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

Xing Wei1, Christopher J Potter1,2, Liqun Luo1 & Kang Shen1

We established a transcription-based binary gene expression system in *Caenorhabditis 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 can be relieved by the nontoxic small molecule quinic acid. We used QF, QS and quinic acid to achieve temporal and spatial control of transgene expression in various tissues in *C. elegans*. We also developed a split Q system, in which we separated QF into two parts encoding its DNA-binding and transcription-activation domains. Each domain showed negligible transcriptional activity when expressed alone, but expression of both reconstituted QF activity, providing additional combinatorial power to control gene expression.

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–tetracycline response element system in mammals1 and the GAL4–UAS-GAL80 system in *Drosophila melanogaster*2,3. Despite its success in *D. melanogaster*, to our knowledge 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-FRT4 or Cre-LoxP5 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 expression6. Although 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 *D. melanogaster* and mammalian cells based on regulatory genes from the *Neurospora crassa qa* gene cluster7. The transcriptional activator QF binds to a 16 base pair (bp) sequence (called QUAS) and activates expression of target genes under the control of QUAS sites. Expression can be efficiently suppressed by the transcriptional repressor QS, and the transcriptional suppression can be relieved by feeding worms quinic acid, a nontoxic small molecule (Fig. 1a). Here we adapted the Q system to *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* (Fig. 1). A-type motor neurons (DA and VA neurons) are cholinergic, excitatory and responsible for backward movement8. The cell bodies of both DA and VA neurons are located in the ventral nerve cord, and DA neurons send their axonal commissures to the dorsal nerve cord, whereas 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 monomeric (m)Cherry under the control of the *unc-4* promoter, similarly to the internal ribosomal entry site in the vertebrate system10.

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 *Apes-10* minimal promoter11 supported strong expression with the Q system in *C. elegans*. We created a transgenic strain (wyEx3661) in which the QF construct was expressed together with QUAS::Apes-10::GFP (S65C) (called QUAS::GFP hereafter). As expected, GFP was robustly expressed in DA and VA neurons in worms that expressed both constructs (Fig. 1c–f) but not in lines that expressed the constructs individually (Supplementary Fig. 1a–d). The fraction of worms showing expression with the QF system was comparable to

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1Howard Hughes Medical Institute, Department of Biology, Stanford University, Stanford, California, USA. 2The Johns Hopkins University School of Medicine, The Solomon H. Synder Department of Neuroscience, Baltimore, Maryland, USA. Correspondence should be addressed to K.S. (kangshen@stanford.edu).

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that seen with direct promoter fusion (Fig. 1o). When we crossed the two single construct–expressing transgenic strains, we also obtained robust GFP expression in both DA and VA neurons in worms with both transgenes (Supplementary Fig. 1e,f); this excluded the possibility that GFP fluorescence was due to recombination between the unc-4 promoter and QUAS::GFP during the generation of the extrachromosomal array12. To test whether the action of QF is repressible, we generated a transgenic line (wyEx4048) that expressed QS, QF and QUAS::GFP in A-type neurons. In these worms, GFP expression in DA and VA neurons was efficiently suppressed (Fig. 1g–i,o). Finally, to test whether quinic acid can derepress 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 h, which increased over time (Supplementary Fig. 2) and was saturated after 24 h of drug application (Fig. 1k–o). The effective concentration of quinic acid (7.5 mg ml−13, similar to the doses used in D. melanogaster and Neurospora crassa) did not cause noticeable abnormalities in transgenic worms (Online Methods), and was lower than the concentration of quinic acid naturally present in cranberry juice (>1%)13. The derepression effect of quinic acid in nematodes was more rapid than in flies7 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 Fig. 3a). GFP expression was effectively suppressed by also expressing QS in body wall muscles, and the suppression was relieved when we fed the worms with quinic acid (Supplementary Fig. 3b,c).

We investigated whether we could use this system to express nonfluorescent transgenes. The dpv-20 gene encodes a nematode-specific zinc-finger protein, which is expressed and required in hypodermal cells for normal body morphology14. dpv-20 (e1282ts) mutant worms raised at a restrictive temperature (25 °C) exhibited a Dpy (shortened body length) phenotype (body length: wild-type, 1,052 μm ± 40 μm; dpv-20, 922 μm ± 14 μm; (±s.e.m.) n = 40)14.

dpv-20 (e1282ts) mutants carrying only the QUAS::dpv-20 transgene still showed the Dpy phenotype (Supplementary Fig. 4a; body length: 920 μm ± 17 μm; n = 40), but additional expression of QF in hypodermal cells using dpy-7 promoter rescued the phenotype (Supplementary Fig. 4b; body length: 1,085 μm ± 94 μm). Furthermore, rescue was suppressed when we expressed QS in hypodermal cells (Supplementary Fig. 4c and Fig. 1p; body length: 917 μm ± 25 μm) and the suppression was relieved when we allowed the worms 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 used to refine spatial control. In C. elegans, although some promoter elements are highly specific for a single cell or few cells, most promoters are expressed in many cells15. 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 when using the unc-4 promoter, whereas a truncated unc-4 promoter (unc-4c) drives expression only in DA neurons in the ventral nerve cord (M. Vanhoven and K.S., unpublished results; Supplementary Fig. 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 express simultaneously unc-4::QF (expression in VA and DA neurons), unc-4c::QS (DA neurons only) and QUAS::GFP. In the same line, we also used an SL2::mCherry cassette fused to QF and QS to label both DA and VA neurons. In 96% (98/102) of worms of these transgenic strains, we detected GFP only in VA neurons, evident from the lack of commissures in the GFP channel, whereas we detected mCherry signals in both VA and DA neurons (Fig. 2b–d). Therefore, expression of the QS in DA neurons limited activity of QF to only VA neurons.

Refining expression patterns with an ‘and’ gate

In D. melanogaster, the DNA-binding domain (BD) and transcription-activation domain (AD) domains from GAL4 can be independently expressed using different promoters, and transcriptional activity can be reconstituted in the intersectional subset of two promoters16. QF has an analogous organization and contains a BD, a putative dimerization domain (DM) and an AD17 (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 to each domain to enhance the reconstitution efficiency of active QF18. We found that the putative DM domain was required for reconstituted activity (Supplementary Table 1). The reconstituted activity of the optimal pair was 42% of intact QF driven by the same promoter, measured as the expression of QUAS::GFP in these transgenic strains. Transgenic lines only expressing the individual domains of QF had 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 construct driven by the mig-13 promoter was expressed in DA9 and VA12 neurons19 whereas the construct driven by the unc-4c promoter was expressed in DA7, DA8 and DA9 neurons (Fig. 3d). All the neurons were labeled by the SL2::mCherry cassette fused to sequences encoding 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–g), and no worms (0/103) showed GFP labeling in other tail neurons. Transgenic lines only expressing mig-13::QF-AD displayed no GFP signal in DA9 neurons (data not shown). Moreover, the reconstituted activity of QF was suppressed completely in 98% of worms (202/207) by also expressing unc-4c::QS introduced by sequential injection (wyEx4355;wyEx4409).

Q system functions effectively with single-copy transgenes

Standard transgenic technique in C. elegans involves microinjecting of exogenous DNA into the gonad, which results in complex extrachromosomal arrays containing high-copy-number transgenes. Although this method is convenient and frequently used, it does not provide stable transgenes or reliable expression because of silencing effects20. An alternative transgenic method, Mos1-mediated single-copy insertion (MosSCI), uses the transposon Mos1 to introduce a single-copy, stably inherited transgene21. We tested whether the Q system was functional when its

Figure 3 | The split Q system. (a) Schematic of QF-BD, putative DM and QF-AD with amino acid positions for each domain indicated. (b) Schematic of the split Q system. The QF-BD-DM and QF-AD are driven using promoters P1 and P2. Zip+ and Zip– are leucine zippers that heterodimerize. The transgene X is expressed only at the intersection of P1 and P2 promoters. ∩ 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 of DA and VA neurons in the tail region (left view). The mig-13 promoter is expressed in DA9 and VA12 neurons, and the unc-4c promoter is expressed in DA7, DA8 and DA9 neurons. Yellow and white arrows indicate the commissures of DA8 and DA9, respectively. White asterisks denote ectopic gut fluorescence caused by SL2::mCherry. Yellow asterisk denotes occasional ectopic gut fluorescence resulting from QUAS::GFP. Scale bar, 20 μm.
components were integrated as 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. 4a–g). However, worms 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–j). In addition, when QS driven by the 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-expressing neuron in 97% of worms (126/130) (Fig. 4k–n). These results demonstrate that Q system components are functional when expressed from both array transgenes as well as from integrated, single-copy transgenes.

**DISCUSSION**

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, and combines spatial and temporal control. Second, using a 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 an important pillar of the *C. elegans* toolbox as more and more strains containing QF and QUAS-effector transgenes become available.

Finally, our split Q system affords a high degree of control and can achieve expression even at single-cell resolution. Owing 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. 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 more precisely control transgene expression.

**METHODS**

Methods and any associated references are available in the online version of the paper at [http://www.nature.com/naturemethods/](http://www.nature.com/naturemethods/).

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**AUTHOR CONTRIBUTIONS**

X.W. and K.S. designed the experiments and wrote the paper. X.W. performed all experiments and data analysis. L.L. and C.J.P. provided unpublished information on the Q system and guided experimental design.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Expression constructs. Expression clones were made in the pSM vector, with extra cloning sites (S. McCarroll and C.I. Bargmann; Rockefeller University), gpd-2 SL2::mCherry was PCR-amplified from pBALU12 (ref. 23) in which the kanamycin cassette and N-terminal nuclear localization sequence (NLS) were removed. QF was amplified from pCaspEpr4. QS was amplified from pACPL-QS and 5xQUAS was amplified from pQUAS-CD8-GFP. Δpes-10 minimal promoter was amplified from pPD97.78 and myo-3 promoter was from pPD122.66 (A. Fire). The unc-4 promoter (4 kb)39, the unc-4c promoter (bashed unc-4 promoter, ~1 kb, M. Vanhoven and K.S., unpublished results), mig-13 promoter (3.4 kb)19 and dpy-7 promoter (218 bp)33 were amplified from N2 genomic DNA. The dpy-20 genomic DNA (3 kb, including the entire dpy-20 gene from initial ATG to stop codon) was amplified from fosmid (gift from M. Chalfie; Columbia University) 25, and was fused with extra cloning sites (S. McCarroll and C.I. Bargmann; Rockefeller University).

Strains and transformation. Wild-type worms were C. elegans Bristol strain N2. All mutants used in the paper were provided by Caenorhabditis Genetics Center. Strains were maintained using standard methods28, and worms 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. Transgenic arrays were generated in N2 background except lines containing split Q constructs and dpy-20 (e1282ts) used for Dpy rescue experiments. For each transformation, at least two transgenic lines were obtained following similar results. MosSCI transformation was performed based on the protocol described in http://sites.google.com/site/jorgensenmossci. The MosSCI 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 M. Nonet (http://thalamus.wustl.edu/nonetlab/ResourcesF/PCR%20of%20MosSCI%20transgenic.pdf). Strain information is available in Supplementary Note 1. Vector maps are available in Supplementary Note 3.

Quinic acid desuppression treatment. The fresh quinic acid stock solution (300 mg/ml) was prepared from d-(−)-quinic acid (Sigma-Aldrich, 98%) in sterile Milli-Q water. The stock can be kept in 4 °C for at least one month. Neutralized with 5 M NaOH to pH 6–7, the quinic acid stock solution can be added into nematode growth medium (NGM) agar (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 5 M NaOH to pH 6.0–7.5. pH was tested by EMD pH-indicator strips, pH 5.0–10.0). Worms were synchronized by hypochlorite bleaching and were cultured on NGM plates with OP50. They were transferred onto quinic acid plates with OP50 6 h, 12 h, 24 h or 30 h before taking images. Worms 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 worms using Plan-Apochromat 40×, 1.3 numerical aperture (NA) objective except the images in Figure 3e–g (using Plan-Apochromat 63×, 1.4 NA objective), and images in Supplementary Figures 3 and 4 (Plan-Apochromat 10×, 0.47 NA objective) on a Zeiss LSM710 confocal microscope (Carl Zeiss). Worms 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. Images in Supplementary Figure 3 are overlays of single fluorescent single plane image and differential interference contrast (DIC) image, and images in 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 Figure 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 40× objective. The total fluorescence intensity of GFP in each cell body was determined using Image J (US National Institutes of Health) and Excel (Microsoft) by integrating pixel intensity across the cell body region. Forty 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).

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