A Novel Strategy for Cell-Autonomous Gene Knockdown in *Caenorhabditis elegans* Defines a Cell-Specific Function for the G-Protein Subunit GOA-1

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ABSTRACT We developed a novel knockdown strategy to examine cell-specific gene function in *Caenorhabditis elegans*. In this strategy a null mutation in any gene is replaced with a genetically stable transgene that contains a wild-type copy of the gene fused to a 3' tag that targets the mRNA transcript for degradation by the host nonsense-mediated decay (NMD) machinery. In NMD-defective animals, tagged transgene mRNA is expressed at levels similar to the endogenous gene it replaced and is translated into wild-type protein that fully rescues gene function. Cell-specific activation of NMD cell autonomously knocks down transgene expression in specific cell types without affecting its expression or function in other cells of the organism. To demonstrate the utility of this system, we replaced the *goa-1* gene, encoding the pan-neuronally expressed G-protein subunit GOA-1, with a degradation-tagged transgene. We then knocked down expression of the transgene from only two neurons, the hermaphrodite-specific neurons (HSNs), and showed that GOA-1 acts cell autonomously in the HSNs to inhibit egg-laying behavior.

EXPERIMENTAL strategies that reduce protein activity, such as the use of genetic mutations or small molecule protein inhibitors, have been very useful in elucidating the functional roles of proteins. However, in multicellular organisms these strategies cause global effects on protein activity that can obscure the cell-specific function of a protein. This is a particular concern when investigating the function of proteins that are widely expressed or that have different functions in different cell types or at different times during development. To understand the cell-specific function of these proteins it is necessary to reduce or eliminate their activity in individual cell types, without affecting their activity in other cells of the organism.

In *Caenorhabditis elegans*, double-stranded RNA interference (dsRNAi), using either promoter-driven sense and antisense transgenes or hairpin RNAs, has been used to reduce

protein function in specific cell types (Tavernarakis *et al.* 2000; Johnson *et al.* 2005; Esposito *et al.* 2007). However, intermediates generated during processing of dsRNAs can be transported between cells of the organism and thus even transgenes driven by cell-specific promoters can cause global knockdown effects (Jose *et al.* 2009, 2011). In addition, extrachromosomal transgenes typically used to drive expression of dsRNAs are randomly lost during cell division, leading to mosaic knockdown effects within individual animals of a population (Stinchcomb *et al.* 1985).

We have developed a method to knock down the expression of any gene in any cell type in *C. elegans* that is both cell autonomous and genetically stable. In this strategy, an endogenous gene is replaced by an integrated single-copy transgene containing the endogenous gene's promoter and coding sequence tagged with a unique 3'-UTR that targets transgene mRNA for destruction by the host cell's nonsense-mediated decay (NMD) machinery. In NMD-defective animals, the tagged transgene is expressed at levels comparable to that of the endogenous gene and is able to restore wild-type gene function. Spatial control of knockdown is achieved by cell-specific restoration of NMD activity. Using appropriate cell-specific promoters to control NMD activity, one can restrict

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the knockdown of transgene expression to individual cell types in the animal without affecting its expression in any other cells. To demonstrate the utility of this strategy, we used a tagged transgene to investigate the cell-specific function of the G-protein subunit *GOA-1* and found that selective removal of *GOA-1* from the two hermaphrodite-specific neurons (HSNs) (but not from other cells of the organism) was sufficient to cause *goa-1* null egg-laying defects. Thus *GOA-1* acts cell autonomously in the HSNs to inhibit egg-laying behavior.

This cell-autonomous method of gene knockdown can be used to examine the cell-specific function of any protein, eliminating the confounding effects caused by the global reduction of protein function typical of other knockdown strategies.

Materials and Methods

Transgenes

To generate *let-858* tagged transgenes 4188 bp of *let-858* sequence was amplified from pPD118.60 (L3808, Addgene), using primers *SbfI*-*let-858F* and *XhoI*-*let-858R* and inserted into *SbfI*/*XhoI* sites of pCFJ151 to generate pCL123 (see list of primers in Supporting Information, Table S1). Promoter and coding sequences were then inserted into the *AvrII*/*SbfI* sites of pCL123 to generate tagged transgenes. For mCherry, a 2105 bp region including *rab-3* promoter and mCherry coding sequence was amplified from pGH8, using primers *AvrII*-pRAB-3-F and *SbfI*-mCherry-R to generate pCL124. For *goa-1*, a 9618 bp region including 5,972 bp of promoter and the entire *goa-1* coding region was amplified from genomic DNA, using primers *goa-1*-pro-*NheI*-F and *goa-1*-*SbfI*-R to generate pCL136. For *unc-4*, a 5205 bp region including 2807 bp of promoter and the entire *unc-4* coding region was amplified from genomic DNA, using primers *unc-4* pro-*AvrII*-F and *unc-4* *SbfI*-R to generate pCL145.

To generate NMD rescue transgenes, a 1971 bp region of *smg-5* genomic sequence and 3'-UTR was amplified from genomic DNA, using primers *smg-5*-*AvrII*-F and *smg-5*-3'-*utr*-*KpnI*-R, and inserted into the *AvrII*/*KpnI* sites of pCFJ178 (Addgene) to generate pCL119. Cell-specific promoters were then inserted into the *SbfI*/*AvrII* sites of pCL119. To restore NMD in cholinergic cells, 3249 bp of *unc-17* promoter sequence was amplified using primers *unc-17*-*SbfI*-F and *unc-17*-pro-*AvrII*-R to generate pCL121. To restore NMD in all *goa-1*-expressing cells 5972 bp of promoter *goa-1* sequence was amplified using primers *goa-1*-pro-*SbfI*-F and *goa-1*-pro-*NheI*-R to generate pCL137. To restore NMD in HSNs, 3124 bp of *tph-1* promoter sequence was amplified using primers *tph-1*-pro-*SbfI*-F and *tph-1*-pro-*AvrII*-R to generate pCL140. To restore NMD in all *unc-4*-expressing cells 2804 bp of *unc-4* promoter sequence was amplified using the primers *unc-4*-pro-*SbfI*-F and *unc-4*-pro-*AvrII*-R to generate pCL147.

Sense/antisense *unc-4* transgenes were made as described using 2912 bp of *unc-4* promoter (Esposito *et al.*

2007). The sense promoter used primers *unc-4*-pro-outer-F and *unc-4*-pro-fusion-sense-R. The antisense promoter used primers *unc-4*-pro-outer-F and *unc-4*-pro-fusion-R-AS. The RNAi target sequence was amplified using *unc-4*-seq-exon-4-F and *unc-4*-ex6-outer-R primers. Using these DNAs as template, the fusion sense transgene was made using primers *unc-4*-pro-inner-F and *unc-4*-ex6-inner-R and the fusion antisense transgene was made using primers *unc-4*-pro-inner-F and *unc-4*-ex4-inner-F to generate 3525 bp products. PCR transgenes were purified before injection.

C. elegans strains

Worm strains were generated and maintained using standard methods and conditions (Brenner 1974). The wild-type strain was Bristol N2. The boundaries of the *goa-1*(n363) deletion mutation were determined to be 5'-AGAACAATATAGAAG-TAGTGCTTAG-ACGCAACTTTTCCAATTGGC-3', resulting in a 15,217 bp deletion that removed the entire coding sequence of *goa-1*. Strains analyzed in this study were as follows:

Figure 1: XP495 *ndEx135*, XP492 *smg-5*(r860) I; *ndEx132*, XP487 *smg-5*(r860) I; *ndEx126*, XP445 *smg-5*(r860) I; *ndSi1* IV; *ndEx115*.

Figure 2: N2, XP384 *smg-5*(r860) I, KO98 *goa-1*(n363) I, XP447 *goa-1*(n363) I; *ndSi7* II, XP466 *smg-5*(r860) *goa-1*(n363) I, XP467 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II.

Figure 3: N2, KO98 *goa-1*(n363) I, XP467 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II, XP469 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II; *ndSi5* IV, XP468 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II; *ndSi6* IV.

Figure 4: N2, KO98 *goa-1*(n363) I, XP467 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II, XP469 *smg-5*(r860) *goa-1*(n363) I; *ndSi7* II; *ndSi5* IV.

Table 1: N2, VC1453 *unc-4*(gk668) II, XP470 *smg-5*(r860) I; *unc-4*(gk668) II, XP481 *smg-5*(r860) I; *unc-4*(gk668) *ndSi19* II, XP503 *smg-5*(r860) I; *unc-4*(gk668) *ndSi19* II; *ndSi20* IV, XA4254 *qals4254*, KP3948 *eri-1*(mg366) IV, *lin-15b*(n744) X, XP508 *ndEx149*, XP509 *ndEx150*, XP510 *ndEx151*, XP511 *ndEx152*, XP512 *ndEx153*.

Construction of transgenic strains

Strains expressing extrachromosomal arrays were generated by coinjecting transgenes at the following concentrations: XP495, pRab100 (*rab-3p*::GFP) (50 ng/μl) and pCL124 (50 ng/μl) into N2 animals (array designation: *ndEx135*); XP492, pRab100 (50 ng/μl) and pCL124 (50 ng/μl) into XP384 *smg-5*(r860) animals (array designation: *ndEx132*); XP487, pCL124 (50 ng/μl) and pCL30 (*unc-17p*::GFP containing 3249 bp of *unc-17* promoter sequence) (50 ng/μl) into XP384 *smg-5*(r860) animals (array designation: *ndEx126*); and XP445, pCL124 (50 ng/μl) and pCL30 (50 ng/μl) into XP380 (*smg-5*(r860); *ndSi1*) animals (array designation: *ndEx115*). XP508, XP509, XP510, XP511, and XP512 animals were generated by injecting N2 animals with sense/antisense

Figure 1 Expression of degradation-tagged mCherry transgene is NMD dependent. (A) Schematic representation of degradation-tagged mCherry transgene. Boxes represent exons and lines between boxes are introns. Positions of stop codons are indicated. Bar, 1 kb. (B) Expression of tagged (*rab-3p::mCherry::let-858*) and untagged (*rab-3p::GFP*) transgenes in *smg-5* mutants (top panels) and wild-type animals (bottom panels). The *rab-3* promoter drives expression in all neurons. mCherry protein is detected in all neurons of NMD-defective (*smg-5*) mutants but is not detected in wild-type animals while GFP is easily detected in all neurons of both mutant and wild-type animals. Bars, 50 μ m. (C) Expression of tagged (*rab-3p::mCherry::let-858*) and untagged (*unc-17p::GFP*) transgenes in GABAergic and cholinergic ventral cord motor neurons of *smg-5* mutants that either lack (left panels) or express (right panels) the *unc-17p::SMG-5* rescue transgene. The *unc-17* promoter drives expression in all cholinergic cells, and thus all green cells are cholinergic. mCherry protein is detected in both cholinergic and GABAergic neurons in *smg-5* mutants lacking the rescue transgene (left panels) while mCherry protein is detected only in GABA neurons in *smg-5* mutants that express the rescue transgene (right panels). Note the presence of yellow cells in the bottom left panel, indicating expression of the tagged mCherry transgene in cholinergic cells, and the lack of yellow cells in the bottom right panel, indicating degradation of tagged transgene in NMD-rescued cholinergic cells. GABA neuron cell bodies are indicated by arrows. Bars, 20 μ m.

unc-4 transgenes (50 ng/ μ l each) with pGH8 (10 ng/ μ l) (array designations: *ndEx149*, *ndEx150*, *ndEx151*, *ndEx152*, and *ndEx153*, respectively).

Transgene integration at Mos1 alleles *cxTi10882* or *ttTi5605* (strains EG5003 and EG4322, respectively) was done as described in Frøker-Jensen *et al.* (2008). Single-copy insertions were confirmed by PCR and enzyme digest, using a protocol developed by M. Nonet (<http://thalamus.wustl.edu/nonetlab/ResourcesF/Resources.html>). Briefly, genomic DNA was purified from putative integrants and the transgene insertion was amplified with primers that flanked the recombination region. Primers to verify insertions at *cxTi10882* were Chr4-SC-Int-OF-2 and Ti10882-Chr4right-OR. Primers for *ttTi5605* were NM3880 and NM3884. Appropriately sized PCR products were digested with *DraI* to confirm their identity. *ndSi1*, *ndSi5*, *ndSi6*, and *ndSi20* were generated by recombination of pCL121, pCL137, pCL140, and pCL147, respectively at Mos1 allele *cxTi10882*. *ndSi7* and *ndSi19* were generated by recombination of pCL136 and pCL145, respectively, at Mos1 allele *ttTi5605*.

Behavioral assays

Locomotion assays were performed as described in Koelle and Horvitz (1996). Briefly, staged L4 animals were placed

on NGM plates containing a thin lawn of *OP50* bacteria and allowed to develop for 24 hr at 20° and then assayed. The number of body bends was counted over a 3-min period for 30 animals per strain. Reversal assays were performed on animals staged as L4 larvae 24 hr before the assay. They were then transferred to NGM plates containing a thin lawn of *OP50* bacteria and left undisturbed for 3 min before assay. The number of spontaneous reversals made by each of 30 animals was counted for a 3-min period to determine reversals per minute. A reversal was scored when an animal changed its direction of movement from forward to reverse and the animal reversed by at least one body bend (a body bend was counted when a point in the body immediately posterior to the pharynx passed through a minimum or maximum amplitude).

Egg-laying assays were performed as described in Koelle and Horvitz (1996). Briefly, animals were staged as L4 hermaphrodites and assayed after developing an additional 30 hr at 20°. Individual adult animals were incubated in 1% sodium hypochlorite solution to dissolve the bodies and the number of eggs that remained was counted for a total of 30 animals per strain.

For nose touch response, staged L4 animals were placed on NGM plates containing a lawn of *OP50* bacteria and

Figure 2 Expression of a degradation-tagged transgene rescues endogenous gene function in NMD-defective but not NMD-competent animals. (A–C) Quantification of (A) locomotion rate, (B) spontaneous reversal frequency, and (C) egg-lying behavior. Genotypes and transgenes expressed for each strain are indicated at the bottom of each panel. The behavior of each of 30 animals was recorded. The mean and standard deviation are shown.

allowed to develop for 24 hr at 20° before assay. Adult animals were moved to an unseeded NGM plate and forward-moving animals were tapped lightly on the head with a platinum wire. *unc-4(gk668)* mutants do not reverse when tapped but instead contract their midbody, causing the animal to coil, defined as the head curling back and touching a region of the body posterior to the head. One hundred animals were assayed per strain. Animals expressing the extrachromosomal *unc-4* sense/antisense transgenes were selected by the presence of a coinjected *rab-3p::mCherry* marker. Nose touch response in these transgenic strains was determined by assaying ≥ 50 animals from each of five individually isolated transgenic lines for a total of ≥ 250 animals. XA4254 embryos were heat-shocked for 4 hr to induce expression of the *unc-4* hairpin RNA as described in Johnson *et al.* (2005) and assayed for their ability to reverse as adults. For feeding dsRNA, KP3948 *eri-1(mg366)*; *lin-15b(n744)* animals were fed bacteria expressing either *unc-4* dsRNA or the control pL4440 strain as described in Wani *et al.* (2012) and F₁ generation animals were assayed for reversal behavior as young adults.

Quantitative RT-PCR

To prepare total RNA, mixed-staged animals were washed from three 6 cm plates and cleaned using a 30% sucrose solution to generate an ~ 100 μ l worm pellet. Worms were flash frozen in liquid nitrogen, ground in a mortar, and dissolved in 1 ml Trizol reagent. RNA was then purified by standard methods (typical yield, 100–250 μ g). Five micrograms total RNA was treated with DNaseI (Promega, Madison, WI) and purified using an RNA concentrator kit (Zymo Research). Five hundred nanograms of purified RNA was used to

generate cDNA, using the Superscript III first-strand synthesis kit (Invitrogen, Carlsbad, CA) with random hexamers. Quantitative real-time PCR assays were performed using Brilliant III SYBR green and ROX master mix (Agilent Technologies) in a Stratagene (La Jolla, CA) Mx3000P thermocycler. Primers *goa-1*-qPCR-F, *goa-1*-qPCR-R, and *cdc-42*-qPCR-F, *cdc-42*-qPCR-R were used to measure *goa-1* and *cdc-42* expression, respectively.

Worm lysates and Western blotting

Worms were grown in 125 ml liquid cultures as described in Hofler and Koelle (2011). Animals were cleaned using a 30% sucrose solution and washed twice with 0.1 M NaCl. The worm pellet was flash frozen in liquid nitrogen and stored at -80° . Worm protein extracts were made by boiling 100 μ l worm pellets for 5 min in 1 ml sample buffer (100 mM Tris, pH 6.8, 2% SDS, 5% β -mercaptoethanol, 15% glycerol) followed by centrifugation to remove worm debris. Fifty micrograms of total protein of each sample was analyzed by 10% SDS-PAGE. Proteins were transferred to nitrocellulose membrane and the membrane was incubated with rabbit anti-UNC-64 antibody (Ab940, dilution 1:20,000) and rabbit anti-GOA-1 (dilution 1:300). The secondary antibody used was goat anti-rabbit HRP-conjugated antibody (dilution 1:3000; Bio-Rad, Hercules, CA) and proteins were detected using the ImmunoStar HRP Chemiluminescence Kit (Bio-Rad).

Statistical analysis

We used a two-tailed Mann–Whitney test to compare behaviors in Figures 2 and 3 and a two-tailed, unpaired Student's *t*-test to compare RNA abundances between wild-type and transgenic animals shown in Figure 4.

Figure 3 NMD-dependent knockdown of tagged transgenes is robust and can be restricted to individual cell types. (A–C) Quantification of (A) locomotion rate, (B) spontaneous reversal frequency, and (C) egg-laying behavior. Genotypes and transgenes expressed for each strain are indicated at the bottom of each panel. Rescue of each behavior by expression of the *goa-1p::GOA-1* tagged transgene is dependent on NMD. NMD-dependent knockdown of the tagged transgene is cell autonomous as expression of SMG-5 in HSNs by the *tph-1p::SMG-5* transgene caused egg-laying defects similar to those observed in *goa-1* null animals but did not cause defects in either locomotion rate or reversal frequency. The behavior of each of 30 animals was recorded. The mean and standard deviation are shown.

Results

Protein expression from a tagged transgene is greatly reduced by NMD

In *C. elegans*, at least seven proteins (SMG-1 through SMG-7) are required for the degradation of aberrant mRNA transcripts that contain a premature termination codon (PTC) (Pulak and Anderson 1993; Cali *et al.* 1999). While the precise signals that trigger NMD in *C. elegans* are not known, the efficiency with which a PTC-containing transcript is degraded is correlated with the distance of the PTC from the normal stop codon, with distances >500 nt causing robust degradation (Pulak and Anderson 1993; Longman *et al.* 2007). To determine whether NMD could be used to regulate gene expression, we generated a tripartite mCherry transgene that contained the pan-neuronal *rab-3* promoter placed upstream of mCherry coding sequence followed by a degradation tag to act as an NMD trigger (Figure 1A). The degradation tag we chose consisted of several exons and introns from the *let-858* gene with its normal stop codon and 3'-UTR. This segment of *let-858* has been shown previously to be capable of targeting chimeric mRNA transcripts for NMD-dependent degradation in *C. elegans* (Link *et al.* 2003). In transcripts from the tripartite transgene, the stop codon of mCherry should be recognized as a PTC by the NMD machinery as it occurs >500 nt upstream of the stop codon of *let-858*. We injected equal concentrations of this tagged *rab-3p::mCherry::let-858* transgene together with an untagged *rab-3p::GFP* transgene into wild-type and *smg-5*

(NMD-defective) mutant animals. Coinjected transgenes concatenate in the *C. elegans* gonad to form large multicopy arrays and thus coinjection of the mCherry and GFP transgenes ensured their coexpression in transgenic progeny (Mello *et al.* 1991). We found that while GFP was easily detected in most or all neurons of both wild-type and *smg-5* transgenic animals, mCherry protein was detectable only in *smg-5* mutant animals, suggesting that the tagged mCherry mRNA was stable in NMD-defective animals and was degraded by NMD in wild-type animals (Figure 1B).

Since the expression of the mCherry protein was dependent upon NMD activity, we next determined whether spatial control of NMD activity could be used to reduce mCherry protein expression cell specifically. To cell-specifically control NMD activity we generated an *unc-17p::SMG-5* transgene to rescue NMD activity in cholinergic cells (Alfonso *et al.* 1993). To ensure that NMD activity was restored in all cholinergic cells, we integrated the *unc-17p::SMG-5* transgene at single copy at the *Mos1* allele *cxTi10882* by *Mos1*-mediated single-copy insertion (MosSCI) (Frøker-Jensen *et al.* 2008). *cxTi10882* is located within an intergenic region of chromosome IV and does not disrupt the function of nearby genes or exert adverse position effects on gene expression (Frøker-Jensen *et al.* 2008). To test for cell-specific knockdown of mCherry, we coinjected *smg-5* and *smg-5; unc-17p::SMG-5* animals with the pan-neuronally expressed tagged *rab-3p::mCherry::let-858* and an untagged *unc-17p::GFP* transgene and examined expression of mCherry and GFP in ventral cord motor

Figure 4 Levels of *goa-1* expression from tagged transgenes are similar to those of wild-type animals and are dramatically reduced by NMD. (A) Quantification of *goa-1* mRNA levels by qRT-PCR in wild-type and transgenic animals. All values represent the average from three independent RNA preparations for each strain and were normalized to *cdc-42* expression. Error bars represent SEM. Comparisons shown are to *goa-1* mRNA levels in the wild type. (B) Analysis of GOA-1 protein expression by Western blot. Worm lysates were probed with GOA-1 (top) and control UNC-64 (bottom) antibodies. Genotypes and transgenes expressed for each strain are indicated at the bottom of each panel.

neurons (Figure 1C). The cell bodies of only two neuron types are found in the ventral cord in *C. elegans*: GABAergic and cholinergic neurons. In our transgenic animals GFP expression marks the cholinergic neurons. We found that all *smg-5* mutants that lacked the *unc-17p::SMG-5* NMD-rescuing transgene expressed mCherry in all neurons, including both GABAergic and cholinergic neurons of the ventral cord (Figure 1C, left). In contrast, mCherry was easily detected only in GABAergic neurons of *smg-5; unc-17p::SMG-5* animals (Figure 1C, right). The absence of mCherry in GFP-expressing cholinergic cells of *unc-17p::SMG-5* rescued animals indicated that the mCherry mRNA was efficiently degraded by NMD in these cells. Thus NMD can be used in *C. elegans* to restrict knockdown of transgene expression to specific cell types.

A single-copy integrated tagged transgene can rescue function of an endogenous gene

To functionally replace an endogenous gene, a transgene must be expressed at the same levels and in the same cells as the gene it replaces. To ensure that our transgenes met these criteria, we used full-length endogenous promoters to drive expression of all transgenes used in this study and we integrated the transgenes in single copy at well-characterized Mos1 alleles that did not cause adverse effects on gene expression (Frøkær-Jensen *et al.* 2008).

We chose to determine whether a tagged transgene could replace the function of *goa-1*, which encodes the most abundant G-protein subunit (GOA-1) in *C. elegans* and shares 80% amino acid identity to the human G-protein subunit $G\alpha_o$. We chose the *goa-1* gene for four reasons. First, the *goa-1(n363)* null deletion removes the entire coding sequence of *goa-1*. Because no *goa-1* mRNA or protein is made in a *goa-1(n363)* deletion mutant, it would serve as an ideal genetic background for expression of a degradation-tagged *goa-1* transgene. Any *goa-1* mRNA or protein made in a *goa-1(n363)* transgenic strain must be from the tagged transgene. Second, GOA-1 is highly expressed in most or all neurons of wild-type animals and is also expressed in many muscle cells (Mendel *et al.* 1995; Ségalat *et al.* 1995). Since our gene replacement strategy would use the endogenous *goa-1* promoter to drive expression of the tagged transgene, we would expect similarly high levels of GOA-1 expression in *goa-1(n363)* null, NMD-defective transgenic animals. Additionally, robust expression from the *goa-1* promoter would allow us to measure large changes in transgene mRNA and protein abundance that might occur as the result of NMD-dependent knockdown. Third, GOA-1 functions in different cell types to modulate different worm behaviors, including locomotion and egg laying, and these behaviors are highly reproducible and easily measured (Mendel *et al.* 1995; Ségalat *et al.* 1995). Thus we could use behavioral assays as a qualitative measure of the ability of the tagged transgene to rescue the function of the *goa-1(n363)* null mutation. *goa-1(n363)* null mutants move faster than wild-type animals (Mendel *et al.* 1995; Ségalat *et al.* 1995) (Figure 2A). While the specific cells that control locomotion rate have not been formally identified, GOA-1 is expressed in the motor neurons that innervate body wall muscles and in the body muscles themselves, making these cell types the likely sites of GOA-1 action (Mendel *et al.* 1995; Ségalat *et al.* 1995). *goa-1(n363)* mutants reverse more frequently than wild-type animals (Figure 2B). In *C. elegans* reversal behavior is controlled by the command interneurons and thus GOA-1 likely functions in the command interneurons to control reversal frequency (Chalfie *et al.* 1985). *goa-1(n363)* null mutants lay eggs more frequently than wild-type animals (Mendel *et al.* 1995; Ségalat *et al.* 1995). This leads to a reduction of the steady-state number of eggs retained in the uterus of *goa-1(n363)* null mutants compared to wild-type animals (Figure 2C). Because GOA-1 is

Table 1 Comparison of knock down efficiency between gene replacement/NMD and dsRNA interference strategies.

Genotype and Knock Down Strategy	Nose Touch Response (% of Prodded Animals)			
	Normal sinusoidal backing behavior	Defective Backing Behavior		Total percent animals with backing defect
		Midbody contraction resulting in partial coil	Midbody contraction resulting in full coil	
No knock down (n = 100 for all strains)				
1. wildtype	100	0	0	0
2. <i>unc-4</i>	0	8	92	100
3. <i>smg-5; unc-4</i>	0	11	89	100
NMD replacement and knock down (n = 100 for all strains)				
4. <i>smg-5; unc-4 ndSi19 [unc-4p::UNC-4::let-858 3'UTR]</i>	100	0	0	0
5. <i>smg-5; unc-4 ndSi19 [unc-4p::UNC-4::let-858 3'UTR]; ndSi20 [unc-4p::SMG-5]</i>	0	72	28	100
Hairpin dsRNAi (n = 100 for all strains)				
6. <i>qals4254</i>	94	3	3	6
dsRNAi Feeding (n = 100 for all strains)				
7. <i>eri-1; lin-15</i> ; pL4440 control	100	0	0	0
8. <i>eri-1; lin-15; unc-4(RNAi)</i>	38	52	10	62
Injection of sense/antisense dsRNA (>50 animals per line)				
9. line 1	41	12	47	59
10. line 2	39	4	57	61
11. line 3	18	39	43	82
12. line 4	46	8	46	54
13. line 5	12	49	39	88
Average response of five transgenic lines	31.2	22.4	46.4	68.8

expressed in both the egg-laying muscle cells and the two HSNs that innervate them, the abnormal egg-laying behavior of *goa-1(n363)* mutants could be due to loss of **GOA-1** function in either of these cell types. In experiments designed to determine the site of **GOA-1** function in egg-laying behavior, Moresco and Koelle (2004) expressed a transgene encoding a constitutively active **GOA-1** protein separately in the egg-laying muscles and in HSN neurons. They found that expression of the mutant **GOA-1** protein in the HSN neurons, but not in the egg-laying muscles, inhibited egg-laying behavior. While the mutant **GOA-1** protein used in these studies may have inhibited egg-laying behavior by activating nonphysiological signaling pathways, these experiments do suggest that **GOA-1** function is necessary in the HSN neurons and not in the egg-laying muscles to control egg-laying behavior. The cell-specific effects of **GOA-1** activity on *C. elegans* behaviors would provide a means for us to evaluate cell-specific gene knockdown, using assays for locomotion rate, reversal frequency, and egg-laying behaviors. The fourth reason for choosing *goa-1* was that global overexpression of wild-type **GOA-1** causes locomotion and egg-laying defects that are opposite to those observed in the *goa-1(n363)* null mutant (Mendel *et al.* 1995; Ségalat *et al.* 1995). Thus animals that overexpress wild-type **GOA-1** move slower and lay eggs less frequently than wild-type animals (Mendel *et al.* 1995; Ségalat *et al.* 1995). The sensitivity of these behaviors to *goa-1* dosage would allow a qualitative assessment of tagged transgene expression levels.

We generated a *goa-1* transgene (*goa-1p::GOA-1::let-858*) that included the full rescuing *goa-1* promoter (Ségalat *et al.* 1995) and coding sequence fused to the *let-858* degradation tag and integrated this tagged transgene at single copy at the Mos1 *ttTi5605* allele. This integrated strain was then used to generate *goa-1*; *goa-1p::GOA-1::let-858* and *smg-5 goa-1*; *goa-1p::GOA-1::let-858* transgenic animals, whose locomotion and egg-laying behaviors were compared to those of control strains (Figure 2). Importantly, the loss of NMD had no effect on the three behaviors tested as wild-type and *smg-5* mutants moved at similar rates, showed similar reversal frequencies, and retained a similar number of eggs *in utero* (Figure 2, A–C). We found that in NMD-competent animals, the *goa-1p::GOA-1::let-858* transgene was unable to replace **GOA-1** function to control any of the three behaviors (compare wild-type animals to *goa-1* mutants that express the *goa-1p::GOA-1::let-858* transgene). In contrast, we found that in NMD-defective animals the *goa-1p::GOA-1::let-858* transgene was able to fully replace **GOA-1** function for all three behaviors (compare wild-type animals or *smg-5* mutants to *smg-5 goa-1* double mutants that express the *goa-1p::GOA-1::let-858* transgene). Because **GOA-1** functions in different cell types to control locomotion rate, reversal frequency, and egg-laying behaviors, we conclude that mRNA from the tagged transgene was degraded in most or all neurons of NMD-competent animals and, in contrast, was stably expressed in most or all neurons of NMD-defective animals. Notably, no

intermediate phenotypes were observed in any of the transgenic animals tested, indicating that transgene expression was tightly controlled by NMD in all animals and in all cell types and that knockdown was robust. Finally, we note that the *goa-1p::GOA-1::let-858* transgene did not cause overexpression phenotypes in *smg-5 goa-1* double-mutant animals, suggesting that it was expressed at levels that were similar to those of the endogenous *goa-1* gene in wild-type animals.

Knockdown of mRNA expressed from a single-copy tagged transgene

The results described so far showed that expression of a single-copy, integrated tagged transgene could replace the function of an endogenous gene to rescue null mutant phenotypes to wild-type behavior. We also demonstrated that the rescuing activity of the tagged transgene is NMD dependent. We next wanted to determine whether we could knock down the expression of the tagged transgene both globally and in specific cell types by selective activation of NMD. To do this we generated two NMD-rescuing transgenes. The first was an untagged *goa-1p::SMG-5* transgene that we integrated at the Mos1 *cxTi10882* allele of *smg-5 goa-1* double mutants that expressed the integrated *goa-1p::GOA-1::let-858* tagged transgene. Expression of the untagged *goa-1p::SMG-5* transgene should restore NMD activity in all cells that express the *goa-1p::GOA-1::let-858* tagged transgene, leading to global degradation of its transcript. Indeed we found that, unlike control strains that lacked the *goa-1p::SMG-5* transgene, these animals were defective in locomotion, reversal frequency, and egg-laying behavior like *goa-1(n363)* null mutants (Figure 3, A–C).

To demonstrate that cell-specific activation of NMD could restrict transgene knockdown to individual cell types, we generated a second untagged transgene (*tph-1p::SMG-5*) and integrated it at the Mos1 *cxTi10882* allele of *smg-5 goa-1* double mutants that also expressed the integrated *goa-1p::GOA-1::let-858* tagged transgene. In these transgenic animals NMD activity would be restored in only four neurons: the two pharyngeal neurosecretory motor (NSM) neurons and the two HSNs where the *tph-1* promoter is active (Moresco and Koelle 2004). The NSM neurons are located in the head of the animal and do not affect egg-laying behavior while the HSNs are located near the vulva, express *GOA-1*, and directly innervate the egg-laying muscles. As expected, we found that expression of *SMG-5* in the HSNs had no measurable effect on locomotion rate or reversal behavior (Figure 3, A and B). In contrast, however, expression of *SMG-5* in the HSNs caused defects in egg-laying behavior that were as dramatic as those observed in *goa-1(n363)* null mutants (Figure 3C). That these transgenic animals showed wild-type locomotion rate and reversal frequency, but showed null-like egg-laying behavior, indicates that the *goa-1p::GOA-1::let-858* tagged transgene was expressed at wild-type levels in all cells of these transgenic animals except in the two HSNs where its transcript was specifically degraded by NMD. The ability of our trans-

gene replacement strategy to remove protein expression/function from individual cell types without affecting function in other cells allows us to unambiguously assign cell-specific function(s) to proteins. For example, these results demonstrate that *GOA-1* normally functions in the HSNs to inhibit egg-laying behavior.

Measurement of tagged transgene expression and knockdown efficiency

From our behavioral analyses we conclude that the *goa-1* tagged transgene was expressed at levels near those of the endogenous gene and that tagged transcripts could be reduced or eliminated by NMD to generate null-like behavioral defects. However, we wanted to directly measure the expression level of the tagged transgene and the efficiency of knockdown by NMD. Therefore, we measured the abundance of tagged *goa-1* transgene mRNA in *smg-5 goa-1* double-mutant animals by quantitative RT-PCR and compared it to *goa-1* mRNA expression in wild-type and *goa-1(n363)* null animals. We found that *goa-1p::GOA-1::let-858* transgene mRNA in *smg-5 goa-1* animals was expressed at levels that were indistinguishable from those of endogenous *goa-1* mRNA found in wild-type animals (Figure 4A, compare bars 1 and 3). We also found that *GOA-1* protein levels were similar in these two strains, confirming that the tagged transgene was translated into wild-type *GOA-1* protein (Figure 4B, compare protein levels in lanes 1 and 3). In stark contrast, when we restored NMD activity in *smg-5 goa-1* animals to all cells that also expressed the tagged transgene, using an integrated untagged *goa-1p::SMG-5* transgene, we found that the abundance of tagged transgene mRNA was reduced by >87% compared to *goa-1p::GOA-1::let-858* mRNA levels in *smg-5 goa-1* double mutants that did not express the untagged *goa-1p::SMG-5* rescue transgene (Figure 4A, compare bars 3 and 4). We observed a similarly dramatic reduction in *GOA-1* protein levels in this strain (Figure 4B, compare protein levels in lanes 3 and 4). Indeed, expression of the untagged *goa-1p::SMG-5* transgene reduced *GOA-1* mRNA and protein expression to near null levels, which correlated well with null-like behaviors observed in this strain as shown in Figure 3 (Figure 4A, compare bars 2 and 4 and protein levels in Figure 4B, lanes 2 and 4). Thus NMD is able to degrade tagged transgene mRNAs and maintain them at near null levels, preventing expression of the encoded protein.

Comparison of NMD-dependent knockdown efficacy to that of other knockdown strategies

Our results demonstrate that NMD can be used to restrict knockdown of gene expression to specific cell types in *C. elegans*. This is a major advantage of the NMD-dependent knockdown strategy over dsRNAi knockdown approaches. We also showed that the strategy could dramatically reduce the expression of extrachromosomal transgenes and we used quantitative PCR to show it could reduce the expression of at least one endogenous gene by >87% to generate

null-like phenotypes. To ask whether this strategy could be effectively adapted to other genes we wanted to test the efficacy of NMD-dependent knockdown on a gene that has been difficult to knock down by conventional dsRNA knockdown approaches. We chose *unc-4* for this test gene as it has proved to be resistant to several dsRNA-based silencing strategies (Simmer *et al.* 2003; Johnson *et al.* 2005). *unc-4* encodes a homeodomain protein expressed in VA neurons and is required for proper synaptic input choice (Miller *et al.* 1992; White *et al.* 1992). VA neurons normally receive synaptic input from interneurons that control backward locomotion, but in *unc-4* mutants these VA neurons instead receive synaptic input from command interneurons that control forward locomotion (Miller *et al.* 1992; White *et al.* 1992). As a result, *unc-4* mutants are defective in backing behavior. Whereas wild-type animals back freely in a sinusoidal motion when prodded on the head, *unc-4* null mutants do not back but instead contract their midbody tightly, causing a dorsal flexure that often becomes so extreme that the head and tail of the animal touch, placing the body in a coiled position. (Table 1, lines 1–3, and [File S1](#) and [File S2](#)). We found that when prodded, 92% of *unc-4* null mutants contracted their midbody fully to form a tight coil while the other 8% contracted their midbody but did not fully coil. *smg-5* had no significant effect on backing behavior (Table 1, compare lines 2 and 3). While we do not understand why some *unc-4* mutants contract their bodies to form a full coil and others do not fully coil when tapped on the head, we suspect that this might be at least partially dependent on the location or strength of the prodding stimulus.

We generated a tagged *unc-4p::UNC-4::let-858* transgene and integrated it at single copy at the Mos1 *ttTi5605* allele of *smg-5*; *unc-4* double mutants. This transgene fully rescued *unc-4* backing behavior (Table 1, compare lines 3 and 4, [File S3](#)). We then integrated an untagged *unc-4p::SMG-5* transgene into this strain to restore NMD activity in all cells that expressed the tagged transgene. We found that unlike single-transgenic animals, 100% of the double-transgenic animals were defective in backing like *unc-4* null animals (Table 1, compare lines 4 and 5, [File S4](#)). We note that a higher proportion of double-transgenic animals did not fully coil after midbody contraction when compared to *unc-4* null mutants (Table 1, compare lines 3 and 5). However, in each case, 100% of the animals were defective in backing. While coiling may be dependent upon the location or strength of the prodding stimulus, the higher levels of noncoiling we observed in the double-transgenic animals compared to *unc-4* null animals might also be due to residual *unc-4* expression in the transgenic animals.

To compare the knockdown of *unc-4* function that we observed using tagged transgenes to dsRNA knockdown strategies, we delivered *unc-4* dsRNA to animals by three different methods. First, we induced the expression of an integrated *unc-4* hairpin dsRNA by heat shock. Since this hairpin construct is integrated into the genome, it should

not be susceptible to mosaic effects that might reduce the efficacy of extrachromosomal transgenes. However, as measured by nose touch response, and as described previously (Johnson *et al.* 2005), we found that hairpin dsRNA was not an effective means to knock down *unc-4* expression with only 6% of heat-shocked animals showing a backing defect (Table 1, line 6). Second, we fed animals bacteria expressing *unc-4* dsRNA. Previous attempts to knock down *unc-4* expression by feeding dsRNA-expressing bacteria failed to cause the expected uncoordinated phenotype (Simmer *et al.* 2003; Johnson *et al.* 2005). Because *unc-4* is expressed in neurons, which are refractory to the effects of RNAi, we used an RNAi-sensitive strain for these experiments. Control animals fed bacteria containing empty expression plasmid (pL4440) did not show an uncoordinated phenotype. In contrast, we found that 62% of animals fed *unc-4* dsRNA-expressing bacteria showed defects in backing (Table 1, compare lines 7 and 8). Like the knockdown observed using NMD-dependent transgenes, a high proportion of these backing-defective animals showed the contraction but non-coiling phenotype. Finally, we coinjected *unc-4* sense and antisense transgenes driven by the *unc-4* promoter together with a *rab-3p::mCherry* transgene. While extrachromosomal transgenes are often lost during cell division, we attempted to reduce these mosaic effects by selecting transgenic animals that showed strong mCherry fluorescence in the nervous system. We recovered five independent transgenic lines and found that 69% (range 54–88%) of all mCherry-positive animals from these lines showed backing defects that were similar to those of *unc-4* null animals (Table 1, lines 9–13) (>50 animals tested per line). Again, the backing defects were distributed between partial and full coiling behavior. However, 31% (range 12–41%) of sense/antisense-expressing transgenic animals showed wild-type behavior, suggesting that the sense/antisense transgenes were either lost or expressed at low levels in at least some neurons in these animals. From these results we conclude that our gene replacement and NMD-mediated knockdown strategy is capable of causing robust knockdown effects that are sufficient to generate null-like behavioral effects in 100% of treated animals, even for genes that are normally recalcitrant to conventional knockdown strategies.

Discussion

We developed a novel strategy that has the potential to knock down the expression of any gene in any cell type in *C. elegans*. The strategy combines three emerging experimental tools in *C. elegans*:

1. NMD-dependent degradation of engineered PTC-containing mRNA transcripts (Link *et al.* 2003): Tagging transgenes with an artificial 3'-UTR creates PTC-containing transcripts that are recognized and degraded by NMD.
2. Mos1-mediated single-copy insertion of transgenes (Frøker-Jensen *et al.* 2008): Integration of single-copy tagged transgenes at well-characterized Mos-1 alleles ensures that the

transgenes are expressed at levels comparable to those of the endogenous gene they replace.

3. Cell-specific promoters: Cell-type-specific restoration of NMD activity provides spatial control of transgene expression.

Together, these tools allowed us to functionally replace an endogenous gene with its tagged counterpart and to knock down the expression of the tagged transgene in either all cells or in specific cell types to generate global or cell-specific null behavioral phenotypes.

Comparison of NMD strategy to knockdown by dsRNA interference

Our knockdown strategy provides several advantages over the three commonly used dsRNA interference strategies, including (1) feeding animals bacteria that express dsRNA (Timmons and Fire 1998), (2) direct gonad injection of dsRNA or dsRNA-encoding constructs to generate extrachromosomal arrays (Fire *et al.* 1998; Esposito *et al.* 2007), and (3) *in vivo* expression of dsRNA from hairpin constructs (Tavernarakis *et al.* 2000; Johnson *et al.* 2005). First, and most importantly, because NMD is a cell-autonomous process (Weischenfeldt *et al.* 2008), knockdown by our method is absolutely cell specific. In contrast, dsRNA or intermediates produced during processing of dsRNA are exported from cells in *C. elegans* to produce systemic knockdown effects (Jose *et al.* 2009, 2011). Second, because our method uses integrated transgenes, we were able to generate large populations of isogenic animals where every animal in the population showed consistent effects of transgene expression and knockdown. This is most clearly seen in Figures 2 and 3, where we plot the behavior of 30 individual animals of each genotype. In contrast, dsRNAs encoded on extrachromosomal arrays or delivered by feeding worms bacteria are not stably inherited. Consistent with the results of others, we found that all three dsRNA approaches caused variable knockdown effects with phenotypes observed in only a portion of all treated animals (Table 1) (Montgomery *et al.* 1998; Tavernarakis *et al.* 2000; Simmer *et al.* 2003). We suspect that the knockdown effects of dsRNA transgenes could be improved by integrating the transgene to eliminate mosaic knockdown effects; however, the integrated hairpin RNA transgene was the least effective knockdown approach tested in this study. Third, knockdown effects using our strategy are robust. As measured by mRNA abundance, our approach reduced gene expression by 87%. For the two *C. elegans* genes tested (*goa-1* and *unc-4*), this was sufficient to cause null-like behavioral defects in all animals of the population. In comparison, all three dsRNA interference strategies caused less dramatic behavioral defects with many animals in the population showing no knockdown effects (Table 1). This could either be due to reduced efficacy of the RNAi mechanism itself, which we did not assess, or, at least in the case of dsRNA injections, be due to mosaic loss of extrachromosomal transgenes. Fourth, since the cell-specific NMD rescue transgenes are stably integrated in our approach, a collection of different cell-

specific NMD-rescue strains could be easily generated and archived for sharing among the research community. With these strains in hand, the cell-specific function of any gene could be investigated by generating just one MosSCI-integrated transgene containing the gene of interest and the degradation tag and crossing this strain to the shared cell-specific NMD rescue strains. Finally, we note that our strategy could be expanded to also include temporal control of cell-specific knockdown. Since seven *smg* genes are required for NMD activity, temporal control could be achieved by placing the expression of a second *smg* gene under inducible control such as that provided by a heat-shock promoter or the Q repressible binary expression system (Jones *et al.* 1989; Wei *et al.* 2012). Alternatively, temporal control could be achieved by regulating the activity of a second *smg* gene, using a temperature-sensitive *smg* allele such as *smg-1(cc546ts)* and switching animals from the restrictive to the permissive temperature (Link *et al.* 2003).

Uses and limitations of the NMD-dependent knockdown strategy

Because the lack of NMD does not affect viability in *C. elegans*, we believe this gene replacement strategy will be useful to study the function of most or all proteins in this organism. However, there may be some genes whose function cannot be examined using this strategy. First, it is possible that NMD might not function equally well in all cell types at all stages of development. While not analyzed in *C. elegans*, it has recently been shown that a developmentally regulated microRNA (miR-128) found in vertebrates can repress NMD activity in mammalian neural cells, suggesting that NMD activity might be under developmental control at least in some cell types (Bruno *et al.* 2012). Second, some genes normally express PTC-containing isoforms and these isoforms may accumulate in NMD-defective cells, possibly causing expression a truncated protein. In rare cases, such a truncated protein might interfere with the ability to analyze protein function in neighboring, NMD-competent, cells. It has been estimated that ~13–25% of *C. elegans* genes are alternatively spliced (Zahler 2005; Ramani *et al.* 2011) and that ~34% of these genes express alternatively spliced mRNAs that naturally contain PTCs (Barberan-Soler *et al.* 2009). Thus it is possible that as many as 4–8% of *C. elegans* genes could not be analyzed by our methods for this reason. We also note that ~10% of *C. elegans* genes are regulated post-transcriptionally by sequences in their 3'-UTR (Lall *et al.* 2006). Our strategy should still work for these genes if the tagged transgene is modified to include the 3'-UTR of the gene under study in place of the 3'-UTR of *let-858*. Such a tagged transgene would contain the regulatory sequences found in the 3'-UTR of the gene under study but would still trigger NMD of transgene mRNA.

The ability to knock down gene expression in single cell types should provide new insights into protein function. This strategy will be particularly useful for proteins that are expressed in multiple tissue types, for proteins that have

different functions in different cell types, and for proteins for which null mutations are lethal.

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Literature Cited

- Alfonso, A., K. Grundahl, J. S. Duerr, H. P. Han, and J. B. Rand, 1993 The *Caenorhabditis elegans* unc-17 gene: a putative vesicular acetylcholine transporter. *Science* 261: 617–619.
- Barberan-Soler, S., N. J. Lambert, and A. M. Zahler, 2009 Global analysis of alternative splicing uncovers developmental regulation of nonsense-mediated decay in *C. elegans*. *RNA* 15: 1652–1660.
- Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71–94.
- Bruno, I. G., R. Karam, L. Huang, A. Bhardwaj, C. H. Lou *et al.*, 2012 Identification of a microRNA that activates gene expression by repressing nonsense-mediated RNA decay. *Mol. Cell* 42: 500–510.
- Cali, B. M., S. L. Kuchma, J. Latham, and P. Anderson, 1999 smg-7 is required for mRNA surveillance in *Caenorhabditis elegans*. *Genetics* 151: 605–616.
- Chalfie, M., J. E. Sulston, J. G. White, E. Southgate, J. N. Thomson *et al.*, 1985 The neural circuit for touch sensitivity in *Caenorhabditis elegans*. *J. Neurosci.* 4: 956–964.
- Esposito, G., E. Di Schiavi, C. Bergamasco, and P. E. Bazzicalupo, 2007 Efficient and cell specific knock-down of gene function in targeted *C. elegans* neurons. *Gene* 395: 170–176.
- Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. A. Driver *et al.*, 1998 Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391: 806–811.
- Frøker-Jensen, C., M. W. Davis, C. E. Hopkins, B. J. Newman, J. M. Thummel *et al.*, 2008 Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat. Genet.* 40: 1375–1383.
- Hofler, C., and M. R. Koelle, 2011 AGS-3 alters *Caenorhabditis elegans* behavior after food deprivation via RIC-8 activation of the neural G protein G α . *J. Neurosci.* 31: 11553–11562.
- Johnson, N. M., C. A. Behm, and S. C. Trowell, 2005 Heritable and inducible gene knockdown in *C. elegans* using Wormgate and the ORFeome. *Gene* 359: 26–34.
- Jones, D., D. K. Dixon, R. W. Graham, and E. P. Candido, 1989 Differential regulation of closely related members of the hsp16 gene family in *Caenorhabditis elegans*. *DNA* 8: 481–490.
- Jose, A. M., J. J. Smith, and C. P. Hunter, 2009 Export of RNA silencing from *C. elegans* tissues does not require the RNA channel SID-1. *Proc. Natl. Acad. Sci. USA* 106: 2283–2288.
- Jose, A. M., G. A. Garcia, and C. P. Hunter, 2011 Two classes of silencing RNAs move between *Caenorhabditis elegans* tissues. *Nat. Struct. Mol. Biol.* 18: 1184–1188.
- Koelle, M. R., and H. R. Horvitz, 1996 EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell* 84: 115–125.
- Lall, S., D. Grün, A. Krek, K. Chen, Y. L. Wang *et al.*, 2006 A genome-wide map of conserved microRNA targets in *C. elegans*. *Curr. Biol.* 16: 460–471.
- Link, C. D., A. Taft, V. Kapulkin, K. Duhke, S. Kim *et al.*, 2003 Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiol. Aging* 24: 397–413.
- Longman, D., R. H. Plasterk, I. L. Johnstone, and J. F. Cáceres, 2007 Mechanistic insights and identification of two novel factors in the *C. elegans* NMD pathway. *Genes Dev.* 9: 1075–1085.
- Mello, C. C., J. M. Kramer, D. Stinchcomb, and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* 12: 3959–3970.
- Mendel, J. E., H. C. Korswagen, K. S. Liu, Y. M. Hajdu-Cronin, M. I. Simon *et al.*, 1995 Participation of the protein G α in multiple aspects of behavior in *C. elegans*. *Science* 267: 1652–1655.
- Miller, D. M., M. M. Shen, C. E. Shamu, T. R. Bürglin, G. Ruvkin *et al.*, 1992 *C. elegans* unc-4 gene encodes a homeodomain protein that determines the pattern of synaptic input to specific motor neurons. *Nature* 355: 841–845.
- Montgomery, M. K., S. Xu, and A. Fire, 1998 RNA as a target of double-stranded RNA-mediated genetic interference in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* 95: 15502–15507.
- Moresco, J. J., and M. R. Koelle, 2004 Activation of EGL-47, a G α -coupled receptor, inhibits function of hermaphrodite-specific motor neurons to regulate *Caenorhabditis elegans* egg-laying behavior. *J. Neurosci.* 24: 8522–8530.
- Pulak, R., and P. Anderson, 1993 mRNA surveillance by the *Caenorhabditis elegans* smg genes. *Genes Dev.* 10: 1885–1897.
- Ramani, A. K., J. A. Calarco, Q. Pan, S. Mavandadi, Y. Wang *et al.*, 2011 Genome-wide analysis of alternative splicing in *Caenorhabditis elegans*. *Genome Res.* 21: 342–348.
- Ségalat, L., D. A. Elkes, and J. M. Kaplan, 1995 Modulation of serotonin-controlled behaviors by G α in *Caenorhabditis elegans*. *Science* 267: 1648–1651.
- Simmer, F., C. Moorman, A. M. van der Linden, E. Kuijk, P. V. van den Berghe *et al.*, 2003 Genome-wide RNAi of *C. elegans* using the hypersensitive *rrf-3* strain reveals novel gene functions. *PLoS Biol.* 1: 77–84.
- Stinchcomb, D. T., J. E. Shaw, S. H. Carr, and D. Hirsh, 1985 Extrachromosomal DNA transformation of *Caenorhabditis elegans*. *Mol. Cell. Biol.* 12: 3484–3496.
- Tavernarakis, N., S. L. Wang, M. Dorovkov, A. Ryazanov, and M. Driscoll, 2000 Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nat. Genet.* 2: 180–183.
- Timmons, L., and A. Fire, 1998 Specific interference by ingested dsRNA. *Nature* 395: 854.
- Wani, K. A., M. Catanese, R. Normantowicz, M. Herd, K. N. Maher *et al.*, 2012 D1 dopamine receptor signaling is modulated by the R7 RGS protein EAT-16 and the R7 binding protein RSBP-1 in *Caenorhabditis elegans* motor neurons. *PLoS ONE* 7: e37831.
- Wei, X., C. J. Potter, L. Luo, and K. Shen, 2012 Controlling gene expression with the Q repressible binary expression system in *Caenorhabditis elegans*. *Nat. Methods* 9: 391–395.
- Weischenfeldt, J., I. Damgaard, D. Bryder, K. Theilgaard-Mönch, L. A. Thoren *et al.*, 2008 NMD is essential for hematopoietic stem and progenitor cells and for eliminating by-products of programmed DNA rearrangements. *Genes Dev.* 22: 1381–1396.
- White, J. G., E. Southgate, and J. N. Thomson, 1992 Mutations in the *Caenorhabditis elegans* unc-4 gene alter the synaptic input to ventral cord motor neurons. *Nature* 355: 838–841.
- Zahler, A. M., 2005 Alternative splicing in *C. elegans* (September 26, 2005), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.31.1, <http://www.wormbook.org>.

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GENETICS

Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.149724/-/DC1>

A Novel Strategy for Cell-Autonomous Gene Knockdown in *Caenorhabditis elegans* Defines a Cell-Specific Function for the G-Protein Subunit GOA-1

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Files S1-S4
Supporting Movies

Available for download at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.149724/-/DC1>.

File S1: Response of wild-type animals to nose touch. Wild-type animals reverse normally when tapped on the nose.

File S2: Response of *unc-4* mutants to nose touch. *unc-4* mutants are unable to reverse.

File S3: Response of *smg-5; unc-4 ndSi19* [*unc-4p::UNC-4::let-858*] animals to nose touch. The tagged *unc-4* transgene restores normal backing behavior in NMD-defective animals.

File S4: Response of *smg-5; unc-4 ndSi19* [*unc-4p::UNC-4::let-858*]; *ndSi20* [*unc-4p::SMG-5*] animals to nose touch. Activation of NMD in all *unc-4* expressing cells causes degradation of the *unc-4* transgene and restores *unc-4* backing defects.