Dual-function quorum-sensing systems in bacterial pathogens and symbionts

Kelsey Barrasso, Samit Watve, Chelsea A. Simpson, Logan J. Geyman, Julia C. van Kessel, Wai-Leung Ng

1 Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, United States of America, 2 Program in Molecular Microbiology, Graduate School of Biomedical Sciences, Tufts University, Boston, Massachusetts, United States of America, 3 Department of Biology, Indiana University, Bloomington, Indiana, United States of America

☯ These authors contributed equally to this work.
*jcvk@indiana.edu (JCVK); wai-leung.ng@tufts.edu (WLN)

Introduction

Quorum-sensing (QS) systems, which rely on the production and detection of chemical signals called autoinducers (AIs) made by the bacteria themselves, are classically thought to be employed as a means to sense “self,” ensuring that bacteria cooperate and share resources to benefit their kin. Thus, most QS receptors are found to be specific for their cognate AIs. Although stringent signal specificity is considered fundamental to the fidelity of QS, receptors that respond broadly to non-self AIs have been identified. These “promiscuous” QS receptors are thought to function as interspecies signaling systems that are implicated in both competition and cooperation between microbes in polymicrobial communities [1,2].

Additional signal-sensing strategies have evolved for the QS systems in pathogenic and symbiotic bacteria which need to interact intimately with their hosts. Here, we discuss the organization and functions of QS circuits that harbor a dual-sensing function by detecting both endogenously produced AIs as well as chemical cues present inside the host. Co-opting the use of QS circuits to incorporate both microbial and host-derived information into their sensing repertoire allows proper spatial and temporal regulation of the expression of determinants critical for pathogenesis and symbiosis.

AI-3/epinephrine sensing by EHEC QseC

The QseC histidine kinase (HK) of enterohemorrhagic Escherichia coli O157:H7 (EHEC) is the earliest reported example of a QS receptor detecting both bacteria-made AIs and host-generated signals [3]. EHEC colonizes the human colon, and virulence is dependent on Shiga toxin, flagella/motility, and a type III secretion system (T3SS). Expression of the genes encoding these factors are all activated by QseC upon detection of various signals [4]. QseC is required for EHEC motility by modulating the phosphorylation state of its cognate response regulator (RR) QseB. Phosphorylated QseB binds to the regulatory region of flhDC (the master regulator of the flagellar regulon) (Fig 1) [5]. The dephosphorylation of QseB by QseC is critical to derepress flhDC and maintain motility gene expression, particularly since another HK PmrB also phosphorylates QseB [6,7]. QseC also phosphorylates 2 additional RRs QseF and KdpE, and together, these 2 RRs activate the expression of T3SS genes on a pathogenicity island called locus of enterocyte effacement (LEE) as well as the Shiga toxin gene stx2 (Fig 1) [8,9].

The kinase activity of QseC is modulated by multiple signals (Fig 1). QseC is activated by a self-produced autoinducer AI-3, which consists of a group of molecules belonging to the...
pyrazinone family, whose biosynthesis depends on threonine dehydrogenase (TDH) [10]. Synthetic AI-3 compounds added to EHEC cells activate virulence gene expression through QseC with varying potencies and specificities; however, a direct ligand binding interaction between AI-3 and QseC has not been demonstrated [10]. QseC also separately detects human adrenergic hormones epinephrine (Epi) and norepinephrine (NE) [3,4,11]. Epi/NE directly activates the kinase activity of QseC in vitro [11] and QseC-dependent virulence gene expression in EHEC [4]. The in vivo role of QseC sensing of Epi/NE in host colonization was studied using Citrobacter rodentium carrying a LEE island similar to that from EHEC [12]. C. rodentium is deficient for colonizing dopamine β-hydroxylase knockout (Dbh−/−) mice, which do not produce Epi/NE. Similarly, qseC null mutants were also impaired for colonizing the mouse intestine, highlighting the importance of host signal sensing during host colonization [12]. Overall, these studies have established that QseC acts a crucial link integrating both host-derived signals (Epi and NE) and self-produced AI molecules (AI-3). It should also be noted that the exact regulatory mechanisms of QseC on target gene regulation are diverse among different E. coli subtypes. For example, in uropathogenic E. coli (UPEC), QseB phosphorylation state can be cross-regulated by another HK PmrB in response to iron to confer polymyxin resistance [7,13,14]. While the role of Epi/NE sensing may not be universal among different E. coli strains and subtypes, QseC signaling has been shown to be critical for virulence in many strains of enteric pathogens such as Salmonella, UPEC, and enteroaggregative E. coli (EAEC) [4,15,16].
**Vibrio QS systems that detect host-generated signals**

Many *Vibrio* species including *Vibrio cholerae*, *Vibrio harveyi*, and *Vibrio fischeri* spend part of their life cycle inside animal hosts either as a pathogen or as a symbiont. These species use multiple QS systems to regulate the expression of the genes involved in host colonization [17]. Emerging evidence suggests that *Vibrio* species, similar to EHEC, also integrate host-derived chemical cues to modulate their overall QS responses. To illustrate this idea, we first focus on the canonical QS circuit of *V. cholerae* composed of 4 HK receptors CqsS, LuxPQ, CqsR, and VpsS [18] (Fig 2). At low cell density (LCD), these 4 HKs function in parallel to phosphorylate RR LuxO through an intermediate phosphotransfer protein LuxU. Phosphorylated LuxO promotes and inhibits the production of master transcriptional regulators AphA and HapR, respectively, resulting in the activation of virulence and biofilm gene expression at LCD, which is critical for *V. cholerae* host colonization [18]. At high cell density (HCD), binding of the cognate signals to the receptors leads to kinase activity inhibition, resulting in dephosphorylation of LuxO and expression of HCD QS genes. Some of the AIs detected by these QS receptors are

![Fig 2. Vibrio signaling pathways for QS and host sensing.](https://doi.org/10.1371/journal.ppat.1008934.g002)
well characterized: CqsS and LuxPQ detect the Vibrio-specific signal CAI-1 (S-3-hydroxytridecan-4-one) and the “universal” signal AI-2 in its cyclic, borated form (S-2-methyl-2,3,4-tetrahydroxytetrahydrofuran-borate), respectively. AI-2 is made by many bacteria via the enzyme LuxS and is considered an interspecies signal [19]. The 2 additional V. cholerae QS receptors, CqsR and VpsS, have been demonstrated to respond to self-made chemicals present in spent culture media; however, the identities of these signals remain unknown [18] (Fig 2). Similar parallel circuit architecture is found in other Vibrio species; however, the receptors used for signal perception can be variable. For example, the LuxN HKs in V. harveyi and Vibrio para-haemolyticus, and AinR HK in V. fischeri, which are all absent in V. cholerae, detect acyl homo-serine lactones (AHLs; Fig 2) [19] and are distinct from the cytosolic LuxR AHL receptor in V. fischeri. Here, we will discuss how 2 host-derived signals, ethanolamine and nitric oxide (NO), are detected and integrated into these parallel HK-based QS systems.

Integration of ethanolamine sensing into the QS circuit

Ethanolamine is a common intestinal metabolite generated during host and bacteria membrane turnover. In an unbiased chemical screen, ethanolamine was found to specifically interact with the periplasmic ligand-binding domain of CqsR. In V. cholerae mutants expressing only CqsR but not the other 3 QS receptors, ethanolamine induces a premature HCD QS response to inhibit virulence gene expression and limit host colonization [20]. Yet, V. cholerae mutant defective in producing ethanolamine is still proficient in QS, suggesting ethanolamine functions only as an external cue for CqsR, and additional signals must be endogenously made by V. cholerae and detected by CqsR. While the exact physiological function of ethanolamine sensing by CqsR remains unclear, the ethanolamine concentration is notably higher in the large intestine than that in the small intestine [20], and therefore, ethanolamine could be used as a proxy for niche identification. Interestingly, previous studies have demonstrated that ethanolamine both positively and negatively affects host colonization and virulence during infection with other enteric pathogens [21,22].

Integration of NO sensing into the QS circuit

NO is produced by a variety of animal cells as an antibacterial mechanism. Upon NO sensing, some bacteria express a set of nitrosative response genes to counteract this toxic compound [23]. Heme NO/O₂ binding (H-NOX) proteins are a broadly conserved family of sensor proteins that bind NO within an Fe(II)-heme domain [24]. H-NOX modulates the activity of a HK called H-NOX-associated QS kinase (HqsK) encoded in the same operon as H-NOX [25–27]. In V. harveyi and V. para-haemolyticus, HqsK feeds into the parallel QS circuitry made of LuxPQ, CqsS, and LuxN described above (Fig 2). In the absence of NO, HqsK phosphorylates LuxO via LuxU. When NO is present, it binds to H-NOX, and this complex inhibits the kinase activity of HqsK. This decreases the pool of phosphorylated LuxU and LuxO, resulting in a premature HCD QS response (e.g., increase in light production) in V. harveyi [25,26]. In V. fischeri, H-NOX/NO inhibits the HqsK homolog, HahK (Fig 2), resulting in decreased biofilm formation via inhibition of syp transcription [28] and decreased expression of genes encoding hemin transport [29]. It is not yet clear if H-NOX influences HKs in the V. fischeri QS circuit, although LuxPQ and the downstream components are conserved in V. fischeri (Fig 2).

NosP proteins are another class of NO-sensing proteins that are widely conserved in bacteria and are also encoded in operons with cognate signaling proteins [30]. In V. cholerae, NosP (also called VpsV) binds NO and inhibits the autokinase activity of VpsS (encoded in the same operon) in vitro [30] (Fig 2). In this way, V. cholerae NosP bound to NO appears to function analogously to V. harveyi H-NOX to inhibit phosphorylation of LuxU and could potentially...
feed into the QS pathway. However, the exact physiological role of NO sensing by NosP/VpsV in \textit{V. cholerae} QS gene regulation is unclear.

Because there are no identified NO synthase genes encoded in these \textit{Vibrio} species, it is hypothesized that NO acts as an interkingdom signaling molecule between bacterium and host. For example, in \textit{V. fischeri}, during early stages of colonization of the light organ in the bobtail squid \textit{Euprymna scolopes}, the surface epithelium of the squid secretes mucus that contains NO [31]. \textit{V. fischeri} cells first adhere to the mucus and form aggregates, and then the bacteria disperse and migrate through pores to eventually colonize the crypts of the light organ. NO inhibits aggregation and biofilm formation, and other signals such as calcium positively influence biofilm formation [28]. Thus, the integration of both NO and calcium signaling may balance aggregation and biofilm formation to a level that enables the bacteria to disperse from the aggregates to colonize the light organ. Additionally, the repression of hemin transport by HahK likely prepares the bacteria for the iron limited environment of the host light organ. In tandem, these results indicate that the \textit{V. fischeri} bacteria rely on NO host signaling to colonize and adjust to the environment in the light organ.

\textbf{Concluding remarks and further perspectives}

It is now clear that the bacterial QS response is not only regulated by self-made AIs, and the boundary between “self-sensing” and other signaling networks becomes blurry. Combining information about the environment together with QS, especially when the system is governed by a positive feedback loop, is proposed to be critical to coordinate bacterial group behaviors within a heterogeneous environment [32]. In addition to the examples discussed above, certain microbiota species affect \textit{V. cholerae} virulence in an AI-2-dependent but LuxP-independent manner [33]. Upon attack by bacteria, mammalian cells produce a molecule that is structurally distinct but functionally similar to AI-2. In turn, this host-produced AI-2 mimic is detected by the bacterial LuxPQ and LsrB QS receptors [34]. Yet, the importance of this reciprocal interkingdom communication pathway in pathogenesis and symbiosis is not clearly defined. Moreover, \textit{V. cholerae} possesses an additional QS circuit detecting an AI called 3,5-dimethyl-pyrazin-2-ol (DPO) with the receptor VqmA, separate from the multi-HK receptor pathway [35] (Fig 2). Interestingly, both DPO and AI-3 depend on TDH for biosynthesis, and DPO is a structural isomer of one of the compounds in the EHEC AI-3 family [10]. The VqmA/DPO system has been proposed as a bypass mechanism to optimize QS functions within specific niches, such as within the host. Whether the Vqm system also responds to host-derived signals remains to be studied.

What is the driving force for pathogenic and symbiotic bacteria to evolve to integrate both self-made AIs and host-derived chemical cues into their QS circuit? We envision that although the initial interactions between the host-derived signals and the QS receptors could be coincidental as some of these host-derived compounds structurally resemble the cognate self-made signal(s), one intriguing possibility is that these receptors might have evolved to serve as dual-function sensors to use the host-derived signal as a proxy for locating different regions in the animal host, as we have discussed in some of the \textit{Vibrio} QS systems. Further investigation into the binding capacity and modulatory effects of host metabolites with other QS receptors present in different species could test this idea.

\textbf{References}

1. Pereira CS, Thompson JA, Xavier KB. AI-2-mediated signalling in bacteria. FEMS Microbiol Rev. 2013; 37 (2):156–181. https://doi.org/10.1111/j.1574-6976.2012.00345.x PMID: 22712853
2. Wellington S, Greenberg EP. Quorum Sensing Signal Selectivity and the Potential for Interspecies Cross Talk. MBio. 2019; 10(2).
3. Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB. Bacteria–host communication: The language of hormones. Proc Natl Acad Sci. 2003; 100(15):8951–8956. https://doi.org/10.1073/pnas.1537100100 PMID: 12847292

4. Hughes DT, Clarke MB, Yamamoto K, Rasko DA, Sperandio V. The QseC adrenergic signaling cascade in Enterohemorrhagic E. coli (EHEC). PLoS Pathog. 2009; 5(8):e1000553. https://doi.org/10.1371/journal.ppat.1000553 PMID: 19696934

5. Clarke MB, Sperandio V. Transcriptional regulation of flhDC by QseBC and sigma (FliA) in enterohemorrhagic Escherichia coli. Mol Microbiol. 2005; 57(6):1734–1749. https://doi.org/10.1111/j.1365-2958.2005.04792.x PMID: 16135237

6. Kostakioti M, Hadjifrangiskou M, Pinkner JS, Hultgren SJ. QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic Escherichia coli. Mol Microbiol. 2009; 73(6):1020–1031. https://doi.org/10.1111/j.1365-2958.2009.06826.x PMID: 19703104

7. Guckes KR, Kostakioti M, Breland EJ, Gu AP, Shaffer CL, Martinez CR 3rd, et al. Strong cross-system interactions drive the activation of the QseB response regulator in the absence of its cognate sensor. Proc Natl Acad Sci U S A. 2013; 110(41):16592–16597. https://doi.org/10.1073/pnas.1315320110 PMID: 24062463

8. Njoroge J, Sperandio V. Enterohemorrhagic Escherichia coli virulence regulation by two bacterial adrenergic kinases. QseC and QseE Infect Immun. 2012; 80(2):688–703. https://doi.org/10.1128/IAI.05921-11 PMID: 22144490

9. Reading NC, Rasko DA, Torres AG, Sperandio V. The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. Proc Natl Acad Sci U S A. 2009; 106(14):5889–5894. https://doi.org/10.1073/pnas.0811409106 PMID: 19298831

10. Kim CS, Gatsios A, Cuesta S, Lam YC, Wei Z, Chen H, et al. Characterization of Autoinducer-3 Structure and Biosynthesis in E. coli. ACS Central Science. 2020; 6(2):197–206. https://doi.org/10.1021/acscentsci.9b01076 PMID: 32123737

11. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. The QseC sensor kinase: A bacterial adrenergic receptor. Proc Natl Acad Sci. 2006; 103(27):10420–10425. https://doi.org/10.1073/pnas.0604343103 PMID: 16803956

12. Moreira CG, Russell P, Mishra AA, Narayanan S, Ritchie JM, Waldor MK, et al. Bacterial Adrenergic Sensors Regulate Virulence of Enteric Pathogens in the Gut mBio. 2016; 7(3):e00826–e00816. https://doi.org/10.1128/mBio.00826-16 PMID: 27273829

13. Guckes KR, Breland EJ, Zhang EW, Hanks SC, Gill NK, Algood HM, et al. Signaling by two-component system noncognate partners promotes intrinsic tolerance to polymyxin B in uropathogenic Escherichia coli. Sci Signal. 2017; 10(461).

14. Hadjifrangiskou M, Kostakioti M, Chen SL, Henderson JP, Greene SE, Hultgren SJ. A central metabolic circuit controlled by QseC in pathogenic Escherichia coli. Mol Microbiol. 2011; 80(6):1516–1529. https://doi.org/10.1111/j.1365-2958.2011.07660.x PMID: 21542668

15. Kostakioti M, Hadjifrangiskou M, Pinkner JS, Hultgren SJ. QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic Escherichia coli. Mol Microbiol. 2009; 73(6):1020–1031. https://doi.org/10.1111/j.1365-2958.2009.06826.x PMID: 19703104

16. Hadjifrangiskou M, Kostakioti M, Chen SL, Henderson JP, Greene SE, Hultgren SJ. A central metabolic circuit controlled by QseC in pathogenic Escherichia coli. Mol Microbiol. 2011; 80(6):1516–1529. https://doi.org/10.1111/j.1365-2958.2011.07660.x PMID: 21542668

17. Ball AS, Chaparian RR, van Kessel JC. Quorum Sensing Gene Regulation by LuxR/HapR Master Regulators in Vibrios. J Bacteriol. 2017; 199(19).

18. Jung SA, Chapman CA, Ng WL. Quadruple quorum-sensing inputs control Vibrio cholerae virulence and maintain system robustness. PLoS Pathog. 2015; 11(4):e1004837. https://doi.org/10.1371/journal. ppat.1004837 PMID: 25974462

19. Hawver LA, Jung SA, Ng W-L. Specificity and complexity in bacterial quorum-sensing systems. FEMS Microbiol Rev. 2016; 40(5):738–752. https://doi.org/10.1093/femsre/fuw014 PMID: 27354348

20. Wasse S, Barrasso K, Jung SA, Davis KJ, Hawver LA, Khataoka A, et al. Parallel quorum-sensing system in Vibrio cholerae prevents signal interference inside the host. PLoS Pathog. 2020; 16(2): e1008313. https://doi.org/10.1371/journal.ppat.1008313 PMID: 32059031

21. Anderson CJ, Clark DE, Adli M, Kendall MM. Ethanolamine Signaling Promotes Salmonella Niche Recognition and Adaptation during Infection. PLoS Pathog. 2015; 11(11):e1005278. https://doi.org/10. 1371/journal.ppat.1005278 PMID: 26656973
22. Garsin DA. Ethanolamine utilization in bacterial pathogens: roles and regulation. Nat Rev Microbiol. 2010; 8(4):290–295. https://doi.org/10.1038/nrmicro2334 PMID: 20234377

23. Spiro S. Regulators of bacterial responses to nitric oxide. FEMS Microbiol Rev. 2007; 31(2):193–211. https://doi.org/10.1111/j.1574-6976.2006.00061.x PMID: 17313521

24. Iyer LM, Anantharaman V, Aravind L. Ancient conserved domains shared by animal soluble guanylyl cyclases and bacterial signaling proteins. BMC Genomics. 2003; 4(1):5. https://doi.org/10.1186/1471-2164-4-5 PMID: 12590654

25. Henares BM, Higgins KE, Boon EM. Discovery of a nitric oxide responsive quorum sensing circuit in Vibrio harveyi. ACS Chem Biol. 2012; 7(8):1331–1336. https://doi.org/10.1021/cb300215t PMID: 22606970

26. Henares BM, Xu Y, Boon EM. A nitric oxide-responsive quorum sensing circuit in Vibrio harveyi regulates flagella production and biofilm formation. Int J Mol Sci. 2013; 14(8):16473–16484. https://doi.org/10.3390/ijms140816473 PMID: 23965964

27. Ueno T, Fischer JT, Boon EM. Nitric Oxide Enters Quorum Sensing via the H-NOX Signaling Pathway in Vibrio parahaemolyticus. Front Microbiol. 2019; 10:2108. https://doi.org/10.3389/fmicb.2019.02108 PMID: 31620101

28. Thompson CM, Tischler AH, Tarnowski DA, Mandel MJ, Visick KL. Nitric oxide inhibits biofilm formation by Vibrio Fischeri via the nitric oxide sensor HnoX. Mol Microbiol 2019; 111(1):187–203. https://doi.org/10.1111/mmi.14147 PMID: 30299554

29. Wang Y, Dufour YS, Carlson HK, Donohue TJ, Marletta MA, Ruby EG. H-NOX-mediated nitric oxide sensing modulates symbiotic colonization by Vibrio Fischeri. Proc Natl Acad Sci U S A. 2010; 107(18):8375–8380 https://doi.org/10.1073/pnas.1003571107 PMID: 20404170

30. Hossain S, Nisbett L-M, Boon EM. Discovery of Two Bacterial Nitric Oxide-Responsive Proteins and Their Roles in Bacterial Biofilm Regulation. Acc Chem Res. 2017; 50(7):1633–1639. https://doi.org/10.1021/acs.accounts.7b00095 PMID: 28605194

31. Davidson SK, Koropatkinski TA, Kossmehl R, Syucro L, McFall-Ngai MJ. NO means 'yes' in the squid-vibrio symbiosis: nitric oxide (NO) during the initial stages of a beneficial association. Cell Microbiol. 2004; 6(12):1139–1151. https://doi.org/10.1111/j.1462-5822.2004.00429.x PMID: 15527494

32. Stabb EV. Could Positive Feedback Enable Bacterial Pheromone Signaling To Coordinate Behaviors in Response to Heterogeneous Environmental Cues? MBio. 2018; 9(3).

33. Hsiao A, Ahmed AM, Subramanian S, Griffin NW, Drewry LL, Petri WA Jr, et al. Members of the human gut microbiota involved in recovery from Vibrio cholerae infection. Nature. 2014; 515(7527):423–426. https://doi.org/10.1038/nature13738 PMID: 25231861

34. Ismail AS, Valastyan JS, Bassler BL. A Host-Produced Autoinducer-2 Mimic Activates Bacterial Quorum Sensing. Cell Host Microbe. 2016; 19(4):470–480. https://doi.org/10.1016/j.chom.2016.02.020 PMID: 26996306

35. Papenfort K, Stüpe JE, Schramma KR, Cong JP, Seyedsayamdost MR, Bassler BL, A Vibrio cholerae autoinducer-receptor pair that controls biofilm formation. Nat Chem Biol. 2017; 13(5):551–557. https://doi.org/10.1038/nchembio.2336 PMID: 28319101