY or $VanY_B$) that contributes to glycopeptide resistance by cleaving the C-terminal D-Ala of late membrane-bound peptidoglycan precursors (3). The latter precursors originate from incomplete hydrolysis of D-Ala–D-Ala by the VanX or $VanX_B$ D,D-dipeptidases (34). Finally, the *vanA* and *vanB* clusters also contain two genes of unknown function (*vanZ* and *vanW*, respectively) that do not have significant sequence similarity to previously sequenced genes or to each other (17). The VanZ protein was found to confer low-level resistance to teicoplanin only (6), whereas the role of VanW in resistance has not been investigated.

Expression of the resistance genes of the *vanA* and *vanB* clusters is regulated by the VanRS and $VanR_BS_B$ two-component regulatory systems, each composed of a membrane-associated sensor kinase (VanS or $VanS_B$) and a cytoplasmic response regulator (VanR or $VanR_B$) that acts as a transcriptional activator (Fig. 1) (9, 17). The regulatory and resistance genes are transcribed from distinct promoters that appear to be coordinately regulated. Namely, the VanRS regulatory system controls transcription of the *vanRS* and *vanHAXYZ* operons at the P_R and P_H promoters (5). Likewise, the $VanR_BS_B$ system controls transcription of the $vanR_BS_B$ and $vanY_BWH_BBX_B$ operons at the P_{RB} and P_{YB} promoters (17, 35). The present review is focused on how the regulation of these promoters determines inducible expression of VanA- and VanB-type glycopeptide resistance. Background information on regulation of bacterial genes by two-component regulatory systems can be found elsewhere (25).

OVERVIEW OF EVENTS THAT LEAD TO INDUCTION OF RESISTANCE

The membrane-associated domains of VanS and $VanS_B$ are thought to sense the presence of glycopeptides in the culture medium by an unknown mechanism (Fig. 1). The signal is transduced to the cytoplasmic catalytic domain of the sensors, leading to kinase stimulation, autophosphorylation of a specific histidine residue, and transfer of the phosphoryl group to a specific aspartate residue of the partner response regulators. The phosphorylated regulators bind to the regulatory region of the target promoters, resulting in transcriptional activation of the regulatory and resistance genes. Ultimately, induction brings about a switch from synthesis of precursors ending in D-Ala–D-Ala to precursors ending in D-Ala–D-Lac.

INDIVIDUAL COMPONENTS OF SIGNAL TRANSDUCTION PATHWAY IN VanA-TYPE ENTEROCOCCI

Phosphotransfer reactions catalyzed by VanS and VanR in vitro. VanR and the cytoplasmic kinase domain of VanS fused to the maltose binding protein have been overerproduced in *Escherichia coli*, purified, and assayed for phosphotransfer reactions (40). VanS was found to catalyze ATP-dependent autophosphorylation of a histidine residue. Upon addition of VanR, the phosphoryl group was transferred from the phosphohistidine residue of VanS to an aspartate residue of the response regulator. In the absence of VanS, dephosphorylation of the phosphorylated form of VanR (phospho-VanR) was extremely slow compared to that of related response regulators, with a half-life of 10 h. VanS stimulated this reaction, indicating that the sensor has a so-called phosphatase activity.

Phospho-VanS was also shown to transfer its phosphoryl group to the PhoB response regulator of the *E. coli* Pho regulon (phosphorus assimilation) (18). Kinetic comparison revealed a 10^4-fold preference for phosphotransfer to VanR

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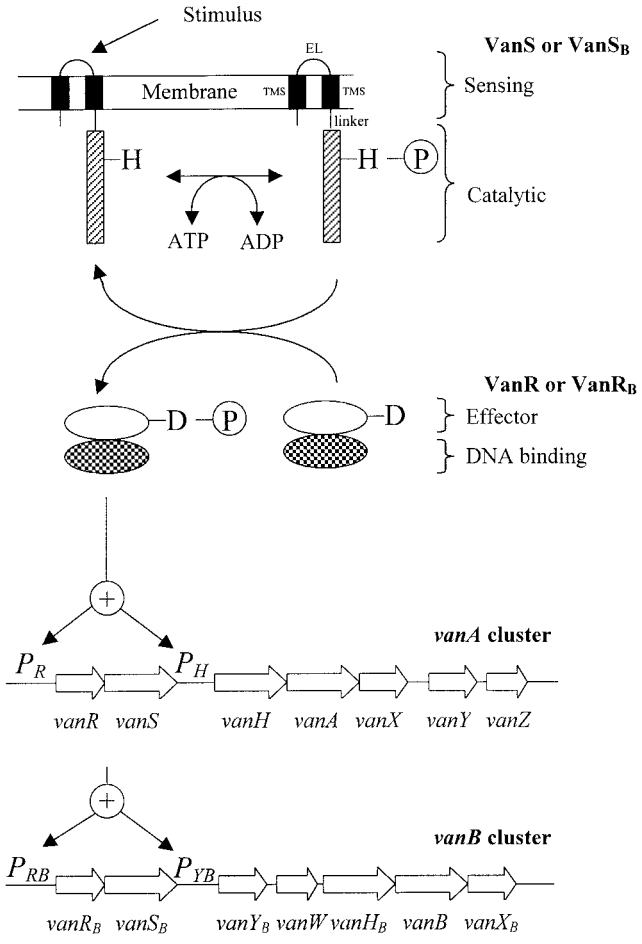


FIG. 1. Events leading to transcriptional activation of the *vanA* and *vanB* gene clusters. The Van sensors contain two *trans*-membrane segments (TMS) that delineate an external loop (EL). The sequence of these regions of VanS and VanS$_B$ are not related, as is found for sensors that recognize different signals (25). A linker connects the membrane-associated domain to the catalytic cytoplasmic domain (hatched). The catalytic domain is conserved in all sensors and contains a conserved histidine residue (H) which is autophosphorylated. The phosphoryl group (P) is then transferred to an aspartate residue (D) of the effector domain of VanR or VanR$_B$. The effector domain is also conserved in all response regulators (25). Phosphorylation of the effector domain activates the DNA binding domain leading to increased affinity for the regulatory regions of the target promoters. The DNA binding domains of VanR and VanR$_B$ are related to those of response regulators belonging to the OmpR subfamily (25).

compared to PhoB (19). Nonetheless, VanS was able to activate PhoB in vivo, leading to a 500-fold increase in transcription from a PhoB-activated promoter (18). Finally, VanR catalyzes its own phosphorylation in the absence of any kinase by using acetyl-phosphate as a substrate (26).

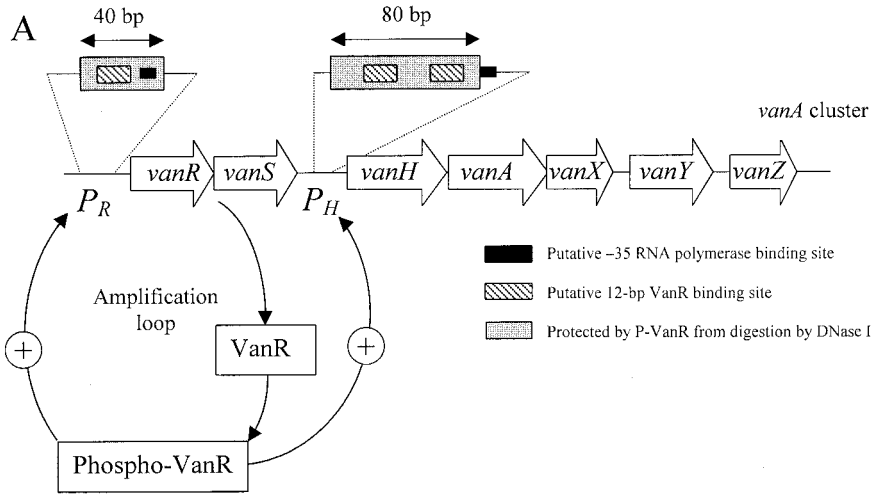
In vitro binding of VanR to the regulatory region of P_R and P_H promoters. VanR and phospho-VanR bind to a similar 80-bp region of the regulatory region of the P_H promoter that contains two putative 12-bp VanR-binding sites (26) (Fig. 2A). The P_R promoter contains a single 12-bp binding site, and phosphorylation of VanR increases the size of the protected region from 20 to 40 bp. Phosphorylation of VanR increases the affinity of the response regulator for both promoters (Fig. 2B). The affinity is higher for P_H than for P_R, and the difference is more important for phospho-VanR than for VanR. These observations suggest that phospho-VanR may cooperatively bind to the P_H promoter.

In vivo activation of the P_R and P_H promoters in *Enterococcus faecalis*. Activation of the P_H and P_R promoters has been studied using various types of transcriptional fusions with reporter genes. Parallel determinations of D,D-dipeptidase activity and of the cytoplasmic pool of peptidoglycan precursors showed that expression of glycopeptide resistance is mainly, if not exclusively, regulated at the level of transcriptional initiation at these promoters (8). The P_H and P_R promoters were found to have a similar strength and to be similarly regulated (Fig. 2C) (5). The promoters are inactive in the absence of VanR and VanS, constitutively activated by VanR in the absence of VanS, and inducible by glycopeptides if the host produces both VanR and VanS. These results indicate that VanR is a transcriptional activator required for initiation at both promoters. VanS is not necessary for full activation of the promoters, because VanR can be activated (phosphorylated) independently from its partner sensor, presumably by a heterologous kinase encoded by the host chromosome. In contrast, VanS is required for negative control of the promoters in the absence of glycopeptides. The sensor is therefore thought to act as a phosphatase under noninducing conditions, preventing accumulation of phospho-VanR.

In vivo evidence for phosphatase activity of VanS. Sensor kinases of two-component regulatory systems display a highly conserved amino acid motif, called the H box, that contains the phosphorylated histidine residue (Fig. 1) (25). Replacement of this histidine by glutamine abolishes autophosphorylation of the sensors and, consequently, transfer of phosphoryl groups to the response regulators (27). However, the modified sensors retain phosphatase activity. Production of VanSH$_{164}$Q, i.e., VanS which carries a histidine (H)-to-glutamine (Q) substitution at the putative autophosphorylation site (position 164), prevented transcriptional activation in the absence of glycopeptides, indicating that the protein was acting as a phosphatase (Table 1) (4). VanSH$_{164}$Q also prevented transcription of the resistance genes in the presence of glycopeptides, suggesting that the phosphatase activity of this protein was not negatively modulated by the antibiotics. Negative control of VanR by VanS$_{164}$Q confirmed that the phosphorylated form of the response regulator is responsible for transcriptional activation.

Amplification of signal. As indicated above, phospho-VanR binds to the P_R promoter and activates transcription of the *vanR* and *vanS* genes. Thus, regulation of the *vanA* gene cluster involves not only a modulation of the relative concentration of VanR and phospho-VanR by the kinase and phosphatase activities of VanS but also a modulation of the absolute concentration of the response regulator (4, 5). An amplification loop results from binding of phospho-VanR to the P_R promoter, increased expression of *vanR*, and accumulation of phospho-VanR following phosphorylation (Fig. 2A). This may account for the high-level transcription of the resistance genes observed in *vanS* null mutants, since the amplification loop, in combination with the long half-life of phospho-VanR, may compensate for inefficient phosphorylation of the response regulator by the putative host kinase (5).

Disruption of the amplification loop was obtained in a *vanS*



B. *in vitro* binding EC_{50} (μM)

Form of VanR	P_R	P_H
Phospho-VanR	1.6	0.040
VanR	100	20

C. *in vivo* activity of P_R and P_H

VanR VanS	inducible
VanR	constitutive
none	inactive

FIG. 2. Regulation of the P_R and P_H promoters. (A) Schematic representation of the *vanA* gene cluster showing details of the regulatory region of P_R and P_H. An amplification loop results from binding of phospho-VanR to P_R, increased transcription of *vanR*, and accumulation of phospho-VanR following phosphorylation (4). (B) Determination of the effective concentrations of phospho-VanR and VanR required to saturate at 50% (EC_{50}) DNA fragments carrying the P_R and P_H promoters based on gel shift experiments indicated that phosphorylation increases the affinity of the response regulator for DNA at both promoters and that the affinity is higher for P_H than for P_R (26). In spite of these differences, the P_R and P_H promoters were found to be coordinately regulated in vivo (C) and to have a similar strength (5).

null mutant by introducing a multicopy plasmid carrying the P_H promoter (4). Introduction of the plasmid prevented transcription of the resistance genes in the absence of glycopeptides, indicating that the high-affinity VanR binding sites of P_H titrated phospho-VanR, thereby preventing the activation of P_R. Expression of the resistance genes was inducible by glycopeptides, suggesting that stimulation of a putative host kinase by these antibiotics can compensate for titration of phospho-VanR (4). This surprising result implies that the chromosome of susceptible *E. faecalis* encodes a glycopeptide sensor.

ACQUISITION OF TEICOPLANIN RESISTANCE BY VanB-TYPE ENTEROCOCCI

Enterococci harboring clusters of the *vanB* class remain susceptible to teicoplanin, since this antibiotic does not trigger induction of the resistance genes (8, 17). However, teicoplanin selects mutants resistant to this antibiotic that belong to one of three phenotypic classes (11, 12) due to three distinct alterations of $VanS_B$ functions (13, 14) (Table 1). Mutations leading to teicoplanin resistance also confer low-level resistance to the investigational glycopeptide LY333328 (7).

Inducible phenotype. Mutations leading to inducible expression of vancomycin and teicoplanin resistance introduced amino acid substitutions in the sensor domain of $VanS_B$ (13). A minority of the substitutions were located between the two putative *trans*-membrane segments of $VanS_B$ (Fig. 1). This portion of the sensor is located at the outer surface of the membrane and may therefore interact directly with ligands, such as glycopeptides, which do not penetrate into the cytoplasm. The majority of the substitutions were located in the linker that connects the membrane-associated domain to the cytoplasmic catalytic domain. Based on analogies with related sensor kinases, these substitutions may affect signal transduction (13). The analysis of the inducible mutants provides experimental evidence that the N-terminal domain of $VanS_B$ is involved in signal recognition and also provides one of the rare examples of modification of specificity of a sensor kinase.

Constitutive phenotype. Mutations responsible for constitutive expression of the *vanB* cluster led to amino acid substitutions at two specific positions located on either side of the histidine at position 233, which corresponds to the putative autophosphorylation site of $VanS_B$ (13) (Table 1). Constitutive expression of glycopeptide resistance is most probably due to impaired dephosphorylation of $VanR_B$ by $VanS_B$, as similar substitutions affecting homologous residues of related sensor kinases impair the phosphatase but not the kinase activity of the proteins (13).

Expression of the *vanB* cluster was inducible by vancomycin in a mutant in which site-directed mutagenesis was used to introduce glutamine instead of histidine at the phosphorylation

TABLE 1. Phenotype associated with alterations of VanS and $VanS_B$ functions in *E. faecalis*

Function alteration and allele (cluster)	Expression of resistance genes
None	
Wild-type *vanS* (*vanA*)	Inducible by VAN, TEC, and MOE[a]
Wild-type $vanS_B$ (*vanB*)	Inducible by VAN
Loss of all functions	
Null allele of *vanS (vanA)*	Constitutive (inducible by VAN, TEC, and MOE if P_H titrates phospho-VanR)
Null allele of $vanS_B$ *(vanB)*	Clonal variation, inducible by VAN, TEC, and MOE
Loss of kinase activity due to substitutions at autophosphorylation site	
vanS $H_{164}Q$ (*vanA*)	Repressed under all conditions
$vanS_B$ $H_{233}Q$ (*vanB*)	Inducible by VAN
Alteration of signal recognition or transduction due to substitution in sensing domain[b]	
$vanS_B$ $A_{30}G$ (*vanB*)	Inducible by VAN and TEC
$vanS_B$ $D_{168}Y$ (*vanB*)	Inducible by VAN and TEC
Impaired phosphatase activity due to substitutions in H box	
$vanS_B$ $S_{232}Y$ (*vanB*)	Constitutive
$vanS_B$ $Y_{237}M$ or K (*vanB*)	Constitutive

[a] MOE, moenomycin; TEC, teicoplanin; VAN, vancomycin.
[b] The substitutions were located in the external loop (e.g., $A_{30}G$) or in the linker (e.g., $D_{168}Y$) of $VanS_B$.

site at position 233 (Table 1) (4). Taken together, these observations confirmed that dephosphorylation of $VanR_B$ is required to prevent transcription of the resistance genes and indicated that the phosphatase activity of $VanS_B$ is negatively regulated by vancomycin independently from autophosphorylation at H_{233} (4, 13).

Heterogeneous phenotype. The heterogeneous mutants most probably harbor null alleles of $vanS_B$ since the mutations introduced translation termination codons at various positions of the gene (Table 1). The antibiotic disk diffusion assay revealed the presence of inhibition zones containing scattered colonies that grew predominantly in 48 h (11). Since this phenotype suggested the presence of a mixture of susceptible and resistant bacteria, the P_{YB} promoter (Fig. 1) was fused to a green fluorescent protein reporter gene in order to analyze gene expression in single cells based on fluorescence microscopy and flow cytometry (14). Use of this reporter system revealed that cultures of heterogeneous mutants grown in the absence of glycopeptides contain a major nonfluorescent subpopulation and a smaller sub population with various fluorescence intensities. Thus, $VanR_B$ was activating transcription of the resistance genes only in a minority of the bacteria. Attempts to grow fluorescent and nonfluorescent bacteria as pure cultures were unsuccessful, indicating that expression of the resistance genes was reversibly turned on and off under noninducing conditions, a phenomenon referred to as clonal variation (39). Vancomycin and teicoplanin induced expression of the reporter gene in bacteria of the major nonfluorescent subpopulation (14). These observations imply that clonal variation and induction both result from activation of $VanR_B$ by a host kinase which is stimulated by glycopeptides.

The amplification loop that results from binding of phospho-$VanR_B$ to the P_{RB} promoter and activation of $vanR_B$ transcription may be critical for clonal variation (14, 39). If the total concentration of $VanR_B$ is low in a bacterium, inefficient phosphorylation of the protein by the putative host kinase may not be adequate to provide sufficient phospho-$VanR_B$ for activation of P_{RB} and production of $VanR_B$. Expression of the resistance genes may tend to remain turned off in the progeny of this susceptible bacterium since the concentration of $VanR_B$ is expected to remain low following cell division. In contrast, if the concentration of $VanR_B$ is high in a bacterium, enough phospho-$VanR_B$ may be produced by inefficient phosphorylation by the host kinase so that auto-activation of $vanR_B$ transcription occurs and persists over several generations. According to this model, stimulation of the host kinase by glycopeptides results in induction, since this would result in efficient phosphorylation of $VanR_B$ in all bacteria. It is worth nothing that the phenomenon of clonal variation observed in $vanS_B$ null mutants may be a particular type of adaptative response that enables genetically identical bacteria to express different sets of genes.

In vivo emergence of teicoplanin resistance. Teicoplanin-resistant mutants have been observed in a patient (24) and in rabbits with experimental endocarditis treated with vancomycin or teicoplanin (11). In the animal model, the combination of gentamicin and teicoplanin was effective in reducing the number of surviving VanB-type *E. faecalis* organisms in cardiac vegetations and prevented the emergence of resistant mutants (11). Subsequent work revealed that two mutations were required to abolish the synergistic activity of teicoplanin and gentamicin against wild VanB-type strains: an initial mutation that allows expression of teicoplanin resistance by one of the three mechanisms discussed above and a second mutation conferring a moderate (two to fivefold) increase in the intrinsic level of gentamicin resistance (30). Simultaneous acquisition of the two types of mutations is probably a rare event that was not observed in the animal or in vitro (30). These laboratory investigations suggest that emergence of teicoplanin resistance may be responsible for therapeutic failure, although the use of drug combinations may prevent the selection of resistant mutants (11, 30).

Acquisition of teicoplanin resistance in two steps. VanB-type enterococci requiring vancomycin for growth (vancomycin-dependent phenotype) are readily selected by vancomycin in vitro (13), in a rabbit model of aortic endocarditis (11), and

in patients (20, 37). These mutants harbor mutations in the host D-Ala–D-Ala ligase gene and depend entirely on the VanB ligase for peptidoglycan synthesis (13). Impaired D-Ala–D-Ala ligase activity accounts for vancomycin dependence, since the VanB protein is only produced under conditions of induction by this antibiotic. Impaired D-Ala–D-Ala ligase also results in increased resistance to vancomycin, since incorporation of D-Ala–D-Ala into peptidoglycan precursors is abolished (13). Mutations in $vanS_B$ that lead to constitutive expression of the resistance genes are readily selected in vancomycin-dependent bacteria, not only on teicoplanin, but also on media devoid of antibiotics, since such mutations allow growth in the absence of the inducer (13, 37). Thus, vancomycin may indirectly select for constitutive teicoplanin resistance in two steps. This may account for the emergence of teicoplanin resistance in a patient treated with vancomycin (24) and may also account for constitutive expression of *vanB*-related gene clusters in clinical isolates of *E. faecalis* that harbor null mutations in the *ddl* D-Ala–D-Ala ligase gene (16).

CROSS TALK AND REGULATION IN HETEROLOGOUS HOSTS

The chromosome of most bacteria encodes several related two-component regulatory systems, and analysis of the unfinished *E. faecalis* genome sequence available from The Institute for Genomic Research reveals the presence of at least 20 pairs of sensor kinases and response regulators. Sequence conservation in the kinase domain of the sensors and in the effector domain of response regulators can allow inefficient phosphotransfer reactions between nonpartner sensors and response regulators (25, 38). In vivo, such cross-reactivities (cross talk) usually lead to activation of response regulators by heterologous kinases in mutants that do not produce the partner sensor kinase. We shall compare activation of VanR and $VanR_B$ by partner and nonpartner sensors in *E. faecalis*, *E. coli*, and *Bacillus subtilis*.

***E. faecalis*.** Expression of the resistance genes in *E. faecalis* is inducible by glycopeptides in $vanS_B$ and *vanS* null mutants if the latter harbor P_H on a multicopy plasmid (see above and Table 1) (4, 14). Induction occurs with the same set of antibiotics, indicating that the response regulators may be activated by the same kinase (Table 1) (4). These observations raise the possibility that *E. faecalis* harbors, as of yet, unidentified genes expressed under the control of this putative kinase in response to inhibition of peptidoglycan synthesis (4).

***E. coli*.** The P_H and P_R promoters of the *vanA* gene cluster are specifically activated by VanR in *E. coli* (35). Likewise, the P_{YB} and P_{RB} promoters are specifically activated by $VanR_B$ (35). Phosphorylation is required for promoter activation by both response regulators (22, 35). VanR and $VanR_B$ belong to the OmpR subfamily of response regulators based on sequence similarity in the DNA binding domains of the proteins (9). Response regulators of this subfamily activate promoters that are recognized by the major form of RNA polymerase corresponding to $E_{\sigma 70}$ in *E. coli* (25). Therefore, it is perhaps not surprising that there are the same requirements for promoter activation in distantly related bacteria such as *E. coli* and *E. faecalis*.

In the absence of VanS, VanR was activated by several mechanisms involving the PhoB and CreB kinases of the Pho regulon and acetyl-phosphate synthesis by the AckA-Pta pathway (22, 35). VanR catalyzes in vitro its own phosphorylation, using acetyl phosphate as a substrate (26), and this reaction may also occur in vivo (22). Deletion of *phoR*, *creB*, *ackA*, and *pta* prevented activation of the P_R and P_H promoters by cross talk and allowed investigators to establish that VanS can activate VanR in *E. coli* (22, 35). Stimulation of VanS by glycopeptides could not be tested in this expression system since the antibiotics do not penetrate the *E. coli* outer membrane. Thus, in the absence of the known stimulus, VanS appears to act as a kinase in *E. coli* (35) but as a phosphatase in *E. faecalis* (5).

The P_{RB} and P_{YB} promoters of the *vanB* cluster were also activated by $VanR_B$ in the absence of $VanS_B$ in *E. coli* (35). Activation by cross talk was observed in the absence of the *phoB*, *creB*, *ackA*, and *pta* genes, indicating that VanR and $VanR_B$ are activated by different pathways. $VanS_B$ prevented activation of $VanR_B$ by cross talk, revealing that the sensor acts as a phosphatase in the absence of the stimulus, as was shown in *E. faecalis*.

***B. subtilis*.** Regulation of the P_H promoter was analyzed in *B. subtilis* hosts producing VanR, VanR and VanS, or neither (36). As expected, VanR was required for promoter activation. Glycopeptides induced P_H both in the presence and absence of VanS, although induction required higher drug concentrations in the absence of this sensor. Thus, activation of VanR by cross talk is regulated by glycopeptides in *B. subtilis*, indicating that this host produces a glycopeptide sensor as does *E. faecalis*. Of note, a glycopeptide-inducible transcriptional fusion was obtained by screening random insertions of a Tn*917* derivative carrying a promoterless *lac* reporter gene in *B. subtilis* (28). Expression of the transcriptional fusion might be controlled by the kinase responsible for activation of $VanR_B$ in the absence of $VanS_B$.

Cross talk between VanRS and $VanR_BS_B$ regulatory systems. Production of the VanS sensor restored homogeneous high-level glycopeptide resistance of a heterogenous $vanS_B$ null mutant and significantly increased synthesis of the $VanX_B$ D, D-dipeptidase (4). These observations suggest that VanS can phosphorylate $VanR_B$. Activation of $VanR_B$ by VanS could not be tested in *E. coli* since cross talk led to high-level activation of this response regulator (35).

Activation of VanR by $VanS_B$ could not be tested in either *E. faecalis*, since cross talk led to high-level activation of the P_H and P_R promoters (4), or *E. coli*, since $VanS_B$ a phosphatase in this host, and stimulation by glycopeptides could not be tested (35). Finally, VanS, $VanSH_{164}Q$, $VanS_B$, and $VanS_BH_{233}Q$ did not prevent promoter activation by cross talk in *E. coli* or *E. faecalis*, suggesting that the Van sensors cannot dephosphorylate nonpartner Van response regulators (4, 35).

NATURE OF THE STIMULUS

The nature of the signal recognized by the VanS and $VanS_B$ sensors is unknown. In fact, it has never been established that signal recognition involves a direct interaction between the sensors and a specific ligand such as glycopeptides or peptidoglycan precursors. Such specific interactions may not exist if signal recognition depends upon a physical constraint imposed on the membrane by the inhibition of peptidoglycan synthesis

(36). The issue was addressed indirectly by determining which compounds, mostly antibiotics, trigger induction. There are discrepancies between the conclusions of different groups that used different reporter systems in different hosts, although there is a consensus that the *vanA* gene cluster is inducible by subinhibitory concentrations of all antibiotics that inhibit the transglycosylation reaction, i.e., the transfer of disaccharide-pentapeptide subunits from lipid intermediate II to the nascent peptidoglycan at the outer surface of the membrane. These antibiotics include glycopeptides and moenomycin, which are structurally unrelated and inhibit the transglycosylases by different mechanisms (12, 23).

Three groups found that VanA-type resistance is inducible by glycopeptides and moenomycin but not by drugs that inhibit the reactions preceding (e.g., ramoplanin) or following (e.g., bacitracin and penicillin) transglycosylation (12, 23, 31). This narrow specificity suggests that accumulation of lipid intermediate II, resulting from inhibition of transglycosylation, may be the signal recognized by the VanS sensor. This would account for induction by antibiotics that inhibit the same step of peptidoglycan synthesis but have different structures (12, 23). In contrast, the $VanS_B$ sensor may interact directly with vancomycin since teicoplanin is not an inducer (12). Amino acid substitutions in the sensor domain of $VanS_B$ allowed induction by teicoplanin but not by moenomycin (see above and Table 1) (12, 13). The primary amino acid sequence of the sensor domains of VanS and $VanS_B$ are unrelated (17). These observations indicate that VanS and $VanS_B$ may sense the presence of glycopeptides by different mechanisms (13).

Two groups reported induction of VanA-type resistance by inhibitors of late (e.g., glycopeptides, moenomycin, bacitracin, and ramoplanin) but not of early (e.g., fosfomycin and D-cycloserine) stages of peptidoglycan synthesis (1, 21). The broader specificity observed by these authors supports the concept that inhibition of peptidoglycan polymerization is critical for induction but does not imply that accumulation of a specific precursor is involved.

Lastly, one group analyzed regulation of the P_H promoter in *B. subtilis* instead of in enterococcal hosts and reported induction by bacitracin, penicillin, fosfomycin, and D-cycloserine as well as by treatment with lysosyme, mutanolysin, and lysostaphin (36). The authors concluded that it is unlikely that VanS is stimulated by the accumulation of peptidoglycan precursors.

Attempts to correlate antibacterial activity and induction using a large number of glycopeptide antibiotics indicated that binding to the peptidyl-D-Ala–D-Ala target and structural features of the antibiotic may both be important for induction (21). This observation may imply that regulation involves recognition of more than one signal (21) but is also compatible with the possibility that VanS interacts with a lipid intermediate II-glycopeptide complex at the outer surface of the membrane. Of note, two large-scale screenings identified 0.1% (29) and 1% (31) of inducers among 6,800 and 8,000 compounds, respectively, revealing induction of VanA-type resistance by a few additional compounds, including some membrane-active agents such as polymyxin B.

In vivo analysis is clearly not sufficient to associate an inducing signal with a specific kinase, since response regulators may be stimulated by cross talk. For example, it is possible that activation of VanR in response to moenomycin in wild VanA-type strains depends not upon a modification of the signaling status of VanS but upon stimulation of the putative heterologous kinase that was revealed by the analysis of *vanS* null mutants (Table 1). Cross talk is expected to vary in different hosts, and this may account for some of the discrepancies concerning the induction specificity of the VanS sensor.

CONCLUSIONS

Several aspects of regulation of the *vanA* and *vanB* gene cluster, including the phosphotransfer reactions (Fig. 1) and promoter activation (Fig. 2), are well characterized based on in vivo analysis of the impact of mutations in the regulatory genes (Table 1) and in vitro analysis of protein functions. In contrast, our understanding of signal recognition remains limited. Likewise, the importance of cross talk in regulation of glycopeptide resistance has been revealed by numerous analyses, although host factors involved in this process remain to be identified. Detection of inducible expression of glycopeptide resistance genes in the absence of the VanS or $VanS_B$ sensors suggests that regulation of the *vanA* and *vanB* cluster may be more integrated in host regulation than was initially anticipated. This observation also suggests that specific host genes may be turned on in response to glycopeptides in susceptible bacteria.

Acquisition of teicoplanin resistance by VanB-type strains, in particular constitutive expression of the resistance genes associated with impaired host D-Ala–D-Ala ligase activity, appears to be a potential trend in the evolution of VanB-type resistance. This may ultimately lead to exclusive production of D-Lac-ending precursors in enterococci. However, production of such precursors is associated with hypersusceptibility to β-lactam antibiotics in some enterococci, presumably because certain low-affinity penicillin binding proteins are unable to efficiently function with D-Lac-ending precursors (2, 13). This could contribute to counterselection of bacteria expressing glycopeptide resistance constitutively.

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