PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Review

Cite this article: Wang F-F, Qian W. 2019 The roles of histidine kinases in sensing host plant and cell–cell communication signal in a phytopathogenic bacterium. *Phil. Trans. R. Soc. B* **374**: 20180311.
http://dx.doi.org/10.1098/rstb.2018.0311

Accepted: 11 October 2018

One contribution of 19 to a theme issue 'Biotic signalling sheds light on smart pest management'.

Subject Areas:
microbiology, biochemistry

Keywords:
bacteria, histidine kinase, plant pathogen, receptor, phosphorylation, two-component signal transduction

Author for correspondence:
Wei Qian
e-mail: qianw@im.ac.cn

THE ROYAL SOCIETY PUBLISHING

The roles of histidine kinases in sensing host plant and cell–cell communication signal in a phytopathogenic bacterium

Fang-Fang Wang and Wei Qian

State Key Laboratory of Plant Genomics, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

WQ, 0000-0002-7041-5354

It has long been known that phytopathogenic bacteria react to plant-specific stimuli or environmental factors. However, how bacterial cells sense these environmental cues remains incompletely studied. Recently, three kinds of histidine kinases (HKs) were identified as receptors to perceive plant-associated or quorum-sensing signals. Among these kinases, HK VgrS detects iron depletion by binding to ferric iron via an ExxE motif, RpfC binds diffusible signal factor (DSF) by its N-terminal peptide and activates its autokinase activity through relaxation of autoinhibition, and PcrK specifically senses plant hormone–cytokinin and elicits bacterial responses to oxidative stress. These HKs are critical sensors that regulate the virulence of a Gram-negative bacterium, *Xanthomonas campestris* pv. *campestris*. Research progress on the signal perception of phytopathogenic bacterial HKs suggests that inter-kingdom signalling between host plants and pathogens controls pathogenesis and can be used as a potential molecular target to protect plants from bacterial diseases.

This article is part of the theme issue 'Biotic signalling sheds light on smart pest management'.

1. Introduction

Discriminating 'self' and 'non-self' is the primary step during host–microbe interaction [1,2]. Eukaryotic cells employ the so-called pattern recognition receptors (PRRs) to detect the pathogen-associated molecular pattern to elicit immune responses, whereas pathogenic prokaryotes must monitor and respond to hostile environments or various stresses in the host tissues [3,4]. Studies in this area became more popular in the areas of both animal and plant diseases after 1990. Rapid progress was made and in 2011, when Bruce A. Beutler and Jules A. Hoffmann won the Nobel Prize in Physiology and Medicine for their discoveries concerning the activation of innate immunity in animals via TLR-like receptors sensing bacterial lipopolysaccharides [5,6], which emphasizes the importance of these investigations.

In the past two decades, scientists studying plant–microbe interactions have identified a number of plant PRRs that function in the recognition of microbial pathogens and modulate innate immune responses [7], including FLS2-perceiving flg22, a short peptide of bacterial flagella [8]; EFR-sensing bacterial elongation factor Tu [9]; CERK1-detecting chitin of fungal cells [10]; LYM-sensing peptidoglycan [11] and LORE-sensing lipopolysaccharides [12]. Although it is well known that plant pathogenic bacteria react to various stimulations of host plants [13,14], the biochemical mechanism by which bacterial cells sense plant chemicals or plant-derived cues remains largely unknown. Especially, the specific receptor–ligand interaction between host and pathogen has not been well established through studies that combine multi-discipline approaches. In fact, knowledge about bacterial sensing will provide insights into the molecular mechanism of disease pathogenesis, which facilitates the rational design of novel approaches to fight these important pathogens.

2. Histidine kinases of *Xanthomonas campestris* pv. *campestris*

With the exception of species belonging to the genus *Mycoplasma*, most bacteria use several to hundreds of two-component signal transduction systems (TCS) as the predominant mechanism to detect and respond to environmental stimuli [15,16]. TCS typically contains a membrane-bound histidine kinase (HK) and a cytosolic response regulator (RR). A typical HK has a variable N-terminal sensor domain that detects a specific signal and a C-terminal that contains a conserved transmitter domain to hydrolyze ATP and that can be autophosphorylated. Upon detection of a stimulus, HK is phosphorylated, and then the phosphoryl group is transferred to the receiver (REC) domain of its cognate RR. The RR performs downstream regulation via its C-terminal output domain, mainly acting as transcription factors [17]. Additionally, a hybrid type of HK (HyHK) that contains the REC domain is widely distributed in various bacteria [18]. HyHK is involved in a multiple-step phosphorelay with other HKs and RRs, adding more regulatory checkpoints to the signal transduction [19–21]. The first TCS was identified in 1986 [22]. After 30 years of research, there is now a full understanding on the biochemistry of the phosphoryltransfer process and the regulatory function of RRs. However, how HK senses environmental stimuli is incompletely understood. Only a few studies have reported the biological functions of HK–signal or HK–ligand interactions [23,24]. For example, in enterohaemorrhagic *Escherichia coli*, HKs QseE and QseC perceive animal hormone epinephrine [25], and PhoQ detects the concentrations of Mg^{2+} and Ca^{2+}, antimicrobial peptides and pH [26,27]. These HKs modulate bacterial virulence. In phytopathogenic bacteria, although a number of HKs are involved in regulating virulence, with the exception of the study cases reviewed here, the signals detected by these HKs remain unclear.

Various scenarios can explain this finding: (i) in contrast to the receptor serine/tyrosine/threonine kinases of eukaryotes, phosphorylated HK is typically unstable and has a very short half-life [15,28], which makes it technically difficult to test the phosphorylated states of HKs *in vivo* by means of Western blot using specific antibodies or Phos-tag gels [29]. Generally, the phosphorylation of HKs or RRs is studied *in vitro* using radio-autography. (ii) The majority of HKs have hydrophobic transmembrane regions that function in signal perception. The standard study strategy that removes transmembrane regions to obtain recombinant, soluble proteins precludes the possibility of investigating HK–signal interactions [23]. (iii) The nature of transmembrane proteins makes it difficult to express and purify recombinant HKs unless full-length HKs are embedded into lipid bilayer [30].

Xanthomonas campestris pv. *campestris* is one of the model organisms used in studies of plant–pathogen interactions given that it is the causative agent of black rot disease in cruciferous vegetables, it is genetically amendable and a Gram-negative, aerobic, rod-shaped bacterium. This bacterium causes serious yield loss worldwide and is listed as one of the disastrous pathogens in agricultural production. The complete genomes of several strains of *X. campestris* pv. *campestris* were reported in the early years of the new millennium [31–33], thus providing genetic information to conduct in-depth research.

The genome of *X. campestris* pv. *campestris* encodes 53 HKs and 54 RRs [34,35], including a HWE-family (histidine–tryptophan–glutamate) HK that was recently identified [36]. These HKs are mainly classified into three major structural groups based on their membrane topologies [34]: Group I, membrane-bound HK with a well-defined periplasmic sensor domain to perceive extracytoplasmic stimuli; Group II, membrane-bound HK without an apparent periplasmic sensory domain, which is believed to employ an unidentified sensory domain and transmembrane regions to perceive extracytoplasmic stimuli or detect membrane-associated stimuli derived from membrane integrity factors, such as ion gradients and electric potential; Group III, soluble, cytoplasmic HK without recognizable transmembrane helices. These HKs of *X. campestris* pv. *campestris* encode a limited number of sensor domains, including Per–Arnt–Sim domain (PAS)/PAC, KdpD, AAA, CHASE, CHASE3, GAF and HAMP domains, prompting an interesting question on how these HKs detect numerous stimuli when the bacterium is free-living or survives in the host environment. Among them, RpfC (Group II), VgrS (or named ColS), PhoQ, PcrK and HpaS (Group I) are critical to regulate virulence of *X. campestris* pv. *campestris* [35,37–40]. Thus, exploration of the stimuli sensed by these virulence-associated HKs is important for the understanding of the molecular pathogenesis of this bacterial pathogen.

3. Techniques for the study of biochemistry of full-length histidine kinases

Most of the HKs of *X. campestris* pv. *campestris* contain 1–12 transmembrane helices and are located in the inner membrane of bacterial cells [34]. As previously mentioned, to investigate the enzymatic activities of HKs, including autokinase, phosphatase and phosphotransferase, full-length HKs must be expressed and embedded into lipid bilayers to reconstruct correct conformations. Currently, three approaches can be used, including inverted membrane vesicle (IMV), liposome and nanodisc. Among them, the IMV is a vesicle formed by fractioned plasma membrane with an inverted direction [41]. The vesicle is extracted from the membrane fraction of *E. coli* cells expressing HKs by ultracentrifugation. Although IMV is the simplest method among these approaches, it has a limitation in the purity of the HK. In fact, IMV is typically a mixture of various proteins embedded in the membrane. The purity of HK ranges from 5% to 30% in total protein, and affinity chromatograph can occasionally be used to increase the purity of HK embedded in IMV [42].

The level of purity obtained with liposomes and nanodiscs is improved compared with IMV, but these systems are more experimentally complicated. A liposome is an artificial spherical vesicle with at least one lipid bilayer that could be inserted by transmembrane HKs to construct a membrane system [43,44]. Full-length HKs are purified, dissolved in appropriate detergents and then integrated into the phospholipid bilayer after self-assembly and dialysis. To study HK biochemistry, one of the considerations for the use of liposomes is the direction of HKs in the lipid bilayer. Some of the HKs are incorrectly embedded in the liposome such that their sensor regions are located in the 'ball' of

phospholipid, which blocks the interaction between sensor regions and chemicals. However, this problem is solved by nanodiscs. Nanodisc is also a synthetic membrane system with a phospholipid bilayer with a planar structure [45]. Two membrane-scaffolding proteins are used to assist the assembly of membrane proteins into the phospholipid bilayer. In addition, selection of appropriate scaffolding proteins can control the number of protein molecules that are integrated into the membrane, thus producing various oligomers, such as dimers, trimers and tetramers [46]. The application of these membrane protein techniques is a prerequisite to study the perception function of bacterial HKs.

4. Bacterium employs the VgrSR system to sense iron depletion and iron repletion

Iron is an essential metal for cellular organisms that participate in crucial biological processes, including photosynthesis, respiration, enzymatic activity and redox reactions [47]. In addition, excess iron is toxic to cells given that it catalyses the Fenton reaction in the presence of oxygen to deliver reactive oxygen species that are harmful to multiple biomacromolecules [48]. Although iron is the fourth most abundant element in the Earth's crust, upon infection, host plants and animals tend to govern iron by chelating the metal or retaining it in macromolecules. Therefore, pathogenic bacteria must survive in an iron-depletion niche after infection. To maintain iron homeostasis, these pathogens must sense extracellular iron depletion to absorb iron into the cells and sense an intracellular iron-replete environment to avoid the destructive role of iron excess.

VgrS–VgrR of *X. campestris* pv. *campestris* is a regulatory TCS of virulence [35,49]. VgrS is a HK that has a transmembrane region and a putative periplasmic sensor, whereas VgrR is an OmpR-family RR that contains a C-terminal helix-turn-helix output region. VgrR exhibits transcription factor activity to bind an AT-rich, palindromic motif in the promoter regions of approximately 400 genes as revealed by chromatin immunoprecipitation (ChIP-seq) analysis [50]. VgrS–VgrR constitutes a typical TCS given that the full-length VgrS can be autophosphorylated and then phosphorylates VgrR. Given that the half-life of the phosphorylated VgrR is as short as approximately 10 s, whether VgrS exhibits phosphatase activity is currently unknown for technical reasons. Inactivation of *vgrR* in various *Xanthomonas* species resulted in significant attenuation of virulence, and *vgrS* mutations caused a slight reduction in virulence. In addition, *vgrS–vgrR* mutations caused deficiencies in bacterial stress responses, metal tolerance, bacterial growth and the ability to elicit hypersensitivity on non-host plants [51,52]. VgrS–VgrR is an important, evolutionarily conserved virulence regulator in pathogenic bacteria belonging to *Xanthomonas*.

A recent study revealed that VgrS–VgrR is involved in the critical regulatory system in sensing and responding to iron repletion and depletion [50]. VgrS harbours a typical ExxE (EPQE) motif within its periplasmic sensor region to specifically bind ferric iron rather than ferrous iron. The Fe^{3+}–VgrS interaction remarkably inhibits VgrS autophosphorylation. In iron-depleted environments, dissociation of Fe^{3+} from the sensor domain of VgrS thus activates VgrS autokinase activity and promotes phosphotransfer from VgrS to VgrR (figure 1). Consequently, the phosphorylated VgrR regulates the transcription of hundreds of genes, especially those involved in iron uptake from the host plant. Interestingly, phosphorylated VgrR behaves as a transcriptional repressor to inhibit the transcription of *tdvA*, a TonB-dependent receptor gene that is detrimental to iron uptake and virulence. Genetic inactivation of *tdvA* significantly increased bacterial virulence and iron uptake, suggesting that TdvA must exhibit a special function that differs from most of the TonB-dependent receptors [50].

Otherwise, when the intracellular iron is in excess, Fe^{2+} binds to the RR VgrR to dissociate it from the promoters and VgrS. This process inactivates the transcription factor activity of VgrR and leads to the transcription initiation of *tdvA*. Expression of *tdvA* exhibits a negative effect on iron uptake of *X. campestris* pv. *campestris*. In contrast to VgrS, VgrR could bind ferrous iron (Fe^{3+}). However, the physiological significance of VgrR–Fe^{3+} binding is unknown given that Fe^{3+} is generally reduced to Fe^{2+} within cells (figure 1) [50].

To our knowledge, VgrS is the first biochemically identified iron-sensing HK in phytopathogenic bacteria. Its function is more like the ExxE motif-carrying iron receptor of *Pseudomonas aeruginosa* BqsS [53], given that Fe^{3+} binding by BqsS and VgrS decreased their autokinase activities. This performance is quite different from the PmrB of *Salmonella enterica*. PmrB autophosphorylation levels are increased in the presence of high iron concentrations, which might be beneficial to bacteria surviving in the intestine of animals [54]. VgrS and PmrB contain iron-binding ExxE motifs. PmrB can bind both Fe^{3+} and Fe^{2+}, but VgrS specifically interacts with Fe^{3+}. The molecular mechanism determining the specificity remains unknown.

5. Bacterium employs the RpfCG system to sense the quorum-sensing signal

Quorum sensing is an ecological process by which microbes measure their population size and elicit crucial physiological responses that are different from the planktonic lifestyle. A number of chemical signals used in bacterial cell–cell communication were identified, including homoserine lactones, quinolones and peptides [55]. In *Xanthomonas* spp. and their close relatives, a group of moderate chain, unsaturated fatty acids act as chemical signals to regulate quorum-sensing [56]. These fatty acids were named diffusible signal factors (DSFs), including DSF (*cis*-11-methyl-2-dodecenoic acid), BDSF (*cis*-2-dodecenoic acid), CDSF (*cis*,*cis*-11 methyldodeca-2,5-dienoic acid), IDSF (*cis*-10-methyl-2-dodecenoic acid) and SDSF (*trans*-2-decenoic acid). DSF family signals not only regulate the quorum sensing of *Xanthomonas* spp. but also are produced or detected by other organisms, such as *Xylella fastidosa*, *Stenotrophomonas maltophilia*, *Burkholderia cenocepacia*, *P. aeruginosa* and even the fungus *Candida albicans*, suggesting a role in inter-kingdom signalling. The biological roles and turnover of DSF family chemicals have been extensively reviewed elsewhere and will not be discussed here [57–60].

In *X. campestris* pv. *campestris*, it has long been predicted that the HK RpfC is the receptor of DSF. RpfC is a HyHK with additional REC and histidine-containing phosphotransfer (HPt) domains [61,62]. RpfC has five transmembrane helices, so it belongs to the Group II HKs. The cognate RR of RpfC is RpfG, a histidine–aspartic acid–glycine–tyrosine–proline (HD-GYP) domain-containing protein that

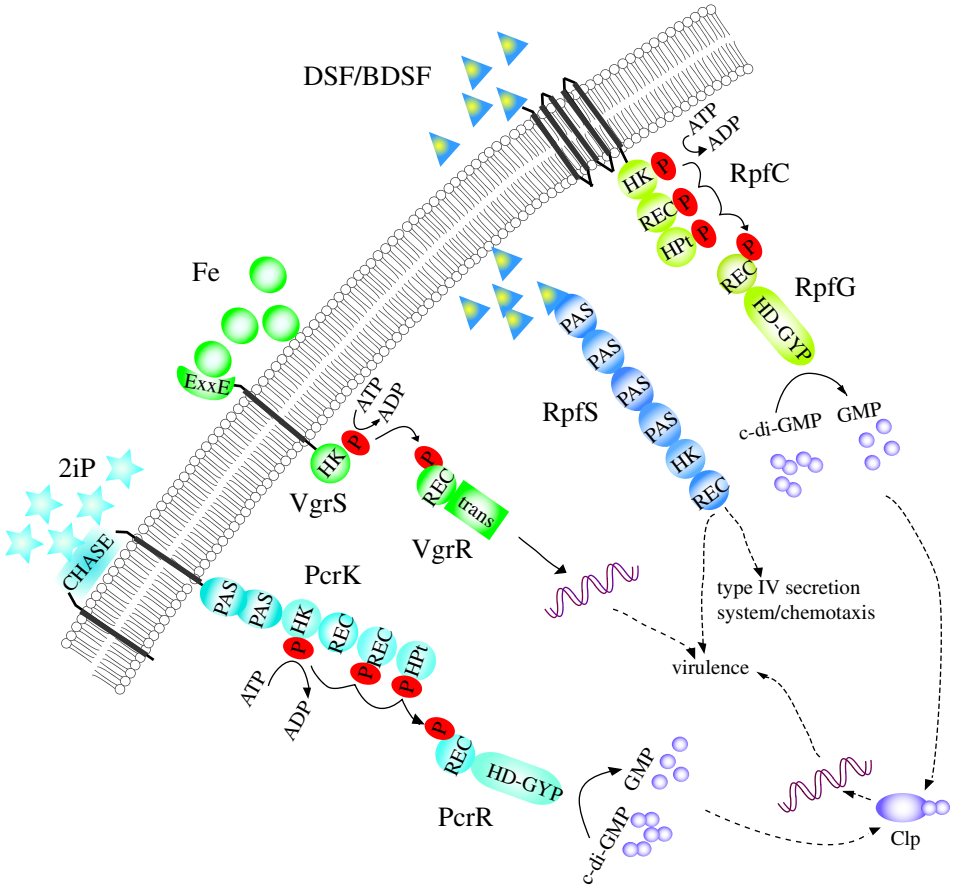

Figure 1. Biochemical mechanisms underlying histidine kinase-mediated perception of host plant and environmental signals of *Xanthomonas campestris* pv. *campestris*. VgrSR, RpfCG, RpfS and PcrKR systems are depicted. P in red circles indicate phosphoryl group. Domains are reported based on the Pfam and SMART databases. HD-GYP, histidine–aspartic acid–glycine–tyrosine–proline (HD-GYP) domain; HK: HisKA domain; pfam acc. no. PF07730; PAS, Per–Arnt–Sim domain; REC: response reg domain, PF00072; HPt: HPT domain, PF01627; CHASE: CHASE domain, PF03924; PAS: PAS/PAS4/PAS8 domain, PF00989/08448/13188; HD-GYP: HD-GYP domain, SM000471; Trans: trans_reg_C domain, SM000862. The known signals and regulation pathways were designated.

exhibits phosphodiesterase activity to degrade the second messenger cyclic di-GMP into GMP. RpfC–RpfG is also a central system to modulate bacterial virulence. Inactivation of *rpfC* or *rpfG* severely decreases bacterial virulence, production of extracellular polysaccharides and various extracellular enzymes, but increases the production of DSF [58,61]. In addition to the aforementioned problems in studying DSF–RpfC interactions, the chemical nature of DSF, i.e. a fatty acid, made it more difficult to determine the HK–fatty acid interaction. For example, two proteins RpfS and RpfR were identified as DSF receptors [63,64]. However, these two proteins lack transmembrane regions and might act as intracellular receptors of DSF.

Recently, Cai *et al*. [65] successfully constructed an IMV and liposome that embedded the full-length RpfC. DSF stimulation remarkably doubled the autophosphorylation level of RpfC liposomes compared with the negative control. A series of deletion analyses revealed that both the transmembrane region and an N-terminal, short sensor of RpfC that is 22 amino acids in length, are important in DSF perception. The study then combined several biophysics methods, including microscale thermophoresis (MST), nanodifferential scanning fluorimetry (NanoDSF) and circular dichroism (CD) spectra, to demonstrate that DSF binds to the 22-amino acid peptide of RpfC (figure 1). The binding affinity measured by MST analysis is at the nanomole level, suggesting a relatively strong interaction between DSF and RpfC. In addition, critical amino acid residues impacting DSF binding were identified, revealing five residues near the first transmembrane helix. In addition, the study used alanine-scanning mutagenesis to identify the conserved juxtamembrane region of RpfC, which links the transmembrane region and the transmitter region of RpfC and autoinhibits its autokinase activity in the absence of DSF. DSF stimulation releases this autoinhibition in an allosteric manner and activates the RpfC. In the juxtamembrane region, substitutions of two residues, Leu^{172} and Ala^{178}, could constitutively activate RpfC autophosphorylation regardless of the absence or presence of DSF stimulation [65]. Therefore, these results demonstrate that RpfC is the *bona fide* receptor of DSF and that the juxtamembrane region of HK exhibits an autoinhibition function, which is similar to that noted in the eukaryotic tyrosine kinases (figure 1). The establishment of the DSF–RpfC relationship and related research approaches will promote in-depth investigations on the specificity of other DSF family signals in various bacteria.

6. Bacterium employs the PcrKR system to sense plant hormones

In pathogenic bacteria infecting animals, perception of host signalling chemicals by HKs was discovered, revealing that inter-kingdom signalling is critical in the regulation of bacterial virulence. For example, *E. coli* senses animal adrenaline or epinephrine via the HK QseC and senses

enteric fucose via the HK FusK [25,66]. Will plant pathogenic bacteria in parallel sense host-derived signalling chemicals, such as plant hormones?

In plants, two types of hormone receptors are HKs [67]: cytokinin and ethylene receptors (such as AHK2/AHK3/AHK4 and ETR1/ETR2/EIN4 of *Arabidopsis thaliana*). This finding prompts an interesting question given that plant pathogenic bacteria have the potential ability to sense these two hormones, especially considering that there are a large number of HKs in the bacterial cell. To challenge this hypothesis, Wang *et al*. identified the HK PcrK from a protein expression library containing all of the 52 HKs of *Xanthomonas campestris* pv. *campestris* [39]. PcrK is a HyHK with an experimentally confirmed periplasmic CHASE domain, two additional REC domains and a HPt domain. PcrK and its cognate RR PcrR control bacterial virulence (figure 1). *In vitro* phosphorylation assays revealed that plant cytokinin 2-isopentenyladenine (2iP) remarkably inhibited PcrK autokinase activity. This inhibition is specific given that other plant cytokinins, such as *trans*-zeatin (*t*ZT) and *cis*-zeatin (*c*ZT) and kintin, did not exhibit a similar effect. In addition, 2iP binds firmly to the periplasmic CHASE domain of PcrK with a dissociation constant at the nanomole level [39]. The 2iP–PcrK interaction promoted bacterial resistance to oxidative stress via the 3′,5′-cyclic diguanylic acid (c-di-GMP) regulatory pathway. Briefly, 2iP stimulation decreased PcrK–PcrR phosphorylation levels, whereas dephosphorylated PcrR exhibits increased phosphoesterase activity to degrade c-di-GMP, which elicits the expression of more than 50 genes (figure 1). Among them, a TonB-dependent receptor CtrA is critical in the oxidative stress response [58].

PcrK is the first experimentally identified prokaryotic receptor of plant hormones [68,69]. This result thus prompts two questions. Do other microbial HKs sense plant cytokinin? PcrK contains a CHASE domain as the plant cytokinin receptor. CHASE-like domains are widely distributed protein motifs in prokaryotes, including a number of plant-associated bacteria. The CHASE domain is differentiated into CHASE, CHASE2, CHASE3 and CHASE4 domains [70]. Therefore, it is possible that other CHASE domain-containing HKs also sense cytokinins. For example, although the receptor remains unidentified, cytokinin is important for the virulence of the animal pathogen *Mycobacterium tuberculosis* [71]. The second question is associated with evolution. How did pathogenic bacteria evolve receptors to detect plant cytokinin? Cytokinins are adenine derivatives that have an ancient evolutionary history, whereas the genus of *Xanthomonas* originated before 1600 million years ago, which is much earlier than the appearance of the original plants in the Earth. We tend to believe that PcrK-like receptors originated early to detect cytokinins, and these receptors then coevolved and hijacked cytokinin signalling after the bacteria–host plant relationship was established in evolutionary history. Future studies are needed to clarify these problems.

7. Concluding remarks and future perspectives

One of the fundamental scientific questions that puzzles plant pathologists is how phytopathogenic bacteria recognize host plants or plant-associated environmental factors. Recent biochemical studies on the relationships between VgrS-iron depletion, RpfC-DSF and PcrK-cytokinin then revealed that bacterial HKs not only monitor and detect environmental stimuli but also sense important plant chemicals, suggesting that inter-kingdom communication between host plant and bacteria is mutual. Although most of the studies were performed in *X. campestris* pv. *campestris*, it is anticipated that other cases will soon be reported given the diversity of plant–bacteria interactions. The results of such studies not only decipher the molecular pathogenesis of pathogens but also can identify lead compounds and ideal molecular targets to develop creative approaches to combat bacterial pathogens.

Data accessibility. This article has no additional data.

Authors' contributions. F.-F.W. and W.Q. conceived, designed and drafted the original manuscript and approved the final version to be published.

Competing interests. The authors declare that they have no competing interests.

Funding. This work was financially supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDB11040700 to W.Q.), the National Science Foundation of China (grant nos 31870119 and 31400071 to F.-F.W. and 31671989 and 31671989 to W.Q.), the Ministry of Science and Technology of China (grant no. 2016YFD0100602) and the State Key Laboratory of Plant Genomics.

Acknowledgements. We thank members of our laboratory for their helpful comments on this manuscript.

References

1. Ronald PC, Beutler B. 2010 Plant and animal sensors of conserved microbial signatures. *Science* **330**, 1061–1064. (doi:10.1126/science.1189468)
2. Jones JD, Dangl JL. 2006 The plant immune system. *Nature* **444**, 323–329. (doi:10.1038/nature05286)
3. Zipfel C. 2014 Plant pattern-recognition receptors. *Trends Immunol.* **35**, 345–351. (doi:10.1016/j.it.2014.05.004)
4. Smale ST. 2012 Transcriptional regulation in the innate immune system. *Curr. Opin. Immunol.* **24**, 51–57. (doi:10.1016/j.coi.2011.12.008)
5. O'Neill LA, Golenbock D, Bowie AG. 2013 The history of Toll-like receptors — redefining innate immunity. *Nat. Rev. Immunol.* **13**, 453–460. (doi:10.1038/nri3446)
6. Volchenkov R, Sprater F, Vogelsang P, Appel S. 2012 The 2011 Nobel Prize in physiology or medicine. *Scand. J. Immunol.* **75**, 1–4. (doi:10.1111/j.1365-3083.2011.02663.x)
7. Couto D, Zipfel C. 2016 Regulation of pattern recognition receptor signalling in plants. *Nat. Rev. Immunol.* **16**, 537–552. (doi:10.1038/nri.2016.77)
8. Gomez-Gomez L, Boller T. 2000 FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell* **5**, 1003–1011. (doi:10.1016/S1097-2765(00)80265-8)
9. Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G. 2006 Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* **125**, 749–760. (doi:10.1016/j.cell.2006.03.037)
10. Miya A *et al*. 2007 CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **104**, 19 613–19 618. (doi:10.1073/pnas.0705147104)
11. Willmann R *et al*. 2011 *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. *Proc. Natl Acad. Sci. USA* **108**, 19 824–19 829. (doi:10.1073/pnas.1112862108)

12. Ranf S *et al.* 2015 A lectin S-domain receptor kinase mediates lipopolysaccharide sensing in *Arabidopsis thaliana*. *Nat. Immunol.* **16**, 426–433. (doi:10.1038/ni.3124)
13. Venturi V, Fuqua C. 2013 Chemical signaling between plants and plant-pathogenic bacteria. *Annu. Rev. Phytopathol.* **51**, 17–37. (doi:10.1146/annurev-phyto-082712-102239)
14. Glick BR. 2012 Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* **2012**, 963401. (doi:10.6064/2012/963401)
15. Stock AM, Robinson VL, Goudreau PN. 2000 Two-component signal transduction. *Annu. Rev. Biochem.* **69**, 183–215. (doi:10.1146/annurev.biochem.69.1.183)
16. Hoch JA. 2000 Two-component and phosphorelay signal transduction. *Curr. Opin Microbiol.* **3**, 165–170. (doi:10.1016/S1369-5274(00)00070-9)
17. Bourret RB, Silversmith RE. 2010 Two-component signal transduction. *Curr. Opin Microbiol.* **13**, 113–115. (doi:10.1016/j.mib.2010.02.003)
18. Khorchid A, Ikura M. 2006 Bacterial histidine kinase as signal sensor and transducer. *Int. J. Biochem. Cell Biol.* **38**, 307–312. (doi:10.1016/j.biocel.2005.08.018)
19. Thomason P, Kay R. 2000 Eukaryotic signal transduction via histidine-aspartate phosphorelay. *J. Cell Sci.* **113**, 3141–3150.
20. Herivaux A, So YS, Gastebois A, Latge JP, Bouchara JP, Bahn YS, Papon N. 2016 Major sensing proteins in pathogenic fungi: the hybrid histidine kinase family. *PLoS Pathog.* **12**, e1005683. (doi:10.1371/journal.ppat.1005683)
21. Defosse TA *et al.* 2015 Hybrid histidine kinases in pathogenic fungi. *Mol. Microbiol.* **95**, 914–924. (doi:10.1111/mmi.12911)
22. Ninfa AJ, Magasanik B. 1986 Covalent modification of the GlnG product, Nri, by the Glnl product, NRii, regulates the transcription of the GlnAlg operon in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **83**, 5909–5913. (doi:10.1073/pnas.83.16.5909)
23. Mascher T. 2014 Bacterial (intramembrane-sensing) histidine kinases: signal transfer rather than stimulus perception. *Trends Microbiol.* **22**, 559–565. (doi:10.1016/j.tim.2014.05.006)
24. Jung K, Fried L, Behr S, Heermann R. 2012 Histidine kinases and response regulators in networks. *Curr. Opin Microbiol.* **15**, 118–124. (doi:10.1016/j.mib.2011.11.009)
25. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. 2006 The QseC sensor kinase: a bacterial adrenergic receptor. *Proc. Natl Acad. Sci. USA* **103**, 10 420–10 425. (doi:10.1073/pnas.0604343103)
26. Groisman EA. 2001 The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* **183**, 1835–1842. (doi:10.1128/Jb.183.6.1835-1842.2001)
27. Park SY, Groisman EA. 2014 Signal-specific temporal response by the *Salmonella* PhoP/PhoQ regulatory system. *Mol. Microbiol.* **91**, 135–144. (doi:10.1111/mmi.12449)
28. Trajtenberg F *et al.* 2014 Allosteric activation of bacterial response regulators: the role of the cognate histidine kinase beyond phosphorylation. *mBio* **5**, e02105. (doi:10.1128/mBio.02105-14)
29. Scharf BE. 2010 Summary of useful methods for two-component system research. *Curr. Opin Microbiol.* **13**, 246–252. (doi:10.1016/j.mib.2010.01.006)
30. Pfluger T *et al.* 2018 Signaling ammonium across membranes through an ammonium sensor histidine kinase. *Nat. Commun.* **9**, 164. (doi:10.1038/s41467-017-02637-3)
31. da Silva ACR *et al.* 2002 Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* **417**, 459–463. (doi:10.1038/417459a)
32. Qian W *et al.* 2005 Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome Res.* **15**, 757–767. (doi:10.1101/gr.3378705)
33. Vorholter FJ *et al.* 2008 The genome of *Xanthomonas campestris* pv. *campestris* B100 and its use for the reconstruction of metabolic pathways involved in xanthan biosynthesis. *J. Biotechnol.* **134**, 33–45. (doi:10.1016/j.jbiotec.2007.12.013)
34. Qian W, Han ZJ, He C. 2008 Two-component signal transduction systems of *Xanthomonas* spp.: a lesson from genomics. *Mol. Plant Microbe Interact.* **21**, 151–161. (doi:10.1094/MPMI-21-2-0151)
35. Qian W, Han ZJ, Tao J, He C. 2008 Genome-scale mutagenesis and phenotypic characterization of two-component signal transduction systems in *Xanthomonas campestris* pv. *campestris* ATCC 33913. *MPMI* **21**, 1128–1138. (doi:10.1094/MPMI-21-8-1128)
36. Karniol B, Vierstra RD. 2004 The HWE histidine kinases, a new family of bacterial two-component sensor kinases with potentially diverse roles in environmental signaling. *J. Bacteriol.* **186**, 445–453. (doi:10.1128/JB.186.2.445-453.2004)
37. Tang JL, Liu YN, Barber CE, Dow JM, Wootton JC, Daniels MJ. 1991 Genetic and molecular analysis of a cluster of *Rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. *Mol. Gen. Genet.* **226**, 409–417.
38. Peng BY, Pan Y, Li RJ, Wei JW, Liang F, Wang L, Wang FF, Qian W. 2017 An essential regulatory system originating from polygenic transcriptional rewiring of PhoP-PhoQ of *Xanthomonas campestris*. *Genetics* **206**, 2207–2223. (doi:10.1534/genetics.117.200204)
39. Wang FF, Cheng ST, Wu Y, Ren BZ, Qian W. 2017 A bacterial receptor PcrK senses the plant hormone cytokinin to promote adaptation to oxidative stress. *Cell Rep.* **21**, 2940–2951. (doi:10.1016/j.celrep.2017.11.017)
40. Li RF *et al.* 2014 Identification of a putative cognate sensor kinase for the two-component response regulator HrpG, a key regulator controlling the expression of the *hrp* genes in *Xanthomonas campestris* pv. *campestris*. *Environ. Microbiol.* **16**, 2053–2071. (doi:10.1111/1462-2920.12207)
41. Rosen BP, Mcclees JS. 1974 Active-transport of calcium in inverted membrane-vesicles of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **71**, 5042–5046. (doi:10.1073/pnas.71.12.5042)
42. Jung K, Tjaden B, Altendorf K. 1997 Purification, reconstitution, and characterization of KdpD, the turgor sensor of *Escherichia coli*. *J. Biol. Chem.* **272**, 10 847–10 852. (doi:10.1074/jbc.272.16.10847)
43. Reading NC, Rasko D, Torres AG, Sperandio V. 2010 A transcriptome study of the QseEF two-component system and the QseG membrane protein in enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology-SGM* **156**, 1167–1175. (doi:10.1099/mic.0.033027-0)
44. Reading NC, Rasko DA, Torres AG, Sperandio V. 2009 The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc. Natl Acad. Sci. USA* **106**, 5889–5894. (doi:10.1073/pnas.0811409106)
45. Bayburt TH, Sligar SG. 2010 Membrane protein assembly into Nanodiscs. *FEBS Lett.*. **584**, 1721–1727. (doi:10.1016/j.febslet.2009.10.024)
46. Ritchie TK, Grinkova YV, Bayburt TH, Denisov IG, Zolnerciks JK, Atkins WM, Sligar SG. 2009 Reconstitution of membrane proteins in phospholipid bilayer nanodiscs. *Method Enzymol.* **464**, 211–231. (doi:10.1016/S0076-6879(09)64011-8)
47. Cassat JE, Skaar EP. 2013 Iron in infection and immunity. *Cell Host Microbe* **13**, 510–520. (doi:10.1016/j.chom.2013.04.010)
48. Nairz M, Haschka D, Demetz E, Weiss G. 2014 Iron at the interface of immunity and infection. *Front. Pharmacol.* **5**, 152. (doi:10.3389/Fphar.2014.00152)
49. Zhang SS *et al.* 2008 A putative colR(XC1049)-colI(XC1050) two-component signal transduction system in *Xanthomonas campestris* positively regulates *hrpC* and *hrpE* operons and is involved in virulence, the hypersensitive response and tolerance to various stresses. *Res. Microbiol.* **159**, 569–578. (doi:10.1016/j.resmic.2008.06.010)
50. Wang L, Pan Y, Yuan ZH, Zhang H, Peng BY, Wang FF, Qian W. 2016 Two-component signaling system VgrRS directly senses extracytoplasmic and intracellular iron to control bacterial adaptation under iron depleted stress. *PLoS Pathog.* **12**, e1006133. (doi:10.1371/journal.ppat.1006133)
51. Xu RQ *et al.* 2008 AvrAC(Xcc8004), a type III effector with a leucine-rich repeat domain from *Xanthomonas campestris* pathovar *campestris* confers avirulence in vascular tissues of *Arabidopsis thaliana* ecotype Col-0. *J. Bacteriol.* **190**, 343–355. (doi:10.1128/Jb.00978-07)
52. Yan Q, Wang NA. 2011 The ColR/ColS two-component system plays multiple roles in the pathogenicity of the citrus canker pathogen *Xanthomonas citri* subsp *citri*. *J. Bacteriol.* **193**, 1590–1599. (doi:10.1128/Jb.01415-10)
53. Kreamer NN, Wilks JC, Marlow JJ, Coleman ML, Newman DK. 2012 BqsR/BqsS constitute a two-component system that senses extracellular Fe(II) in *Pseudomonas aeruginosa*. *J. Bacteriol.* **194**, 1195–1204. (doi:10.1128/JB.05634-11)
54. Chen HD, Groisman EA. 2013 The biology of the PmrA/PmrB two-component system: the major

regulator of lipopolysaccharide modifications. *Annu. Rev. Microbiol.* **67**, 83–112. (doi:10.1146/annurev-micro-092412-155751)

55. Rutherford ST, Bassler BL. 2012 Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harbor Perspect. Med.* **2**, a012427. (doi:10.1101/cshperspect.a012427)
56. Wang LH *et al.* 2004 A bacterial cell–cell communication signal with cross-kingdom structural analogues. *Mol. Microbiol.* **51**, 903–912. (doi:10.1046/j.1365-2958.2003.03883.x)
57. He YW, Zhang LH. 2008 Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiol. Rev.* **32**, 842–857. (doi:10.1111/j.1574-6976.2008.00120.x)
58. Deng YY, Wu JE, Tao F, Zhang LH. 2011 Listening to a new language: DSF-based quorum sensing in Gram-negative bacteria. *Chem. Rev.* **111**, 160–173. (doi:10.1021/cr100354f)
59. Zhou L, Zhang LH, Camara M, He YW. 2017 The DSF family of quorum sensing signals: diversity, biosynthesis, and turnover. *Trends Microbiol.* **25**, 293–303. (doi:10.1016/j.tim.2016.11.013)
60. Ryan RP, An SQ, Allan JH, McCarthy Y, Dow JM. 2015 The DSF family of cell–cell signals: an expanding class of bacterial virulence regulators. *PLoS Pathog.* **11**, e1004986. (doi:10.1371/journal.ppat.1004986)
61. He YW, Wang C, Zhou L, Song HW, Dow JM, Zhang LH. 2006 Dual signaling functions of the hybrid sensor kinase RpfC of *Xanthomonas campestris* involve either phosphorelay or receiver domain-protein interaction. *J. Biol. Chem.* **281**, 33 414–33 421. (doi:10.1074/jbc.M606571200)
62. Slater H, Alvarez-Morales A, Barber CE, Daniels MJ, Dow JM. 2000 A two-component system involving an HD-GYP domain protein links cell–cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol. Microbiol.* **38**, 986–1003. (doi:10.1046/j.1365-2958.2000.02196.x)
63. Deng YY *et al.* 2012 *Cis*-2-dodecenoic acid receptor RpfR links quorum-sensing signal perception with regulation of virulence through cyclic dimeric guanosine monophosphate turnover. *Proc. Natl Acad. Sci. USA* **109**, 15 479–15 484. (doi:10.1073/pnas.1205037109)
64. An SQ, Allan JH, McCarthy Y, Febrer M, Dow JM, Ryan RP. 2014 The PAS domain-containing histidine kinase RpfS is a second sensor for the diffusible signal factor of *Xanthomonas campestris*. *Mol. Microbiol.* **92**, 586–597. (doi:10.1111/mmi.12577)
65. Cai Z, Yuan ZH, Zhang H, Pan Y, Wu Y, Tian XQ, Wang FF, Wang L, Qian W. 2017 Fatty acid DSF binds and allosterically activates histidine kinase RpfC of phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* to regulate quorum-sensing and virulence. *PLoS Pathog.* **13**, e1006304. (doi:10.1371/journal.ppat.1006304)
66. Pacheco AR, Curtis MM, Ritchie JM, Munera D, Waldor MK, Moreira CG, Sperandio V. 2012 Fucose sensing regulates bacterial intestinal colonization. *Nature* **492**, 113–117. (doi:10.1038/nature11623)
67. Vanstraelen M, Benkova E. 2012 Hormonal interactions in the regulation of plant development. *Annu. Rev. Cell Dev. Biol.* **28**, 463–487. (doi:10.1146/annurev-cellbio-101011-155741)
68. Kabbara S, Schmulling T, Papon N. 2018 CHASEing cytokinin receptors in plants, bacteria, fungi, and beyond. *Trends Plant Sci.* **23**, 179–181. (doi:10.1016/j.tplants.2018.01.001)
69. Naseem M, Bencurova E, Dandekar T. 2018 The cytokinin-activating LOG-family proteins are not lysine decarboxylases. *Trends Biochem. Sci.* **43**, 232–236. (doi:10.1016/j.tibs.2018.01.002)
70. Anantharaman V, Aravind L. 2001 The CHASE domain: a predicted ligand-binding module in plant cytokinin receptors and other eukaryotic and bacterial receptors. *Trends Biochem. Sci.* **26**, 579–582. (doi:10.1016/S0968-0004(01)01968-5)
71. Samanovic MI *et al.* 2015 Proteasomal control of cytokinin synthesis protects *Mycobacterium tuberculosis* against nitric oxide. *Mol. Cell* **57**, 984–994. (doi:10.1016/j.molcel.2015.01.024)