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GUEST COMMENTARY

Layers of Signaling in a Bacterium-Host Association

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Quorum sensing, the monitoring of population density by bacteria, is used to coordinately control gene expression and therefore particular behaviors under conditions of high cell density. Such group behaviors provide advantages to organisms under certain conditions, such as during pathogenic colonization when virulence traits are induced by a group of bacteria. In the accompanying paper, Lupp and Ruby describe the requirement for a quorum-sensing system at relatively low cell density during colonization by *Vibrio fischeri* of its symbiotic host, *Euprymna scolopes* (18).

Quorum-sensing systems in *Vibrio fischeri* and *Vibrio harveyi*. Quorum sensing is a field built from laboratory-based investigations of luminous marine bacteria, namely, *Vibrio fischeri* and *Vibrio harveyi* (25). It was in *V. fischeri* that the quorum-sensing regulators LuxR (a transcriptional activator) and LuxI (a signal synthase) first were discovered (reviewed in reference 6). The LuxI-produced signaling molecule, an acyl homoserine lactone (acyl-HSL), diffuses out of and then (under conditions of high cell density) back into the cell, where it activates LuxR and thus the *lux* (luminescence) genes, which are under LuxR control (11, 14). Homologs of these proteins have been found in most gram-negative quorum-sensing bacteria studied to date.

Studies of quorum sensing in *V. harveyi* subsequently revealed a much more complex system for *lux* control (Fig. 1) (reviewed in reference 28). This organism uses three sensor kinase proteins to detect its three quorum-sensing molecules (one of which is an acyl-HSL) (1, 2, 13). At low cell density, the sensor proteins autophosphorylate. The phosphate is sequentially transferred to LuxU, a phosphotransferase protein, and LuxO, a transcriptional activator. Phosphorylated LuxO activates the transcription of five small RNA (sRNA) genes (16). The resulting sRNAs work in conjunction with the Hfq protein to destabilize the transcript encoding $LuxR_{VH}$, a transcriptional regulator that is not homologous to *V. fischeri* LuxR (16). The consequence is that $LuxR_{VH}$ is not synthesized, and thus the *lux* genes are not transcribed. Increasing population densities sequentially signal the three sensor proteins to switch from kinases to phosphatases. The consequence is dephosphorylation of LuxO, loss of sRNA synthesis, increased translation of LuxR, and thus transcription of *lux* and production of light.

For some time, it has been known that *V. fischeri* encodes a second acyl-HSL synthase, termed AinS (8). AinS is homologous to LuxM of *V. harveyi* yet produces a distinct acyl-HSL (8, 15). More recently, homologs of the other *V. harveyi* quorum-sensing components have been identified (Fig. 1) (7, 17, 19, 24). Although only a few of these components have been examined at a molecular level, the results to date suggest that this second *V. fischeri* system functions like that of *V. harveyi*. The *ain* system is integrated with the *lux* system at LitR, a $LuxR_{VH}$ homolog that is controlled by LuxO and itself controls transcription of *luxR* (Fig. 1) (7, 24), thereby controlling *lux* expression.

Roles for AinS and LuxO in symbiotic initiation. Lupp and Ruby report the novel finding that the acyl-HSL synthase AinS is required for initiation of symbiotic colonization of the squid *E. scolopes* by *V. fischeri* (18). Loss of *ainS* delayed both colonization and the luminescence emission that results from the symbiotic association (Fig. 2A). No such delay of colonization was observed for *lux* mutants, suggesting that AinS may control factors other than *lux* that are required for symbiotic initiation. Surprisingly, a mutation in *luxO*, which might be expected to counteract the consequences of an *ainS* mutation, also prevented normal symbiotic initiation. Together, these data suggested that an optimal level of AinS/LuxO-controlled (non-*lux*) target gene transcription is necessary for symbiotic initiation.

To identify potential AinS-controlled genes, Lupp and Ruby used microarray analysis (the first such published for the recently sequenced *V. fischeri* [27]). Their screen yielded 30 positively and negatively controlled genes, including, notably, negatively controlled motility genes (18). The effect of the AinS signal on motility was supported experimentally by the finding that the *ainS* mutant migrated through soft agar faster than the wild-type strain. Is the increase in motility sufficient to account for the colonization defect? Perhaps. Previous work has demonstrated that motility is essential for symbiotic colonization: nonmotile bacteria fail to colonize, while hypermotile mutants exhibit a severe delay (9, 22). Interestingly, a *luxO* mutant also displayed a defect in motility, in this case a decreased rate of migration through soft agar. These data support the idea that mutations in *ainS* and *luxO* can differentially unbalance regulation of a downstream target. Besides motility genes, however, there were a number of other, equally interesting, targets identified that could be the cause of the initiation defect observed and which will presumably be the focus of future investigations.

Sequential activation of two quorum-sensing systems. In laboratory culture, induction of *lux* depends primarily upon *ainS*. Whereas *luxI* mutants exhibit near-wild-type levels of light emission, no luminescence can be detected from *ainS* mutants (19, 29). During symbiotic colonization, the opposite is true: *luxI* mutants fail to produce any detectable light, while

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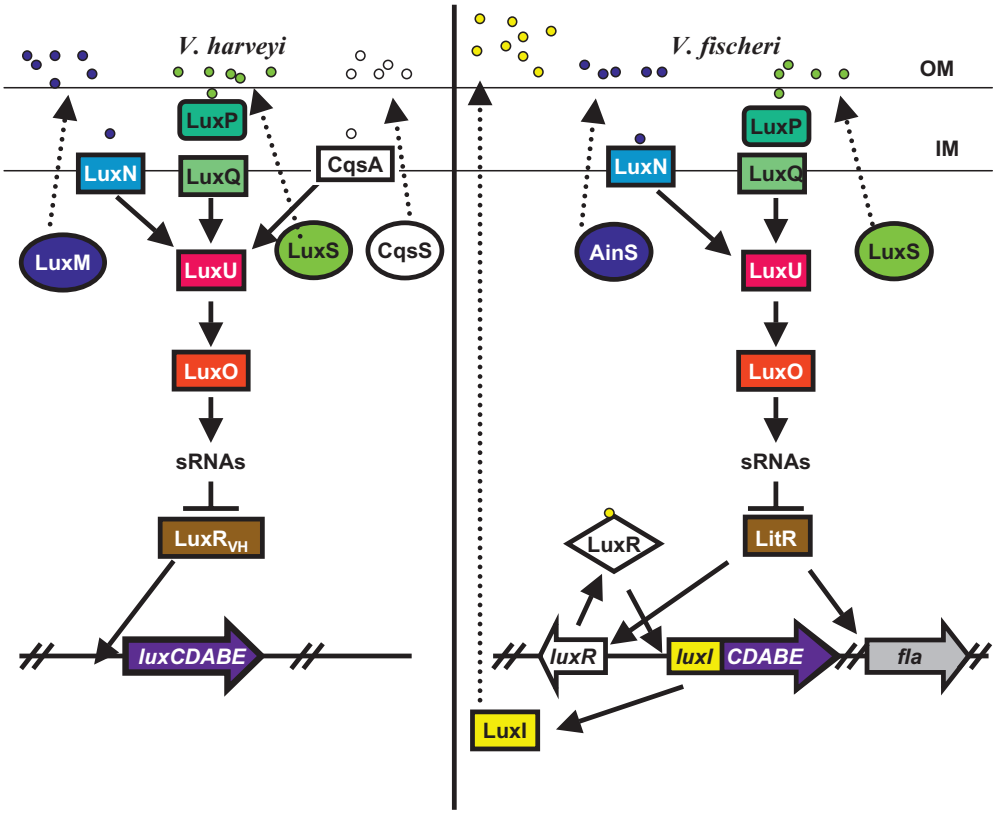

FIG. 1. A comparison of the *V. harveyi* and *V. fischeri* quorum-sensing systems. Parts of the pathway in *V. fischeri* are modeled after those described for *V. harveyi* and are described further in the text. The colors reflect proteins that are homologous between the two species. The acyl-HSL synthases are depicted as ovals. Although the *V. fischeri* AinS and LuxS proteins are homologous to LuxM and LuxS of *V. harveyi*, the acyl-HSL signaling molecules (represented by small circles) produced by the different bacteria are distinct. It is not known whether sRNAs are involved in bioluminescence control in *V. fischeri*. OM, outer membrane; IM, inner membrane.

ainS mutants exhibit only a small decrease in light. These data led Lupp and Ruby to suggest a model in which the AinS signal promotes luminescence at low cell densities, which occur in culture and the early stages of symbiotic colonization; their results that *ainS*, but not *luxI*, is required for initiation support this low cell density role for AinS. At higher cell densities, which occur in the symbiotic light organ, LuxI produces the signal necessary for high-level light production.

Colonization by *V. fischeri* occurs in a series of stages that occur immediately after hatching of the juvenile squid (Fig. 2) (reviewed in reference 26). First, *V. fischeri* cells in the seawater aggregate in mucus on the surface of the symbiotic (light) organ (Fig. 2A). Next, after a short period of time, during which signaling likely occurs between the bacteria themselves (as suggested by the results of Lupp and Ruby) as well as between the bacteria and their host, motile bacteria migrate into the light organ. After entry into nutrient-rich crypts, the bacteria begin to multiply to high cell density, induce the *lux* genes, and lose their flagella (Fig. 2B). The last stage, termed persistence, occurs once the animals are fully colonized (Fig. 2C). This stage requires the adaptation of the microbe to a changing environment: certain host developmental events are triggered by the bacteria, including an increase in size of the epithelial cells lining the bacterium-containing crypts. Furthermore, 90% of *V. fischeri* cells are expelled each morning, while the remaining 10% regrow to high cell density.

At the persistence stage of colonization, mutants defective for one of the quorum regulators, *luxR* and *luxI*, or the LuxR/I target gene, *luxA* (encoding one subunit of the luciferase enzyme), fail to achieve the same levels of colonization (regrowth) as the wild-type strain (29) (Fig. 2C). Furthermore, normal host development requires *luxA*, as *luxA* mutants fail to induce the typical epithelial cell swelling (29). It is not yet understood how the function (or lack thereof) of this quorum-sensing-controlled gene is communicated to the host.

Lupp et al. (19) previously reported that the acyl-HSL synthase AinS is similarly required for symbiotic persistence (Fig. 2C). Whereas mutations in *luxI* abolish symbiotic bioluminescence, mutations in *ainS* only slightly decrease light levels. Thus, although the colonization defect of the *luxI* mutant can be attributed to control of *lux* transcription, it is not clear that the same is true for the defect of the *ainS* mutant. Indeed, in culture, this mutant is unable to achieve the same growth yield as the wild-type strain, suggesting that an AinS-controlled factor other than *lux* may be involved in symbiotic persistence. The array experiments performed by Lupp and Ruby thus may also yield a (non-*lux*) factor required for symbiotic persistence as well. Taken together, these studies support sequential roles

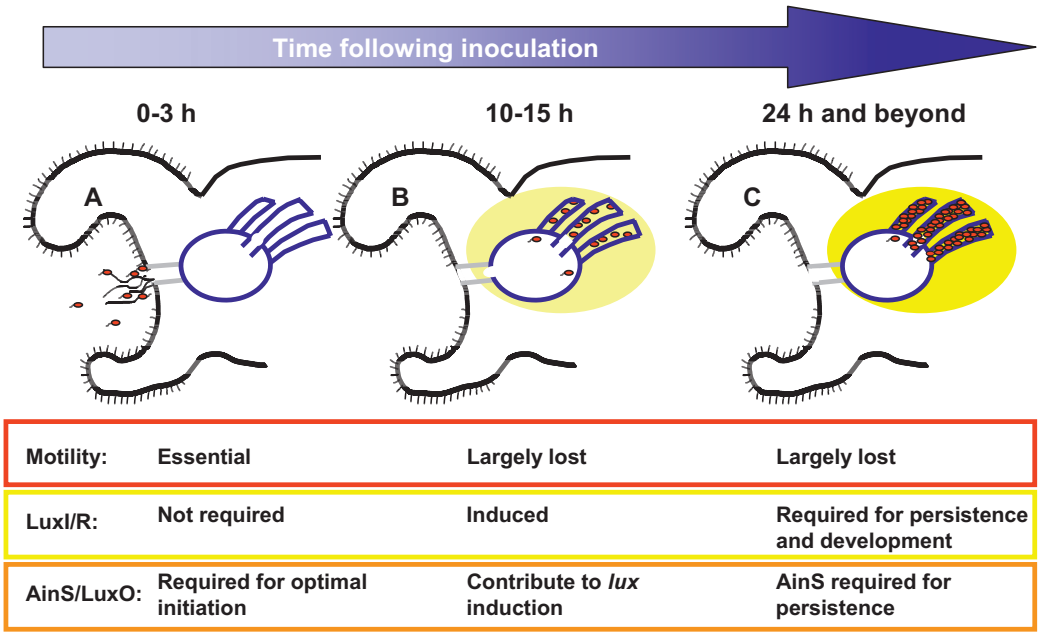

FIG. 2. Time course of bacterial colonization in the *V. fischeri-E. scolopes* symbiosis. Panels A to C depict a one side of the bilaterally symmetric light organ from juvenile *E. scolopes*, containing one of three crypts (outlined in blue). Each panel represents a different stage of symbiotic colonization. *V. fischeri* bacteria are shown as red ovals, with or without flagella. The yellow ovals surrounding the crypt spaces represent bioluminescence emission from the bacteria. The states of motility and of LuxI/R and AinS/LuxO activity are indicated below each stage. The cartoon reflects data reviewed in reference 26.

in symbiotic colonization for the AinS-produced signal (early control of a *lux*-independent target and later control of *lux* and/or a *lux*-independent target) and the LuxI-produced signal (later control of *lux*) (Fig. 2). Thus, it has been shown, for the first time, that sequential signaling by two acyl-HSLs occurs, and is important, during colonization of host tissue.

Quorum sensing in *Vibrio* spp. Although LuxI/LuxR homologs have been found in most gram-negative quorum-sensing organisms studied to date, homologs of the *V. harveyi* quorum-sensing components have been found only in other *Vibrio* species, including *V. cholerae* (10, 21, 30), *V. vulnificus* (3), *V. parahaemolyticus* (12, 20), *V. anguillarum* (4, 5, 23), and *V. fischeri* (17, 19). Of the four sequenced *Vibrio* species (*V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. fischeri*), only *V. fischeri* appears to encode both LuxR/LuxI- and LuxO-based systems of quorum sensing. Why are the vibrios distinct from other gram-negative bacteria in the mechanism by which quorum sensing occurs, and why does *V. fischeri* contain both systems? These questions await further study, including genomic sequencing of additional *Vibrio* strains (including other luminescent isolates), but insights into these questions may be gained from research into the roles of the two systems in *V. fischeri*.

In summary, this work represents an important leap forward in terms of elucidating factors necessary for the *V. fischeri*-squid symbiosis. Significantly, the dissection by Lupp and Ruby of the relative roles of two distinct quorum-sensing systems at specific stages of colonization, and in particular during an early, low-cell-density stage, provides important insights for the larger field of quorum sensing as well.

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