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Host/Microbe Interactions Revealed Through "Omics" in the Symbiosis Between the Hawaiian Bobtail Squid *Euprymna scolopes* and the Bioluminescent Bacterium *Vibrio fischeri*

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Abstract. The association between *Euprymna scolopes*, the Hawaiian bobtail squid, and *Vibrio fischeri*, a bioluminescent bacterium, has served as a model for beneficial symbioses for over 25 years. The experimental tractability of this association has helped researchers characterize many of the colonization events necessary for symbiosis. Recent technological advances, such as the sequenced genome of *V. fischeri*, DNA microarrays, and high-throughput transcriptomics and proteomics, have allowed for the identification of host and symbiont factors that are important in establishing and maintaining specificity in the association. We highlight some of these findings pertaining to quorum sensing, luminescence, responses to reactive oxygen and nitrogen species, recognition of microbe-associated molecular patterns by the innate immune system of the host, and a diel rhythm that helps regulate the symbiont population. We also discuss how comparative genomics has allowed the identification of symbiont factors important for specificity and why sequencing the host's genome should be a priority for the research community.

Introduction: Squid/*Vibrio* symbiosis

The association between the Hawaiian bobtail squid *Euprymna scolopes* and the bioluminescent marine bacterium *Vibrio fischeri* is an important natural model system for understanding host-bacterial interactions, including mechanisms of colonization and host-symbiont specificity and signaling between the innate immune system and symbiotic bacteria (McFall-Ngai, 2002, 2008: Nyholm and McFall-Ngai, 2004; Mandel, 2010; McFall-Ngai *et al.,* 2010). *V. fischeri* colonizes the epithelium-lined crypt spaces of a bilobed light organ that is located in the mantle cavity of the host (to 10^9 colony-forming units [CFUs]; Fig. 1A, B; Lee and Ruby, 1994; Nyholm and McFall-Ngai, 1998, 2004). This association is horizontally transmitted; thus newly hatched squid are colonized by environmental *V. fischeri* from a background of largely nonsymbiotic bacteria (approx. 10^6 cells/ml) in the surrounding seawater (Nyholm and McFall-Ngai, 2004). The symbiosis is also highly specific because only *V. fischeri* can colonize the light organ. This specificity begins in host-derived mucous secretions where *V. fischeri* is able to out-compete other bacteria before its cells travel through ciliated pores and ducts to reach the light organ crypts and grow to a density that promotes light production (luminescence) (Nyholm and McFall-Ngai, 2003, 2004). The host likely uses this light in an anti-predatory behavior termed counterillumination when it is foraging on tropical reefs at night (Jones and Nishiguchi, 2004). Once it has colonized the host, the symbiont initiates morphogenesis of the light organ whereby a superficial ciliated epithelium that secretes mucus and promotes colonization undergoes apoptosis and regression (Fig. 1C; McFall-Ngai and Ruby, 1991). In addition, colonization initiates a diel rhythm of the symbiont population that is hypothesized to help regulate the association and seed the environment with *V. fischeri* for future generations of hosts (Fig. 1D; Lee and Ruby, 1994; Boettcher *et al.,* 1996; Nyholm and McFall-Ngai, 1998). In 25 years of research, a number of symbiont signals that lead to distinct

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Abbreviations: MAMP, microbe-associated molecular pattern.

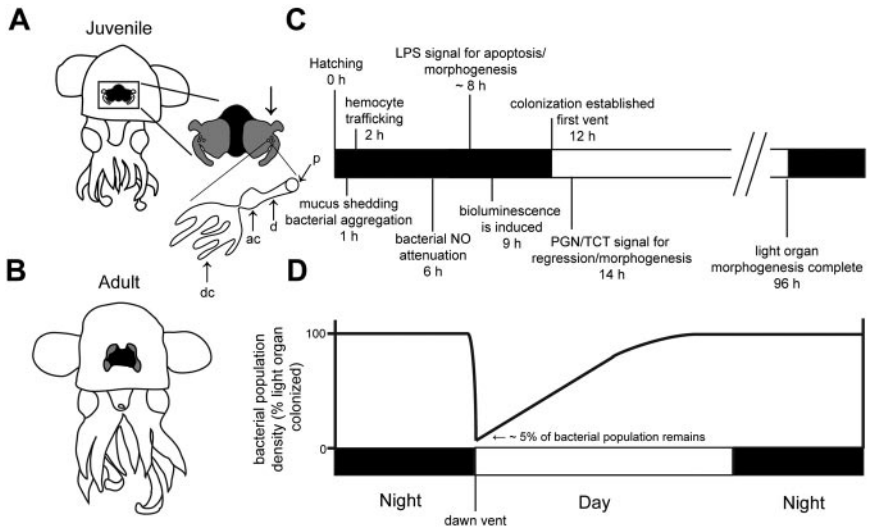


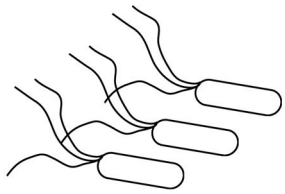
Figure 1. The *Euprymna scolopes*/*Vibrio fischeri* symbiosis. (A) Cartoon of the ventral surface of a juvenile squid. Suspended in the mantle cavity and enlarged is the nascent light organ, including the superficial ciliated epithelial fields (gray). Further enlarged is the interior architecture of the juvenile light organ of one crypt system, including the pore (p), duct (d), antechamber (ac), and the deep crypts (dc). (B) Cartoon of the ventral surface of an adult squid. Suspended in the mantle cavity is the mature light organ. (C) Timeline of some important early colonization events, starting with hatching and progressing through the first 96 h of the association. Hash marks show change in time scale. (D) Representation of the diel cycle exhibited by the light organ over a 24-h day/night period. During the evening, when the host is active and is engaged in counterillumination, the light organ has a full complement of *V. fischeri*. Each day at dawn, 95% of the symbionts are vented/expelled and the remaining 5% divide over the day to repopulate the organ.

host phenotypes have been described (Fig. 1C; Foster *et al.,* 2000; Koropatnick *et al.,* 2004; Nyholm and McFall-Ngai, 2004; McFall-Ngai *et al.*, 2012). This research has been facilitated by the relative ease with which the partners can be manipulated independently in the laboratory and mutant *V. fischeri* can be generated, and by the size and morphology of the juvenile host. These attributes make possible a variety of microscopy techniques that have allowed researchers to follow colonization *in vivo* (Nyholm and McFall-Ngai, 2004; Lee *et al.,* 2009). Recent advances in "omics" technologies have expanded the repertoire of methods being used to investigate this experimentally tractable association. Genomics, microarrays, transcriptomics, and proteomics have all been applied to the system and have yielded new discoveries. We highlight some of the recent findings using these methods and offer an opinion on how omics technologies might be applied to the squid/*Vibrio* association in the future (Fig. 2).

Vibrio fischeri—Quorum Sensing and Regulation of Luminescence

Quorum sensing, or density-dependent bacterial cell-cell communication, was first discovered in *V. fischeri* and plays a major role in regulating the light production that is critical for a functional symbiosis (reviewed in Miyashiro and Ruby, 2012). Quorum sensing in gram-negative bacteria relies on the secretion and accumulation of small signaling molecules, N-acyl homoserine lactones (also termed autoinducers), that initiate gene expression when a quorum (critical density of *V. fischeri* cells) is present. Luminescence, which is required for persistence of the symbiosis, is perhaps one of the best-studied phenotypes in the squid/*Vibrio* association (Miyashiro and Ruby, 2012). In *V. fischeri*, luminescence is regulated by multiple quorum-sensing systems (reviewed in Miyashiro and Ruby, 2012). Two of these, termed LuxR-LuxI (based on the expression of the *lux* operon containing the canonical genes for luminescence) and AinS-AinR, sequentially activate luminescence, with the *ain* system induced at moderate cell densities and positively regulating the *lux* system to maximally produce luminescence at high cell densities (Lupp *et al.,* 2003). In addition to regulation of the canonical light-producing genes, quorum sensing also allows for a coordinated expression of factors that are necessary for effective colonization of the host (Visick *et al.,* 2000; Lupp *et al.,* 2003; Lupp and Ruby, 2005).

Investigating whether this stepwise activation of quorum sensing was functional in the symbiosis, Lupp and Ruby (2005) found that knocking out *ainS*, the gene that encodes an *N*-octanoyl-homoserine lactone autoinducer, resulted in a colonization defect, suggesting that AinS regulates colonization factors. To address this, they used microarrays rep-



Host		Symbiont	
Technique	**Tissue**	**Technique**	**Strain**
Transcriptomics		Genomics	ES114, MJ11, SR5
EST libraries	Juvenile light organs		
454 cDNA library	Adult circulating hemocytes	Transcriptomics	
		Microarrays	ES114
Microarrays	Juvenile light organs		Adult light organ
	Adult light organ epithelia		populations
Proteomics	Adult light organ epithelia	Proteomics	Adult light organ exudate
	Adult light organ exudate		
	Adult circulating hemocytes		

Figure 2. A variety of "omics" methods have been applied to the squid/*Vibrio* association. The *V. fischeri* ES114 genome has furnished a wealth of information and provided targets for studying genes essential for symbiosis as well as allowing different strains of *V. fischeri* to be compared. Microarrays offer a method for whole-transcriptome analysis with emphasis on differential gene expression. Taking advantage of genomics and transcriptomics, microarrays have allowed squid/*Vibrio* researchers to identify both host and symbiont factors that are important for colonization and maintenance of the symbiosis, as well as to identify colonization targets for future research. Liquid chromatography-mass spectrometry (LC-MS/MS) has helped foster a new proteomics age in biology. LC-MS/MS and variations of this technique like multidimensional protein identification (MudPIT) made it cost-effective to identify proteins from complex biological samples. These techniques have been applied to identify host and symbiont proteins in the light organ as well as in the host's macrophage-like hemocytes. The host's genome has not yet been sequenced, so large expressed sequence tag (EST) libraries and transcriptomes of light organ tissues and hemocytes have provided critical host information.

resenting approximately 95% of the *V. fischeri* genome to compare transcriptomes of wild type and *ainS* mutant strains. Their results showed that expression of genes involved with motility, transcriptional regulation, metabolism, and exopolysaccharide production, and other genes of unknown function were either enhanced or repressed by *ain* quorum sensing. A later DNA microarray study performed by Antunes *et al.* (2007) identified a number of novel genes that were controlled by LuxR (the transcriptional activator that is induced by the *V. fischeri N*-3-oxohexanoyl-homoserine lactone autoinducer encoded by *luxI*). The addition of this *V. fischeri* autoinducer to wild type cultures was used in order to identify genes regulated by LuxR. Along with genes known to be involved with light production, 15 genes previously unknown to be regulated by autoinducer were also identified. Among these were proteases and peptidases, ABC-type transporters, a mechanosensitive ion channel, a peptidoglycan-binding protein, outer membrane proteins, and a bacterial immunoglobulin-like protein (Antunes *et al.,* 2007). While some of the genes identified in these studies—for example, those involved with motility—had previously been described as important for the symbiosis, others have revealed potential targets that have not been as well-characterized and may be important novel colonization determinants.

A recent study of the light organ crypt proteome also revealed a number of symbiont proteins previously identified from culture-grown cells (Schleicher and Nyholm, 2011). Among the symbiont proteins identified was a LuxR-regulated periplasmic protein, QsrP (originally described by Callahan and Dunlap, 2000), one of the most abundant symbiont proteins detected in the light organ but not in un-induced culture-grown *V. fischeri*. Also present was a putative surface protein with bacterial immunoglobulin-like domains, a homolog of which has been implicated in mediating host cell adhesion in pathogenic associations (Lin *et al.,* 2010). Although these proteins remain functionally uncharacterized, they do represent targets of interest and highlight the strength of applying multiple omics strategies to identify and confirm gene and protein expression.

In an effort to understand how *V. fischeri* luminescence influences the expression of host genes important for early colonization events, Chun *et al.* (2008) used microarrays containing the 13,962 unique host transcripts identified from a database of light organ expressed sequence tags (ESTs) (Chun *et al.,* 2006) to characterize gene expression

from juvenile light organs. They harvested light organ tissues from hosts at 18-h post-colonization, when the bacterial population is dense and brightly luminescent. They compared a number of different conditions: the wild type symbiotic state, the uncolonized (aposymbiotic) state, colonization with a non-luminescent *V. fischeri luxA* mutant, colonization with a *luxI* mutant that does not produce autoinducer, and supplementation with exogenous *lux* autoinducer. These gene expression comparisons showed a hierarchy of host transcriptional responses depending on the colonization state. The most changes in host gene expression were in response to colonization by wild type *V. fischeri*, followed by the symbiont's ability to produce light, and finally the presence of exogenous autoinducer. Interestingly, the addition of exogenous autoinducer had the most dramatic effect on host transcription when the light organ was already colonized with *V. fischeri*, suggesting that the host may respond to differing concentrations of autoinducer.

Underscoring the importance of luminescence to the host, previous studies showed that *V. fischeri lux* mutants deficient in light production can colonize the host but do not persist in the light organ (Visick *et al.,* 2000; Bose *et al.,* 2008). Furthermore, these dark mutants are outcompeted by wild type *V. fischeri* in mixed colonization assays. Taken together, these results suggest that the host can somehow perceive and respond to bacterial bioluminescence, perhaps out of a necessity to prevent strains of light-deficient "cheaters" from persisting in the light organ. Exposure to *lux* mutants leads to altered host gene expression and also a failure to fully induce a number of host phenotypes normally associated with colonization by wild type *V. fischeri*, including hemocyte trafficking and light organ morphogenesis (reviewed in McFall-Ngai *et al.,* 2012). A study using transcriptomic analyses of light organs from juvenile squid revealed visual transduction proteins necessary to receive and respond to light (Tong *et al.,* 2009). Electroretinograms and immunocytochemistry confirmed that the light organ tissues do indeed respond directly to light stimuli and express phototransduction proteins in the crypt spaces. These findings provide a possible molecular mechanism by which the host monitors the efficiency of its symbionts, as measured by light production. How or if the host regulates and removes "cheaters" remains to be determined, but colonization with a *lux* mutant did lead to a dramatic decrease in light organ hemocyanin, the main oxygen-binding protein of the host, and may be a contributing factor by which unproductive symbionts are selected against (Chun *et al.,* 2008).

Vibrio fischeri—Response to Reactive Nitrogen and Oxygen Species (RNS and ROS)

Nitric oxide (NO) has powerful antimicrobial and signaling activities and can mediate bacterial responses to varying conditions in hosts. Many bacteria contain a gene, *h-nox*, that belongs to a family of putative NO sensors. During the initial colonization of the squid, the symbiont is exposed to host-derived NO and nitric oxide synthase (NOS), the enzyme responsible for producing NO both outside the host in the secreted mucus and inside the ducts and antechamber leading to the light organ crypt spaces (Davidson *et al.,* 2004). Colonization by *V. fischeri* or exposure to specific bacterial cell wall and outer membrane components also causes an attenuation of both NO and NOS in the light organ (Davidson *et al.,* 2004; Altura *et al.,* 2011). The *V. fischeri* genome contains an *h-nox* homolog, and a recent study showed that H-NOX$_{vf}$ protein binds NO *in vitro* (Wang *et al.,* 2010). Microarrays were used to determine if NO sensing by H-NOX$_{vf}$ initiates a NO-dependent transcriptional response. Transcripts from a wild type strain of *V. fischeri* were compared to an *h-nox*$_{vf}$ mutant, in the presence and absence of exogenous NO. Genes that are involved in detoxification of NO were upregulated in both the wild type and *h-nox*$_{vf}$ mutant strains, suggesting that *h-nox*$_{vf}$ is not required for defense against NO. However, a suite of differentially expressed genes were either upregulated or downregulated in the presence of NO in the wild type strain but not in the mutant strain, suggesting that these genes are *h-nox*$_{vf}$-dependent. It was further shown that 8 out of 10 of the downregulated genes contained a promoter sequence motif similar to the master iron-responsive regulator Fur (ferric uptake regulator) of *Escherichia coli*. A search of the *V. fischeri* genome for other genes with putative Fur-binding sites revealed 48 Fur targets that showed expression patterns similar to each other, suggesting that expression of the Fur regulon is responsive to NO in an H-NOX$_{vf}$-dependent manner (Wang *et al.,* 2010). Surprisingly, the *h-nox*$_{vf}$ mutant also displayed increased symbiotic competence in comparison with wild type *V. fischeri*. Taken together, these data suggest that the role of H-NOX$_{vf}$ binding to NO may be to sense the external environment and modulate iron acquisition.

In addition to RNS, the host has other ROS that are thought to help regulate specificity of the squid/*Vibrio* association. For example, a halide peroxidase (EsHPO) that was previously found in the light organ is thought to help generate hypohalous acid, a potent microbicidal compound (Weis *et al*., 1996). Besides EsHPO, several other peroxidases were found in the proteome of the light organ exudate (Schleicher and Nyholm, 2011), confirming that the crypts are likely an oxidatively stressful microenvironment. To counteract these host chemical assaults, the symbiont produces a periplasmic catalase (KatA) that may reduce hydrogen peroxide availability for host EsHPO (Visick and Ruby, 1998). KatA was also detected in the light organ proteome, along with a number of other putative antioxidant enzymes, suggesting that *V. fischeri* can respond to the host's ROS challenges (Schleicher and Nyholm, 2011).

Interactions With the Innate Immune System of the Host

Increasing evidence shows that the host responds to microbe-associated molecular patterns (MAMPs) through all stages of the symbiosis (Koropatnick *et al.,* 2004; Nyholm and McFall-Ngai, 2004; McFall-Ngai *et al.,* 2010; Krasity *et al.,* 2011). One of the striking host phenotypes induced by the symbiont is the apoptosis and regression of superficial ciliated fields that assist *V. fischeri* in colonizing the nascent light organ (McFall-Ngai and Ruby, 1991). Years of research culminated with the discoveries that the MAMPs tracheal cytotoxin (TCT; a derivative of peptidoglycan [PGN, a bacterial cell wall component]) and lipopolysaccharide (LPS, an outer membrane component of gram-negative bacteria) trigger the morphogenetic program of the host (Fig. 1; Foster *et al.,* 2000; Koropatnick *et al.,* 2004). How does the host respond to these MAMPs? Transcriptomic and proteomic analyses have identified a number of pattern recognition receptors (PRRs) in the host (Goodson *et al.,* 2005; Chun *et al.,* 2006; Troll *et al.,* 2009, 2010; Krasity *et al.,* 2011; Schleicher and Nyholm, 2011; Collins *et al.,* 2012). An initial EST database composed of juvenile light organs identified members of the evolutionarily conserved Toll/NFκB pathway, including a Toll-like receptor and four peptidoglycan recognition proteins (Goodson *et al.,* 2005). Microarray analyses of symbiotic and aposymbiotic light organs at 18-h post-inoculation also identified a number of transcripts differentially regulated by the presence of the symbiont (Chun *et al.,* 2008). These included transcription factors or components of the signal transduction pathways, such as the NF-κB and MAP-kinase pathways. Specifically, LPS binding protein (LBP) and PGN recognition proteins (PGRP) EsLBP and EsPGRP1 and 2 were upregulated by the presence of the symbiont. Given that the morphogenesis of the light organ is triggered by these two MAMPs, it is not surprising that putative host receptors were also upregulated. Downregulated transcripts included those involved in the synthesis and maintenance of ciliated epithelia and in the visual transduction cascade. Further analyses have shown a diversity of host PRRs. Three LBPs have now been proposed in *E. scolopes* (Krasity *et al.,* 2011). Two are predicted to be secreted and one expressed in the nucleus and/or cytosol (Krasity *et al.,* 2011). Furthermore, in colonized juveniles, EsLBP1 mRNA expression was upregulated and the protein was also localized to the symbiont-containing crypt spaces (Chun *et al.*, 2008; Krasity *et al.,* 2011). EsPGRPs are also diverse and may play multiple roles in the association (Troll *et al.,* 2009, 2010). For example, EsPGRPs in juvenile light organs change cellular localization after colonization. Several of these proteins also have predicted and, in the case of EsPGRP2, demonstrated amidase activity and are likely capable of degrading bacterial peptidoglycan and/or TCT. A gel-based proteomic study of aposymbiotic and symbiotic juveniles over the first 96 h of the association revealed a number of symbiont-induced changes in the soluble light organ proteome (Doino Lemus and McFall-Ngai, 2000). Although specific proteins were not identified, applying techniques based on liquid chromatography-mass spectrometry may reveal further components of the innate immune system and other factors involved with mediating morphogenesis of the light organ.

The host's major innate cellular defense is the macrophage-like hemocyte, a cell-type that has been shown to be important in mediating interactions with *V. fischeri* (Nyholm and McFall-Ngai, 1998; Koropatnick *et al.,* 2007; Nyholm *et al.,* 2009). Hemocytes migrate into the superficial epithelium of the light organ in response to the symbiont and have also been found in the light organ crypt spaces. Adult hemocytes can differentiate between symbiotic and nonsymbiotic bacteria, and colonization state influences the ability of these cells to specifically recognize *V. fischeri,* suggesting that the host's immune system develops symbiont "tolerance" (Nyholm *et al.,* 2009). To begin to understand the molecular basis of these interactions, both a transcriptome and proteome were analyzed for adult circulating hemocytes (Collins *et al.,* 2012). This study revealed a number of innate immunity genes and proteins, including a fifth PGRP (EsPGRP5) that was among the most abundant hemocyte transcripts. EsPGRP5 is predicted to be intracellular and possibly even secreted. It has conserved amino acid residues for amidase activity, suggesting EsPGRP5 may interact with and degrade bacterial peptidoglycan both inside and outside the cell. The hemocyte proteome also revealed a number of complement-like proteins, including the previously described EsC3 (an essential component of all complement-like systems; Castillo *et al.,* 2009) that may also serve an important role in the symbiosis. Quantitative RT-PCR showed that several innate immunity transcripts, including EsPGRP5, EsC3, and EsNOS, are differentially expressed in hemocytes from fully symbiotic *versus* cured (symbiont-free) hosts, suggesting that *V. fischeri* can regulate gene expression of the host's innate immune system (Collins *et al.,* 2012).

Understanding the Diel Rhythm

As part of the daily rhythm of the symbiosis, the host expels up to 95% of the bacterial population each day at dawn (Fig. 1; Boettcher *et al.,* 1996; Nyholm and McFall-Ngai, 1998). During the day, the remaining bacteria grow in numbers such that there is a full complement of bioluminescent symbionts when the host is active during the night. This diel cycle is a hallmark of the squid/*Vibrio* association and is thought to be a product of communication between the partners. Perhaps one of the most exciting uses of microarrays in the squid/*Vibrio* symbiosis was an analysis

of the transcriptomes of both the host light organ epithelia and the symbionts over a 24-h day/night cycle (Wier *et al.,* 2010). In an effort to understand how the host epithelium coordinates the growth and metabolic activity of the symbiont while maintaining a stable population, four time points were chosen for microarray analyses over a 24-h period, including directly before and after the dawn venting. In the host, the time interval before dawn and the interval after dawn showed the highest differential gene expression. The symbiont, while also having the highest differential gene expression during these two time intervals, displayed a more uniform pattern of up- or downregulated genes. Both symbiont and host showed changes in the expression of a number of metabolism and signaling genes during the same time intervals, suggesting that the hours immediately before and after dawn represent a dynamic period of interaction between the partners.

Among the host genes most dramatically influenced were those involved with immune and stress responses. There was also an enrichment of gene expression related to cytoskeletal proteins, and microscopy of the light organ epithelium revealed that, in the hours surrounding dawn, the epithelia became effaced, with the apical surfaces blebbed into the crypt spaces. The epithelium recovered, however, and was restored within hours after venting. These data suggest that the diel fluctuation in the symbiont population is coordinated with a dramatic restructuring of the light organ epithelium. In the time interval before the dawn expulsion, the symbiont increased expression of genes required for the catabolism of chitin, which has recently been reported to be present in circulating adult hemocytes and juvenile light organ ducts (Heath-Heckman and McFall-Ngai, 2011; Mandel *et al*., 2012). In contrast, after the expulsion event, chitin utilization genes were downregulated while symbiont genes involved in catabolism of glycerol were upregulated. This finding suggests that the symbiont may dynamically regulate metabolic strategies, perhaps cycling between the fermentation of chitin and the anaerobic respiration of glycerol. These data also suggest that the host provides different substrates to the symbiont during different times of the day. This study has given unprecedented insight into the daily interactions between the host and symbiont, and has led researchers to start developing hypotheses for understanding how the association is maintained (Troll *et al.,* 2010; Rader *et al.,* 2012).

Many of the transcripts identified as being differentially regulated in Wier *et al*. (2010) were confirmed in a recent proteomics study that analyzed both the host and symbiont proteins in the exudate expelled at dawn (Schleicher and Nyholm, 2011). These analyses putatively identified 1581 proteins (870 from the host and 711 from the symbiont). Many of these proteins were also found in the transcriptome—for example, innate immunity proteins like PGRPs and host and symbiont factors involved with generating and responding to ROS and RNS. As seen from this and other studies, dawn represents a dramatic transition in this symbiosis for both the host and symbiont. While the host's crypt epithelium undergoes cellular changes, the symbiont transitions from the microenvironment of the light organ to a free-living bacterium in seawater. Surprisingly, a number of symbiont proteins involved with bacterial flagellar production were also detected, suggesting that the symbionts may anticipate the transition from the squid host to the free-living environment by switching from a non-motile light organ stage to a free-living motile stage. Transcript analyses of free-living *versus* symbiotic bacteria from two other bioluminescent squids (*Euprymna tasmanica* and *Uroteuthis chinensis*) also suggest that light organ bacteria exhibit different patterns of gene expression depending on their host or free-living environments (Guerrero-Ferreira and Nishiguchi, 2010; Jones and Nishiguchi, 2006).

Using Comparative Genomics to Elucidate Specificity Determinants

Understanding of the relationships between symbiotic bacteria and their metazoan hosts has been greatly enhanced by genomic analyses. *Vibrio fischeri* is a cosmopolitan bacterium, and many free-living and symbiotic strains have been isolated from a number of geographical locations and from different squid and fish hosts (Thompson *et al.,* 2004). To date, three *V. fischeri* genomes have been reported: the *E. scolopes* light organ symbiont strain ES114; strain MJ11 isolated from the Japanese pinecone fish (*Monocentris japonica*); and most recently, SR5, which was isolated from the Mediterranean sepiolid squid *Sepiola robusta* (Ruby *et al.,* 2005; Mandel *et al.,* 2008, 2009; Gyllborg *et al.,* 2012). The complete genome of *V. fischeri* ES114 was a tremendous resource for researchers in the squid/*Vibrio* field, providing insight into a number of genes known to be involved with the establishment and maintenance of the symbiosis and also revealing some surprises—for example, a number of putative toxins and other factors normally associated with pathogenic vibrios (Ruby *et al.,* 2005). Sequencing the genome of this strain also allowed for future comparisons between other *V. fischeri* genomes. One of the strengths of comparative genomics is that such analyses can lead to the detection of genes that may be necessary for responding to different environments, which for symbiotic bacteria may be different host types. In an elegant study, Mandel *et al.* (2009) compared the genome of *V. fischeri* ES114 to MJ11 (a poor colonizer of *E. scolopes*) to detect genes that may be important in regulating specificity of a squid *versus* fish host. They discovered that *rscS*, a two-component sensor kinase that acts upstream of the response regulator *sypG* (Visick and Skoufos, 2001; Hussa *et al.,* 2008) and enables *V. fischeri* to form biofilms and aggregate in the mucus of the juvenile squid, was missing from the MJ11 genome.

Introduction of the ES114 *rscS* gene enabled MJ11 to not only form biofilms, but also colonize the light organs of *E. scolopes* with the same efficiency as ES114. The ability to change symbiont host range by the insertion of a single regulatory gene highlights ways in which strains might adapt to expand their environmental range or change host niches. Given that a number of *V. fischeri* strains show differing abilities to colonize different sepiolid squid hosts (Nishiguchi *et al.,* 1998), comparative genomics will likely continue to be a powerful tool for understanding the foundations of symbiont specificity.

Host Genomics

Although transcriptomics and proteomics have revealed a number of genes and proteins essential to the functional symbiosis, a sequenced genome for the host is lacking. Like other cephalopods, *E. scolopes* has a large genome (C-value 3.75 pg $\approx$ 3.67 Gbp as estimated by Feulgen Image Analysis Densitometry; Gregory, 2001, 2012). In fact, no cephalopod genome has been published or released to date, and this omission has largely been due to technological and cost constraints. Recent advances in DNA sequencing and bioinformatics technologies, however, have dramatically lowered costs and increased efficiencies of large genome sequencing. Cephalopods represent an important invertebrate group for the study of a number of fields in biology, including developmental biology, host/microbe interactions, neurobiology, ecology, and evolution (Ruby, 1999; Grant *et al.,* 2006; Lee *et al.,* 2009; Mathger *et al.,* 2009; Leigh, 2010; Partridge, 2012). Besides serving as important biomedical models, members of this group are also critical to global fisheries and the food chains of marine ecosystems (Rodhouse and Nigmatullin, 1996; Smale, 1996; Sims *et al.,* 2001). There are a growing number of researchers who currently use *E. scolopes* as a model, not only for the squid/*Vibrio* symbiosis (16 laboratories in the United States alone) but also in fields such as developmental biology, neurobiology, and vision research (Lee *et al.,* 2009; McFall-Ngai *et al.,* 2012). Having accessible genomes for both the host and symbiont would provide a powerful tool for future research. For example, sequencing of the pea aphid genome (*Acyrthrosiphon pisum*) along with the genome of its primary bacterial endosymbiont (*Buchnera aphidicola*) helped reveal how this nutritional symbiosis is coupled and produced some surprising results (*e.g.,* a reduced immune system in the host), thus giving researchers in that field tools to form testable hypotheses about how the association functions (Wernegreen, 2002; Gerardo *et al.,* 2010; MacDonald *et al.,* 2011). We would argue that sequencing the genome of *E. scolopes* is now within reach and would be a valuable resource for researchers involved in a number of diverse areas of biological research.

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