



# Microbial Telesensing: Probing the Environment for Friends, Foes, and Food

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## Microbial Telesensing: Probing the environment for friends, foes and food

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### Abstract

Bacterial sensing circuits may be triggered by molecules originating from the environment (e.g., nutrients, chemoattractants). Bacteria also actively probe the environment for information, by releasing molecular probes to measure conditions beyond the cell surface -- telesensing. Perceiving the environment beyond is achieved by sensing environmentally induced changes in those probes, such as occurs when a siderophore chelates an iron atom, or a quorum sensing signal is inactivated by a specific enzyme or adsorbent. This information, captured by chemical and physical changes induced in specifically produced molecules transiting through the environment, enable bacteria to mount a contextually appropriate response.

### Introduction

We now understand that bacteria are highly sentient organisms with a sophisticated and diverse array of systems for sensing their surroundings. Rapidly accumulating genomic information shows that microbes in fluctuating environments are rich in two-component signaling and other sensing systems. For example, *Pseudomonas aeruginosa* strains possess as many as 53 two-component systems (Kiil et al., 2005). Contact sensing systems function when a molecule originating from the environment directly collides with a cell surface or periplasmic receptor, as in the binding of nutrients or chemoattractants. Other mechanisms for analyzing the environment, such as quorum sensing, require the microbe to generate a probe to query conditions beyond the cell surface. Local versus distance sensing systems are not unique to bacteria – it is at the heart of subjects as diverse as the debate over the need for manned space flight, where concepts of “touch sensing” and “telesensing” are well established (Genta and Perino, 2005).

Quorum sensing is a specific type of telesensing, and new variations on the theme are continually discovered (Svenningsen et al., 2009; Swem et al., 2008). There are several outstanding recent reviews of quorum sensing (Camilli and Bassler, 2006; Williams et al., 2007). The goal of quorum sensing is to count siblings or close relatives, by measuring a

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molecule that has diffused from a distance. Other telesensing systems probe the environment for specific external conditions, and when that condition is found, trigger a contextually appropriate response.

As one of the most thoroughly studied types of telesensing, it is of value to briefly examine quorum sensing so that it can be contrasted and compared to other telesensing systems. Telesensing involves the production, release and diffusion of a signal, followed by subsequent recognition of an environmentally imposed change in that signaling molecule. In the case of quorum sensing, the environment dictates the fate of the quorum molecule, including the rate of its accumulation to a threshold concentration, which then triggers a contextually appropriate genetic program. In its simplest form, quorum sensing enables bacteria to coordinate gene expression according to local population density. As a bacterial population increases, the concentration of the quorum molecule, or autoinducer, in the external environment increases proportionally. Thus, by controlling gene expression in response to a chemical that is synthesized, released, and either exchanged with a sibling or returns to the producer, bacteria query the environment beyond the cell surface for information on the population of siblings, and alter their behavior accordingly. Logically, the behaviors that are triggered are social involving community structure in biofilms, group expression of bioluminescence, sporulation, conjugation, motility, competence, and secretion of virulence factors (Miller and Bassler, 2001).

Quorum sensing mechanisms appear to have evolved along three tracks – acylhomoserine lactone-based (AHL) signaling systems of Gram-negative bacteria, peptide-based systems in Gram-positive bacteria, and a furanone-based system common to both (Hardie and Heurlier, 2008). AHL circuits are typically homologous to the LuxI/LuxR system of *Vibrio fischeri* (Engebrecht et al., 1983). LuxI catalyzes the formation of specific fatty acylhomoserine lactones (AHLs) that diffuse through the membrane. Once a threshold level is reached, AHLs bind to a LuxR-type regulator, which then induces or represses expression of target genes (Lazdunski et al., 2004; Miller and Bassler, 2001). In contrast, Gram-positive bacteria typically use oligopeptides, such as the Agr system of *Staphylococcus aureus* (Lyon and Novick, 2004), to actively probe the environment. These are generically referred to as autoinducing peptides (AIPs). Once exported, these AIPs interact with the environment, then are sensed in most cases by the external domains of membrane bound sensor kinase proteins. This interaction induces a phosphorylation cascade that leads to the activation of target genes. Autoinducers enable specific intraspecies communication. More universal communication is achieved between microbes of various species through the autoinducer 2 (AI2) system, which is shared by both Gram-positive and Gram-negative bacteria (Xavier and Bassler, 2003).

Common to all quorum sensing systems is the release of specific, microbially synthesized molecules into the environment. In an inert environment, the accumulation of a monotonically produced signal becomes a direct reflection of a) the number of microbes present, b) the length of time a closed environment has been occupied by the signal producing cell, or a combination of a) and b). Some highly co-evolved systems, such as the *Vibrio fischeri*/sepiolid squid light organ, approximate a closed environment with reproducible diffusion characteristics. Other environments with consistent, reproducible diffusion characteristics include the internal milieu of a monospecies bacterial colony. In a bacterial colony, a telesensing system may function just as a morphogen does in the development of higher organisms: A signaling molecule is produced by one cell in the population, which then diffuses outward. Rapid dilution into the environment from the surface of a colony creates a zone of reduced concentration at the colony/environment interface, whereas limited solvent movement between cells leads to locally high accumulation internally, overall resulting in a concentration gradient across the colony. Cells along that gradient express varying developmental programs, which in higher eukaryotes, results in the creation of complex tissue and organ architectures (Kicheva et al., 2007). The

discovery of a quorum-regulated *Streptococcus pneumoniae* system that causes lysis of neighboring sibling cells in a colony along a concentration gradient (Guiral et al., 2005), led to a proposed mechanism and role for autolysis in the release of DNA and formation of higher order architecture in stable microbial communities, such as biofilms (Gilmore and Haas, 2005). In a variation on signal-induced fratricide, the soil bacterium *Paenibacillus dendritiformis* secretes a lethal factor that diffuses out from the colony. If another colony of the same strain is in close proximity, the concentration of the lethal factor can exceed the inhibitory threshold, producing a distinct zone of inhibition and cell death where the colonies approach each other (Be'er et al., 2009).

However, not all environments into which quorum or other sensing molecules are released have consistent diffusion or chemical characteristics, and the importance of the of external environment in altering telesensing signals is beginning to be appreciated (Horswill et al., 2007). Telesensing processes are now known to be influenced by environmental cues including temperature, ligand concentration, pH and water and oxygen availability (Bollinger et al., 2001; Bose et al., 2007; Dulla and Lindow, 2008; Hasegawa et al., 2005; Jensen et al., 2006; Latour et al., 2007; McGowan et al., 2005; Palmer et al., 2007; Pessi and Haas, 2000; Shrout et al., 2006; Surette and Bassler, 1998; Wagner et al., 2006). These observations highlight the importance of telesensing systems being contextually sensitive. Here, we review several well characterized telesensing systems that have evolved contextual sensitivity. Common to all of these systems is the production and release of a telesensing molecule by organisms that may inhabit diverse niches. If the molecule is released in one biologically relevant environment, the molecule is specifically affected by conditions within that environment, and this dictates the decision algorithm of the microbe. In another environment where the molecule may not be affected and returns unaltered, or simply increased in concentration behaving purely as a quorum sensor, another behavioral decision is made by the microbe. In short, we review here telesensing systems that have the characteristic of being environmentally responsive.

## Environmentally responsive telesensing systems

Telesensing may be used by microbes to sense conditions in animal or plant niches, or occurring at large in the environment. Telesensing may regulate traits that dictate the relationship in a host niche, leading the microbe to break out of a stable commensal relationship and cause disease. Alternatively, telesensing may be used by a microbe to sense when it has crossed a line from stable colonization to being in direct conflict with the host, and down-regulate potentially pathogenic traits to promote a return to a stable host-commensal relationship. There is increasing evidence in some cases to support this more nuanced latter view.

### A. Telesensing signals that are modified by host factors

**1-The *Enterococcus faecalis* cytolysin signal senses target cells, and produces a toxin in response—***Enterococcus faecalis* is a common resident of the gastrointestinal tract of a wide variety of hosts, ranging from mammals to insects. Moreover, it is a leading cause of hospital-acquired infection, and also the main cause of sub-acute endocarditis in the community ((McCormick et al., 2001). Highly virulent strains of *E. faecalis* produce a two subunit toxin termed the cytolysin. This toxin has broad activity against both eukaryotic and prokaryotic cells, and contributes to the severity of infection in all models tested (Coburn and Gilmore, 2003; Garsin et al., 2001). Cytolysin is a variable trait of *E. faecalis*, and is usually encoded within pheromone-responsive plasmids (plasmids that encode the ability to recognize molecules released into the environment by a potential recipient bacterium, and in response upregulate the conjugation machinery that transfers a plasmid copy into that recipient), or on a pathogenicity island (Shankar et al., 2002). The cytolysin consists of two small peptides, CylL<sub>L</sub> and CylL<sub>S</sub>. Both are posttranslationally modified and secreted into the extracellular environment by accessory proteins encoded within the cytolysin operon. Once activated by a

final proteolytic step outside of the cell, the mature toxin subunits, termed CylL<sub>L</sub>" and CylL<sub>S</sub>", form a complex in eukaryotic or prokaryotic target cell membranes that results in membrane rupture, and in the case of erythrocytes which are often used as targets in vitro, hemoglobin release. Eight genes are involved in expression and regulation of cytolysin production. Six of these genes are transcribed from the promoter, P<sub>Lys</sub>, and are required for toxin production, maturation, secretion, and immunity. Two additional divergently transcribed genes, expressed from the promoter P<sub>Reg</sub>, encode regulatory proteins CylR1 and CylR2.

Cytolysin expression is regulated by an environmentally influenced telesensing system (Coburn et al., 2004). The smaller of the two toxin subunits, CylL<sub>S</sub>", autoinduces expression of the toxin operon through a quorum sensing-like mechanism (Haas et al., 2002). Local accumulation of mature CylL<sub>S</sub>" leads to autoinduction. Enigmatically, little cytolysin is expressed in liquid *E. faecalis* cultures, even though it is readily produced on blood agar leading to clear zones of hemolysis. Because of this, the cytolysin was originally termed a "pseudohemolysin" (Todd, 1934). More recently it was observed that when erythrocytes are added to broth culture, cytolysin activity is produced at high levels (Coburn et al., 2004). It was therefore of interest to determine the mechanism by which *E. faecalis* was able to sense the presence of target cells in the environment, and to express the cytolysin when target cells were sensed.

It was found that in the absence of target cells, the larger cytolysin subunit, CylL<sub>L</sub>", interacts with the smaller subunit/autoinducer, CylL<sub>S</sub>", to form an oligomeric complex. This very stable complex is devoid of cytolytic activity, and sequesters CylL<sub>S</sub>" in a way that prevents its induction of the cytolysin operon (Coburn et al., 2004). In other words, CylL<sub>L</sub>" titrates back the level of free CylL<sub>S</sub>" in solution, and holds it below the threshold necessary to trigger high-level cytolysin expression. However, in the presence of target cells it was found that CylL<sub>L</sub>" binds to lipid membranes with a 6 – 7 fold greater affinity than CylL<sub>S</sub>". CylL<sub>L</sub>" binds membranes in way that prevents its sequestration of CylL<sub>S</sub>", allowing a pool of free CylL<sub>S</sub>" to accumulate, at least transiently, and induce high-level cytolysin expression (Figure 1). Stated another way, when target cells are absent, the two cytolysin subunits CylL<sub>S</sub>" and CylL<sub>L</sub>" form inactive aggregates, but in the presence of target cells, CylL<sub>L</sub>" rapidly adsorbs to the target cell surface leaving behind a pool of free CylL<sub>S</sub>", which is capable of autoinduction. Using this mechanism, *E. faecalis* is able to sense cytolysin target cells and produce cytolysin only when target cells are present. Whether this regulatory system evolved to sense competing Gram-positive bacterial cells in the competitive GI tract (in addition to being a toxin, it is also a bacteriocin specific for Gram-positive bacteria), or perhaps single-celled eukaryotic organisms in the external environment, or cells of more complex life forms such as mammals, where it invariably contributes to virulence (Coburn and Gilmore, 2003), is currently unknown.

**2-Neutralization of a pheromone inhibitor enables pheromone accumulation, leading to binding to a potential plasmid recipient, or to bacterial accumulation on host tissues in infection—**Pheromone-responsive conjugative plasmids are widely distributed among enterococci where they contribute to the proliferation and distribution of antimicrobial resistance and other traits. Well characterized pheromone-responsive conjugation systems have been identified on plasmids pAD1 and pCF10. Expression of the large set of gene products required for conjugative transfer is controlled by communication between plasmid-free recipients and plasmid-carrying donor cells. Potential recipient cells release 7 – 8 amino acid oligopeptides, which are generally encoded within and processed from the C-terminal signal sequence of lipoprotein precursors, that act as pheromone attractants (Clewett et al., 2000). The pheromone peptide from a potential recipient cell is internalized by the potential donor *E. faecalis* cell (Dunny, 2007), where it induces expression of a plasmid-encoded cell surface protein. This protein, named aggregation substance, mediates stable mating pair-formation between donor and recipient, and if not carefully controlled, can also

lead to donor clumping or autoaggregation. (Donor cells also produce the pheromone. However, donor cells additionally produce a plasmid-encoded surface protein as well as a plasmid-encoded inhibitor peptide that prevent donor-produced pheromone uptake by a molecular mechanism yet to be precisely defined). The plasmid-encoded pheromone inhibitor is a small peptide, which, like the pheromone, is synthesized as a precursor. Blockage of the pheromone by these donor cell activities prevents autoaggregation and futile activation of the transfer pathway. Expression of additional pheromone by the potential recipient, however, exceeds the capacity of the inhibitory activities, resulting in local pheromone concentrations that tip the balance in favor of mounting a response and expression of aggregation substance. Disturbing the balance of pheromone to pheromone antagonizing activities, even in a pure culture of plasmid-containing donor cells, can lead to autoaggregation.

The surface protein that mediates autoaggregation, originally termed aggregation substance prior to determination of its molecular character, in addition to mediating effective mating pair formation leading to plasmid transfer, also contributes to virulence in most infection models (Wirth, 2000). This suggested that aggregation substance is somehow expressed in the absence of recipient cells in sites of infection. Using pCF10, it was found that a host factor, most likely an albumin/lipid complex, selectively sequesters or degrades the pheromone inhibitor, resulting in a local pool of free pheromone even in the absence of recipient cells. This leads to expression of aggregation substance on the surface of *E. faecalis* in an infection (Chandler et al., 2005), where it results in larger vegetations on heart valves, and likely also to clumping making phagocytic clearance more difficult. Whether or not this behavior at the site of infection is the result of selective pressure in this setting is an interesting question. By far, enterococcal existence is mainly as a commensal in the GI tract in nearly all animals, despite its occurrence as a leading cause of multiple antibiotic resistant hospital acquired infection. Nevertheless, this mechanism of producing an aggregation effector along with an inhibitor, allows enterococci to sense the presence of either bacterial partners or its occurrence at the site of infection, and in response induce the expression of aggregation substance leading to attachment to recipient bacterial cells or accumulation on host tissue (Figure 2).

### **3-Evidence for détente between *Staphylococcus aureus* and man, mediated in part by environmentally responsive telesensing**—

Nearly all humans asymptotically carry *Staphylococcus aureus* as a member of the commensal flora, yet it is the leading bacterial cause of invasive infection in the developed world, causing more deaths annually in the US than HIV/AIDS (Bancroft, 2007; Fridkin et al., 2005; Klevens et al., 2007). *S. aureus* virulence is centrally regulated by the *agr* system and other regulators (Novick and Geisinger, 2008). The *agr* locus is transcribed from two promoters, P<sub>2</sub> and P<sub>3</sub>. Promoter P<sub>2</sub> initiates transcription of a 4-gene operon, *agrB*, *D*, *C* and *A*. AgrD encodes the precursor of the signal molecule, which is trimmed and secreted by AgrB to generate the autoinducer signal peptide, a 7-9 mer peptide containing a 5 membered thiolactone ring. The secreted, cyclized autoinducer then binds the sensor kinase, AgrC, on the surface of *S. aureus*, which then activates the AgrA response regulator via a cascade of phosphorylation. Once phosphorylated, AgrA upregulates expression from the P<sub>2</sub> promoter, as well as the P<sub>3</sub> promoter, which initiates transcription of a regulatory RNA molecule, termed RNAIII. RNAIII is the effector of the *agr* regulon.

RNAIII down regulates expression of genes encoding surface adhesins while it up regulates capsule, toxins and protease production (Novick and Geisinger, 2008). This phenotypic conversion from an adhesive and colonizing behavior to a tissue damaging/invasive form, is central to the current understanding of the pathogenesis of systemic infection (Novick, 2003). Suggesting that there may be important complexities to this understanding, Rothfork and colleagues (Rothfork et al., 2004) made the surprising observation that phagocyte NADPH oxidase, myeloperoxidase or inducible nitric oxide synthetase inactivate the type I Agr

signaling thiolactone. This results in inhibition of bacterial communication, and limits the upregulation of the virulence traits that constitute the virulent form. Oxidation of the C-terminal methionine of the Agr thiolactone was primarily responsible for the loss of activity. The use of oxygen radical generating defense and predation systems is common among most eukaryotes, and clearly predates the evolution of modern primates. Moreover, other amino acids are known to be functional at this position. Nevertheless, this microbe still employs a virulence signaling system that is inactive in the oxidative intracellular milieu of a phagocytic cell. Therefore, in interacting with the human host, a quorum of *S. aureus* behaves in a virulent way in some human niches, but in an avirulent way when it is exposed to host produced reactive oxygen and nitrogen species. Although this oxidative inactivation was only shown for Agr type I, the oxidized methionine is conserved in Agr type IV, and also in the *E. faecalis* gelatinase-regulating system, *fsr* (Lyon and Novick, 2004), which would be predicted to be similarly inactivated at sites of reactive oxygen and nitrogen species generation. Interestingly, the Agr homologs of coagulase-negative staphylococci, as well as Agr types II and III of *S. aureus*, lack this C-terminal methionine and have other terminal amino acids instead.

The Agr telesensing system is not the first evidence suggesting that *S. aureus* has struck a careful balance between virulence and host colonization. The Skaar laboratory (Torres et al., 2007) initially observed, perhaps counterintuitively, that when *S. aureus* detected the presence of heme (presumably a signal of its presence in an invasive infection) through the HssRS two component system, virulence was down-regulated. Inactivation of this two component system led to increased virulence (Torres et al., 2007). This indicated that in the bloodstream or other site of invasive infection where heme is abundant, *S. aureus* down regulates the factors necessary for the translation to the virulent form.

Supporting the concept of managed virulence by *S. aureus*, organisms that much more often colonize than infect, others observed that in conditions where the *codY* system of was likely to be active, such as in the bloodstream, *S. aureus* virulence was again attenuated (Camargo and Gilmore, 2008; Majerczyk et al., 2008). The *codY* global regulatory system of low G+C Gram-positive bacteria connects nutritional status, through sensing extracellular concentrations of branched chain amino acids and intracellular GTP levels, with regulation of metabolic and virulence genes. Further support for the management of *S. aureus* virulence in the bloodstream derives from the following: Whereas components of the bloodstream induce an imbalance in *E. faecalis* donor pheromone homeostasis, resulting in the autoaggregation of donor cells and exacerbation of virulence; VLDL and LDL lipoproteins antagonize Agr signaling and induction of virulence, very likely by a direct adsorption mechanism (Peterson et al., 2008).

All of these observations are consistent with an evolutionary model (Camargo and Gilmore, 2008) that proposes that *S. aureus* down-regulates virulence in a well nourished, immunologically intact host (that is, one capable of oxidatively inactivating the Agr thiolactone within phagocytic cells and clearing *S. aureus* from the bloodstream before sheer bacterial numbers tip the balance away from the additional suppression of virulence mediated by LDL/VLDL adsorption of the autoinducer, or the self-suppression of virulence by the *codY* regulon and by the sensing of heme). The net effect is that *S. aureus* is of limited virulence to a healthy host, but may contribute to the demise of a weakened, malnourished, immune compromised host, such that the microbe is first to the table to benefit from host demise. By culling the herd of weakened individuals, such a model may be net positive in selecting for host fitness, as well as advantageous for the microbe that can proliferate on the culled individual. These observations argue strongly for a much more nuanced view of virulence and the dynamic between hosts and microbes near the fine line that separates commensal from pathogen. Moreover, introduction of strong selective pressures into the human ecology, such the use of antibiotics, may promote the destabilization of this finely balanced host-microbe homeostasis,

by selecting for the introduction of mobile genetic elements into the *S. aureus* genome that have the potential to inadvertently disrupt highly co-evolved gene expression patterns.

Expressing a telesensing signal that is conditionally responsive within various host niches is certainly not unique to *S. aureus*. Chun *et al.* (Chun *et al.*, 2004) demonstrated that differentiated human airway epithelia have the ability to inactivate the N-(3-oxododecanoyl)-L-homoserine lactone signal of *P. aeruginosa*. However, in this case the argument would have to be made that the vast majority of *P. aeruginosa* human association is in the form of asymptomatic commensal colonization, and that this colonization/carriage, and not pathogenic infection, was the main selective pressure in evolving the C<sub>12</sub> lactone signal of *P. aeruginosa*. While this relationship is clearer for *S. aureus*, commensal colonization is currently an area of substantial controversy for *P. aeruginosa* and other microbes, as new microbiome technologies reveal previously unappreciated species associations with the healthy human host (Fierer *et al.*, 2008). Whether inactivating a quorum molecule is a host response (in which case the point would have to be argued that the host is prevailing despite the ability of bacteria to rapidly out-replicate the host and evolve), or a compromise reached between host and microbe, is currently unknown. A conceptually similar question exists in microbial ecology where, for example, several *Bacillus* species produce a lactonase, named AiiA, that inactivates among other things, acyl homoserine lactones (Dong *et al.*, 2002). Whether this activity nonspecifically inactivates signaling by Gram-negative microbes, or by inactivating signaling, promotes specific microbe-microbe associations between lactonase-producing *Bacillus* species and Gram-negative microbes that otherwise would not occur, is likely to vary on a case by case basis. Interestingly, it was shown that 3-oxo-N-acylhomoserine lactones spontaneously hydrolyze to antimicrobial, antioxidant, iron-binding tetramic acid derivatives. The production of a lactonase by *Bacillus* and other organisms may simply represent a form of self defense (Kaufmann *et al.*, 2005). Nevertheless, the tools for using telesensing to foster stable associations between microbe and host (or pathogenic relationships between microbes and host) likely derived from those used to establish cooperative and predatory relationships between microbes in the environment.

## B. Telesensing probes that incorporate environmental molecules

**1-“Ferrimones”: siderophores and hemophores detect and mediate responses to the presence of iron in the environment**—Iron is an essential element for almost all organisms, but is generally not soluble in aerobic environments at neutral pH. Thus, despite its abundance on earth, there is little free iron to satisfy microbial requirements. Iron availability is further limited in mammalian hosts by being complexed to protein carriers, which protect cells against iron toxicity due to Fenton reactions. Pathogens must be able to compete for these limited supplies of available iron to colonize and cause disease.

In response to iron deprivation, many bacteria synthesize and release into the environment compounds that bind ferric iron (siderophores) or heme (hemophores) with high affinity (Wandersman and Delepelaire, 2004). The complexes diffuse back and bind to specific receptors on the cell surface, and the ferri-siderophore or heme is transported into the cell. Thus they serve as both iron acquisition and iron sensing molecules. Regulation of expression of the synthesis and transport of these compounds is accomplished by an iron binding repressor, Fur or DtxR, which recognizes a conserved sequence in the promoters of iron-regulated genes (Hantke, 2001). In the absence of iron, the repressor is inactive and the genes are expressed, but when iron is abundant, the repressor binds DNA and prevents expression of the iron acquisition genes, thus avoiding iron toxicity. Under iron starvation conditions *in vitro*, a pathogen may express all of its iron transport systems, and many pathogens have large numbers of systems. *V. cholerae*, for example, has genes for synthesis, secretion and transport of the siderophore vibriobactin (Wyckoff *et al.*, 2007). It also has three transport systems for heme

(Mey and Payne, 2001), at least three systems for transport of siderophores made by other bacteria (Wyckoff et al., 2007), and two ferrous and one ferric iron transporter (Wyckoff et al., 2006). The genes encoding these systems make up more than 1% of the *V. cholerae* genome and many more genes, including some encoding virulence factors, are regulated by the concentration of iron in the cell (Mey et al., 2005). Derepressing all these systems simultaneously is metabolically expensive, and forcing derepression by mutating *fur* inhibits growth or is lethal (Barton et al., 1996; Mey et al., 2005). The picture that is emerging is one in which the bacteria produce basal levels of the various chelators to probe the environment, and then up regulate or downregulate the various systems depending on which iron sources are available as revealed by which environmental probe is chemically altered. This has been termed ferrimone sensing (Brickman and Armstrong, 2009), and is another well studied class of telesensing.

*Bordetella pertussis*, the agent of whooping cough, has multiple iron transport systems. Three of these, production and transport of the siderophore alcaligen, transport of the catechol siderophore enterobactin, and heme transport, have been characterized at the genetic level (Brickman and Armstrong, 2009). Each of these is positively regulated by a transcription factor interacting with the cognate, iron-containing compound. This allows the bacterium to sense the iron sources available at a given site within the host and to monitor changes in iron sources over time. Temporal expression patterns of these systems, all of which are required for virulence, have been shown in vivo (Brickman et al., 2008). The siderophore biosynthesis and transport genes are induced early during infection while heme transport is induced late. Alcaligin is secreted, binds iron from the host and is transported into the bacterial cell, where it interacts with the AraC-type transcriptional regulator AlcR, which induces high levels of alcaligin gene expression (Brickman et al., 2001). A second siderophore receptor gene, *bfeA*, is also up-regulated early in infection. This receptor is induced by the enteric siderophore enterobactin, but its ligand in the respiratory tract appears to be neuroendocrine catecholamines including norepinephrine (Anderson and Armstrong, 2006). The catecholamines can bind iron and can be used as iron sources by *B. pertussis*. Thus, *B. pertussis* can monitor and respond to the presence of catecholamine released by the host as a result of damage to the respiratory epithelium. The heme transport system is induced late in infection. Heme binding to the heme receptor BhuR initiates signaling through an N-terminal extension of the receptor that is similar to extensions in proteins involved in iron-inducible extracytoplasmic function (ECF) sigma factor dependent regulators (Vanderpool and Armstrong, 2003). In the absence of heme, basal expression of the *bhu* operon results from read-through from an upstream promoter in low iron conditions. If heme is present in the environment, heme bound to BhuR results in binding of the anti-sigma membrane sensor HurR which allows release or activation of sigma factor HurI. HurI allows increased expression of BhuR from the *bhu* promoter.

*P. aeruginosa* secretes two different siderophores – pyoverdine and pyochelin. Pyoverdine contributes to *P. aeruginosa* virulence in multiple animal models (Meyer et al., 1996; Takase et al., 2000), and has been isolated from sites of *P. aeruginosa* human infection (Haas et al., 1991). Lamont and co-workers (Beare et al., 2003; Lamont et al., 2002) have shown that pyoverdine can stimulate its own biosynthesis, and also influences production of at least two other *P. aeruginosa* virulence factors, exotoxin A and PrlP protease. Activation of the expression of these genes is mediated via the alternative sigma factor, PvdS, which recruits RNA polymerase to target promoters (Figure 3).

Some Gram-negative pathogens secrete hemophores, heme- or hemopexin (a high affinity heme-binding protein in mammalian serum)-binding proteins that act as telesensing molecules for heme. *Haemophilus influenzae* HxuA is an example of a hemopexin-binding hemophore (Cope et al., 1995). Heme-binding hemophore systems have been identified in *Serratia marcescens*, *P. aeruginosa*, and *Yersinia pestis*, among others. Synthesis of the hemophore,

HasA, by *S. marcescens* is mediated via an autoinduction control mechanism (Cescau et al., 2007; Wandersman and Delepelaire, 2004). The HasA outer membrane receptor, HasR, can bind heme-free (Apo-HasA) and heme-loaded (Holo-HasA) hemophores with similar affinity. However, only Holo-HasA activates *has* gene transcription (Cescau et al., 2007; Rossi et al., 2003). Since siderophores and hemophores not only deliver nutrients to the microbe, but also differentially induce specific genetic response programs when ligand is bound, they fulfill the criteria for environmentally responsive telesensing molecules.

### **2-Rhodopseudomonas palustris incorporates potentially host produced molecules into a telesensing signal**

The fatty acyl group of fatty acyl-homoserine lactones provides signal specificity, but the variety of signal structures is limited. *R. palustris*, a Gram-negative plant saprophyte, possesses a LuxRI-like quorum sensing circuit consisting of RpaI and RpaR. Schaefer and co-workers (Schaefer et al., 2008) discovered that *rpaI* expression increased specifically in the presence of *p*-coumarate, a phenolic component of plant lignin. Further investigation showed that the acyl-homoserine lactone signal incorporated a *p*-coumaroyl group into its structure, generating a novel *p*-coumaroyl-homoserine lactone. RpaI was shown to catalyze the synthesis of *p*-coumaroyl-homoserine lactone from S-adenosylmethionine and *p*-coumaryl coenzyme A. Transcriptome analyses demonstrated that expression of 17 genes, including *rapI*, were significantly altered when bacteria were grown in the presence of either this modified autoinducer or *p*-coumarate (Cooley et al., 2008). Rather than sending out a telesensing molecule and responding differentially to it after it is altered by the environment, this system first takes up a specific plant metabolite and uses it to generate a telesensing quorum signal, which could thereby mediate specific interactions with a plant host. Thus, the use of *p*-coumarate results in a signal that perceives both cell-density and an environmental cue. Production of *p*-coumaroyl derivatives of homoserine lactones is not unique to *R. palustris*, in that it was found to occur in at least two other species of soil bacteria, *Bradyrhizobium s.p* and *Silicibacter pomeroyi* (Schaefer et al., 2008).

## **C. Telesensing system integration with host signalling**

### ***P. aeruginosa*: The host responds to microbial signals and the microbe responds to host signals**

The opportunistic pathogen *P. aeruginosa* possesses two AHL-type quorum sensing systems, *las* and *rhl*, consisting of the LuxRI homologues, LasRI and RhIRI (Whiteley and Greenberg, 2001). Both systems contribute to *P. aeruginosa* pathogenicity in different animal models (Smith and Iglewski, 2003) by regulating expression of virulence determinants, including the galactophilic *P. aeruginosa* lectin (LecA) and pyocyanin (de Kievit and Iglewski, 2000).

Human host cells express a variety of pattern recognition receptors and other mechanisms for detecting bacteria. Wu *et al.* (Wu et al., 2005) hypothesized that bacteria themselves might possess specialized receptors that in turn recognize and respond to the host immune activation. They observed that the upregulation of *lecA* was substantially enhanced in the presence of exogenous interferon- $\gamma$  (IFN- $\gamma$ ). Moreover, this effect was specific for this particular cytokine, and was abrogated in *rhl* mutant strains. IFN- $\gamma$  was found to bind specifically to OprF, an outer membrane protein, which in turn activated the *rhl* quorum sensing machinery by an unknown mechanism. The result, however, was increased expression of virulence factors including PAI lectin and pyocyanin (Wu et al., 2005). In addition to its signaling function, the autoinducer produced by the *lasRI* quorum sensing system was shown to act on host signaling pathways, including stimulating IFN- $\gamma$  production by T-cells establishing a potential feedback loop (Smith and Iglewski, 2003) (Figure 4). In this case, the microbe induces a host response, to which the microbe responds in a way that induces further host response.

## Conclusions and perspectives

It is increasingly clear that many systems have evolved to be specifically responsive to particular variations in the environment. Bacterial telesensing systems enable a microbe to actively probe the environment beyond the cell surface, and based on changes induced in the environmental probe by environmental factors, respond in a manner that is contextually appropriate. Main subcategories of telesensing include 1) quorum sensing in its many forms, 2) iron or “ferrimone” signaling, and 3) variations on these themes often related to virulence factor expression by opportunists. Contextually sensitive telesensing systems are diverse and occur in both Gram-positive and Gram-negative bacteria. These systems are central to bacterial decision making, promoting host/microbe stability in some cases, and promoting parasitism in others, especially in situations where the microbe senses stress or host compromise. Telesensing inputs are integrated into complex multilayered signal transduction networks that overlap with additional pathways involved in environmental sensing and response. Examples of multilayered systems include *Vibrio harveyi* bioluminescence, which is not only cell density dependent, but is also influenced by MetR and CRP nutritional sensors (Chatterjee et al., 2002). Other organisms, such as *Xanthomonas campestris*, regulate virulence by both quorum sensing and the RavS/RavR two-component regulatory system (He et al., 2009). It is clear that tremendous complexity and sophistication has evolved in the mechanisms microbes possess for sensing the environment, even at a distance, and making contextually appropriate decisions. More research will be required to fully understand the algorithms used by microbes to integrate sensory inputs and make the contextually appropriate decisions that lead to perpetuation of the species.

## Acknowledgments

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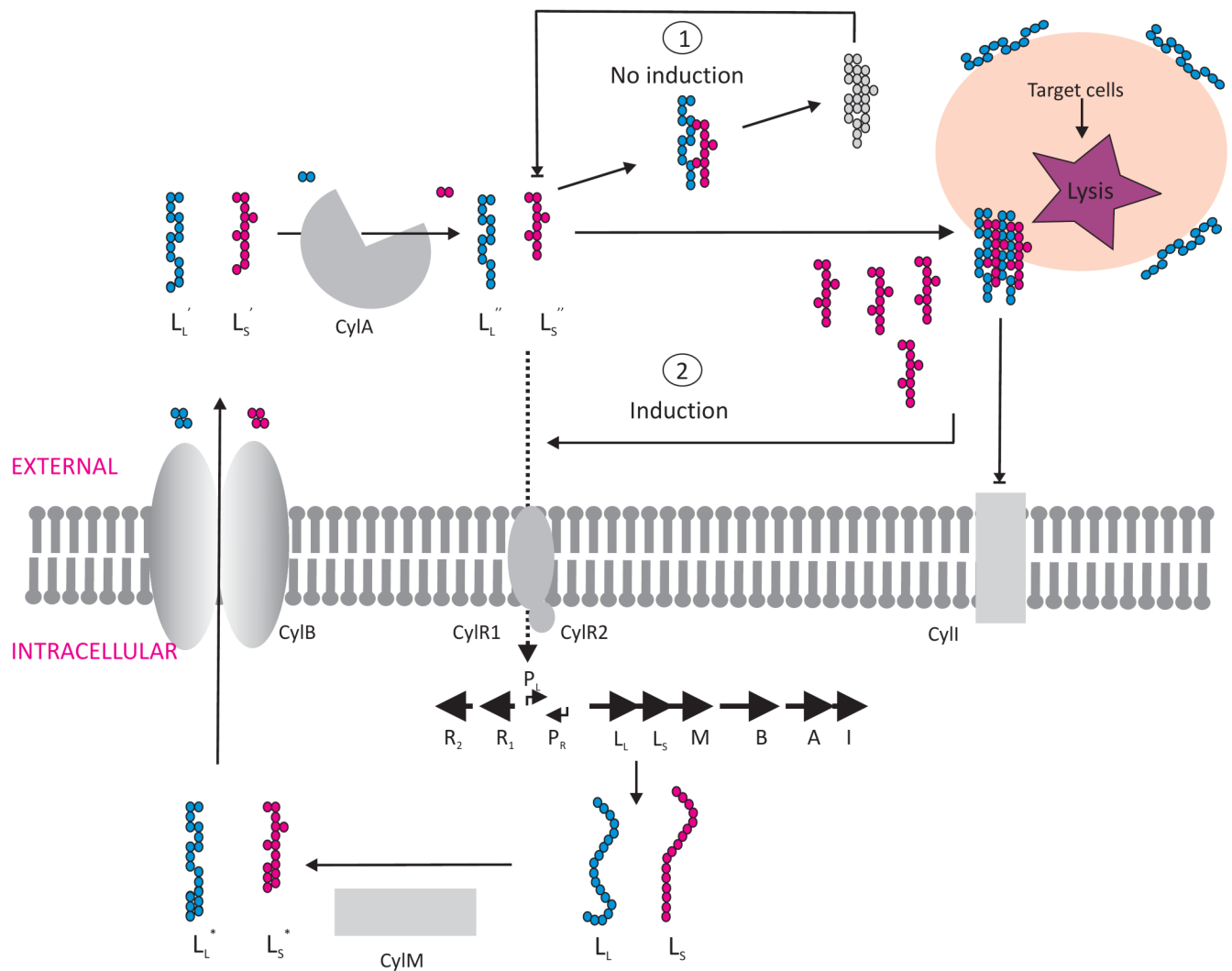
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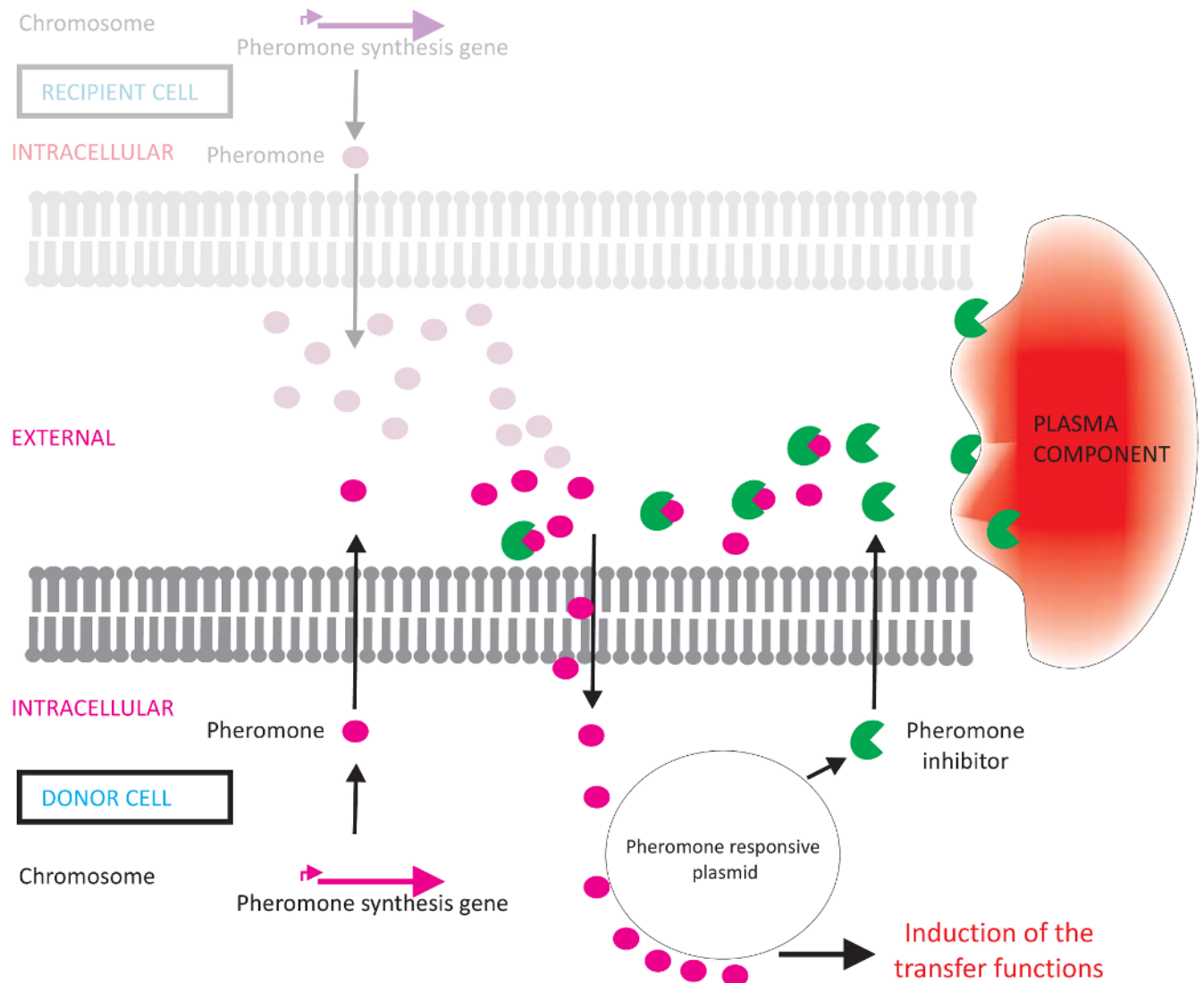
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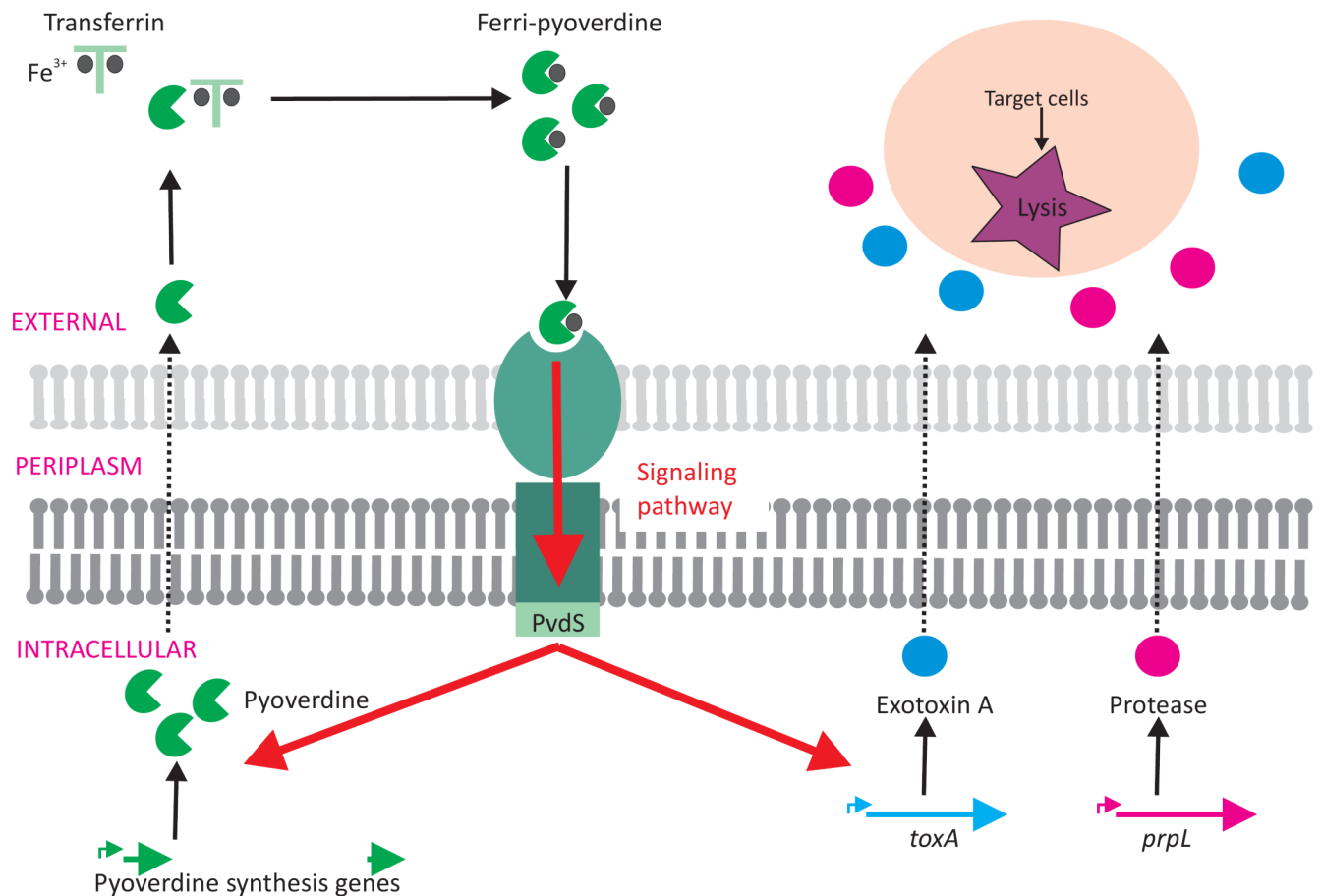
**Figure 1. The *Enterococcus faecalis* cytolsin senses and destroys target cells**

The two subunits of the toxin,  $CyIL_L$  and  $CyIL_S$  are modified and secreted in the extracellular environment. Cytolsin expression is regulated by  $CyIL_S''$  through a quorum sensing-like mechanism. In absence of target cells, the mature subunit  $CyIL_L''$ , interacts with  $CyIL_S''$  to form an inactive oligomeric complex (1) that is not able to induce expression of the cytolsin operon. In the presence of target cells,  $CyIL_L''$  binds them preferentially, creating a transient pool of free  $CyIL_S''$  (2) to induce high-level cytolsin expression, prior to the slow reaction of  $CyIL_S''$  joining the pore complex.



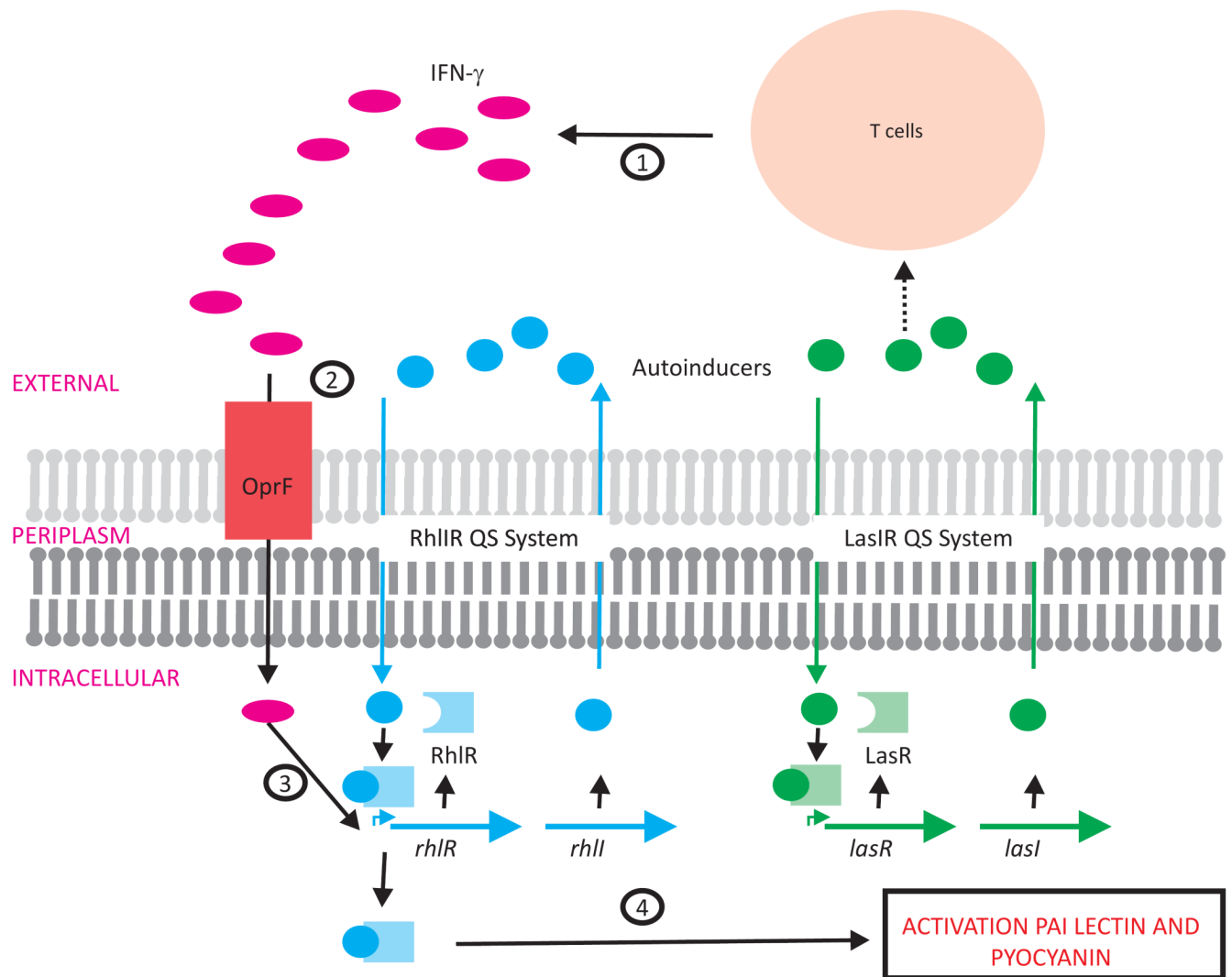
**Figure 2. Pheromone inducible plasmids enable *Enterococcus faecalis* to sense either recipients or conditions in the bloodstream**

Chromosomally encoded pheromones activate plasmid encoded factors that promote transfer of pheromone-responsive plasmids (e.g., pAD1 and pCF10) by conjugation from donor to recipient. Both donor and recipient cells produce pheromones. In donor cells, however, pheromone activity is held in check via a plasmid encoded cell wall protein (not shown) and a pheromone inhibitor also encoded by the plasmid. An increase in external pheromone concentration can be driven by additional pheromone production by a potential recipient (grayed image), or by the presence of substances that selectively neutralize the inhibitor, as occurs in blood.



**Figure 3. Pyoverdine "ferrimone" signaling**

Ferri-pyoverdine, having extracted a ferric ion from the environment, complexes with a specific outer-membrane receptor inducing a signaling pathway ultimately sensed by the PvdS alternative sigma factor. Activated PvdS recruits RNA polymerase to target promoters leading to activation of gene expression including genes involved in pyoverdine production, *prpL* encoding a protease and indirectly *toxA* coding for the exotoxin A.



**Figure 4. *Pseudomonas aeruginosa* recognizes the host immune response**

Interferon  $\gamma$  (IFN- $\gamma$ ) activates expression of *P. aeruginosa* *rhlIR* QS genes. (1) Cells of the innate immunity system sense bacteria and secrete IFN- $\gamma$ . Autoinducers produced via the LasIR QS system can induce IFN- $\gamma$  release. (2) INF- $\gamma$  binds to the OprF membrane protein leading to (3) activation of the *rhl* genes by an unknown mechanism. (4) This leads to RhlIR QS system induced activation of virulence factors, such as PAI lectin and pyocyanin.