The STAR protein QKI-6 is a translational repressor

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The signal transduction and activation of RNA (STAR) family of RNA-binding proteins, whose members are evolutionarily conserved from yeast to humans, are important for a number of developmental decisions. For example, in the mouse, *quaking* proteins (QKI-5, QKI-6, and QKI-7) are essential for embryogenesis and myelination , whereas a closely related protein in *Caenorhabditis elegans*, germline defective-1 (GLD-1), is necessary for germline development. Recently, GLD-1 was found to be a translational repressor that acts through regulatory elements, called TGEs (for *tra-2* and *GLI* elements), present in the 3′ untranslated region of the sex-determining gene *tra-2*. This gene promotes female development, and repression of *tra-2* translation by TGEs is necessary for the male cell fates. The finding that GLD-1 inhibits *tra-2* translation raises the possibility that other STAR family members act by a similar mechanism to control gene activity. Here we demonstrate, both *in vitro* and *in vivo*, that QKI-6 functions in the same manner as GLD-1 and can specifically bind to TGEs to repress translation of reporter constructs containing TGEs. In addition, expression of QKI-6 in *C. elegans* wild-type hermaphrodites or in hermaphrodites that are partially masculinized by a loss-of-function mutation in the sex-determining gene *tra-3* results in masculinization of somatic tissues, consistent with QKI-6 repressing the activity of *tra-2*. These results strongly suggest that QKI-6 may control gene activity by operating through TGEs to regulate translation. In addition, our data support the hypothesis that other STAR family members may also be TGE-dependent translational regulators.

quaking | myelination | mouse | translation control | *tra-2* and *GLI* element (TGE)

The STAR family of RNA-binding proteins is required for a number of important developmental decisions in both vertebrates and invertebrates (1). STAR family members contain a conserved KH domain as well as two conserved domains called QUA1 and QUA2. STAR proteins are necessary for embryogenesis and myelination in the mouse (2, 3), muscle development in *Drosophila* (4, 5), notochord differentiation in *Xenopus* (6), and germ-line development in *Caenorhabditis elegans* (7, 8). Although over 30 STAR family members have been identified, for most, it is not clear how they function to regulate gene activity.

Recently, GLD-1 was shown to inhibit the translation of the *C. elegans* sex-determining gene *tra-2* (9). *C. elegans* has two sexes: hermaphrodites and males. Hermaphrodites are essentially female animals that make sperm and then oocytes. *tra-2* is required for female development and is predicted to encode a large transmembrane protein, called TRA-2A, that is necessary to inhibit downstream male determinants (10–12). GLD-1 is germ line specific and plays multiple roles in hermaphrodite germ cell development. In the hermaphrodite, GLD-1 is essential for oogenesis (7) and is necessary for hermaphrodite spermatogenesis and inhibition of premeiotic proliferation (7).

tra-2 is translationally regulated by two elements, called TGEs (for *tra-2* and *GLI* elements), located in the 3′ untranslated region (3′UTR) (13, 14). Translational repression of *tra-2* is required for hermaphrodite spermatogenesis (13, 15). GLD-1 specifically binds the TGEs and represses translation of TGE-containing RNAs both *in vivo* and *in vitro* (9). These findings suggest that GLD-1 controls hermaphrodite spermatogenesis by

repressing *tra-2* translation (9). The TGE control is a conserved process that is present in nematodes and mammals (14), raising the possibility that other STAR family members act through TGE-like elements to control translation. We addressed this idea by asking whether the mouse *quaking* proteins (QKI) are TGE-dependent translational regulators.

The *qk* gene produces at least three major transcripts that are 5 (*qkI-5*), 6 (*qkI-6*), and 7 (*qkI-7*) kb long (2). The three transcripts produce very similar proteins, QKI-5, QKI-6, and QKI-7, respectively, that differ only at the carboxyl terminus. All three proteins contain the identical KH RNA-binding domain and QUA1 and QUA2 domains. Northern blot analyses indicate that *qkI-5* mRNA is the major form present in early embryos, and that *qkI-6* and *qkI-7* mRNA are found in late development when myelination begins (16). Immunocytochemistry experiments indicate that QKI proteins are expressed in oligodendrocytes and astrocytes in the central nervous system as well as Schwann cells in the peripheral nervous system. QKI-5 is nuclear, although it shuttles between the nucleus and cytoplasm, whereas QKI-6 and QKI-7 are predominantly cytoplasmic (16, 17).

Recessive viable mutations in *quaking* (qk^v) result in hypomyelination, and as a result the qk^v homozygotes develop a rapid tremor by postnatal day 10. Homozygotes also suffer chronic/tonic seizures (18). The myelin deficiency is most pronounced in the brain, less in the spinal cord, and even less in the peripheral nervous system (19). In the qk^v allele, QKI-6 and QKI-7 are absent from oligodendrocytes and Schwann cells (16), possibly explaining the tremors in mutant animals. Other *qk* alleles, embryonic lethal mutations induced by ethylnitrosourea (qk^{kt4}), have been isolated (20–22). The terminal phenotype is one of arrested growth with generalized abnormalities by embryonic day 9, but the precise cause of lethality is not known (3). The mice die more than 10 days before myelination begins, indicating that QKI proteins may affect multiple cellular processes.

In this paper, we find that like GLD-1, the QKI-6 protein can act through TGEs to repress translation. This finding is supported by the observation that QKI-6 specifically binds TGEs, represses translation of reporter RNAs both *in vivo* and *in vitro*, and promotes the fate of male cells in *C. elegans*. Our findings indicate that a number of STAR family proteins may control gene expression by repressing translation through TGEs.

Methods

General Procedures and Strains. Routine maintenance was as described in ref. 23. All strains were raised at 20°C unless otherwise noted. The following mutant alleles were used in this study: LGII, *tra-2(e2020 gf)* (15); LGIV, *unc-24(e138)fem-3(e1996lf)*; LGIV, *tra-3(e1107)/nT1* (24). The balancer *nT1* (previously called DnT1) suppresses recombination over much of chromosome IV (25).

DEVELOPMENTAL BIOLOGY

RNA Gel Shift Analysis. A construct encoding the entire QKI-6 or a mutant QKI-6 that carries the qk^{kt4} mutation (amino acid number 48 has been changed from glutamic acid to glycine) with an amino-terminal His tag (His-QKI-6 or His-QK^{kt4}), were expressed in bacteria and purified by using the His-Binding Buffer Kit (Novagen) (17). RNA gel shifts were performed as described (13). ^{32}P-labeled and unlabeled *tra-2(+)* and *tra-2(−108)* 3′UTR probes were made by standard methods as described (26). Small ^{32}P-labeled RNAs (EBG-9, EBG-11, EJ-19, EJ-32, EJ-35, EJ-38, EMP-9, and EMP-10) were produced by using the method of Milligan and Uhlenbeck (27). See below for sequences.

Transgenic Analysis. *hsp*::*QKI-6* (also called pBG102) was made by amplifying the *qkI-6* coding region by PCR with primers EMP-7 and EMP-8. The PCR product was cut with *Sal*I and cloned into the same site of pBG518. pBG518 contains the *C. elegans*-inducible heat shock promoter, *hsp16.41*, and the *C. elegans unc-54* 3′UTR (9). The construction of pBG3 [*lacZ*::*Ce-tra-2(-32)3′UTR*] and pBG4 [*lacZ*::*Ce-tra-2(−60)3′UTR*] are described elsewhere (28).

Transgenic *C. elegans* animals were generated by using standard methods (29): 25 ng/μl of *hsp*::*QKI-6* with 50 ng/μl of *emb-9*::*GFP* were injected into animals carrying either *lacZ*::*tra-2(−32)3′UTR* or *lacZ*::*tra-2(−60)3′UTR. emb-9*::*GFP* contains the coding region of green fluorescent protein (GFP) controlled by the *emb-9* promoter. For analysis of the ability of QKI-6 to promote the fate of male cells in the soma, *tra-3(e1107)/nt1* hermaphrodites were injected with 100 ng/μl of *hsp*::*QKI-6* and 100 ng/μl of pRF4 which encodes the collagen *rol-6* gene.

In Vitro Yeast Translational Assay. Yeast lysates were produced as described (30) with modifications (31). The *in vitro* translation assay was performed as described (30). Capped RNAs containing the luciferase (*luc*) gene, and different 3′UTRs were produced by a standard *in vitro* transcription reaction (26). The ratio of cap analog to GTP was 5 to 1. pBG51 and pBG53 are described in ref. 9 and correspond to constructs containing the *luc* gene and wild-type *tra-2(+)* 3′UTR or mutant *tra-2(−108)* 3′UTR in which both TGEs and some flanking sequences were deleted, respectively. The two plasmids contain a poly(A)$_{65}$ tract downstream of the 3′UTRs followed by an *Nsi*I site. The constructs were linearized by using *Nsi*I and transcribed by using a Promega SP6 Ribomax kit. RNAs were quantitated by spectrophotometry and checked for purity on an agarose gel. RNA was added to the yeast lysates to a final concentration of 6 nM, and His-QKI-6 or His-QK^{kt4} was added to a final concentration of 60 nM. At specific time points, aliquots were taken. Each aliquot was divided into two; half was frozen in liquid nitrogen and processed for luciferase activity and the other half added to TRIZOL (GIBCO/BRL) for Northern analysis. Luciferase activity was assayed by using a Monolight 2010 Luminometer. RNA levels were quantitated by Northern blot analysis (Storm 860 phosphoimager).

Oligonucleotide Sequences. EMP-9: 5′-TGCAGCTCCCCCAAT-TTTTCTGGAAGGATCCCCTTAGGAAATGCGATCTGT-GATGGATGAGATTCCCTCGCCCTATAGTGAGTCGTA-TTA-3′.

EMP-10: 5′-TGCAGCTCCCCCAATTTTTCTGGAAGGA-TCCCCTTAGGAAATGCGATCTGTGATGGCACAGATT-CCCTCGCCCTATAGTGAGTCGTATTA-3′.

EBG-9: 5′-TGGACGATTAGATATGAGATGATAAGAA-ATTAAATATGAGTAGATATGAGTAGATAAGAAATT-AAATAATGAAATGGAAATTGTCGCCCTATAGTGAG-TCGTATTA-3′.

EBG-11: 5′-TGGACGATTATGAAATGGAAATTGTAC-AAATAATAGAAACGAAAATGAGTAAGAAATGAAA-

TTTTGGAACCAAATTCTCGCCCTATAGTGAGTCGTA-TTA-3′.

EJ-19: 5′-TGTGTTCAGAAAACTAGGCAGGAAAGTA-GGAAAAGATCTGTTAATCGCCCTATAGTGAGTCGTA-TTA-3′.

EJ-24: 5′-GGAAGGATAGAAACCCCTTAGGAAATGC-GATCTGTGATGGATGAGATTCCCTCGCCCTATAGTG-AGTCGTATTA-3′.

EJ-32: 5′-TACAAGATCTGTGTTCCTAGGCAGGAAAG-TAGGAAACACAGATCTGTTCGCCCTATAGTGAGTCG-TATTA-3′.

EJ-35: 5′-GAATTCTCGAGTACAAGATCTGTGTTCCT-AGGCAGGAAAGTAGGAAAGTGAGATCTGTTCGCCC-TATAGTGAGTCGTATTA-3′.

EJ-38: 5′-TGCAGCTCCCCCAATTTTTCTGGAAGGAT-AGAAACCCCTTAGGAAATGCGATCTGTGATGGATG-AGATTCCCTCGCCCTATAGTGAGTCGTATTA-3′.

Results and Discussion

To test whether other STAR proteins, like GLD-1, are translational repressors, we asked whether the murine QKI proteins could control translation in a TGE-dependent manner. By using RNA gel shift analysis, we examined the different QKI proteins (QKI-5, QKI-6, and QKI-7) for specific binding to the *C. elegans tra-2* TGEs. Purified bacterial-expressed proteins were incubated with radiolabeled RNAs that contain either the wild-type *tra-2* 3′UTR [*tra-2(+)3′UTR*] or a mutant 3′UTR in which both TGEs were deleted [*tra-2(−108)3′UTR*] and electrophoresed on a nondenaturing polyacrylamide gel. The QKI proteins contained a His tag at their amino terminus and encoded the entire QKI protein. We found that QKI-6 bound to RNAs that carried TGEs but not to ones in which the TGEs were removed (Fig. 1 *A* and *D*). The binding of QKI-6 to the TGEs was specific because complex formation was abolished by an excess of unlabeled *tra-2(+)* 3′UTR RNA but not by an excess of unlabeled *tra-2(−108)* 3′UTR RNA (data not shown). We also examined the binding of QKI-5 and QKI-7 to the different RNAs in gel shift assays and found little difference in complex formation (data not shown). Because the cytoplasmic localization of QKI-6 and QKI-7 is more consistent with a role in translational control, and there is little sequence difference between QKI-6 and QKI-7 protein, we focused our studies on QKI-6.

To further test the binding specificity of QKI-6, we assayed for binding of the protein to a small RNA that contained the *C. elegans tra-2* TGEs (EBG-9) or to one in which the TGEs were deleted (EBG-11; see *Methods* for sequences). We found that similar to GLD-1, QKI-6 bound EBG-9 but not EBG-11 (Fig. 1 *B* and *D*). Previously, we identified TGEs in the 3′UTRs of the *Caenorhabditis briggsae tra-2* and the human *GLI* genes, and demonstrated that GLD-1 specifically associates with these elements (9); therefore, we asked whether QKI-6 also bound the wild-type *C. briggsae tra-2* (EJ-19) and wild-type *GLI* (EJ-38) TGEs but not mutant TGEs (for mutant *C. briggsae tra-2*: EJ-32 and EJ-35 and for mutant *GLI*: EMP-9 and EMP-10). EJ-35 and EMP-9 contain a 6-nt deletion, and EJ-32 and EMP-10 contain the same 6-nt deletion plus three altered nucleotides. Similar to GLD-1, QKI-6 binds EJ-19 and EJ-38 but not EJ-32, EJ-35, EMP-9, or EMP-10 (Fig. 1 *C* and *D*). These results show that similar to GLD-1, QKI-6 specifically associates with wild-type TGEs.

As mentioned above, the qk^{kt4} mutation is embryonic lethal and likely represents a strong loss-of-function mutation. This mutation changes a glutamic acid at position 48 in the QUA1 domain to glycine (2). QKI-5 protein carrying this mutation binds RNA but does not form a homodimer, raising the possibility that the interaction of the protein with itself or another protein is required for function (32). We tested whether the QK^{kt4} protein could bind TGEs by assaying the ability of this
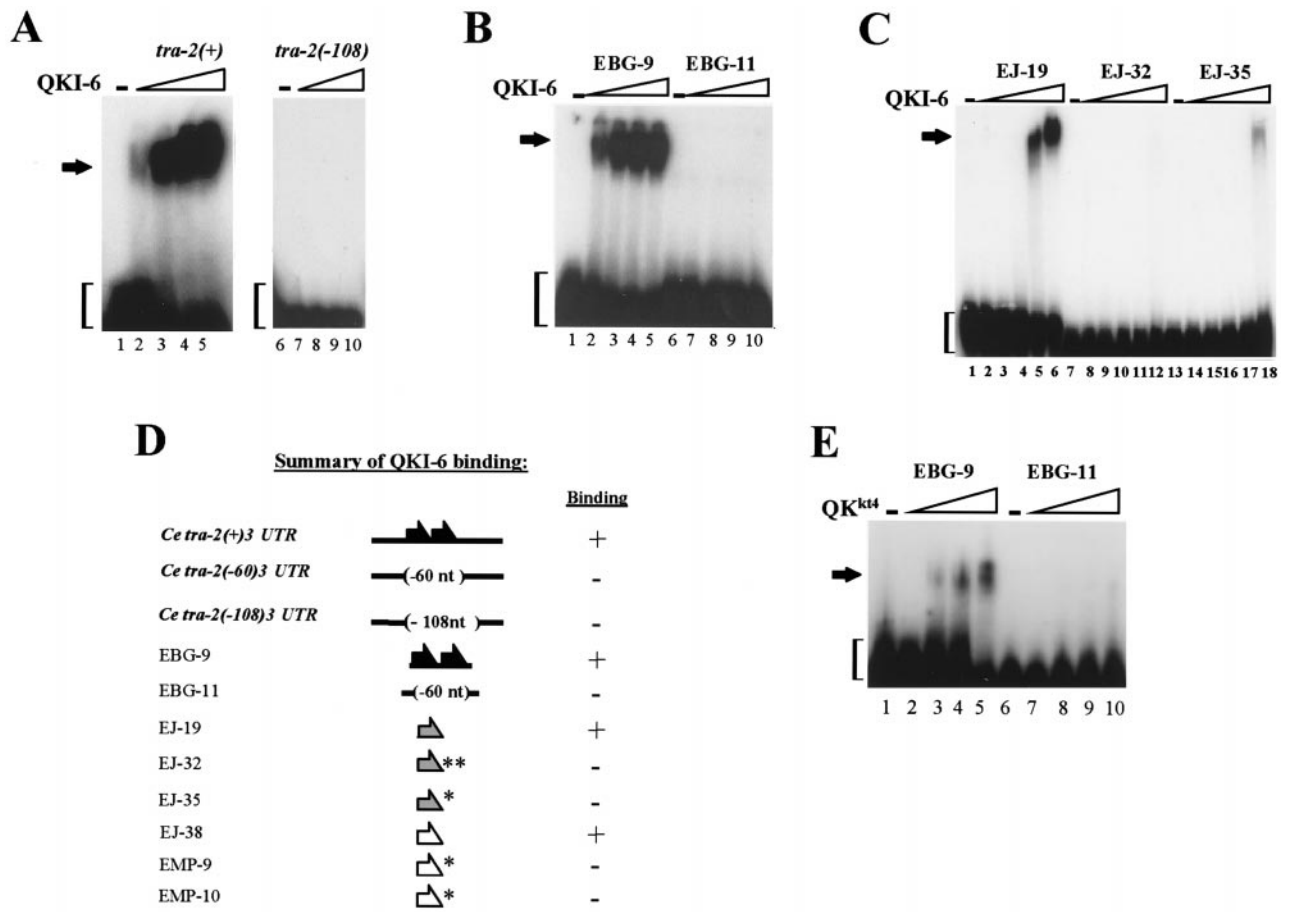
Fig. 1. QKI-6 as well as QK[kt4] specifically bind the TGEs. The binding of QKI-6 and QK[kt4] to the TGEs was tested by using RNA gel shift analysis. (*A*) One femtomole of ^{32}P-labeled *C. elegans tra-2(+)* 3′UTR (lanes 1–5) or mutant *tra-2(−108)* 3′UTR (lanes 6–10) in which the TGEs have been deleted were incubated alone or with increasing amounts of purified bacterially expressed His-QKI-6 protein (0, 0.25, 0.5, 0.75, or 1 µg). Reactions were electrophoresed on a 3.75% nondenaturing polyacrylamide gel. Slower migrating bands represent complex formation (arrow); the faster migrating bands indicate free probe (brackets). (*B*) One femtomole of ^{32}P-labeled *C. elegans tra-2* TGE (EBG-9; lanes 1–5) or mutant *tra-2* TGE (EBG-11; lanes 6–10; see *Methods* for sequences) were incubated alone or with increasing amounts of purified bacterially expressed His-QKI-6 protein (0, 0.25, 0.5, 0.75, or 1 µg). (*C*) One femtomole of ^{32}P-labeled *C. briggsae tra-2* TGE (EJ-19; lanes 1–6) or mutant *C. briggsae tra-2* TGEs (EJ-32, lanes 7–12 and EJ-35, lanes 13–18) were incubated with increasing amounts of His-QKI-6 (0, 0.16, 0.32, 0.64, 0.80, or 3.5 µg). (*D*) Summary of QKI-6 binding. Binding of His-QKI-6 was determined by using RNA gel shifts. (*Left*) Names of RNAs (for sequences, see *Methods*). (*Middle*) Diagrams of RNAs. Black arrows represent *C. elegans tra-2* TGEs, stippled arrows represent *C. briggsae tra-2* TGEs, and white arrows represent the *GLI* TGEs. Asterisks indicate mutant *C. briggsae tra-2* or *GLI* TGE sequences. Deletions are indicated in brackets. (*Right*) RNAs were scored for the ability (plus sign) or inability (minus sign) to bind QKI-6. (*E*) One femtomole of ^{32}P-labeled *C. elegans tra-2* TGEs (EBG-9, lanes 1–5) or mutant *tra-2* TGEs (EBG-11, lanes 6–10; see *Methods* for sequences) were incubated alone or with increasing amounts of purified bacterially expressed His-QK[kt4] protein (0, 0.25, 0.5, 0.75, or 1 µg). In every case, QKI-6 and QK[kt4] formed complexes with RNAs containing wild-type TGEs but not those with mutant elements.

protein to bind EBG-9 but not EBG-11. Interestingly, we find that QK[kt4] bound EBG-9, though less efficiently than wild type, and that QK[kt4] did not bind EBG-11 (Fig. 1*E*). These findings are consistent with earlier findings that indicated this mutation in the QUA1 domain does not abolish RNA binding.

To test whether QKI-6 can act as a TGE translational repressor *in vivo*, we assayed for its ability to repress the activity of a reporter transgene that contains the TGEs or one in which the TGEs have been deleted. We expressed QKI-6 in *C. elegans* animals carrying a reporter transgene that contained the *tra-2* 3′UTR lacking one TGE [*lacZ::tra-2(−32)3′UTR*] or a 3′UTR missing both TGEs [*lacZ::tra-2(−108)3′UTR*]. The presence of a single TGE within the 3′UTR only partially controls translation and provides a sensitive assay for regulation. Consequently, for analysis of the ability of QKI-6 to regulate translation through the TGEs, we used the *lacZ::tra-2(−32)3′UTR* construct (28). Both reporter transgenes encode β-galactosidase (β-gal) and are controlled by the inducible heat-shock promoter (*hsp16–41*).

The expression of QKI-6 dramatically decreased the number of animals with intestinal β-gal staining. In the absence of QKI-6, 51% of transgenic animals carrying *lacZ::tra-2(−32)3′UTR* had β-gal staining in intestinal cells (Fig. 2*A*, Table 1). In contrast, when QKI-6 was expressed in the soma, 8% of *lacZ::tra-2(−32)3′UTR* transgenic animals had intestinal β-gal staining (Fig. 2*B*, Table 1). Ectopic expression of QKI-6 had little effect on the β-gal expression of *lacZ::tra-2(−108)3′UTR* animals (Fig. 2 *C* and *D*, Table 1). These results indicate that QKI-6 can repress the activity of the reporter transgene in a TGE-dependent manner.

As discussed above, the TGE regulation is necessary to repress the translation of *tra-2* which allows for the specification of male cell fates. Previously, we found that mis-expression of GLD-1 in the soma resulted in masculinization of somatic tissues in hermaphrodite animals (9). We reasoned that if QKI-6 is behaving in a similar manner, expression of QKI-6 in the hermaphrodite soma should also result in the development of male cell fates. To examine this, we heat-shocked wild-type
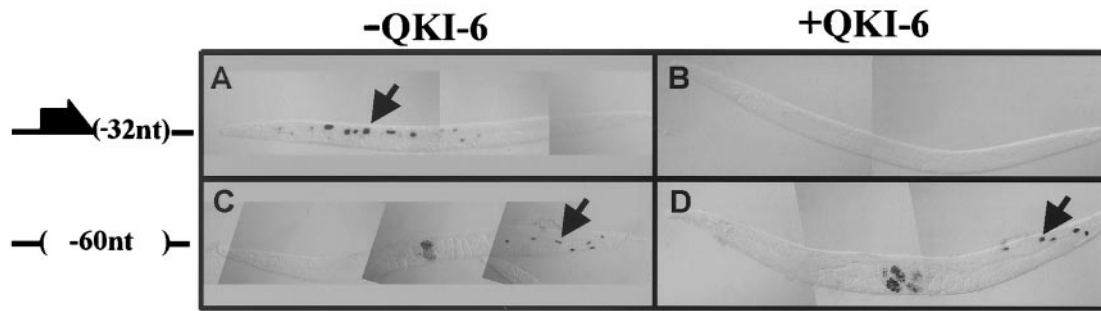
Fig. 2. QKI-6 represses the activity of reporter transgenes through the TGEs *in vivo*. Lateral views of *C. elegans* adult animals with anterior to the left and ventral down. Two reporter transgenes were used. Both encode the *lacZ* gene and are driven by the *C. elegans* heat shock promoter (*hsp16-41*) (37). *lacZ::tra-2(−32)3′UTR* carries a *tra2 3′UTR lacking one TGE*, and *lacZ::tra-2(−60)3′UTR* has a *tra-2 3′*UTR in which both TGEs have been deleted. Somatic expression of QKI-6 (*hsp::QKI-6*) was accomplished by expressing the entire QKI-6 cDNA by using the heat shock promoter. For *lacZ(−32)3′UTR, (A)* in the absence of QKI-6, more animals had intestinal β-gal staining [β-gal activity is detected in 11 intestinal cells (arrow)] than (B) in the presence of QKI-6. For *lacZ(−60)3′UTR*, (C) the absence or (D) presence of QKI-6 did not affect the number of animals with intestinal β-gal staining [β-gal staining is detected in seven and five intestinal cells, respectively (arrows)]. (*Left*) Cartoon of the different *tra-2 3′UTRs* inserted downstream of the *lacZ* reporter gene.

hermaphrodites carrying *hsp::QKI-6* throughout development and assayed for masculinization. We found that 28% ($n = 122$) of the XX hermaphrodite animals expressing QKI-6 produced truncated tails, indicative of masculinization (Fig. 3 *C* and *D*); however, truncated tails were not detected in animals not expressing QKI-6 ($n = 200$; Fig. 3*A*). These data indicate that QKI-6 can act similarly to GLD-1 to repress *tra-2* translation *in vivo*.

Because QKI-6 only weakly masculinized wild-type hermaphrodites, we used a second *in vivo* assay to ask whether QKI-6 could masculinize animals that were intersexual. To do this, we heat-shocked *tra-3(lf)* animals carrying *hsp::QKI-6* throughout development and assayed for masculinization. *tra-3*, like *tra-2*, is required for female development (10) and genetically appears to act upstream of *laf-1* to regulate *tra-2* translation (28). *tra-3(lf)* animals are incomplete males. As expected, less than 0.5% of *tra-3(e1107lf)* animals expressing QKI-6 produced vulva ($n = 256$), indicative of male development, compared with 10% of the *tra-3(lf)* animals not expressing QKI-6 ($n = 193$). The further masculinization of *tra-3(lf)* animals after overexpression of QKI-6 is consistent with our initial conclusion that QKI-6 can repress *tra-2* translation to inhibit female development. We also found that expression of GLD-1 and QKI-6 in the soma resulted in some embryonic lethality (data not shown), possibly indicating

that these proteins are disrupting the activity of an essential gene(s).

To test whether the observed masculinization after expression of QKI-6 was acting on *tra-2*, QKI-6 was expressed in the soma of *fem-3(lf)* animals by using the *hsp::QKI-6* construct. *fem-3* activity is required for male somatic development and *tra-2* is thought to promote female development by directly inhibiting *fem-3* activity (for review, see ref. 33). QKI-6 was expressed in XX *fem-3(lf)* animals by using the *hsp::QKI-6* construct. In contrast to wild-type animals, *fem-3(lf)* animals expressing QKI-6 did not develop truncated tails ($n = 192$), consistent with QKI-6 acting upstream of *fem-3* in a genetic hierarchy. In addition, to determine whether QKI-6 was regulating *tra-2* directly through the TGEs, we expressed QKI-6 in *tra-2(e2020 gf)* animals. The *tra-2(e2020 gf)* mutation completely removes the TGEs from the *tra-2* 3′UTR. We observed that in contrast to

Table 1. QKI-6 represses TGE control *in vivo*

Reporter transgene*	QKI-6 transgene†	Animals with intestinal β-gal staining, %‡
lacZ::tra-2(−32)3′UTR	None	51 ($n = 47$)
	hsp::QKI-6	8 ($n = 37$)
lacZ::2(−108)3′UTR	None	62 ($n = 58$)
	hsp::QKI-6	69 ($n = 51$)

*Reporter transgene containing the *C. elegans* heat shock promoter (hsp-16.41) controlling the *lacZ* gene. Mutant *tra-2* 3′UTRs were inserted downstream of the *lacZ* gene. In all experiments, adult transgenic worms were heat-shocked 2 h at 33°C and allowed to recover for an additional 2 h at 20°C before being fixed and stained for β-gal activity.

†Transgenic *C. elegans* animals containing different reporter transgenes were injected with *hsp::QKI-6*. *hsp::QKI-6* is controlled by the heat-shock promoter (hsp-16-41) and carries the *unc-54* 3′UTR and the coding region for QKI-6.

‡Trangenic animals were scored as positive if blue precipitate was detectable in intestinal cells at ×630 magnification. Intestinal cells were scored genetic evidence indicates the TGE regulation is present in these cells (15). Percentiles represent the values of one typical transgenic line. Other lines gave similar results. $n = $ total number of animals scored from at least four different experiments.
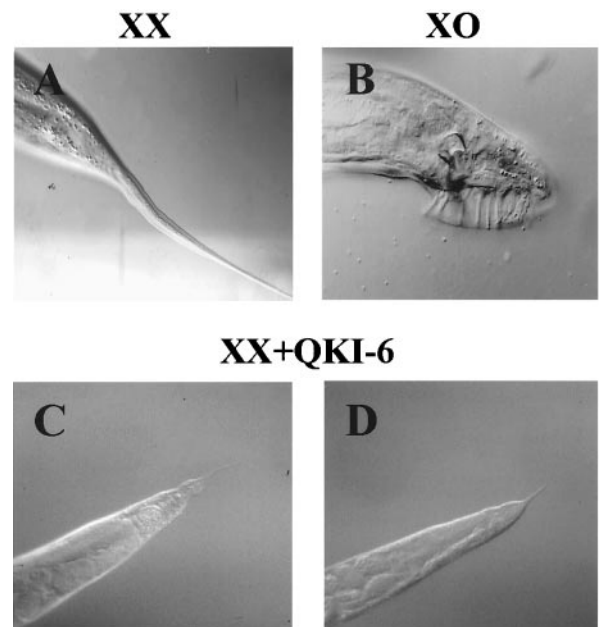

Fig. 3. Expression of QKI-6 causes masculinization of somatic tissues. (*A*) Adult wild-type XX hermaphrodite and (*B*) XO male tails (C and D). Transgenic XX hermaphrodite animals carrying the *hsp::QKI-6* transgene were heat shocked once a day from embryogenesis to adults. The truncated tail is indicative of somatic masculinization.
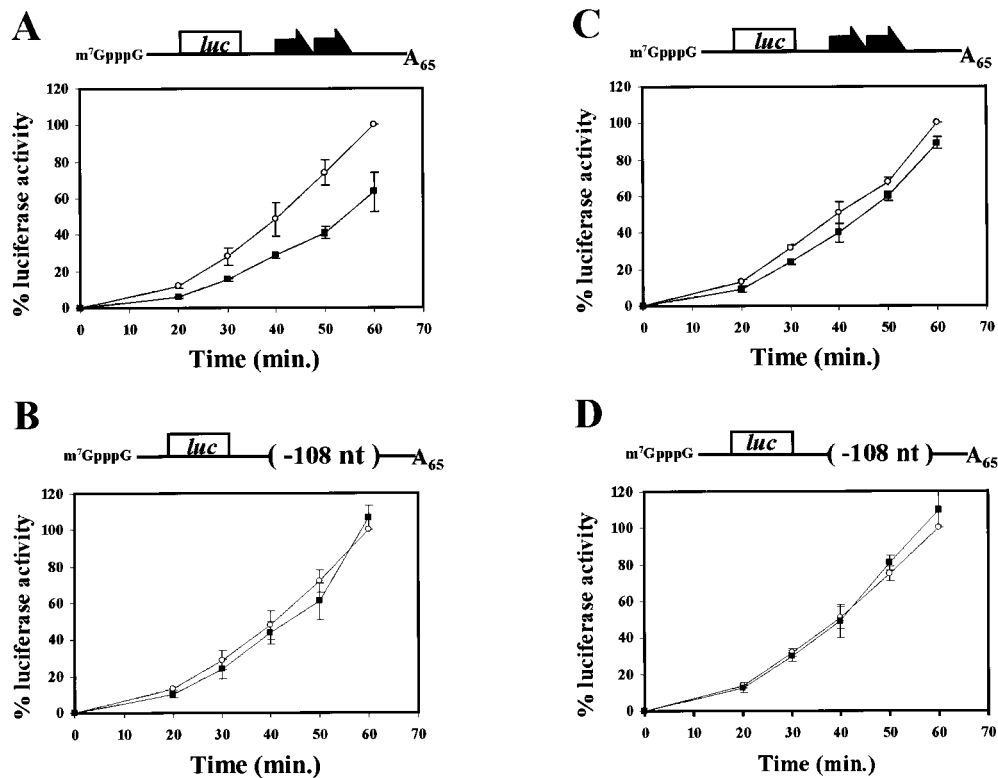
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Fig. 4. QKI-6 represses translation through the TGEs *in vitro*, whereas QK^kt4 containing a glutamic acid to glycine mutation at position 48 does not repress translation. Translation of reporter RNAs were assayed in yeast extracts. (*A*) A reporter luciferase RNA (6 nM) carrying the *tra-2(+)* 3′UTR or (*B*) the *tra-2(−108)* 3′UTR lacking the TGEs was incubated with 60 nM His-QKI-6 protein (closed squares) and compared with incubations in which protein was not added (open circles). (*C*) A reporter luciferase RNA (6 nM) carrying the *tra-2(+)* 3′UTR or (*D*) the *tra-2(−108)* 3′UTR was incubated with 60 nM His-QK^kt4 protein (closed squares) and compared with incubations in which protein was not added (open circles). The 3′UTRs present within the reporter RNAs are depicted above each graph. Aliquots of the reaction mixture were taken at the indicated time points. Data are plotted as a percent of total luciferase activity at the $t = 60$ min when only the reporter RNAs were present. All presented data are averages and standard deviations of at least three independent experiments. The absolute luciferase values were similar for each of the reporter RNAs in the absence of His-QKI-6 or His-QK^kt4 proteins.

wild-type and *tra-3(lf)* animals, all *tra-2(gf)* expressing QKI-6 had wild-type tails and developed vulva ($n = 132$). These findings suggest QKI-6 was acting through the TGEs to repress *tra-2* translation.

Previously, GLD-1 was shown to repress translation of reporter RNAs that carried TGEs *in vitro* (9). We examined whether QKI-6 could inhibit translation in a TGE-dependent manner *in vitro* by using yeast translation extracts and assaying the translation of reporter RNAs containing different 3′UTRs. The reporter RNAs encoded luciferase and carried a wild-type *tra-2* 3′UTR [*tra-2(+)*] or a mutant *tra-2* 3′UTR [*tra-2(−108)*] in which both TGEs as well as some flanking sequence was deleted. The RNAs were capped and contained a poly(A) tail of 65 adenosine residues. The reporter RNAs were added to the yeast extract with or without a 10-fold molar excess of purified His-QKI-6 protein, and the luciferase activity was quantitated at specific times. The addition of His-QKI-6 protein to reactions containing RNAs carrying *tra-2(+)* 3′UTR resulted in an approximately 40–50% decrease in luciferase activity (Fig. 4*A*, closed squares); however, incubation of His-QKI-6 protein with RNAs carrying *tra-2(−108)* 3′UTR did not significantly affect luciferase activity (Fig. 4*B*, closed squares). Northern analysis showed that the differences in luciferase activity were not the result of changes in RNA levels (data not shown). Neither GLD-1 nor QKI-6 fully represses translation in the yeast *in vitro* translation assay. Similar to QKI-6, GLD-1 represses translation by about 50%. It is possible that full repression by QKI-6 and GLD-1 requires other factors not present in the yeast extract. Alternatively, it is possible that 50% repression is sufficient for

normal development, because many processes are dosage sensitive. We next examined whether QK^kt4 could repress translation in the yeast *in vitro* assay. Although QK^kt4 still binds RNA (see above), it was not able to repress translation of a reporter RNA that contained the wild-type *tra-2(+)* 3′UTR [Fig. 4*C*; compare a tenfold molar excess of QK^kt4 (open circles) with no protein (closed squares)]. These data indicate it is possible to separate RNA binding from translational control. Perhaps the disruption of the ability of QKI-6 to interact with itself or another factor by the *qk*^kt4 mutation within the QUA1 domain significantly reduces its ability to repress translation.

In conclusion, the mouse QKI-6, like the *C. elegans* GLD-1, can repress translation both *in vivo* and *in vitro*, and this repression is dependent on TGEs within the 3′UTR. QKI-6 protein is required for proper myelination in the mouse. How QKI-6 controls development and the nature of its RNA target(s) is unclear. Our findings raise the possibility that QKI-6 regulates development in the mouse by controlling the translation of TGE-containing RNA(s). Immunocytochemistry indicates that QKI-6 is mostly cytoplasmic (16); this is consistent with it acting as a translational regulator. It is also not known how QKI-5 and QKI-7 control gene activity. QKI-5 is predominantly nuclear and shuttles between the nucleus and cytoplasm, suggesting it may act by a different mechanism to control gene expression (16, 17). Perhaps QKI-5 is involved in splicing like the STAR family member SF1/BBP (34, 35). QKI-7 has the same tissue distribution as QKI-6 and is also cytoplasmic (16), and may operate in the same manner as QKI-6. Because STAR proteins are thought to homo- as well as heterodimerize (6, 36), one specu-

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lative model is that QKI-7 and QKI-6 interact, and in so doing alter their activity and/or RNA specificity.

The TGE regulation and the STAR proteins are found in both invertebrates and vertebrates (1, 9). Our analysis of *C. elegans* GLD-1 and the mouse QKI-6 proteins suggests that at least these two STAR family members control gene expression by acting through TGEs to repress translation. Interestingly, sequence analysis of the STAR proteins indicates that they fall into subfamilies (1). For example, GLD-1 and QKI-6 are more similar to the *Drosophila* WHO/HOW, *Xenopus* XQUA, and the human HQUA proteins than the mammalian SF1/BPP and SAM-68. It is possible that STAR protein subfamilies function similarly and those proteins most similar to GLD-1 and QKI-6 may also control development by acting as TGE translational regulators.

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