Communicative & Integrative Biology 6:6, e26633; November/December 2013; © 2013 Landes Bioscience

Decision making in *C. elegans* chemotaxis to alkaline pH

Competition between two sensory neurons, ASEL and ASH

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Monitoring of environmental and tissue pH is critical for animal survival. The nematode, *Caenorhabditis elegans* (*C. elegans*), is attracted to mildly alkaline pH, but avoids strongly alkaline pH. However, little is known about how the behavioral switching or decision making occurs. Genetic dissection and Ca^{2+} imaging have previously demonstrated that ASEL and ASH are the major sensory neurons responsible for attraction and repulsion, respectively. Here we report that unlike *C. elegans* wild type, mutants deficient in ASEL or ASH were repelled by mildly alkaline pH, or were attracted to strongly alkaline pH, respectively. These results suggest that signals through ASEL and ASH compete to determine the animal's alkaline-pH chemotaxis. Furthermore, mutants with 2 ASEL neurons were more efficiently attracted to mildly alkaline pH than the wild type with a single ASEL neuron, indicating that higher activity of ASEL induces stronger attraction to mildly alkaline pH. This stronger attraction was overridden by normal activity of ASH, suggesting that ASH-mediated avoidance dominates ASEL-mediated attraction. Thus, *C. elegans* chemotactic behaviors to alkaline pH seems to be determined by signal strengths from the sensory neurons ASEL and ASH, and the behavior decision making seems to be the result of competition between the 2 sensory neurons.

Keywords: Alkalinity sensing, *C. elegans*, GCY-14, OSM-9, OCR-2

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Submitted: 08/27/2013; Revised: 09/25/2013; Accepted: 09/26/2013

Citation: Murayama T, Maruyama IN. Decision making in C. elegans chemotaxis to alkaline pH: Competition between two sensory neurons, ASEL and ASH. Communicative & Integrative Biology 2014; 7:e26633; http://dx.doi.org/10.4161/cib.26633

Addendum to: Murayama T, Takayama J, Fujiwara M, Maruyama IN. Environmental alkalinity sensing mediated by the transmembrane guanylyl cyclase GCY-14 in C. elegans. Curr Biol 2013; 23:1007–12; http://dx.doi.org/10.1016/j.cub.2013.04.052; PMID:23664973

Animals survive within a narrow pH range by monitoring pH of their environments and body fluids. While acid sensor molecules, such as acid-sensing ion channels (ASICs) and transient receptor potential vanilloid type (TRPV) ion channels, have been identified in sensory neurons and other cells,[1] little is known about how organisms sense extracellular, environmental alkalinity. Neurons sensitive to extracellular alkalinization have been found in insects and mammals.[2-4] However, the means by which neurons sense extracellular alkaline pH remain to be explored, although ion channels have recently found to monitor intracellular alkalinization.[5-7] *C. elegans* is attracted to alkaline pH ≤ 10,[3,8,9] and avoids pH higher than 10.5.[10] We have previously identified ASEL and ASH gustatory neurons as sensors for mildly and strongly alkaline pH, respectively. Imaging of in vivo Ca^{2+} concentrations, $[Ca^{2+}]$, in these neurons revealed that they respond differently to environmental alkalinization. ASEL is activated by raising pH from 6.8 to 8.1–10.9 with a maximum peak at pH 10.0.[9] The increase of $[Ca^{2+}]$ in ASEL resulting from environmental alkaline-pH stimulation was transient, and the $[Ca^{2+}]$ returned to baseline within a minute, even during alkaline pH stimulation. In contrast, $[Ca^{2+}]$ in ASH neurons slowly increased upon environmental alkalinization, and the elevated $[Ca^{2+}]$ remained constant during stimulation with strongly alkaline pH.[10]

These distinct responses of ASEL and ASH to environmental alkalinization may be due to distinct properties and regulation of sensor molecules and/or ion channels for Ca^{2+} entry into the neurons. A transmembrane-type guanylyl cyclase, GCY-14, acts as a mildly alkaline-pH sensor molecule in ASEL.[9] Upon stimulation with strongly alkaline pH, a TRPV channel consisting of OSM-9 and OCR-2 subunits is required for Ca^{2+} entry into

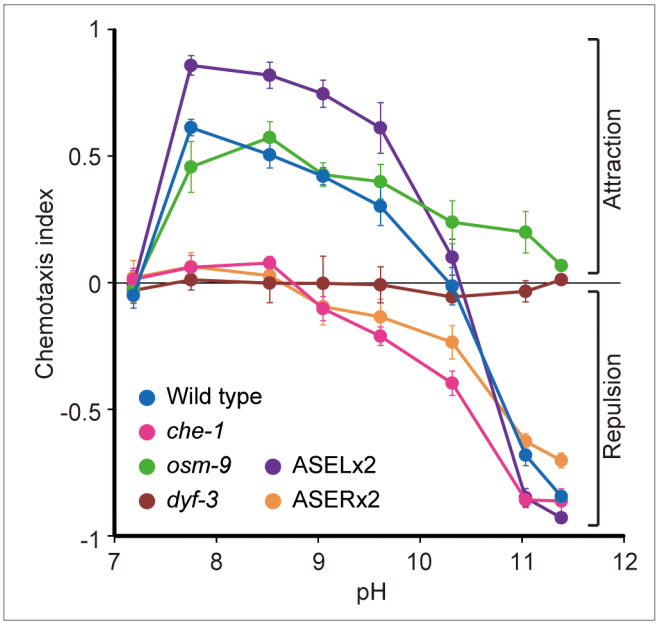

Figure 1. Chemotaxis assay of *C. elegans* to alkaline pH. Chemotaxis assays were performed using agar plates in petri dishes, 10 cm in diameter, divided into 4 quadrants containing either neutral or alkaline pH, as described previously.[10] After being washed twice with deionized H_2O, animals were placed at the center of assay plates, and allowed to move freely for 8 min. A chemotaxis index (CI) was calculated using the equation $CI = (N_{alkaline} - N_{neutral}) / (N_{alkaline} + N_{neutral})$, in which $N_{alkaline}$ and $N_{neutral}$ are the numbers of animals in alkaline and neutral pH areas, respectively. The following mutant stains were used: OF226 *che-1*(*p679*) I, CX10 *osm-9*(*ky10*) IV, SP1603 *dyf-3*(*m185*) IV, OH7805 *otIs204*[*ceh-36::lsy-6;elt-2::gfp*] as ASELx2, OH2535 *lsy-6*(*ot71*) V as ASERx2. Error bars indicate the SEM (n = 6–8 assays).

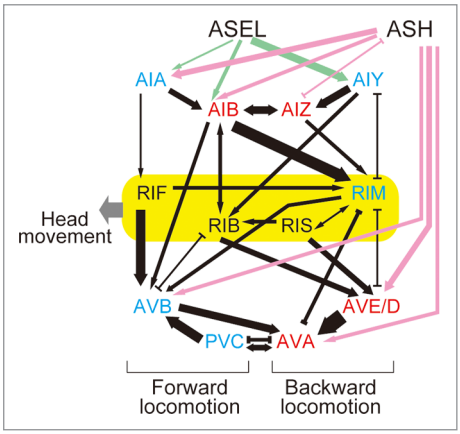

Figure 2. Model neural networks that may be involved in alkaline pH sensing. Interneurons that regulate motor neurons for head movement are indicated by a yellow background, and interneurons that are involved in forward and backward locomotion are shown in blue and red, respectively.[19-23] Arrows and I-shaped bars represent chemical synapses and gap junctions, respectively. Thickness of arrows and I-shaped bars indicate relative strengths of the connections.

ASH.[10,11] Although it has not been experimentally shown, the TRPV channel is a candidate for a strongly alkaline-pH sensor molecule in ASH since TRPV1, a mammalian homolog of OSM-9 and OCR-2, is activated by intracellular alkalinization.[5] Alternatively, strongly alkaline pH may be sensed by an unidentified sensor molecule that subsequently activates TRPV channels. In olfactory AWA neurons, polyunsaturated fatty acids mediate intracellular signaling from an olfactory receptor to OSM-9/OCR-2 channels.[12]

Like *C. elegans*, fish also exhibit a preference for alkaline water ranging from pH 9–10, but they avoid pH higher than 10.0.[13,14] To understand how organisms respond to alkaline pH and regulate their behavior, we analyzed chemotactic responses of *C. elegans* mutants using agar plates, as described previously.[10] *dyf-3* animals, defective in the sensory structure of gustatory neurons, which include ASE and ASH, were insensitive to alkaline pH (**Fig. 1**). Interestingly, *osm-9* mutants were attracted to not only mildly alkaline pH, but also strongly alkaline pH (**Fig. 1**), indicating that *osm-9* functions in ASH, but not in ASEL. Whereas *che-1* mutants, which lack both ASE neurons (ASEL and ASER),[15,16] were not at all attracted to mildly alkaline pH, the mutants instead avoided pH 9. These results are consistent with the concept that the ASEL gustatory sensory neuron and ASH multimodal nociceptors are responsible for alkalinity sensation at mildly and strongly alkaline pH, respectively. It was particularly interesting that *osm-9* was attracted to strongly alkaline pH. This led us to hypothesize that ASEL and ASH activities might compete with each other to regulate the behaviors.

To test the hypothesis described above, we analyzed chemotactic behaviors of *lsy-6* mutants, which have 2 ASER neurons due to genetic transformation of ASEL into ASER.[17] We also analyzed behaviors of animals that have 2 ASELs, which were created by expressing the wild-type *lsy-6* gene in both ASE neurons.[18] Interestingly, animals with two ASERs were repelled by mildly alkaline pH (9–10), to which the wild type is attracted (**Fig. 1**). This indicates that in the absence of ASEL, ASH nociceptors are activated by mildly alkaline pH and induce avoidance behaviors even against mildly alkaline pH. In contrast, animals with 2 ASELs were more strongly attracted to mildly alkaline pH. This result also suggests that enhanced activation of ASEL induces stronger attraction to mildly alkaline pH. However, animals with 2 ASELs showed normal avoidance behavior from strongly alkaline pH, indicating that ASH-mediated signals are dominant over ASEL-mediated signals. As described above, the dominance of ASH over ASEL may be explained by long-lasting Ca^{2+} entry into ASH during alkaline-pH stimulation, while Ca^{2+} entry into ASEL is transient.[9,10] Neural networks transmitting signals from ASH and ASEL may also play roles in regulating the behaviors. As shown in Figure 2, both ASEL and ASH send signals to primary layer interneurons consisting of AIA, AIB, AIY, and AIZ, and in addition, ASH directly sends signals to command interneurons such as AVA and AVE/D, which provoke backward movement of the animals through directly activating motor neurons.[19] This direct connection between ASH and command interneurons

may more efficiently function over ASEL signals sequentially transmitted through the primary, secondary, and command interneurons. Toward an understanding of how the nervous system regulates the behaviors, we need to elucidate functional networks through which ASH and ASEL signals are transmitted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Holzer P. Acid sensing by visceral afferent neurones. Acta Physiol (Oxf) 2011; 201:63-75; http://dx.doi.org/10.1111/j.1748-1716.2010.02143.x; PMID:20456281
2. Merivee E, Ploomi A, Milius M, Luik A, Heidemaa M. Electrophysiological identification of antennal pH receptors in the ground beetle *Pterostichus oblongopunctatus.* Physiol Entomol 2005; 30:122-33; http://dx.doi.org/10.1111/j.1365-3032.2005.00435.x
3. Ward S. 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 1973; 70:817-21; PMID:4351805; http://dx.doi.org/10.1073/pnas.70.3.817
4. Bryant BP. Mechanisms of somatosensory neuronal sensitivity to alkaline pH. Chem Senses 2005; 30:i196-7; http://dx.doi.org/10.1093/chemse/bjh182; PMID:15738112
5. Dhaka A, Uzzell V, Dubin AE, Mathur J, Petrus M, Bandell M, Patapoutian A. TRPV1 is activated by both acidic and basic pH. J Neurosci 2009; 29:153-8; http://dx.doi.org/10.1523/JNEUROSCI.4901-08.2009; PMID:19129393
6. Fujita F, Uchida K, Moriyama T, Shima A, Shibasaki K, Inada H, Sokabe T, Tominaga M. Intracellular alkalization causes pain sensation through activation of TRPA1 in mice. J Clin Invest 2008; 118:4049-57; http://dx.doi.org/10.1172/JCI35957; PMID:19033673
7. Lishko PV, Kirichok Y, Ren D, Navarro B, Chung JJ, Clapham DE. The control of male fertility by spermatozoan ion channels. Annu Rev Physiol 2012; 74:453-75; http://dx.doi.org/10.1146/annurev-physiol-020911-153258; PMID:22017176
8. Dusenbery DB. Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. J Exp Zool 1974; 188:41-7; PMID:4822548; http://dx.doi.org/10.1002/jez.1401880105
9. Murayama T, Takayama J, Fujiwara M, Maruyama IN. Environmental alkalinity sensing mediated by the transmembrane guanylyl cyclase GCY-14 in *C. elegans.* Curr Biol 2013; 23:1007-12; http://dx.doi.org/10.1016/j.cub.2013.04.052; PMID:23664973
10. Sassa T, Murayama T, Maruyama IN. Strongly alkaline pH avoidance mediated by ASH sensory neurons in *C. elegans.* Neurosci Lett 2013; 13: http://dx.doi.org/10.1016/j.neulet.2013.06.001; PMID:23769685
11. Tobin D, Madsen D, Kahn-Kirby A, Peckol E, Moulder G, Barstead R, Maricq A, Bargmann C. Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. Neuron 2002; 35:307-18; http://dx.doi.org/10.1016/S0896-6273(02)00757-2; PMID:12160748
12. Kahn-Kirby AH, Dantzker JL, Apicella AJ, Schafer WR, Browse J, Bargmann CI, Watts JL. Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. Cell 2004; 119:889-900; http://dx.doi.org/10.1016/j.cell.2004.11.005; PMID:15607983
13. Hartwell SI, Jin HJ, Cherry DS, Cairns J. Evaluation of Statistical-Methods for Avoidance Data of Schooling Fish. Hydrobiologia 1986; 131:63-76; http://dx.doi.org/10.1007/BF00008325
14. West DW, Boubee JAT, Barrier RFG. Responses to pH of nine fishes and one shrimp native to New Zealand freshwaters. New Zeal J Mar Fresh 1997; 31:461-8; http://dx.doi.org/10.1080/00288330.1997.9516779
15. Uchida O, Nakano H, Koga M, Ohshima Y. The *C. elegans che-1* gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development 2003; 130:1215-24; http://dx.doi.org/10.1242/dev.00341; PMID:12588839
16. Lewis JA, Hodgkin JA. Specific neuroanatomical changes in chemosensory mutants of the nematode *Caenorhabditis elegans*. J Comp Neurol 1977; 172:489-510; http://dx.doi.org/10.1002/cne.901720306; PMID:838889
17. Johnston RJ, Hobert O. A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans.* Nature 2003; 426:845-9; http://dx.doi.org/10.1038/nature02255; PMID:14685240
18. Ortiz CO, Faumont S, Takayama J, Ahmed HK, Goldsmith AD, Pocock R, McCormick KE, Kunimoto H, Iino Y, Lockery S, et al. Lateralized gustatory behavior of *C. elegans* is controlled by specific receptor-type guanylyl cyclases. Curr Biol 2009; 19:996-1004; http://dx.doi.org/10.1016/j.cub.2009.05.043; PMID:19523832
19. Chalfie M, Sulston JE, White JG, Southgate E, Thomson JN, Brenner S. The neural circuit for touch sensitivity in *Caenorhabditis elegans.* J Neurosci 1985; 5:956-64; PMID:3981252
20. Gray JM, Hill JJ, Bargmann CI. A circuit for navigation in *Caenorhabditis elegans.* Proc Natl Acad Sci U S A 2005; 102:3184-91; http://dx.doi.org/10.1073/pnas.0409009101; PMID:15689400
21. Piggott BJ, Liu J, Feng Z, Wescott SA, Xu XZ. The neural circuits and synaptic mechanisms underlying motor initiation in *C. elegans.* Cell 2011; 147:922-33; http://dx.doi.org/10.1016/j.cell.2011.08.053; PMID:22078887
22. Tsalik EL, Hobert O. Functional mapping of neurons that control locomotory behavior in *Caenorhabditis elegans.* J Neurobiol 2003; 56:178-97; http://dx.doi.org/10.1002/neu.10245; PMID:12838583
23. Wakabayashi T, Kitagawa I, Shingai R. Neurons regulating the duration of forward locomotion in *Caenorhabditis elegans.* Neurosci Res 2004; 50:103-11; http://dx.doi.org/10.1016/j.neures.2004.06.005; PMID:15288503