Corrigendum to “STAR proteins quaking-6 and GLD-1 regulate translation of the homologues GLI1 and tra-1 through a conserved RNA 3′ UTR-based mechanism” [Dev. Biol. 287 (2005) 98–110]

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Because of some ambiguity in the origin of the images used in Figs. 1d and 1e of Lakiza et al. (2005), the authors undertook the verification of these data and the conclusions related to them with independent methodologies. The figure was reported to establish that the translational regulation of tra-1 by GLD-1 was due to binding of the GLD-1 protein to the 3′ UTR of tra-1, the homolog of mammalian Gli1, and that GLD-1 and tra-1 formed an RNA–protein complex in vivo. To validate these conclusions we obtained transgenic C. elegans animals (a kind gift from Tim Schedl, Washington University School of Medicine, St. Louis) that produced GLD-1 protein tagged with GFP and FLAG. We then used anti-FLAG antibody to pull down GLD-1 from cytosol extracts of the worms followed by RT-PCR using primers that could identify tra-1 RNA (Fig. 1). The results unequivocally establish the presence of GLD-1 protein-tra-1 RNA complexes. We next used biotin-labeled RNA sequences from the tra-1 3′ UTR (YY2 and YY2sc) mixed with streptavidin-labeled magnetic beads and cytosol extracts from the transgenic worms to determine the binding of GLD-1 to the 3′ UTR of tra-1 (Fig. 2). The beads were collected, and the proteins bound to the RNA sequences were interrogated with western blots. By using anti-FLAG antibody we established that the GLD-1 bound RNA sequences as originally described (Lakiza et al., 2005). Thus, we conclude that GLD-1 forms an RNP complex with tra-1 RNA in vivo and that it binds to the 3′ UTR of tra-1. The legend to Fig. 1 in Lakiza et al. (2005) describes RNA quantification by determining RNA ratios using GAPDH as the reference gene when the reference gene was actually ran-1. The authors have identified the original data for the remaining data in Fig. 1. The results presented here confirm the conclusions reached in the published paper.

Material and methods

RNA probes

Synthetic biotinylated RNAs were obtained from Invitrogen (Carlsbad, CA).

YY2 sense: 5′-UCACCCCCCUCACACUAUUUGGA-3′
YY2 sc sense: 5′-UCACCCCCCUCACACUAUUUGGA-3′

Mouse mutant mGli1 probe (putative mammalian binding site is deleted), sense orientation: 5′-GUAUUAUGCUACAGUAUGA-3′

The italics are wild-type (YY2) or mutant scrambled (YY2sc) binding sites.

Antisense YY2 sequence: 5′-UCCAAAACUAUGAGAGGGGGGUGA-3′
Antisense YY2sc sequence: 5′-UCCAAAACUAUGAGAGGGGGGUGA-3′
The italics are the wild-type (YY2) or mutant (YY2sc) relaxed consensus GLD-1 binding sites.

Ribonucleoprotein (RNP) complex analysis

Cytosol extracts, immunoprecipitation RT-PCR and the biotin pull-down assays were performed essentially as described by Lee and Schedl (2001), except that worms were fragmented using a mortar and pestle and Sample Grinding Kit (Amersham Biosciences). The IP was performed with anti-FLAG M2 agarose affinity gel, which is a monoclonal antibody against FLAG covalently attached to

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Fig. 1. (A) RT-PCR performed with RNA isolated from RNP complexes generated by immunoprecipitation (IP) of FLAG-tagged GLD-1 from cytosol extracts of transgenic worms expressing GLD-1∷GFP∷FLAG fusion protein. IP was performed using anti-FLAG Ab M2 beads (Sigma) and RT-PCR was done using *tra-1* specific primers. Lanes 1–3, anti-FLAG Ab M2 beads + independent cytosol extracts (CE); lanes 4–6, Sepharose-protein A beads (no anti-FLAG antibody) + CE; lane 7, RNA isolated directly from GLD-1∷GFP∷FLAG transgenic worms without immunoprecipitation (positive control showing integrity of the RNA in CE and identifying the *tra-1* band). (B) Additional RT-PCR controls performed as described for A. Lane 1, 100-bp ladder; lane 2, anti-FLAG Ab M2 beads + CE, *tra-1* specific primers; lane 3, negative control beads, anti-BrdU Ab attached to CL-4B, *tra-1* specific primers; lane 4, no reverse transcriptase control, anti-FLAG Ab M2 beads + CE, *tra-1*-specific primers; lane 5, specificity control, anti-FLAG Ab M2 beads + CE, *ran-1*-specific primers; lane 6, positive control for *ran-1* primers, total RNA, *ran-1*-specific primers.

Fig. 2. Western blot analysis using anti-FLAG antibody. Biotinylated 3′ UTR YY2 (wild-type) and YY2sc (mutant) RNA fragments were incubated with CE prepared from adult transgenic worms expressing GLD-1∷GFP∷FLAG fusion protein. Biotinylated RNA was isolated using streptavidin magnetic beads (Promega, Madison, WI), and bound proteins were subjected to SDS-PAGE and western analysis with anti-FLAG antibody (Rockland Immunochemicals, Inc., Gilbertsville, PA). Two membranes prepared identically at the same time and on the same film are shown. Lane 1, 150 μl CE + YY2sc biotinylated RNA, antisense orientation; lane 2, 100 μl CE + YY2sc biotinylated RNA, antisense orientation; lane 3, 50 μl CE + YY2sc biotinylated RNA, antisense orientation; lane 4, 150 μl CE + YY2sc biotinylated RNA, sense orientation; lane 5, 100 μl CE + YY2sc biotinylated RNA, sense orientation; lane 6, 50 μl CE + YY2sc biotinylated RNA, sense orientation; lane 7, 150 μl CE, no RNA added (negative control); lane 8, 150 μl CE + *mGli1* biotinylated RNA probe (negative control); lane 9, 15 μl CE without pull-down (positive control); lane 10, 150 μl CE + YY2 biotinylated RNA, antisense orientation; lane 11, 100 μl CE + YY2 biotinylated RNA, antisense orientation; lane 12, 50 μl CE + YY2 biotinylated RNA, antisense orientation; lane 13, 150 μl CE + YY2 biotinylated RNA, sense orientation; lane 14, 100 μl CE + YY2 biotinylated RNA, sense orientation; lane 15, 50 μl CE + YY2 biotinylated RNA, sense orientation; lane 16, 150 μl CE, no RNA added (negative control); lane 17, 150 μl CE + *mGli1* biotinylated RNA probe (negative control); lane 18, 15 μl CE without pull-down (positive control). This assay shows the expected specificity, as FLAG-tagged GLD-1 is captured by YY2 *tra-1* RNA probe that bears the predicted GLD-1 binding sites. When we used the same concentration of biotinylated *tra-1* mRNA that contained the mutant 3′ UTR fragment (YY2sc probe), the interaction of the *tra-1* 3′ UTR with GLD-1∷GFP∷FLAG fusion protein was dramatically reduced, indicating that GLD-1 is ineffective in binding the mutant *tra-1* 3′ UTR fragment. The antisense probe binds specifically but at lower affinity, presumably through a relaxed consensus sequence that appears in this sequence.
agarose CL-4B beads. The immunoprecipitate was washed and RNA was isolated from ribonucleoprotein complex bound to beads using RNeasy Mini Kits (Qiagen, Valencia, CA). RT-PCR was done using the one-step RT-PCR Kit (Qiagen). In the biotin pull-down experiment 1 μg of each synthetic biotinylated RNA probe (Invitrogen) was used. Either 150, 100 or 50 μl of cytosol extract was added to the streptavidin magnetic beads. The proteins were washed and subjected to western analysis using anti-FLAG antibody to identify GLD-1 bound to the RNA probes.

*tra-1*-specific primers:

tra-1 F 6′-CAAACGACCACAAAAACACGCTGCC-3′
tra-1 R 2′-ATCACATCTTCCACTCAACGGC-3′

*ran-1*-specific primers:

ran-1 5′-AGTTCCACCACCTTGTCTTCCACAC-3′
ran-1 5′-GCCTTCACCTTTACGTCCTTGAC-3′

The experiments were repeated three times with different protein extract samples, and the results were the same with minor variations in background.

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**References**
