Published in final edited form as:

Annu Rev Genet. 2017 November 27; 51: 311–333. doi:10.1146/annurev-genet-120116-023507.

Genetic and Structural Analyses of RRNPP Intercellular Peptide Signaling of Gram-Positive Bacteria

Matthew B. Neiditch[1], **Glenn C. Capodagli**[1], **Gerd Prehna**[2], and **Michael J. Federle**[3]

[1]Department of Microbiology, Biochemistry, and Molecular Genetics, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, New Jersey 07103

[2]Center for Structural Biology, Research Resources Center and Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, Illinois 60607

[3]Department of Medicinal Chemistry and Pharmacognosy and Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, Illinois 60607

Abstract

Bacteria use diffusible chemical messengers, termed pheromones, to coordinate gene expression and behavior among cells in a community by a process known as quorum sensing. Pheromones of many gram-positive bac, such as *Bacillus* and *Streptococcus*, are small, linear peptides secreted from cells and subsequently detected by sensory receptors such as those belonging to the large family of RRNPP proteins. These proteins are cytoplasmic pheromone receptors sharing a structurally similar pheromone-binding domain that functions allosterically to regulate receptor activity. X-ray crystal structures of prototypical RRNPP members have provided atomic-level insights into their mechanism and regulation by pheromones. This review provides an overview of RRNPP prototype signaling; describes the structure–function of this protein family, which is spread widely among gram-positive bacteria; and suggests approaches to target RRNPP systems in order to manipulate beneficial and harmful bacterial behaviors.

Keywords

quorum sensing; signal transduction; peptide pheromone; Rap phosphatase; repeat domain

INTRODUCTION: A FAMILY OF CYTOPLASMIC PROTEINS THAT SERVE AS PHEROMONE RECEPTORS

Intercellular chemical communication (quorum sensing) provides a means to coordinate gene expression and behavior among bacteria. By coordinating behaviors, bacterial communities can enhance their ability to adapt to nutrient-limited conditions, defend against assaults from competing microorganisms or host immune systems, and improve their ability

matthew.neiditch@rutgers.edu.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

to acquire new genetic material that could potentially lead to antibiotic resistance. Chemical signals, here referred to as pheromones, are small secreted molecules employed by bacterial communities. Pheromones found most commonly in gram-positive bacteria are ribosomally produced, secreted peptides. Either bacteria detect extracellular peptides at the cell surface by membrane-spanning signal-transduction proteins [exemplified by ComP and AgrC of *Bacillus subtilis* and *Staphylococcus aureus,* respectively (52, 82)] or, as is the case for the large family of signaling systems known as RRNPP, pheromones are detected by receptors located within the cytoplasm. Features of the RRNPP family are reviewed here with emphasis on recent findings that indicate how these signaling pathways provide specificity in signaling and versatility in mechanisms of regulation.

The RRNPP family is named for the prototypical members, Rap, Rgg, NprR, PlcR, and PrgX, and the term was first coined upon discovery that peptide signaling systems found in distantly related gram-positive bacteria utilized peptide receptors that displayed remarkably similar structures despite relatively low levels of sequence homology (37). With at least one X-ray crystal structure now elucidated for each prototype, the domain directly interacting with the peptide pheromone defines the unifying feature of the RRNPP proteins. These C-terminal regions of the proteins adopt a tetratricopeptide repeat (TPR) domain-like conformation, described as a superhelical structure that binds its ligand on an inner concave surface (14). Also, consistent among RRNPP systems is that their signaling peptides are linear, are unmodified (with the exception that proteolytic cleavage determines their mature length), and exhibit mature lengths between 5 and 10 amino acids. Pheromone biosynthesis follows a conventional track of ribosomal translation, processing, and secretion, but factors contributing in later steps (cleavage and secretion) have not been fully elucidated for all systems. A limited understanding also remains regarding pheromone stability and the existence of enzymes responsible for peptide turnover. RRNPP family receptors are located within the cell; therefore, pheromones must be translocated from outside to inside by oligopeptide permeases and, in some species, assisted by accessory proteins shown to have high selectivity for pheromone peptides (e.g., PrgZ of *Enterococcus*) (68, 95, 101).

As detailed below, considering their many common structural features, it is particularly striking that RRNPP peptide receptors employ different allosteric or, in some cases, catalytic mechanisms to regulate gene expression. Because these receptors are being targeted for therapeutic interventions aimed at manipulating bacterial behaviors, atomic-resolution descriptions of the receptors and the conformational changes triggered by interactions with pheromones, DNA, or other target proteins provide valuable structural insights and are guiding strategies to interfere with normal receptor function.

PHYLOGENY OF CYTOPLASMIC PEPTIDE RECEPTORS

The RRNPP family is substantial in size and widespread among *Firmicutes.* Using protein sequences of archetypical members of the family (*Bacillus thuringiensis* NprR and PlcR, *B. subtilis* RapH, *Enterococcus faecalis* conjugative plasmid pCF10 PrgX, and four Rgg paralogs of *Streptococcus pyogenes),* we conducted Domain Enhanced Lookup Time Accelerated–Basic Local Alignment Search Tool (DELTA–BLAST) (17) searches of the US National Center for Biotechnology Information nonredundant (nr) protein database. For each

protein query, we identified between 1,200 and 6,200 hits using default thresholds, except in the case of PrgX, which generated only 187 hits (see Supplemental Appendix). As some species are overrepresented in the nr database, representation bias was observed as expected. Though the RRNPP family exhibits conserved structural commonalities (detailed below), there was a surprisingly low level of overlap in BLAST results between some of the groups, as indicated by Jaccard index values that compare the similarity between sets of the returned hits for each query (Supplemental Table 1). In particular, Rap and NprR homologs were predominantly found in the order *Bacillales*, whereas homologs of Rgg and PrgX were present in *Lactobacillales*. Interestingly, PlcR homologs were nearly equally split between the two orders, supporting the notion, raised by Declerck et al. (37), that PlcR presents an evolutionary bridge between the groups. Small numbers of other classes of *Firmicutes* were represented in BLAST results; for example, homologs of NprR, PlcR, and Rgg were identified in *Clostridia*. Results indicated that homologs are present, but few in number, in species outside the *Firmicutes*.

A recent report identified a tentative RRNPP system encoded by a temperate bacteriophage and demonstrated that the affiliated signaling peptide coordinated lysis–lysogeny decisions (42). Our recent phylogenetic analysis of the RRNPP family did not identify this peptide receptor, AimR, as its alignment to RRNPP members did not meet our defined minimal homology criteria. However, the structure–prediction server Protein Homology/analogY Recognition Engine V 2.0 (Phyre2) (60), which incorporates Position-Specific Iterated–BLAST and secondary-structure analysis to generate a hidden Markov model that is then matched to a database of hidden Markov models consisting of sequences of known structure, suggests that AimR is likely to share structural features of the RRNPP family. Further, homology searches with an AimR query revealed numerous proteins, primarily from *Bacillus,* with high degrees of similarity to AimR but low homology with other RRNPP hits (M.B. Neiditch, G.C. Capodagli, G. Prehna & M.J. Federle, unpublished data). Thus, AimR may constitute another subgroup of the protein family and raises the question whether other members could exist in mobile genetic elements or phage genomes that have yet to be investigated.

Not only are RRNPP proteins widespread among the *Firmicutes*, but in many cases individual genomes contain multiple paralogs. This is best illustrated in *B. subtilis*, which encodes 11 Rap paralogs, and in *S. pyogenes,* which contains four different Rgg proteins (31, 93). Functional overlap and redundancy are apparent in some cases (for example, RapA, RapB, RapE, RapH, and RapJ are Spo0F phosphatases; see the section titled RRNPP Structure–Function), but independent signaling pathways are also observed and exist in parallel with other intercellular signaling pathways (for example, competence and bacteriocin signaling in *Streptococcus mutans*; (61) and provide bacteria with multiple channels for communication (43).

PHEROMONE CHARACTERISTICS, TRANSPORT, AND STABILITY

RRNPP pheromones originate from at least three recognized gene categories, all of which lead to the secretion of linear peptides comprising 5 to 10amino acids (Figure 1). Peptides recognized by Rap, NprR, and PlcR receptors are encoded by small open reading frame

(sORF) genes, where the inactive pre-peptides are generally 40–50 amino acids in length. They contain recognizable secretion signal sequences (81) and are therefore considered substrates of the Sec-dependent secretory pathway (92, 99). However, processing of the precursor into the active (mature) peptide pheromone involves a cleavage event beyond that provided by the signal peptidase to release the mature product (109). In *B. subtilis,* one of several serine proteases under the control of alternative sigma factor H (σ^H) liberates the active Phr pentapeptides (Figure 1a) (66). Three proteases were identified, subtilisin, Vpr, and Epr, and produced mature forms of Phr peptides, with exception of PhrE, which has additional C-terminal residues and is deduced to be processed further by an unidentified enzyme. In contrast, the metalloprotease NprB was identified in *Bacillus cereus* as being responsible for the maturation of PapR (Figure 1c) (98), indicating that the RRNPP pheromone-maturation proteases vary among gram-positive species.

The Rgg pheromone genes identified thus far are also sORFs, but they encompass a second category, as they are even shorter than the Rap/Npr/PlcR ligands and they follow a different maturation pathway. Predicted coding sequences are fewer than 35 residues and can be as short as 15 amino acids in length, as seen for *comS* of *Streptococcus bovis* (77). Although some features of secretion signals are apparent in these genes (displaying a basic N terminus), factors considered critical for Sec-dependent membrane integration and subsequent proteolysis by signal peptidase (hydrophobic span followed by a polar region) are not clearly present (34, 81). Instead, an ABC-type transporter, termed PptAB, was recently identified as being responsible for exporting peptides affiliated with Rgg-like receptors. For *Enterococcus* and *Streptococcus* signaling, PptAB—likely working with the integral membrane zinc protease Eep—produces sex pheromones and short hydrophobic peptide (SHP) pheromones (Figure 1d,e) (29, 97, 114). However, alternatives to PptAB are also likely to exist, as it was found that PptAB was not essential for transport of the SigX-inducing peptide (XIP) in *S. mutans*; strains containing *pptAB* deletions remained capable of secreting XIP, albeit at low efficiency (29).

Conjugative transfer of several plasmids in *Enterococcus* is mediated by sex pheromones that are recognized by PrgX-like receptor proteins. The activity of these receptors is modulated by two kinds of pheromones, inducers and inhibitors. Conjugation inducers (referred to as c, such as in cCF10 and cAD1, which incorporate the names of the plasmids they specifically regulate, here exemplified by pCF10 and pAD1) originate from the N-terminal signal-sequence domain of lipoproteins encoded in the genome. Thus, these pheromones originate from within coding sequences of larger proteins and therefore provide a third categorical example of a pheromone source. The c peptides are released from the signal sequence by two proteases: signal peptidase II and Eep (5, 6, 28). Conjugation is induced when c binds to the RRNPP receptor (PrgX in the case of conjugative plasmid pCF10, or TraA for plasmid pAD1). However, conjugation is inhibited by another peptide called i (e.g., iCF10 and iAD1) that functions as a competitive inhibitor of c (33, 80). As detailed below, further studies determined that i and c compete for the same binding pocket of PrgX and induce modest structural differences in the receptor (65). Unlike c peptides that originate from lipoprotein signal sequences, the inhibitor peptide is encoded by a sORF on the conjugative plasmid. Therefore, the pheromone receptor is allosterically regulated by

two opposing peptides that are encoded by unlinked genes whose product pheromones are generated by different mechanisms.

Few studies have sought to examine the stability or endurance of peptide pheromones, especially in native environmental conditions; however, reliability of pheromone turnover is likely to be as important as signal production in maintaining sensory fidelity. We recently reported that SHP pheromones of the Rgg2/3 signaling pathway of *S. pyogenes* are rapidly degraded by the endopeptidase PepO (Figure 1e), which is expressed when the bacteria are challenged with the antimicrobial peptide LL-37 that targets the bacterial membrane (116). It appears that signaling by the Rgg2/3 pathway is silenced under conditions in which the bacterium faces severe membrane stress, indicating that Rgg2/3 signaling is perhaps disadvantageous under such conditions.

Though pheromone genes of the prototypical RRNPP members are well characterized, identifying coding sequences of pheromones for the considerably large number of RRNPP homologs present in genome databases remains a significant challenge. Most sORFs of sequenced genomes remain unannotated, and rules for what constitutes a functional pheromone are still rather vague. Yet bioinformatic prediction of pheromone candidates, supported by rigorous experimental testing, continues to generate pheromone discovery. In one bioinformatic study, researchers analyzed genomes of 20 species of gram-positive bacteria to identify functional sORFs and defined the sORFs as likely to be transcribed (having a recognizable promoter) and translated (having an appropriately positioned Shine–Dalgarno sequence) (57). The compiled results led to the identification of a proximal relationship between sORFs encoding hydrophobic peptides and adjacent *rgg* genes. This study led to the first reports indicating that Rgg-type proteins controlled gene expression in response to SHPs (44, 46). In addition to illustrating that the in silico method could predict functional sORFs, these reports helped in the realization that Rgg proteins are a subclass of RRNPP proteins. However, many other recognized RRNPP proteins remain without identified cognate ligands. The first characterized Rgg-type proteins, Rgg of *Streptococcus gordonii* and RopB of *S. pyogenes,* have been long known as regulators of transcription, but their inducing signals have not been identified in the literature, and; thus, these examples are considered orphan receptors (70, 72, 111). Orphans of the Rap subfamily are also well documented and conserved (43, 88). Robust genetic studies coupled with phenotype-driven (forward-genetic) approaches are likely to provide the best routes to ligand identification. For example, a recent report identified a peptide encoded in the signal sequence of a *Listeria monocytogenes* lipoprotein (similar to the genes encoding c factors in plasmid conjugation in *E. faecalis*; see above) to have an important role in bacterial escape from host-cell vacuoles. Ironically in this case, it is the pheromone receptor, and not the pheromone, that has yet to be identified. Nevertheless, the report demonstrates the power of a good genetic system and relevant phenotypic assay (118).

RRNPP STRUCTURE-FUNCTION

A defining characteristic of the RRNPP family members is the presence of a C-terminal peptide-pheromone-binding domain. These domains consist of helix-turn-helix (HTH) repeats that form right-handed superhelical structures containing convex outer surfaces and a

concave pheromone-binding inner surface (Figure 2) (120). The Rap, NprR, and PlcR pheromone-binding domains are TPR domains containing canonical or degenerate TPR sequences (36, 37, 50). TPR domains have been adapted by bacteria for a myriad of purposes, including outer membrane protein assembly and virulence (11, 27). In contrast, the repeats of the Rgg and PrgX family proteins do not contain recognizable TPR sequences but adopt TPR-like folds. The lack of bona fide TPR sequences indicates that PrgX and Rgg proteins are perhaps the most recently evolved RRNPP family members. Here, we refer to the structurally similar TPR and non-TPR C-terminal pheromone-binding domains common to all RRNPP proteins as repeat domains. In contrast to structures of the C-terminal domain, the N-terminal region is where RRNPP protein architecture most varies. Here, Rgg, PrgX, and PlcR proteins have HTH DNA-binding domains (DBDs); Rap proteins have 3-helix bundles (3HBs); and NprR proteins contain both DBDs and 3HBs (Figure 2, Table 1). Consistent with these structural differences in their N-terminal regions, and despite the similar domain architectures of the Rgg, PrgX, and PlcR proteins in particular, peptide–pheromone binding to the repeat domains differentially regulates the activity of each RRNPP family member.

Rap Proteins Use Catalytic and Noncatalytic Mechanisms to Regulate the Activity of Structurally Diverse Target Proteins

B. subtilis sporulation, biofilm formation, and genetic competence are regulated by five sensory histidine kinases (KinA–E) (58) whose autophosphorylation is controlled by changing cellular conditions including, among others, the ratio of NAD^+ to NADH (63) and potassium leakage (Figure 1a) (69). Phosphoryl groups are transferred via a phosphorelay pathway from the histidine kinases to the stand-alone response regulator protein Spo0F, and then, in sequence, from Spo0F to a histidine phosphotransferase, Spo0B, and from Spo0B to the transcription factor Spo0A (Figure 1a) (23). Phosphorylated Spo0A directly activates or represses its target gene promoters, triggering spore development, biofilm formation, and genetic competence (48, 74, 76, 110, 115). Phosphoryl flow along the phosphorelay pathway is reversible; i.e., Spo0B can transfer phosphoryl groups from Spo0A, and Spo0F can transfer phosphoryl groups from Spo0B. It is important to note that Spo0F can also transfer phosphoryl groups to water in autohydrolysis reactions, effectively draining phosphoryl groups from the phosphorelay pathway. The rate of Spo0F dephosphorylation is determined not only by autohydrolysis activity intrinsic to Spo0F but also by the activity of a subset of Rap phosphatases that dephosphorylate Spo0F (58, 89, 93, 107). Rap phosphatases that dephosphorylate Spo0F, e.g., the genomically encoded proteins RapA, RapB, RapE, RapH, and RapJ, are antagonists of sporulation, biofilm formation, and genetic competence (Figure 1a). The activity of these Rap phosphatases is directly inhibited by Phr peptide pheromones; e.g., PhrA and PhrH inhibit RapA and RapH function, respectively (93, 107). These Phr peptide pheromones that inhibit Rap phosphatases are, therefore, agonists of sporulation, biofilm formation, and genetic competence.

To reveal how Rap proteins such as *B. subtilis* RapA, RapB, RapE, RapH, and RapJ function mechanistically to dephosphorylate Spo0F and inhibit sporulation (58, 89, 94, 107), and, more fundamentally, to experimentally determine the overall shape of a Rap protein, researchers determined the *B. subtilis* RapH–Spo0F complex X-ray crystal structure (89).

This structure revealed, as predicted from its primary sequence, that the C-terminal domain of the Rap proteins are TPR HTH folds (Figure 2). Unexpectedly, the N-terminal domain was found to be a 3HB, which is connected to the C-terminal domain by a flexible linker region. This crystal structure also showed that the Rap protein 3HB and repeat domain together form the Spo0F-binding surface. A conserved glutamine (RapH Gln47) inserts into the Spo0F active-site pocket, and this residue is catalytic and conserved as Gln or Glu in all Rap proteins known to dephosphorylate Spo0F (88). Interestingly, RapH binds to a surface of Spo0F previously shown to be important for binding both KinA and Spo0B (113), and it was shown that RapH could sterically interfere with kinase and phosphotransferase access to the Spo0F active site (89).

Additional regulation of *B. subtilis* genetic competence is provided by the ComP–ComA two-component signaling pathway (Figure 1a). The histidine kinase ComP autophosphorylates in response to binding the quorum-sensing signal ComX (71). ComP transfers phosphoryl groups to the transcription factor ComA (71). ComA drives transcription of the *srfA* operon, which, in turn, triggers expression of the late-stage competence genes (79). Numerous Rap proteins including genomically encoded RapC, RapF, and RapH function as transcriptional antiactivators, binding to the ComA DBD (ComAc) and inhibiting its interaction with target gene promoters, e.g., the *srfA* promoter (16, 36, 107). Therefore, these Rap proteins antagonize genetic competence development, and the Phr peptide pheromones that inhibit the activity of these Rap proteins stimulate the development of genetic competence.

To determine how Rap proteins such as *B. subtilis* RapC, RapF, and RapH function as antiactivators that bind to $ComA_C$ and inhibit the development of genetic competence gene expression (16, 36, 84, 107), the X-ray crystal structure of *B. subtilis* RapF–$ComA_C$ was determined (12). Comparison of RapF–$ComA_C$ and RapH–Spo0F revealed that when bound to their target, these Rap proteins are structurally similar; however, the target binding surfaces are different and nonoverlapping. In fact, to bind $ComA_C$, RapF employs the opposite face of the 3HB and the linker region connecting the 3HB and repeat domain (Figure 2). RapF inhibition of ComA DNA binding is explained by the facts that (*a*) a surface of RapF mimics the shape and charge of DNA to bury six of the seven $ComA_C$ DNA-binding residues at the RapF–$ComA_C$ interface (12), and (*b*) RapF causes dissociation of ComA dimers, which are the transcriptionally functional stoichiometric form (12, 51, 117).

Finally, to determine how Phr peptide pheromones function to inhibit Rap protein activity and, in turn, stimulate sporulation, biofilm formation, and genetic competence, researchers examined the structures of *B. subtilis* Rap proteins alone (RapF and RapI) and in complex with Phr peptide (RapF–PhrF and RapJ–PhrC) (49, 87). Comparison of these structures to RapH–Spo0F and RapF–ComAc showed that Rap proteins undergo regulatory conformational changes. In the Phr peptide-bound conformation, Rap proteins are compressed along their TPR superhelical axes, and their N-terminal 3HBs and linker regions flip and merge with the C-terminal portions of the repeat domains to form single extended repeat domains (Figure 2). The first two repeats of the extended repeat domains originate from the repacking of the 3HB and linker region, and this mechanism was dubbed

conformational change-induced repeat domain expansion (87). It was clear from the Phr-bound structures how peptide pheromone inhibits Rap protein function. Phr-peptide binding to the concave surface of the repeat domain causes allosteric conformational changes that bury Rap protein residues critical to ComA binding. Furthermore, the Spo0F binding surface formed by residues on the 3HB and TPR domain is dismantled. More specifically, the Phr-induced conformational change disjoins portions of the Spo0F-binding surface. In the Phr-bound conformation, portions of the Spo0F-binding surface are positioned on opposite sides of the protein, where it would be impossible for them to simultaneously interact with Spo0F.

It is important to note that the structural basis of activity is still unknown for some Rap proteins, for example, RapI, RapG, and Rap60. The *rapI–phrI* cassette is encoded on the *B. subtilis* integrative and conjugative element, ICEBs1 (7). ImmR regulates the expression of genes required for excision and transfer of ICEBs1 (7). RapI has been proposed to increase the specific activity of the protease ImmA, which cleaves ImmR; thus, RapI activity stimulates ICEBs1 excision and transfer (21) (Figure 1a). PhrI antagonizes RapI, and cells expressing PhrI could intercellularly inhibit ICEBs1 mobility (9). How RapI functions mechanistically to regulate ImmA is unknown.

In addition, like the Rap protein transcriptional antiactivators that target ComA (Figure 1a), *B. subtilis* RapG has been shown to function as a transcriptional antiactivator targeting the response regulator DegU (84). DegU regulates the transcription of *aprE* and *comK*, encoding for secreted alkaline protease and the master regulatory transcription factor of *B. subtilis* genetic competence, respectively (53, 78). The structural basis of the Rap–DegU interaction has not been described.

Finally, although *B. subtilis* plasmid-encoded Rap60 appears to function like RapH, employing a catalytic glutamine to dephosphorylate Spo0F, it was also shown that Rap60 functions within Rap60–ComA–DNA complexes to inhibit transcription, and Rap60 inhibits KinA autophosphorylation (15). In the absence of Rap60–ComA, Rap60–ComA–DNA, and Rap60–KinA crystal structures, how Rap60 functions mechanistically to regulate ComA and KinA will likely remain largely unknown (15).

PlcR and NprR Work Sequentially to Control Virulence, Necrotrophism, and Sporulation

The examples of PlcR and NprR provide an interesting study of contrasts between RRNPP family members, both in function and in structure. These pheromone receptors are located within genomes of the *B. cereus* group, which includes the commercially marketed biological pesticide *B. thuringiensis.* The life cycle of *B. thuringiensis* involves infection and death of an insect larval host, a necrophytic stage of growth within the insect cadaver, and an ability to form spores to enhance survival and spread to new hosts (40). Remarkably, PlcR and NprR are quorum-sensing regulators that are utilized sequentially to drive and adapt to stages of the cycle.

PlcR.—During the infection process of the insect larvae, PlcR stands as a primary transcriptional activator of virulence factor expression, including *plcA* (phosphatidylinositol-specific phospholipase C) and other degradative enzymes, cell-surface proteins, and toxins (2). For transcriptional activation, PlcR must bind to its cognate ligand, the heptapeptide

pheromone PapR (PapR7) (Figure 1b) (22, 106). On the basis of structural comparison of apo–PlcR, PlcR–PapR7–DNA (as well as a complex of PlcR with a shorter peptide variant, PapR5), and analysis of PlcR mutants that are transcriptionally active in the absence of peptide, an elegant mechanistic understanding of PlcR regulation has been formulated (Figure 2) (37, 50). Specifically, it was proposed that the dimeric apo–PlcR conformation is stabilized by interactions between the linker helices across the dimer interface (i.e., the Tyr64–Tyr64 stacking interaction and the Ile68–Ile68 stacking interaction) (50). PapR binding to the TPR domain triggers local conformational changes in the TPR domain and capping helix that in turn destabilize not only the Tyr64–Tyr64 stacking interaction between the linker helices but also the interaction of the DBDs with the linker regions. DNA binding appears to trigger a conformational change that breaks the Ile68–Ile68 interaction and kinks the linker helices to a degree that each linker helix in effect becomes two smaller helices. This enables the large reorientation of the PlcR DBDs required for their asymmetric interaction with the imperfect-palindrome half sites of PlcR-box DNA.

NprR.—Once *B. thuringiensis* has killed the larval host, the bacterium must transition to a physiological state that allows it to survive on the decaying organic material and compete with other opportunistic saprophytes. Necrotrophism, or growth in the dead host, is not possible for *B. thuringiensis* without NprR and the pheromone it binds, NprX, because genes expressed under their control—which includes the highly abundant extracellular neutral protease NprA—provide a means to acquire nutrients and sustain viability in the cadaver (Figure 1c) (24, 25, 40, 90, 91). NprR–NprX mutants are also ineffective in sporulation development. However, NprR's ability to regulate the sporulation process is based on a mechanism separate from its ability to function as a transcriptional regulator. Thus, NprR contains a second regulatory function, also controlled by the NprX pheromone, whose action was revealed through structural comparisons to Rap and PlcR.

X-ray crystal structures have been determined for NprR alone and in complex with the NprX octapeptide (Figure 2) through the use of a truncated *B. thuringiensis* NprR protein lacking its DBD, NprR(AHTH) (91, 121). Together with biochemical, genetic, and additional biophysical studies, these crystal structures revealed that NprR is a transcriptionally inactive dimer in the absence of NprX and a transcriptionally active tetramer when bound to NprX (90, 91, 121).

Inserted between its N-terminal DBD and its C-terminal NprX-binding repeat domain, NprR contains a region of sequence and structural similarity to the Rap protein 3HB (12, 90, 99). This observation led to the proposition that NprR is an evolutionary intermediate between Rap proteins and the RRNPP proteins that contain DBDs but lack the 3HB (37). It was the structural similarity of Rap and NprR proteins, genetic studies connecting NprR to sporulation (1, 40, 100, 119), and the observation that Rap protein Spo0F-binding residues are conserved and surface-exposed in the NprR(AHTH)-NprX structure that led to the hypothesis that—like the Rap phosphatases—NprR interacts with Spo0F (24).

Indeed, NprR–Spo0F binding was confirmed by two groups who reached opposite conclusions regarding the role of this interaction (24, 25, 91). Cabrera and colleagues (24) found that NprR–NprX (also known as NprR–NprRB) positively affects sporulation; i.e.,

NprR–NprX interacts with Spo0F to promote sporulation, and NprR lacking its DBD retained this activity. Subsequently they demonstrated that both NprR and the NprR–NprX complex interact with Spo0F, and that NprX lowered NprX–Spo0F binding affinity but only in a NprR–NprX complex formed in the absence of Spo0F (25). Although it is unclear how the interaction of NprR with Spo0F has a positive effect on sporulation, they proposed a model in which NprR interacts with Spo0F at both low and high NprR/NprX ratios and in which NprR interacts with promoter DNA only at high NprR/NprX ratios (25).

In contrast to the studies showing that NprR–NprX positively regulates sporulation (24, 25), Perchat and colleagues (91) found that NprR inhibits sporulation. This effect was independent of promoter DNA binding, as NprR proteins containing nonfunctional DBDs negatively affected sporulation. Comparison of the NprR(ΔHTH) crystal structures with that of RapH identifies NprR residues in positions previously demonstrated to interact with Spo0F in the RapH–Spo0F structure (89). NprR proteins containing alanine substitutions at any of these positions did not inhibit sporulation, like that seen for wild-type NprR (91). The authors then demonstrated in vitro with purified proteins that NprR interacts with Spo0F, and although bona fide NprR phosphatase activity was not demonstrated, it was shown in vitro that NprR inhibits phosphotransfer from *B. subtilis* KinA to Spo0F (91).

PrgX Activity Is Controlled by Two Opposing Pheromones

As described above, the *E. faecalis* RRNPP protein PrgX is the most thoroughly characterized sex pheromone receptor regulating enterococcal conjugative plasmid transfer (Figure 1d). PrgX is a transcriptional repressor encoded on the tetracycline-resistant plasmid pCF10 (55). PrgX transcriptional repressor function is enhanced and inhibited by peptide pheromones iCF10 and cCF10, respectively (13, 65). A model for PrgX regulation was formulated, as described below, from extensive genetic and biochemical studies as well as comparative analysis of X-ray crystal structures of PrgX and PrgX mutants bound to i or c peptides (10, 26, 32, 64, 65, 105).

In brief, PrgX tetramers repress transcription of the pCF10 *prgQ* operon, which encodes components important to the conjugation process, such as aggregation factor Asc10 (59), as well as the inhibitory pheromone iCF10 (80). iCF10 binds to PrgX, stabilizing the PrgX tetramer interface and, in turn, securing *prgQ* operon repression (65). cCF10 activates PrgX by competing with iCF10 for a shared surface on the concave portion of the PrgX repeat domain and destabilizing PrgX tetramers, triggering derepression of the *prgQ* operon and expression of the conjugation genes in donor cells (65, 105). In the absence of cCF10 contributed by recipient cells, the ratio cCF10:iCF10 is insufficient to inhibit PrgX repression of the *prgQ* operon. It is the recipient cells lacking pCF10 that produce the additional cCF10 required to outcompete iCF10 in the plasmid donor cells and derepress expression of the *prgQ* operon.

PrgX is structurally similar to PlcR, Rgg, and ComR; i.e., they contain an N-terminal DBD connected by a linker region to a pheromone-binding C-terminal repeat domain and form domain-swapped dimers (Figure 2) (37, 86, 104, 105, 112). In the absence of cCF10, PrgX dimers form tail-to-tail tetramers, linking the two PCF10 operator sites (O1 and O2), causing the region to loop, and increasing the occupancy of PrgX at O1 and O2 (26). The PrgX–

iCF10 structure shows that iCF10 interacts with PrgX residues 312–314 to form a two-stranded β-sheet. It is proposed that these interactions stabilize the tetramer interface (specifically the orientation of the capping helix) and the PrgX repressor conformation (65). In contrast, the PrgX–cCF10 structure shows that cCF10 interacts with PrgX residues 296–298 to form a three-stranded β-sheet, and it is proposed that this interaction translates into tetramer interface destabilization via reconfiguration of the capping helix and ultimately derepression of the *prgQ* operon (105). Finally, it is worthwhile to note that an alternative model is proposed in Reference 41, whereby pheromone binding regulates PrgX activity by triggering conformational changes in the PrgX tetramer rather than driving its dissociation to dimers.

Rgg Proteins Regulate Various Behaviors, Including Natural Competence and Virulence

Among members of the RRNPP family, Rgg proteins have been identified as pheromone receptors most recently (Figure 1e). Though Rgg proteins had been regarded as stand-alone transcription regulators for several years, studies conducted initially in *Streptococcus thermophilus, S. pyogenes,* and *S. mutans* demonstrated that pheromone-dependent transcriptional activity was occurring in several species (31, 44, 46, 73). For instance, in *S. thermophilus,* Rgg1358, when bound to its cognate pheromone termed SHP1358, regulates expression of a radical SAM enzyme gene and another sORF, *ster1357* (44). The radical SAM enzyme catalyzes an unprecedented chemical linkage between lysine and tryptophan residues of STER1357 to generate a cyclical peptide termed streptide (102). The activity of streptide is not yet known but stands as a first example of a novel secondary metabolite class produced by streptococci (103). Studies of pheromone regulation of Rgg proteins in other species, such as *Streptococcus agalactiae, Streptococcus pneumoniae,* and *S. pyogenes,* report roles in host–pathogen interactions and stress responses (19, 97, 122). However, the best characterized Rgg-pheromone system described to date is ComRS, which regulates expression of the SigX alternative sigma factor and master regulator of competence genes in all salivarius, pyogenic, mutans, and bovis species of *Streptococcus* (comprising over 30 species) (46, 47, 73).

Though ComRS is present in a majority of streptococci, conserved motifs within the mature pheromone XIP suggest that some residues are critical to the signal's function, whereas other positions that vary between species may provide specificity to avoid crosstalk between members of the genus. More precisely, all XIP peptides encoded by pyogenic, mutans, and bovis species contain a double-tryptophan motif (WW), whereas salivarius strains contain a double-aromatic residue motif of Y/F-F. A third pattern, seen only in *Streptococcus suis,* contains a split-tryptophan (WXXW). Residues surrounding these conserved motifs vary between species, as do sequences of the ComR pheromone receptors. Signal specificity was tested for 10 ComR-XIP pairs in a recent study, and although some receptors could respond to more than one XIP type, receptors of the bovis class responded to nearly all other XIP variants, whereas ComR of *S. mutans* responded to only its cognate ligand. Thus, both stringent and promiscuous activities were exemplified by different ComR alleles, but the benefit provided to an organism displaying one or the other level of specificity remains unclear. Perhaps *S. mutans* benefits by isolating itself from outside signals, whereas *S. bovis* is better off by eavesdropping on another organism's communications. Further testing that

utilizes multispecies coculturing techniques would be a powerful approach to follow up on these observations.

Recent biophysical and structural studies of ComR from *S. thermophilus* ($ComR_{St}$) and *S. suis* ($ComR_{suis}$) have helped to elucidate the mechanism of ComR activation, the first for any Rgg-type protein (104, 112). Upon binding XIP, ComR dimerizes, as observed by dynamic light scattering for $ComR_{suis}$ (104) and size exclusion chromatography with inline multiangle light scattering for $ComR_{St}$ (112). Ligand diversity among all ComR–XIP pairs is reflected by sequence alignments of residues comprising the binding pocket of ComR, which displays a conserved face and variable face. The conserved face provides critical mechanistic contacts with the ligand, such as with $ComR_{St}$ residues T90 and K100 (112), whereas the variable face aids in the discrimination of peptide sequences (104). The pheromone-induced monomer-to-dimer transition of $ComR_{St}$ is a key element in its regulatory mechanism. In the apo conformation, helix 3 and several key residues (R35, R39, and R51) of the DBD that are required to contact DNA in the active conformation (112) are instead packed against the repeat domain in an interface stabilized by extensive hydrogen bonding (Figure 2) (104). Upon binding of the pheromone in the repeat domain, key contacts induce the release of the DBD. The C-terminal XIP residue L24 side chain makes hydrophobic contacts with F171 and Y174 on helix 10, which influences both the conformations of helices 9 and 10 and the loop region between helices 8 and 9 (104, 112). These conformational changes are also thought to encourage dimer formation in the repeat domain to make a flexible dimer intermediate that can then bind DNA (Figure 2) (112).

Comparison of the $ComR_{St}$–XIP complex in relation to other receptor-ligand interactions of the RRNPP family reveals an important difference in activation mechanism, particularly in how the ligand engages the receptor. Superficially, the complexed structures of RapF (49), RapJ (87), NprR (121), PlcR (50), and PrgX (65) (Figure 2 and Supplemental Table 11) show the pheromone in an extended conformation, in contrast with the XIP mode of binding. XIP adopts a partially helical structure that is perhaps needed to induce the observed large conformational change (39, 112). Further examination of Rap/NprR/PlcR/PrgX repeat domains and their corresponding peptide contacts show that conserved asparagine residue(s) provide hydrogen bonding contacts with the pheromone's main chain (38, 85). Though an asparagine residue (N208) does contribute in ligand binding between $ComR_{St}$ and XIP, its role in other ComR repeat domains is not as clear. Although it would provide a similar role, the equivalent position of N208 in $ComR_{suis}$ is an aspartic acid (D213). Additionally, the only asparagine in $ComR_{sius}$, pheromone-binding pocket (N220) is also not conserved in other ComR alleles, and instead N220 was shown to be critical for $ComR_{suis}$ to discriminate between XIP alleles (104). Furthermore, a homology model for the ComR of 5. *pyogenes* ($ComR_{pyo}$) predicts that its peptide binding pocket may not even contain a surface-exposed asparagine residue (104). Speculatively, this divergence from other RRNPP family members is perhaps reflective of the extensive catalog of XIP sequences and evolutionarily directed decisions about crosstalk between streptococcal species in the context of shared niches and the exchange of DNA.

Structures of two other Rgg proteins, RopB and Rgg2, from the pathogenic streptococci *S. pyogenes* and *Streptococcus dysgalactiae,* respectively, are also now available, albeit without

bound pheromones (72, 86). RopB controls expression of the secreted cysteine protease SpeB, which contributes substantially in *S. pyogenes* virulence (56). Although a ligand that would account for RopB's activation has not been identified, a solved structure of the RopB repeat domain indicates a ligand binding pocket and provides a compelling argument that RopB's activity as a transcriptional activator is allosterically regulated (72). Aside from RopB and ComR, two other Rgg proteins, Rgg2 and Rgg3, are present in all sequenced genomes of *S. pyogenes.* Rgg2 and Rgg3 display a high level of similarity to each another (55% identical), they bind identical DNA sites, and they work in tandem to regulate surface properties of the bacterium that affects biofilm development and lysozyme susceptibility (30, 31, 67). Orthologs of Rgg2 and Rgg3 have been studied in other related species, such as *S. agalactiae* and *S. dysgalactiae* (35, 45), and all respond to the hydrophobic pheromone SHP (small hydrophobic peptide, (Table 1) (35, 44–46). The X-ray crystal structure of Rgg2 from *S. dysgalactiae* (Rgg2$_{Sdys}$) revealed an unusual characteristic that is absent from other RRNPP proteins. An intermolecular disulfide bond between Rgg2 homodimers was identified between cysteine residues at position 45, located within the DBD (86). A cysteine is conserved at this position among more than 140 Rgg2 and Rgg3 orthologs from 20 different species of *Streptococcus* and *Lactobacillus,* and its conservation suggests an important function in dimer stabilization. The possibility that a disulfide bond could provide a sensory mechanism for the redox state of the cell is not without precedent for transcription factors (41–43), but prior to the Rgg2 structure, disulfide bonds have not been described in any other bacterial pheromone receptors. Substitution of Cys45 for Ser desensitized Rgg2 to SHP only modestly under laboratory growth conditions; therefore, its role in signaling remains unclear.

TARGETED MODULATION OF RRNPP SIGNALING

With roles in virulence, natural competence, sporulation, biofilm formation, and other activities, RRNPP signaling pathways stand as attractive targets for treatments aimed at manipulating bacterial behaviors. The development of small molecules that disrupt signaling pathway activities may be a feasible approach to alter the course of a bacterial infection or the integrity of a biofilm. Given that an inherent challenge to targeting RRNPP receptors is the fact they are located within the cytoplasm and that any successful modulator must overcome the natural barrier of the cell membrane, an ideal inhibitor would be a peptide because the oligopeptide permease transporter is integral to RRNPP signaling. As described above, inhibitory peptides are a natural regulatory component of plasmid conjugation in *Enterococcus.* When natural inhibitor molecules outcompete the inducer, the expression of genes leading to conjugation is blocked (65). However, until recently, there has been no thorough characterization of natural or synthetic RRNPP antagonists, other than enterococcal peptide inhibitors. In a recent study, a chemical library composed of FDA-approved drugs was screened for inhibitory activity against Rgg2 of *S. pyogenes* and ComR of *S. mutans* (3). The best hit against Rgg2 was the lipid-soluble, cyclic peptide cyclosporin A (CsA), which is an effective immunosuppressant drug that is produced by the soil fungus *Tolypocladium inflatum* (18). The crystal structure of Rgg2$_{Sdys}$ bound to CsA indicates that the inhibitor occupies the predicted pheromone docking site and holds Rgg2$_{Sdys}$ in a nearly identical conformation as when the pheromone is unbound (86). CsA competes with

pheromone binding to the receptor with nearly identical affinity as the native ligand. Application of an immunosuppressant drug is not an ideal strategy to disrupt microbial behaviors in a host setting; a structural analog of CsA lacking immunosuppressive activity, termed valspodar, was equally effective in blocking streptococcal signaling.

To date, no other studies have reported deliberate efforts to target RRNPP signaling pathways, but identifying signaling modulators would also be beneficial in facilitating a deeper understanding of communication mechanisms. Compounds that disrupt or enhance pheromone responses are also likely to target non-RRNPP components, perhaps by blocking oligopeptide transporters or enzymes used in the maturation of pheromones. Pursuing such chemical probes would enhance our knowledge of the pathways and constituents of signaling while also providing possible new methods to modulate bacterial behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Support for this work comes from National Institutes of Health grants AI125452 and AI091779, and the Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Diseases.

LITERATURE CITED

1. Aceves-Diez AE, Robles-Burgueño R, de la Torre M. 2007 SKPDT is a signaling peptide that stimulates sporulation and crylAa expression in Bacillus thuringiensis but not in Bacillus subtilis. Appl. Microbiol. Biotechnol. 76:203–9 [PubMed: 17486337]
2. Agaisse H, Gominet M, Økstad OA, Kolstø AB, Lereclus D. 1999 PlcR is a pleiotropic regulator of extracellular virulence factor gene expression in Bacillus thuringiensis. Mol. Microbiol. 32:1043–53 [PubMed: 10361306]
3. Aggarwal C, Jimenez JC, Lee H, Chlipala GE, Ratia K, Federle MJ. 2015 Identification of quorum-sensing inhibitors disrupting signaling between Rgg and short hydrophobic peptides in streptococci. mBio 6:e00393–15
4. Aggarwal C, Jimenez JC, Nanavati D, Federle MJ. 2014 Multiple length peptide-pheromone variants produced by Streptococcus pyogenes directly bind Rgg proteins to confer transcriptional regulation. J. Biol. Chem. 289:22427–36 [PubMed: 24958729]
5. An FY, Sulavik MC, Clewell DB. 1999 Identification and characterization of a determinant (eep) on the Enterococcus faecalis chromosome that is involved in production of the peptide sex pheromone cAD1. J. Bacteriol. 181:5915–21 [PubMed: 10498702]
6. Antiporta MH, Dunny GM. 2002 ccfA, the genetic determinant for the cCF10 peptide pheromone in Enterococcus faecalis OG1RF. J. Bacteriol. 184:1155–62 [PubMed: 11807076]
7. Auchtung JM, Lee CA, Garrison KL, Grossman AD. 2007 Identification and characterization of the immunity repressor (ImmR) that controls the mobile genetic element ICEBs1 of Bacillus subtilis. Mol. Microbiol. 64:1515–28 [PubMed: 17511812]
8. Auchtung JM, Lee CA, Grossman AD. 2006 Modulation of the ComA-dependent quorum response in Bacillus subtilis by multiple Rap proteins and Phr peptides. J. Bacteriol. 188:5273–85 [PubMed: 16816200]
9. Auchtung JM, Lee CA, Monson RE, Lehman AP, Grossman AD. 2005 Regulation of a Bacillus subtilis mobile genetic element by intercellular signaling and the global DNA damage response. PNAS 102:12554–59 [PubMed: 16105942]

10. Bae T, Kozlowicz B, Dunny GM. 2002 Two targets in pCF10 DNA for PrgX binding: their role in production of Qa and prgX mRNA and in regulation of pheromone-inducible conjugation. J. Mol. Biol. 315:995–1007 [PubMed: 11827471]
11. Bakelar J, Buchanan SK, Noinaj N. 2016 The structure of the β-barrel assembly machinery complex. Science 351:180–86 [PubMed: 26744406]
12. Baker MD, Neiditch MB. 2011 Structural basis of response regulator inhibition by a bacterial anti-activator protein. PLOS Biol. 9:e1001226
13. Bensing BA, Manias DA, Dunny GM. 1997 Pheromone cCF10 and plasmid pCF10-encoded regulatory molecules act post-transcriptionally to activate expression of downstream conjugation functions. Mol. Microbiol. 24:285–94 [PubMed: 9159516]
14. Blatch GL, Lassle M. 1999 The tetratricopeptide repeat: a structural motif mediating protein–protein interactions. BioEssays 21:932–39 [PubMed: 10517866]
15. Boguslawski KM, Hill PA, Griffith KL. 2015 Novel mechanisms of controlling the activities of the transcription factors Spo0A and ComA by the plasmid-encoded quorum sensing regulators Rap60-Phr60 in Bacillus subtilis. Mol. Microbiol. 96:325–48 [PubMed: 25598361]
16. Bongiorni C, Ishikawa S, Stephenson S, Ogasawara N, Perego M. 2005 Synergistic regulation of competence development in Bacillus subtilis by two Rap–Phr systems. J. Bacteriol. 187:4353–61 [PubMed: 15968044]
17. Boratyn GM, Schäffer AA, Agarwala R, Altschul SF, Lipman DJ, Madden TL. 2012 Domain enhanced lookup time accelerated BLAST. Biol. Direct 7:12 [PubMed: 22510480]
18. Borel JF, Feurer C, Gubler HU, Stähelin H. 1976 Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 6:468–75 [PubMed: 8969]
19. Bortoni ME, Terra VS, Hinds J, Andrew PW, Yesilkaya H. 2009 The pneumococcal response to oxidative stress includes a role for Rgg. Microbiology 155:4123–34 [PubMed: 19762446]
20. Bose B, Auchtung JM, Lee CA, Grossman AD. 2008 A conserved anti-repressor controls horizontal gene transfer by proteolysis. Mol. Microbiol. 70:570–82 [PubMed: 18761623]
21. Bose B, Grossman AD. 2011 Regulation of horizontal gene transfer in Bacillus subtilis by activation of a conserved site-specific protease. J. Bacteriol. 193:22–29 [PubMed: 21036995]
22. Bouillaut L, Perchat S, Arold S, Zorrilla S, Slamti L, et al. 2008 Molecular basis for group-specific activation of the virulence regulator PlcR by PapR heptapeptides. Nucleic Acids Res. 36:3791–801 [PubMed: 18492723]
23. Burbulys D, Trach KA, Hoch JA. 1991 Initiation of sporulation in B. subtilis is controlled by a multicomponent phosphorelay. Cell 64:545–52 [PubMed: 1846779]
24. Cabrera R, Rocha J, Flores V, Vázquez-Moreno L, Guarneros G, et al. 2014 Regulation of sporulation initiation by NprR and its signaling peptide NprRB: molecular recognition and conformational changes. Appl. Microbiol. Biotechnol. 98:9399–412 [PubMed: 25256619]
25. Cabrera R, Rodríguez-Romero A, Guarneros G, de la Torre M. 2016 New insights into the interaction between the quorum-sensing receptor NprR and its DNA target, or the response regulator Spo0F. FEBS Lett. 590:3243–53 [PubMed: 27543719]
26. Caserta E, Haemig HA, Manias DA, Tomsic J, Grundy FJ, et al. 2012 In vivo and in vitro analyses of regulation of the pheromone-responsive prgQ promoter by the PrgX pheromone receptor protein. J. Bacteriol. 194:3386–94 [PubMed: 22544272]
27. Cerveny L, Straskova A, Dankova V, Hartlova A, Ceckova M, et al. 2013 Tetratricopeptide repeat motifs in the world of bacterial pathogens: role in virulence mechanisms. Infect. Immun. 81:629–35 [PubMed: 23264049]
28. Chandler JR, Dunny GM. 2008 Characterization of the sequence specificity determinants required for processing and control of sex pheromone by the intramembrane protease Eep and the plasmid-encoded protein PrgY. J. Bacteriol. 190:1172–83 [PubMed: 18083822]
29. Chang JC, Federle MJ. 2016 PptAB exports Rgg quorum-sensing peptides in Streptococcus. PLOS ONE 11:e0168461
30. Chang JC, Jimenez JC, Federle MJ. 2015 Induction of a quorum sensing pathway by environmental signals enhances group A streptococcal resistance to lysozyme. Mol. Microbiol. 97:1097–113 [PubMed: 26062094]

31. Chang JC, LaSarre B, Jimenez JC, Aggarwal C, Federle MJ. 2011 Two group A streptococcal peptide pheromones act through opposing Rgg regulators to control biofilm development. PLOS Pathog. 7:e1002190
32. Chatterjee A, Johnson CM, Shu CC, Kaznessis YN, Ramkrishna D, et al. 2011 Convergent transcription confers a bistable switch in Enterococcus faecalis conjugation. PNAS 108:9721–26 [PubMed: 21606359]
33. Clewell DB, Pontius LT, An FY, Ike Y, Suzuki A, Nakayama J. 1990 Nucleotide sequence of the sex pheromone inhibitor (iAD1) determinant of Enterococcus faecalis conjugative plasmid pAD1. Plasmid 24:156–61 [PubMed: 2128961]
34. Cook LC, Federle MJ. 2014 Peptide pheromone signaling in Streptococcus and Enterococcus. FEMS Microbiol. Rev. 38:473–92 [PubMed: 24118108]
35. Cook LC, LaSarre B, Federle MJ. 2013 Interspecies communication among commensal and pathogenic streptococci. mBio 4:e00382–13
36. Core L, Perego M. 2003 TPR-mediated interaction of RapC with ComA inhibits response regulator-DNA binding for competence development in Bacillus subtilis. Mol. Microbiol. 49:1509–22 [PubMed: 12950917]
37. Declerck N, Bouillaut L, Chaix D, Rugani N, Slamti L, et al. 2007 Structure of PlcR: insights into virulence regulation and evolution of quorum sensing in Gram-positive bacteria. PNAS 104:18490–95 [PubMed: 17998541]
38. Diaz AR, Core LJ, Jiang M, Morelli M, Chiang CH, et al. 2012 Bacillus subtilis RapA phosphatase domain interaction with its substrate, phosphorylated Spo0F, and its inhibitor, the PhrA peptide. J. Bacteriol. 194:1378–88 [PubMed: 22267516]
39. Do H, Kumaraswami M. 2016 Structural mechanisms of peptide recognition and allosteric modulation of gene regulation by the RRNPP family of quorum-sensing regulators. J. Mol. Biol. 428:2793–804 [PubMed: 27283781]
40. Dubois T, Faegri K, Perchat S, Lemy C, Buisson C, et al. 2012 Necrotrophism is a quorum-sensing- regulated lifestyle in Bacillus thuringiensis. PLOS Pathog. 8:e1002629
41. Dunny GM, Berntsson RP. 2016 Enterococcal sex pheromones: evolutionary pathways to complex, two-signal systems. J. Bacteriol. 198:1556–62 [PubMed: 27021562]
42. Erez Z, Steinberger-Levy I, Shamir M, Doron S, Stokar-Avihail A, et al. 2017 Communication between viruses guides lysis–lysogeny decisions. Nature 541:488–93 [PubMed: 28099413]
43. Even-Tov E, Bendori SO, Pollak S, Eldar A. 2016 Transient duplication-dependent divergence and horizontal transfer underlie the evolutionary dynamics of bacterial cell-cell signaling. PLOS Biol. 14:e2000330
44. Fleuchot B, Gitton C, Guillot A, Vidic J, Nicolas P, et al. 2011 Rgg proteins associated with internalized small hydrophobic peptides: a new quorum-sensing mechanism in streptococci. Mol. Microbiol. 80:1102–19 [PubMed: 21435032]
45. Fleuchot B, Guillot A, Mezange C, Besset C, Chambellon E, et al. 2013 Rgg-associated SHP signaling peptides mediate cross-talk in streptococci. PLOS ONE 8:e66042
46. Fontaine L, Boutry C, de Frahan MH, Delplace B, Fremaux C, et al. 2010 A novel pheromone quorum-sensing system controls the development of natural competence in Streptococcus thermophilus and Streptococcus salivarius. J. Bacteriol. 192:1444–54 [PubMed: 20023010]
47. Fontaine L, Wahl A, Flechard M, Mignolet J, Hols P. 2015 Regulation of competence for natural transformation in streptococci. Infect. Genet. Evol. 33:343–60 [PubMed: 25236918]
48. Fujita M, Gonzalez-Pastor JE, Losick R. 2005 High- and low-threshold genes in the Spo0A regulon of Bacillus subtilis. J. Bacteriol. 187:1357–68 [PubMed: 15687200]
49. Gallego del Sol F, Marina A. 2013 Structural basis of Rap phosphatase inhibition by Phr peptides. PLOS Biol. 11:e1001511
50. Grenha R, Slamti L, Nicaise M, Refes Y, Lereclus D, Nessler S. 2013 Structural basis for the activation mechanism of the PlcR virulence regulator by the quorum-sensing signal peptide PapR. PNAS 110:1047–52 [PubMed: 23277548]
51. Griffith KL, Grossman AD. 2008 A degenerate tripartite DNA-binding site required for activation of ComA-dependent quorum response gene expression in Bacillus subtilis. J. Mol. Biol. 381:261–75 [PubMed: 18585392]

52. Grossman AD. 1995 Genetic networks controlling the initiation of sporulation and the development of genetic competence in Bacillus subtilis. Annu. Rev. Genet. 29:477–508 [PubMed: 8825484]
53. Hahn J, Kong L, Dubnau D. 1994 The regulation of competence transcription factor synthesis constitutes a critical control point in the regulation of competence in Bacillus subtilis. J. Bacteriol. 176:5753–61 [PubMed: 8083167]
54. Hayashi K, Kensuke T, Kobayashi K, Ogasawara N, Ogura M. 2006 Bacillus subtilis RghR (YvaN) represses rapG and rapH, which encode inhibitors of expression of the srfA operon. Mol. Microbiol. 59:1714–29 [PubMed: 16553878]
55. Hedberg PJ, Leonard BA, Ruhfel RE, Dunny GM. 1996 Identification and characterization of the genes of Enterococcus faecalis plasmid pCF10 involved in replication and in negative control of pheromone- inducible conjugation. Plasmid 35:46–57 [PubMed: 8693026]
56. Hollands A, Aziz RK, Kansal R, Kotb M, Nizet V, Walker MJ. 2008 A naturally occurring mutation in ropB suppresses SpeB expression and reduces M1T1 group A streptococcal systemic virulence. PLOS ONE 3:e4102 [PubMed: 19116661]
57. Ibrahim M, Nicolas P, Bessieres P, Bolotin A, Monnet V, Gardan R. 2007 A genome-wide survey of short coding sequences in streptococci. Microbiology 153:3631–44 [PubMed: 17975071]
58. Jiang M, Grau R, Perego M. 2000 Differential processing of propeptide inhibitors of Rap phosphatases in Bacillus subtilis. J. Bacteriol. 182:303–10 [PubMed: 10629174]
59. Kao SM, Olmsted SB, Viksnins AS, Gallo JC, Dunny GM. 1991 Molecular and genetic analysis of a region of plasmid pCF10 containing positive control genes and structural genes encoding surface proteins involved in pheromone-inducible conjugation in Enterococcus faecalis. J. Bacteriol. 173:7650–64 [PubMed: 1938961]
60. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. 2015 The Phyre2 web portal for protein modeling, prediction and analysis. Nat. Protoc. 10:845–58 [PubMed: 25950237]
61. Khan R, Rukke H, Høvik H, Åmdal H, Chen T, et al. 2016 Comprehensive transcriptome profiles of Streptococcus mutans UA159 map core streptococcal competence genes. mSystems 1:e00038–15
62. Koetje EJ, Hajdo-Milasinovic A, Kiewiet R, Bron S, Tjalsma H. 2003 A plasmid-borne Rap–Phr system of Bacillus subtilis can mediate cell-density controlled production of extracellular proteases. Microbiology 149:19–28 [PubMed: 12576576]
63. Kolodkin-Gal I, Elsholz AK, Muth C, Girguis PR, Kolter R, Losick R. 2013 Respiration control of multicellularity in Bacillus subtilis by a complex of the cytochrome chain with a membrane-embedded histidine kinase. Genes Dev. 27:887–99 [PubMed: 23599347]
64. Kozlowicz BK, Bae T, Dunny GM. 2004 Enterococcus faecalis pheromone-responsive protein PrgX: genetic separation of positive autoregulatory functions from those involved in negative regulation of conjugative plasmid transfer. Mol. Microbiol. 54:520–32 [PubMed: 15469521]
65. Kozlowicz BK, Shi K, Gu ZY, Ohlendorf DH, Earhart CA, Dunny GM. 2006 Molecular basis for control of conjugation by bacterial pheromone and inhibitor peptides. Mol. Microbiol. 62:958–69 [PubMed: 17038121]
66. Lanigan-Gerdes S, Briceno G, Dooley AN, Faull KF, Lazazzera BA. 2008 Identification of residues important for cleavage of the extracellular signaling peptide CSF of Bacillus subtilis from its precursor protein. J. Bacteriol. 190:6668–75 [PubMed: 18689487]
67. LaSarre B, Aggarwal C, Federle MJ. 2013 Antagonistic Rgg regulators mediate quorum sensing via competitive DNA binding in Streptococcus pyogenes. mBio 3:e00333–12
68. Leonard BA, Podbielski A, Hedberg PJ, Dunny GM. 1996 Enterococcus faecalis pheromone binding protein, PrgZ, recruits a chromosomal oligopeptide permease system to import sex pheromone cCF10 for induction of conjugation. PNAS 93:260–64 [PubMed: 8552617]
69. López D, Fischbach MA, Chu F, Losick R, Kolter R. 2009 Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis. PNAS 106:280–85 [PubMed: 19114652]
70. Lyon WR, Gibson CM, Caparon MG. 1998 A role for Trigger Factor and an Rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of Streptococcus pyogenes. EMBO J. 17:6263–75 [PubMed: 9799235]

71. Magnuson R, Solomon J, Grossman AD. 1994 Biochemical and genetic characterization of a competence pheromone from B. subtilis. Cell 77:207–16 [PubMed: 8168130]
72. Makthal N, Gavagan M, Do H, Olsen RJ, Musser JM, Kumaraswami M. 2016 Structural and functional analysis of RopB: a major virulence regulator in Streptococcus pyogenes. Mol. Microbiol. 99:1119–33 [PubMed: 26714274]
73. Mashburn-Warren L, Morrison DA, Federle MJ. 2010 A novel double-tryptophan peptide pheromone controls competence in Streptococcus spp. via an Rgg regulator. Mol. Microbiol. 78:589–606 [PubMed: 20969646]
74. Mirouze N, Desai Y, Raj A, Dubnau D. 2012 Spo0A~P imposes a temporal gate for the bimodal expression of competence in Bacillus subtilis. PLOS Genet. 8:e1002586
75. Mirouze N, Parashar V, Baker MD, Dubnau DA, Neiditch MB. 2011 An atypical Phr peptide regulates the developmental switch protein RapH. J. Bacteriol. 193:6197–206 [PubMed: 21908671]
76. Molle V, Fujita M, Jensen ST, Eichenberger P, González-Pastor JE, et al. 2003 The Spo0Aregulon of Bacillus subtilis. Mol. Microbiol. 50:1683–701 [PubMed: 14651647]
77. Morrison DA, Guédon E, Renault P. 2013 Competence for natural genetic transformation in the Streptococcus bovis group streptococci S. infantarius and S. macedonicus. J. Bacteriol. 195:2612–20 [PubMed: 23543718]
78. Mukai K, Kawata M, Tanaka T. 1990 Isolation and phosphorylation of the Bacillus subtilis degS and degU gene products. J. Biol. Chem. 265:20000–6 [PubMed: 2123196]
79. Nakano MM, Zuber P. 1989 Cloning and characterization of srfB, a regulatory gene involved in surfactin production and competence in Bacillus subtilis. J. Bacteriol. 171:5347–53 [PubMed: 2507521]
80. Nakayama J, Ruhfel RE, Dunny GM, Isogai A, Suzuki A. 1994 The prgQ gene of the Enterococcus faecalis tetracycline resistance plasmid pCF10 encodes a peptide inhibitor, iCF10. J. Bacteriol. 176:7405–8 [PubMed: 7545961]
81. Nielsen H, Engelbrecht J, Brunak S, von Heijne G. 1997 Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. 10:1–6
82. Novick RP, Geisinger E. 2008 Quorum sensing in staphylococci. Annu. Rev. Genet. 42:541–64 [PubMed: 18713030]
83. Ogura M, Fujita Y. 2007 Bacillus subtilis rapD, a direct target of transcription repression by RghR, negatively regulates srfA expression. FEMS Microbiol. Lett. 268:73–80 [PubMed: 17227471]
84. Ogura M, Shimane K, Asai K, Ogasawara N, Tanaka T. 2003 Binding of response regulator DegU to the aprE promoter is inhibited by RapG, which is counteracted by extracellular PhrG in Bacillus subtilis. Mol. Microbiol. 49:1685–97 [PubMed: 12950930]
85. Omer Bendori S, Pollak S, Hizi D, Eldar A. 2015 The RapP-PhrP quorum-sensing system of Bacillus subtilis strain NCIB3610 affects biofilm formation through multiple targets, due to an atypical signal-insensitive allele of RapP. J. Bacteriol. 197:592–602 [PubMed: 25422306]
86. Parashar V, Aggarwal C, Federle MJ, Neiditch MB. 2015 Rgg protein structure-function and inhibition by cyclic peptide compounds. PNAS 112:5177–82 [PubMed: 25847993]
87. Parashar V, Jeffrey PD, Neiditch MB. 2013 Conformational change-induced repeat domain expansion regulates Rap phosphatase quorum-sensing signal receptors. PLOS Biol. 11:e1001512
88. Parashar V, Konkol MA, Kearns DB, Neiditch MB. 2013 A plasmid-encoded phosphatase regulates Bacillus subtilis biofilm architecture, sporulation, and genetic competence. J. Bacteriol. 195:2437–48 [PubMed: 23524609]
89. Parashar V, Mirouze N, Dubnau DA, Neiditch MB. 2011 Structural basis of response regulator dephosphorylation by Rap phosphatases. PLOS Biol. 9:e1000589
90. Perchat S, Dubois T, Zouhir S, Gominet M, Poncet S, et al. 2011 A cell–cell communication system regulates protease production during sporulation in bacteria of the Bacillus cereus group. Mol. Microbiol. 82:619–33 [PubMed: 21958299]
91. Perchat S, Talagas A, Poncet S, Lazar N, Li de la Sierra-Gallay I, et al. 2016 How quorum sensing connects sporulation to necrotrophism in Bacillus thuringiensis. PLOS Pathog. 12:e1005779
92. Perego M 1997 A peptide export–import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. PNAS 94:8612–17 [PubMed: 9238025]

93. Perego M, Glaser P, Hoch JA. 1996 Aspartyl-phosphate phosphatases deactivate the response regulator components of the sporulation signal transduction system in Bacillus subtilis. Mol. Microbiol. 19:1151–57 [PubMed: 8730857]

94. Perego M, Hanstein C, Welsh KM, Djavakhishvili T, Glaser P, Hoch JA. 1994 Multiple proteinaspartate phosphatases provide a mechanism for the integration of diverse signals in the control of development in B. subtilis. Cell 79:1047–55 [PubMed: 8001132]

95. Perego M, Higgins CF, Pearce SR, Gallagher MP, Hoch JA. 1991 The oligopeptide transport system of Bacillus subtilis plays a role in the initiation of sporulation. Mol. Microbiol. 5:173–85 [PubMed: 1901616]

96. Perego M, Hoch JA. 1996 Cell–cell communication regulates the effects of protein aspartate phosphatases on the phosphorelay controlling development in Bacillus subtilis. PNAS 93:1549–53 [PubMed: 8643670]

97. Pérez-Pascual D, Gaudu P, Fleuchot B, Besset C, Rosinski-Chupin I, et al. 2015 RovS and its associated signaling peptide form a cell-to-cell communication system required for Streptococcus agalactiae pathogenesis. mBio 6:e02306–14

98. Pomerantsev AP, Pomerantseva OM, Camp AS, Mukkamala R, Goldman S, Leppla SH. 2009 PapR peptide maturation: role of the NprB protease in Bacillus cereus 569 PlcR/PapR global gene regulation. FEMS Immunol. Med. Microbiol. 55:361–77 [PubMed: 19159431]

99. Pottathil M, Lazazzera BA. 2003 The extracellular Phr peptide-Rap phosphatase signaling circuit of Bacillus subtilis. Front. Biosci. 8:d32–45 [PubMed: 12456319]

100. Rocha J, Flores V, Cabrera R, Soto-Guzmán A, Granados G, et al. 2012 Evolution and some functions of the NprR–NprRB quorum-sensing system in the Bacillus cereus group. Appl. Microbiol. Biotechnol. 94:1069–78 [PubMed: 22159892]

101. Rudner DZ, LeDeaux JR, Ireton K, Grossman AD. 1991 The spo0K locus of Bacillus subtilis is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. 173:1388–98 [PubMed: 1899858]

102. Schramma KR, Bushin LB, Seyedsayamdost MR. 2015 Structure and biosynthesis of a macrocyclic peptide containing an unprecedented lysine-to-tryptophan crosslink. Nat. Chem. 7:431–37 [PubMed: 25901822]

103. Schramma KR, Seyedsayamdost MR. 2017 Lysine-tryptophan-crosslinked peptides produced by radical SAM enzymes in pathogenic streptococci. ACS Chem. Biol. 12:922–27 [PubMed: 28191919]

104. Shanker E, Morrison DA, Talagas A, Nessler S, Federle MJ, Prehna G. 2016 Pheromone recognition and selectivity by ComR proteins among Streptococcus species. PLOS Pathog. 12:e1005979

105. Shi K, Brown CK, Gu ZY, Kozlowicz BK, Dunny GM, et al. 2005 Structure of peptide sex pheromone receptor PrgX and PrgX/pheromone complexes and regulation of conjugation in Enterococcus faecalis. PNAS 102:18596–601 [PubMed: 16339309]

106. Slamti L, Lereclus D. 2002 A cell-cell signaling peptide activates the PlcR virulence regulon in bacteria of the Bacillus cereus group. EMBO J. 21:4550–59 [PubMed: 12198157]

107. Smits WK, Bongiorni C, Veening JW, Hamoen LW, Kuipers OP, Perego M. 2007 Temporal separation of distinct differentiation pathways by a dual specificity Rap–Phr system in Bacillus subtilis. Mol. Microbiol. 65:103–20 [PubMed: 17581123]

108. Solomon JM, Lazazzera BA, Grossman AD. 1996 Purification and characterization of an extracellular peptide factor that affects two different developmental pathways in Bacillussubtilis. Genes Dev. 10:2014–24 [PubMed: 8769645]

109. Stephenson S, Mueller C, Jiang M, Perego M. 2003 Molecular analysis of Phr peptide processing in Bacillus subtilis. J. Bacteriol. 185:4861–71 [PubMed: 12897006]

110. Stragier P, Losick R. 1996 Molecular genetics of sporulation in Bacillussubtilis. Annu. Rev. Genet. 30:297–341 [PubMed: 8982457]

111. Sulavik MC, Tardif G, Clewell DB. 1992 Identification of a gene, rgg, which regulates expression of glucosyltransferase and influences the Spp phenotype of Streptococcus gordonii Challis. J. Bacteriol. 174:3577–86 [PubMed: 1534326]

112. Talagas A, Fontaine L, Ledesma-Garca L, Mignolet J, Li de la Sierra-Gallay I, et al. 2016 Structural insights into streptococcal competence regulation by the cell-to-cell communication system ComRS. PLOS Pathog. 12:e1005980

113. Tzeng YL, Hoch JA. 1997 Molecular recognition in signal transduction: the interaction surfaces of the Spo0F response regulator with its cognate phosphorelay proteins revealed by alanine scanning mutagenesis. J. Mol. Biol. 272:200–12 [PubMed: 9299348]

114. Varahan S, Harms N, Gilmore MS, Tomich JM, Hancock LE. 2014 An ABC transporter is required for secretion of peptide sex pheromones in Enterococcus faecalis. mBio 5:e01726–14

115. Vlamakis H, Chai Y, Beauregard P, Losick R, Kolter R. 2013 Sticking together: building a biofilm the Bacillus subtilis way. Nat. Rev. Microbiol. 11:157–68 [PubMed: 23353768]

116. Wilkening RV, Chang JC, Federle MJ. 2016 PepO, a CovRS-controlled endopeptidase, disrupts Streptococcus pyogenes quorum sensing. Mol. Microbiol. 99:71–87 [PubMed: 26418177]

117. Wolf D, Rippa V, Mobarec JC, Sauer P, Adlung L, et al. 2016 The quorum-sensing regulator ComA from Bacillus subtilis activates transcription using topologically distinct DNA motifs. Nucleic Acids Res. 44:2160–72 [PubMed: 26582911]

118. Xayarath B, Alonzo F III, Freitag NE. 2015 Identification of a peptide-pheromone that enhances Listeria monocytogenes escape from host cell vacuoles. PLOS Pathog. 11:e1004707

119. Yang H, Sikavi C, Tran K, McGillivray SM, Nizet V, et al. 2011 Papillation in Bacillus anthracis colonies: a tool for finding new mutators. Mol. Microbiol. 79:1276–93 [PubMed: 21205011]

120. Zeytuni N, Zarivach R. 2012 Structural and functional discussion of the tetra-trico-peptide repeat, a protein interaction module. Structure 20:397–405 [PubMed: 22404999]

121. Zouhir S, Perchat S, Nicaise M, Perez J, Guimaraes B, et al. 2013 Peptide-binding dependent conformational changes regulate the transcriptional activity of the quorum-sensor NprR. Nucleic Acids Res. 41:7920–33 [PubMed: 23793817]

122. Zutkis AA, Anbalagan S, Chaussee MS, Dmitriev AV. 2014 Inactivation of the Rgg2 transcriptional regulator ablates the virulence of Streptococcus pyogenes. PLOS ONE 9:e114784

SUMMARY POINTS

1. Gram-positive bacteria utilize peptides as secreted signals that are detected either at the cell surface or within the cytoplasm. All cytoplasmic peptide pheromone receptors described to date are members of the RRNPP family, which directly bind to small (5–10 amino acids), linear peptides.
2. RRNPP (Rap/Rgg/NprR/PlcR/PrgX) homologs are widespread in *Firmicutes* and are most prevalent in *Bacillales* and *Lactobacillales,* and, to a lesser extent, in *Clostridia.* A recent report indicates the presence of RRNPP proteins in bacteriophage.
3. All RRNPP proteins contain structurally similar peptide-binding repeat domains. Pheromone binding triggers conformational changes regulating receptor functions including, among others, DNA binding (activator/repressor), phosphatase, and protein sequestration (antiactivator function). In some cases, the repeat domains allosterically regulate receptor oligomerization in response to pheromone binding.
4. RRNPP proteins are viable targets for small molecule modulation. Identified RRNPP inhibitors (e.g., iCF10 and CsA) competitively inhibit pheromone binding to the concave surface in the receptor repeat domains.

FUTURE ISSUES

1. With substantial evidence of widespread RRNPP representation in genome databases, it remains to be seen what types of behaviors are most commonly coordinated by these quorum-sensing systems. Do all RRNPP receptors recognize peptides, or might they bind other small molecules? What new in silico, in vitro, and in vivo techniques can be used to identify peptide pheromones?
2. Will RRNPP proteins prove to be useful therapeutic targets for treating diseases or in modulating bacterial behaviors that could benefit industrial, agricultural, or environmental processes? Development of small molecule modulators is in its very early stages.
3. Additional X-ray crystal structures that would provide critical mechanistic insight into RRNPP function include the following: PrgX in complex with DNA and iCF10, PrgX in complex with DNA, Rgg2 bound to SHP2 or SHP3, Rgg3 bound to SHP2 or SHP3, Rgg2 bound to DNA, Rgg2 bound to DNA and SHP2 or SHP3, Rgg3 bound to DNA, Rap proteins in complex with cellular targets other than Spo0F and ComA, and RopB (Rgg1) in complex with a ligand.

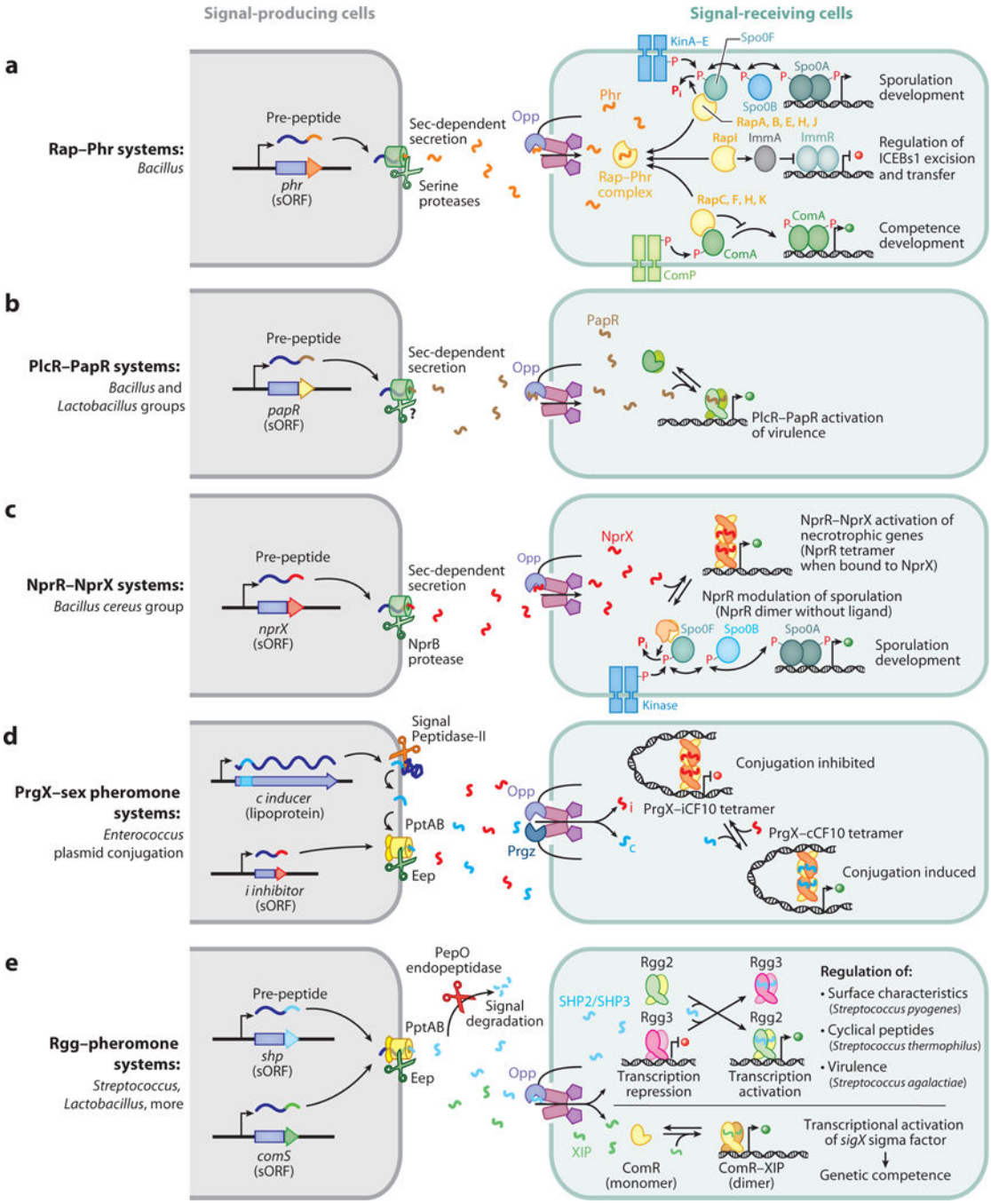

Figure 1.

Examples of RRNPP pheromone signaling. (*Left*) Pheromone source genes, secretion pathways, and processing enzymes. (*Right*) Cells receive pheromones along one or more regulatory pathways. (*a*) Rap–Phr systems. Phr peptides are encoded by small open reading frames (sORFs), translocated by the Sec-dependent secretion system, and matured by serine proteases. The oligopeptide permease (Opp) complex imports Phr peptides into the cell, as is true for all RRNPP pathways (95, 101). Rap proteins modulate three main signal transduction pathways: sporulation, integrative and conjugative element (ICE) transfer, and

competence. RapA, B, E, H, and J are each capable of dephosphorylating Spo0F, thus interrupting the phosphorelay from membrane kinases KinA–E to Spo0A, which is a transcriptional regulator of sporulation genes (23). RapI increases ImmA-dependent degradation of ImmR, promoting ICEBs1 excision and transfer (7). RapC, F, H, and K bind to the DNA-recognition domain of ComA, preventing its ability to regulate transcription of competence-dependent genes (71). In each case, Phr binding to the corresponding Rap protein disrupts the ability of Rap proteins to engage their protein targets. (*b*) PlcR–PapR systems. As seen for Phr peptides above, PapR pheromones are encoded by sORFs and thought to be secreted by the Sec-dependent pathway. Upon binding PapR, PlcR undergoes a conformational change to favor DNA binding and transcriptional activation of genes associated with virulence (37, 50). (*c*) NprR–NprX systems. NprX peptide maturation proceeds along the Sec-dependent pathway, and in *Bacillus cereus*, processing occurs by the NprB neutral protease (98). Upon entering the cell, NprX binds NprR and promotes the receptor to adopt a tetrameric structure that enables DNA binding and transcriptional activation of genes necessary for a necrotrophic phase of growth (90, 91, 121). In the absence of NprX, NprR forms dimers and does not bind to DNA; instead, like Rap, it functions as a phosphatase of Spo0F (25, 91). (*d*) PrgX–sex pheromone systems. Pheromones that induce conjugation, labeled c (*light blue*), are integral components of lipoproteins that are processed by Signal Peptidase-II and Eep and are exported by the ABC-type transporter PptAB. Inhibitory peptides, labeled i (*red*), are encoded by sORFs and are also transported and processed by PptAB/Eep (28, 114). PrgZ, a homolog of the OppA substrate-binding lipoprotein, enhances pheromone importation through the oligopeptide transporter (68). PrgX regulates the conjugative transfer of plasmid pCF10 by acting as a transcriptional repressor of conjugation genes. Repression is favored when i is bound to PrgX, which forms PrgX–iCF10 tetramers and binds at two sites to form a DNA loop that occludes RNA polymerase. Repression is disrupted when c binds to form PrgX–cCF10 tetramers, thus altering PrgX's conformation and allowing transcription to proceed (41, 105). (*e*) Rgg–pheromone systems. Several Rgg pathways have been described, but only two are illustrated here. Rgg pheromones are encoded by sORFs and follow a maturation path like that of *Enterococcus* sex pheromones, by way of the PptAB/Eep transport system (29, 114). Short hydrophobic peptides (SHPs) are degraded by the endopeptidase PepO, which is the first described pheromone-degradation enzyme for RRNPP systems (116). Two pheromones of *Streptococcus pyogenes,* SHP2 and SHP3, are functionally equivalent; either can bind to Rgg2 or Rgg3 (4). Rgg3, without bound ligand, is a transcriptional repressor of genes that affect the surface characteristics of *S. pyogenes* and cause enhanced lysozyme resistance and cellular aggregation (30, 31, 67). When SHP2 or SHP3 binds to the receptors, Rgg3 is displaced from DNA, allowing access for Rgg2 to serve as a transcriptional activator. In other species, Rgg2 and Rgg3 orthologs regulate expression of various behaviors, including production of a cyclical peptide in *Streptococcus thermophilus* and virulence genes in *Streptococcus agalactiae* (44, 97, 102). Another Rgg system is ComR–ComS. The sORF *comS* encodes the mature pheromone called XIP, which when bound to ComR, induces transcription of the alternative sigma factor gene *sigX,* which controls expression of competence-related genes (46, 47, 73).

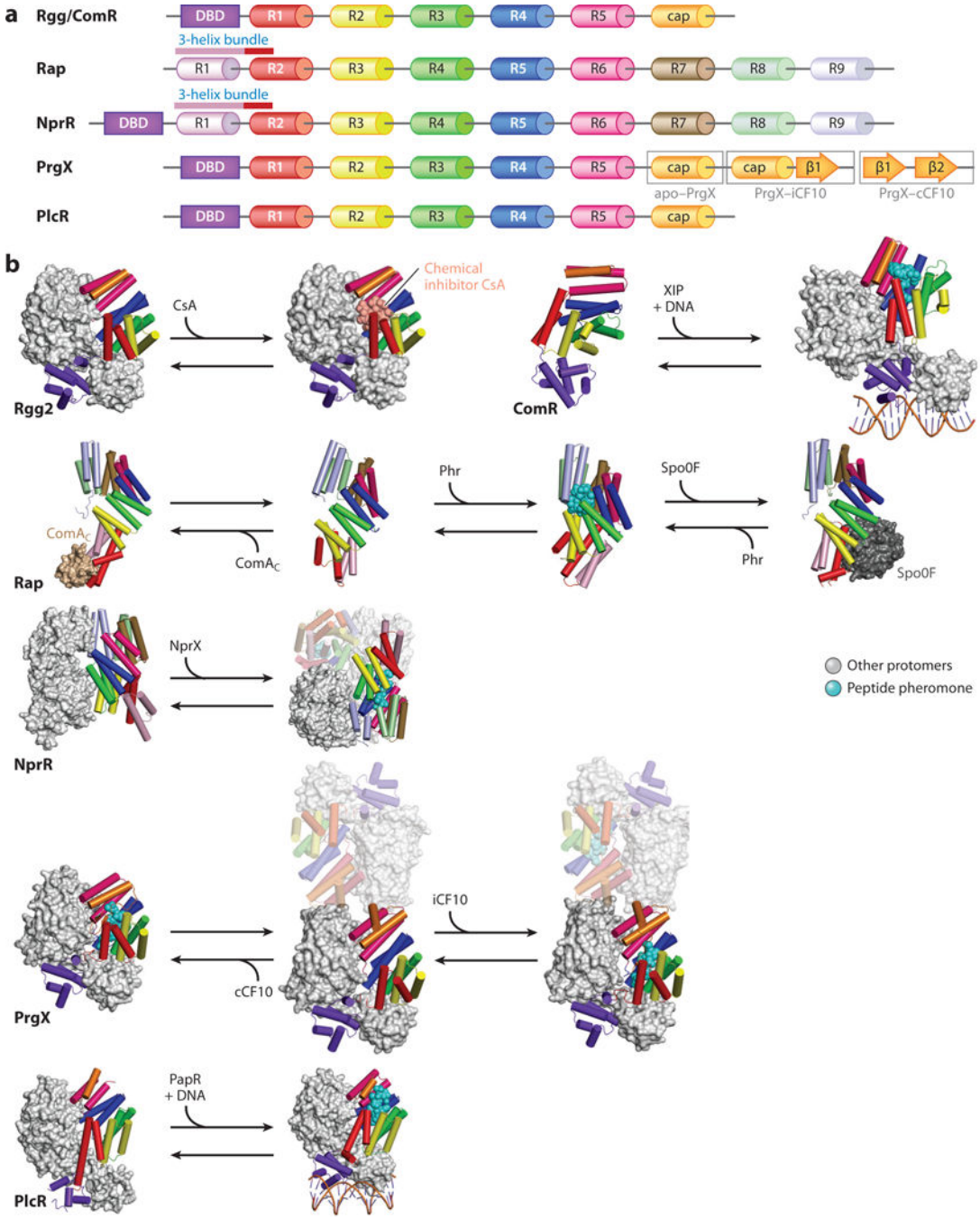

Figure 2.

RRNPP protein domain architecture and structure–function. (*a*) The RRNPP protein domain architecture consists of DNA-binding domains (DBDs) and helix-turn-helix repeats (here, R1–9). When Rap R1 and R2 bind to Spo0F or $ComA_C$ (12, 89), and when NprR R1 and R2 bind to Spo0F (91), these regions are thought to adopt a 3-helix bundle conformation (*lavender* and *red lines*). Different secondary structures are adopted at the PrgX C terminus in the apo-, iCF10-, and cCF10-bound PrgX crystal structures (*gray rectangles*). (*b*) A selection of representative RRNPP protein structures (see Supplemental Table 11 for

relevant Protein Data Bank identifiers) in their apo conformations or in a complex with a peptide pheromone, inhibitor cyclosporine A (CsA), DNA, Spo0F, or $ComA_C$. The structures are shown in their biologically relevant stoichiometric configurations. For simplicity, one protomer is shown in color; the remaining protomers are rendered in gray. NprR crystal structures lack the DBDs. PrgX–cCF10 may be tetrameric (41).

Table 1

Selected examples of RRNPP receptors and peptides

Prototype	Examples	Species	Pheromone name	Mature pheromone sequence	Biological relevance	Reference(s)
Rap	RapA	*Bacillus subtilis*	PhrA	ARNQT	Spo0F phosphatase, sporulation regulator	94, 96
	RapB		PhrC	ERGMT	Spo0F phosphatase, sporulation regulator	92, 94
	RapC		PhrC	ERGMT	Antiactivator of ComA, competence regulator	108
	RapD		ND	ND	Negative regulator of *srfA* expression	83
	RapE		PhrE	SRNVT	Spo0F phosphatase, sporulation regulator	58
	RapF		PhrF	QRGMI	Antiactivator of ComA, competence regulator	16
	RapG		PhrG	EKMIG	Antiactivator of DegU, negative regulator of *srfA* expression	54, 84
	RapH		PhrH	DRNTT	Spo0F phosphatase, sporulation regulator; antiactivator of ComA, competence regulator	54, 75, 107
	RapI		PhrI	DRVGA or ADRVGA	Regulator of ICEBs1 excision and transfer	20, 75
	RapJ		PhrC	ERGMT	Spo0F phosphatase, sporulation regulator	8, 87
	RapK		PhrK	ERPVG	Negative regulator of *srfA* expression	8
	Rap60		Phr60	SRNAT or ASRNAT	Spo0F phosphatase, sporulation regulator, antiactivator of ComA, competence regulator	15, 62
PlcR	PlcR-I	*Bacillus cereus group*	PapR-I	ADLPFEF	Regulator of virulence during insect host infection	22, 106
	PlcR-II		PapR-II	SDMPFEF	Regulator of virulence during insect host infection	22, 106
	PlcR-III		PapR-III	NEVPFEF	Regulator of virulence during insect host infection	22, 106
	PlcR-IV		PapR-IV	SDLPFEH	Regulator of virulence during insect host infection	22, 106
NprR	NprR-I	*Bacillus cereus group*	NprX-I	SKPDIVG	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
	NprR-II		NprX-II	SKPDTYG	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
	NprR-III		NprX-III	SNPDIYG	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
	NprR-IV		NprX-IV	SRPDVLT	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
	NprR-V		NprX-V	WTSDIYG	Transcriptional activator of necrotrophic growth and regulator of sporulation	90

Prototype	Examples	Species	Pheromone name	Mature pheromone sequence	Biological relevance	Reference(s)
	NprR-VI		NprX-VI	WKPD[N/V/T][Y/L]G	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
	NprR-VII		NprX-VII	WRPDMSI	Transcriptional activator of necrotrophic growth and regulator of sporulation	90
PrgX	PrgX	*Enterococcus faecalis*	cCF10	LVTLVFV	Transcriptional repressor of conjugation; cCF10 breaks repression	28, 33, 80
	PrgX		iCF10	AITLIFI	Transcriptional repressor of conjugation; iCF10 favors repression	28, 33, 80
	TraA		cAD1	LFSLVLAG	Transcriptional repressor of conjugation; cAD1 breaks repression	28, 33, 80
	TraA		iAD1	LFVVTLVG	Transcriptional repressor of conjugation; iAD1 favors repression	28, 33, 80
Rgg	Ster1357	*Streptococcus thermophilus*	SHP1357	EGIIVIVVG	Transcriptional activator of radical SAM enzyme that produces streptide	44, 57, 102
	Ster1299	*Streptococcus thermophilus*	SHP1299	DIIIFPPFG	Transcriptional activator, targets not fully elucidated	45
	RopB	*Streptococcus pyogenes*	ND	ND	ND	70, 72
	Rgg2	*Streptococcus pyogenes*	SHP2, SHP3	DILIIVGG, DIIIIVGG	Transcriptional activator, controlling surface attributes	4, 31
	Rgg3	*Streptococcus pyogenes*	SHP2, SHP3	DILIIVGG, DIIIIVGG	Transcriptional repressor, controlling surface attributes	4, 31
	RovS	*Streptococcus agalactiae*	ND	DILIIVGG	Transcriptional activator of virulence-related genes	45, 97
	Rgg1509	*Streptococcus mutans*	SHP1509	ETIIIIGGG	ND	45
	$ComR_{pyo}$	*Streptococcus pyogenes*	XIP_{pyo}	SAVDWWRL	Transcriptional activator of *sigX* sigma factor and inducer of competence	73, 104
	$ComR_{St}$	*Streptococcus thermophilus*	XIP_{St}	PYFAGCL	Transcriptional activator of *sigX* sigma factor and inducer of competence	46, 112
	$ComR_{Smu}$	*Streptococcus mutans*	XIP_{Smu}	GLDWWSL	Transcriptional activator of *sigX* sigma factor and inducer of competence	73
	$ComR_{suis}$	*Streptococcus suis*	XIP_{suis}	WGTWVEE	Transcriptional activator of *sigX* sigma factor and inducer of competence	104
	$ComR_{Sbo}$	*Streptococcus bovis*	XIP_{Sbo}	LTAWWGL	Transcriptional activator of *sigX* sigma factor and inducer of competence	77

Abbreviation: ND, not determined.