Controlling gene expression with the Q repressible binary expression system in *Caenorhabditis elegans*

Xing Wei[1], **Christopher J. Potter**[1,2], **Liqun Luo**[1], and **Kang Shen**[1]

[1]Howard Hughes Medical Institute, Department of Biology, Stanford University, Stanford, CA, USA

[2]The Johns Hopkins University School of Medicine, The Solomon H. Synder Department of Neuroscience, Baltimore, MD, USA

Abstract

We establish the first transcription-based binary gene expression system in *C. elegans* using the recently developed Q system. This system, derived from genes in *Neurospora crassa*, uses the transcriptional activator QF to induce the expression of target genes. Activation can be efficiently suppressed by the transcriptional repressor QS, and suppression in turn can be relieved by the non-toxic small molecule, quinic acid (QA). We used QF/QS and QA to achieve temporal and spatial control of transgene expression in various tissues in *C. elegans*. We further developed a Split Q system, in which we separated QF into two parts encoding its DNA-binding and transcription-activation domains. Each domain shows negligible transcriptional activity when expressed alone, but co-expression reconstitutes QF activity, providing additional combinatorial power to control gene expression.

INTRODUCTION

The capability to regulate the expression of engineered transgenes has revolutionized the study of biology in multicellular genetic model organisms. One popular and powerful strategy is using a binary expression system such as the tetracycline-regulated tTA/TRE system in mammals[1] and the GAL4/UAS/GAL80 system in *Drosophila*[2, 3]. Despite its success in *Drosophila*, so far there is no transcription-based binary expression system reported in the nematode *C. elegans*.

An alternative method in *C. elegans* is to use DNA recombinase systems such as FLP/FRT[4] or Cre/LoxP[5] to remove regulatory elements in transgene constructs to control gene expression. However, the action of the recombinases is not reversible or repressible, and the expression pattern integrates the developmental history of the promoter that drives

recombinase expression. Another strategy is to combine heat-shock control and a tissue-specific promoter; in an *hsf-1* mutant background that is defective for the heat-shock response, the combination of cell autonomous rescue of *hsf-1* and a heat-shock promoter-driven transgene can achieve spatial and temporal control of gene expression[6]. While this method has many advantages, it requires the transgenes to be expressed in the *hsf-1* mutant background. In addition, because worms cannot tolerate extended heat-shock, this method can only achieve gene expression with transient onset and offset.

Recently, a repressible binary expression system, the Q system, was established in *Drosophila* and mammalian cells based on regulatory genes from the *Neurospora crassa qa* gene cluster[7]. The transcriptional activator QF binds to a 16 bp sequence (named *QUAS* hereafter) and activates expression of target genes under the control of *QUAS* sites. Expression can be efficiently suppressed by its transcriptional repressor QS and the transcriptional suppression in turn can be relieved by feeding animals quinic acid, a non-toxic small molecule (Fig. 1a). Here we adapted the Q system to the nematode *C. elegans*, and demonstrated its utility for controlling transgene expression with temporal and spatial precision. We also developed the "Split Q system" by separating the transcriptional activator QF into two parts, to achieve intersectional control of gene expression.

RESULTS

Characterization of the Q system in *C. elegans*

We used the Q system to label A-type motor neurons in C. elegans. A-type motor neurons (DAs and VAs) are cholinergic, excitatory and responsible for backward movement[8]. The cell bodies of both DAs and VAs are located in the ventral nerve cord, and DA neurons send their axonal commissures to the dorsal nerve cord, while VA neurons extend their axons exclusively in the ventral nerve cord (Fig. 1b). We used the *unc-4* promoter (expressed in both DA and VA neurons)[9] to drive the expression of QF and monitored expression of QF with a *SL2∷mCherry* cassette. The trans-spliced leader sequence SL2 permits the bicistronic expression of QF and mCherry under the control of the *unc-4* promoter in a manner similar to the Internal Ribosomal Entry Site (IRES) in the vertebrate system[10].

The transcriptional machinery of *C. elegans* requires a minimal promoter to initiate transcription. After trying several such sequences (data not shown), we found that the *pes-10* minimal promoter[11] supports strong expression with the Q system in *C. elegans*. We created a transgenic strain (*wyEx3661*) that co-express the *QF* construct together with the *QUAS∷pes-10∷GFP* (shortened as *QUAS∷GFP* hereafter) construct. As expected, GFP was robustly expressed in DA and VA neurons in co-expressing lines (Fig. 1c-1f) but not in lines expressing the constructs individually (Supplementary Figure 1a-1d). The fraction of animals showing expression with the QF system is comparable to that seen with direct promoter fusion (Fig. 1o). When the two single-expressing transgenic strains were crossed together, we also obtained robust GFP expression in both DAs and VAs in animals bearing both transgenes (Supplementary Figure 1e-1f), excluding the possibility that GFP fluorescence is due to recombination between the *unc-4* promoter and *QUAS∷GFP* during the generation of the extrachromosomal array[12].

To test whether the action of QF is repressible, we generated a transgenic line (*wyEx4048*) coexpressing QS, QF and *QUAS::GFP* in A-type neurons. In these animals, the expression of GFP in DA and VA neurons was efficiently suppressed (Fig. 1g-1j and Fig. 1o). Finally, to test if quinic acid can de-repress the QS inhibition of QF, we applied the drug to the same *QS* transgenic strain. Transgenic larvae fed on quinic acid showed detectable GFP signal after 6 hr, which increased over time (Supplementary Figure 2) and was saturated after 24 hr of drug application (Fig. 1k-1o). The effective concentration of quinic acid (7.5 mg/ml, similar to the doses used in *Drosophila* and *Neurospora crassa*) does not cause noticeable abnormalities of animals (Online Methods), and is lower than the concentration of quinic acid naturally present in cranberry juice (>1%)[13]. Interestingly, the de-repression effect of quinic acid in *C. eleagns* is more rapid than in flies[7] and may be useful for temporally regulating QF-driven transgene expression.

Application of Q system in various tissues

We expressed QF in body wall muscles of *QUAS::GFP* transgenic worms using the *myo-3* promoter, and it robustly activated the expression of GFP in this tissue (Supplementary Figure 3a). GFP expression was effectively suppressed by co-expressing QS in body wall muscles and the suppression was relieved when animals were fed with quinic acid (Supplementary Figure 3b,c).

We investigated whether we could use this system to express non-fluorescent transgenes. The *dpy-20* gene encodes a nematode specific zinc finger protein, which is expressed and required in hypodermal cells for normal body morphology[14]. *dpy-20 (e1282ts)* mutant animals raised at restrictive temperature (25°C) exhibit a Dpy (shortened body length) phenotype (body length: wild-type 1052±40 μm, *dpy-20* 922±14 μm; *n* =40)[14]. *dpy-20 (e1282ts)* mutants carrying only the *QUAS::dpy-20* transgene still showed the Dpy phenotype (Supplementary Figure 4a; body length: 920±17 μm; *n* =40), but co-expression of QF in hypodermal cells using *dpy-7* promoter rescued the phenotype (Supplementary Figure 4b; body length: 1085±94 μm). Furthermore, rescue was suppressed when QS was expressed in hypodermal cells (Supplementary Figure 4c and Fig. 1p; body length: 917±25 μm) and the suppression was relieved when the animals were allowed to develop in the presence of quinic acid (data not shown).

Refining expression patterns with a NOT gate

In addition to permitting precise spatial and temporal control of transgene expression in various tissues, the Q system can also be utilized to refine spatial control. In *C. elegans*, while some promoter elements are highly specific for a single cell or few cells, most promoters are expressed in many cells[15]. It is desirable to develop specific labeling schemes for the reproducible marking of small subsets of cells. The repressible Q system can meet this need by combining QF and QS into a NOT gate. For instance, for A-type motor neurons, DA and VA neurons are both labeled by the *unc-4* promoter while a truncated *unc-4* promoter (*unc-4c*) is expressed only in DA neurons in the ventral nerve cord (M. Vanhoven and K. Shen, unpublished results; Supplementary Figure 5). However, there is no available promoter to only label VA neurons (Fig. 2a). To achieve specific expression in VA neurons, we created transgenic strains that coexpress *unc-4::QF* (VA + DA neurons),

unc-4c∷QS (DA neurons only) and *QUAS∷GFP* simultaneously. In the same line, we also used a *SL2∷mCherry* cassette fused with *QF* and *QS* to label both the DA and VA neurons. In 96% (98/102) animals of these transgenic stains, we found that GFP could only be detected in the VA neurons, evident from the lack of commissures in the GFP channel, while the mCherry signals could be detected in both VA and DA neurons (Fig. 2b-2d). Therefore, expression of the QS in DA neurons limited activity of QF to only VA neurons.

Refining expression patterns with an AND gate

In *Drosophila*, the DNA binding (BD) and transcription activation (AD) domains from GAL4 can be independently expressed using different promoters, and transcriptional activity can be reconstituted within the intersectional subset of two promoters[16]. QF has an analogous organization and contains a DNA binding (BD) domain, a putative dimerization (DM) domain and a transcription activation (AD) domain[17] (Fig. 3a). We tested whether QF can be similarly divided into two modules that can be used for intersectional labeling (Fig. 3b). We fused a heterodimerizing leucine zipper fragment with each domain to enhance the reconstitution efficiency of active QF[18]. We found that the putative dimerization domain (DM) is required for reconstituted activity (Supplementary Table 1). The reconstituted activity of the optimal pair is 42% of intact QF driven by the same promoter, measured as the expression level of *QUAS∷GFP* in these transgenic strains. Transgenic lines only expressing the individual domains of QF displayed minimal transcriptional activity (Fig. 3c).

To test whether the Split Q system can be applied to label the intersectional group of two different promoters, we expressed QF-AD from the *mig-13* promoter and QF-BD-DM from the *unc-4c* promoter (*wyEx4355*). In the tail region, the *mig-13* promoter is expressed in DA9 and VA12 neurons[19] while the *unc-4c* promoter is expressed in DA7, DA8 and DA9 neurons (Fig. 3d). All the neurons were labeled by the *SL2∷mCherry* cassette fused with the two halves of QF. We found that *QUAS∷GFP* was only expressed in the DA9 neuron (61%, 62/103 in this line), which is at the intersection of the expression patterns of *mig-13* and *unc-4c* promoters (Fig. 3e-3g), and no animals (0/103) show GFP labeling in other tail neurons. Transgenic lines only expressing *mig-13∷QF-AD* displayed no GFP signal in DA9 (data not shown). Moreover, the reconstituted activity of QF was suppressed completely in 98% of animals (202/207) by co-expressing *unc-4c∷QS* introduced by sequential injection *(wyEx4355;wyEx4409)*.

The Q system functions effectively with single copy transgenes

Standard transgenic technique in *C. elegans* involves microinjection of exogenous DNA into the gonad, which results in complex extrachromosomal arrays containing high copy number transgenes. While this method is convenient and frequently used, it does not provide stable transgenes or reliable expression levels due to silencing effects[20]. An alternative transgenic method, *Mos1* mediated Single Copy Insertion (*MosSCI*), uses the transposon *Mos1* to introduce a single-copy, stably inherited transgene[21]. We tested whether the Q system was functional when its components were integrated as single copy *MosSCI* transgenes. We created two *MosSCI* transgenes that contain *QUAS∷GFP*(*wySi374*) and *Pmig-13∷QF∷SL2∷mCherry* (*wySi377*), respectively. Neither transgene alone yielded a GFP signal (Fig. 4b-4g and Fig. 4o). However, animals carrying both transgenes

consistently showed robust expression of GFP in VA12 and DA9 neurons (100%, 120/120), reflecting the promoter activity of *Pmig-13* (Fig. 4h-6j and Fig. 4o). In addition, when QS driven by *unc-4c* promoter (expressed in all DA neurons including DA9) was introduced into this strain, the transcriptional activation in DA9 neuron was specifically suppressed by QS, leaving VA12 neuron as the only GFP positive neuron in 97% animals (126/130) (Fig. 4k-4o). These results demonstrate that Q system components are functional when expressed from both array transgenes as well as integrated, single-copy transgenes.

DISCUSSION

Here we show that the Q repressible binary expression system functions efficiently in the nematode *C. elegans*, similar to its application in the mammalian cells and *Drosophila*. It enables greatly improved spatial and temporal control of transgene expression in various tissues. Compared to transgene expression driven directly by promoters, the repressible Q binary system offers several advantages. First, it is difficult to "turn off" or "turn down" gene expression by direct promoter fusion methods. In contrast, QS expression provides an efficient approach to suppress gene expression. With the regulatory role of quinic acid, the Q system can "turn on" gene expression at any desired time point, and combines spatial and temporal control. Second, using combination of promoters, the Q system can refine transgene expression to more specific subsets of cells. Third, although it is routine to generate transgenic C. elegans lines with direct promoter-fusion methods, using new promoters or effectors in each case necessitates that the transgenes must be transformed (and possibly integrated) again. By comparison, a library of single copy insertion strains containing transgenes expressing various QF drivers and/or *QUAS effectors* can be systematically combined by genetic crosses to generate reproducible expression patterns. The Q system could become a significant pillar of the *C. elegans* toolbox as more and more strains containing QF and *QUAS-effector* transgenes become available.

Finally, our newly introduced Split Q system affords a higher degree of control and can achieve expression even at single cell resolution. Due to the complexity and heterogeneity of the nervous system, one bottleneck in understanding neural circuits and behavior is genetic access to specific neurons or groups of neurons, such that one can reproducibly label them with anatomical or developmental markers, express genetically-encoded indicators of activity, or selectively silence or activate specific neurons[22]. The Split Q system should greatly increase the precision of genetic access to specific neuronal populations. This system could also be applied to other model organisms to achieve more precise control of transgene expression.

ONLINE METHODS

Expression constructs

Expression clones were made in the pSM vector, a derivative of pPD49.26 (A. Fire) with extra cloning sites (S. McCarroll and C. I. Bargmann, personal communication). *gpd-2 SL2: mCherry* was PCR amplified from pBALU12[23] in which the kanamycin cassette and N-terminus nuclear localization sequence (NLS) were removed. The QF was amplified from pCaSpeR4, the QS was amplified from pACPL-QS and 5xQUAS was amplified from

pQUAS-CD8-GFP[7]. pes-10 minimal promoter was amplified from pPD97.78 and *myo-3* promoter was from pPD122.66 (A. Fire). The *unc-4* promoter (4kb)[9], the *unc-4c* promoter (bashed *unc-4* promoter, ~1 kb, M. Vanhoven and K. Shen, unpublished results), *mig-13* promoter (3.4kb)[19] and *dpy-7* promoter (218bp)[24] were amplified from N2 genomic DNA. The *dpy-20* genomic DNA (3kb, including the whole *dpy-20* gene from initial ATG to stop codon) was amplified from fosmid WRM0616CH07. QF-AD was the C-terminus part (650-816aa) of QF[17], adding GSGSGSGSGSGT linker sequence at 5' and SV40 NLS at 3'. The sequence of Zip(-) antiparallel leucin zipper (AQLEKKLQALEKKLAQLEWKNQALEKKLAQ) was amplified from CZ-CED-3 (a gift from M. Chalfie)[25], and was fused with QF-AD fragment by overlapping PCR, adding NheI sites at 5' and 3'ends. QF-BD-DM was the N-terminus part (1-650aa) of QF, adding SV40 NLS at 5' and GSGSGSGSGSGSA linker sequence at 3'. The sequence of Zip(+) antiparallel leucin zipper (ALKKELQANKKELAQLKWELQALKKELAQ) was amplified from CED-3-NZ (a gift from M. Chalfie)[25], and was fused with QF-BD-DM fragment by overlapping PCR, adding NheI sites at 5' and 3' ends. Details about primer sequences and constructs are available in Supplementary Note 1. All plasmids are available upon request.

Strains and transformation

Wild-type animals were *C. elegans* Bristol strain N2. All mutants used in the paper were provided by Caenorhabditis Genetics Center. Strains were maintained using standard methods[26], and animals were grown at 20°C except lines containing Split Q constructs and *dpy-20 (e1282ts)*, which were grown at 25°C. Normal transgenic lines were made using standard protocols[12]. Transgenic arrays were generated in N2 background except *dpy-20 (e1282ts)* used for Dpy rescue experiments. For each transformation, at least two transgenic lines were obtained showing similar results. *MosSCI* transformation was performed based on the protocol described in http://sites.google.com/site/jorgensenmossci/[21]. The Mos1 insertion strains EG4322 or EG5003 were used for injection. These single copy insertions were verified by following the protocol of long-fragment PCR provided by Michael Nonet. (http://thalamus.wustl.edu/nonetlab/ResourcesF/PCR%20of%20MosSCI%20transgenes.pdf) Strains information in detail is available in Supplementary Note 2.

Quinic acid de-suppression treatment

The fresh quinic acid stock solution (300 mg/ml) was prepared from D- (–)-quinic acid (Sigma-Aldrich, 98%, Catalog # 138622) in sterile Milli-Q water. The stock can be kept in 4°C for at least one month. Neutralized with 5M NaOH to pH 6-7, the quinic acid stock solution can be added into NGM agar medium (7.5 mg/ml) before pouring into petri plates or onto NGM plates seeded with OP50 directly (per 60 mm × 15 mm petri dish, 40 μl M9 buffer with ~300 μl quinic acid stock solution, adding ~60-70 μl 5M NaOH to pH 6.0-7.5. pH is tested by EMD pH-indicator strips, pH 5.0-10.0, Cat.9588). Animals were synchronized by hypochlorite bleaching and were cultured on NGM plates with OP50. They were transferred onto quinic acid plates with OP50 6 hr, 12 hr, 24 hr or 30 hr before taking images. Animals kept on seeded NGM plates containing quinic acid for five generations exhibited no noticeable abnormalities of morphology, development, brood size, egg-laying behavior, and touch response.

Confocal imaging and image quantification

Images of fluorescent proteins were captured in live animals using Plan-Apochromat 40X/ 1.3NA objective except Fig. 3e-3g (using Plan-Apochromat 63X/1.4NA objective), and Supplementary Figure 3-4 (Plan-Apochromat 10X/10.47NA objective) on a Zeiss LSM710 confocal microscope (Carl Zeiss). Animals were immobilized on 2% agarose pad using 10 mM levamisole (Sigma-Aldrich) and oriented anterior to the left and dorsal up. For imaging and measuring body length 0.3 M 2,3-butanedione monoxime (Sigma-Aldrich) was used. Supplementary Figure 3 are overlays of single fluorescent single plane image and DIC image, and Supplementary Figure 4 are DIC images. All images were taken using Zen2009 (Carl Zeiss) and confocal images were rendered in three dimensions by maximum intensity projection method. Images were adjusted as necessary in Photoshop (Adobe) using cropping and thresholding tools, and assembled into figures using Illustrator (Adobe). For quantification of fluorescence intensity in Fig. 3c, fluorescence images of ventral nerve cord neurons labeled by *mig-13* promoter (DD and DA neurons) were captured using the same parameters across groups with a 40X objective. The total fluorescence intensity of GFP in each cell body was determined using Image J (NIH) and Excel (Microsoft) by integrating pixel intensity across the cell body region. 40 neurons from 20 early L4 larvae were used to calculate the average fluorescence intensity for each group. The activity was normalized to the percentage of the activity measured in strains containing intact QF (*wyEx4212*).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Gossen M, Bujard H. Tight Control of Gene-Expression in Mammalian-Cells by Tetracycline-Responsive Promoters. P Natl Acad Sci USA. 1992; 89:5547–5551.

2. Brand AH, Perrimon N. Targeted Gene-Expression as a Means of Altering Cell Fates and Generating Dominant Phenotypes. Development. 1993; 118:401–415. [PubMed: 8223268]

3. Lee T, Luo LQ. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron. 1999; 22:451–461. [PubMed: 10197526]

4. Macosko EZ, et al. A hub-and-spoke circuit drives pheromone attraction and social behaviour in C. elegans. Nature. 2009; 458:1171–1175. [PubMed: 19349961]

5. Davis MW, Morton JJ, Carroll D, Jorgensen EM. Gene activation using FLP recombinase in C. elegans. Plos Genet. 2008; 4:e1000028. [PubMed: 18369447]

6. Bacaj T, Shaham S. Temporal control of cell-specific transgene expression in Caenorhabditis elegans. Genetics. 2007; 176:2651–2655. [PubMed: 17603102]

7. Potter CJ, Tasic B, Russler EV, Liang L, Luo LQ. The Q System: A Repressible Binary System for Transgene Expression, Lineage Tracing, and Mosaic Analysis. Cell. 2010; 141:536–548. [PubMed: 20434990]

8. White JG, Southgate E, Thomson JN, Brenner S. The Structure of the Nervous-System of the Nematode Caenorhabditis-Elegans. Philos T Roy Soc B. 1986; 314:1–340.

9. Miller DM, Niemeyer CJ. Expression of the Unc-4 Homeoprotein in Caenorhabditis-Elegans Motor-Neurons Specifies Presynaptic Input. Development. 1995; 121:2877–2886. [PubMed: 7555714]

10. Spieth J, Brooke G, Kuersten S, Lea K, Blumenthal T. Operons in C-Elegans - Polycistronic Messenger-Rna Precursors Are Processed by Transsplicing of Sl2 to Downstream Coding Regions. Cell. 1993; 73:521–532. [PubMed: 8098272]

11. Seydoux G, Fire A. Soma-Germline Asymmetry in the Distributions of Embryonic Rnas in Caenorhabditis-Elegans. Development. 1994; 120:2823–2834. [PubMed: 7607073]

12. Mello C, Fire A. DNA transformation. Methods Cell Biol. 1995; 48:451–482. [PubMed: 8531738]

13. Nollet, LML. Food analysis by HPLC. Vol. 100. CRC; 2000.

14. Clark DV, Suleman DS, Beckenbach KA, Gilchrist EJ, Baillie DL. Molecular cloning and characterization of the dpy-20 gene of Caenorhabditis elegans. Mol Gen Genet. 1995; 247:367–378. [PubMed: 7770042]

15. Zhang S, Ma C, Chalfie M. Combinatorial marking of cells and organelles with reconstituted fluorescent proteins. Cell. 2004; 119:137–144. [PubMed: 15454087]

16. Luan H, Peabody NC, Vinson CR, White BH. Refined spatial manipulation of neuronal function by combinatorial restriction of transgene expression. Neuron. 2006; 52:425–436. [PubMed: 17088209]

17. Giles NH, Geever RF, Asch DK, Avalos J, Case ME. The Wilhelmine E. Key 1989 invitational lecture. Organization and regulation of the qa (quinic acid) genes in Neurospora crassa and other fungi. J Hered. 1991; 82:1–7. [PubMed: 1825499]

18. Ghosh I, Hamilton AD, Regan L. Antiparallel leucine zipper-directed protein reassembly: Application to the green fluorescent protein. J Am Chem Soc. 2000; 122:5658–5659.

19. Sym M, Robinson N, Kenyon C. MIG-13 positions migrating cells along the anteroposterior body axis of C. elegans. Cell. 1999; 98:25–36. [PubMed: 10412978]

20. Hsieh J, Fire A. Recognition and silencing of repeated DNA. Annual Review of Genetics. 2000; 34:187–204.

21. Frokjaer-Jensen C, et al. Single-copy insertion of transgenes in Caenorhabditis elegans. Nat Genet. 2008; 40:1375–1383. [PubMed: 18953339]

22. Luo L, Callaway EM, Svoboda K. Genetic dissection of neural circuits. Neuron. 2008; 57:634–660. [PubMed: 18341986]

23. Tursun B, Cochella L, Carrera I, Hobert O. A toolkit and robust pipeline for the generation of fosmid-based reporter genes in C. elegans. PLoS One. 2009; 4:e4625. [PubMed: 19259264]

24. Gilleard JS, Barry JD, Johnstone IL. cis regulatory requirements for hypodermal cell-specific expression of the Caenorhabditis elegans cuticle collagen gene dpy-7. Mol Cell Biol. 1997; 17:2301–11. [PubMed: 9121480]

25. Chelur DS, Chalfie M. Targeted cell killing by reconstituted caspases. P Natl Acad Sci USA. 2007; 104:2283–2288.

26. Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77:71–94. [PubMed: 4366476]
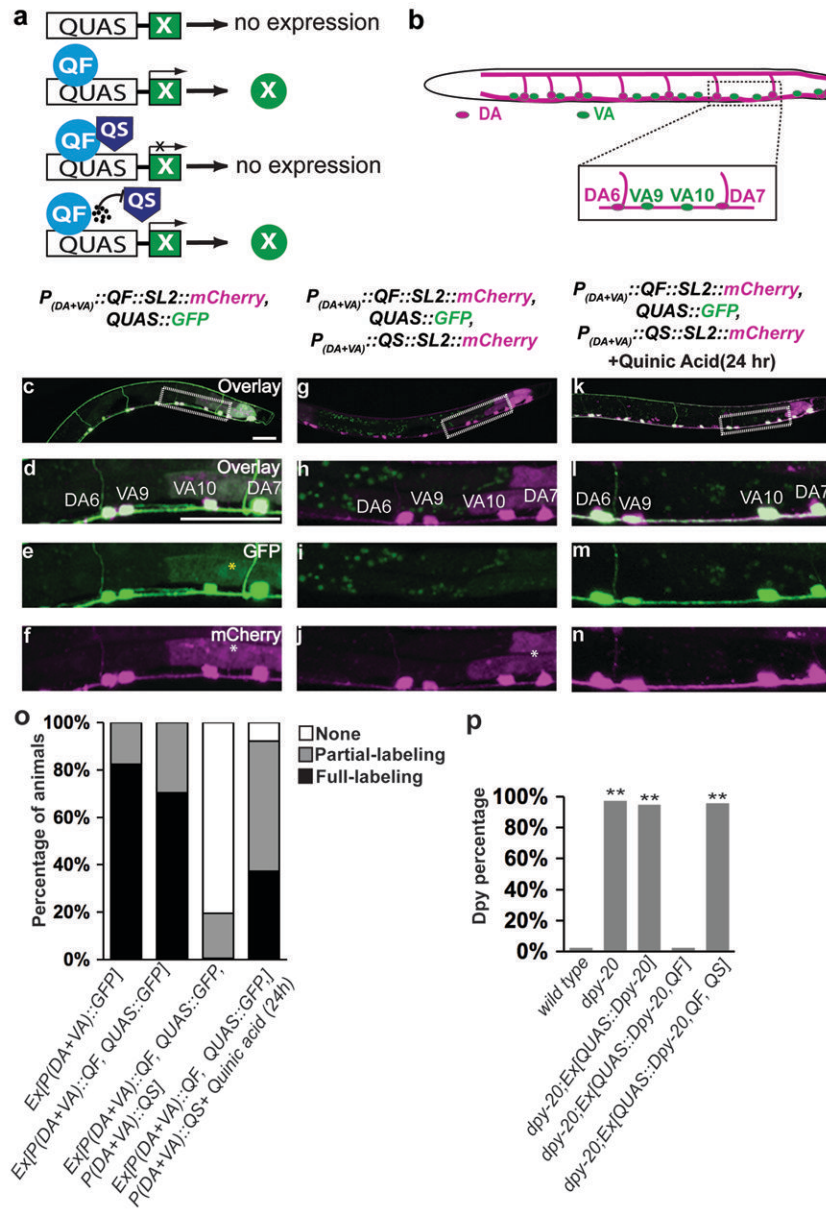
Fig. 1. The repressible Q binary system functions effectively in *C. elegans*

(**a**) Schematic of the Q system. X indicates transgene. Black circles at the bottom indicate quinic acid.

(**b**) Schematic diagram of VA and DA motor neurons in the ventral nerve cord (VNC). The boxed region is magnified to show VA9 and VA10 neurons in green and DA6 and DA7 in magenta. (**c-n**) The micrographs show transgenic worms (L3 larvae) expressing the indicated transgenes, with or without treatment with quinic acid for 24h. An overview of the VNC (**c, g, k**) and magnification of the boxed region (**d-n**) are shown. GFP expression is shown green, mCherry expression is shown in magenta. P, promoter; SL2, trans-spliced leader sequence. White asterisks denote ectopic gut fluorescence caused by *SL2::mCherry* cassette (X. Wei and K. Shen, unpublished results). Yellow asterisks denote occasional ectopic gut fluorescence due to *QUAS::GFP*. Scale bars, 20 μm. (**o**) Quantification of

labeling efficiency in (**c-n**). Late L3 or early L4 stage larvae were scored (*n*>200 for each strain). Animals are divided into 3 categories: None (no A-type neurons labeled), Full-labeling (all A-type neurons labeled) and Partial-labeling (between None and Full-labeling). (**p**) Quantification of Dpy rescue efficiency with Q system in Supplementary Figure 4. *n* =40 for each group; **P 0.0001 (versus wild type animals), χ^2 test. Animals with a body length longer than 972 μm are scored as wild type. Animals with a body length shorter than 972 μm are scored as Dpy mutants.
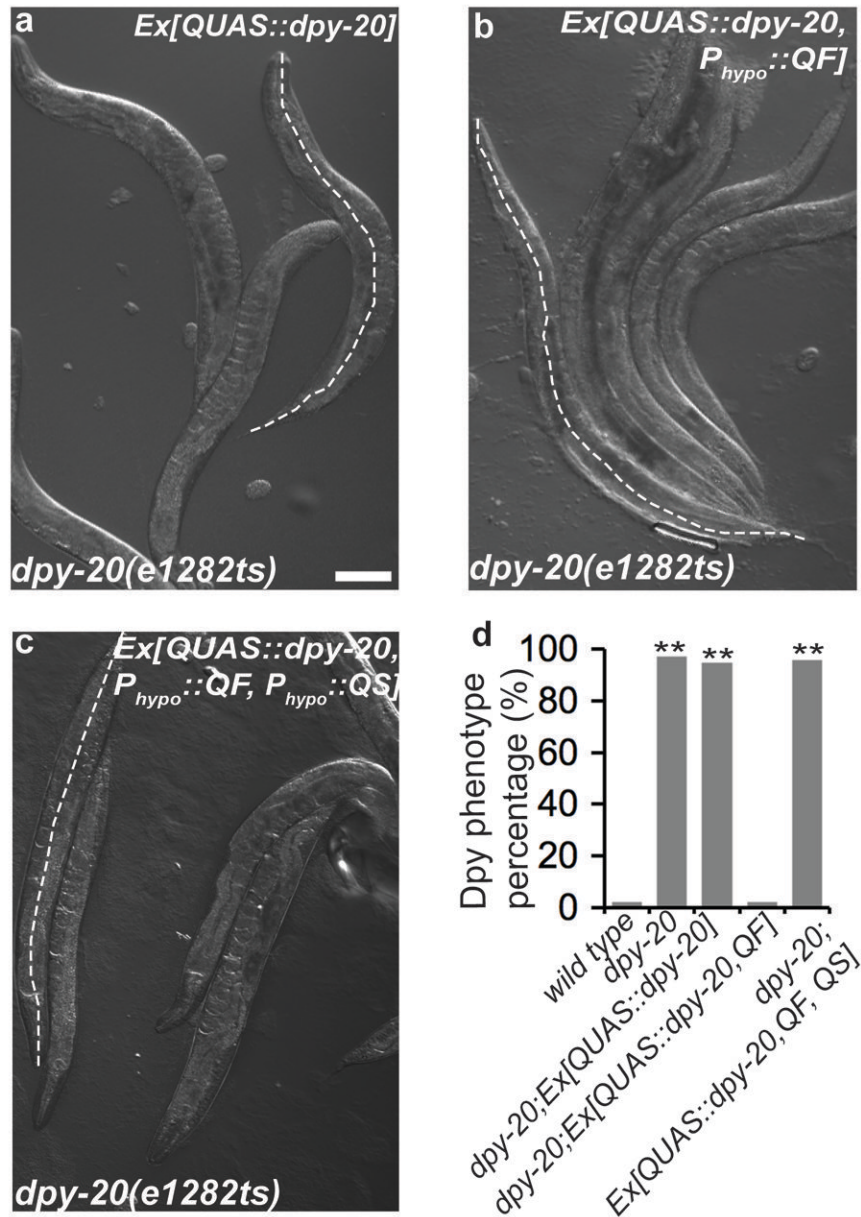
Fig. 2. Refining expression patterns in VA motor neurons with a NOT gate
(**a**) Schematic diagram of VA and DA motor neurons in the posterior region of the VNC.
DA neurons extend axonal commissures (magenta arrowheads) to the dorsal nerve cord. The
unc-4 promoter is expressed in VA and DA neurons while the *unc-4c* promoter is only
expressed in DA neurons. (**b-d**) The micrographs show the same region as schematically
depicted in (**a**) in an early L4 larva expressing the indicated transgenes. Magenta shows
mCherry expression and green shows GFP expression. White arrowheads indicate the
commissures of DA neurons. The asterisk denotes ectopic gut fluorescence caused by
SL2∷mCherry cassette. Scale bar, 20 μm.
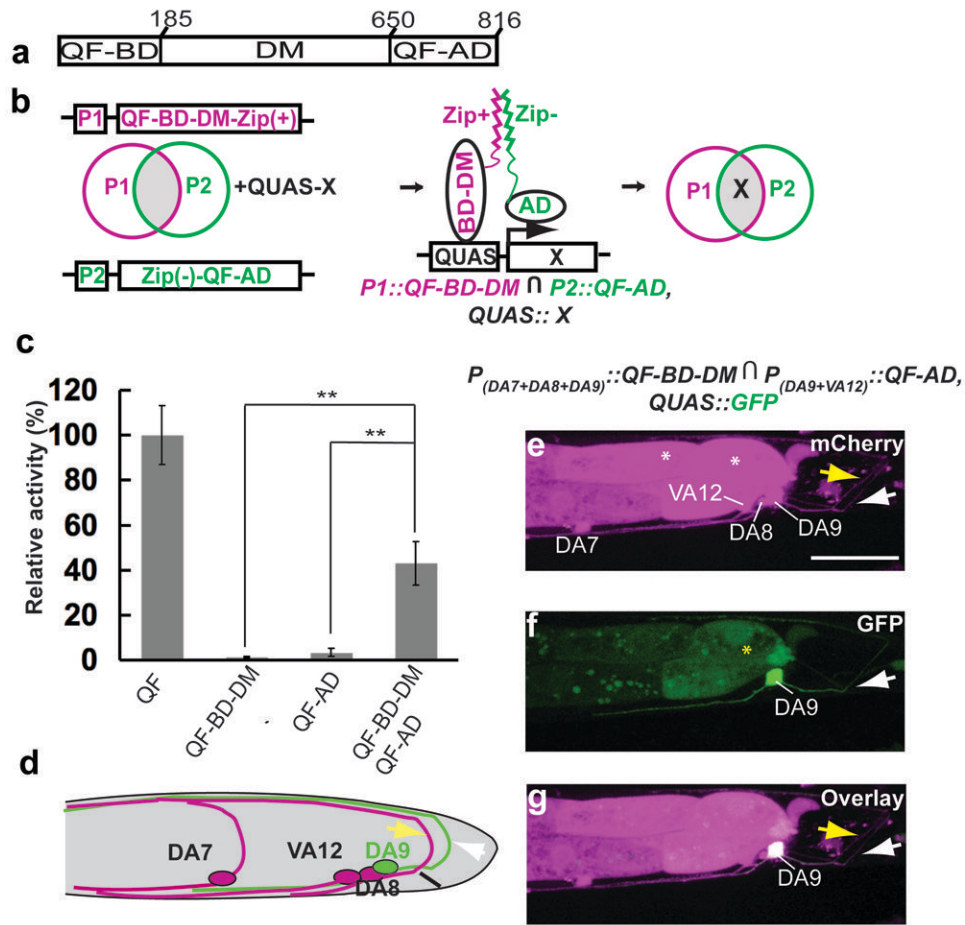
Fig. 3. The Split Q system
(**a**) Schematic showing the DNA binding domain (QF-BD), the putative dimerization domain (DM) and the transcription activation domain (QF-AD) of QF. The amino acid positions for each domain are indicated. (**b**) Schematic depiction of the Split Q system. The QF-BD-DM and QF-AD are driven using different promoters (P1 and P2). Zip+ and Zip- are a pair of heterodimerization leucine zippers. The transgene X is expressed only at the intersection of P1 and P2 promoters (gray area). ∩ denotes the intersection of P1 and P2. (**c**) Relative transcriptional activities (measured as GFP fluorescence intensity) of the indicated Split Q constructs, normalized to the activity measured in strains containing intact QF. All constructs were driven by the same promoter (*mig-13*). Error bars, S.E.M. **P<0.01, *n*=40, one-tailed t-test. (**d**) Schematic diagram of DA and VA neurons in the tail region (left view). The *mig-13* promoter is expressed in DA9 and VA12 neurons while the *unc-4c* promoter is expressed in DA7, DA8 and DA9 neurons. The yellow and white arrows indicate the commissures of DA8 and DA9 respectively. (**e-g**) The micrographs show an early stage 4 larva expressing the indicated transgenes. Green shows GFP fluorescence, magenta shows mCherry fluorescence. The yellow and white arrows indicate the commissures of DA8 and DA9 respectively. White asterisks denote ectopic gut fluorescence caused by *SL2::mCherry*. The yellow asterisk denotes occasional ectopic gut fluorescence due to *QUAS::GFP*. Scale bar, 20 μm.
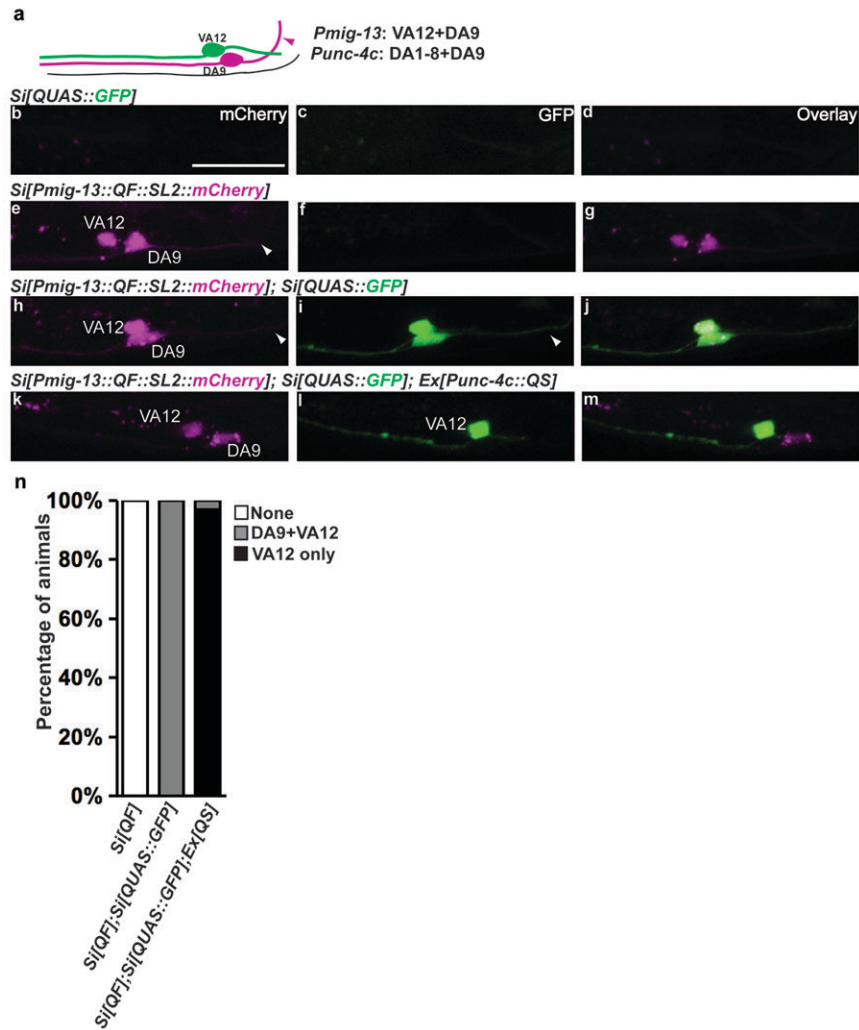
Fig. 4. The Q system functions effectively with single copy transgene
(**a**) Schematic diagram of DA9 and VA12 neurons in the tail region. The *mig-13* promoter expresses in DA9 (in magenta) and VA12 (in green) neurons while the *unc-4c* promoter expresses in the DA9 neuron. The magenta arrowhead indicates the commissure of DA9. (**b-m**) The images show the same region as schematically depicted in (**a**) in transgenic worms (L4 larvae) containing the indicated transgenes. GFP expression is shown green and mCherry expression is shown in magenta. Si, single insertion. The white arrowheads indicate the commissures of DA9. Scale bar, 20 μm. (**n**) Quantification of labeling efficiency in (**b-m**). Late L3 or early L4 stage larvae were scored (*n*>100 for each strain). Animals are divided into 3 categories: None (neither DA9 nor VA12 neurons are labeled by GFP), DA9+VA12 (both neurons are labeled), VA12 only (only VA12 neuron is labeled).