Caenorhabditis elegans Recognizes a Bacterial Quorum-sensing Signal Molecule through the AWCON Neuron*

Kristen M. Werner‡, Lark J. Perez§, Rajarshi Ghosh¶, Martin F. Semmelhack¶, and Bonnie L. Bassler‡**1

From the ‡Department of Molecular Biology and §Department of Chemistry, Princeton University, Princeton, New Jersey 08544, ¶Department of Chemistry and Biochemistry, Rowan University, Glassboro, New Jersey 08028, and the **Howard Hughes Medical Institute, Chevy Chase, Maryland 20815

Background: The nematode Caenorhabditis elegans consumes bacteria as its sole food source. The bacterium Vibrio cholerae produces a quorum-sensing signal molecule called CAI-1, which C. elegans detects through the AWCON chemosensory neuron.

Results: The bacterium Vibrio cholerae prefers V. cholerae to other bacterial species. CAI-1 is the dominant signal in V. cholerae (7–10).

Conclusion: V. cholerae uses bacterial-produced molecules as cues, and these molecules are also physiologically significant to bacteria.

Significance: The V. cholerae molecule CAI-1 enables cross-kingdom chemical interaction.

Bacterial group behaviors are governed by quorum sensing (QS),1 in which bacteria produce, release, and detect extracellular signal molecules called autoinducers (AIs). Vibrios, which are the model organisms for QS analyses, produce multiple AIs, some of which enable intraspecies communication, some are for intragenera communication, and others promote interspecies communication (1–7).

In a process known as quorum sensing, bacteria use chemicals called autoinducers for cell-cell communication. Population-wide detection of autoinducers enables bacteria to orchestrate collective behaviors. In the animal kingdom detection of chemicals is vital for success in locating food, finding hosts, and avoiding predators. This behavior, termed chemotaxis, is especially well studied in the nematode Caenorhabditis elegans. Here we demonstrate that the Vibrio cholerae autoinducer (S)-3-hydroxytridecan-4-one, termed CAI-1, influences chemotaxis in C. elegans. C. elegans prefers V. cholerae that produces CAI-1 over a V. cholerae mutant defective for CAI-1 production. The position of the CAI-1 ketone moiety is the key feature driving CAI-1-directed nematode behavior. CAI-1 is detected by the C. elegans amphid sensory neuron AWCON. Laser ablation of the AWCON cell, but not other amphid sensory neurons, abolished chemotaxis to CAI-1. These analyses define the structural features of a bacterial-produced signal and the nematode chemosensory neuron that permit cross-kingdom interaction.

†To whom correspondence should be addressed: Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544. Tel: 609-258-2875; E-mail: bbassler@princeton.edu.

‡The abbreviations used are: QS, quorum sensing; AI, autoinducer; C.I., chemotaxis index.

**1 This work was supported, in whole or in part, by National Institutes of Health Grant 5R01GM065859 (to B. L. B.). This work was also supported by the Howard Hughes Medical Institute and National Science Foundation Grant MCB-0348321 (to B. L. B.)

10.1074/jbc.M114.573832

EXPERIMENTAL PROCEDURES

Chemotaxis Assays—Nematodes were grown at 20 °C on Escherichia coli strain HB101 under well-fed and uncrowded conditions (23). Chemotaxis assays were performed on square plates containing 10 ml of 1.6% agar, 5 mM potassium phosphate, 1 mM calcium chloride, and 1 mM magnesium sulfate (24). Plates were divided into six equal sectors labeled A–F. For population assays, 1 µl each of stimulus and 1 µl of 1 M sodium azide were added in two spots in sector A, and 1 µl each of control diluent (DMSO, LB, or ethanol) and 1 µl of 1 M sodium azide were added in two spots in sector F. Adult animals were washed twice with S-Basal buffer (23) and once with chemotaxis buffer, placed in the center of the assay plate (between sectors C and D) and counted after 1 h. 100–200 worms were assayed per plate, with the exception of the laser ablation studies, in which 15–30 worms were assayed per plate. Each assay was performed in triplicate, with at least three independent experiments. The chemotaxis index was calculated using the formula,
was acquired from Professor Jean Schwarzbauer. To obtain the tax-2, tax-4 double mutant, tax-2(ks10) and tax-4(p678) animals were mated. The presence of each mutation was confirmed by PCR in the F1 and F2 generations.

**Chemical Synthesis**—All compounds were synthesized using previously reported procedures and were consistent with the published characterization data for these molecules. (C8)-CAI-1, (C9)-CAI-1, CAI-1, (C11)-CAI-1, and 1–6 were prepared according to procedures described in Bolitoh et al. (28). (C4)-HSL, (C6)-HSL, (C8)-HSL, and (C10)-HSL were prepared using established procedures from L-homoserine lactone hydrochloride and the appropriate carboxylic acid (29). (C8)-CAI-1, CAI-1, (C4)-HSL, (C6)-HSL, (C8)-HSL, and (C12)-HSL were analyzed as enantiopure (S) isomers. (C9)-CAI-1 and (C11)-CAI-1 were tested as racemic mixtures. The chemotaxis response to Compound 2 (Fig. 3, c and d) demonstrates that the hydroxyl group does not influence chemotaxis; therefore, the racemic compounds did not require further purification for individual analysis.

### RESULTS

**C. elegans Chemotact toward V. cholerae**—*C. elegans* chemotaxis occurs to a variety of organic compounds as well as to different species of bacteria (30). The notion is that chemotaxis toward certain bacterial-derived compounds enables *C. elegans* to locate food sources, whereas chemotaxis away from noxious bacteria allows *C. elegans* to avoid danger. Two pathogens, *P. aeruginosa* and *B. nematocida*, are more attractive to *C. elegans* than are harmless laboratory *E. coli* strains (22, 31). In the case of *P. aeruginosa*, *C. elegans* is initially attracted to the bacteria but through associative learning subsequently avoids them (31). It is not understood how *C. elegans* distinguishes attractive and aversive bacterial-produced cues and translates detection of these chemicals into meaningful behavioral responses. We chose to investigate whether QS AIs are involved in *C. elegans* chemotactic behavior using the nematode pathogen *V. cholerae*.

We first tested whether *C. elegans* can distinguish between its standard laboratory food source, *E. coli* HB101, and *V. cholerae* using volatile chemotaxis assays (24). *C. elegans* displayed a preference for *V. cholerae* over *E. coli* (C.I. = 0.83, leftmost bar in Fig. 1a) that is comparable to its preference for *P. aeruginosa* over *E. coli* (31). Higher level chemotactic behavior also occurred to *V. cholerae* cell-free culture fluids compared with those prepared from *E. coli* HB101 (C.I. = 0.87, leftmost bar in Fig. 1b).

We sought to identify the compounds that mediate this potent attraction. Obvious candidates are bacterial AIs. We examined the *C. elegans* chemotactic response to purified, synthetic AIs with varying chemical structures, including the intraspecific *Vibrio* signal CAI-1, the interspecies AI, AI-2, the intraspecies AI from the related bacterium *Vibrio harveyi*, N-(3-hydroxybutanoyl)-l-homoserine lactone (HAI-1), and the *P. aeruginosa* intrastrspecies AI, N-(3-oxodecanoyl)-l-homoserine lactone (PAI-1) (Fig. 2a, and structures are shown in Fig. 2e). Among these molecules, *C. elegans* was most potently attracted to CAI-1, had a far weaker response to AI-2, and the response to PAI-1 was insignificant (C.I. = 0.19, S.D. = 0.15). The nematodes were weakly repulsed by HAI-1. With respect to CAI-1,

### TABLE 1

**Bacterial strains and genotypes**

| Strain     | Organism       | Genotype | Reference |
|------------|----------------|----------|-----------|
| C6706str  | *V. cholerae*  | Wild type| (27)      |
| WN1380    | *V. cholerae*  | ΔgpaC    | (9)       |
| DH54c     | *E. coli*      | Wild type| (50)      |
| WN1940    | *E. coli*      | pDH5spG4 on pEV1141, Kan<sup>a</sup> |
| HB101     | *E. coli*      | Wild type| (51)      |

### TABLE 2

**C. elegans strains and genotypes**

| Strain | Relevant mutation | Genotype |
|--------|------------------|----------|
| N2     | tax-2, tax-4     | Wild type|
| FG125  | ocr-2, osm-9     | osm-2(ak47) osm-9(ks10) IV |
| FK311  | ceh-36           | ceh-36(ks86) X |
| PR674  | che-1            | che-1(p674) I |
| CX5161 | nsy-5            | nsy-1(ky324) I |
| CX5998 | nsy-1            | nsy-1(ky397) II |
| CX3695 | str-2:GFP        | ky3140 [str-2:GFP + lin-15(+)] I |
| OH3679 | che-1:GFP       | och116 [lin-6p-GFP + rol-6(su1006)] |
| OH3192 | gcy-5:GFP       | och116[ex1262[lin-15(+); gcy-5;GFP] |
| OH3351 | gcy-6:GFP       | och116[ex6-6:GFP + lin-15(+)] |

\[
\text{Cl} = \frac{(A + B) - (E + F)}{N} \quad \text{(Eq. 1)}
\]

where A, B, E, and F are the number of animals in plate sectors A, B, E, and F, respectively, N is the total number of animals in all six sectors of the plate. Positive control odorant dilutions were 1:100 (isoamyl alcohol) and 1:200 (benzaldehyde). All chemotactic responses were verified in multiple experiments. All assays were quantified by two independent investigators. Data for chemotaxis assays were compared using unequal two-tailed t tests.

**Laser Ablations**—Laser ablations were performed on anesthetized early L4 animals (N2 strain) expressing str-2:GFP using a 2-photon microscope. Only one cell in each animal expressed cytoplasmic GFP. Using GFP as a marker for AWCON neurons allowed to recover on standard NGM plates with overnight before assay for chemotaxis. All worms were verified for target cell gfp expression before behavioral assays.

**Bacterial Strains**—All *V. cholerae* strains are derivatives of wild-type C6706str (27). *V. cholerae* and *E. coli* mutant and recombinant strains were generated in the Bassler group (Table 1).

**C. elegans Strains**—Strains were acquired from the Caenorhabditis Genetics Center (CGC) (Table 2) and include CGC strain numbers and genotypes. The wild-type strain, Bristol N2, is the wild-type stock. The double mutant, tax-2(ks10); tax-4(ks678) animals were mated. The presence of each mutation was confirmed by PCR in the F1 and F2 generations.

**Chemical Synthesis**—All compounds were synthesized using previously reported procedures and were consistent with the published characterization data for these molecules. (C8)-CAI-1, (C9)-CAI-1, CAI-1, (C11)-CAI-1, and 1–6 were prepared according to procedures described in Bolitoh et al. (28). (C4)-HSL, (C6)-HSL, (C8)-HSL, and (C10)-HSL were prepared using established procedures from L-homoserine lactone hydrochloride and the appropriate carboxylic acid (29). (C8)-CAI-1, CAI-1, (C4)-HSL, (C6)-HSL, (C8)-HSL, and (C12)-HSL were analyzed as enantiopure (S) isomers. (C9)-CAI-1 and (C11)-CAI-1 were tested as racemic mixtures. The chemotaxis response to Compound 2 (Fig. 3, c and d) demonstrates that the hydroxyl group does not influence chemotaxis; therefore, the racemic compounds did not require further purification for individual analysis.

**RESULTS**

**C. elegans Chemotact toward V. cholerae**—*C. elegans* chemotaxis occurs to a variety of organic compounds as well as to different species of bacteria (30). The notion is that chemotaxis toward certain bacterial-derived compounds enables *C. elegans* to locate food sources, whereas chemotaxis away from noxious bacteria allows *C. elegans* to avoid danger. Two pathogens, *P. aeruginosa* and *B. nematocida*, are more attractive to *C. elegans* than are harmless laboratory *E. coli* strains (22, 31). In the case of *P. aeruginosa*, *C. elegans* is initially attracted to the bacteria but through associative learning subsequently avoids them (31). It is not understood how *C. elegans* distinguishes attractive and aversive bacterial-produced cues and translates detection of these chemicals into meaningful behavioral responses. We chose to investigate whether QS AIs are involved in *C. elegans* chemotactic behavior using the nematode pathogen *V. cholerae*.

We first tested whether *C. elegans* can distinguish between its standard laboratory food source, *E. coli* HB101, and *V. cholerae* using volatile chemotaxis assays (24). *C. elegans* displayed a preference for *V. cholerae* over *E. coli* (C.I. = 0.83, leftmost bar in Fig. 1a) that is comparable to its preference for *P. aeruginosa* over *E. coli* (31). Higher level chemotaxis also occurred to *V. cholerae* cell-free culture fluids compared with those prepared from *E. coli* HB101 (C.I. = 0.87, leftmost bar in Fig. 1b). We sought to identify the compounds that mediate this potent attraction. Obvious candidates are bacterial AIs. We examined the *C. elegans* chemotactic response to purified, synthetic AIs with varying chemical structures, including the intraspecific *Vibrio* signal CAI-1, the interspecies AI, AI-2, the intraspecies AI from the related bacterium *Vibrio harveyi*, N-(3-hydroxybutanoyl)-l-homoserine lactone (HAI-1), and the *P. aeruginosa* intraspecies AI, N-(3-oxodecanoyl)-l-homoserine lactone (PAI-1) (Fig. 2a, and structures are shown in Fig. 2e). Among these molecules, *C. elegans* was most potently attracted to CAI-1, had a far weaker response to AI-2, and the response to PAI-1 was insignificant (C.I. = 0.19, S.D. = 0.15). The nematodes were weakly repulsed by HAI-1. With respect to CAI-1,
we observed a striking dose-dependent response to the \textit{V. cholerae} CAI-1 autoinducer: C.I. = 0.39 at 10 \( \mu M \) CAI-1 and C.I. = 0.56 at 100 \( \mu M \) CAI-1 (Fig. 2b). In \textit{V. cholerae} bacterial cultures, however, 1 \( \mu M \) CAI-1 saturates the QS response (7), so it was unclear whether chemotaxis to these concentrations of CAI-1 represented a biologically relevant behavior. To address this we adapted the chemotaxis protocol described in Choe et al. (32) (termed the two-spot assay) such that CAI-1 is applied to a lawn of \textit{E. coli} HB101 and the amount of time each worm spends in the CAI-1 region is compared with the control region. This assay showed that 1 \( \mu M \) CAI-1 is indeed sufficient for \textit{C. elegans} attraction, as the worms spent 3 times longer in the CAI-1 region than in the control region (Fig. 2c).

Although \textit{C. elegans} chemotacts toward synthetic, purified CAI-1, it was possible that CAI-1 was not a major contributor to \textit{C. elegans} attraction to \textit{V. cholerae} bacterial cultures. To examine whether CAI-1 is the attractant, we assayed chemotaxis to the wild-type \textit{V. cholerae} strain that produces CAI-1 and compared that to a mutant strain that is incapable of CAI-1 production as well as to the cell-free fluids from the same two strains (\textit{V. cholerae} cqsA, Fig. 1, \text{a} and \text{b}, middle bars). In addition, we assayed cultures of and cell-free fluids from \textit{E. coli} carrying the \textit{cqsA} gene encoding the CAI-1 synthase (\textit{E. coli/ PcqS\text{A}}) and compared those preparations to \textit{E. coli} carrying the empty vector (Fig. 1, \text{a} and \text{b}, rightmost bars). We showed previously that introduction of \textit{cqsA} into recombinant \textit{E. coli} is sufficient for high level production of CAI-1 (7, 33). In all cases \textit{C. elegans} preferred bacteria that produce CAI-1 to those that do not, and \textit{C. elegans} preferred fluids collected from bacterial cultures containing CAI-1 compared with those lacking CAI-1. We note, however, that the absence of CAI-1 does not completely eliminate \textit{C. elegans} attraction to \textit{V. cholerae} bacterial cultures or cell-free fluids. Therefore, \textit{V. cholerae} must produce chemotactants in addition to CAI-1.

\textit{C. elegans} Recognizes Key Structural Features of CAI-1—Previous structure-activity relationship analyses demonstrate that the \textit{V. cholerae} CAI-1 receptor, CqsS, can be activated by a set of CAI-1 variants (9, 33). We wondered whether \textit{C. elegans} also recognizes structural variants of CAI-1. To explore this, we tested enamino-CAI-1, a naturally-occurring CAI-1 variant that potently activates CqsS, and we tested phenyl-CAI-1, which is a CqsS antagonist (9, 33) (see the structures in Fig. 2e). \textit{C. elegans} displayed statistically similar recognition of each molecule (Fig. 2d), suggesting that the head group of the molecule is not playing a role in specifying the structural requirements for chemotact.

To further examine the specificity of CAI-1 detection by \textit{C. elegans}, we tested molecules with variations in the carbon tail length. For example, \textit{V. cholerae} CAI-1 has a 10-carbon tail, whereas \textit{V. harveyi} produces a CAI-1-type molecule with an 8-carbon side chain, (C8)-CAI-1. In addition to assaying these natural molecules, we synthesized and tested (C9)-CAI-1 and (C11)-CAI-1. Analyses of this series of CAI-1 molecules demonstrate that 9- and 10-carbon chain CAI-1 variants produce the most robust \textit{C. elegans} chemotactic response (Fig. 3a, molecules shown in Fig. 3b). Specifically, we found the order of chemotactic preference to be: CAI-1 = (C9)-CAI-1 > (C8)-CAI-1 = (C11)-CAI-1. Interestingly, this order of preference for tail length exactly parallels the CqsS receptor preference (9, 28, 33). To assess the generality of this response to other bacterial signaling molecules containing fatty acid tails, we examined the responses of \textit{C. elegans} to a series of acylated homoserine lactone AIs. We observed a similar trend within this series of molecules (Fig. 3, \text{a} and \text{b}). Specifically, a homoserine lactone with a C10 tail induced high-level chemotaxis, whereas molecules with shorter carbon chains resulted in reduced attraction (\textit{i.e.} (C6)-HSL) or repulsion (\textit{i.e.} (C4)-HSL). A molecule harboring a longer chain, (C12)-HSL, also elicited weak chemotraction (Fig. 3, \text{a} and \text{b}). Consistent with this result, Fig. 2a shows that \textit{C. elegans} exhibits similarly low attraction to PAI-1, which is a homoserine lactone harboring a 12-carbon tail and a 4-ketone moiety (Fig. 2, \text{a} and \text{e}). Together, these data suggest that, similar to the CAI-1 series, a range of carbon chain lengths enable chemotaxis to homoserine lactone AIs with C10 being the optimal length.
In our effort to define the structural features of the CAI-1 molecule that are important for chemotaxis in *C. elegans*, we prepared a series of related compounds that vary in the position of the hydroxyl or ketone moieties and tested them in the chemotaxis assay. The compounds analyzed can be broadly categorized into molecules that maintain chemoattractant properties (Fig. 3, compounds 1 and 2) and neutral molecules (Fig. 3, compounds 3–6). Shifting the positions of the hydroxyl and ketone groups from C3 and C4 to C2 and C3, respectively, reduced the chemoattractive potency of CAI-1 (Fig. 3c, compound 1), whereas removal of the hydroxyl group from CAI-1 while maintaining the 4-ketone functionality had minimal effect on activity (Fig. 3c, compound 2). Compound 2 was previously assayed for agonist activity in *V. cholerae* QS and had no activity at concentrations up to 50 μM (28). Exchanging the ketone position with the hydroxyl group (compound 3),
moving the ketone by one carbon atom to make a 3-ketone molecule (compound 2 versus compound 5), or removal of the ketone (compounds 4 and 6) eliminated chemoattraction (Fig. 3). In summary, although moderate alterations in the length of the carbon chain and dramatic changes in the head group are tolerated, the presence of a 4-ketone functionality is necessary for robust CAI-1 chemoattractive activity.

C. elegans Detects CAI-1 as an Attractant through the AWCON Sensory Neuron—To determine how C. elegans detects CAI-1, we investigated the role of the amphid sensory neurons, which detect many chemical stimuli (30). Most chemosensory neurons in C. elegans use either cGMP-gated channels or TRPV channels for sensory transduction (30). We found that animals with mutations in the cGMP-gated channel encoded by tax-2 and tax-4 are defective in attraction to CAI-1, as they fail to respond to 100 μM synthetic CAI-1 (Fig. 4a). By contrast, animals mutant for the TRPV channels encoded by osm-9 and ocr-2 chemotact to CAI-1-like wild type (Fig. 4a). This result suggests that sensory neurons expressing tax-4 and tax-2 are required to detect CAI-1, whereas neurons expressing osm-9 and ocr-2 are dispensable. tax-2 and tax-4 function in eight classes of amphid chemosensory neurons, including AWB, AWC, and ASE neurons. To pinpoint the cell or cells that mediate the C. elegans response to V. cholerae CAI-1, we next assayed ceh-36 mutant animals, which lack functional AWC and ASE neurons (34, 35). ceh-36 animals do not chemotact to
CAI-1 (Fig. 4a). This result narrows candidate neurons to ASEL and the two AWC cells. che-1 mutants are defective in the development of both ASEL and ASER sensory neurons (34, 36, 37). These mutant animals exhibited wild-type attraction to CAI-1, eliminating the ASE neuron as having a role in the CAI-1 response (Fig. 4a). Finally, to differentiate between the two AWC neurons, we examined nsy-5 mutants. These mutants fail to develop the AWCON neuron, as defined by the expression of str-2::GFP (38), resulting in the formation of two AWCOFF neurons (39, 40). nsy-5 mutant animals do not chemotact toward CAI-1 (Fig. 4a). Conversely, nsy-1 mutants, which fail to develop the AWCOFF neuron, maintain the ability to detect CAI-1 (Fig. 4a). Together, these results suggest that the AWCON neuron is essential for attraction to CAI-1, but the AWCOFF neuron is not.

To verify the role of AWCON in CAI-1 detection, we tested transgenic animals in which AWCON neurons expressing GFP (str-2::GFP) were laser-ablated. AWCON-ablated animals were not attracted to CAI-1 (Fig. 4b), whereas animals that were mock ablated or defective in development of neurons other than AWCON, i.e. either defective for the ASE neuron (che-1::GFP), ASER (gcy-5::GFP), or ASEL (gcy-6::GFP), demonstrated wild-type attraction to CAI-1 (Fig. 4b). In addition, AWCON-ablated animals were able to chemotact toward benzaldehyde (Fig. 4b and Ref. 41), demonstrating that ablation of AWCON did not generally impair detection of stimuli or chemotaxis behavior. Thus, AWCON is the neuron that mediates C. elegans attraction to V. cholerae CAI-1. In an analogous series of experiments we show that, surprisingly, animals lacking the AWCON cell (nsy-5 mutants) remained capable of chemotaxis toward (C10)-HSL, whereas animals lacking the AWCOFF cell (nsy-1 mutants) were impaired (Fig. 4c). Therefore, AWCOFF, rather than AWCON, mediates chemoattraction to (C10)-HSL. There are precedents for opposite behaviors for the two AWC neurons. Indeed, our results are consistent with prior studies in which the AWCON neuron plays the primary role in sensation of particular chemicals, such as butanone, whereas the AWCOFF neuron is critical for detection of a different set of stimuli (41). By contrast, in the case of high salt chemotaxis, both the AWCON and AWCOFF neurons are essential (42).

**DISCUSSION**

Nematodes have behavioral as well as developmental strategies for coping with environmental stresses. Success depends on correctly interpreting the information encoded in the chemical environments in which they reside. One crucial environmental factor for nematode survival is the availability of non-pathogenic bacteria as food. In this study we have demonstrated that C. elegans can intercept bacterial-produced signals;
specifically, *C. elegans* detects the QS AI, CAI-1. We additionally observed chemotaxis to another class of QS signaling molecules, the homoserine lactone AIs. The maximal response to both classes of bacterial signals was to molecules containing 10-carbon fatty acid tails; however, other chain length tails are tolerated. Assessment of the other structural features of CAI-1 reveals that the 4-ketone moiety is an important feature for recognition. CAI-1 appears to mediate only a portion of the tolerated. Assessment of the other structural features of CAI-1 molecules, the homoserine lactone AIs. The maximal response to another class of QS signaling molecules containing short carbon chains was attractants (Fig. 3a). Small molecules control dauer formation, chemoavoidance, and chemotraction. In each case the molecules are detected by distinct neural control dauer formation, chemoavoidance, and chemotraction. In each case the molecules are detected by distinct neural overall influence by the length of the carbon chain on the small molecule (47–49). Possibly, sensory neuron subtypes distinguish molecules, at least in part, based on carbon chain length. Our findings in the present study suggest that bacteria and *C. elegans* can detect similar chemical cues, and we demonstrate a mechanism underpinning how bacterial QS can influence the behavior of potential host animals. The intimate relationship between *C. elegans* and bacteria, as their food source, could require that *C. elegans* rely on the detection and interpretation of bacterial-produced cues. For example, while many of the nematode genes involved in dauer formation have been characterized, *C. elegans* uses an unknown bacterial cue or cues to exit the metabolically arrested dauer phase and continue through development (43). Similarly, in a recent characterization of L1 nematode metabolites, it was found that the *C. elegans* avoidance response to L1-produced small molecules is overridden by the addition of bacteria to the assay (49). In these cases of bacterial-regulated *C. elegans* development and behavior, the specific bacterial-produced stimuli remain mysterious. We have begun to examine the relationship between *C. elegans* behavior and specific bacterial exoproducts. We determined the key chemical moieties (4-ketone and a long carbon tail) along with the nematode sensory neuron (AWC\textsuperscript{ON}) required for cross-kingdom interaction between *C. elegans* and *V. cholerae*. These relationships provide a promising platform to study prokaryote-eukaryote interactions at the molecular level, and they may reveal how eukaryotes detect and interpret information about nutrition and the microbes that shape their life cycles.

Acknowledgments—We thank the Caenorhabditis Genetics Center and Professor Jean Schwarzauer for providing C. elegans strains; Kevin T. O’Brien, Samantha Sustek, and Kevin C. Lyman for assistance in the synthesis of Compounds 1–6; Dr. Stephan Thibege for assistance in the two-photon facility at Princeton University; Professor Corinna Bargmann and her group for expertise and training in performing chemotaxis assays and analyses; the Bassler group for insightful comments and discussion.

REFERENCES

1. Surette, M. G., and Bassler, B. L. (1998) Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. U.S.A.* 95, 7046–7050

2. Surette, M. G., Miller, M. B., and Bassler, B. L. (1999) Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1639–1644

3. Miller, M. B., Skorupski, K., Lenz, D. H., Taylor, R. K., and Bassler, B. L. (2002) Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. *Cell* 110, 303–314

4. Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B. L., and Hughson, F. M. (2002) Structural identification of a bacterial quorumsensing signal containing boron. *Nature* 415, 545–549

5. Mok, K. C., Wingreen, N. S., and Bassler, B. L. (2003) *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. *EMBO J.* 22, 870–881

6. Henke, J. M., and Bassler, B. L. (2004) Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J. Bacteriol.* 186, 6902–6914

7. Higgins, D. A., Pomianek, M. E., Kraml, C. M., Taylor, R. K., Semmelhack, M. F., and Bassler, B. L. (2007) The major *Vibrio cholerae* autoinducer and its role in virulence factor production. *Nature* 450, 883–886

8. Kelly, R. C., Bolitho, M. E., Higgins, D. A., Lu, W., Ng, W.-L., Jeffrey, P. D., Rabinowitz, J. D., Semmelhack, M. F., Hughson, F. M., and Bassler, B. L. (2009) The *Vibrio cholerae* quorum-sensing autoinducer CAI-1: analysis of the biosynthetic enzyme CqsA. *Nat. Chem. Biol.* 5, 891–895

9. Ng, W.-L., Perez, L. J., Wei, Y., Kraml, C. M., Semmelhack, M. F., and Bassler, B. L. (2011) Signal production and detection specificity in *Vibrio CqsA/CqsS* quorum-sensing systems. *Mol. Microbiol.* 79, 1407–1417

10. Wei, Y., Perez, L. J., Ng, W.-L., Semmelhack, M. F., and Bassler, B. L. (2011) Mechanism of *Vibrio cholerae* autoinducer-1 biosynthesis. *ACS Chem. Biol.* 6, 356–363

11. Schauder, S., Shokat, K., Surette, M. G., and Bassler, B. L. (2001) The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol. Microbiol.* 41, 463–476

12. Waters, C. M., Lu, W., Rabinowitz, J. D., and Bassler, B. L. (2008) Quorum sensing controls biofilm formation in *Vibrio cholerae* through modulation of cyclic di-GMP levels and repression of vpsT. *J. Bacteriol.* 190, 2527–2536

13. Hammer, B. K., and Bassler, B. L. (2009) Distinct sensory pathways in *Vibrio cholerae* El Tor and classical biotypes modulate cyclic dimeric GMP levels to control biofilm formation. *J. Bacteriol.* 191, 169–177

14. Ward, S. (1973) Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. *Proc. Natl. Acad. Sci. U.S.A.* 70, 817–821

15. Dusenbery, D. B. (1974) Analysis of chemotaxis in the nematode *Caenorhabd-
C. elegans Detects Cross-kingdom Chemical Signals

abditis elegans by countercurrent separation. J. Exp. Zool. 188, 41–47
16. Horvitz, H. R., Challie, M., Trent, C., Sulston, J. E., and Evans, P. D. (1982) Serotonin and octopamine in the nematode Caenorhabditis elegans. Science 216, 1012–1014
17. Avery, L., and Horvitz, H. R. (1990) Effects of starvation and neuroactive drugs on feeding in Caenorhabditis elegans. J. Exp. Zool. 253, 263–270
18. Thomas, J. H. (1990) Genetic analysis of defecation in Caenorhabditis elegans. Genetics 124, 855–872
19. Bargmann, C. I., Hartwig, E., and Horvitz, H. R. (1993) Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell 74, 515–527
20. Beale, E., Li, G., Tan, M.-W., and Rumbaugh, K. P. (2006) Caenorhabditis elegans senses bacterial autoinducers. Appl. Environ. Microbiol. 72, 5135–5137
21. Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C. I., and Ewbank, J. J. (2007) Detection and avoidance of a natural product from the pathogenic bacterium Serratia marcescens by Caenorhabditis elegans. Proc. Natl. Acad. Sci. U.S.A. 104, 2295–2300
22. Niu, Q., Huang, X., Zhang, L., Xu, J., Yang, D., Wei, K., Niu, X., An, Z., Bennett, J. W., Zhou, C., Yang, J., and Zhang, K.-Q. (2010) A Trojan horse mechanism of bacterial pathogenesis against nematodes. Proc. Natl. Acad. Sci. U.S.A. 107, 16631–16636
23. Brenner, S. (1974) The genetics of Caenorhabditis elegans. Genetics 77, 71–94
24. Troemel, E. R., Kimmel, B. E., and Bargmann, C. I. (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell 91, 161–169
25. Denk, W., Strickler, J. H., and Webb, W. W. (1990) Two-photon laser scanning fluorescence microscopy. Science 248, 73–76
26. Pologruto, T. A., Sabatini, B. L., and Svoboda, K. (2003) ScanImage: flexible software for operating laser scanning microscopes. Biomed. Eng. Online 2, 13
27. Thelin, K. H., and Taylor, R. K. (1996) Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by Vibrio cholerae O1 El Tor biotype and O139 strains. Infect. Immun. 64, 2853–2856
28. Bolitho, M. E., Perez, L. J., Koch, M. J., Ng, W.-L., Bassler, B. L., and Semmelhack, M. F. (2011) Small molecule probes of the receptor binding site in the Vibrio cholerae CAI-1 quorum sensing circuit. Bioorg. Med. Chem. 19, 6906–6918
29. Swem, L. R., Swem, D. L., O’Loughlin, C. T., Gatmaitan, R., Zhao, B., Ulrich, S. M., and Bassler, B. L. (2009) A quorum-sensing antagonist targets both membrane-bound and cytoplasmic receptors and controls bacterial pathogenicity. Mol. Cell 35, 143–153
30. Bargmann, C. I. (2006) Chemosensation in C. elegans. WormBook 1–29
31. Zhang, Y., Lu, H., and Bargmann, C. I. (2005) Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. Nature 438, 179–184
32. Choe, A., Chuman, T., von Reuss, S. H., Dossey, A. T., Yim, J. J., Ajreditin, R., Kolawa, A. A., Kaplan, F., Albom, H. T., Teal, P. E., Schroeder, F. C., Sternberg, P. W., and Edison, A. S. (2012) Sex-specific mating pheromones in the nematode Panagrellus redivivus. Proc. Natl. Acad. Sci. U.S.A. 109, 20949–20954
33. Ng, W.-L., Wei, Y., Perez, L. J., Cong, J., Long, T., Koch, M., Semmelhack, M. F., Wingreen, N. S., and Bassler, B. L. (2010) Probing bacterial transmembrane histidine kinase receptor-ligand interactions with natural and synthetic molecules. Proc. Natl. Acad. Sci. U.S.A. 107, 5575–5580
34. Chang, S., Johnston, R. J., Jr., and Hobert, O. (2003) A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of C. elegans. Genes Dev. 17, 2123–2137
35. Lanjuin, A., VanHoven, M. K., Bargmann, C. I., Thompson, J. K., and Sengupta, P. (2003) Otx/oth homeobox genes specify distinct sensory neuron identities in C. elegans. Dev. Cell 5, 621–633
36. Lewis, J. A., and Hodgkin, J. A. (1977) Specific neuroanatomical changes in chemosensory mutants of the nematode Caenorhabditis elegans. J. Comp. Neurol. 172, 489–510
37. Uchida, O., Nakano, H., Koga, M., and Ohshima, Y. (2003) The C. elegans che-1 gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development 130, 1215–1224
38. Chang, C.-F., Vanhoven, M. K., Fetter, R. D., Verselis, V. K., and Bargmann, C. I. (2007) An innexin-dependent cell network establishes left-right neuronal asymmetry in C. elegans. Cell 129, 787–799
39. Troemel, E. R., Sagasti, A., and Bargmann, C. I. (1999) Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in C. elegans. Cell 99, 387–398
40. Sagasti, A., Hisamoto, N., Hyodo, J., Tanaka-Hino, M., Matsumoto, K., and Bargmann, C. I. (2001) The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. Cell 105, 221–232
41. Wes, P. D., and Bargmann, C. I. (2001) C. elegans odour discrimination requires asymmetric diversity in olfactory neurons. Nature 410, 698–701
42. Leinwand, S. G., and Chalasani, S. H. (2011) Neuropeptide signaling re-models chemosensory circuit composition in Caenorhabditis elegans. Nat. Neurosci. 16, 1461–1467
43. Golden, J. W., and Riddle, D. L. (1984) The Caenorhabditis elegans dauer larva: developmental effects of pheromone, food, and temperature. Dev. Biol. 102, 368–378
44. Jeong, P.-Y., Jung, M., Yim, Y.-H., Kim, H., Park, M., Hong, E., Lee, W., Kim, Y. H., Kim, K., and Paik, Y.-K. (2005) Chemical structure and biological activity of the Caenorhabditis elegans dauer-inducing pheromone. Nature 433, 541–545
45. Butcher, R. A., Fujita, M., Schroeder, F. C., and Clardy, J. (2007) Small-molecule pheromones that control dauer development in Caenorhabditis elegans. Nat. Chem. Biol. 3, 420–422
46. Srivivasan, J., Kaplan, F., Ajreditin, R., Zachariah, C., Albom, H. T., Teal, P. E., Malik, R. U., Edison, A. S., Sternberg, P. W., and Schroeder, F. C. (2008) A blend of small molecules regulates both mating and development in Caenorhabditis elegans. Nature 454, 1115–1118
47. Butcher, R. A., Ragains, J. R., Li, W., Ruvkin, G., Clardy, J., and Mak, H. Y. (2009) Biosynthesis of the Caenorhabditis elegans dauer-prolonging pheromone. Proc. Natl. Acad. Sci. U.S.A. 106, 1875–1879
48. Zagoriy, V., Matyash, V., and Kurzchalia, T. (2010) Long-chain O-acetyls-glucosyl-alkanediols are constitutive components of Caenorhabditis elegans but do not induce dauer larva formation. Chem. Biodivers. 7, 2016–2022
49. Artyukhin, A. B., Yim, J. J., Srivivasan, J., Izrayelit, Y., Bose, N., von Reuss, S. H., Jo, Y., Jordan, J. M., Baugh, L. R., Cheong, M., Sternberg, P. W., Avery, L., and Schroeder, F. C. (2013) Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in C. elegans. J. Biol. Chem. 288, 18778–18783
50. Hanahan, D. (1985) DNA Cloning: A practical approach, pp. 109–135, IRL Press, Oxford, UK
51. Boyer, H. W., and Rouland-Dussoix, D. (1969) A complementation analysis of the restriction and modification of DNA in Escherichia coli. J. Mol. Biol. 41, 459–472