Ligand-Gated Chloride Channels Are Receptors for Biogenic Amines in C. elegans

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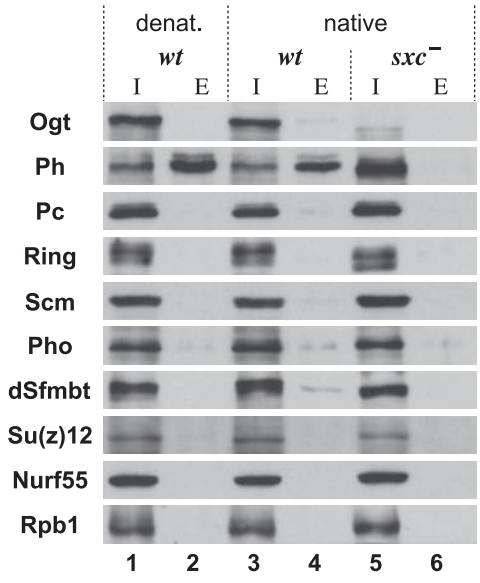

Fig. 4. Sxc/Ogt glycosylates Ph in *Drosophila*. Extracts of wild-type (WT) or sxc^{1}/sxc^{7} (sxc^{-}) mutant larvae were subjected to affinity-purification with WGA-agarose under native or denaturing (denat.) conditions (*3*) and probed with antibodies to PRC1 subunits Ph, Pc, Ring, and Scm; PhoRC subunits dSfmbt and Pho; PRC2 subunits Su(z)12 and Nurf55; the large RNA polymerase II subunit Rpb1; and Ogt. "I" indicates 0.5% of input extract and "E" indicates 10% of affinity-purified material. Ph, but none of the other proteins, is strongly enriched in WGA affinity–purified material (lanes 2 and 4); no enrichment of any protein is seen in material purified from *sxc* mutant larvae (lane 6). Weak enrichment of dSfmbt under native (lane 4) but not denaturing conditions (lane 2) probably reflects association with GlcNAc-modified Ph. Levels of Ph are increased in *sxc* mutant larvae as compared with those in WT larvae (compare lane 5 with lanes 1 and 3), possibly reflecting a failure to downregulate Ph expression by the PcG system through PREs in the Ph gene (*23*).

mice, in which it is required for the viability of embryonic stem cells (*17*, *18*), but dispensable for the normal development of *Caenorhabditis elegans* (*19*). In contrast, *Drosophila* mutants lacking Sxc/Ogt and O-GlcNAcylation display a specific phenotype: loss of Polycomb repression. *sxc* mutants show no other obvious developmental defects, suggesting that PcG repression is the main process that critically depends on O-GlcNAcylation in *Drosophila*. We provide evidence that Ph is GlcNAcylated. Although it remains to be determined whether Ph is indeed the relevant Sxc/Ogt substrate in PcG repression, it is tempting to speculate that the function of Sxc/Ogt in gene silencing may be to GlcNAcylate Ph. The *sxc* null phenotype is not as severe as that of other PcG mutants, notably that of *ph* [(*1*, *20*, *21*) and this study]. Thus, if GlcNAcylation of Ph contributes to its function, Ph still retains partial repressor activity in the absence of this modification. One possibility would be that GlcNAcylation of Ph is needed for efficient anchoring of Ph to PREs and/or for the capacity of PRE-tethered Ph to maintain a repressed chromatin state at target genes.

All *Drosophila* PcG proteins are conserved in vertebrates, and the PcG system represses a large set of orthologous developmental regulator genes both during *Drosophila* development and in mammalian embryonic stem cells (*13*, *22*). Thus, GlcNAcylation of Polyhomeotic homologs, or perhaps other PcG proteins, may also be an evolutionary ancient and essential function of Ogt in vertebrates.

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Supporting Online Material

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Ligand-Gated Chloride Channels Are Receptors for Biogenic Amines in *C. elegans*

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Biogenic amines such as serotonin and dopamine are intercellular signaling molecules that function widely as neurotransmitters and neuromodulators. We have identified in the nematode *Caenorhabditis elegans* three ligand-gated chloride channels that are receptors for biogenic amines: LGC-53 is a high-affinity dopamine receptor, LGC-55 is a high-affinity tyramine receptor, and LGC-40 is a low-affinity serotonin receptor that is also gated by choline and acetylcholine. *lgc-55* mutants are defective in a behavior that requires endogenous tyramine, which indicates that this ionotropic tyramine receptor functions in tyramine signaling in vivo. Our studies suggest that direct activation of membrane chloride conductances is a general mechanism of action for biogenic amines in the modulation of *C. elegans* behavior.

Biogenic amines function in diverse neuronal circuits as neurotransmitters and neuromodulators. Therapeutics for many psychiatric disorders, including major depression, schizophrenia, and bipolar affective disorder, target signaling pathways of such biogenic amines as serotonin, dopamine, and noradrenaline (*1*). Biogenic amine signaling pathways are also targets of drugs of abuse (*1*). Almost all known biogenic amine receptors are G protein–coupled receptors (GPCRs) that signal though the activation of heterotrimeric guanine nucleotide–binding proteins (G proteins), which activate second-messenger signaling pathways. However, there exists a second type of biogenic amine receptor:

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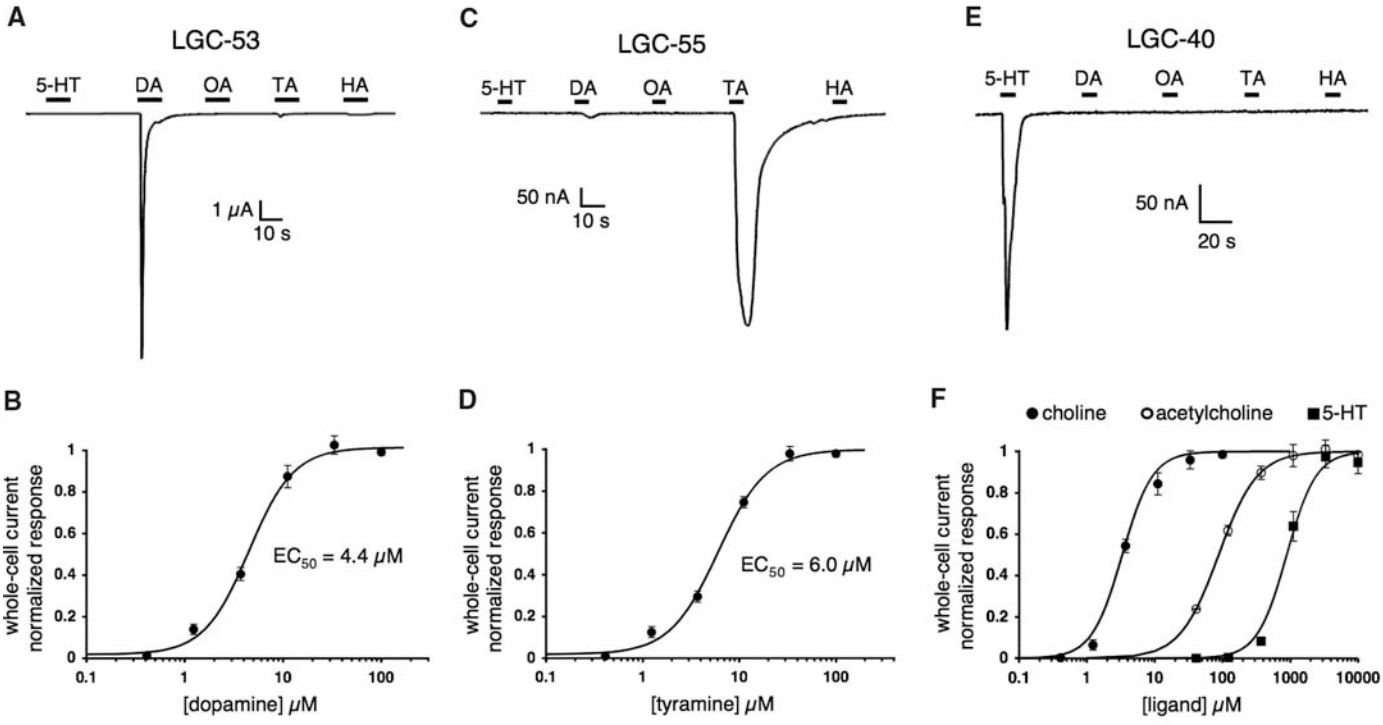

Fig. 1. Biogenic amines evoke currents in *Xenopus* oocytes expressing LGC-53, LGC-55, or LGC-40. (**A**, **C**, and **E**) Whole-cell currents recorded from *Xenopus* oocytes expressing LGC-53, LGC-55, or LGC-40 during application of serotonin (5-HT), dopamine (DA), octopamine (OA), tyramine (TA), and histamine (HA). The concentrations of applied neurotransmitters were 10 μM in (A) and (B) and 100 μM in (C). (**B** and **D**) Dose-response curves for dopamine- and tyramine-evoked currents in *Xenopus* oocytes expressing LGC-53 and LGC-55, respectively. Each point (± SEM) represents the average of three to five recordings. Dose-response data were fitted to the Hill equation and normalized to the maximum amplitude of the current (I_{max}). The EC_{50} of dopamine for LGC-53 was 4.4 μM, and the estimated Hill coefficient was 1.9. The EC_{50} of tyramine for LGC-55 was 6.0 μM, and the estimated Hill coefficient was 1.8. (**F**) Dose-response curves for serotonin-, acetylcholine-, and choline-evoked currents in *Xenopus* oocytes expressing LGC-40. Dose-response data were fitted to the Hill equation and normalized to I_{max}. $n \geq 5$. The EC_{50} of serotonin for LGC-40 was 905 μM, and the estimated Hill coefficient was 2.7. The EC_{50} values of acetylcholine and choline for LGC-40 were 87 μM and 3.4 μM, respectively, and the estimated Hill coefficients were 1.5 and 1.9, respectively.

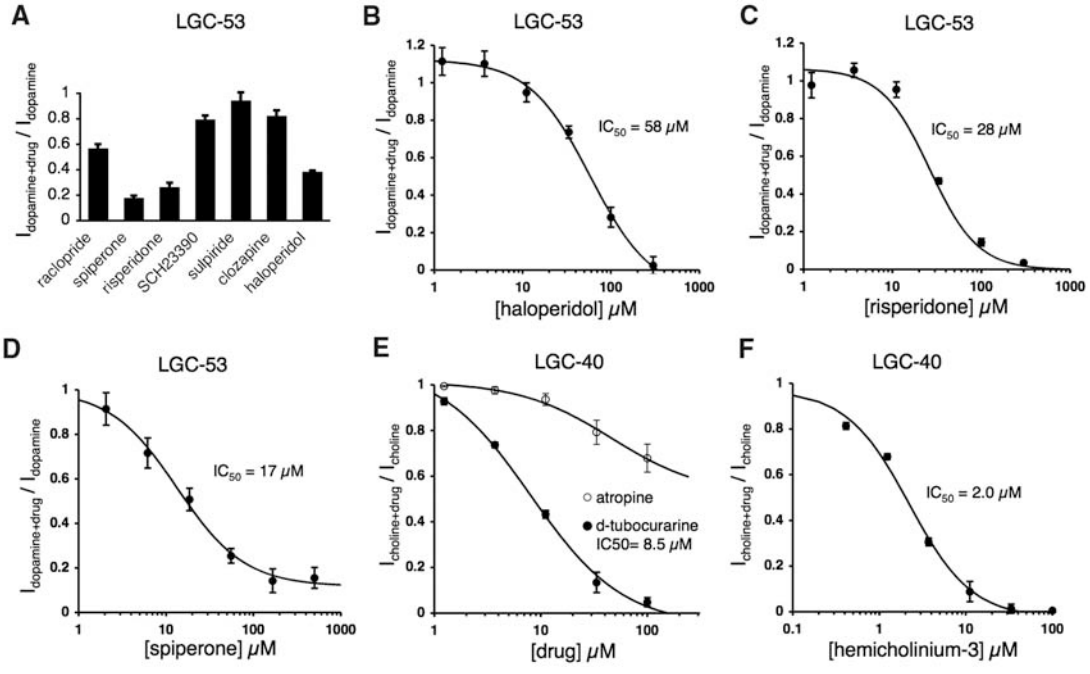

Fig. 2. Pharmacological characterizations of the ionotropic dopamine receptor LGC-53 and the ionotropic choline receptor LGC-40. (**A**) Inhibition of LGC-53 currents by dopamine-receptor antagonists. The mean ratios (± SEM) of the peak currents evoked in the presence and absence of the indicated dopamine receptor antagonist are shown. $n \geq 5$. Drugs were tested at a concentration of 100 μM, and 5 μM dopamine was used to evoke LGC-53 currents. (**B** to **D**) Dose-response curves for the inhibition by haloperidol, risperidone, and spiperone of LGC-53 currents. Dopamine (5 μM) was used to evoke currents in oocytes expressing LGC-53 in the presence of different concentrations of dopamine receptor antagonists. Currents were normalized to the current evoked by 5 μM dopamine in the absence of receptor antagonists. $n \geq 5$. (**E** and **F**) Dose-response curves for the inhibition by atropine, *d*-tubocurarine, and hemicholinium-3 of LGC-40 currents. Currents were evoked by 2 μM choline in oocytes expressing LGC-40 in the presence of different concentrations of compounds. Currents were normalized to the current evoked by 2 μM choline in the absence of any compounds. $n \geq 5$.

biogenic amine-gated ion channels. The vertebrate 5-hydroxytryptamine (serotonin) receptor 3A (5-HT_3 receptor) is a serotonin-gated cation channel (*2*, *3*). Two arthropod histamine receptors and the *Caenorhabditis elegans* MOD-1 serotonin receptor are biogenic amine–gated chloride channels (*4*–*8*). These biogenic amine–gated channels from diverse phyla suggest a mechanism of action for their cognate ligands: fast excitation or inhibition analogous to the response to the activation of nicotinic acetylcholine receptors or to the activation of γ-aminobutyric acid type A (GABA_A) receptors, respectively. We report that such a mechanism of action for biogenic amines is more general: a single species, *C. elegans*, expresses multiple ion channels gated by biogenic amines.

The known biogenic amine–gated ion channels are members of the Cys-loop family of ion channels. Using database searches of proteins encoded by the *C. elegans* genome, we identified 26 presumptive Cys-loop family ion channels that are highly similar to the MOD-1 serotonin-gated chloride channel (*9*) [E values reported by the BLAST algorithm range from 10^{-35} to 10^{-94} (table S1)]; 23 have sequences in their pore-forming M2 transmembrane domains, predicted to confer chloride selectivity (*10*). We expressed these 26 receptors individually in *Xenopus laevis* oocytes and tested them for receptor activity using a two-electrode voltage clamp to monitor whole-cell currents evoked by application of a panel of agonists, including the biogenic amines serotonin, dopamine, octopamine, tyramine, and histamine (*11*). We identified three genes that encode ion channels activated by biogenic amines: *lgc-40*, *lgc-53*, and *lgc-55*. LGC-53 was activated with the highest efficacy by dopamine (Fig. 1A), with a median effective concentration (EC_{50}) of 4.4 μM (Fig. 1B). LGC-55 was activated with the highest efficacy by tyramine with an EC_{50} of 6.0 μM (Fig. 1, C and D). LGC-40 was activated only by high concentrations of serotonin (EC_{50} = 905 μM) (Fig. 1, E and F). We tested whether other potential ligands could be more effective and found that choline and acetylcholine gated LGC-40 at lower concentrations (choline EC_{50} = 3.4 μM, acetylcholine EC_{50} = 87 μM) (Fig. 1F) (*12*). Compounds that block ion channels endogenous to *Xenopus* oocytes failed to block agonist-evoked whole-cell currents in oocytes expressing LGC-40, LGC-53, and LGC-55, which indicates that such endogenous channels do not mediate the agonist-evoked currents (fig. S1).

Ligand binding by Cys-loop family ion channels is mediated by the amino-terminal extracellular domains of channel subunits (*13*). We aligned the extracellular domain sequences of LGC-40, LGC-53, and LGC-55 with those of MOD-1 and 5-HT_3 receptor subunits and of other Cys-loop family receptors, but did not identify any amino acids that were conserved specifically among amine-gated channels (fig. S2).

All previously characterized dopamine receptors are GPCRs (*1*). We tested whether antagonists of G protein–coupled dopamine receptors can inhibit the LGC-53 dopamine-gated ion channel. Of seven antagonists tested (Fig. 2A), three acted with a median inhibitory concentration (IC_{50}) of less than 100 μM: risperidone (IC_{50} = 40 μM), haloperidol (IC_{50} = 32 μM), and spiperone (IC_{50} = 38 μM) (Fig. 2, B to D) (*11*). Risperidone, haloperidol, and spiperone all are in clinical use as antipsychotics (*1*). Because these drugs also block LGC-53, we suggest that some of their actions as therapeutics might be through inhibition of a yet-to-be-identified human dopamine receptor that, like LGC-53, is composed of Cys-loop family channel subunits.

We tested *d*-tubocurarine and atropine, antagonists of ionotropic and G protein–coupled acetylcholine receptors, respectively (*1*), and hemicholinium-3, a blocker of the high-affinity choline transporter (*14*), for effects on choline-evoked currents in oocytes expressing LGC-40

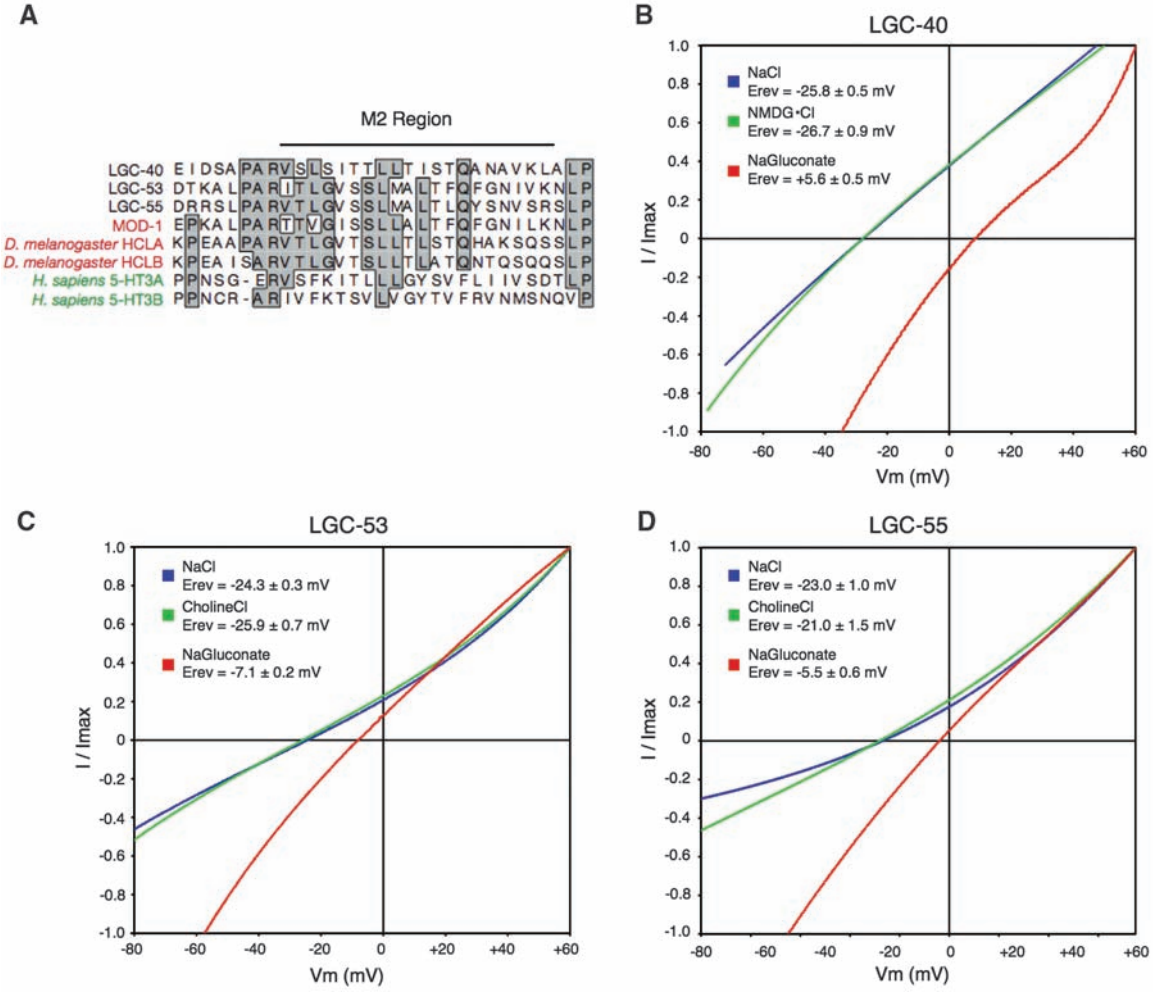

Fig. 3. LGC-40, LGC-53, and LGC-55 are chloride channels. (**A**) Alignment of sequences from the presumptive M2 region of amine-gated ion channels, which determines ion selectivity of Cys-loop family ion channels (*10*, *13*, *29*). Cation-selective 5-HT_3 receptor subunits are labeled in green, and anion-selective MOD-1, HCLA, and HCLB subunits are labeled in red. (**B** to **D**) Current-voltage relations (*I-V* curves) of LGC-40, LGC-53, and LGC-55 in ND96 medium (which contains 96 mM sodium and 104 mM chloride), sodium-free medium (0 mM sodium and 104 mM chloride), or low-chloride medium (96 mM sodium and 8 mM chloride). The mean reversal potential ± SEM from four to five experiments under each condition is shown.

(Fig. 2, F and G). LGC-40 currents were blocked by low concentrations of *d*-tubocurarine (IC_{50} = 8.3 μM) but were relatively insensitive to atropine (Fig. 2F). Hemicholinium-3 was the most effective antagonist of the LGC-40 channel, with an IC_{50} of 2.0 μM (Fig. 2G). Thus, LGC-40 is sensitive to both a canonical antagonist of acetylcholine-gated ion channels and an inhibitor of the high-affinity choline transporter.

The presumptive M2 regions, which are predicted to determine the ion selectivity of Cys-loop family ion channels, of LGC-40, LGC-53, and LGC-55 are more similar to the M2 regions of known chloride channels than to those of known cation channels (Fig. 3A) (*10*, *13*). To test whether these receptors are chloride channels, we measured the reversal potentials of agonist-evoked currents in *Xenopus* oocytes expressing LGC-40, LGC-53, and LGC-55 (Fig. 3, B through D). In solutions containing 96 mM Na^+ and 104 mM Cl^-, ligand-evoked currents reversed at –23 to –26 mV, close to the reversal potential for chloride in *Xenopus* oocytes (*15*). Replacement of sodium ions with *N*-methyl-D-gluconate (NMDG) or choline did not alter the reversal potentials of the evoked currents. Replacement of chloride ions with gluconate shifted the reversal potentials positively by 17 to 31 mV (Fig. 3, B to D) (*16*). These data indicate that LGC-40, LGC-53, and LGC-55 are chloride channels. The observed shift in reversal potential was less than predicted for channels that are perfectly selective for chloride over other anions (our unpublished observations); perhaps these channels might also pass the anions we used to replace chloride.

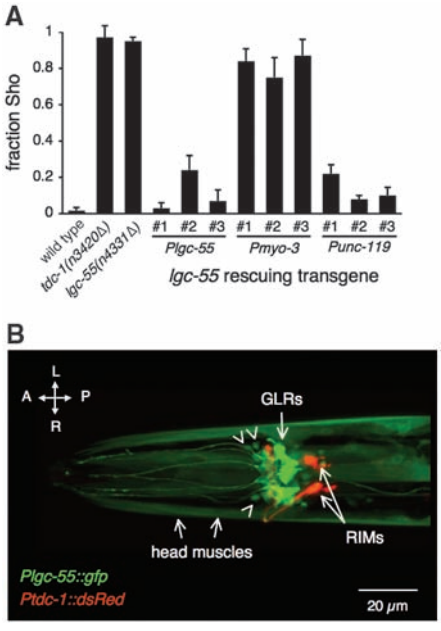

Fig. 4. LGC-55 is required for the tyraminergic modulation of head movements by *C. elegans* and is expressed in the GLR glia-like cells and head muscles. (**A**) The fractions of animals that have the Sho phenotype and cannot suppress head oscillations are plotted for the wild type, *tdc-1* and *lgc-55* mutants, and *lgc-55* mutants carrying rescuing transgenes that express the *lgc-55* cDNA using its own promoter, the pan-neuronal *unc-119* promoter, or the pan-muscle *myo-3* promoter. In each experiment, we tested 20 individuals of each genotype for the Sho phenotype. The mean fraction of animals with the Sho phenotype ± SEM is plotted, $n \geq 3$. Three independent lines (labeled #1 to 3) carrying each transgene were assayed. (**B**) Expression of *lgc-55*. An *lgc-55::gfp* reporter transgene is expressed in the GLR glia-like cells and head muscles (*17*). Arrowheads indicate some of the unidentified head neurons that express the reporter transgene. The tyraminergic RIM neurons, which provide the tyramine that inhibits head movements during reversals, are labeled with a *tdc-1::dsRed* reporter transgene.

To identify biological functions of these amine-gated chloride channels, we isolated *lgc-40*, *lgc-53*, and *lgc-55* deletion mutants (fig. S3). *lgc-55* mutants, which lack the tyramine-gated chloride channel, had a behavioral defect that indicates an in vivo function for this receptor in tyraminergic signaling. Tyramine from the RIM motor neurons suppresses small rapid head movements during touch-evoked locomotory reversals (*17*). (RIM neurons are a pair of inter–motor neurons that innervate muscles in the head via neuromuscular junctions in the nerve ring.) Animals lacking endogenous tyramine fail to suppress these head oscillations and exhibit the Sho phenotype (suppression of head oscillations–defective), as do animals lacking the tyraminergic RIM motor neurons (*17*) (Fig. 4A). Similarly, *lgc-55* mutants continued to execute head movements during touch-evoked reversals (Fig. 4A). The Sho phenotype of *lgc-55* mutants was rescued by transgenes containing the *lgc-55* cDNA fused to *lgc-55* promoter sequences and by transgenes containing the *lgc-55* cDNA fused to the *unc-119* promoter, which drives expression broadly in the nervous system (*18*). To identify cells that express LGC-55, we created transgenic animals expressing green fluorescent protein under the control of the *lgc-55* promoter (*18*). We observed expression of this reporter transgene in head muscles and in the glia-like GLR cells, which are connected to the head muscles by gap junctions (*19*), and weaker expression in many unidentified head neurons (Fig. 4B). We also observed strong expression in the ALM and AVM mechanosensory neurons (not shown). Expression of *lgc-55* in muscles, including head muscles, did not rescue the Sho phenotype of *lgc-55* mutants (Fig. 4A). Our findings suggest that LGC-55 can act in the GLR cells or neurons to control head movements and establish that the LGC-55 tyramine receptor functions in vivo in the tyraminergic control of head movements. No behavioral abnormalities were detected in mutants that carry deletions in *lgc-40* and *lgc-53* (*20*).

We conclude that, including MOD-1, there exist in *C. elegans* at least four ligand-gated chloride channels that can be activated by biogenic amines. Our results indicate that ligand-gated chloride channels constitute a class of receptor for biogenic amines and that direct activation of membrane chloride conductances is a general mechanism of biogenic amine action in *C. elegans*. Biogenic amine–gated chloride channels might exist in the nervous systems of animals other than *C. elegans* and arthropods. In mollusks, synaptic dopamine rapidly activates a chloride conductance that is blocked by picrotoxin, an antagonist of ligand-gated chloride channels (*21*–*24*). In the mammalian brain, histaminergic neurons project to the hypothalamus and use rapid inhibitory synaptic signals that (i) are not mediated by G protein signaling, (ii) are picrotoxin-sensitive, and (iii) are mediated by chloride conductances (*25*). The molecular identity of the histamine receptor in this synapse is not known, but the recent demonstration that $GABA_A$ receptor subunits can form histamine-gated chloride channels in vitro suggests that an ionotropic histamine receptor might contain known ligand-gated chloride channel subunits (*26*). If direct activation of ligand-gated chloride channels is a mechanism of biogenic amine action in the mammalian brain, such receptors might be therapeutic targets, given the many links between aminergic signaling and psychiatric disorders (*1*, *27*).

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11. Descriptions of how we expressed *lgc-40, lgc-53*, and *lgc-55* in *Xenopus laevis* oocytes are in (*9*). We recorded whole-cell currents using a two-electrode voltage clamp (Warner Instruments). The ground electrode was connected to the recording chamber (Warner Instruments model RC-3Z) using an agar bridge. Data were acquired with Clampex 8.0 (Molecular Devices) and analyzed offline with Clampfit (Molecular Devices). Oocyte membrane potential was held at –60 mV for all experiments. To identify new Cys-loop family biogenic amine receptors, oocytes expressing candidate channels were sequentially superfused with ND96 (96 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, and 5 mM Hepes, pH 7.6) containing GABA (100 μM), glycine (100 μM), glutamate (100 μM), histamine (100 μM), serotonin (1 mM), dopamine (100 μM), octopamine (100 μM), or tyramine (100 μM). Candidate receptors not activated by these ligands were also tested with ivermectin (10 μM), which can activate many ligand-gated chloride channels (*28*).
12. The following compounds were tested for LGC-40 agonist activity: *N*-acetyl 5-hydroxytryptamine, adenosine, adenosine triphosphate, carnosine, epinephrine, FLRFamide (FLFR is Phe-Leu-Arg-Phe-NH_2) (*29*), FMRFamide, 5-hydroxytryptophan, homocarnosine, melatonin, methoxytyramine, norepinephrine, *O*-methyl

5-hydroxytryptamine, *o*-methoxylphenylethylamine, phenylethylamine, tryptamine. All chemicals were purchased from Sigma and tested at 1 mM concentrations, except for the FLRFamide and FMRFamide peptides, which were tested at 10 μM.
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16. For ion replacement experiments, oocytes expressing LGC-40, LGC-53, or LGC-55 were held at a resting potential of −90 mV. LGC-40, LGC-53, or LGC-55 currents were evoked with 2 μM choline, 5 μM dopamine, and 5 μM tyramine, respectively, in ND96 medium, sodium-free medium (96 mM cholineCl or NMDG-Cl replacing NaCl) or low-chloride medium (96 mM NaGluconate replacing NaCl). After 5 s, a voltage ramp of 20 mV/s from −90 mV to +60 mV was applied. Currents from voltage ramps recorded in the absence of ligands were subtracted from these current traces.
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29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
30. We thank Y. Kohara for *lgc-40*, *lgc-53*, and *lgc-55* cDNA clones, A. Fire for expression vectors, R. O'Hagan for help with electrophysiology, A. Hellman for deletion screening, M. Alkema for discussions, J. Kehoe for bringing to our attention reports of dopamine-activated chloride conductances in mollusks, and D. Denning and B. Galvin for suggestions concerning the manuscript. This work was supported by NIH grant GM24663. N.R. received support from the Howard Hughes Medical Institute, the Life Sciences Research Foundation and The Medical Foundation. H.R.H. is an Investigator of the Howard Hughes Medical Institute.

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Materials and Methods
Figs. S1 to S3
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LXR Regulates Cholesterol Uptake Through Idol-Dependent Ubiquitination of the LDL Receptor

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Cellular cholesterol levels reflect a balance between uptake, efflux, and endogenous synthesis. Here we show that the sterol-responsive nuclear liver X receptor (LXR) helps maintain cholesterol homeostasis, not only through promotion of cholesterol efflux but also through suppression of low-density lipoprotein (LDL) uptake. LXR inhibits the LDL receptor (LDLR) pathway through transcriptional induction of Idol (inducible degrader of the LDLR), an E3 ubiquitin ligase that triggers ubiquitination of the LDLR on its cytoplasmic domain, thereby targeting it for degradation. LXR ligand reduces, whereas LXR knockout increases, LDLR protein levels in vivo in a tissue-selective manner. Idol knockdown in hepatocytes increases LDLR protein levels and promotes LDL uptake. Conversely, adenovirus-mediated expression of Idol in mouse liver promotes LDLR degradation and elevates plasma LDL levels. The LXR-Idol-LDLR axis defines a complementary pathway to sterol response element–binding proteins for sterol regulation of cholesterol uptake.

The low-density lipoprotein (LDL) receptor (LDLR) is central to the maintenance of plasma cholesterol levels (*1*). Mutations in this receptor are the leading cause of autosomal dominant hypercholesterolemia, characterized by elevated plasma cholesterol levels, and increased risk of cardiovascular disease (*2*, *3*). In line with its pivotal role in cholesterol homeostasis, expression of the LDLR is tightly regulated. Transcription of the *LDLR* gene is coupled to cellular cholesterol levels through the action of the sterol response element–binding protein (SREBP) transcription factors (*4*, *5*). Enhanced processing of SREBPs to their mature forms when cellular sterol levels decline leads to increased *LDLR* transcription (*6*). Posttranscriptional regulation of LDLR expression is also a major determinant of lipoprotein metabolism. Genetic studies have identified mutations in the genes encoding the LDLR adaptor protein 1 (*LDLRAP1/ARH*) (*7*, *8*) and the SREBP target gene proprotein convertase subtilisin/kexin 9 (*PCSK9*) that result in altered stability, endocytosis, or trafficking of the LDLR (*9*–*13*).

The liver X receptors (LXRs) are also important transcriptional regulators of cholesterol metabolism. LXRα (NR1H3) and LXRβ (NR1H2) are sterol-dependent nuclear receptors activated in response to cellular cholesterol excess (*14*). LXR target genes such as *ABCA1* and *ABCG1* promote the efflux of cellular cholesterol and help to maintain whole-body sterol homeostasis (*15*, *16*). Mice lacking LXRs accumulate sterols in their tissues and manifest accelerated atherosclerosis, whereas synthetic LXR agonists promote reverse cholesterol transport and protect mice against atherosclerosis (*17*–*19*). The coordinated regulation of intracellular sterol levels by the LXR and SREBP signaling pathways led us to investigate whether LXRs control the uptake as well as efflux of cholesterol.

We initially tested the ability of LXRs to modulate LDL uptake in HepG2 human liver cells and primary mouse macrophages (*20*). Treatment with synthetic LXR ligand (GW3965 or T1317) decreased binding and uptake of boron-dipyrromethene (BODIPY)-labeled LDL (Fig. 1A). The LXR ligands modestly induced changes in LDLR mRNA expression (fig. S1A); however, they decreased LDLR protein levels rapidly and in a dose-dependent manner, and this effect was independent of cellular sterol levels (Fig. 1, B to D). Levels of ABCA1 protein, an established target of LXR, were reciprocally increased by LXR ligands (Fig. 1, B to D). LXR ligands had no effect on LDLR levels in macrophages or mouse embryonic fibroblasts (MEFs) lacking LXRα and LXRβ (Fig. 1E and fig. S1B). LXR activation also decreased LDLR protein but not mRNA levels in human SV589 fibroblasts (fig. S1, C and D) (*21*).

To investigate the link between endogenous LXR ligands and LDLR expression, we used an adenovirus vector encoding oxysterol sulfotransferase (Sult2b1) (*22*, *23*). Depletion of oxysterol agonists by Sult2b1 in SV589 cells led to increased LDLR protein, and this effect was reversed by synthetic ligand (fig. S1E). We further tested the effect of LXR agonists on LDLR produced from a transfected vector (i.e., not subject to endogenous SREBP regulation). In HepG2 cells stably expressing an LDLR–green fluorescent protein (GFP) fusion protein, LDLR-GFP expression was localized primarily on the plasma membrane (Fig. 1F). Ligand activation of LXR decreased LDLR-GFP

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