4-Quinolones: Smart Phones of the Microbial World

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1. Introduction

The cellular telephone has revolutionized the way in which humans communicate. Since its invention, this device has evolved into the multifunctional "smart phone". The smart phone provides us with not only a means of communication but also a useful toolkit for managing our daily lives. For example, the smart phone enables us to check email, take photographs, and get directions, although it remains primarily a communication device. This review will discuss small molecules that serve as bacterial "smart phones", allowing bacteria to not only communicate but also monitor their external environment.

The finding that bacteria communicate to organize group behaviors has revolutionized the way we view these unicellular organisms. The mechanism of bacterial communication was elucidated in the marine bacterium *Vibrio fischeri* and since has been likened to communication mechanisms used by eukaryotes. Bacterial communication, or quorum sensing (QS), occurs via the sequential production, accumulation, and sensing of small hormone-like molecules, resulting in altered gene expression at defined cell densities. By using QS to regulate gene expression, bacteria coordinate group activities that may be more effective when carried out by a large population. QS is both ecologically and medically

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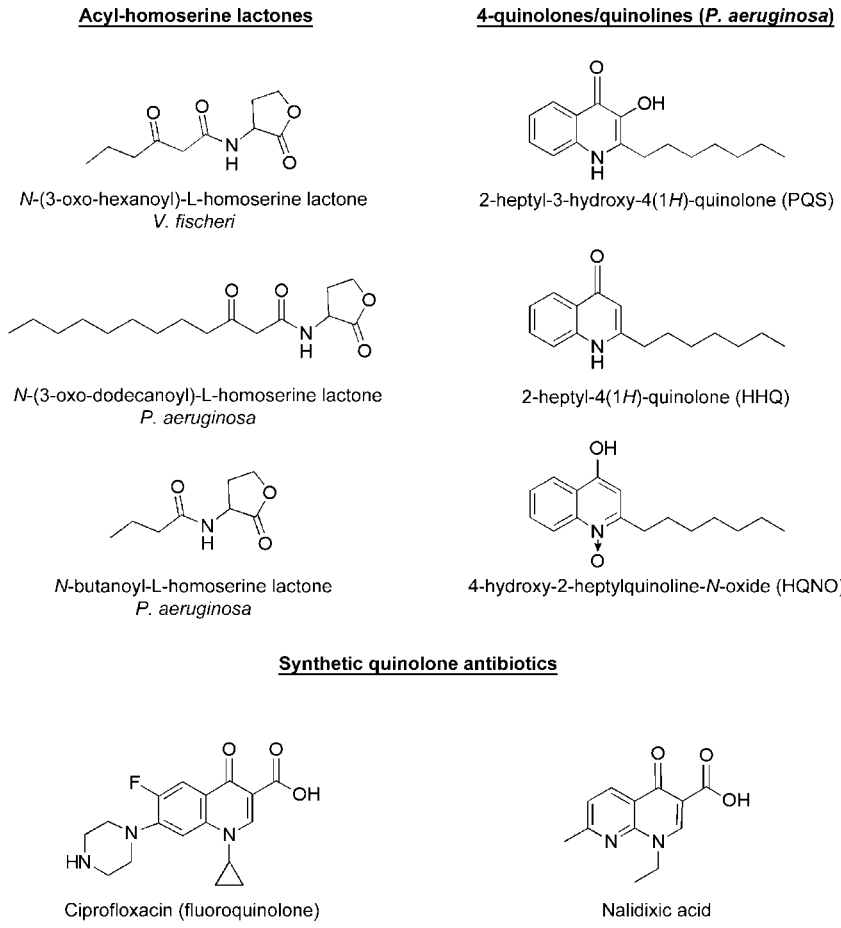

Figure 1. Structures of common QS signals and synthetic quinolone antibiotics.

relevant. For example, QS controls behaviors as diverse as bioluminescence and virulence factor production (as reviewed in ref 2).[1,2]

One major group of QS signaling molecules is the heterocyclic 4-quinolone/quinolines (4Qs). 4Qs have been primarily studied in the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa*. *P. aeruginosa* produces two primary 4Q QS molecules, 2-heptyl-4(1*H*)-quinolone (HHQ)[3−5] and 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal; PQS; Figure 1).[6] In addition to HHQ and PQS, *P. aeruginosa* produces >50 other 4Qs,[3,7] many of which remain functionally uncharacterized. Interestingly, 4Qs produced by *P. aeruginosa* share the basic 4-quinolone backbone of commercially synthesized quinolone antibiotics[8,9] (Figure 1). Indeed, *P. aeruginosa* produces 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO), a 4Q that possesses antibiotic activity,[10,11] though its mechanism of action differs from synthetic quinolone antibiotics.[12] The purpose of this review is to discuss 4Q-based QS systems, biosynthesis of 4Qs, regulation of 4Q biosynthesis, 4Qs and virulence, and 4Qs in biological roles other than signaling. We will not discuss how 4Qs interact with iron since this topic has been recently reviewed (see refs 13−15). Additionally, since bacterial 4Qs have been recently reviewed (see refs 14 and 16), we will also describe properties of commercial 4Q antibiotics and discuss recent literature that implicates these molecules in signaling.[17,18] Finally, we will speculate on new biological roles for 4Qs. This review covers literature published between 1945 and 2009.

2. Understanding the Smart Phone Basics: Introduction to Quorum Sensing

Because of its ecological and medical relevance, QS is an intensely studied topic and has been extensively reviewed in the past two decades (see refs 2, 14−16, and 19−22 for reviews). The QS paradigm is as follows: bacteria produce small signaling molecules at constitutive levels that are secreted into the environment. As the cell number within a population increases, the signal accumulates. When the signal reaches a threshold concentration, it is bound by a transcriptional regulator, which leads to global changes in gene expression.[2] QS molecules are termed autoinducers (AIs) because they often induce genes responsible for their biosynthesis. Many QS molecules are strain-specific, but there are examples of interspecies and interdomain signaling systems.[23−26] Since many bacterial pathogens use QS to control virulence, it has been proposed as a therapeutic target.[27,28]

While QS as a means of cell density-dependent gene regulation is widely accepted, two additional models have called into question its comprehensiveness and offer alternative explanations as to why bacteria evolved diffusible signaling molecules.[29,30] The first of these models, diffusion sensing, proposes that signals are inexpensive probes that allow a bacterium to assess the flux and mass transfer of an environment.[30] This enables a cell to determine whether the production of certain molecules, such as extracellular proteins and secondary metabolites, is economical.[30] The second

model, efficiency sensing, combines the basic tenets of QS and diffusion sensing.[29] Efficiency sensing states that flux, mass transfer, cell density, and spatial distribution are important for signaling.[29]

3. Blackberries, iPhones, and Droids: Quorum Sensing Systems

QS systems are diverse and widespread throughout the eubacterial domain. Bacteria produce a variety of signals, each with its own characteristic hydrophobicity, stability, and redox potential.[2] These chemical signals are often produced by dedicated AI synthases and bound by cognate transcriptional regulators.[2] A well-described class of QS signals is the acyl-homoserine lactones (acyl-HSLs) produced by Gram-negative bacteria. Acyl-HSLs are generally described as intraspecies signals, with species specificity conferred by the length, saturation, and degree of substitution on the acyl side chain.[2] For example, *P. aeruginosa* utilizes two distinct acyl-HSLs to control gene expression, each with a different acyl chain length and substitution[31−34] (Figure 1). The first, *N*-(3-oxododecanoyl)-L-HSL (3-oxo-C_{12}-HSL), has a 12-carbon acyl chain with a carbonyl on the 3-position. This molecule is produced and sensed by LasI/R. The LasI/R system exerts control over the second system, RhlI/R, which produces and senses *N*-butanoyl-L-HSL (C_4-HSL), a molecule with a 4-carbon acyl chain. Both the Las and Rhl systems regulate transcription of numerous genes encoding virulence factors, and in some cases both systems directly regulate transcription of a single gene.[2,31,32,34]

Unlike Gram-negative bacteria, Gram-positive bacteria produce small signaling peptides that can be linear or cyclic.[19,21] Similar to acyl-HSLs, these oligopeptides can be customized by chemical modifications that confer specificity.[19,21] However, in contrast to acyl-HSLs, the cyclic peptides are perceived by two-component regulatory systems that act via phosphorelay to induce changes in gene expression.[19,21]

In addition to intraspecies QS molecules such as acyl-HSLs, bacteria are capable of producing interspecies and interdomain signaling molecules. Autoinducer-2 (AI-2) is an interspecies signaling molecule produced by many bacterial species.[19,21] The term AI-2 describes a group of interconvertible furanones derived from dihydroxypentanedione,[35] which undergo spontaneous rearrangements. It is thought that different species respond to distinct forms of AI-2.[36] Acyl-HSLs may also play roles in interspecies communication, as different species can produce the same signal.[20] Some bacteria also produce interdomain signals. Autoinducer-3 (AI-3) is a unique epinephrine-like molecule involved in host−microbe interactions.[26] The pathogen *Escherichia coli* serotype O157:H7 produces and responds to AI-3;[26] however, the AI-3 system also responds to host epinephrine/norepinephrine, resulting in increased virulence factor production and altered motility.[26]

4. Getting to Know Your Smart Phone: 4-Quinolone Quorum Sensing Systems

In 1999, Pesci et al. identified a molecule that was capable of inducing the acyl-HSL regulated gene, *lasB,* in an acyl-HSL-deficient strain of *P. aeruginosa*.[6] The signal was identified as PQS, revealing a new class of QS molecules, the 4Qs.[6] Interestingly, PQS had previously been identified in 1959 by Takeda from *P. aeruginosa* culture supernatants, although its biological roles were not known.[37] Following the discovery by Pesci et al., it was shown that HHQ, the direct precursor of PQS, also acts as a signaling molecule.[3,4] In addition to PQS and HHQ, *P. aeruginosa* produces over 50 4Qs including molecules with antimicrobial properties, though most remain uncharacterized.[3,7,10,38−41] Although a signal, modification of HHQ to the N-oxide derivative HQNO radically alters its biological activity, from a QS signal to an antimicrobial involved in microbial competition.[3,11]

P. aeruginosa is not the only bacterium to produce 4Qs. Recent experiments using mass spectrometry have shown that numerous *Burkholderia* species also produce 4Q signals,[42,43] and HHQ production has been reported in *Pseudomonas putida*.[42] Interestingly, 4Qs produced by *Burkholderia* spp. are methylated at the 3-position, and this methyl group is required for signaling.[43] Here, we discuss the biosynthesis and regulation of 4Qs and their roles in QS and virulence.

4.1. How to Initiate the Call: Biosynthesis of 4Qs

Studies of 4Q biosynthesis began in the 1950s, when it was suggested that 4Qs were formed from the condensation of the central metabolite anthranilate and a β-keto-fatty acid.[40] Since then, experiments utilizing radiolabeled metabolites and genetics have confirmed this reaction.[27,44,45] 4Q biosynthesis is complex, as anthranilate can be obtained from three distinct metabolic pathways. One pathway involves the *phnAB* operon, which encodes an anthranilate synthetase that produces anthranilate from chorismate.[3,46,47] The second pathway involves degradation of tryptophan to anthranilate via the kynurenine pathway using enzymes encoded by *kynA*, *kynB*, and *kynU*.[48] Recent evidence shows that the kynurenine pathway can provide anthranilate when cells are grown in rich medium, while the *phnAB* pathway is used when cells are grown in minimal medium.[48] In addition, the *trpEG* genes encode proteins that normally synthesize anthranilate for tryptophan biosynthesis but could potentially be used for 4Q biosynthesis.[49] As might be expected, the nutritional environment significantly affects 4Q production. Similar to growth in the presence of tryptophan, 4Q production is enhanced in the presence of the aromatic amino acids tyrosine (tyr) and phenylalanine (phe).[50] As chorismate is a precursor to both 4Qs and phe/tyr, it is likely that the presence of phe/tyr allows increased flux of chorismate to 4Q production. These data suggest that 4Q biosynthesis is highly linked to central metabolism and the nutritional environment (Figure 2).

4.2. 4Q Biosynthetic Enzymes

Multiple enzymes and regulatory proteins are involved in 4Q biosynthesis in *P. aeruginosa*. These proteins are encoded by the *pqsABCDE* operon (*pqs* operon),[3,46] *pqsH*,[46] *pqsL*,[7,51] and *pqsR*.[3,46,52] The functions of each are described below.

4.2.1. PqsA

PqsA was identified as an anthranilate−coenzyme A ligase.[46,53] This enzyme activates anthranilate to form anthraniloyl−coenzyme A in the first step of 4Q biosynthesis. A *pqsA* mutant does not produce HHQ, PQS, or other 4Qs[3].

4.2.2. PqsB, PqsC, and PqsD

PqsB, PqsC, and PqsD are classified as 3-oxoacyl-(acyl carrier protein) synthases.[46] Though their direct roles in 4Q

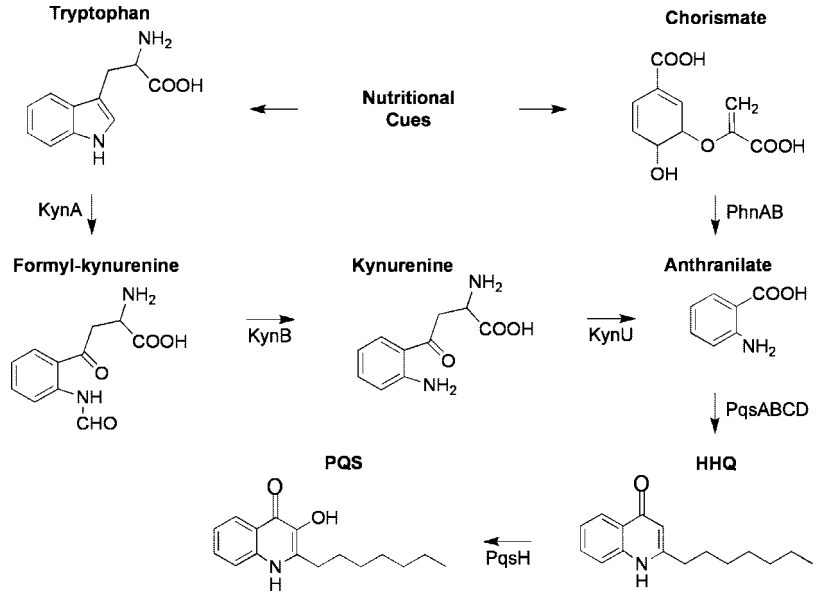

Figure 2. Biosynthetic pathway of HHQ, PQS, and other 4Qs. Nutritional cues determine which pathway is used for 4Q biosynthesis. Anthranilate is converted to HHQ and other 4Qs via PqsABCD. HHQ is converted to PQS by PqsH.

biosynthesis are unknown, these enzymes, along with PqsA, mediate the conversion of anthranilate to HHQ.[3,46]

4.2.3. PqsH

PqsH is involved in the final step of PQS biosynthesis. It is a putative flavin-dependent monooxygenase proposed to hydroxylate HHQ at the 3-position, forming PQS;[3,46] however, the enzymatic activity of PqsH has not been demonstrated.

4.2.4. PqsL

PqsL is a putative monooxygenase.[54] Though the enzymatic activity of PqsL has also not been characterized, it is involved in the synthesis of 4Q-*N*-oxides, as a *pqsL* mutant is deficient in their production.[7] In *P. aeruginosa* strain PAO1, mutation of *pqsL* leads to an overproduction of PQS.[51] This may be due to a blocked 4Q-*N*-oxide pathway that results in HHQ accumulation.[3,7] Interestingly, PQS and HHQ accumulation has been associated with autolysis in *P. aeruginosa*, although the phenotypes are strain specific.[51,55,56]

4.3. Regulation of 4Q Biosynthesis

4.3.1. PqsR

PqsR (also known as MvfR) is a LysR-type transcriptional regulator that directly activates the *pqsABCDE* operon.[46,52] PqsR binds the *pqsA* promoter in the absence of PQS, but binding is enhanced in the presence of PQS and HHQ, although PQS is ~100-fold more potent.[5,57] HHQ and PQS, therefore, act as coinducers to direct transcription of *pqsABCDE*. PqsR is required for full virulence, as a *pqsR* mutant does not produce 4Qs and consequently numerous virulence factors.[46,52]

4.3.2. PqsE

PqsE is a predicted cytoplasmic protein that possesses a metallo-β-lactamase fold.[58,59] This protein is not required for PQS biosynthesis[46] but is involved in the cellular response to PQS.[46,59,60] Farrow et al.[59] demonstrated that PqsE can act independently of 4Qs to upregulate pyocyanin and rhamnolipids.[59]

4.3.3. Las/Rhl

PQS is produced during logarithmic growth and reaches its maximum concentration during late stationary phase.[61] The LasI/R system positively regulates *pqsR* and *pqsH*,[57] and the RhlI/R system negatively regulates PQS production,[62] establishing a regulatory system in which the ratio of 3-oxo-C_{12}-HSL to C_4-HSL controls PQS signaling.[63]

5. 4Qs and Virulence

Several studies propose that 4Qs are important during infection. *pqsR* and *pqsE* mutants display reduced virulence factor production, including pyocyanin[46,52,60] and elastase.[52,60] Additionally, a *pqsE* mutant produces less rhamnolipid[60] and lectin.[60] A *pqsR* mutant is reduced for virulence in nematodes,[46] plants,[52] and mice.[52,64] Mutations in other 4Q genes, *pqsC*, *pqsD*, *pqsE*, and *pqsH*, result in reduced nematode killing.[46] In a burn-wound mouse model, *pqsA*[5,65] and *pqsE*[65] mutants were reduced for killing compared to wild-type, but a *pqsH* mutant was not.[5] This suggests that 4Qs are important for virulence; however, since a *pqsH* mutant is reduced for nematode killing[46] but not for pathogenesis in a burn-wound model,[5] there is some debate about the importance of PQS for virulence.

P. aeruginosa is a common cause of infection in immunocompromised patients (see refs 66 and 67 for reviews). In particular, *P. aeruginosa* causes chronic respiratory infections in individuals with the heritable disease cystic fibrosis (CF).[66,67] *P. aeruginosa* CF clinical isolates produce 4Qs, including HHQ,[11] HQNO,[11] and PQS,[68,69] and 4Qs are important for growth as a biofilm, which is the proposed mode of growth in the CF lung.[70] In *P. aeruginosa*, DNA is an important component of the biofilm extracellular polymeric substance (EPS).[71] Biofilms formed by a 4Q-deficient *pqsA* mutant are thin, flat, and more detergent-sensitive compared to wild-type,[72] likely due to decreased levels of extracellular DNA.[72] In contrast, *pqsL* mutant cultures produce more extracellular DNA.[72] Reduced and increased extracellular DNA was also shown for *pqsA* and *pqsL* mutants, respectively, when grown in media supplemented with 5 uM iron.[73] A final consideration that 4Qs may be

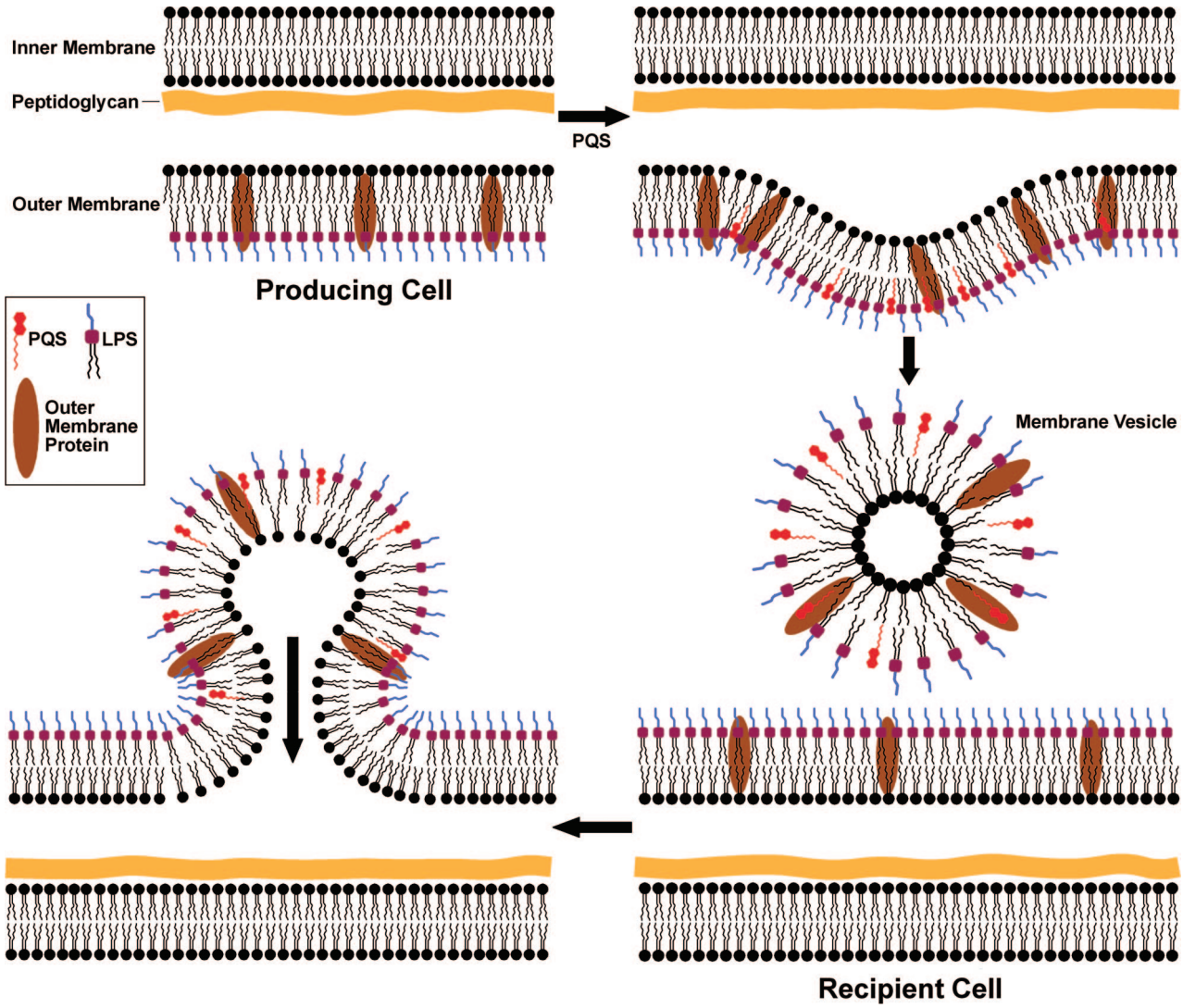

Figure 3. Membrane vesicle formation and fusion to recipient cells. Clockwise from left: Depiction of a Gram-negative cell envelope. PQS interacts with LPS, which leads to asymmetric growth of the outer leaflet of the outer membrane. Asymmetric growth leads to vesicle formation. It is unknown how membrane vesicles fuse to recipient cells.

important in the CF lung is that media mimicking the nutritional environment of the CF lung has been shown to enhance *P. aeruginosa* 4Q production.[50,74]

6. There's an App for That: Other Biological Roles of 4Qs

The 4Qs are a unique group of molecules because they display a multitude of functions. Here, we discuss some of these functions and potential new roles for 4Qs.

6.1. 4Qs and Membrane Vesicle Trafficking

Unlike many QS signals, PQS is only slightly soluble in aqueous solutions, posing an interesting question as to how it is trafficked between cells. Calfee et al.[75] demonstrated that PQS is solubilized by rhamnolipids in vitro, which are QS-regulated biosurfactants produced by *P. aeruginosa*.[75] Later, it was demonstrated by our laboratory that PQS is packaged and potentially trafficked by outer-membrane vesicles[76] (MVs). MVs are small, spherical, bilayered structures that "pinch off" from the Gram-negative outer membrane.[77,78] *P. aeruginosa* MVs contain ~90% of the PQS produced, and other 4Qs, including HHQ and HQNO, are also found associated with MVs.[76] In addition to their role in 4Q trafficking, MVs contain bacterial toxins, DNA, and proteins, suggesting that these delivery vehicles are involved in other processes besides signaling[77−79] (reviewed in refs 80−82). Packaging cargo into MVs may allow trafficking of vesicle contents, including 4Qs, to recipient cells and potentially allow concentration or sequestration of vesicular contents[82] (Figure 3).

Interestingly, PQS is required for MV production in *P. aeruginosa*, independent of its signaling function.[76] A mutant strain deficient in PQS production is reduced for MV formation,[76,83] and exogenous addition of PQS restores MV formation.[76] Recently, experiments using biophysical techniques showed that PQS interacts with lipopolysaccharide (LPS), the major lipid component of the outer leaflet of the Gram-negative outer membrane. The hydroxyl group at the 3-position of PQS is important for interactions with the 4′-phosphate of the lipid A anchor of LPS[84] (Figure 3). The alkyl chain at the 2-position of PQS also interacts with the lipid A acyl chains, resulting in a less fluid membrane.[84] These studies suggest a model whereby PQS interacts with LPS, leading to asymmetric growth of the outer leaflet of

the outer membrane and thus budding of the outer membrane away from the cell as MVs.[84]

MVs isolated from *P. aeruginosa* incorporate into the outer membrane of other cells,[79] but very little is known about the mechanism of MV fusion. In addition, it is not known whether MV fusion is required to transduce PQS signaling, what factors are involved in fusion, or furthermore, how PQS is transported into target cells to bind PqsR and initiate signaling.

6.2. Antibacterial Properties of 4Qs

Although remarkable signals, 4Q compounds were initially described indirectly in the late 1880s when mixed infection studies with *P. aeruginosa* and *Bacillus anthracis* showed that *P. aeruginosa* secretes agents that kill other bacteria (see references in refs 16 and 39). These molecules were later described in the 1940s and the 1950s as "Pyo" compounds.[39−41,85] In 2004, Deziel et al.[3] showed that extracts of *pqsA* and *pqsR* mutants, which are deficient for 4Q biosynthesis, display little or no antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis,* while wild-type extracts clearly inhibit both species.[3] The authors attributed this antibiotic activity to HQNO.[3] In addition, purified outer-membrane vesicles isolated from *P. aeruginosa* were shown to be biologically active against *S. epidermidis*,[76] implicating MVs as 4Q (potentially HQNO) delivery vehicles.

While naturally produced 4Qs are studied for their roles in QS and microbial competition, synthetic 4Q antibiotics are important for treating infectious diseases (Figure 1). Unlike the natural 4Q antibiotic HQNO, which targets cellular respiration,[10,11] the synthetic quinolones target the essential DNA replication enzymes topoisomerase II in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria (reviewed in ref 86).[87] When the first synthetic quinolone, nalidixic acid, was characterized in 1962,[88] this new line of drugs was only used to treat urinary tract infections caused by Gram-negative bacteria (reviewed in refs 12 and 89).[88] However, the addition of a piperazine at the 7-position and fluorine at the 6-position greatly enhanced antibacterial activity against Gram-positive organisms.[12,89] Today, synthetic quinolones like ciprofloxacin and levofloxacin are widely used because they are well-tolerated by patients and they possess potent, broad-spectrum activity.[12,89]

6.3. Quinolone Antibiotics As Signaling Molecules?

Despite its role as an antimicrobial, in nonlytic doses HQNO has been shown to trigger heritable changes in *S. aureus*,[90] including formation of small colony variants.[90] Small colony variants are more resistant to the aminoglycoside antibiotic tobramycin[90] and have been implicated as important mediators of *S. aureus* persistence in polymicrobial infections. Thus, HQNO not only serves as a potent antimicrobial for *P. aeruginosa* but also can elicit strong responses from neighboring microbes at subinhibitory levels.

Recently, it has been hypothesized that many antibiotics may not reach inhibitory concentrations in the environment and may therefore act more like signals or cues, effectively altering gene expression of a microbial population.[91,92] Studies have shown that synthetic quinolone antibiotics affect gene expression at subinhibitory levels.[17,18] For example, 5% of *P. aeruginosa* genes are differentially expressed in response to subinhibitory concentrations of ciprofloxacin,[18] including the expression of QS genes and QS-regulated virulence factors.[17] Most of the changes are outside the expected response to treatment with quinolone antibiotics,[17,18] such as the DNA damage response,[93] implying effects beyond those associated with the antibiotic mechanism of action.

6.4. Role of PQS in Redox Homeostasis

Besides serving as a QS signal, PQS has been shown to act as a pro-oxidant, sensitizing cells to killing by hydrogen cyanide and antibiotics.[94] Interestingly, it can also act as an antioxidant, lowering the intracellular concentration of reactive oxygen species.[94] It is proposed that the antioxidant activity of PQS increases resistance to ultraviolet radiation by fragmenting DNA and activating an important stress response.[94] The antioxidant and pro-oxidant activities of PQS may enable a "survival of the fittest" strategy for coping with environmental stresses.[94] PQS seemingly has beneficial (antioxidant) and deleterious (pro-oxidant) effects, thus shaping the community structure and its response to the environment.[94]

PQS also influences redox homeostasis through regulation of phenazines. Phenazines are redox-active, pigmented, heterocyclic compounds (as reviewed in ref 95). Perhaps the best-known phenazine in *P. aeruginosa* is pyocyanin, which is responsible for producing the characteristic blue color displayed by this bacterium in culture.[95] Similar to PQS, phenazines are multifunctional. They were originally studied for their antibiotic properties, but it is now appreciated that phenazines participate in a variety of biological activities,[95,96] including QS signaling and redox homeostasis.[97] At low oxidant concentrations, phenazines may act as electron acceptors to allow NAD^+ regeneration, which is essential for central metabolism.[95,97] The reduced phenazines may be shuttled out of the cell, where they donate electrons to an external acceptor, and the oxidized phenazine can reenter the cell to capture more electrons.[97] The redox activity of phenazines may be particularly important in multicellular biofilms where oxygen and oxidants can become limited.[95,97]

7. Metabolism and Degradation of 4Qs

Microorganisms have the ability to catabolize quinolones/quinolines.[98−100] Bacteria isolated from multiple environments use the synthetic quinolone antibiotics ciprofloxacin, levofloxacin, and nalidixic acid as sole carbon and energy sources.[101] Some species can also catabolize the common fluoroquinolone and soil pollutant danofloxacin.[102] In addition, there is some mechanistic evidence suggesting that bacteria could use ring cleavage to degrade HHQ, PQS, or other naturally produced 4Qs (ref 103 reviews ring cleavage via oxygenases).[104,105] *Arthrobacter ilicis* strain Rü61a and *Pseudomonas putida* strain 33/1 each produce a dioxygenase that degrades 1*H*-3-hydroxy-4-oxoquinaldine and 1*H*-3-hydroxy-4-oxoquinoline, respectively.[103−105] These substrates, which resemble HHQ and PQS, are converted to *N*-acetylanthranilic acid or *N*-formylanthranilic acid by the incorporation of two oxygen atoms at C2 and C4 of the N-heteroaromatic ring.[103−105] Interestingly, the two products are degraded via anthranilate, which is a substrate for 4Q biosynthesis in *P. aeruginosa*.[3,27,40] Could organisms capable of degrading synthetic quinolones also degrade PQS? A recent report by Pustelny et al.[106] demonstrated that Hod, a dioxygenase from *Arthrobacter nitroguajacolicus* strain

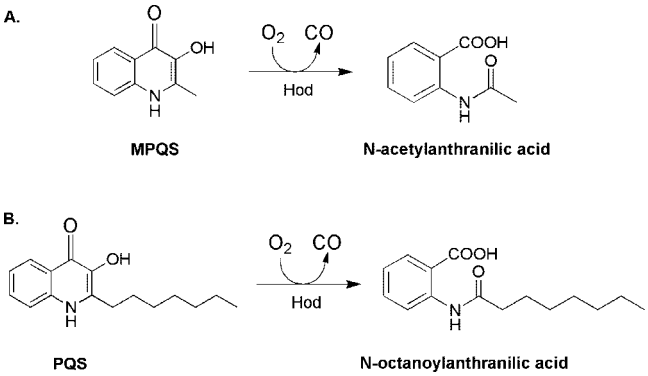

Figure 4. PQS can be degraded by Hod, a dioxygenase from *Arthrobacter nitroguajacolicus* strain Rü61a. (A) The natural substrate of Hod is 3-hydroxy-2-methyl-4(1*H*)-quinolone (MPQS), which is converted to *N*-acetylanthranilic acid. (B) Hod converts PQS to *N*-octanoylanthranilic acid.

Rü61a, can convert PQS to *N*-octanoylanthranilic acid and carbon monoxide[106] (Figure 4). This reaction is not specific to PQS, as Hod also has activity toward 2-ethyl-, 2-propyl-, and 2-pentyl-4(1*H*)-quinolone. The authors showed that the addition of exogenous Hod to *P. aeruginosa* cultures could reduce expression of *pqsA* and PQS-controlled virulence factors, and virulence was reduced in a plant model of infection.[106] These results are encouraging for the development of new therapeutics that target quinolone signaling.

8. What Will They Think of Next? Conclusions on 4Qs Quorum Sensing

While much has been learned regarding the regulation and biosynthesis of PQS and other 4Qs produced by *P. aeruginosa*, we are only beginning to understand the biological functions of these molecules. Many unanswered questions remain in 4Q signaling: what are the functions of the remaining proteins in the 4Q biosynthetic pathway; how is PQS imported into recipient cells; and how widespread are 4Q QS systems? Finally, what are the roles of the nearly 50 other 4Qs produced by *P. aeruginosa*? Studies addressing these questions may reveal novel functions for 4Qs.

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