Communicative & Integrative Biology 6:6, e26668; November/December 2013; © 2013 Landes Bioscience

A G-protein α subunit, GOA-1, plays a role in *C. elegans* avoidance behavior of strongly alkaline pH

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Keywords: alkalinity sensing, chemotaxis, nociceptor, G protein, TRPV channels

The ability of animals to avoid strongly alkaline pH is critical for survival. However, the means by which they sense high pH has not been determined. We have previously found that the nematode *Caenorhabditis elegans* (*C. elegans*) avoids environmental pH above 10.5. Detection involves ASH nociceptive neurons as the major sensors. Upon stimulation, transient receptor potential vanilloid-type (TRPV) ion channels encoded by *osm-9* and *ocr-2* play an essential role in Ca^{2+} entry into ASH. Here we report that *C. elegans* mutants deficient in a G-protein α subunit, GOA-1, failed to avoid strongly alkaline pH with normal Ca^{2+} influx into ASH. These results suggest that GOA-1 regulates signal transmission downstream of Ca^{2+} influx through OSM-9/OCR-2 TRPV channels in ASH.

Survival requires that animals monitor environmental pH. Mammalian trigeminal neurons respond to pH ranging from 7.8 to 10.0.[1] Fish,[2] shrimp,[3] insects,[4] and nematodes[5] are also sensitive to environmental alkalinity. Secretion of the peptide hormone, gastrin, in the stomach is promoted by luminal alkalinization,[6] and capsaicin-sensitive afferent neurons seem to be involved in this process.[7] However, little is known about how sensory neurons detect it. *C. elegans* has proven extremely useful for dissecting the molecular bases of behavior. The nervous system of adult hermaphrodites comprises 302 neurons, including 12 pairs of amphid sensory neurons with ciliated dendrites.[8] Among the sensory neurons, 3 pairs, ASH, ADL, and AWB, are responsible for sensing chemical repellents.[9,10] Stimulation of these neurons triggers reverse locomotion. In particular, ASH is polymodal, and is required for nociception; mechanosensation, osmosensation, and chemosensation.[11,12] TRPV ion channels, which consist of OSM-9 and OCR-2 subunits, are expressed in ASH, and are involved in sensation of strongly alkaline pH.[13] Mutations in *ocr-2* reduce all the 3 forms of nociception.[14] Both osmosensation and mechanosensation require ODR-3, a G-protein α-subunit, in ASH.[15] These observations suggest that OSM-9 and OCR-2 are not directly activated by mechanical stimuli,[16] and that the signals detected by unknown sensor molecules may be transmitted to OSM-9/OCR-2 channels through ODR-3.

Our previous study on the aversion of *C. elegans* to strongly alkaline pH showed that the nematode senses pH higher than 10.5 as a noxious stimulus via ASH nociceptive sensory neurons, in which OSM-9/OCR-2 TRPV channels play an essential role.[13] Furthermore, it is known that ODR-3 appears to be generally important for the cellular response to most, if not all, repellents sensed by ASH.[17] To determine whether any upstream factors regulate OSM-9/OCR-2 channels in strongly alkaline-pH sensation, we analyzed behavior of *C. elegans* mutants deficient in G-protein α-subunits using a chemotaxis assay. Details of the experiments were described in our previous study[13] on the aversion of *C. elegans* to strongly alkaline pH. ASH is known to express at least 10 G-protein α-subunits: EGL-30, GOA-1, GPA-1, GPA-3, GPA-11, GPA-13, GPA-14, GPA-15, GSA-1, and ODR-3.[15,18,19] Among mutants defective in these genes, only *goa-1* mutants were unable to avoid strongly alkaline pH (**Fig. 1A**). All the other mutants, including *odr-3,* retreated from the noxious stimulus. However, Ca^{2+} transients in ASH of *goa-1* mutants were clearly observed at levels similar to those of wild-type N2 (**Fig. 1B**), indicating that GOA-1 functions downstream of OSM-9/OCR-2 channels in intracellular signaling, perhaps in synaptic exocytosis, or in downstream neurons of ASH. Indeed, it has previously been shown that GOA-1 does not affect neuronal depolarization in response to aversive stimuli such as high osmolality and quinine, but acts in ASH to modulate downstream transmission of intracellular signals.[20]

As described above, ODR-3 appears to be generally important for the cellular response to most, if not all, repellents sensed by ASH,[17] and the OSM-9/OCR-2 TRPV channel appears to be the signal generation channel downstream of G-protein coupled receptors (GPCRs) and ODR-3/GPA-3 G-protein signaling.[21] As shown in the present study, however, mutant animals deficient

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Submitted: 09/19/2013; Revised: 10/01/2013; Accepted: 10/01/2013
Citation: Sassa T, Maruyama IN. A G-protein α subunit, GOA-1, plays a role in C. elegans avoidance behavior of strongly alkaline pH. Communicative & Integrative Biology 2014; 7:e26668; http://dx.doi.org/10.4161/cib.26668

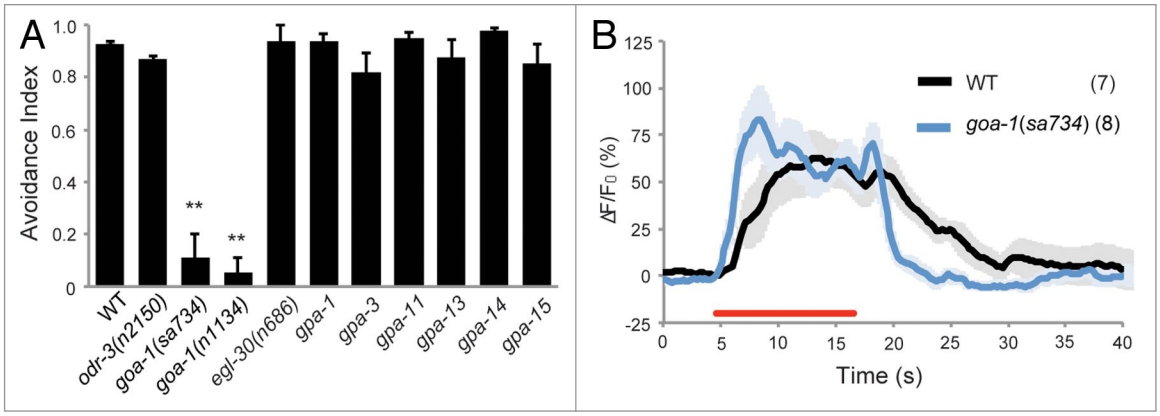

Figure 1. Behavior and imaging analyses of mutants deficient in a G protein α subunit. (**A**) Avoidance indices of wild-type and mutant animals. Assays were performed using petri dishes with 4 quadrants as described previously.[13] Error bars indicate the SEM of 5 independent assays. **p < 0.01. (**B**) Ca^{2+} imaging of ASH in wild-type and *goa-1* animals upon stimulation with pH 11.2. The red line represents the period of time during which animals were stimulated with pH 11.2 buffer. Numbers of recordings are shown in parentheses, and light color shading denotes the SEM.

in *odr-3* or *gpa-3* showed similar avoidance indices to those of wild-type animals. This suggests that the OSM-9/OCR-2 channel may be a direct sensor molecule for strongly alkaline pH, and that the channel may not be regulated by upstream GPCRs. Indeed, ammonia and intracellular alkalinization directly activate TRPV1 in cultured cells, via a mechanism that involves a cytoplasmic histidine residue of the channel.[22] Thus, the present study suggests that OSM-9/OCR-2 TRPV channels may serve as sensor molecules for strongly alkaline pH, and that GOA-1 may act to modulate downstream signaling of TRPV channels in ASH. However, these results do not rule out possibilities that other molecules than GPCRs may act as a sensor, or that the G-proteins may act redundantly as sensors for strongly alkaline pH.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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