$G_o\alpha$ regulates olfactory adaptation by antagonizing $G_q\alpha$-DAG signaling in *Caenorhabditis elegans*

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The heterotrimeric G protein G_o is abundantly expressed in the mammalian nervous system and modulates neural activities in response to various ligands. However, G_o's functions in living animals are less well understood. Here, we demonstrate that GOA-1 $G_o\alpha$ has a fundamental role in olfactory adaptation in *Caenorhabditis elegans*. Impairment of GOA-1 $G_o\alpha$ function and excessive activation of EGL-30 $G_q\alpha$ cause a defect in adaptation to AWC-sensed odorants. These pathways antagonistically modulate olfactory adaptation in AWC chemosensory neurons. Wild-type animals treated with phorbol esters and double-mutant animals of diacylglycerol (DAG) kinases, *dgk-3*; *dgk-1*, also have a defect in adaptation, suggesting that elevated DAG signals disrupt normal adaptation. Constitutively active GOA-1 can suppress the adaptation defect of *dgk-3*; *dgk-1* double mutants, whereas it fails to suppress the adaptation defect of animals with constitutively active EGL-30, implying that GOA-1 acts upstream of EGL-30 in olfactory adaptation. Our results suggest that down-regulation of EGL-30–DAG signaling by GOA-1 underlies olfactory adaptation and plasticity of chemotaxis.

chemotaxis | G protein

The olfactory sensory system can endow animals with abilities to detect food sources and mates and, in some cases, to avoid harmful chemicals and predators. Sensitivity to an odor stimulus can be appropriately adjusted by previous experience, allowing the sensory system to adapt to changeable environments. In mammals, olfactory adaptation (habituation) is known to occur throughout the odorant sensory pathway; for example, olfactory receptor neurons (1), secondary interneurons (2), and primary and higher-order olfactory cortices (3). These adaptation mechanisms appear to allow animals to increase the range of concentrations of odor that can be sensed and to discriminate among multiple odors.

The nematode *Caenorhabditis elegans* has only 302 neurons, and a variety of behaviors have been observed. Of these, olfactory behavior is relatively well studied. Volatile odorants are mainly sensed by five pairs of sensory neurons, AWA, AWB, AWC, ADL, and ASH (4–6), of which AWA and AWC chemosensory neurons mediate attraction behavior (4). In this response, olfactory adaptation has also been observed (7). Animals lacking OSM-9 TRPV channel (7, 8), EGL-4 cGMP-dependent protein kinase (9) or animals overexpressing ODR-1 guanylyl cyclase (10) exhibited defects in olfactory adaptation to AWC-sensed odorants. By contrast, animals with mutated *tax-6*, which encodes calcineurin, exhibited hyperadaptation (11), suggesting that Ca^{2+} and cGMP signaling cascades participate in olfactory adaptation. Moreover, ARR-1 arrestin (12), TBX-2 T-box transcription factor (13), and the Ras–MAPK pathway (14) are also known to act in olfactory adaptation, illustrating that olfactory adaptation in *C. elegans* is modulated by complicated mechanisms consisting of multiple signaling cascades and control of gene expression.

In general, neural activities are modulated by many types of ligands through seven-transmembrane receptors and heterotrimeric G proteins. Of these, G_o is abundantly expressed in the mammalian nervous system (15). G_o-deficient mice displayed a severe impairment of motor control and a hyperalgesic response (16). Another work showed that G_o is localized to the axons of olfactory receptor neurons that project to the main olfactory bulb and is involved in olfactory behavior (17, 18). However, the mode of action of G_o in these behaviors is unclear. *C. elegans* has 21 G protein α-subunit genes (19, 20), and the only orthologue of mammalian $G_o\alpha$ is encoded by the *goa-1* gene (21, 22). Mutations in *goa-1* cause various defects in behaviors, including locomotion and egg laying. The locomotion rate was shown to be antagonistically regulated by GOA-1 $G_o\alpha$ and EGL-30 $G_q\alpha$ signaling cascades (23–27). The stimulation of EGL-30 causes a rise of diacylglycerol (DAG) level through activation of EGL-8 PLCβ, leading to a change in distribution of UNC-13, resulting in facilitation of acetylcholine release at neuromuscular junctions (25, 26). By contrast, GOA-1 is known to negatively regulate the EGL-30 and DAG signaling pathway in response to serotonin (22, 27) or dopamine (28).

To understand how $G_o\alpha$ modulates olfactory responses, genetic approaches were taken in this study in *C. elegans*. We found that GOA-1 $G_o\alpha$ is required for adaptation to AWC-sensed odorants. Furthermore, our results indicate that GOA-1 acts in AWC chemosensory neurons and plays an important role in olfactory adaptation by antagonizing the EGL-30 $G_q\alpha$–DAG signaling pathway.

Results

GOA-1 $G_o\alpha$ Is Required for Olfactory Adaptation to AWC-Sensed Odorants. In our attempts to identify mutants with altered olfactory responses, we found that the *goa-1*(*n1134*) mutants show curious chemotaxis behaviors. *goa-1*(*n1134*), a weak loss-of-function mutant of *goa-1* $G_o\alpha$ (22), exhibited a normal chemotaxis to a wide range of concentrations of AWC-sensed odorants, benzaldehyde, isoamyl alcohol, and butanone, whereas they showed increased chemotaxis to a high concentration of benzaldehyde (Fig. 1*A*). We reasoned that the increased chemotaxis observed in *goa-1*(*n1134*) mutants may result from an adaptation defect, because strong or prolonged odor stimuli are known to cause olfactory adaptation in *C. elegans* (7, 29). Therefore, we examined whether *goa-1*(*n1134*) shows normal adaptation to odor. In so doing, we found that *goa-1*(*n1134*) mutants are defective in olfactory adaptation to AWC-sensed odorants (Fig. 2 *A* and *B*). After preexposure treatments, where animals were soaked in an odorant-containing buffer for 1 h, wild-type animals displayed diminished olfactory responses (to isoamyl alcohol) or aversive responses (to benzaldehyde or butanone). *goa-1*(*n1134*) mutants, however, continued to show a strong chemotaxis to all AWC-sensed odorants we tested (Fig.

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Abbreviations: DAG, diacylglycerol; DGK, DAG kinase; PMA, phorbol 12-myristate 13-acetate.

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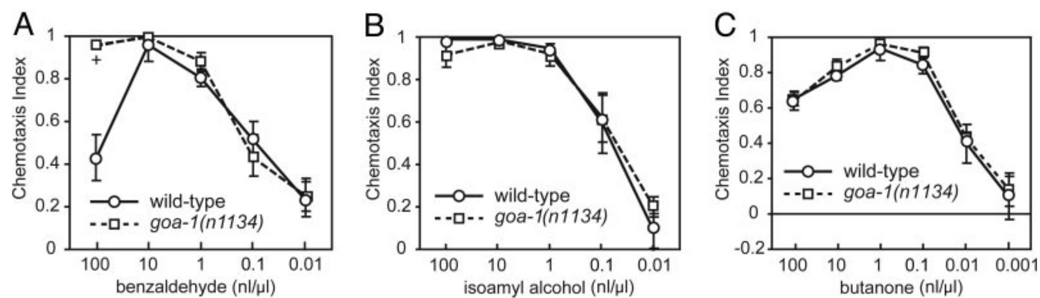

Fig. 1. Olfactory responses of wild-type animals and *goa-1*(*n1134*) mutants. These animals were soaked in assay buffer for 1 h and then tested for chemotaxis to various dilutions of benzaldehyde (*A*), isoamyl alcohol (*B*), and butanone (*C*). *goa-1*(*n1134*) mutants respond normally to a wide range of concentrations of these odorants, whereas they show enhanced chemotaxis to strong (100 nl/μl) benzaldehyde. +, $P < 0.01$.

2 *A* and *B*). Like *goa-1*(*n1134*) mutants, *goa-1* null mutants *goa-1*(*sa734*) and *goa-1*(*n363*) also have severe defects in adaptation to benzaldehyde (Fig. 2*A* and data not shown). Furthermore, the benzaldehyde-adaptation defect of *goa-1*(*n1134*) mutants was rescued by introduction of the *goa-1* transgene, in which *goa-1* cDNA was expressed under the control of *goa-1*'s own promoter (Fig. 2*A*). Thus, we conclude that GOA-1 is required for adaptation to AWC-sensed odorants.

In contrast to AWC-sensed odorants, *goa-1* mutants were defective in chemotaxis to the AWA-sensed odorants diacetyl and pyrazine, even after treatment with odor-free buffer (Fig. 2*C*). Therefore, we could not judge whether *goa-1* is involved in adaptation to AWA-sensed odorants.

In addition to olfactory adaptation, we found that *goa-1* mutants are also defective in the plasticity of salt chemotaxis (30) (Fig. 2*D*). Wild-type animals previously soaked in NaCl-containing buffer showed an aversive response to chemoattractive NaCl. In contrast, *goa-1* mutants were still attracted to NaCl, even after preexposure to NaCl. These results suggest that GOA-1 modulates multiple sensory responses to control behavioral plasticity.

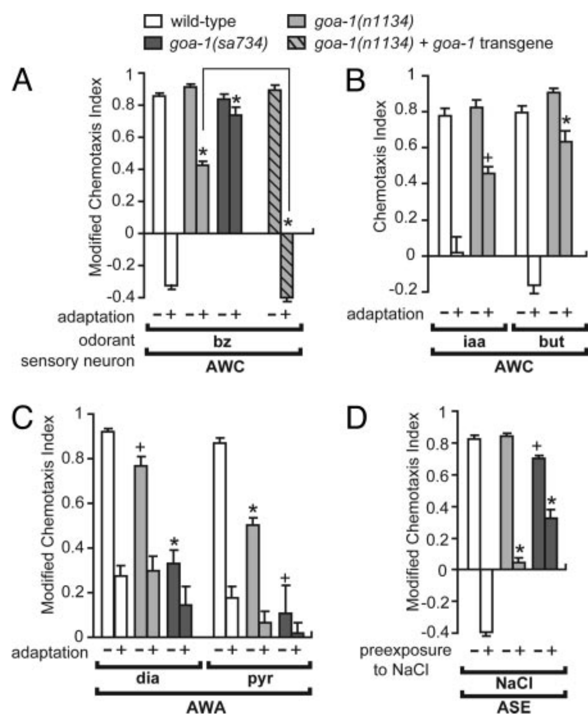

Fig. 2. *goa-1* $G_o\alpha$ mutants are defective for adaptation to AWC-sensed odorants and plasticity of chemotaxis to NaCl. (*A*–*C*) Animals were soaked in a buffer with (adaptation +) or without (adaptation −) odorant for 1 h and then tested for chemotaxis to the same odorant. (*D*) Animals were soaked in a buffer with (preexposure +) or without (preexposure −) NaCl (20 mM) for 1 h and then tested for chemotaxis to NaCl. (*A*) *goa-1* mutants are defective in adaptation to AWC-sensed benzaldehyde, and adaptation defects of *goa-1*(*n1134*) mutants are fully rescued by introduction of the *goa-1p*::*goa-1* transgene. (*B*) *goa-1* mutants are also defective in adaptation to AWC-sensed isoamyl alcohol and butanone. (*C*) *goa-1* mutants show a defect in chemotaxis to AWA-sensed diacetyl and pyrazine under the conditions of mock preexposure to odor. (*D*) *goa-1* mutants are defective for plasticity of chemotaxis to NaCl. bz, benzaldehyde; iaa, isoamyl alcohol; but, 2-butanone; dia, diacetyl; and pyr, pyrazine. *, $P < 0.001$; +, $P < 0.01$.

GOA-1 Acts in AWC Chemosensory Neurons for Olfactory Adaptation. To identify the neurons in which GOA-1 acts for olfactory adaptation, we expressed *goa-1* cDNA under the control of various promoters. The promoters of *odr-3*, *gpa-13*, and *odr-1* drive the expression in AWC neurons and some other neurons (10, 19, 31, 32). When *goa-1* was expressed in AWC chemosensory neurons of *goa-1*(*n1134*) mutants using these promoters, *goa-1*(*n1134*) mutants were partially rescued for adaptation (Fig. 3*A*), suggesting that GOA-1 acts in AWC chemosensory neurons for adaptation.

AIY and AIZ interneurons are known to have fundamental roles in thermotaxis behavior (33) and olfactory response (34, 35). Furthermore, the Ras–MAPK pathway acts in AIY interneurons to regulate early adaptation (14). Given the importance of interneurons in *C. elegans* behaviors, it is necessary to examine the possibility that *goa-1* also functions in interneurons. To do this, *goa-1* was expressed in various interneurons, including AIY, AIA, AIZ, or command interneurons. As a result, we found that *goa-1* expression in interneurons failed to rescue the adaptation defect of *goa-1*(*n1134*) mutants (Fig. 3*A*), suggesting that the function of GOA-1 in interneurons is not important for olfactory adaptation.

To examine the molecular function of GOA-1 further, we expressed a constitutively active form of GOA-1, GOA-1(Q205L), in AWC neurons by using the *odr-1* promoter. Expression of GOA-1(Q205L) caused not only enhanced adaptation compared with wild-type (Fig. 3*B*, gray bars), but also reduced chemotaxis, even without preexposure to odor (Fig. 3*B*, white bars). This result indicates that enhanced activation of GOA-1 represses the function of AWC neurons regardless of preexposure to odor.

Enhanced EGL-30 $G_q\alpha$-DAG Signaling Disrupts Normal Olfactory Adaptation. Previous reports have shown that GOA-1 and EGL-30 antagonistically regulate acetylcholine release at neuromuscular junctions (24–27). Hence, we hypothesized that EGL-30 also has an effect opposite to GOA-1 in adaptation. In fact, we found that gain-of-function mutants of *egl-30*, *egl-30(js126)* (36), and animals expressing the *egl-30* transgene at a high level, *syIs36*[*egl-*

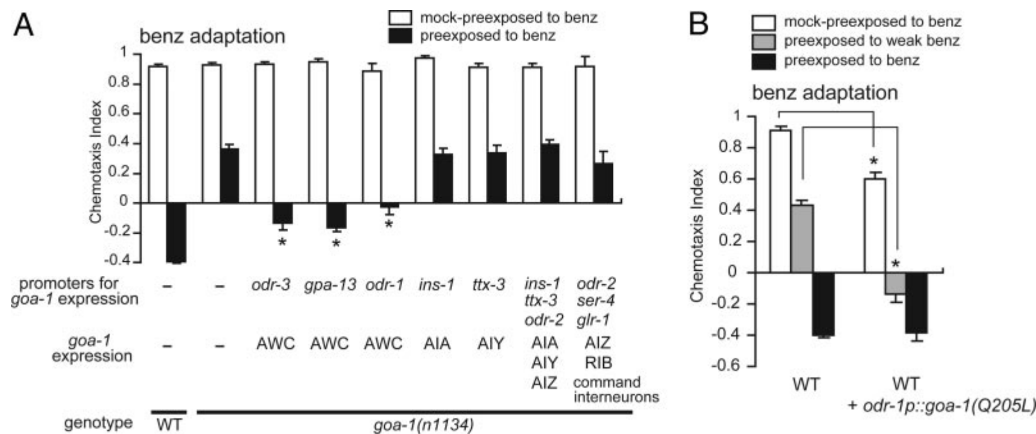

Fig. 3. GOA-1 $G_o\alpha$ modulates olfactory adaptation in AWC chemosensory neurons. (*A*) *goa-1* cDNA was fused to various promoters that drive cell-specific expression, and resulting constructs were injected into *goa-1*(*n1134*) mutants. *goa-1* expression in AWC sensory neurons partially restores adaptation to *goa-1*(*n1134*) mutants. (*B*) Expression of a constitutively active form of GOA-1, GOA-1(Q205L), in AWC neurons impairs the chemotaxis responses of wild-type animals after mock preexposure to benzaldehyde (white bars) or preexposure to weak benzaldehyde (gray bars). *, $P < 0.001$.

30(+)] (23, 24), exhibited severe defects in adaptation (Fig. 4*A*). Moreover, expression of a constitutively active form of EGL-30, EGL-30(Q205L), in AWC neurons is sufficient to cause an adaptation defect (Fig. 4*A*). Similar to *goa-1* mutants, these transgenic animals showed an enhanced chemotaxis to a high concentration of benzaldehyde, whereas their sensitivity to a low concentration of benzaldehyde was almost normal (see Fig. 6*A*, which is published as supporting information on the PNAS web site). These results indicate that EGL-30 negatively modulates olfactory adaptation in AWC chemosensory neurons.

EGL-30 regulates locomotory behavior through activation of EGL-8 PLCβ (25, 26). Because PLCβ produces DAG by hydrolyzing PIP_2, we asked whether the effect of EGL-30(Q205L) in olfactory adaptation is mediated by elevated production of DAG. To test this hypothesis, we added the phorbol ester phorbol 12-myristate 13-acetate (PMA) a DAG analogue, during preexposure treatment in an adaptation assay. We found that addition of PMA made wild-type animals defective in adaptation, mimicking the effect of EGL-30(Q205L) (Fig. 4*B*). This result suggests that EGL-30 regulates olfactory adaptation through DAG. In addition, the addition of PMA also made GOA-1(Q205L)-expressing animals defective in adaptation (see Fig. 7, which is published as supporting information on the PNAS web site), which would be expected if GOA-1 negatively regulates DAG levels.

In locomotion and egg-laying behaviors, GOA-1 and EGL-30 are negatively regulated by regulator of G protein signaling (RGS) proteins EGL-10 and EAT-16, respectively (24, 37). GPB-2, which is a *C. elegans* orthologue of mammalian $G\beta_5$, is necessary for both EGL-10 and EAT-16 functions for these behaviors (38, 39). Therefore, we tested the participation of these RGS proteins and GPB-2 in adaptation. As a result, two *eat-16* alleles, *eat-16(ce71)* (40) and *eat-16(sa609)* (24), which are putative null and loss-of-function mutants, respectively, and animals carrying a high-copy array of the *egl-10* gene *nIs51* (37) showed modest defects in adaptation (Fig. 4*C*). These results imply that EGL-10 and EAT-16 also act as RGS proteins for GOA-1 and EGL-30, respectively, to negatively regulate G protein signaling in olfactory adaptation. In addition, two *gpb-2* alleles, *gpb-2*(*vs23*) (38) and *gpb-2*(*ad541*) (41, 42), which are putative null and loss-of-function mutants, respectively, exhibited slight adaptation defects (Fig. 4*C*). To examine the effect of *gpb-2* mutation on the EGL-10 function in adaptation, we expressed the *egl-10* gene in wild-type and *gpb-2*(*vs23*) mutants at high levels and performed adaptation assays. In the wild-type background, overexpression of *egl-10* powerfully disrupted normal adaptation (Fig. 4*D*). By contrast, in the *gpb-2* null background, only a small effect of high-copy *egl-10* array was observed, suggesting that at least some of the EGL-10 function depends on GPB-2 in adaptation.

DGK-1 DGKθ and DGK-3 DGKβ Are Required for Adaptation. DAG kinase (DGK) reduces DAG levels by converting DAG to phosphatidic acid. Loss-of-function mutants of *dgk-1*, which encodes a diacylglycerol kinase θ (DGKθ), show hyperactive locomotion and hyperactive egg laying similar to *goa-1* mutants (24, 26, 27). However, we found that the *dgk-1* putative null mutant *dgk-1*(*sy428*) (24, 43) does not exhibit an adaptation defect (Fig. 5*A*). This unexpected result suggests possibilities that either DGK does not regulate olfactory adaptation or that another DGK is also involved in adaptation. To explore the latter possibility, we examined the *C. elegans* genome database and found four DGK-encoding genes in addition to *dgk-1*. Of these, *dgk-2* and *dgk-3* encode *C. elegans* orthologues of mammalian DGKε and DGKβ, respectively. We found, however, that neither *dgk-2* mutants nor *dgk-3* mutants exhibited adaptation defects (Fig. 5*A*). Furthermore, *dgk-1 dgk-2* double mutants and *dgk-3*; *dgk-2* double mutants did not show adaptation defects either (Fig. 5*A*). By contrast, *dgk-3*; *dgk-1* double mutants are defective in adaptation (Fig. 5*A*), suggesting that the presence of either wild-type DGK-1 or DGK-3 is sufficient for normal olfactory adaptation, but lack of both isoforms causes an adaptation defect. On the other hand, there was no significant difference between sensitivities of *dgk-1* mutants and *dgk-3*; *dgk-1* double mutants to a low concentration of benzaldehyde (Fig. 6*B*). This result implies that the adaptation defect of *dgk-3*; *dgk-1* double mutants was not simply caused by high sensitivity to odor. We conclude that DGK-1 and DGK-3 redundantly act to reduce DAG levels and that down-regulation of DAG signaling appears to be important for olfactory adaptation.

GOA-1 $G_o\alpha$ May Antagonize DAG Signaling by Repression of EGL-30 $G_q\alpha$ in Olfactory Adaptation. Our genetic analysis suggests that GOA-1 antagonizes the EGL-30–DAG pathway in adaptation, whereas it is unknown how GOA-1 affects the EGL-30–DAG signaling pathway. One model would be that GOA-1 stimulates DGK-1 and/or DGK-3 to promote depletion of the accumulated DAG. Otherwise, GOA-1 would repress the activity of EGL-30 to reduce DAG production. To test these models, we examined the effect of GOA-1(Q205L) on adaptation of *dgk-3*; *dgk-1*

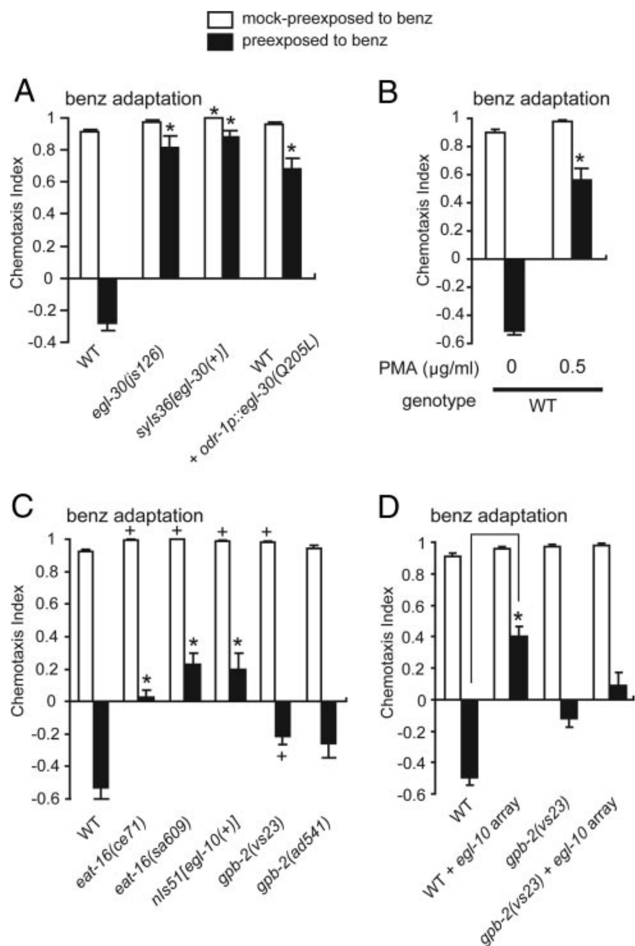

Fig. 4. Excessive activation of EGL-30 $G_q\alpha$ and elevated DAG signals inhibit olfactory adaptation. (*A*) Adaptation of wild-type animals, *egl-30*(*js126*) mutants, *syIs36*[*egl-30*(+)] animals, and wild-type animals carrying *odr-1p*::*egl-30*(*Q205L*) transgene to benzaldehyde. All mutants are defective for adaptation. (*B*) Wild-type animals treated with phorbol ester PMA (0.5 μg/ml) show a severe adaptation defect. (*C*) Adaptation of *eat-16* mutants, *gpb-2* mutants, and animals carrying a high-copy *egl-10* array to benzaldehyde. *eat-16* mutants and *egl-10*-overexpressing animals show a modest defect, and *gpb-2* mutants exhibit a slight defect in adaptation. (*D*) Overexpression of the *egl-10* gene in *gpb-2* mutants hardly causes an additive defect in olfactory adaptation. Chemotaxis indices of two transgenic lines were averaged. *, $P < 0.001$; +, $P < 0.01$.

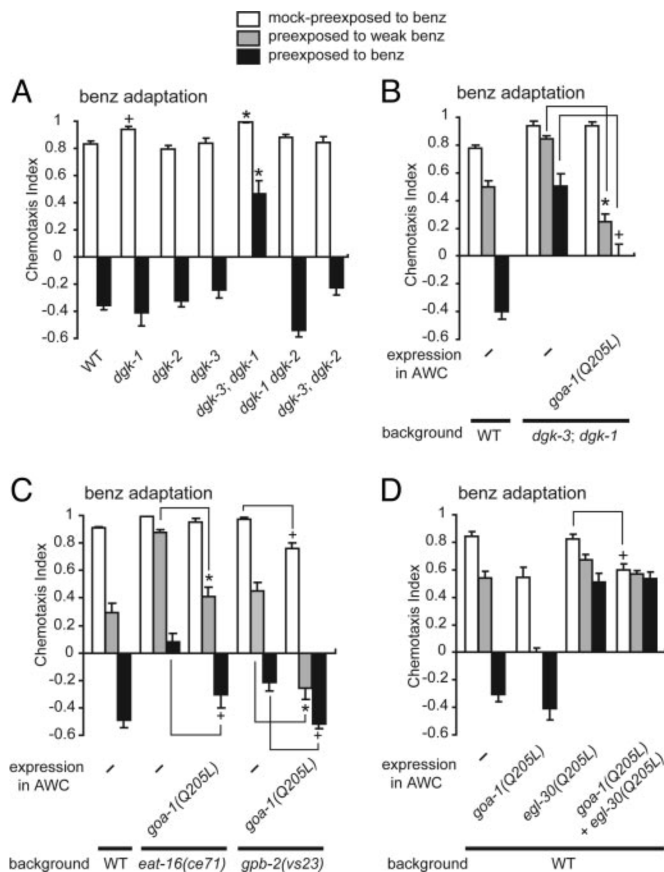

Fig. 5. GOA-1 $G_o\alpha$ appears to antagonize the EGL-30 $G_q\alpha$–DAG signaling pathway by down-regulation of EGL-30 $G_q\alpha$ in olfactory adaptation. (*A*) Adaptation of DGK mutants to benzaldehyde. *dgk-3*; *dgk-1* double mutants show a defect in adaptation. (*B*) Adaptation defect observed in *dgk-3*; *dgk-1* double mutants is suppressed by the expression of *goa-1*(*Q205L*) in AWC chemosensory neurons. (*C*) Adaptation defects observed in *eat-16*(*ce71*) and *gpb-2*(*vs23*) mutants are suppressed by the expression of *goa-1*(*Q205L*) in AWC chemosensory neurons. (*D*) GOA-1 may act upstream of EGL-30 in olfactory adaptation. Animals carrying *odr-1p*::*goa-1*(*Q205L*) were crossed to animals carrying *odr-1p*::*egl-30*(*Q205L*) to generate animals carrying both constructs. There is no significant difference between responses of animals expressing both constructs and that of animals expressing only *egl-30*(*Q205L*) after preexposure to weak (gray bars) or normal benzaldehyde (black bars) ($P = 0.04$ or $P = 0.79$, respectively). However, the expression of *goa-1*(*Q205L*) in AWC neurons inhibits chemotaxis, even if *egl-30*(*Q205L*) is expressed together (white bars). *, $P < 0.001$; +, $P < 0.01$.

double putative null mutants and animals expressing a constitutively active form of EGL-30, EGL-30(Q205L). Interestingly, we observed distinct phenotypes in these two kinds of animals: the adaptation defect of *dgk-3*; *dgk-1* double mutants was suppressed by the expression of GOA-1(Q205L) in AWC neurons (Fig. 5*B*). On the other hand, the adaptation defect of EGL-30(Q205L)-expressing animals was not suppressed (Fig. 5*D*). These results support the latter model that GOA-1 $G_o\alpha$ negatively modulates the activity of EGL-30 $G_q\alpha$ to control olfactory adaptation. However, the adaptation defects of *eat-16*(*ce71*) and *gpb-2*(*vs23*) mutants were effectively suppressed by the expression of GOA-1(Q205L) in AWC neurons (Fig. 5*C*), suggesting that GOA-1 does not act through EAT-16 to down-regulate EGL-30, or, if it does, EAT-16 is not the only target of the action. Taken together, our results suggest that GOA-1 acts for olfactory adaptation by negatively regulating EGL-30 through EAT-16-indepenent mechanisms. It is also noteworthy that GOA-1(Q205L) expression in AWC could not impair the chemotaxis of *dgk-3*; *dgk-1* double mutants to benzaldehyde (Fig. 5*B*, open bars, and Fig. 6*B*) but could impair the chemotaxis of EGL-30(Q205L)-expressing animals (Fig. 5*D*, open bars). These results suggest that, in nonadapted animals, some of the GOA-1 effect would be mediated by DGK-1 and DGK-3.

Discussion

Hyperactivation of $G_q\alpha$-DAG Signaling Causes an Adaptation Defect. Through our analysis of adaptation, we suggested that elevated DAG signaling results in a defect in olfactory adaptation. Three lines of evidence support this proposal. First, animals in which EGL-30 $G_q\alpha$ was excessively activated showed an adaptation defect. Because $G_q\alpha$ is known to produce DAG through activation of PLCβ, activated EGL-30 signaling is likely to bring about overproduction of DAG. Second, exogenously added phorbol ester PMA effectively disrupted olfactory adaptation. Third, double-mutant animals with mutations in two DGK genes, *dgk-1* and *dgk-3*, also displayed a defect in adaptation. Because DGK converts DAG to phosphatidic acid, elimination

of DGK activity is likely to result in elevated DAG levels. Furthermore, our results showed that the expression of EGL-30(Q205L) in AWC neurons was sufficient to cause an adaptation defect. Recently, it was reported that *dgk-3* is expressed in a subset of amphid sensory neurons, including AWC neurons (44), and we also confirmed that *dgk-3* is strongly expressed in AWC neurons (data not shown). Therefore, these results suggest that hyperactivation of $G_q\alpha$-DAG signaling, specifically in AWC neurons, causes an adaptation defect.

EGL-8 PLCβ was reported to be widely expressed in the nervous system and to act downstream of EGL-30 $G_q\alpha$ to produce DAG in motoneurons (25, 26). Although it is necessary to test whether EGL-8 acts downstream of EGL-30 in adaptation, we were unable to test the participation of EGL-8 in olfactory behavior because of the strong locomotion defects of the mutants.

Down-Regulation of $G_q\alpha$-DAG Signaling by $G_o\alpha$ Is Crucial for Olfactory Adaptation. As in mammals, GOA-1 $G_o\alpha$ is ubiquitously expressed in the nervous system in *C. elegans* and reported to be involved in many aspects of behavior (21, 22). In our study, we demonstrated that GOA-1 modulates sensory responses, specifically, olfactory adaptation (and salt chemotaxis plasticity) in *C. elegans*. Furthermore, the adaptation defect observed in *dgk-3*; *dgk-1* double mutants was suppressed by hyperactivation of GOA-1 in AWC neurons. This result suggests that GOA-1 antagonizes DAG signaling in AWC neurons in olfactory adaptation, probably not through positive regulation of DGK. In contrast to this result, the adaptation defect observed in EGL-30(Q205L)-expressing animals was not suppressed by the expression of GOA-1(Q205L) in AWC neurons. These results lead us to propose a model that GOA-1 antagonizes DAG signaling through down-regulation of EGL-30 signaling, and it is crucial for olfactory adaptation. This model is consistent with genetic studies on locomotion behavior that indicated that GOA-1 may act upstream of EGL-30 to modulate this behavior (24, 26). However, in both cases, the mechanisms of GOA-1 in the negative regulation of EGL-30 are unknown, except that the results of GOA-1(Q205L) expression in *eat-16* mutants suggest that EAT-16 regulator of G protein signaling cannot be the sole mediator of the regulation of EGL-30 by GOA-1. Thus, additional studies will be required to clarify the molecular mechanism for the regulation.

Factors that Act Downstream of the EGL-30 $G_q\alpha$-DAG Signaling Pathway. What acts downstream of the DAG signaling pathway in olfaction? In motoneurons, the distribution of UNC-13, which plays an important role in priming of synaptic vesicles, is regulated by the EGL-30 $G_q\alpha$–DAG signaling pathway (25, 27). In addition, a more recent study indicates that TTX-4 nPKCε/η and TPA-1 nPKCδ/θ positively regulate olfaction in AWA and AWC chemosensory neurons (45). UNC-13 and these nPKCs have DAG-binding domains (C1 domains) and are activated by phorbol esters, providing the possibility that inhibition of the EGL-30 $G_q\alpha$–DAG signaling pathway by GOA-1 $G_o\alpha$ in AWC neurons would cause inactivation of UNC-13 and/or nPKCs, and, as a result, chemotaxis to odor would be impaired.

Adaptation Regulated by Non-Cell-Autonomous Mechanisms. *goa-1* mutants are resistant to the paralytic effect of exogenously added serotonin and dopamine (22, 27, 28). Thus serotonin and dopamine signaling are thought to be mediated by GOA-1. Although D2-like dopamine receptor DOP-3 was reported to mediate dopamine signals through GOA-1 to inhibit locomotion (28), *dop-3* mutants did not show an adaptation defect (data not shown). Intriguingly, serotonin significantly inhibits the olfactory adaptation to benzaldehyde (46). In the case of olfactory adaptation, however, GOA-1 is unlikely to mediate serotonin signaling, because hyperactivation of GOA-1 causes hyperadaptation, whereas the lack of GOA-1 causes adaptation defects, contrary to the effect of serotonin. Therefore, other ligands, such as neuropeptides, may send signals through GOA-1 in adaptation.

goa-1(n1134) mutants had a normal sensitivity to a wide range of concentrations of AWC-sensed odorants, unless they were preexposed to odor, suggesting that GOA-1 would not be continuously activated in AWC chemosensory neurons. Instead, GOA-1 appears to be activated in response to prolonged or strong odor stimuli. In the olfactory bulb, olfactory receptor neurons (ORNs) are thought to be presynaptically inhibited by GABA and dopamine released by juxtaglomerular cells, which receive excitatory glutamatergic input from ORNs in the glomeruli (47–50). By analogy, interneurons located downstream of AWC neurons in *C. elegans* may release an inhibitory signal to repress activity of AWC neurons during olfactory adaptation, and this inhibitory signal may be transmitted by GOA-1 in AWC neurons. Alternatively, chemosensory neurons may be the source of the signal for GOA-1-mediated olfactory adaptation, because benzaldehyde is detected not only by AWC but also by ASH chemosensory neurons (6), which are known to mediate aversive responses.

Olfactory adaptation in *C. elegans* has been intensively studied. These studies have revealed that multiple proteins, most of which are likely to act cell-autonomously in AWC chemosensory neurons, are involved in olfactory adaptation (7, 9–13). Our results on the G_o–G_q signaling pathway strongly suggest that non-cell-autonomous mechanisms act to control olfactory adaptation in *C. elegans.* Although more extensive future studies will be required to reveal the relationships between the G_o–G_q pathway and other adaptation mechanisms, adaptation mechanisms mediated by extracellular signals appear to be important for adjustment of the sensitivity of olfactory neurons in response to diverse environmental conditions and internal states of an organism.

Materials and Methods

Strains and Culture. *C. elegans* strains were cultured by using standard methods (51), except that the *Escherichia coli* strain NA22 was used as food. The following strains were used in this study: wild-type Bristol N2, *eat-16*(*ce71*) I, *eat-16*(*sa609*) I, *egl-30*(*js126*) I, *goa-1*(*n1134*) I, *goa-1*(*sa734*) I, *gpb-2*(*vs23*) I, *gpb-2*(*ad541*) I, *dgk-3*(*gk110*) III, *dgk-1*(*sy428*) X, *dgk-2*(*gk124*) X, *dpy-20*(*e1282*) IV; *syIs36*[*egl-30*(+)], and *lin-15*(*n765*) *nIs51* X.

Chemotaxis and Adaptation Assays. Assays of chemotaxis to odorants were modified from ref. 4. Assays were performed with 9-cm assay plates [2% agar, 5 mM potassium phosphate (pH 6.0), 1 mM $CaCl_2$, 1 mM $MgSO_4$]. Well-fed animals were washed three times with assay buffer [5 mM potassium phosphate (pH 6.0), 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.05% gelatin] and placed at the center of the assay plate. One microliter each of odorant and ethanol (for control) were placed at a spot 1.5 cm from the edge of the plate along with 1 μl of 0.5 M NaN_3 at each spot. Assay time was 1 h for normal chemotaxis assays and 30 min for adaptation assays. Unless otherwise noted, dilutions of odorants in ethanol were 1:200 for benzaldehyde, 1:1,000 for isoamyl alcohol, 1:1,000 for butanone, 1:1,000 for diacetyl, and 10 mg/ml pyrazine. Assays of chemotaxis to NaCl were modified from ref. 30. To form an NaCl gradient on assay plates, an agar plug containing 100 mM NaCl was placed on the assay plate 20 h before assay. The format of assay plates and assay time were the same as those used in the assay of chemotaxis to odorants.

The chemotaxis index was calculated as [(no. of animals within a 2-cm radius of odorant spot) − (no. of animals within a 2-cm radius of control spot)]/(total no. of animals). To roughly estimate the chemotaxis of animals defective in dispersion, such

as *goa-1* null mutants, the modified chemotaxis index was calculated as [(no. of animals within a 2-cm radius of odorant spot) − (no. of animals within a 2-cm radius of control spot)]/[(total no. of animals) − (no. of animals near the center of the plate)].

Adaptation assays were modified from ref. 46. Washed animals were incubated with 650 μl of assay buffer with (preexposure) or without (mock-preexposure) odorant for 1 h, washed once with fresh assay buffer, and tested for chemotaxis as above. Odorant concentrations used for preexposure treatment were 60 or 6 (for weak benzaldehyde) nl/ml benzaldehyde, 8 nl/ml isoamyl alcohol, 30 nl/ml butanone, 30 nl/ml diacetyl, and 1 μg/ml pyrazine. To examine the plasticity of chemotaxis to NaCl, washed animals were incubated with 650 μl of assay buffer with or without 20 mM NaCl for 1 h and tested for chemotaxis.

For each data point, chemotaxis and adaptation assays were independently performed at least five times, and 50–200 animals were used in each assay. Error bars in the figures indicate the SEM, and statistical analysis was performed by using Student's *t* test.

PMA Treatment. Wild-type animals were washed three times with assay buffer and soaked in an odorant-containing buffer with 0.5 μg/ml PMA for 1 h. After the preexposure treatment, animals were washed once with assay buffer and tested for chemotaxis.

Plasmid construction and germ-line transformation are described in *Supporting Methods*, which is published as supporting information on the PNAS web site.

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