Caenorhabditis elegans TRPV Channels Function in a Modality-Specific Pathway to Regulate Response to Aberrant Sensory Signaling

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Manuscript received February 4, 2010
Accepted for publication February 20, 2010

ABSTRACT

Olfaction and some forms of taste (including bitter) are mediated by G protein-coupled signal transduction pathways. Olfactory and gustatory ligands bind to chemosensory G protein-coupled receptors (GPCRs) in specialized sensory cells to activate intracellular signal transduction cascades. G protein-coupled receptor kinases (GRKs) are negative regulators of signaling that specifically phosphorylate activated GPCRs to terminate signaling. Although loss of GRK function usually results in enhanced cellular signaling, *Caenorhabditis elegans* lacking GRK-2 function are not hypersensitive to chemosensory stimuli. Instead, *grk-2* mutant animals do not chemotax toward attractive olfactory stimuli or avoid aversive tastes and smells. We show here that loss-of-function mutations in the transient receptor potential vanilloid (TRPV) channels OSM-9 and OCR-2 selectively restore *grk-2* behavioral avoidance of bitter tastants, revealing modality-specific mechanisms for TRPV channel function in the regulation of *C. elegans* chemosensation. Additionally, a single amino acid point mutation in OCR-2 that disrupts TRPV channel-mediated gene expression, but does not decrease channel function in chemosensory primary signal transduction, also restores *grk-2* bitter taste avoidance. Thus, loss of GRK-2 function may lead to changes in gene expression, via OSM-9/OCR-2, to selectively alter the levels of signaling components that transduce or regulate bitter taste responses. Our results suggest a novel mechanism and multiple modality-specific pathways that sensory cells employ in response to aberrant signal transduction.

TO survive, organisms must be able to recognize and respond appropriately to chemical cues in their environment that indicate the presence or absence of food, reproductive partners, or predators. Chemosensation is the fundamental process by which chemical signals, in the form of gustatory (taste) and olfactory (smell) stimuli, are detected. The sense of taste is particularly vital to ensure survival as it confers the ability to distinguish favorable food sources from hazardous compounds before they are ingested (HERNESS and GILBERTSON 1999; PEREZ *et al.* 2003). Bitter or sour tastes usually indicate the presence of toxic compounds that would be rejected, whereas salty, sweet, and umami (amino acid) reflect the presence of valuable nutrients (HERNESS and GILBERTSON 1999).

Olfaction and gustatory responses to bitter, sweet, and umami stimuli are generally mediated by G protein-coupled signal transduction pathways that are conserved across species (DRYER and BERGHARD 1999; CHANDRASHEKAR *et al.* 2006; PALMER 2007). Signaling is initiated when a ligand (odorant or tastant) binds to a seven-transmembrane

G protein-coupled receptor (GPCR), inducing a conformational change in the receptor that activates the associated heterotrimeric G proteins. The G α subunit exchanges GDP for GTP and, now activated, dissociates from the G β and G γ (G $\beta\gamma$) subunits. Both the free G α -GTP and G $\beta\gamma$ subunits can stimulate intracellular signaling cascades by interacting with downstream effectors such as adenylate cyclases, phospholipases, and ion channels (MCCUDDEN *et al.* 2005).

Following the activation of G protein-coupled signaling, a negative feedback mechanism known as desensitization is initiated (HAUSDORFF *et al.* 1990; METAYE *et al.* 2005). G protein-coupled receptor kinases (GRKs) recognize and phosphorylate activated GPCRs (FREEDMAN and LEFKOWITZ 1996; PITCHER *et al.* 1998; PENN *et al.* 2000; PREMONT and GAINETDINOV 2007). The phosphorylated GPCRs can then be bound by cytosolic arrestin proteins (FREEDMAN and LEFKOWITZ 1996; METAYE *et al.* 2005; PREMONT and GAINETDINOV 2007). GRK phosphorylation and arrestin binding result in the cessation of G protein signaling, even in the continued presence of agonist (FREEDMAN and LEFKOWITZ 1996; PENN *et al.* 2000; PREMONT and GAINETDINOV 2007). This desensitization process is necessary to avoid the potentially harmful effects that can result from excessive stimulation through activated GPCRs (METAYE *et al.* 2005). For example, loss-of-function mutations in human GRK1 (rhodopsin kinase) lead to Oguchi disease (CIDECIYAN *et al.* 1998; YAMADA *et al.* 1999). GRK1 is required for rod

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.110.115188/DC1>.

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recovery after photoactivation, and in patients with this disease, prolonged rod photoreceptor responses and slow recovery following light exposure result in night blindness. In a mouse model for Oguchi disease, loss of GRK1 function leads to retinal degeneration (CHEN *et al.* 1999).

In most instances, the absence of GRK-mediated desensitization causes prolonged, exaggerated responses to GPCR agonists (JABER *et al.* 1996; ROCKMAN *et al.* 1998; GAINETDINOV *et al.* 1999, 2003; PREMONT and GAINETDINOV 2007). However, there are unique situations in which loss of a particular GRK can lead to decreased signaling and responsiveness in a cell-specific manner. For example, while GRK6^{-/-} mice are hypersensitive to psychostimulants such as cocaine (GAINETDINOV *et al.* 2003), T cells from GRK6-deficient mice are significantly impaired in their chemotactic response to CXCL12, a stimulatory chemokine that wild-type T cells migrate toward (FONG *et al.* 2002). Additionally, loss of GRK3, which is highly expressed in mouse olfactory epithelium (SCHLEICHER *et al.* 1993), significantly reduces odorant-induced generation of the second messenger cAMP in cilia preparations (PEPPEL *et al.* 1997), in addition to the expected lack of agonist-induced desensitization following odorant exposure (SCHLEICHER *et al.* 1993; BOEKHOFF *et al.* 1994; PEPPEL *et al.* 1997).

As soil dwelling nematodes, *Caenorhabditis elegans* depend heavily upon their ability to detect volatile (olfactory) and soluble (gustatory) chemicals to find food, avoid noxious environments, develop appropriately, and mate (BARGMANN 2006a). Despite their small nervous system, consisting of just 302 neurons, *C. elegans* have a remarkable chemosensory repertoire. Using a limited number of head and tail sensory neurons, *C. elegans* are able to detect hundreds of chemicals as well as discriminate among multiple chemosensory stimuli when they are presented simultaneously (BARGMANN and HORVITZ 1991; BARGMANN *et al.* 1993; TROEMEL 1999; BARGMANN 2006a). The 11 pairs of chemosensory neurons located in the head each respond to a defined subset of stimuli (BARGMANN 2006a). The AWA and AWC olfactory neurons detect chemicals that *C. elegans* are attracted to and chemotax toward, while the ASH, ADL, AWB, and ASK sensory neurons detect aversive odorants and tastants that *C. elegans* avoid by initiating backward locomotion upon stimulus detection (BARGMANN 2006a). Furthermore, the ASH nociceptive neurons are polymodal and detect a range of aversive stimulants, including the odorant octanol, the bitter tastant quinine, SDS, high osmolarity, heavy metals such as copper, and light touch to the nose (BARGMANN *et al.* 1990; KAPLAN and HORVITZ 1993; HART *et al.* 1999; SAMBONGI *et al.* 1999; TROEMEL 1999; HILLIARD *et al.* 2002, 2004, 2005).

The *C. elegans* genome encodes >500 predicted chemosensory GPCRs (BARGMANN 2006a) and 21 G α , 2 G β , and 2 G γ subunits (JANSEN *et al.* 1999; CUPPEN *et al.* 2003). The G α proteins ODR-3 and GPA-3 have a stimulatory role in chemical detection in AWA, AWC, and ASH

(ROAYAIE *et al.* 1998; TROEMEL 1999; HILLIARD *et al.* 2004; LANS *et al.* 2004; BARGMANN 2006a). Downstream of G proteins, two distinct channels appear to be involved in chemosensory signal transduction. A cyclic nucleotide-gated channel encoded by the *tax-2* and *tax-4* genes is a sensory transduction channel in the AWC neurons (COBURN and BARGMANN 1996; KOMATSU *et al.* 1996). In contrast, the OSM-9 and OCR-2 transient receptor potential vanilloid (TRPV) channel subunits participate in primary signal transduction in the AWA and ASH neurons (COLBERT *et al.* 1997; HILLIARD *et al.* 2002, 2005; TOBIN *et al.* 2002). Accordingly, loss of OSM-9 or OCR-2 results in mild to severe defects in AWA-mediated olfactory responses and ASH-mediated avoidance behaviors (COLBERT *et al.* 1997; TOBIN *et al.* 2002; HILLIARD *et al.* 2004, 2005). In addition, FRET-based imaging revealed that Ca²⁺ transients, which are likely downstream of GPCR signaling in all sensory neurons, are reduced or eliminated in ASH in response to a variety of stimuli (including quinine) in *osm-9* mutants (HILLIARD *et al.* 2005). However, *osm-9* and *ocr-2* mutants retain a substantial behavioral response to the bitter tastant quinine (HILLIARD *et al.* 2004; this work), which is detected primarily by ASH. This indicates that while OSM-9/OCR-2 contribute to bitter taste transduction, other channels are also likely involved.

Although GRKs have classically been described as negative regulators of GPCR signal transduction, *C. elegans* lacking GRK-2 function are not hypersensitive to chemical stimuli due to increased sensory signaling (FUKUTO *et al.* 2004). Instead, *grk-2* mutant animals neither chemotax toward attractive odorants detected by AWA and AWC nor avoid aversive odorants and tastants detected primarily by ASH (FUKUTO *et al.* 2004). Consistent with defective chemosensory behavioral responses, loss of GRK-2 function leads to a decrease in stimulus-evoked Ca²⁺ signaling in the ASH sensory neurons (FUKUTO *et al.* 2004). Taken together, loss of GRK-2 function leads to decreased signaling in *C. elegans* sensory neurons, similar to loss of mammalian GRK3 in olfactory epithelia (PEPPEL *et al.* 1997; FUKUTO *et al.* 2004). However, the mechanism by which loss of mammalian GRK3 leads to decreased stimulus-induced cAMP levels is not known.

It was proposed that loss of *C. elegans* GRK-2 may initially result in excessive chemosensory signaling, but that this activates compensatory mechanisms to down-regulate G protein-coupled signal transduction and terminate signaling (FUKUTO *et al.* 2004) (supporting information, Figure S1). In this model, the inhibitory compensatory mechanism, which serves to protect against the potentially harmful effects of excessive neuronal stimulation, renders *grk-2* mutants unable to respond to a range of chemosensory stimuli. Consistent with this model, loss of the regulator of G protein signaling (RGS) GTPase-activating protein EAT-16, a negative regulator of G α activity, restores *grk-2* chemotaxis to diacetyl

(AWA) (FUKUTO *et al.* 2004). However, loss of EAT-16 has no effect on ASH-mediated behaviors. *grk-2;eat-16* double mutants remain defective for octanol (odorant) and quinine (tastant) avoidance. Furthermore, we found that loss of the other neuronally expressed RGS proteins, EGL-10, RGS-1, RGS-2, RGS-3, RGS-6, or RGS-10/11 (KOELLE and HORVITZ 1996; DONG *et al.* 2000; KUNITOMO *et al.* 2005; FERKEY *et al.* 2007; M. KOELLE, personal communication), does not restore ASH-mediated behaviors (data not shown). These results indicate that there are diverse, cell-specific responses to aberrant G protein-coupled signaling.

We sought to identify mechanisms responsible for regulating chemosensory GPCR signaling in the absence of GRK function in the ASH sensory neurons. Using *C. elegans grk-2* mutant animals, we performed a forward genetic screen to isolate animals with the restored ability to respond to quinine, an aversive bitter tastant detected by ASH (HILLIARD *et al.* 2004). We isolated eight mutants in which the quinine response of *grk-2* animals was restored; three of the mutations were found to be in the TRPV-related channels OSM-9 and OCR-2. Surprisingly, we find that complete loss of OSM-9/OCR-2 channel function restores response of *grk-2* mutants in both a cell-specific and modality-specific manner, as *grk-2;TRPV* double mutants have a wild-type response to bitter tastants, but remain defective for other chemosensory stimuli detected by ASH and AWA.

Downstream of their roles in primary signal transduction, OSM-9 and OCR-2 affect activity-dependent gene expression pathways to regulate the long-term transcriptional levels of sensory genes. Loss of these TRPV channels reduces expression of ODR-10, a GPCR expressed in the AWA olfactory neurons that detects diacetyl (SENGUPTA *et al.* 1996; TOBIN *et al.* 2002) and selectively decreases expression of the serotonin biosynthetic enzyme TPH-1 in the ADF sensory neurons (ZHANG *et al.* 2004). Furthermore, OCR-2 appears to use distinct structural motifs for primary chemosensory signal transduction and modulation of pathways that control transcriptional activity (SOKOLCHIK *et al.* 2005). Specifically, the OCR-2(G36E) N-terminal point mutation, encoded by *ocr-2(yz5)*, results in decreased TPH-1 expression in ADF, but does not diminish AWA olfaction (SOKOLCHIK *et al.* 2005). Unexpectedly, we also find that the OCR-2(G36E) point mutation is sufficient to restore the response of *grk-2* mutants to bitter tastants. This suggests that a unique output of TRPV function, transmitted via the OCR-2(G36) N-terminal structural motif, leads to the bitter taste defects of *grk-2* animals.

MATERIALS AND METHODS

Strains: Strains were maintained under standard conditions on NGM agar plates seeded with OP50 *Escherichia coli* bacteria (BRENNER 1974). Strains used in this study include: N2 Bristol wild type, CB4856 Hawaiian, FG7 *grk-2(gk268)*, FG78 *grk-2(gk268)*;

ocr-2(ud21), FG87 *grk-2(gk268);osm-9(ud23)*, FG91 *grk-2(gk268);osm-9(ud19)*, CX10 *osm-9(ky10)*, FG60 *grk-2(gk268);osm-9(ky10)*, CX4544 *ocr-2(ak47)*, FG99 *grk-2(gk268);ocr-2(ak47)*, LX748 *osm-9(ky10)ocr-2(ak47)*, FG118 *grk-2(gk268);osm-9(ky10)ocr-2(ak47)*, JY243 *ocr-2(yz5)*, FG140 *grk-2(gk268);ocr-2(yz5)*, CX7265 *osm-9(ky10);yzEx53[osm-10::osm-9,elt-2::gfp]*, FG130 *grk-2(gk268);osm-9(ky10);yzEx53[osm-10::osm-9,elt-2::gfp]*, FG166 *udEx15[osm-10::ocr-2,elt-2::gfp]*, FG167 *udEx16[osm-10::ocr-2,elt-2::gfp]*, FG168 *udEx17[osm-10::ocr-2,elt-2::gfp]*, FG169 *grk-2(gk268);ocr-2(yz5);udEx18[osm-10::ocr-2,elt-2::gfp]*, FG170 *grk-2(gk268);ocr-2(yz5);udEx19[osm-10::ocr-2,elt-2::gfp]*, FG171 *grk-2(gk268);ocr-2(yz5);udEx20[osm-10::ocr-2,elt-2::gfp]*, FG173 *udEx22[srb-6::ocr-2,elt-2::gfp]*, FG174 *udEx23[srb-6::ocr-2,elt-2::gfp]*, FG175 *udEx24[srb-6::ocr-2,elt-2::gfp]*, FG176 *grk-2(gk268);ocr-2(yz5);udEx25[srb-6::ocr-2,elt-2::gfp]*, FG177 *grk-2(gk268);ocr-2(yz5);udEx26[srb-6::ocr-2,elt-2::gfp]*, FG179 *grk-2(gk268);ocr-2(yz5);udEx28[srb-6::ocr-2,elt-2::gfp]*, FG180 *grk-2(gk268);ocr-2(ak47);udEx29[osm-10::ocr-2,elt-2::gfp]*, FG181 *grk-2(gk268);ocr-2(ak47);udEx30[osm-10::ocr-2,elt-2::gfp]*, FG182 *grk-2(gk268);ocr-2(ak47);udEx31[osm-10::ocr-2,elt-2::gfp]*, FG184 *grk-2(gk268);ocr-2(ak47);udEx33[srb-6::ocr-2,elt-2::gfp]*, FG185 *grk-2(gk268);ocr-2(ak47);udEx34[srb-6::ocr-2,elt-2::gfp]*, FG186 *grk-2(gk268);ocr-2(ak47);udEx35[srb-6::ocr-2,elt-2::gfp]*, FG187 *udEx36[sra-6::ocr-2,elt-2::gfp]*, FG188 *udEx37[sra-6::ocr-2,elt-2::gfp]*, FG189 *udEx38[sra-6::ocr-2,elt-2::gfp]*, FG190 *grk-2(gk268);ocr-2(ak47);udEx39[sra-6::ocr-2,elt-2::gfp]*, and FG191 *grk-2(gk268);ocr-2(ak47);udEx40[sra-6::ocr-2,elt-2::gfp]*.

Plasmid construction: *osm-10::ocr-2* (pFG14): The *ocr-2* promoter was removed from pAJ35 (gift of Cori Bargmann) using *SphI* and *XmaI*, leaving the *ocr-2* cDNA in the vector backbone. The ~900-bp upstream promoter region of *osm-10* was removed from CR142 (RONGO *et al.* 1998) using *SphI* and *XmaI* and was placed into these sites upstream of the *ocr-2* cDNA in the remaining fragment of pAJ35.

srb-6::ocr-2 (pFG15): The ~1.3 kb *srb-6* promoter was first isolated from pHA#355 (FUKUTO *et al.* 2004) using *PstI* and *BamHI* and inserted into the same sites of Fire vector pPD49.26 to create pFG10. The *srb-6* promoter was then removed from pFG10 using *SphI* and *XmaI* and was placed into these sites upstream of the *ocr-2* cDNA (remaining fragment of pAJ35, as described above).

hsp::ocr-2 (pFG16): The *hsp16-2* promoter was removed from Fire vector pPD49.78 using *SphI* and *XmaI* and was placed into these sites upstream of the *ocr-2* cDNA (remaining fragment of pAJ35, as described above).

sra-6::ocr-2: This construct was the kind gift of Cori Bargmann and was described previously (DE BONO *et al.* 2002).

Genetic analysis: *grk-2(gk268)* animals were mutagenized with EMS (ethyl methanesulfonate) as previously described (BRENNER 1974). Using this deletion allele decreased the likelihood of isolating intragenic suppressors or revertants, as might have been more likely if the *grk-2(rt97)* animals, which contain a single point mutation, were used. F₂ animals were assayed for avoidance of 10 mM quinine using the drop assay with the quinine drop placed in front of the animal (HILLIARD *et al.* 2002; FUKUTO *et al.* 2004). Animals that responded by initiating backward locomotion within 4 sec of encountering the drop were selected. A total of 25,000 F₂ animals were screened. Eight mutant strains were isolated.

To generate the mapping strain, the *grk-2(gk268)* allele (which was in the N2 wild-type background) was crossed into the Hawaiian single nucleotide polymorphism (SNP) strain CB4856. Animals were then extensively recrossed (10 times) to CB4856 and PCR and restriction enzyme digests were used to verify that all of the chromosomes had been replaced by CB4856 DNA, except for the left arm of chromosome III, where *grk-2(gk268)* now resided (map position -26.34). In short, *grk-2(gk268)* was crossed into the CB4856 Hawaiian background. Since the *grk-2(gk268)* allele was in both the

animals that were used in the EMS screen and in the mapping strain, it remained homozygous during all mapping crosses. This allowed the use of the CB4856 restriction fragment length polymorphisms (RFLPs) to map the *grk-2(gk268)* suppressor mutations. In genetic mapping experiments, *ud19* and *ud23* were linked to LG IV near SNP C09G12 and *ud21* was linked to LG IV between the two SNPs C06A6 and D2096. Following genetic linkage analysis, complementation assays using the previously defined alleles of *osm-9(ky10)* and *ocr-2(ak47)* confirmed that *ud19* and *ud23* were alleles of *osm-9*, while *ud21* represented an allele of *ocr-2*. All subsequent behavioral experiments were performed with previously characterized alleles of *osm-9* and *ocr-2*, and the molecular lesions in *ud19*, *ud23*, and *ud21* have not been determined.

Behavioral assays: Well-fed young adult animals were used for analysis, and all behavioral assays were performed on at least 2 separate days, along with controls. Behavioral assays were performed as previously described. Response to octanol was scored as the amount of time it took an animal to initiate backward locomotion when presented with a hair dipped in octanol (TROEMEL *et al.* 1995; HART *et al.* 1999). (Assays were stopped at 20 sec.) Response to soluble tastants was scored as the percentage of animals that initiated backward locomotion within 4 sec of encountering a drop of tastant placed on the agar plate (HILLIARD *et al.* 2002, 2004; FUKUTO *et al.* 2004). Tastants were dissolved in M13, pH 7.4 (WOOD 1988). The drop was placed in front of a forward moving animal. For octanol and taste avoidance assays, animals were tested 10–20 min after transfer to NGM plates lacking bacteria (“off food”). Chemotaxis assays were performed as previously described (BARGMANN *et al.* 1993). After 1 hr, the chemotaxis index (C.I.) was calculated as the number of animals that had accumulated at the attractant, minus the number of animals at the control, divided by the total number of animals (BARGMANN *et al.* 1993). For heat-shock experiments, animals were raised to young adulthood and then shifted to 33° for 2 hr. They were allowed to recover for 4 hr at 25° prior to testing. All data are presented as $\pm$ standard error of the mean (SEM). Student's *t*-test was used for statistical analysis.

RESULTS

Loss of OSM-9 or OCR-2 TRPV channel function restores quinine response to *grk-2* mutant animals: To identify the mechanisms that regulate sensory signaling in the absence of GRK-2 function, we performed a forward genetic screen to identify second-site suppressor mutations that restored the response of *grk-2* animals to quinine. We used *grk-2(gk268)*, a deletion allele that removes 608 nucleotides of the 5' untranslated region and the first three exons of *grk-2* coding sequence (930 additional nucleotides); it is a predicted *grk-2* null and animals are phenotypically identical to the previously characterized *grk-2(rt97)* severe loss-of-function animals (FUKUTO *et al.* 2004).

grk-2(gk268) mutant animals were EMS mutagenized by standard protocol (BRENNER 1974) and second generation (F₂) progeny of mutagenized animals were tested for restoration of normal quinine response. Single nucleotide polymorphism (SNP) mapping (WICKS *et al.* 2001) and genetic complementation analysis identified *ud19* and *ud23* as alleles of *osm-9* and *ud21* as an allele of *ocr-2*. The previously defined null alleles of

these genes, *osm-9(ky10)* (COLBERT *et al.* 1997) and *ocr-2(ak47)* (TOBIN *et al.* 2002), were used in subsequent experiments. Loss of either OSM-9 or OCR-2 TRPV channel function restored the response of *grk-2* mutants to 10 mM quinine to wild-type levels (Figure 1A). In addition, because OSM-9 and OCR-2 require each other for their mutual subcellular localization and function, simultaneous loss of OSM-9 and OCR-2 should affect behavioral responses similarly to the individual loss of these channels (TOBIN *et al.* 2002). Consistent with this prediction, *grk-2;osm-9ocr-2* triple mutants responded to 10 mM quinine similarly to *grk-2;osm-9* and *grk-2;ocr-2* double mutants (Figure 1A).

Loss of TRPV channel function restores bitter taste response generally: *C. elegans* show an avoidance response to several soluble, bitter chemicals in addition to quinine (HILLIARD *et al.* 2004). We therefore tested whether loss of the TRPV channels OSM-9 and OCR-2 selectively restored quinine avoidance (Figure 1A) or whether decreased TRPV channel function could restore *grk-2* bitter taste response more generally. We assayed the response of *grk-2;osm-9*, *grk-2;ocr-2*, and *grk-2;osm-9ocr-2* animals to five additional bitter tastants. Similar to quinine, loss of OSM-9 and OCR-2, alone or in combination, completely or partially restored the response of *grk-2* animals to primaquine (Figure 1B), amodiaquine (Figure 1C), quinacrine, chloroquine, and shikimic acid (data not shown). Taken together, decreased TRPV channel function broadly restored bitter taste response to *grk-2* mutant animals.

Loss of TRPV channel function does not restore *grk-2* response to other ASH-detected stimuli: Loss of OSM-9 or OCR-2 TRPV function in *grk-2* mutant animals restored response to soluble bitter compounds that likely act through G protein-coupled receptors (CHANDRASHEKAR *et al.* 2006; PALMER 2007) expressed in the ASH sensory neurons. Olfactory receptors are also G protein-coupled receptors (DRYER and BERGHARD 1999) and *grk-2* animals are severely defective in their response to the aversive odorant octanol (FUKUTO *et al.* 2004). Octanol is detected primarily by ASH, with contributions from the ADL and AWB neurons (TROEMEL *et al.* 1995; CHAO *et al.* 2004). However, unlike bitter taste response, loss of the OSM-9/OCR-2 TRPV channels did not restore the octanol response of *grk-2* mutants back to wild-type levels (Figure 2A).

grk-2 animals are also partially defective for avoidance of several soluble, ASH-detected stimuli that are not thought to be detected by G protein-coupled receptors (FUKUTO *et al.* 2004), including copper and SDS (SAMBONGI *et al.* 1999; HILLIARD *et al.* 2002, 2005). As bitter tastants are soluble stimuli, we asked whether the TRPV suppression of *grk-2* defects extended to include these additional soluble compounds detected by ASH. Loss of neither OSM-9 nor OCR-2 restored the response of *grk-2* mutants to copper or SDS (Figure 2, B and C). We conclude that loss of TRPV channel selec-

FIGURE 1.—Loss of TRPV channel function restores *grk-2* bitter taste response. While *grk-2* mutant animals do not respond to bitter taste stimuli, loss of OSM-9 and OCR-2 TRPV channel function, alone or in combination, restored response to (A) 10 mM quinine, (B) 10 mM primaquine, and (C) 10 mM amodiaquine ($P \leq 0.001$ when compared to *grk-2* for each). Loss of OSM-9 and OCR-2 also partially or completely restored response to 10 mM quinacrine, 10 mM chloroquine, and 10 mM shikimic acid (not shown). Alleles used: *grk-2(gk268)*, *osm-9(ky10)*, and *ocr-2(ak47)*. WT, the N2 wild-type strain. All tastants were dissolved in M13 buffer,

tively restores the response of *grk-2* animals to soluble, bitter tastants, and does not restore ASH signaling in general.

Loss of TRPV channel function does not restore chemotaxis toward attractive odorants: The ASH and AWA sensory neurons share a common signal transduction pathway, with OSM-9 and OCR-2 being part of the primary signaling cascade in both neurons (COLBERT *et al.* 1997; TOBIN *et al.* 2002; HILLIARD *et al.* 2004). Having established that loss of either OSM-9 or OCR-2 function restored ASH-mediated avoidance of bitter tastants to *grk-2* mutant animals, we wondered whether the loss of either TRPV channel would restore AWA-mediated attractive chemosensory behavior. *grk-2;osm-9* and *grk-2;ocr-2* double mutant animals and *grk-2;osm-9ocr-2* triple mutants were assayed for their ability to chemotax toward the AWA-detected attractive odorants diacetyl and pyrazine (BARGMANN *et al.* 1993). A range of concentrations for each odorant was tested (diacetyl, 10^0 – 10^{-4} ; pyrazine, 100 mg/ml–1 mg/ml). Loss of neither TRPV channel, alone or in combination, restored AWA-mediated chemotaxis to *grk-2* mutant animals at any concentration (Figure 3 and data not shown). We conclude that although the ASH and AWA sensory neurons utilize many of the same primary signal transduction components, including OSM-9 and OCR-2, the mechanism by which decreased TRPV channel function restores chemosensory behavior to *grk-2* mutants is unique to the ASH-mediated avoidance of bitter tastants.

The TRPV channels function in the ASH sensory neurons: The OSM-9 and OCR-2 TRPV channels have very restricted expression patterns (COLBERT *et al.* 1997; TOBIN *et al.* 2002). OSM-9 is expressed in 10 pairs of head neurons, with OCR-2 being coexpressed in only 4 pairs of head sensory neurons: ASH, ADL, ADF, and AWA (TOBIN *et al.* 2002). Although GRK-2 is expressed throughout the *C. elegans* nervous system, it appears to function in the sensory neurons to regulate chemosensory signaling (FUKUTO *et al.* 2004). Importantly, loss of GRK-2 severely diminishes or eliminates stimulus-evoked Ca^{2+} fluxes in the ASH sensory neurons (FUKUTO *et al.* 2004). In addition, laser ablation studies revealed that ASH is the main sensory neuron responsible for quinine detection, although the ASK sensory neurons also contribute (HILLIARD *et al.* 2004).

As GRK-2 and the TRPV channels function in the sensory neurons (COLBERT *et al.* 1997; TOBIN *et al.* 2002; FUKUTO *et al.* 2004; HILLIARD *et al.* 2005), it suggests that loss of TRPV channel function in the ASH neurons themselves may restore quinine avoidance to *grk-2;osm-9* and *grk-2;ocr-2* double mutant animals. To determine where TRPV function contributes to the defective qui-

pH 7.4. The percentage of animals responding is shown. $N \geq 40$. Error bars represent the standard error of the mean (SEM).

FIGURE 2.—Loss of TRPV channel function does not restore *grk-2* response to other ASH-detected stimuli. The ASH sensory neurons also detect the volatile odorant octanol, the heavy metal copper, and the detergent SDS. *grk-2* mutant animals are defective in their avoidance response to each of these stimuli. (A) Loss of OSM-9 and OCR-2 TRPV channel function had only a minimal, although statistically significant ($P \leq 0.01$ when compared to *grk-2*) effect on 100% octanol response. However, *grk-2;osm-9*, *grk-2;ocr-2*, and *grk-2;osm-9ocr-2* responses were not restored to the level of wild-type animals or to that of the TRPV channel mutants ($P \leq 0.00001$). Time to respond is shown. $N \geq 40$. (B) Loss of the OSM-9 or OCR-2 TRPV channels, alone or in combination, did not restore *grk-2*

nine avoidance response of *grk-2* mutant animals, the *osm-10* promoter (HART *et al.* 1999), *srb-6* promoter (TROEMEL *et al.* 1995), and *sra-6* promoter (TROEMEL *et al.* 1995; DE BONO *et al.* 2002) were used to express wild-type *ocr-2* in *grk-2;ocr-2* null mutant animals. ASH is the only sensory neuron in which all three promoters are expressed. While *grk-2;ocr-2* double mutant animals respond robustly to quinine (Figure 4A), double mutant animals expressing the *osm-10::ocr-2*, *srb-6::ocr-2*, or *sra-6::ocr-2* transgene were returned to the *grk-2* quinine response defective phenotype (Figure 4A). Transgene expression had no effect in wild-type animals, indicating that transgene expression did not disrupt ASH function (data not shown). In addition, the *osm-10* promoter (HART *et al.* 1999) was used to express *osm-9* cDNA in the ASH sensory neurons of *grk-2;osm-9* animals. While *grk-2;osm-9* double mutants also respond robustly to quinine (Figure 4B), double mutant animals expressing the *osm-10::osm-9* transgene *yzEx53* (ZHANG *et al.* 2004; CHANG *et al.* 2006) were defective in their response to quinine (Figure 4B). We conclude that TRPV channel function in the ASH sensory neurons is sufficient for the defective quinine response of *grk-2* mutant animals, and that *grk-2;TRPV* animals may have restored bitter tastant chemosensory responses due to changes in ASH sensory neuron signaling in the absence of OSM-9/OCR-2 TRPV channel function.

The OCR-2(G36E) point mutation restores *grk-2* bitter taste response: The *ocr-2(yz5)* mutation encodes a single nucleotide change that creates a glycine-to-glutamate (G36E) substitution in the N-terminal cytoplasmic tail of OCR-2 (ZHANG *et al.* 2004; SOKOLCHIK *et al.* 2005). While the product of the *ocr-2(ak47)* deletion allele cannot form a functional channel, leading to severe disruption of AWA-mediated chemosensory transduction (TOBIN *et al.* 2002), the G36E substitution produces a protein with correct subcellular localization and *ocr-2(yz5)* mutants have wild-type AWA-mediated chemotaxis (SOKOLCHIK *et al.* 2005). Interestingly, the *ocr-2(yz5)* point mutation decreases expression of *tph-1*, which encodes a serotonin biosynthetic enzyme, in the ADF neurons as strongly as the *ocr-2(ak47)* predicted null (ZHANG *et al.* 2004). Thus, the N-terminal G36E mutation appears to selectively disrupt the ability of the OCR-2 TRPV channel to direct changes in gene

response to 10 mM copper ($P \geq 0.1$ when compared to *grk-2*). The percentage of animals responding is shown. $N \geq 40$. (C) Loss of the OSM-9 or OCR-2 TRPV channels, alone or in combination, did not restore *grk-2* response to 0.1% SDS. ($P \geq 0.01$ for *grk-2;osm-9* double mutants when compared to *grk-2*, with the double mutant response being somewhat worse than *grk-2*. $P \geq 0.1$ for *grk-2;ocr-2* and *grk-2;osm-9ocr-2* when compared to *grk-2*). The percentage of animals responding is shown. $N \geq 60$. Alleles used: *grk-2(gk268)*, *osm-9(ky10)*, and *ocr-2(ak47)*. WT, the N2 wild-type strain. Error bars represent the standard error of the mean (SEM).

FIGURE 3.—Loss of TRPV channel function does not restore AWA-mediated chemotaxis. The ASH and AWA sensory neurons share common signaling molecules to transduce chemosensory signals. The OSM-9 and OCR-2 TRPV channels are part of the primary signal transduction cascade in both neurons. (A and B) Loss of the OSM-9 or OCR-2 TRPV channels, alone or in combination, did not restore *grk-2* chemotaxis toward diacetyl over a range of concentrations tested. Chemotaxis to 1 μ l of (A) 1:100 and (B) 1:1000 diacetyl is shown; 100%, 1:10 and 1:10,000 not shown. (C and D) Loss of the OSM-9 or OCR-2 TRPV channels, alone or in combination, did not restore *grk-2* chemotaxis toward any concentration of pyrazine tested. Chemotaxis to 1 μ l of (C) 100 mg/ml and (D) 10 mg/ml pyrazine is shown; 1 mg/ml not shown. Chemotaxis index = (number of animals at odorant – number of animals at control) $\div$ total number of animals on the assay plate. Each bar represents the average of four or more assays with 50–150 animals per trial. Alleles used: *grk-2(gk268)*, *osm-9(ky10)*, and *ocr-2(ak47)*. WT, the N2 wild-type strain. Error bars represent the standard error of the mean (SEM).

expression, while leaving channel function in primary chemosensory signal transduction intact (ZHANG *et al.* 2004; SOKOLCHIK *et al.* 2005). In addition, the OCR-2 N terminus is sufficient to increase TPH-1 expression when it is part of a chimeric channel (SOKOLCHIK *et al.* 2005).

To determine whether the *ocr-2(yz5)* mutation was also able to suppress the bitter tastant avoidance defects of *grk-2* animals, *grk-2(gk268);ocr-2(yz5)* double mutants were assayed for their response to quinine and primaquine. The *ocr-2(yz5)* single mutants have a wild-type response to these bitter tastants, and in the *grk-2* mutants, the *ocr-2(yz5)* point mutation completely restored bitter tastant avoidance (Figure 5, A and B). Importantly, the mechanism by which the OCR-2 N-terminal G36E mutation restores quinine response appears to operate within the ASH sensory neurons. Similar to the ASH rescue of *ocr-2* in *grk-2;ocr-2* null animals (Figure 4A), expression of wild-type *ocr-2* in the ASH neurons of *grk-2(gk268);ocr-2(yz5)* animals resulted in defective quinine responses (Figure 5A). Furthermore, the OCR-2 N-terminal point mutation appears to selectively restore

the response to ASH-detected bitter tastants, as *grk-2(gk268);ocr-2(yz5)* double mutants remained defective for chemotaxis toward diacetyl and pyrazine (Figure 5, C and D), mediated by the AWA neurons, although *ocr-2(yz5)* animals displayed wild-type chemotaxis toward both odorants (SOKOLCHIK *et al.* 2005).

By decreasing expression of TPH-1, mutations in the TRPV channels would also cause a reduction in serotonin levels. To ensure that disruption of TRPV channel function did not restore *grk-2* bitter tastant avoidance by decreasing serotonin levels in a non-cell-autonomous manner, we assayed *tph-1(mg280);grk-2(gk268)* double mutants for their response to quinine. Loss of serotonin synthesis, via the *tph-1* mutation, did not restore the response of *grk-2(gk268)* mutants to quinine (percentage responding to 10 mM quinine: N2 = 85 $\pm$ 2.9%, *grk-2* = 5 $\pm$ 5%, *tph-1* = 75 $\pm$ 2.9%, and *tph-1;grk-2* = 7.5 $\pm$ 4.9%).

Taken together our results suggest that alterations in downstream regulatory pathways that couple to the N terminus of OCR-2 in the ASH sensory neurons may account for the restored response to bitter stimuli in *grk-2;TRPV* double mutant animals.

FIGURE 4.—The OSM-9/OCR-2 TRPV channels function in ASH. The ASH sensory neurons are the primary neurons used to detect quinine. OSM-9/OCR-2 TRPV channel function is required in ASH for the defective quinine avoidance response of *grk-2* animals. (A) The *osm-10* (HART *et al.* 1999), *srb-6* (TROEMEL *et al.* 1995), and *sra-6* (TROEMEL *et al.* 1995; DE BONO *et al.* 2002) promoters were used to drive expression of wild-type *ocr-2* in the quinine-detecting ASH neurons of *grk-2;ocr-2* double mutant animals. The *osm-10* promoter expresses in ASH, ASI, PHA, and PHB, while the *srb-6* promoter drives expression in ASH, ADL, ADF, PHA, and PHB and the *sra-6* promoter expresses in ASH, ASI, and PVQ. ASH is the only sensory neuron common to all three promoters. While *grk-2;ocr-2* animals avoid 10 mM quinine, restoring OCR-2 function in ASH returned *grk-2;ocr-2* animals to the quinine response defective phenotype ($P \leq 0.0001$ for each transgene when compared to *grk-2;ocr-2*). (B) The *osm-10* promoter (HART *et al.* 1999) was used to drive expression of *osm-9* cDNA in the quinine-detecting ASH neurons of *grk-2;osm-9* double mutant animals. While *grk-2;osm-9* animals avoid 10 mM quinine, restoring OSM-9 function in ASH returned *grk-2;osm-9* animals to the *grk-2* quinine response defective phenotype ($P = 0.2$ when compared to *grk-2*). *Ex[osm-10::osm-9]* = the extrachromosomal transgenic array *yzEx53* (ZHANG *et al.* 2004; CHANG *et al.* 2006). The percentage of animals responding is shown. $N \geq 80$ transgenic animals. Alleles used: *grk-2(gk268)*, *ocr-2(ak47)*, and *osm-9(ky10)*. WT, the N2 wild-type strain. Error bars represent the standard error of the mean (SEM).

DISCUSSION

As *C. elegans* lack the ability to see and hear, they have evolved to rely heavily on their ability to detect chemical cues to successfully navigate their environment. This is reflected in the fact that >5% of their genome is dedicated to recognizing environmental chemicals (BARGMANN 2006a). *C. elegans* must properly respond to gustatory and olfactory cues to initiate chemotaxis toward favorable conditions or rapid avoidance to evade harmful environments. Therefore, signals through chemosensory GPCRs must be precisely transduced and regulated to ensure continued survival.

Loss of the GPCR negative regulator GRK-2 results in an intriguing phenotype; *grk-2* mutant animals are defective in chemosensory signaling and behavioral responses both to attractive and aversive chemosensory stimuli (FUKUTO *et al.* 2004). To better understand how loss of receptor regulation can lead to decreased signaling in different cell types, we sought to identify mechanisms responsible for dampening ASH signaling in the absence of GRK-2 function. Three of the mutations found in our *grk-2* suppressor screen were identified as alleles of the TRPV channels encoded by *osm-9* and *ocr-2*. Using the previously characterized null alleles *osm-9(ky10)* and *ocr-2(ak47)*, we found that complete loss of OSM-9/OCR-2 channel function fully restored the response of *grk-2* mutant animals to quinine and other bitter tastants. This suggests that these channels contribute to multiple bitter taste responses, in addition to quinine (HILLIARD *et al.* 2004, 2005). Surprisingly, though, *grk-2;TRPV* mutants remain defective in their avoidance of octanol, an odorant detected by ASH, the same neuron primarily responsible for quinine detection. In addition, loss of these TRPV channels failed to restore *grk-2* responses to attractive chemosensory stimuli detected by the AWA sensory neurons. Together, these results reveal that *C. elegans* TRPV channels can regulate chemosensory signaling in both a cell-specific and modality-specific manner.

Furthermore, the ability of the *ocr-2(yz5)* N-terminal point mutation to restore bitter taste response in *grk-2* mutants suggests that it may not be loss of TRPV channels as primary signal transduction components that restores *grk-2* bitter taste avoidance. Rather, loss of a downstream function or pathway coupled to the N-terminal structural motif of OCR-2 may restore bitter responses in the absence of GRK-2 function. For example, the G36E change may disrupt interactions with adaptor proteins or signaling components required for TRPV-modulated changes in gene expression (SOKOLCHIK *et al.* 2005); to date, no other function has yet been ascribed to this region of OCR-2. Therefore, one possibility is that loss of TRPV channels may decrease the expression of components used in primary signal transduction, thereby reducing the strength of signals being transduced (Figure S1). Reducing neuro-

FIGURE 5.—The OCR-2(G36E) point mutation functions cell autonomously to restore *grk-2* bitter taste responses mediated by ASH. (A and B) The OCR-2(G36E) point mutation, encoded by the *ocr-2(yz5)* allele, restored *grk-2* mutant animals' response to (A) 10 mM quinine and (B) 10 mM primaquine ($P \leq 0.001$ when compared to *grk-2* for each). The *osm-10* (HART *et al.* 1999) and *srb-6* (TROEMEL *et al.* 1995) promoters were used to drive wild-type *ocr-2* expression in the ASH sensory neurons of *grk-2(gk268);ocr-2(yz5)* double mutant animals. Although *grk-2;ocr-2* animals avoid 10 mM quinine, restoring OCR-2 function in ASH returned *grk-2;ocr-2* animals to the *grk-2* quinine response defective phenotype ($P \leq 0.0001$ for each transgene when compared to *grk-2;ocr-2*). $N \geq 130$ transgenic animals. Tastants were dissolved in M13 buffer, pH 7.4. The percentage of animals responding is shown. $N \geq 40$. (C and D) *grk-2;ocr-2(yz5)* mutant animals remained defective for AWA-mediated chemotaxis toward (C) 1:100 diacetyl and (D) 100 (mg/ml) pyrazine ($P \geq 0.08$ when compared to *grk-2*). Chemotaxis index = (number of animals at odorant – number of animals at control) $\div$ total number of animals on the assay plate. Each bar represents the average of four or more assays with 50–150 animals per trial. Alleles used: *grk-2(gk268)* and *ocr-2(yz5)*. WT, the N2 wild-type strain. Error bars represent the standard error of the mean (SEM).

nal activity in this manner could circumvent the activation of inhibitory pathways that act to dampen signaling in the absence of GRK-2, thus restoring behavioral response. The $G\alpha$ proteins are one possible target for transcriptional regulation. ODR-3 $G\alpha$ and GPA-3 $G\alpha$ contribute to a broad range of chemosensory responses, including bitter taste avoidance. However, ODR-3 protein levels are not altered in *grk-2* mutants and loss of neither ODR-3 nor GPA-3 restores *grk-2* response to bitter tastants (FUKUTO *et al.* 2004; FERKEY *et al.* 2007). In addition, TRPV channel-mediated regulation of transcription in the ADF neurons was shown to occur independently of $G\alpha$ activity (ZHANG *et al.* 2004).

As loss of TRPV channel function selectively restored bitter taste response, and not chemosensory responses

in general, it suggests that signaling components specific to bitter taste response may be regulated by the OSM-9/OCR-2 channels in the ASH sensory neurons. For example, the expression levels of the receptors for bitter tastants could be regulated by TRPV channels. If loss of TRPV channel function decreases expression of bitter GPCRs, then perhaps a signal being transduced through a reduced number of GPCRs would no longer be perceived as aberrant. This could also avert activation of compensatory inhibition in the absence of GRK-2 function, thereby restoring behavioral response in *grk-2;TRPV* double mutants.

Modulation of GPCR expression by sensory activity is a well-documented phenomenon in *C. elegans* (LANJUN and SENGUPTA 2002; NOLAN *et al.* 2002; van der LINDEN *et al.* 2008). Unlike vertebrates, *C. elegans* express

multiple receptors in each chemosensory neuron (BARGMANN 2006b). To selectively modify behavioral response to a single chemical, *C. elegans* may rely on changing the expression of a particular chemoreceptor gene, rather than altering signaling efficacy of the entire neuron, which would inadvertently affect the response to many chemicals (PECKOL *et al.* 2001; NOLAN *et al.* 2002). Selectively modulating distinct populations of receptors in this manner may allow *C. elegans* to fine tune their chemosensory GPCR repertoire and respond appropriately to their environment. This may be particularly important in a polymodal sensory neuron like ASH, which detects diverse stimuli including odorants, tastants, high osmolarity, and mechanical touch. Although the *C. elegans* genome encodes ~500 chemosensory GPCRs (BARGMANN 2006a), no receptors have yet been identified as bitter responsive. Thus, we were not able to directly examine the expression levels of this receptor class in animals lacking OSM-9 or OCR-2 function. However, we note that although levels of the diacetyl receptor ODR-10 are regulated by OSM-9/OCR-2 (TOBIN *et al.* 2002), loss of neither TRPV channel restored *grk-2* chemotaxis toward diacetyl (Figure 3).

Another possible target of TRPV-mediated transcriptional regulation is a compensatory pathway. Loss of GRK-2 may initially result in increased, aberrant signaling that is translated, via the TRPV channels, into changes in gene expression of inhibitory molecules (Figure S1). TRPV-mediated transcriptional upregulation of a compensatory pathway, which specifically dampens signals being transduced through bitter GPCRs, would result in the loss of behavioral response to bitter tastants. Loss of TRPV channel-regulated gene transcription could prevent the increased expression of an inhibitory molecule(s), thereby restoring bitter GPCR signal transduction and behavioral response in the absence of GRK-2 function. Interestingly, prior studies have not identified a role for the OSM-9 and OCR-2 TRPV channels in regulating expression of sensory components in ASH (SOKOLCHIK *et al.* 2005). Perhaps TRPV channels do not affect expression of primary signal transduction molecules, but rather only regulatory components necessary to maintain normal signaling. Thus, it may be only in a sensitized background, like *grk-2* where ordinary signaling has been compromised, that a role for the TRPV channels in modulating expression of ASH sensory components can be revealed.

Although regulated expression of sensory signaling components has not yet been described in mammals, exposure to chemosensory stimuli has been shown to activate the transcription factor CREB in both rat olfactory receptor neurons and taste receptor cells (MOON *et al.* 1999; CAO *et al.* 2002). CREB is a key mediator in translating transient neuronal activity into long-term changes in gene expression (Ooi and Wood 2008). Therefore, it has been proposed that in addition to initiating the immediate response of membrane de-

polarization, gustatory and olfactory stimuli also generate a delayed response that may modulate gene transcription in their respective sensory neurons (MOON *et al.* 1999; CAO *et al.* 2002). Thus, in addition to sharing components of chemosensory signal transduction, the long-lasting cellular consequences of odorant and tastant detection may also be conserved among invertebrates and vertebrates.

While it is possible that the N terminus of OCR-2 may have additional roles in cellular events downstream of the TRPV channels that have not yet been identified, our results suggest that misregulated signaling, such as in the absence of *C. elegans* GRK-2 function, may also lead to long-term transcriptional changes that alter signaling levels and behavioral responses. Importantly, temporal rescue of GRK-2 function in adult *grk-2* mutant animals is sufficient to restore chemosensory response to octanol and quinine (FUKUTO *et al.* 2004 and data not shown), indicating that the mechanisms that dampen signaling in the absence of GRK-2 function are under ongoing regulatory control. Similarly, adult rescue of OCR-2 function in *grk-2;ocr-2* double mutants is sufficient to return animals to the *grk-2* quinine response defective phenotype within hours (Figure S2). It will be interesting to determine whether similar TRPV-mediated mechanisms are at work in mammalian cells that show decreased signaling and responsiveness in the absence of GRK function (PEPPEL *et al.* 1997; FONG *et al.* 2002). Future studies to identify OCR-2 interacting proteins and the downstream targets regulated by activity through the TRPV channels will also further our understanding of the modality-specific mechanisms used by cells to modulate intracellular signaling.

We thank Richard Gronostajski, Douglas Portman, Sean M. Rumschik, and Jordan Wood for valuable feedback on this manuscript. We thank Cori Bargmann, Michael Chao, Anne Hart, Andrew Fire, Michael Koelle, Noelle L'Etoile, and the *Caenorhabditis* Genetics Center for reagents, and we are grateful to Marina Ezcurra and William Schafer for help with experiments not included in this manuscript. This work was supported by the National Science Foundation (MCB-0917896, to D.M.F.).

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Communicating editor: R. ANHOLT

GENETICS

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<http://www.genetics.org/cgi/content/full/genetics.109.115188/DC1>

***Caenorhabditis elegans* TRPV Channels Function in a Modality-Specific Pathway to Regulate Response to Aberrant Sensory Signaling**

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DOI: 10.1534/genetics.109.115188

FIGURE S1.—Proposed model for the cellular response to loss of GRK-2 function. (A) Wild-type chemosensory signaling is initiated when a ligand binds to a GPCR expressed by a sensory neuron, activating the associated G proteins. The activated G proteins interact with downstream effectors to generate second messengers and subsequent activation of channels allows Ca²⁺ to enter the cell. Through connections with interneurons and motor neurons, this activity in the sensory neuron is ultimately translated into a behavioral response. Signaling is terminated when GRK-2 phosphorylates the GPCR, rendering it incapable of further G protein activation. (B) It has been proposed that early in the life of the animal, the initial cellular response to sensory stimulation in the absence of GRK-2 function is an increased, exaggerated signal that triggers protective compensatory mechanisms to inhibit signaling (left side of panel B) (FUKUTO *et al.* 2004). The net result of this response to aberrant signaling is loss of chemosensory signal transduction in the sensory neurons of adult animals (right side of panel B), as illustrated by diminished Ca²⁺ fluxes and lack of behavioral response to chemosensory stimuli (FUKUTO *et al.* 2004 and this work). In theory, mutations that decrease primary signal transduction could prevent activation of the compensatory inhibitory mechanisms early on and restore behavioral response to *grk-2* mutant animals. Alternatively, loss of inhibitory proteins/pathways that act in the absence of GRK-2 function could permit sufficient chemosensory signaling to restore behavioral responses (FUKUTO *et al.* 2004).

FIGURE S2.—OCR-2 function in adulthood contributes to the defective quinine avoidance response of *grk-2* mutant animals. To establish when TRPV channel function contributes to the defective quinine response of *grk-2* animals, the wild-type *ocr-2* cDNA was placed under the control of a heat shock inducible promoter (STRINGHAM *et al.* 1992) and temporally expressed in adult *grk-2;ocr-2* null mutant animals. Animals were tested as adults without heat shock (white bars) or with heat shock treatment (gray bars). While *grk-2;ocr-2* double mutants have a wild-type response to quinine, heat shock induced expression of *ocr-2* in adult *grk-2;ocr-2* mutant animals results in a defective quinine response that resembles the *grk-2* phenotype ($p < 0.0001$ when compared to *grk-2;ocr-2* transgenic animals without heat shock). Heat shock induced expression of *ocr-2* did not decrement the responses of wild-type animals expressing the transgene. The percentage of animals responding is shown. $n \geq 51$ transgenic animals. The pooled F2 data of 7 transgenic *grk-2;ocr-2* lines is shown. Alleles used: *grk-2(gk268)* and *ocr-2(ak47)*. WT = the N2 wild-type strain. Error bars represent the standard error of the mean (SEM).