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We're in this together: Sensation of the host cell environment by endosymbiotic bacteria

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Abstract

Bacteria inhabit diverse environments, including the inside of eukaryotic cells. While a bacterial invader may initially act as a parasite or pathogen, a subsequent mutualistic relationship can emerge in which an endosymbiotic bacteria and its host share metabolites. While the environment of the host cell provides improved stability when compared to an extracellular environment, the endosymbiont population must still cope with changing conditions, including variable nutrient concentrations, the host cell cycle, host developmental programs, and host genetic variation. Furthermore, the eukaryotic host can deploy mechanisms actively preventing a bacterial return to a pathogenic state. Many endosymbionts are likely to use two-component systems (TCSs) to sense their surroundings, and expanded genomic studies of endosymbionts are likely to reveal how TCSs may promote bacterial integration with a host cell. We suggest that studying TCS maintenance or loss may be informative about the evolutionary pathway taken toward endosymbiosis, or even toward endosymbiont-to-organelle conversion.

1. Introduction

Numerically, prokaryotes dominate our planet (Whitman et al., 1998) and display metabolic proficiency and flexibility currently unmatched by eukaryotes (Goyal, 2018; Torsvik et al., 2002). To maintain their survival and propagation, all organisms must sense their surroundings. Toward this goal, bacteria have evolved a number of mechanisms that allow responses to their environment, including responses to other microorganisms of the same or different species.

One specific and peculiar environment that bacteria may inhabit is the inside of a eukaryotic cell. Occasionally, an endosymbiotic partnership can form in which two organisms appear to form a mutually beneficial relationship based upon syntrophy, or the sharing of metabolites (Morris et al., 2013). Upon establishment of an endosymbiont within its host, there is often a contraction of genome size prompted by redundancy of gene products (Bennett and Moran, 2015; Moran, 2003; Shigenobu et al., 2000) and small population size (Kuo et al., 2009). Primary bacterial endosymbionts have become firmly ensconced within their hosts and are typically engaged in mutual metabolic dependency with their eukaryotic partner. Secondary endosymbionts have typically initiated a more recent relationship with their host, are more often transmitted horizontally, potentially survive outside of the host cell, and closely skirt the line between parasitism and mutualism that may mark the progression to endosymbiosis (Zachar and Boza, 2020; McCutcheon et al., 2019; Sullivan, 2017; Pérez-Brocal et al., 2013; Sachs et al., 2011). For primary endosymbionts, even full-length host proteins may eventually be put to use by the endosymbiont (Nakabachi et al., 2014; Nowack and Grossman, 2012), and the use of host proteins by the endosymbiont may mark a major transition point that occurs during the rare conversion of an endosymbiont to an organelle (Keeling et al., 2015; McCutcheon and Keeling, 2014).

Should the environment inhabited by the endosymbiont be considered simple or complex? On the one hand, to the potential benefit of the endosymbiont, multiple features of the

environment are stabilized when compared to the environment outside of the eukaryotic host. Strict maternal transmission can limit exposure to phage (Metcalf and Bordenstein, 2012). Moreover, residence inside of a eukaryotic cell may provide protection against predation, consistent with the idea that predation can drive major evolutionary transitions (Herron et al., 2019; Boraas et al., 1998; Stanley, 1973). Ion concentration and pH within the eukaryotic host would be maintained within tight boundaries acceptable to the host, and therefore may be particularly suitable for many bacterial guests. In addition, an obligate endosymbiont can harvest any metabolite for which consumption does not lead to fitness costs for the host and selection against the conglomerate. Taken together, an endosymbiotic life strategy may be regarded as a simplified and hospitable environment.

On the other hand, the intracellular environment of an endosymbiont is not as simple as it may seem. Host and endosymbiont cell cycles are expected to be coordinated with the help of the appropriate bacterial signaling pathways (Catta-Preta et al., 2015), and host-derived antimicrobial peptides (AMPs) can be employed in a delicate dance between host and endosymbiont that prevents re-emergence of pathogenicity (Login et al., 2011). The nutritional status and life stage of its host may fluctuate, and the endosymbiont must regulate its number and behavior accordingly (Darby et al., 2012; Snyder et al., 2012; Stoll et al., 2009; Wilkinson et al., 2007; Fenn and Blaxter, 2004), even if the spectrum and scale of endosymbiont responses to its environment may eventually become diminished (Wilcox et al., 2003). Endosymbionts also regulate their gene expression in a manner concordant with the different tissues in which they may reside or, if ever transmitted between host cells, the extracellular environment (Darby et al., 2012; Bright and Bulgheresi, 2010). Moreover, beyond a more reactive use of sensing mechanisms, endosymbionts may manipulate the germline and somatic activities of their hosts (Foray et al., 2018; Pietri et al., 2016; Landmann et al., 2014; Fast et al., 2011; Serbus and Sullivan, 2007). Divergent host genotypes can present additional variation to which the endosymbiont must adjust its gene expression (Smith and Moran, 2020). Consequently, the habitats of endosymbionts may not be as simple as they may first appear, raising the possibility that robust sensation mechanisms might be maintained by some endosymbionts.

Here, we focus our attention upon two-component systems (TCSs), a versatile set of sensors and effectors used by a wide variety of bacteria to detect and respond to their environment. We highlight the small, but expanding number of studies focused upon endosymbiont sensation, and we argue that knowledge of TCS activities may be informative about the evolutionary histories of, and strategies deployed by, endosymbionts.

2. Fundamental Aspects of Two-Component Systems

TCSs are prominently used by bacteria to sense and respond to the environment (Gao et al., 2019; Jacob-Dubuisson et al., 2018; Zschiedrich et al., 2016). Individual bacterial species can encode tens, or even hundreds of TCSs (Borland et al., 2016), allowing responses to divergent signals that include myriad small molecules, temperature, gasses, and light (Krell et al., 2010). Within the context of a TCS, a histidine kinase (HK) component and a response regulator (RR) serve as a minimal set of polypeptides that can sense cellular conditions, yet this

arrangement can be markedly elaborated by additional regulatory pathway members (Gao et al., 2019; Jacob-Dubuisson et al., 2018; Zschiedrich et al., 2016). HKs involved in sensation are often membrane-bound, with sensor domains extending into the cytoplasm. Other HKs are membrane-inserted, yet lack periplasmic extensions, or can even be wholly cytoplasmic. HK domains used for signal detection are characterized by significant structural diversity, in accordance with the heterogeneous signals sensed by bacteria, but the catalytic core tends to be well conserved. The cytosolic portion of a HK, encompassing the autokinase domain, consists of a Dimerization and Histidine-phosphotransfer domain (DHp) and a Catalytic and ATP-binding domain (CA) connected by a short loop of amino acids. A diverse array of additional domains (Krell et al., 2010) contribute to protein-protein interactions and help to modulate autokinase activity.

HKs are typically found as homodimers for which autophosphorylation prompted by activation can occur in either a *cis*- or *trans*- fashion (Casino et al., 2009, 2014). Upon activation by the stimulus, which can be sensed even at relatively low binding affinities (Krell et al., 2010; Cheung and Hendrickson, 2009), the epsilon nitrogen of a conserved histidine in the DHp domain is phosphorylated by use of ATP (Bhate et al., 2015). Next, phosphotransfer to the appropriate RR is catalyzed, providing tight control of response to the stimulus. The RR is phosphorylated at a conserved aspartate, and the transfer of the phosphoryl group from the key HK histidine is driven primarily by the receiver (REC) domain of the RR (Zschiedrich et al., 2016) (Figure 1).

Specificity of signaling is mostly encoded at the interaction face between a given HK and its cognate RR (Podgornaia and Laub, 2013; Fisher et al., 1996), although specificity is also dependent upon proper stoichiometry of TCS components (Steiner et al., 2018). Not all HKs act exclusively with one RR; several HKs can share a particular RR and phenotypic outcome (Stephenson and Hoch, 2002). Hybrid HKs also exist for which the HK and RR are fused within the same polypeptide (Townsend et al., 2013; Capra et al., 2012), ensuring dedicated phosphorylation of the relevant RR. As well as providing kinase activity, HKs can also act as phosphatases, removing instances of direct RR phosphorylation by cellular acetyl-phosphate (Gao et al., 2019; Podgornaia and Laub, 2013; Klein et al., 2007) and blocking pathway activation when signal reception is concluded (Huynh and Stewart, 2011). Kinase activity of HKs does not simply correspond with the presence of ligand or other stimuli; kinase activity can instead be prompted by the lack of a signaling molecule or environmental condition (Neiditch et al., 2005; Henke and Bassler, 2004). HKs are often, but not always, found in the same operon with their cognate RRs (Capra and Laub, 2012). Of note, there can be additional elaboration upon the standard theme of the TCS, including complicated phosphorelay systems (Francis and Porter, 2019; Dworkin, 2015; Wright and Ulijasz, 2014). Recent evidence also suggests TCS cross-talk by HK phosphorylation of other HKs (Francis and Porter, 2019; Francis et al., 2018)

After the REC domain is phosphorylated, the conformation of RRs, and their multimerization state, can change (Gao et al., 2019; Galperin, 2006). Like HKs, RRs harbor many different functional domains that provide for regulatory complexity under diverse environmental conditions (Galperin, 2006). The majority of RRs bind to DNA, and upon DNA

binding, these factors can regulate transcription by functioning as activators or repressors, or they can block chromosome replication. These RRs can also manifest enzymatic activity and can regulate downstream processes by protein-protein interactions (Gao et al., 2019). Beyond signal shut-off by the phosphatase activity of HKs or by dedicated RR phosphatases (Zschiedrich et al., 2016; Pazy et al., 2010), RRs have the ability to auto-dephosphorylate (Gao et al., 2019).

3. Two-Component Systems of Endosymbionts

As the functions carried out by a bacterium become intertwined with that of the host, its genome becomes eroded as a result of reduced selection and population bottlenecks (Bennett and Moran, 2015; Kuo et al., 2009; Moran, 2003; Shigenobu et al., 2000). Like the more generalized metric of genome size, the number of TCSs may serve as a reflection of the relative duration of endosymbiont association with its host (Kim et al., 2010). Most evidence does indeed suggest that the number of HKs and RRs can be an order of magnitude lower in bacteria exclusively localized with a eukaryotic cell (Christensen and Serbus, 2015; Rikihisa, 2010; Wakeel et al., 2010; Cheng et al., 2006), with some endosymbionts and intracellular pathogens harboring few or no TCS pathways (Capra and Laub, 2012; Ashby, 2004). Those specific TCSs that are maintained the longest within the degenerating genome may be informative about key aspects of endosymbiont evolutionary history or current aspects of the mutualistic relationship between endosymbiont and host. Yet, the roles of TCSs encoded by endosymbionts are, to date, very poorly understood.

Perhaps the earliest study of TCSs in endosymbionts was focused upon *Bradyrhizobium japonicum*, a facultative endosymbiont that can obtain nitrogen from the atmosphere for soybeans and other legumes (Lardi et al., 2016; Ferguson et al., 2010). By a complex process initiated by plant metabolites, *B. japonicum* activates the appropriate transcriptional program to form a nodule within the plant root that becomes a suitable location for nitrogen fixation. An operon that includes the HK NodV and the RR NodW is required for nodulation (Göttfert et al., 1990), and subsequent work demonstrated that the vast majority of *B. japonicum* transcriptional targets activated by the soybean nodulation-promoting signal required phosphorylation of NodW by NodV (Lang et al., 2008). *B. japonicum* is not limited to the endosymbiotic lifestyle, but also inhabits the soil. In agreement with this *B. japonicum* life history, its genome is not diminished when compared to other, free-living bacteria, and, along with NodV and NodW this species can encode tens of additional HKs and approximately one hundred RRs (Kaneko et al., 2011).

To illuminate closer genetic and metabolic interdependencies between host and endosymbiont, efforts have been made to understand TCS signaling in Wolbachia, perhaps the most prominent model system for exploration of host-endosymbiont interactions. Wolbachia are intracellular bacteria from the alpha-proteobacterial Rickettsiae family that are widespread among arthropods and nematodes. While some Wolbachia interact with their hosts in a parasitic or pathogenic manner, other Wolbachia are mutualist endosymbionts required by their host for the provision of metabolites (Sullivan, 2017; Gutzwiller, 2016; Taylor et al., 2013; Darby et al., 2012). Wolbachia is mostly, although not exclusively, transmitted vertically through the female

germline (Werren, 1997), and these endosymbionts can be tightly associated with the ability of some of their pathogenic hosts to cause disease (Christensen and Serbus, 2015; Saint André et al., 2002).

To investigate the landscape of TCS signaling in endosymbionts, a comprehensive search for TCS components has been performed within several Wolbachia species (Christensen and Serbus, 2015). Similar to previous searches within the clade Anaplasmataceae, the number of HKs and RRs recovered by BLAST queries based upon the HK and RR sequences of free-living alpha-proteobacter *Caulobacter crescentus* was exceedingly low. These HKs and RRs were not found within the same operons but were scattered to different chromosomal locations and surrounded by genes for which a functional link to Wolbachia-encoded TCSs was unclear. Specifically, the HK CckA and the RR CtrA were identified in multiple Wolbachia species. These two proteins act within a phosphotransfer cascade controlling cell cycle progression in *C. crescentus* (Biondi et al., 2006; Jacobs et al., 2003), although there appears to be significant divergence among alpha-proteobacterial species when considering targets directly regulated by CtrA (Pini et al., 2015). Additionally, an ortholog of DvlL that lacked a carboxyl-terminal catalytic domain was encoded in a chromosomal location near the *ctrA* locus in several Wolbachia genomes. DvlL is predicted to be a possible potentiator of CckA HK activity, and Wolbachia DvlL harbors multiple Per-Arnt-Sim (PAS) domains, which are common among bacterial polypeptides involved in signal transduction.

Another TCS, consisting of the HK PleC and its target RR PleD, was found among multiple Wolbachia species. While many RRs are DNA-binding proteins (Gao et al., 2019), PleD instead harbors a GGDEF domain, named after a conserved sequence pattern, that may generate bis-(3′-5′)-cyclic diguanylic acid (c-di-GMP) through its diguanylate cyclase activity (Jenal et al., 2017; Lai et al., 2009; Paul et al., 2004). c-di-GMP is an important bacterial second messenger that binds to multiple downstream effectors and controls many processes including cell polarity, transition to biofilm formation, and virulence (Jenal et al., 2017; Valentini and Filloux, 2016; Trampari et al., 2015; Tschowri et al., 2014; Davis et al., 2013; Römling et al., 2013; Moscoso et al., 2012). Interestingly, CckA from *C. crescentus* was found to be directly regulated by c-di-GMP (Lori et al., 2015), suggesting that the co-existence of the CckA/CtrA and PleC/PleD TCSs within the same Wolbachia species is not coincidental and may have functional relevance.

The paucity of Wolbachia TCS components identified in the study described above is quite consistent with a relaxation of selection on, and subsequent loss of, many genes typically required by free-living bacteria. Yet, some TCSs have clearly been maintained, and some evidence supports the idea of positive selection on the PleD ortholog of the *w*Mel strain of Wolbachia pipientis (Brownlie et al., 2007). Moreover, experiments in which gene expression data of *W. pipientis w*Mel were followed during the life cycle of *Drosophila melanogaster* suggest that nearly 8% of Wolbachia genes are differentially expressed in a manner dependent upon sex or developmental stage (Gutzwiller et al., 2015). Intriguingly, one of the genes regulated in a stage-specific manner was CckA (Christensen and Serbus, 2015; Gutzwiller et

al., 2015), consistent with a role for this HK in responding to developmental cues provided by the host.

A consistent feature of endosymbiont establishment and maintenance within host organisms is likely to be a balance between sensitivity and tolerance to host-synthesized AMPs (Mergaert, 2018; Masson et al., 2016). After introduction and the initiation of a mutualistic relationship, endosymbionts may reside within special compartments, such as the bacteriocytes of tsetse flies or the trophosomes of the gutless tube worm *Riftia pachyptila*, and AMPs appear to prevent bacterial escape from some of these special structures (Bing et al., 2017; Masson et al., 2016; Bright et al., 2013; Login et al., 2011). Among other functions, the PhoP-PhoQ TCS, encoded by several gram-negative pathogens, plays a role in sensation of and response to host-synthesized AMPs (Bader et al., 2005), and the modification of lipopolysaccharide prompted by PhoP-PhoQ activation by AMPs can confer pathogen resistance to these antibacterial agents (Dalebroux and Miller, 2014; Groisman and Mouslim, 2006). Interestingly, changes to PhoP-PhoQ activity in endosymbionts can correspond with the transition to endosymbiotic mutualism. *Sodalis glossinidius* is a vertically transmitted gamma-proteobacterial endosymbiont that has only recently become established within its tsetse fly host (Chen et al., 1999). *S. glossinidius* appears to have a perpetually activated PhoP-PhoQ TCS that drives high AMP resistance, suggesting that at an early stage of endosymbiosis, resisting immune functions of the host remains important (Pontes et al., 2011). A sustained endosymbiotic strategy may correspond with a lack of selection for PhoP-PhoQ and consequent loss of this TCS, consistent with the establishment of confident mutualism less subject to reversion to a state of bacterial pathogenicity.

The PhoP HK and PhoQ RR appear again within the context of a different endosymbiont-mediated phenomenon: resistance of the pea aphid *Acyrthosiphon pisum* to the larvae of parasitic wasp *Aphidius ervi*. Defense of *A. pisum* can be provided by its facultative endosymbionts. To understand the genomic basis of this resistance, the genomic contents of two isolates of the endosymbiont *Regiella insecticola* that exhibit disparate capacities to defend against wasp parasites were examined (Hansen et al., 2012). Notably, the PhoP-PhoQ TCS was found to be associated specifically with the isolate that provided parasite resistance. Moreover, the PhoQ transcriptional target PqaA, also encoded by the resistance-conferring *R. insecticola* isolate, has previously been shown to block the activity of parasitoid venom peptides like melittin (Baker et al., 1997), raising the possibility that PhoP-PhoQ-PqaA can act as key modulators of pea aphid resistance. The aphid endosymbiont *Hamiltonella defensa*, which provides protection against parasitoid wasps, also encodes numerous TCS components (Degnan et al., 2009), although their role is not yet characterized.

We performed our own search for HKs and RRs in *Wigglesworthia glossinidia*, an obligate gamma-bacterial endosymbiont producing B vitamins for its tsetse fly host (Rio et al., 2012; Akman et al., 2002). A BLAST search using PFAM seed sequences revealed only an operon containing the HK CpxA (44% identity to *Escherichia coli* along aligned region) and the RR CpxR (75% identity to *Escherichia coli* along aligned region). The CpxA-CpxR-driven response can be prompted by protein folding stress in the inner membrane (Mitchell and

Silhavy, 2019). These findings suggest that changes to conditions within, or demands upon, the endosymbiont within the host may lead to inner membrane proteostasis defects that must be countered by a TCS-mediated transcriptional response.

While a pathway sensing membrane stress may be the last to be maintained by *W. glossinidia*, the genomic sequence of another endosymbiont appears to document the final loss of TCS signaling by destruction of its last HK. An intact, single RR with 93% alignment identity to *E. coli* OmpR is annotated within the genome of the *Cinara cedri* (aphid) endosymbiont *Serratia symbiotica* (*S. symbiotica* SCc), which is almost certainly in the midst of conversion from facultative symbiont to obligate endosymbiont (Lamelas et al., 2011). The OmpR protein is typically partnered in a TCS with the EnvZ protein. However, only a truncated EnvZ protein can be found in the same operon of *S. symbiotica* SCc by BLAST analysis, suggesting that the gene has been pseudogenized and is no longer required by the bacterium. Since this TCS appears to be the last to be lost from *S. symbiotica* SCc, and because OmpR-EnvZ TCS is involved in sensing osmotic stress and acidity, this result suggests that *S. symbiotica* SCc recently circumvented challenges associated with osmotic pressure and/or pH. Of note, the CpxA-CpxR pathway maintained in *W. glossinidia* is functionally connected to the EnvZ-OmpR system (Grabowicz and Silhavy, 2017), potentially indicating a need for further experimental emphasis on membrane biogenesis in endosymbionts. Of course, close examination of TCS loss from related endosymbionts making the same transition among similar host species would be necessary to accurately trace the particular stresses and demands encountered by endosymbionts as they become ever more established within their hosts.

4. Quorum Sensing Mechanisms in Endosymbionts

In order to coordinate collective behavior in response to the demands of the local environment, bacteria must sense and respond to members of the same species by use of quorum sensing mechanisms (Figure 2). Cooperative behavior regulated by quorum sensing includes biofilm production, expression of virulence factors, production of antibiotics, and antibiotic resistance (Abisado et al., 2018; Prüß, 2017; Rutherford and Bassler, 2012). Symbiosis is also modulated by quorum sensing, and indeed, initial efforts to understand quorum sensing focused upon bacterial luminescence by the symbiont *Vibrio fischeri* when it is localized to the light-producing organs of its bobtail squid host (Hastings and Nealson, 1977; Nealson et al., 1970).

Mechanisms of quorum sensing differ between gram-negative and gram-positive bacteria (Mukherjee and Bassler, 2019; Hmelo, 2017; Papenfort and Bassler, 2016). Gram-negative bacteria synthesize one or more acyl-homoserine lactones or “autoinducer 1” (AI-1) ligands when communicating with one another in a more specific manner. More generalized “autoinducer 2” (AI-2) signals, produced by use of the metabolite 4,5-dihydroxy-2,3-pentanedione, appear to allow communication between different species (Pereira et al., 2013). TCSs can play an important role in the detection of specific and general quorum sensing signals in gram-negative bacteria (Papenfort and Bassler, 2016). For example, the general quorum sensing molecule AI-2 binds, at high cell density, to the periplasmic LuxP protein of the

bioluminescent *Vibrio harveyi*. Ligand binding ensures that the phosphatase activity of the hybrid HK LuxQ predominates, resulting in activation of hundreds of genes (Ball et al., 2017). The more species-specific AI-1 appears to act through a different HK in *V. harveyi*, called LuxN. This ligand binds directly to its periplasmic domain and promotes its phosphatase activity, similarly resulting in the transcription of genes activated by elevated cell density.

For gram-positive bacteria, peptide-based ligands are often used for quorum-sensing (Bhatt, 2018; Lyon and Novick, 2004). Ligands are synthesized as pro-peptides and potentially processed before and after secretion. TCSs are often the mediators of these quorum sensing peptides. As examples, the AgrC-AgrA TCS binds the processed AgrD peptide to mediate toxin synthesis and virulence in the opportunistic pathogen *Staphylococcus aureus* (Wang and Muir, 2016), and competence in *Streptococcus pneumoniae* is promoted when peptide used for quorum sensing activates the ComD-ComE TCS, resulting in the upregulation of genes required for DNA uptake (Shanker and Federle, 2017).

Quorum sensing pathways are not limited to bacteria that live outside of a eukaryotic host. Quorum sensing occurs even in endosymbionts. For example, in the secondary endosymbiont *S. glossinidius*, quorum sensing regulates genes involved in the response to oxidative stress (Pontes et al., 2008), which is intriguing given the demonstrated relationship between population density and resistance to reactive oxygen species (Ma and Eaton, 1992). These targets of quorum sensing are also found in the closely related, obligate symbiont inhabiting the rice weevil *Sitophilus oryzae* (Pontes et al., 2008). Proteins involved in quorum sensing have also been identified in *H. defensa* (Degnan et al., 2009), which is mainly, although not exclusively, vertically transmitted (Li et al., 2018). Targets of quorum-sensing pathways can change significantly upon conversion of a free-living bacteria to an endosymbiont, and while quorum sensing is often associated with virulence, quorum sensing pathways may also serve as a check upon virulence to promote establishment of a mutualistic relationship between bacterium and host (Enomoto et al., 2017; Papenfort and Bassler, 2016; Winzer and Williams, 2001). We suggest that the host may even exploit endosymbiont quorum sensing pathways in order to maintain mutualism. Supporting the idea that eukaryotes can control bacterial pathogenicity by exploiting bacterial quorum sensing mechanisms, proliferation of the pathogen *Acinetobacter baumannii* can be hindered by its sensation of a fungus-produced farnesol within the context of a co-infection paradigm (Kobayashi and Crouch, 2009; Peleg et al., 2008).

So far, to our knowledge, TCSs have not been explicitly linked to quorum sensing in an endosymbiont, and ligand sensation by HKs are certainly not strictly required for quorum sensing (Colton et al., 2015; Urbanowski et al., 2004). However, TCSs should be expected to have a prominent role in intraspecies and interspecies communication by endosymbionts. Given the rapid expansion of endosymbiont genomes available and the well-characterized general role of TCSs in quorum sensing, we suggest that TCS involvement in endosymbiont quorum sensing should be a focus of future bioinformatic and experimental attention.

5. Two-Component Systems and Endosymbiont-to-Organelle Transitions

The ability of an endosymbiont to sense, respond to, and potentially defend itself against AMPs, likely mediated by TCSs, may be relevant to the frequency at which endosymbiont-to-organelle conversions may take place. What it means to be an 'organelle' remains ill-defined (Keeling and Archibald, 2008; Theissen and Martin, 2006). However, a bright line between mutualist endosymbiont and organelle is almost certainly crossed when the import of key host proteins into the endosymbiont becomes required for host survival. The question of how such an import mechanism can evolve is difficult, and the rarity of extant organelles derived from endosymbionts suggests that development of the required translocation machinery is not trivial (Cavalier-Smith and Lee, 1985). Recently, several instances in which host proteins are translocated through endosymbiont membranes have been identified (Bublitz et al., 2019; McCutcheon and Keeling, 2014; Nakabachi et al., 2014). Among these intriguing examples, the most prominent may be the import of hundreds of host proteins into the photosynthetic endosymbiont residing within the amoeba *Paulinella chromatophora* (Nowack and Grossman, 2012), which seems to have been captured in the midst of an endosymbiont-to-organelle transition.

Of the proteins imported from the host into the *P. chromatophora* endosymbiont, many substrates were reported to harbor amino-terminal sequences similar in structure to AMPs (Singer et al., 2017), although additional support for the idea that these regions are related to AMPs is warranted (Knopp et al., 2020). However, if these amino-termini do indeed have AMP-like activity, these findings, as well as others focused upon organelle targeting sequences, would raise the possibility that the initial import of host proteins into an endosymbiont may not require pre-existing translocation machinery. Instead, endosymbiont-directed proteins may instead self-translocate into or through membrane barriers by utilizing the biophysical properties of membrane-permeable domains mimicking or derived from AMPs (Mergaert et al., 2017; Wollman, 2016). As mentioned above, TCSs like the PhoP-PhoQ system can play a role in AMP resistance. Consequently, the link between AMP sensation and endosymbiont-to-organelle transitions will remain a topic of high interest for those studying the initial and continuing evolution of the eukaryotic cell.

The level of autonomy that the endosymbiont maintains over its most important activities during the endosymbiont-to-organelle transition may be reflected by the TCSs that it encodes, since any semblance of autonomy would require the ability to respond to the appropriate local signals (Allen, 1993, 2017). Interestingly, *P. chromatophora* encodes at least one HK protein clearly related to the NblS protein of the cyanobacterium *Synechococcus elongatus* (50% identity over alignment region). NblS can be involved in the sensation of multiple stressors (Ashby and Houmard, 2006), is commonly found in cyanobacteria (Morrison et al., 2005) and is linked to regulation of photosynthetic processes (Hsiao et al., 2004; van Waasbergen et al., 2002). The presence of NblS as one of the few TCSs remaining in the *P. chromatophora* endosymbiont is consistent with the idea that this endosymbiont maintains regulatory control over its metabolism and photosynthetic capacity.

6. Concluding Remarks and Perspective

In this chapter, we have described the current status of research into TCS signaling by endosymbionts. Although a number of endosymbiont TCS pathways have been discovered, most of these pathways remain uncharacterized. Yet, given the incredible pace with which new endosymbiont genomes are acquired and characterized, and taking into account the appropriately increasing interest in endosymbionts, we expect an increase in efforts to understand endosymbiont signal reception in the coming years. Moreover, genomic approaches will reveal which TCSs and downstream transcriptional programs might be most easily lost during integration of endosymbionts into their hosts, thereby revealing the stressors and factors most difficult for endosymbionts to circumvent. Finally, instances of host protein import into endosymbionts, implying potential endosymbiont-to-organelle conversion, continue to be identified, and the study of endosymbiont TCSs may be informative regarding organelle evolution.

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Figure Legends

Figure 1: Schematic representation of a typical two-component system structure. A two-component system is comprised of a histidine kinase (HK) and response regulator (RR). Perception of an extracellular signal by the sensor domain leads to the hydrolysis of ATP by the Catalytic and ATP-Binding Domain, and consequent phosphorylation of the central histidine (H) residue in the DHp domain of the HK. This phosphate is then transferred to the Aspartate (D) residue located in the receiver domain of the RR. Activation of the effector domain of the RR can prompt changes in gene expression to bring about an appropriate cellular response.

Figure 2: Quorum sensing allows bacteria to change behavior based upon the number of bacteria within the environment. Bacteria produce signaling molecules (denoted here as red spheres). An increase in the signaling molecule concentration allows the population to sense greater numbers. Upon reaching a particular population density, bacteria can respond with concerted group behavior.

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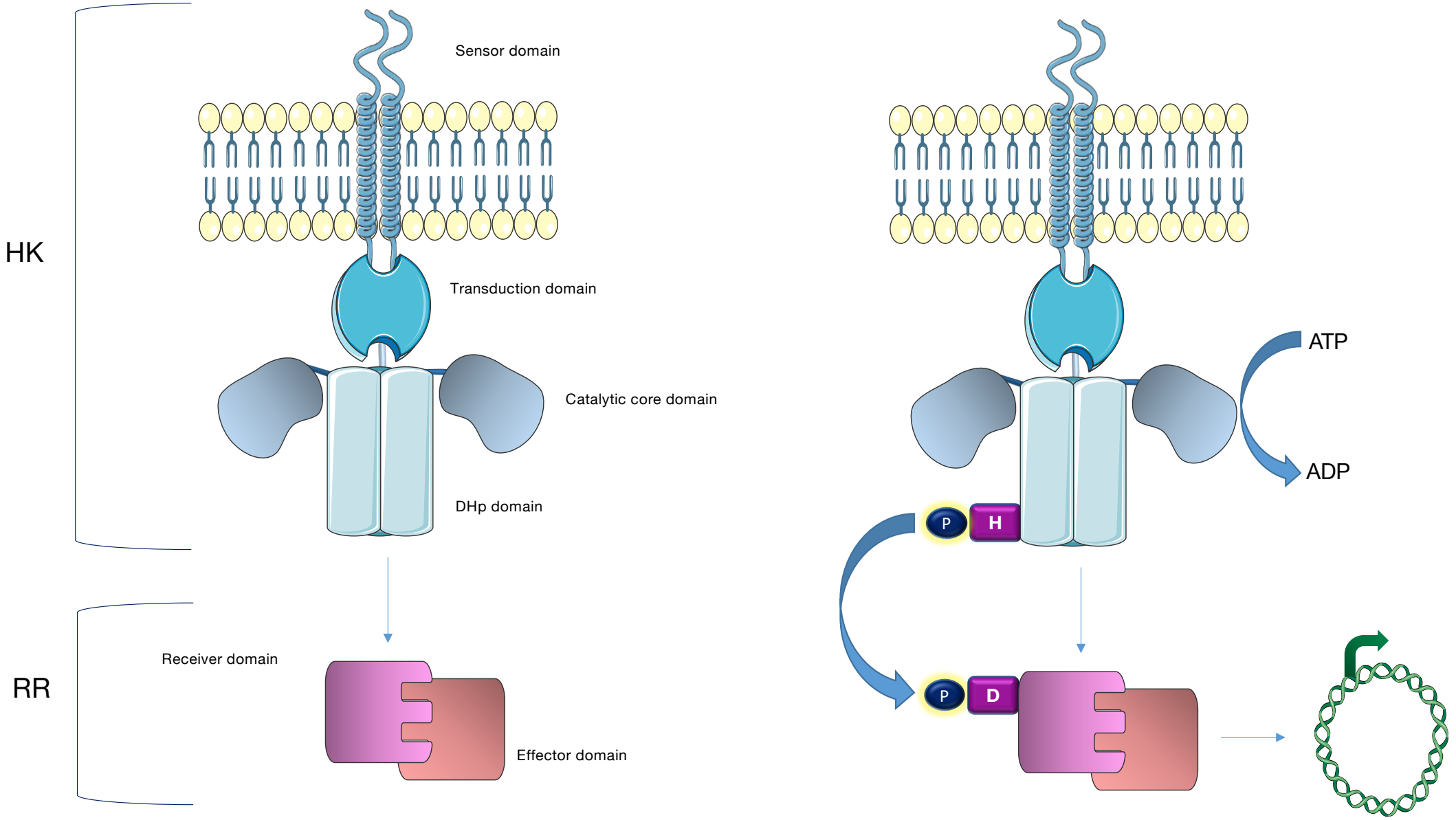
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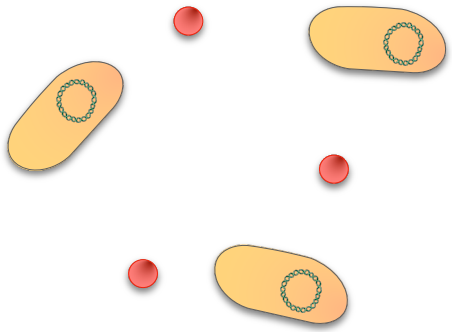
Low cell density



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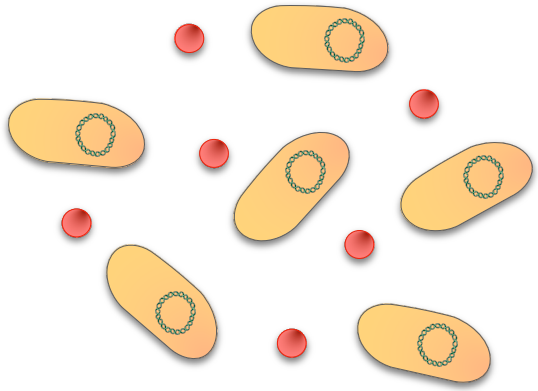
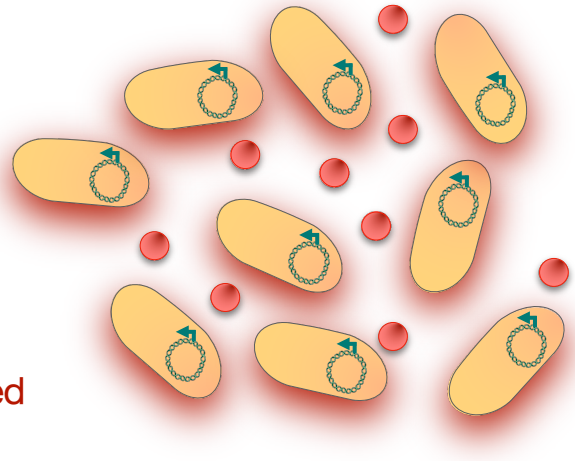


Signaling molecule concentration reaches the necessary threshold

High cell density



Bacteria manifest a concerted response