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Minireview

MINIREVIEW – Pathogens & Pathogenicity

The expanding horizon of alkyl quinolone signalling and communication in polycellular interactomes

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One sentence summary: Deciphering the communication networks that underpin cellular interactions within polymicrobial infections in disease provides a platform for innovative therapeutic development.
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ABSTRACT

Population dynamics within natural ecosystems is underpinned by microbial diversity and the heterogeneity of host–microbe and microbe–microbe interactions. Small molecule signals that intersperse between species have been shown to govern many virulence-related processes in established and emerging pathogens. Understanding the capacity of microbes to decode diverse languages and adapt to the presence of 'non-self' cells will provide an important new direction to the understanding of the 'polycellular' interactome. Alkyl quinolones (AQs) have been described in the ESKAPE pathogen *Pseudomonas aeruginosa*, the primary agent associated with mortality in patients with cystic fibrosis and the third most prevalent nosocomial pathogen worldwide. The role of these molecules in governing the physiology and virulence of *P. aeruginosa* and other pathogens has received considerable attention, while a role in interspecies and interkingdom communication has recently emerged. Herein we discuss recent advances in our understanding of AQ signalling and communication in the context of microbe–microbe and microbe–host interactions. The integrated knowledge from these systems-based investigations will facilitate the development of new therapeutics based on the AQ framework that serves to disarm the pathogenesis of *P. aeruginosa* and competing pathogens.

Keywords: *Pseudomonas aeruginosa*; *Pseudomonas* quinolone signal (PQS); alkylquinolones; anti-infectives; interspecies signalling; quorum sensing

BACKGROUND

Prokaryotes and eukaryotes have coexisted and coevolved for millions of years. Though higher order eukaryotes have evolved an array of communicative mechanisms, small molecule signalling remains an integral element in the microbe–microbe and microbe–host interactomes. Deciphering these molecular interactions, their spatio-temporal dynamics and the hierarchical structure with which they modulate community behaviour remains a major challenge. One of the best studied forms of bacterial small molecular communication is termed quorum sensing (QS, Fuqua, Winans and Greenberg 1994). Initially described as a process characterised by auto-inducing small molecules that govern cellular behaviour in response to a particular cell density or quorum, our understanding of QS signalling has expanded rapidly in recent years (Whiteley, Diggle and Greenberg 2017). In

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describing molecular interactions that govern microbial cell–cell communication, we are required to carefully assign behaviours and distinguish between signalling, coercion and cues (Diggle et al. 2007a). This is particularly true where QS-based interactions are not restricted to bacterial cell–cell communication, but are also involved in communication with higher order eukaryotes and mammalian cells.

Alkyl quinolones (AQs) are a species-specific class of QS molecule that have been described in *Pseudomonas aeruginosa* (Pesci *et al*. 1999; McGrath, Wade and Pesci 2004), and related bacteria including *P. putida* and *Burkholderia* spp. (Diggle *et al*. 2006). More than 55 distinct AQs are produced through the PqsABCDE biosynthetic pathway in *P. aeruginosa*, with the majority of the diversity arising from unsaturation, different alkyl chain lengths and modification of the ring-substituted nitrogen (Deziel *et al*. 2004; Dulcey *et al*. 2013). An insight into the evolutionary basis of AQ diversity has emerged from *Burkholderia thailandensisis* where two AQ analogues (HQNO and a methylated HMNQ) were shown to act synergistically to inhibit bacterial growth (Wu and Seyedsayamdost 2017). AQs exhibit a broad spectrum of functions including cell–cell signalling, redox activity, iron chelation and antimicrobial activity (Deziel *et al*. 2004; Bredenbruch *et al*. 2006; Diggle *et al*. 2007b). The two AQ QS signal molecules have been identified as 2-heptyl-3-hydroxy-4(1H)-quinolone (also known as the *Pseudomonas* quinolone signal, PQS) and its biological precursor 2-heptyl-4(1H)-quinolone (HHQ). PQS is generated from HHQ through the action of the distantly encoded PqsH monooxygenase (Deziel *et al*. 2004). The role of other AQs such as HQNO and DHQ in *P. aeruginosa* physiology and pathogenesis is unclear. In this minireview, we summarise recent advances in understanding the regulation and mode of action of AQs. We discuss new knowledge that has changed perspectives on the interactivity of AQs and discuss innovative initiatives in the design of potent novel anti-infective therapies.

MULTISYSTEM CONTROL OF AQ SIGNALLING

Control of AQ biosynthesis is controlled at the transcriptional level directly through the action of PqsR (referred to as MvfR in the PA14 strain). PqsR is a member of the LysR-type transcriptional regulator (LTTR), of which *P. aeruginosa* encodes ~125 individual proteins, with PqsR being amongst the most evolutionarily constrained (Reen *et al*. 2013). The activity of PqsR is controlled via autoinduction by HHQ and PQS, while the *pqsR* promoter itself has been shown to fall under the control of two separate promoter sites: distal and proximal (Farrow and Pesci 2017). LasR-mediated activation has been shown to occur at a distal promoter site, which can be antagonised by the activation of another LTTR, CysB (Farrow *et al*. 2015). The proximal promoter site also contributes to activation of PqsR, with initiation at this site inhibited by a negative regulatory sequence element, and potentially by the H-NS family members MvaT and MvaU (Farrow and Pesci 2017). The authors propose that this arrangement could allow for dual information processing from both environmental signals and cell–cell communication. Small RNAs (sRNAs), such as PhrS, PrrF and ReaL, are also known to fine tune AQ production in response to environmental signals. PqsR activation is influenced by PhrS, which responds to oxygen levels (Sonnleitner *et al*. 2011). Iron homeostasis in *P. aeruginosa* is maintained in part by the PrrF sRNAs which were recently shown to promote the production of PQS through repression of another LTTR protein AntR, an activator of genes involved in degradation of the AQ precursor anthranilate (Reinhart *et al*. 2015, 2017). Yet another sRNA, ReaL, links Las and PQS signalling through post-transcriptional regulation of PqsC (Carloni *et al*. 2017).

Complex regulation also extends to other elements of the AQ signalling system (Fig. 1). RhlR was found to bind an alternative transcriptional start site to PqsR, resulting in the formation of secondary structure in the 5′ untranslated region of the *pqsA* promoter (Brouwer *et al*. 2014). A novel AraC regulator CdpR (*PA2588*) was found to regulate *pqsH* in addition to itself through interaction with the ClpAS-P system (Zhao *et al*. 2016). A host of other regulatory elements have been shown to influence AQ production. These include RpoN (mediating carbapenem tolerance through PQS and PqsE; Viducic *et al*. 2017), DesB (controlling AQ synthesis through modulation of the MexEF-OprN efflux pump; Kim, Yoon and Choi 2015), the alarmone signal (p)ppGpp (significantly modulating the AHL and PQS QS hierarchy; Schafhauser *et al*. 2014) and QapR (modulating PQS production through the *qapR* operon and *PA5507;* Tipton, Coleman and Pesci 2015). Host factors such as serum, antimicrobial peptides, dynorphin and bile have also been shown to promote PQS production (Zaborina *et al*. 2007; Cummins *et al*. 2009; Reen et al. 2012b, 2016a; Strempel *et al*. 2013; Kruczek *et al*. 2014) as has cigarette and e-cigarette smoke (Gallagher *et al*. 2017). In contrast, the C-natriuretic peptide hormone suppressed PQS levels (Blier *et al*. 2011).

Analysis of global transcriptomics has provided some further insights into the regulation of AQ signalling. PqsE emerged as a key factor involved in the regulation of a repertoire of diverse genes encoding factors involved in biofilm formation and virulence, while there was further evidence for the link between PQS and iron (Rampioni *et al*. 2016). HQNO did not influence transcription, providing further evidence that perhaps it is not a true QS signal molecule (Rampioni *et al*. 2016). Although the function of PqsR has been suggested to be restricted to the *pqsA* promoter, it has recently been suggested that multiple promoters may interact with the AQ master regulator (Maura *et al*. 2016). It is therefore likely that the complexity of the regulation of AQ production in response to cell–cell communication and environmental factors is only beginning to be understood.

AQ CHEMICAL MESSAGING IN THE POLYCELLULAR INTERACTOME

AQ signalling in nosocomial pathogens

AQs are known to control virulence and pathogenesis in *P. aeruginosa* both dependent and independent of the central regulator PqsR. In all, an estimated 12% of the genes present in the *P. aeruginosa* genome are known to fall under the control of the hierarchical QS network (Schuster *et al*. 2003; Deziel *et al*. 2005). The mechanism through which AQs achieve such an exquisite level of control within multicellular aggregates requires intercellular communication. While HHQ is reportedly able to diffuse into the extracellular environment or can be exported through the MexEF-OprN efflux pump (Lamarche and Deziel 2011), export of PQS has been shown to be intrinsically linked to strain-dependent outer membrane vesicle (OMV) formation (Florez *et al*. 2017). Several groups have reported a role for PQS in OMV formation in *P. aeruginosa* and other pathogens (Mashburn-Warren *et al*. 2008; Tashiro *et al*. 2010; Lin *et al*. 2017). Schertzer and co-workers proposed the bilayer-couple model for OMV biogenesis, where PQS intercalates into the outer membrane causing expansion of the outer leaflet and consequently the induction of curvature in a strain-dependent manner (Schertzer

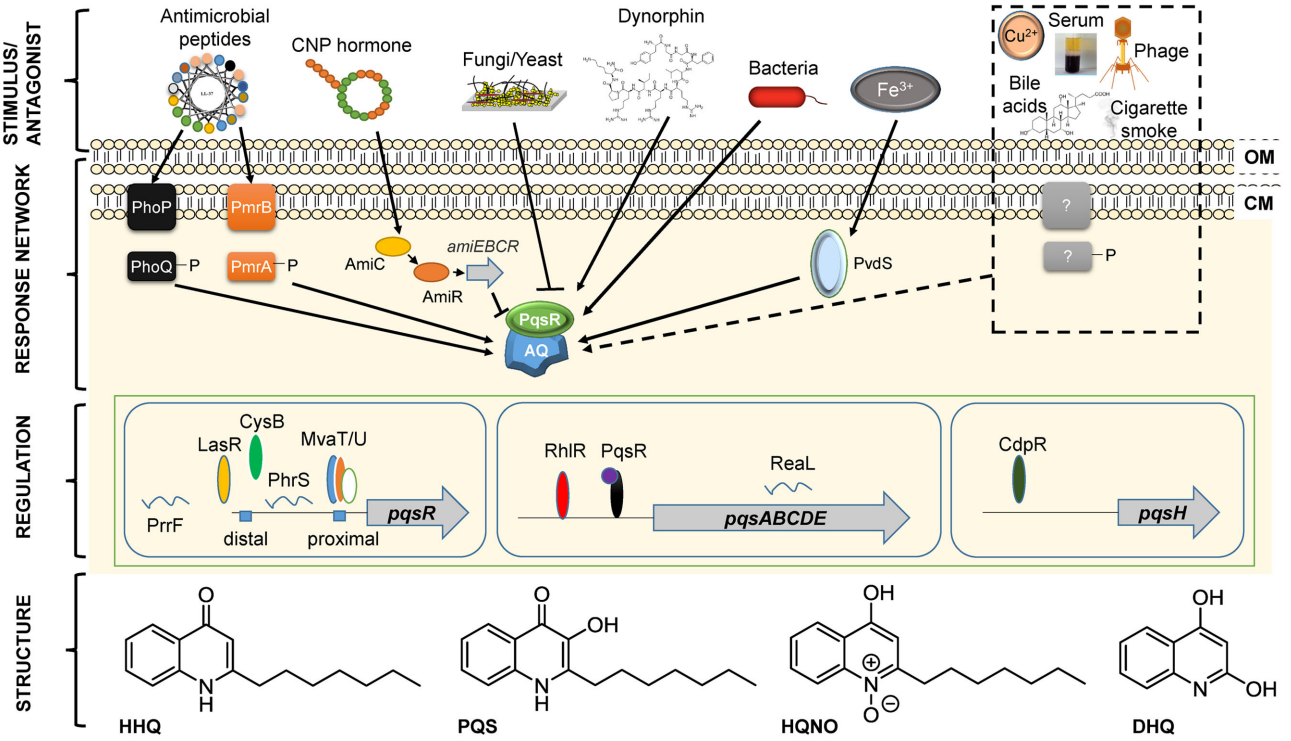

Figure 1. Overview of environmental and host modulators of AQ production in *P. aeruginosa*. The growing complexity of transcriptional and post-transcriptional regulation at the *pqsA-E*, *pqsR* and *pqsH* promoters governs the levels of AQs produced, with HHQ, PQS, HQNO and DHQ being the best studied to date. Dashed arrows indicate stimuli for which the molecular mechanism of AQ activation remains to be elucidated.

and Whiteley, 2012; Florez *et al.* 2017). However, a number of other studies have reported that PQS is not an absolute requirement for MV production in planktonic cultures (Macdonald and Kuehn, 2013; Turnbull *et al.* 2016), particularly under anaerobic conditions (Toyofuku *et al.* 2014).

Much of the attention on AQ signalling in *P. aeruginosa* has focused on the interaction of HHQ and PQS with PqsR (reviewed recently by Sams *et al.* 2016). Interplay between PQS and the QS regulator VqsR has also been reported and shown to mediate carbapenem tolerance through the action of the sigma factor RpoS (Viducic *et al.* 2017). More recently, immobilised PQS probes were used to identify new interacting proteins, with MexG and MgtA being implicated as binding partners of PQS (Hodgkinson *et al.* 2016). In a subsequent study using photoaffinity probes, additional PQS binding partners were identified, including RhlR, PqsD, WbpB and FtsZ (Baker *et al.* 2017). The latter two proteins were also found to interact with HHQ. These studies add a further layer of complexity to the AQ signalling system, and how these new signal–protein interactions impact on the classical models of QS regulation in *P. aeruginosa* remain to be seen.

The role of other AQs in modulating *P. aeruginosa* behaviour has been less extensively studied. The function of HQNO in *P. aeruginosa* cellular physiology remains to be elucidated, as does the mechanism by which self-poisoning is avoided (Rampioni *et al.* 2016). Rather than simply protecting itself from the antirespiratory activity of HQNO, it is possible that subpopulations of *P. aeruginosa* may be targeted in a similar manner to the stochastic effects of PQS that have been described (Haussler and Becker 2008). Hazan *et al.* (2016) have described how autopoisoning of the respiratory chain by HQNO can promote biofilm formation and antibiotic tolerance. It is possible that HQNO may provide *P. aeruginosa* with an evolutionary mechanism to sacrifice the few for the greater benefit of the population.

Unlike PQS, DHQ production does not require oxygen, and therefore its synthesis in low-oxygen environments such as the lungs of patients with cystic fibrosis (CF) would be of particular interest. Lepine *et al.* (2007) reported that DHQ did not influence *pqsA-lacZ* activity in *P. aeruginosa*, while exogenous DHQ had no effect on the production of AQs or the blue phenazine pyocyanin. More recently, Gruber *et al.* (2016) have shown that DHQ binds to PqsR, activating transcription of the *pqs* operon, and influencing pyocyanin production in *P. aeruginosa*. It is worth noting that 100 μM DHQ was required to elicit a 60% increase in *pqsA* expression relative to carrier control in an *Escherichia coli* reporter strain, compared with 110% activation by 1 μM PQS (Gruber *et al.* 2016). The apparent disparity between these studies may be attributed to dose-dependent effects.

AQs as modulators of interspecies behavior

Apart from controlling cell–cell communication within the growing population of *P. aeruginosa* cells, AQs are also proposed to play a prominent role in facilitating the emergence of *P. aeruginosa* within complex microbial communities. PQS and HHQ were found to modulate the virulence behaviour of a range of bacterial pathogens. Swarming motility was affected in response to PQS, while HHQ suppressed biofilm formation in the gram-positive pathogen *Bacillus atropheaus* (Reen *et al.* 2011). HHQ was also shown to be selectively bacteriostatic to several gram-negative species including *Vibrio* spp., although *V. parahaemolyticus* was unaffected. Structure activity relationship (SAR) analysis revealed the C-3 position of HHQ to be important in the antibiofilm activity of this compound (McGlacken *et al.* 2010; Reen et al. 2012a, 2015). Although neither PQS nor HHQ had any effect on the growth of *B. cenocepacia*, the antagonistic activity of *P. aeruginosa* supernatants against this important pathogen

was shown to be dependent on an intact AQ signalling system (Costello *et al.* 2014). Fernandez-Pinar *et al.* (2011) also reported an interspecies dimension to AQ signal molecules with biofilm formation, swarming and iron uptake affected in *P. putida*, while Inaba *et al.* (2015) reported that PQS could inhibit biofilm formation in *Streptococcus mutans*. Furthermore, Toyofuku *et al.* (2010) showed that PQS could affect growth of gram-negative and gram-positive bacteria, owing at least in part to its iron-trap activity. Indeed, the ability of PQS to chelate or trap iron is a central factor in its biological activity, being also implicated in the 'red-death' killing of *Caenorhabditis elegans* (Zaborin *et al.* 2009).

The dominance of *P. aeruginosa* within polymicrobial communities more than likely depends on the action of several AQs. The antimicrobial activity of *P. aeruginosa* against *Staphylococcus aureus* was shown to be enhanced by iron depletion and was dependent on multiple AQ metabolites (Filkins *et al.* 2015; Nguyen *et al.* 2015, 2016). *Pseudomonas aeruginosa* can scavenge iron through AQ-dependent lysis of *S. aureus* (Mashburn *et al.* 2005), with AQ production itself stimulated by resulting peptidoglycan released from *S. aureus* (Korgaonkar *et al.* 2013). *Staphylococcus aureus* can mount its own challenge to *P. aeruginosa* competition through suppression of virulence, antibiotic tolerance and growth, depending on the functionality of the strains QS signalling system (Korgaonkar *et al.* 2013; Frydenlund Michelsen *et al.* 2016). Furthermore, protocooperative interactions between *P. aeruginosa* and *S. aureus* have also been reported (Frydenlund Michelsen *et al.* 2016), perhaps a reflection of the adaptive heterogeneity reported among clinical isolates (Markussen *et al.* 2014; Winstanley *et al.* 2016). Rather than the antagonistic interactions described above, the DK2-P2M24-2003 lung adapted isolate altered its AQ production and protected *S. aureus* from the killing effect of tobramycin. The ability of DK2-P2M24-2003 and *S. aureus* to co-exist could be due to the fact that no pyocyanin, rhamnolipids or HQNO was detected in the former, in contrast to the model strains used in most studies (Frydenlund Michelsen *et al.* 2016). However, Orazi and O'Toole (2017) have subsequently shown that *P. aeruginosa* supernatants protect *S. aureus* from vancomycin challenge, this time implicating HQNO as the active component. Another study using clinical isolates from patients with CF implicated both PQS and HQNO in the stimulation of *S. aureus* biofilm formation, although this effect was lost in co-existing isolates (Fugere *et al.* 2014). Together, these studies suggest that specific adaptations occur within microbial communities, further emphasising the necessity to interrogate interactions in multiple clinical isolates before the dynamics of a particular interaction can be established. They also serve to highlight the impact that interspecies interactions can have on antibiotic efficacy, of critical importance in light of the pending 'perfect storm' of antibiotic resistance and the insufficient antibiotic development pipeline (Cooper and Shlaes 2011).

PQS has also recently been shown to promote the evolution of resistance to parasitic bacteriophages (Moreau, Diggle and Friman 2017). Addition of exogenous PQS improved the growth of non-signalling bacteria in the presence of phage. It follows that the loss of QS within a population could have a previously unforeseen fitness cost whereby cells would be more prone to phage attack. Production of PQS could itself be driven by phage attack, as seen when *P. aeruginosa* was challenged with YuA, 14-1 and LUZ24 phage (De Smet *et al.* 2016). Therefore, *P. aeruginosa* appears to utilise the AQ system as a primed attack response, much in the same way it does when faced with antibiotic challenge or microbial competition.

Evidence for the importance of AQ chemical messages in microbial communities has also come from the observation that co-colonising microbes have developed mechanisms to disarm the threat of AQ signalling and its associated secondary metabolite production (Fig. 2). Pustelny *et al.* (2009) described the action of an *Arthrobacter* dioxygenase HodC against PQS. *Mycobacterium abscessus* and *Rhodococcus erythropolis* were subsequently shown to degrade HHQ and PQS, potentially through the action of a novel *aqd* gene cluster (Muller *et al.* 2015; Birmes *et al.* 2017), as could *Achromobacter xylosoxidans* (Soh *et al.* 2015). *Arthrobacter*, *Rhodococcus* and *S. aureus* were all shown to modify HQNO by incorporation of a hydroxyl group at the C-3 position (Thierbach *et al.* 2017). While quenching may be an attractive antivirulence strategy, there are limitations. PQS quenching by HodC has been shown to increase *P. aeruginosa* biofilm formation as a result of increased iron availability (Tettmann *et al.* 2016), a factor that may also influence the pathogenesis of competing organisms within the polymicrobial community.

An interkingdom and host–microbe dimension to AQ-based communication

The interspecies dimension to AQ communication suggested a central role in the competitiveness of *P. aeruginosa* within the complex polycellular ecosystem that is the human host (Fig. 2). To be truly effective, AQs would require the ability to govern cellular behaviour at the interkingdom level, and studies in *Candida albicans* provided evidence that this may indeed be the case (Cugini *et al.* 2007; McAlester, O'Gara and Morrissey 2008; Cugini, Morales and Hogan 2010). Cugini *et al.* (2007) reported that farnesol, a sesquiterpene compound secreted by *C. albicans*, could suppress AQ signalling in *P. aeruginosa* by binding to PqsR resulting in a non-optimal complex. A follow on study by the same group showed that farnesol could enhance PQS production and restore pyocyanin in a *lasR* mutant, this time through RhlR-dependent stimulation of *pqsH* transcription (Cugini, Morales and Hogan 2010). HHQ was subsequently shown to antagonise *C. albicans* biofilm formation, while PQS elicited a marginal increase (Reen *et al.* 2011). More recently, both HHQ and PQS were shown to suppress biofilm formation in clinical isolates of the respiratory fungal pathogen *Aspergillus fumigatus* (Reen et al. 2016c).

AQ molecules have also been shown to play an important role in the host—microbe interaction, particularly in disrupting immune homeostasis in host cells. PQS was first shown to modulate cell proliferation, the production of interleukin-2 (IL-2) and TNFα in mitogen-stimulated human peripheral blood mononuclear cells (Hooi *et al.* 2004). PQS was later shown to inhibit the production of IL-12 by LPS-stimulated bone marrow-derived dendritic cells (Skindersoe *et al.* 2009). Kim *et al.* (2010) provided some insight into the transcriptional changes elicited by AQs revealing that both HHQ and PQS downregulated the host immune response through inhibition of NF-κB. At the same time, PQS was shown to destabilise the hypoxia inducible factor-1α (HIF-1α) subunit of the HIF-1 which is known to govern the hypoxic response in host cells, in addition to being a key regulatory element of the immune response (Legendre *et al.* 2012). Secretion of PQS may therefore facilitate evasion of host defences and prevent the HIF-1 dependent resolution of acute inflammation (Campbell *et al.* 2014). Mutation of *pqsA* led to a reduction in, but not loss of, HIF-1α destabilisation activity, thus implicating other but as yet uncharacterised AQs in this interaction. (Legendre *et al.* 2012). Loss of *pqsA-E* resulted in attenuation of inflammation, tissue destruction and reduced levels of proinflammatory cytokines at the site of infection in mice (Bala, Chhibber

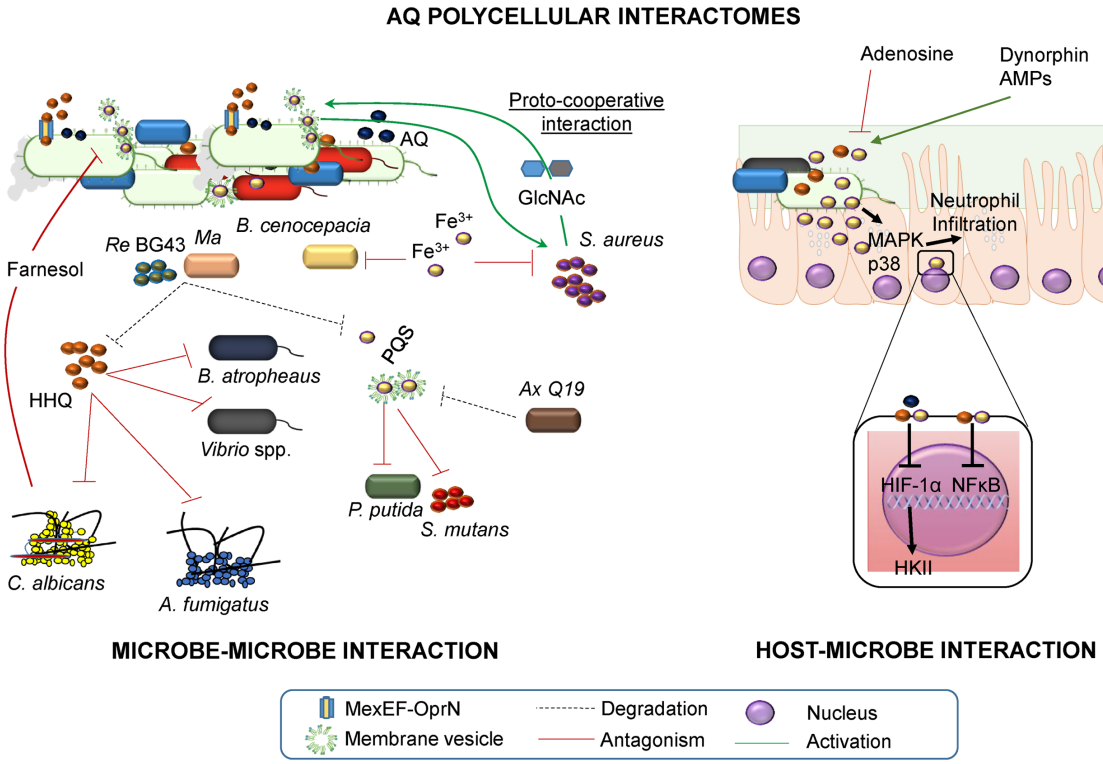

Figure 2. AQ signalling in *P. aeruginosa* interfaces between microbe–microbe and microbial–host interactions. Competition or co-operation between co-colonising pathogens can shape the dynamics of host microbiota, while communication with host cells can subvert the immune response.

and Harjai 2014). Neutrophil infiltration was influenced by PQS via the MAPK and p38 signalling pathways in a concentration-dependent manner (Hansch *et al.* 2014). More recently, PQS was shown to induce oxidative stress and inhibit haeme oxygenase-1 expression (Abdalla *et al.* 2017). The induction of ROS is not a new phenomenon for PQS, with both anti-oxidant and pro-oxidant activities reported in *P. aeruginosa* (Haussler and Becker 2008). Taken together, these studies all support a role for AQ signals in the host environment following acquisition of *P. aeruginosa*.

Understanding the role of AQs in the host—microbe interaction will require us to monitor the production of AQ profiles in clinically relevant samples. Though it is clear from *in vitro* studies that AQs can significantly modulate critical pathways in host cells, the presence of PQS, HHQ and other AQs in clinical samples is yet to be established. While some studies have reported PQS positivity in biological samples (Collier *et al.* 2002; Guina *et al.* 2003; Barr *et al.* 2015), others (including ourselves) have not been successful, even in samples from patients that were culture positive for *P. aeruginosa* (Buzid *et al.* 2016; Quinn *et al.* 2016; Buzid *et al.* 2017). Advances in LC-MS/MS (Turnpenny *et al.* 2017), *lux*-based biosensors (Fletcher *et al.* 2018) and electrovoltammetric-based detection (Zhou *et al.* 2011; Seviour *et al.* 2015; Buzid *et al.* 2016; Buzid *et al.* 2017) will provide more effective technologies for monitoring small molecule signalling both *in vitro* and *in situ*. Rapid and early detection of *P. aeruginosa* would also enhance the clinical management of these infections.

INTEGRATING NEW KNOWLEDGE FOR AQ-BASED THERAPEUTICS

QS has traditionally been viewed as being dispensable for survival in most natural ecosystems. Therefore, it follows that targeting QS would be an attractive anti-infective strategy, being neutral with regard to the selective pressure for the emergence of resistance. This view has been questioned somewhat by recent reports that describe resistance mechanisms against QS inhibitors (Defoirdt, Brackman and Coenye 2013, García-Contreras, Maeda and Wood 2016), while intraspecies heterogeneity (Markussen *et al.* 2014; Winstanley *et al.* 2016) and adaptive co-evolution (Frydenlund Michelsen *et al.* 2016) also have implications for the effectiveness of anti-infective therapeutics (Whiteley, Diggle and Greenberg 2017). Nonetheless, inactivation of AQ signalling in microbial pathogens remains an attractive strategy that continues to receive considerable attention (Fig. 3).

Several enzymes in the PQS biosynthetic pathway have been the target of inhibitor development studies. The fact that PqsA is required for the synthesis of AQs in both *P. aeruginosa* and *B. thailandensis* makes it an attractive drug target, particularly as its interference would not only affect AQ synthesis but also block the accumulation of anthranilic acid and DHQ (Lesic *et al.* 2007; Drees *et al.* 2016; Gruber *et al.* 2016; Witzgall, Ewert and Blankenfeldt 2017). Analogues of anthranilic acid were shown to increase survival and lower bacterial dissemination in mice (Lesic *et al.* 2007). Sulfonyladenosine-based mimics of the anthranilyl-AMP reaction intermediate were shown to decrease HHQ and PQS levels in *P. aeruginosa*, although pyocyanin production was unaffected (Ji *et al.* 2016). A SAR approach to the design of (2-nitrophenyl)methanol derivatives, known to be effective inhibitors of PqsD, resulted in the generation of fluorescent compounds with improved cellulo activity (Storz *et al.* 2014).

Given the central role of PqsR in mediating the signalling effects of the AQ system, it is unsurprising that this protein has been the target of inhibition of several studies. The development of PqsR inhibitors (reviewed recently in Ó Muimhneacháin *et al.* 2018) has benefited from the availability of crystal

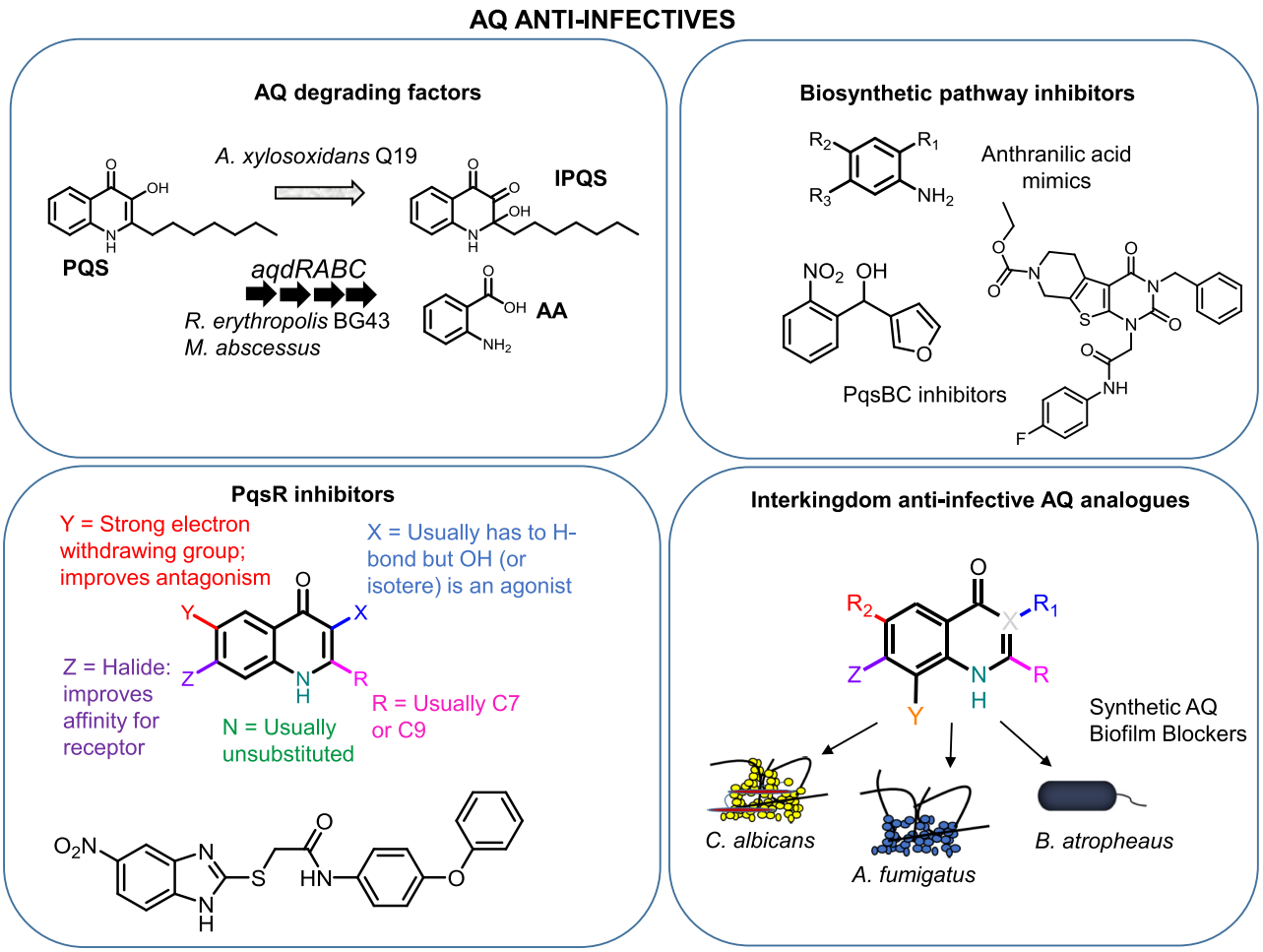

Figure 3. AQ signals as the basis for innovative anti-infective strategies. In light of the current antibiotic shortage, the AQ framework is proving an excellent target for anti-infective development, both against *P. aeruginosa* and other pathogens. Suppression of AQ production, either by targeting the biosynthetic process or its regulation, is a promising anti-infective strategy for the control of *P. aeruginosa* pathogenesis. Synthetic modification of the AQ framework has also significant potential for control of important bacterial and fungal pathogens based on the interkingdom dimension to AQ-based cell–cell communication.

structure (Ilangovan *et al.* 2013), and detailed SAR analyses by several groups (Mashburn-Warren *et al.* 2009; Hodgkinson *et al.* 2010; Lu *et al.* 2014; Shanahan *et al.* 2017). The outcome of these studies suggests that a highly lipophilc alkyl 'tail' and a polar 'head' are essential for effective antagonism, which is further enhanced by the presence of an electron-withdrawing group at C6 and a halide at C7. The therapeutic application of these HHQ/PQS-like antagonists will likely be hampered by their poor aqueous solubility. Efforts are ongoing to overcome this drawback (Lu *et al.* 2014; Nafee *et al.* 2014). The concept of dual target inhibition of both the PqsA-E and PqsR systems has gained traction in recent years. The Hartmann group has adopted this approach for the synthesis of compounds that inhibit PqsR and PqsD (Thomann *et al.* 2016), while Welsh *et al.* (2015) have proposed the design of chemical agents that disrupt crosstalk between the PQS and Rhl pathways. A recent study by Allegretta *et al.* (2017) revealed an interesting divergence between the downstream effects of PqsR and PqsBC inhibitors on AQ compound profiles. PqsR antagonists suppressed the production of 2-AA, HQNO, HHQ, PQS and DHQ (at higher concentrations). In contrast, while PqsBC inhibitors did suppress HHQ and PQS, they also resulted in increased production of 2-AA, DHQ and HQNO. This divergence may underpin the need for a polypharmacology approach described by the Rahme group, whereby both PqsBC and PqsR are targeted in tandem (Maura and Rahme 2017). In addition to targeting both acute and persistence-related functions, this dual approach may also sustain the therapeutic intervention in the face of evolved resistance.

The importance of host-associated microbiota in maintaining homeostasis and health has been highlighted in recent years, as has the structural complexity and heterogeneity (both spatial and species) therein. The integrative 'holobiont' theory has received considerable attention, viewing microbe and host as a single organism or 'hologenome' (Bordenstein and Theis 2015). Thus, the AQ-based anti-*Pseudomonas* strategies described above must be viewed in the context of an interactome that involves bacterial, fungal, viral and host cells. This requires us to look at how interventions influence the orchestra, rather than simply silencing what may be the loudest instrument. As described earlier, several microbes have developed strategies to degrade the AQ signal. While evidence of AQ analogue degradation by competing organisms has not yet been reported, the possible biotransformation of AQ anti-infectives within a community must be considered, and further work is needed in this regard. The impact of PQS inactivation on iron availability within the community could have implications for competing organisms,

as well as on the host cell, one of the key players in the polycellular interactome. The collective influence of AQ signals on HIF-1 and NFκB signalling, oxidative stress and neutrophil stimulation amongst other effects will need to be assessed as part of the implementation strategy of AQ-based therapies.

The ability of HHQ, PQS and their derivatives to modulate the behaviour of bacterial and fungal pathogens, as well as host signalling, may see the AQ signal itself provide a potential therapeutic strategy to control virulence. A suite of AQ analogues were shown to suppress biofilm formation in a range of bacterial and fungal pathogens (Reen *et al.* 2015, 2016). Importantly, these compounds were non-agonists in *P. aeruginosa* and less cytotoxic than the parent molecule (Reen et al. 2016b,c). Further modification of the AQ framework to encompass suppressive activity toward PqsR, while retaining anti-infective activity against competing pathogens, would prove a significant advance toward targeted therapeutic intervention. Combinatorial therapies may ultimately be required, for example, where immunomodulators would be administered in tandem with AQ anti-infectives. Long-term low-dose macrolide antibiotics have been shown to inhibit the expression of *P. aeruginosa lasR* and *pqsA* within the airways of patients with non-CF bronchiectasis, without reducing the bacterial load (Burr *et al.* 2016). The activity of immunomodulatory macrolide therapeutics is the subject of significant interest at the moment, with the basis of anti-inflammatory and anti-infective outcomes poorly understood. Dual targeting of AQ signalling with tandem enhancement of the host immune response may prove to be a particularly attractive therapy. However, significant pharmacological and pharmacokinetic challenges remain before such interventions could become a reality.

SUMMARY

While the challenge of translating these microbial chemical messages into a readable code remains, unlocking the elements that govern cell–cell signalling and communication in natural ecosystems will provide a framework upon which this goal can be pursued. Many important concept rich papers have been published in the sphere of microbial signalling and sociobiology, with clear distinctions drawn between signalling, coercion and cues. While some of the interactions described in this review do not fulfil the strict criteria of evolved signals, they are nonetheless important in understanding the complexity of intercellular interactions. Microbe–microbe and microbial–host communication may be just as prone to dysfunction as their more developed eukaryotic counterparts, whereby community homeostasis is overtaken by subversion of chemical messages resulting in the emergence of a dominant force to the detriment of the common good.

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