Conserved SOL-1 proteins regulate ionotropic glutamate receptor desensitization

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The neurotransmitter glutamate mediates excitatory synaptic transmission by activating ionotropic glutamate receptors (iGluRs). In *Caenorhabditis elegans*, the GLR-1 receptor subunit is required for glutamate-gated current in a subset of interneurons that control avoidance behaviors. Current mediated by GLR-1-containing iGluRs depends on SOL-1, a transmembrane CUB-domain protein that immunoprecipitates with GLR-1. We have found that reconstitution of glutamate-gated current in heterologous cells depends on three proteins, STG-1 (a *C. elegans* stargazin-like protein), SOL-1, and GLR-1. Here, we use genetic and pharmacological perturbations along with rapid perfusion electrophysiological techniques to demonstrate that SOL-1 functions to slow the rate and limit the extent of receptor desensitization as well as to enhance the recovery from desensitization. We have also identified a SOL-1 homologue from *Drosophila* and show that *Dro* SOL1 has a conserved function in promoting *C. elegans* glutamate-gated currents. SOL-1 homologues may play critical roles in regulating glutamatergic neurotransmission in more complex nervous systems.

AMPA receptor | *Caenorhabditis elegans* | TARPs | stargazin | GLR-1

Fast synaptic neurotransmission in the vertebrate central nervous system is mostly mediated by ionotropic glutamate receptors (iGluRs) that are gated by the neurotransmitter glutamate. There are various classes of iGluRs defined by pharmacological criteria (1); of these, the class selectively gated by the ligand α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is of central importance for the processes of learning and memory (2). Biochemical studies in the past 5 years have revealed a large number of proteins that are directly or indirectly associated with AMPA receptors (AMPARs) (3). Genetic studies have provided complementary information about AMPAR-associated proteins that were not identified in previous biochemical or yeast two-hybrid studies. Vertebrate stargazin, the founding member of the transmembrane AMPAR regulatory protein family, is important for multiple aspects of AMPAR function, including the delivery of AMPARs to the cell surface and the modulation of receptor desensitization (4). Stargazin-like proteins have also been identified in invertebrates, including *Drosophila* and *Caenorhabditis elegans* (31). Interestingly, invertebrate stargazins appear to have a major role in regulating receptor gating and only minor effects on cell surface delivery. In addition, a genetic screen in *C. elegans* identified SOL-1, a CUB-domain transmembrane protein that associates with the AMPAR subunit GLR-1 and is required for glutamate-gated currents (5, 6). In *sol-1* mutants, glutamate-gated currents that depend on GLR-1 cannot be detected after pressure application of ligand. However, the mechanism of SOL-1 function is not yet understood.

To study how SOL-1 contributes to AMPAR function, we reconstituted receptor function in heterologous cells by using STG-1, SOL-1, and GLR-1. In *Xenopus* oocytes, we show that the effect of concanavalin-A (Con-A), a drug that slows desensitization of AMPARs, depends on SOL-1. We also show that mutations in GLR-1 that modify desensitization properties are able to partially overcome the dependence of receptor function on SOL-1. To obtain more direct mechanistic insights into SOL-1 and STG-1 function, we turned to reconstitution of receptor function in muscle cells of transgenic *C. elegans*, a tissue that does not normally express iGluRs (7). Rapid perfusion studies of cultured muscle cells from these transgenic worms revealed an essential role for SOL-1 in receptor desensitization, whereas STG-1 appears required for another step in receptor gating.

Results

Con-A and Mutations in GLR-1 Partially Compensate for the Absence of SOL-1. In *sol-1* mutants, GLR-1 is present at the cell surface but glutamate-gated current is disrupted, suggesting that SOL-1 regulates GLR-1 function (5). Indirect support for this idea came from behavioral and electrophysiological studies showing that a point mutation in GLR-1 (A687T), analogous to the mouse *lurcher* mutation, partially restored behavior as well as glutamate-gated currents in neurons of *sol-1* mutants (6). However, in these studies, we were unable to detect the mechanism by which SOL-1 contributed to GLR-1 function. To identify this mechanism, we turned to the study of reconstituted GLR-1 receptors in *Xenopus* oocytes. Coexpression of GLR-1, SOL-1, and STG-1 results in large, rapidly desensitizing currents in response to bath application of 1 mM glutamate, a saturating concentration (Fig. 1*A* and Fig. 6, which is published as supporting information on the PNAS web site) (31). However, if the receptor rapidly desensitized, we would underestimate the true current magnitude because of the relatively slow application of glutamate. We tested this hypothesis using two drugs, cyclothiazide and Con-A, previously shown to modulate the desensitization of non-NMDA-type iGluRs (8). When tested on oocytes expressing SOL-1, STG-1, and GLR-1, we found no effect with cyclothiazide (data not shown). Con-A however, increased the peak magnitude of glutamate-gated current and markedly slowed the kinetics of desensitization (Fig. 1 *A* and *G*). As reported earlier for kainate receptors (9), these effects of Con-A were blocked by first desensitizing receptors with glutamate and then applying Con-A in the continued presence of glutamate (Fig. 1*A*). Typically, we could not detect glutamate-gated current in the absence of SOL-1. However, in some experiments, we were able to record small, rapidly desensitizing currents from oocytes 5–8 days after injection. These currents were only slightly potentiated by Con-A (Fig. 1 *B* and *G*), and did not show obvious changes in desensitization. In the absence of STG-1, we could not detect glutamate-gated current in the presence or absence of Con-A (Fig. 1*C*). These data show that SOL-1 is required for Con-A to prevent desensitization.

Another means to slow desensitization of AMPARs is by introduction of a single amino acid change in the ligand binding domain of the receptor (10). The corresponding mutation in *C. elegans* GLR-1 is glutamine to tyrosine–GLR-1(Q552Y) (11). Glutamate-gated currents recorded from *Xenopus* oocytes that expressed

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Abbreviations: iGluR, ionotropic glutamate receptor; AMPA; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; Con-A, concanavalin-A.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. DQ015970).

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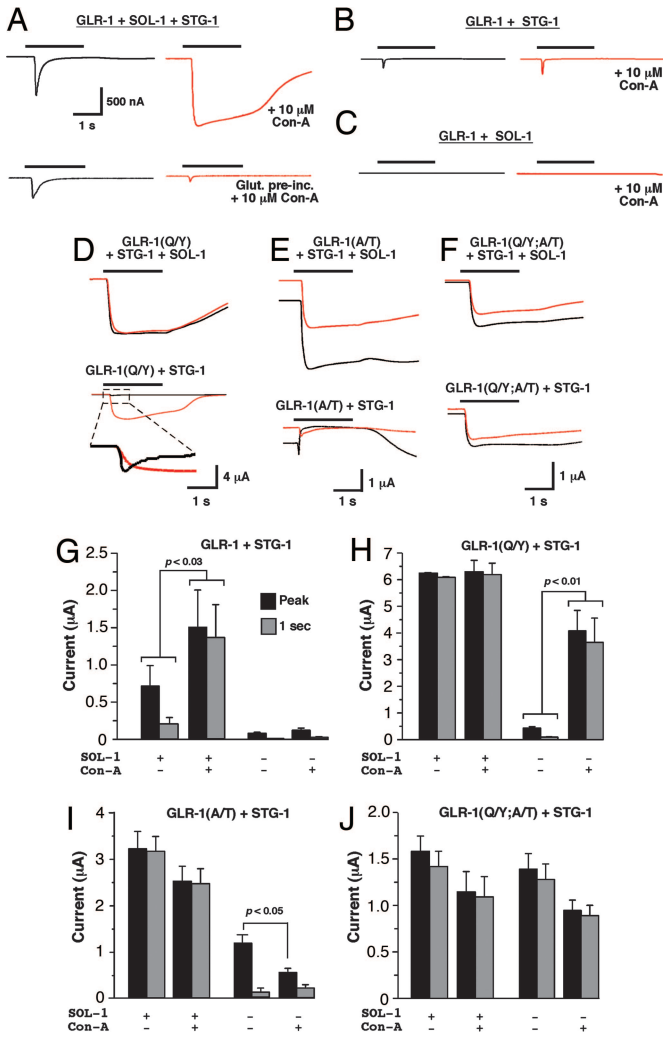

Fig. 1. Glutamate-gated current in the absence of either SOL-1 or STG-1 depends on modifying the desensitization of GLR-1. (*A–C*) Currents measured in response to 1 mM glutamate application in *Xenopus* oocytes coinjected with GLR-1, SOL-1, and STG-1 (*A*), GLR-1 and STG-1 (*B*), or GLR-1 and SOL-1 (C) cRNA before (black) and after (red) preincubation with Con-A. Preincubation in the presence of glutamate is also shown (*A*). (*D–F*) Currents measured in response to 1 mM glutamate application in *Xenopus* oocytes that expressed STG-1 and GLR-1(Q552Y) (*D*), GLR-1(A687T) (*E*), or GLR-1(Q552Y; A687T) (*F*) in the presence or absence of SOL-1 before (black) and after (red) preincubation with 10 μM Con-A. (*A–F*) Oocytes were voltage-clamped at −70 mV. (*G–J*) Average peak current (black) and current amplitude 1 s after the beginning of glutamate application (gray) for currents mediated by GLR-1, GLR-1(Q/Y), GLR-1(A/T), or GLR-1(Q/Y;A/T) coexpressed with STG-1 in the presence or absence of SOL-1.

GLR-1(Q552Y), STG-1, and SOL-1 showed almost no desensitization, and treatment with Con-A caused little change in peak current amplitude (Fig. 1 *D* and *H*). These results suggest that Con-A and the Q552Y mutation function to block the same pathway leading to desensitization. We observed dramatically reduced glutamate-gated currents when GLR-1(Q552Y) was expressed in the absence of SOL-1 (Fig. 1 *D* and *H*). However, in this case, treatment with Con-A increased the glutamate-gated current and slowed desensitization (Fig. 1 *D Inset* and *H*). This finding was in marked contrast to wild-type GLR-1, where Con-A had almost no effect on current amplitude in the absence of SOL-1 (compare Fig. 1 *D* with *B*). Thus, although Con-A's effect is normally dependent on SOL-1, it can be made independent of SOL-1 by introducing a non-desensitizing mutation (Q552Y) into GLR-1. The superadditive effects of Con-A and the Q552Y mutation, neither perturbation alone is sufficient to potentiate current in the absence of SOL-1, suggest that the receptor has parallel paths to a desensitized state(s) in the absence of SOL-1.

We have shown that the behavioral and electrophysiological defects of *sol-1* mutants can be partially suppressed by transgenic expression of the GLR-1(A687T) *lurcher* variant in *C. elegans* (5, 6). We examined the effect of the *lurcher* mutation on GLR-1-dependent currents by expressing GLR-1(A687T) in *Xenopus* oocytes together with SOL-1 and STG-1. We observed two major changes in the glutamate-gated current: the oocytes had a substantial leak current and the kinetics of desensitization were markedly slowed (Fig. 1 *E* and *I*). The GLR-1(A687T)-mediated current showed less dependence on SOL-1 than did GLR-1 or GLR-1(Q552Y). Oocytes that expressed GLR-1(A687T) and STG-1 in the absence of SOL-1 exhibited a significant leak current. The current further increased in response to glutamate but rapidly desensitized to a nonconducting state, such that the net current in the continued presence of glutamate was significantly smaller than the leak current recorded before glutamate application. After washout of glutamate, the current slowly increased in magnitude to the steady-state leak current (Fig. 1*E*). Curiously, Con-A pretreatment blocked the leak current in the presence or absence of SOL-1, but unlike for the Q552Y mutation, it did not potentiate current or have dramatic effects on desensitization in the absence of SOL-1.

We hypothesized that Q552Y and A687T might use distinct mechanisms to effect GLR-1 receptor gating. To test this, we introduced both Q552Y and A687T mutations into GLR-1 and coexpressed GLR-1(Q552Y; A687T), STG-1, and SOL-1 in oocytes. In contrast to oocytes that expressed GLR-1(A687T), these oocytes had reduced leak current. Compared to wild-type GLR-1, the kinetics of desensitization of glutamate-gated current were markedly slowed, and currents were not appreciably altered by pretreatment with Con-A (Fig. 1 *F* and *J*). Furthermore, oocytes that expressed GLR-1(Q552Y; A687T) and STG-1 in the absence of SOL-1 had only small leak currents and slow kinetics of desensitization that were not appreciably modified by Con-A (Fig. 1 *F* and *J*). Thus, GLR-1(Q552Y; A687T) with two different mutations that affect receptor kinetics, resulted in large, non-desensitizing currents. Together, these mutations bypassed the need for SOL-1 and eliminated the effects of Con-A treatment.

***Drosophila* SOL-1 Can Functionally Substitute for *C. elegans* SOL-1.** SOL-1 has an important role in *C. elegans* GLR-1 function. Are functional homologues present in other species? Using a BLAST search, we identified a promising partial predicted sequence (CG31218) in *Drosophila*, and using RACE we isolated a complete cDNA (*Dro* SOL1) with ≈25% amino acid identity to *C. elegans* SOL-1 (Fig. 2*A*). The predicted protein appears to have the same domains as found in *C. elegans* SOL-1, including four CUB domains and a transmembrane domain. To test whether *Dro* SOL1 could substitute for *C. elegans* SOL-1, we recorded currents from oocytes that expressed *C. elegans* GLR-1, STG-1, and either *Ce* SOL-1 or *Dro* SOL1. In the absence of *Ce* SOL-1 or *Dro* SOL1, we could record only small currents. However, the current magnitude increased dramatically with coexpression of either *Ce* SOL-1 or *Dro* SOL1 (Fig. 2 *B* and *C*). In addition, Con-A pretreatment dramatically slowed the desensitization kinetics. These data show that *Drosophila* SOL1 can functionally substitute for *C. elegans* SOL-1 and suggest that SOL-1-like proteins are likely to have important roles in insect glutamatergic neurotransmission.

GLR-1 Function in Transgenic Muscle Cells Depends on STG-1 and SOL-1. Our studies in *Xenopus* oocytes lead to the hypothesis that receptor desensitization is influenced by SOL-1. Unfortunately, further study of desensitization kinetics is not feasible in *Xenopus* oocytes because agonists cannot be rapidly applied to the entire cell membrane. The membrane receptor density was not sufficient for studies of isolated membrane patches, so we sought to study

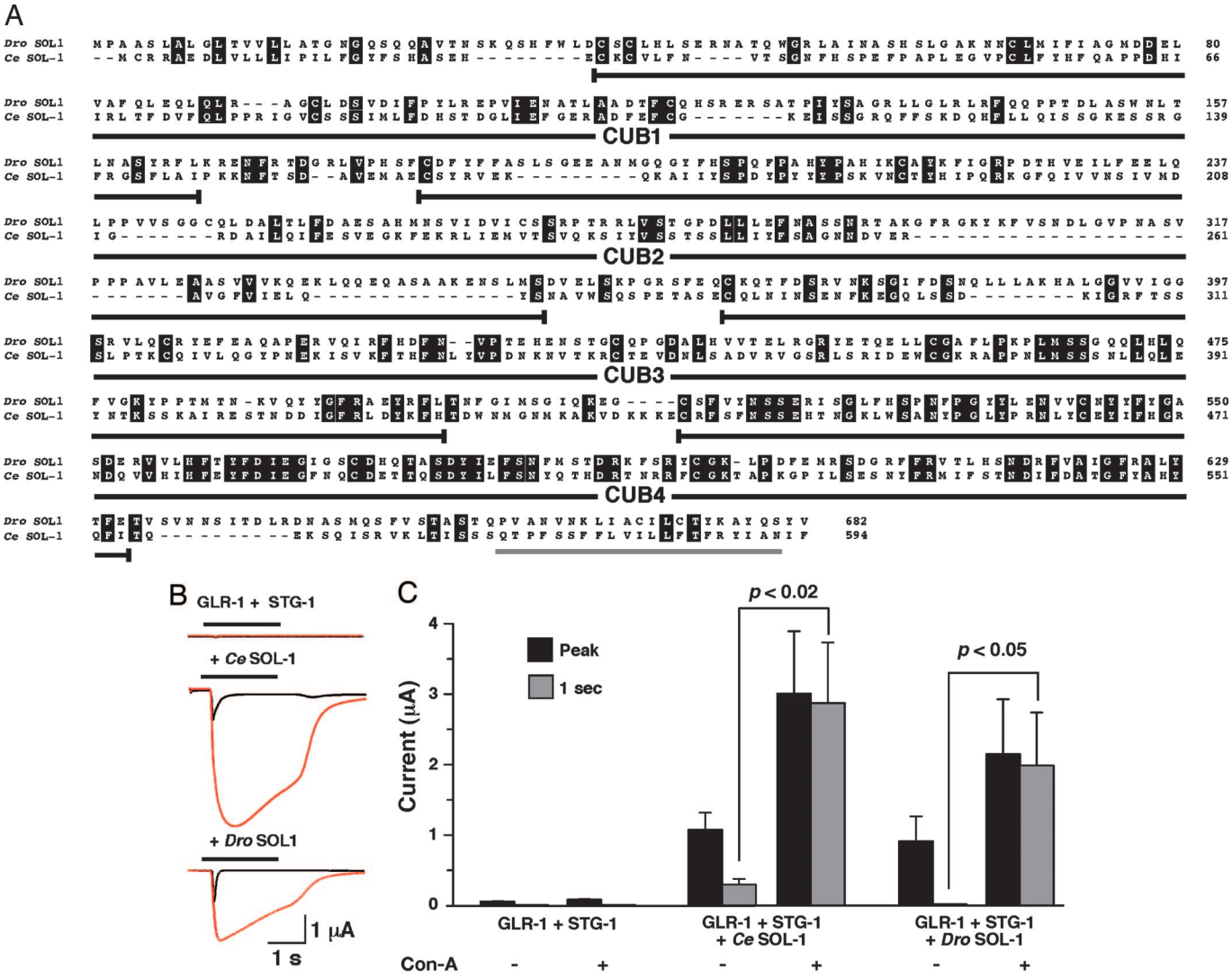

Fig. 2. *Drosophila* SOL1 modulates *C. elegans* GLR-1-mediated current. (*A*) The predicted amino acid sequences encoded by *C. elegans sol-1* and *Drosophila sol1*. Amino acids are numbered beginning with the first predicted methionine. Predicted transmembrane domains at the C terminus and CUB domains are indicated by gray and black underlines, respectively. (*B*) Currents measured in response to 1 mM glutamate application in *Xenopus* oocytes that expressed GLR-1 and STG-1 or GLR-1 and STG-1 coexpressed with either *Ce* SOL-1 or *Dro* SOL1 before (black) and after (red) preincubation with Con-A. Oocytes were voltage-clamped at −70 mV. (*C*) Average peak current (black) and current 1 s after the beginning of glutamate application (gray).

reconstituted receptors in the commonly used HEK 293 tissue culture cells. However, we were unable to record currents from these cells. Therefore, we turned to expression in a more easily accessible cell type in *C. elegans*.

C. elegans muscle cells receive cholinergic and GABAergic inputs (12), but have no known glutamatergic inputs nor do they express GLR-1 (13, 14), the AMPAR subunit GLR-2 (7, 15), SOL-1 (5), or STG-1 (31). Therefore, muscle cells are ideal for reconstitution of GLR-1 function. Moreover, muscle cells offer greater experimental accessibility and a genetic background more easily manipulated than those in typical cell culture lines or *Xenopus* oocytes. When the *C. elegans* iGluR subunits GLR-1 and GLR-2 were expressed in muscle with either SOL-1 or STG-1, we observed no current responses to pressure application of glutamate (Fig. 3 *A* and *B*). However, we found that we could record glutamate-gated currents when GLR-1 and GLR-2 were coexpressed with both SOL-1 and STG-1 (Fig. 3 *C* and *E*). We also found that GLR-2 was not absolutely required for glutamate-gated current (Fig. 3 *D* and *E*).

To determine whether the dramatic increase in glutamate-gated current with coexpression of STG-1 was secondary to an increase in either GLR-1 or SOL-1 surface expression, we assessed the surface expression of functional hemagglutinin-A (HA) and GFP tagged GLR-1 (HA::GLR-1::GFP) and GFP tagged SOL-1 (GFP::SOL-1) in both the absence and presence of STG-1. Under nonpermeabilized conditions and in the absence of STG-1, both GLR-1 and SOL-1 were expressed on the surface of muscles and concentrated at the tips of muscle arms that extend to the ventral nerve cord (Fig. 4 *A–C*). Coexpression of GLR-1 with either STG-1 (Fig. 4*D*) or both STG-1 and SOL-1 (Fig. 4*E*) did not produce an obvious change in GLR-1 surface expression levels. Therefore, the dramatic increase in glutamate-gated current observed when GLR-1 was coexpressed with STG-1 and SOL-1 could not be explained by changes in cell surface expression. We observed no staining when the HA epitope was placed at an intracellular location (Fig. 4*F*). These data suggest that both SOL-1 and STG-1 are required for GLR-1 function independent of surface delivery.

Vertebrate stargazin is known to associate with AMPARs (16, 17). This finding suggests that *C. elegans* STG-1 should colocalize

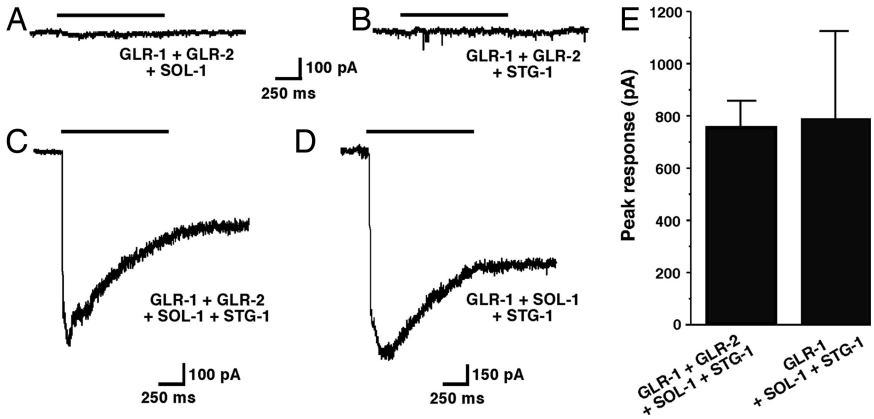

Fig. 3. Glutamate-gated currents in transgenic *C. elegans* muscles require STG-1 and SOL-1. Patch-clamp current records measured in response to pressure application of 1 mM glutamate to body wall muscle cells that expressed GLR-1, GLR-2, and SOL-1 (*A*), GLR-1, GLR-2, and STG-1 (*B*), GLR-1, GLR-2, SOL-1, and STG-1 (*C*), and GLR-1, SOL-1, and STG-1 (*D*). (*E*) Average peak glutamate-gated current amplitude. Muscle cells were voltage-clamped at −60 mV.

with GLR-1 in muscle cells. Coexpression of CFP-tagged GLR-1 and YFP-tagged STG-1 showed that the two molecules colocalized at the tips of muscle arms (Fig. 4*G*), the site of synapse formation, providing additional evidence that STG-1 and GLR-1 may participate in a functional receptor complex (31). The topological arrangement of vertebrate stargazin has the N- and C-terminal regions intracellular and the loop between the first and second transmembrane domains (TMD) extracellular. By tagging STG-1 with GFP at the loop between TMD1 and TMD2 (GFP::STG-1) (Fig. 4*H*), or near the C terminus (STG-1::GFP) (Fig. 4*I*), and using antibody detection of GFP at the cell surface, we show that the topology of *C. elegans* STG-1 is consistent with that of vertebrate transmembrane AMPAR regulatory proteins.

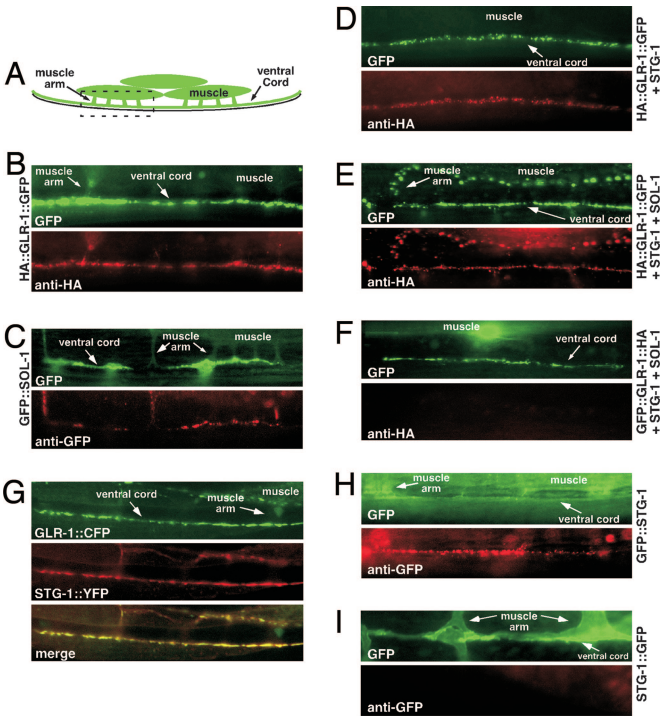

Fig. 4. GLR-1, SOL-1, and STG-1 are expressed on the surface of transgenic body wall muscle cells. (*A*) Schematic of body wall muscles, including muscle arms and the ventral cord. (*B–F*) Images of transgenic worm body wall muscle cells that expressed various combinations of HA::GLR-1::GFP, GFP::GLR-1::HA, GFP::SOL-1, SOL-1, and STG-1 as indicated using the *myo-3* muscle-specific promoter. Transgenic worms were injected with Alexa Fluor 594 conjugated anti-HA or anti-GFP to detect surface expression of GLR-1 or SOL-1. (*G*) Images of a transgenic worm that expressed GLR-1::CFP and STG-1::YFP in body wall muscle cells. (*H* and *I*) Images of transgenic worms injected with Alexa Fluor 594 conjugated anti-GFP that expressed either GFP::STG-1 (*H*) or STG-1::GFP (*I*) in body wall muscle cells.

SOL-1 Slows the Desensitization of GLR-1. Two lines of evidence suggest that SOL-1 has an effect on the desensitization of GLR-1. First, mutations in GLR-1, Q552Y and A687T, known to modify the kinetics of desensitization, lessen the dependence of glutamate-gated currents on SOL-1. Second, Con-A, a drug that slows desensitization, when applied to GLR-1(Q552Y), restores glutamate-gated current in the absence of SOL-1. To directly test the hypothesis that the rate of desensitization depends on SOL-1, we rapidly applied glutamate using piezoelectric switching (18, 19) to cultured muscle cells dissociated from worms (20) that expressed the various combinations of GLR-1, SOL-1, and STG-1. The rise time for solution exchange using cultured cells ranged from 0.6 to 3.0 ms as estimated from open-tip potentials. When all three proteins were coexpressed in cultured muscle cells, we observed a rapidly desensitizing current that decayed to a significant plateau current with a time constant of ≈7 ms (Fig. 5 *A* and *B*). In contrast, the glutamate-gated current from muscle cells that expressed GLR-1 and STG-1 almost completely desensitized within 2 ms, leaving a dramatically reduced plateau current (Fig. 5*A Inset*). We found that the average current magnitude appeared smaller in the absence of SOL-1, but this difference was not statistically significant and may have been a consequence of rapid desensitization (Fig. 5*C*). We found no difference in the recovery from desensitization in the presence or absence of SOL-1 when repeated applications of glutamate were separated by 2- to 3-s intervals (Fig. 7, which is published as supporting information on the PNAS web site), indicating that the receptor does not enter into a long-lived desensitized state. However, the absence of a steady-state current in cells that coexpress GLR-1 and STG-1 suggested that more rapid recovery from desensitization might be affected by SOL-1. In support of this hypothesis, we noted a prolonged time course of recovery from desensitization in the absence of SOL-1 (Fig. 5*E*). Our results demonstrate that GLR-1 can open to a conducting state in the absence of SOL-1, but rapidly and completely desensitizes and is slow to recover from desensitization, thus explaining our failure to record glutamate-gated currents using slower bath or pressure application of glutamate.

Previously, we showed that mutants that expressed a hypomorphic allele of SOL-1 (G323H), had less severe behavioral phenotypes than *sol-1* null mutants (6). In these G323H mutants, we could

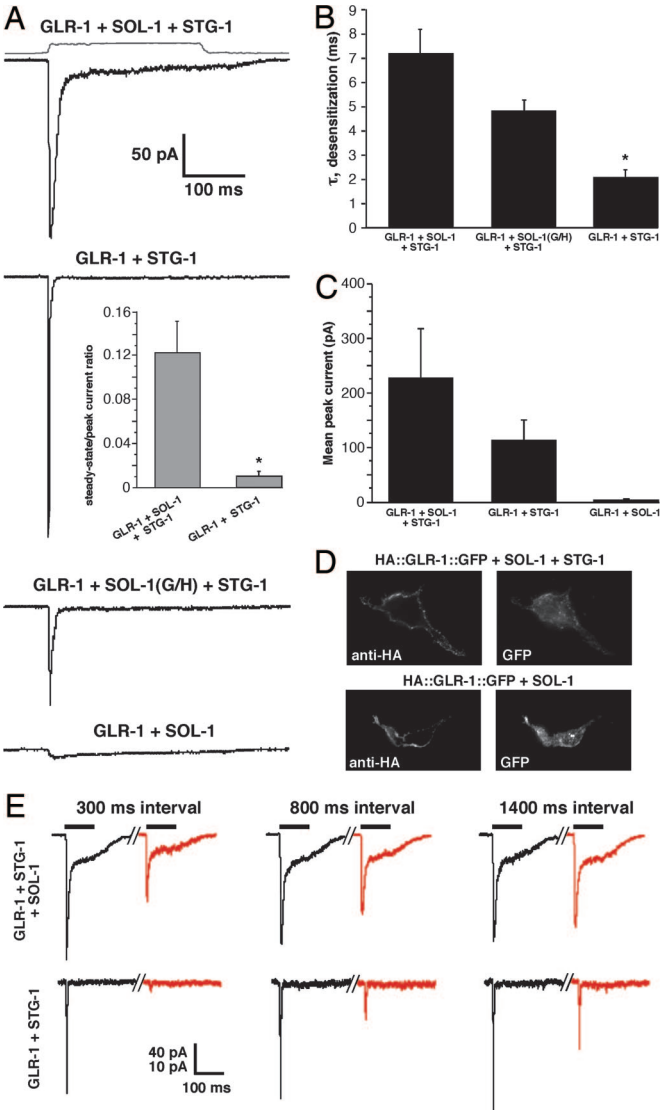

Fig. 5. Rapid glutamate perfusion reveals a rapidly desensitizing current in the absence of SOL-1. (*A*) Currents measured in response to rapid application of 1 mM glutamate to cultured muscle cells that expressed combinations of GLR-1, STG-1, SOL-1, and SOL-1(G323H) as indicated. Traces represent the average of 5–10 consecutive responses. (*Inset*) The average ratio of steady-state to peak current. The steady-state value represents current measured 100 ms after the onset of glutamate application. Asterisk indicates significant difference from cells that expressed GLR-1, SOL-1, and STG-1, $P < 0.01$. (*B*) Average time constant of desensitization of currents measured from cultured muscle cells. Asterisk indicates significantly different from cells that expressed GLR-1, SOL-1 and STG-1; or GLR-1, SOL-1(G/H) and STG-1, $P < 0.01$. (*C*) Average peak current amplitudes. (*D*) Images of muscle cells cultured from transgenic worms that expressed HA::GLR-1::GFP, SOL-1 and STG-1 (*Upper*) or HA::GLR-1::GFP and SOL-1 (*Lower*). Shown are anti-HA staining and HA::GLR-1::GFP fluorescence under nonpermeabilized conditions. (*E*) Currents measured in response to rapid application of 3 mM glutamate (bar) to cultured muscle cells that expressed GLR-1, STG-1 and SOL-1, or GLR-1 and STG-1. Paired current traces (black and red) represent the average of three to five consecutive responses separated by the indicated interval.

also record glutamate-gated currents from the AVA interneurons, although the peak amplitudes were considerably smaller than those measured in wild-type worms. To assess the effects of SOL-1(G323H) on GLR-1 function, we recorded glutamate-gated currents from cultured muscle cells that expressed GLR-1, SOL-1(G323H), and STG-1. These rapid-perfusion experiments revealed an intermediate rate of desensitization compared to muscle cells that expressed all three wild-type proteins or in the absence of SOL-1 (Fig. 5 *A* and *B*).

Even with rapid perfusion, we could not record fast glutamate-gated currents from cultured muscle cells that expressed GLR-1 and SOL-1. However, we sometimes observed a very small, slow current (3.4 ± 1.4 pA; $n = 6$) (Fig. 5*A*). We earlier showed that GLR-1 was expressed at the cell surface in the absence of STG-1 using *in vivo* labeling techniques (Fig. 4). We now show using *in vitro* antibody staining that STG-1 does not obviously affect surface expression of HA::GLR-1::GFP in cultured muscle cells. Thus, changes in surface expression cannot explain the large increase in current caused by coexpression of STG-1 (Fig. 5*D*).

Discussion

We have shown that SOL-1 has an essential role in the desensitization of *C. elegans* GLR-1 receptors. In *Xenopus* oocytes, we showed that the effects of a drug known to reduce iGluR desensitization, the plant lectin Con-A, required coexpression of SOL-1 with GLR-1. Furthermore, we demonstrated that mutations in GLR-1 that are known to slow receptor desensitization bypass the requirement for SOL-1. Based on these results, we hypothesized that SOL-1 regulates GLR-1 desensitization. We tested this hypothesis by using rapid perfusion of glutamate to measure currents from cultured muscle cells expressing combinations of GLR-1, SOL-1, and STG-1. In the absence of SOL-1, the GLR-1 receptors opened, but desensitized rapidly and completely, and recovered more slowly from desensitization.

Our work shows that *C. elegans* muscle cells have significant advantages for the study of ion channel function. We can reconstitute channel function in a defined cell type with well understood gene expression in which we can manipulate the genetic background with relative ease. Using our transgenic approach, we provide direct evidence that SOL-1 primarily acts by slowing the rate of receptor desensitization. In contrast, STG-1's precise role is less clear. In the absence of STG-1, either GLR-1 desensitization occurs too quickly to be detected by our recording techniques or the receptor is not competent to enter into the conventional open state. The lack of glutamate-gated current could not be explained by failure of receptor surface delivery because GLR-1 was found at the surface in the absence of the auxiliary proteins. Thus, both SOL-1 and STG-1 regulate fundamental aspects of iGluR function. A recent study has shown that vertebrate STG primarily interacts with the transmembrane region of iGluRs (16), whereas we have previously shown that the extracellular region of SOL-1 is required for its function (6). Thus, the differential effects of STG-1 and SOL-1 on GLR-1 may result from interactions with distinct structural elements of the receptor.

We also identified a SOL-1 homologue in a distantly related species, *Drosophila*. *Dro* SOL1 could substitute for *C. elegans* SOL-1 to dramatically enhance glutamate-gated currents. Interestingly, coexpression of *Dro* SOL1 appeared to change the kinetics of GLR-1 desensitization when compared to coexpression with *C. elegans* SOL-1. Because we have now identified a SOL-1 homologue in *Drosophila*, we suspect that similar proteins may also function in other species and may play a conserved role in modulating receptor desensitization.

We found that mutations in GLR-1 that slowed desensitization partially bypassed the requirement for SOL-1. Furthermore, the effects of Con-A treatment were critically dependent on the nature of the mutation in GLR-1. When GLR-1(Q552Y) and STG-1 were coexpressed only small currents could be recorded. However, pretreatment with Con-A restored large, non-desensitizing current responses to glutamate application. In contrast, with GLR-1(A687T) and STG-1 coexpression, fast glutamate-gated currents were reliably recorded, but pretreatment with Con-A did not potentiate currents. Interestingly, the currents we measured with the Q552Y;A687T double mutant were nearly the same in the

presence or absence of SOL-1, suggesting that these two mutations affect different pathways that lead to desensitization.

How might SOL-1 change the rate of GLR-1 desensitization? The extracellular domains, S1 and S2, of iGluR subunits (and presumably GLR-1) are arranged in a clamshell like arrangement. After binding of glutamate, these domains undergo a conformational change that by an unknown process leads to the opening of the channel pore (21–23). SOL-1 effects on gating may also modify the apparent receptor affinity for glutamate, but it is now recognized that separating changes in affinity from gating effects is not easily realized (24). After channel opening, the S1 and S2 domains further rearrange while bound to ligand (25), leading to desensitization and closure of the channel. How S1–S2 rearrangement leads to channel closure is still not understood. SOL-1 may stabilize the open state by slowing the rearrangement of the dimer interface, thus increasing the current conducted by the ligand-bound receptor.

Materials and Methods

General Methods and Strains. All strains were raised at 20°C under standard conditions. Germ-line transformation was achieved by using pJM23 as a transformation marker (13). *lin-15(n765ts)* mutants were used in all transgenic experiments and expressed the following extrachromosomal arrays: *akEx357*, pYZ147 (*Pmyo-3*::GFP::SOL-1) + pYZ150 (*Pmyo-3*::GLR-1) + pYZ220 (*Pmyo-3*::GLR-2); *akEx533*, pYZ150 + pYZ220 + pDM743 (*Pmyo-3*::STG-1::GFP); *akEx503*, pYZ147 + pYZ150 + pYZ220 + pDM743; *akEx534*, pYZ147 + pYZ150 + pDM743; *akEx530*, pYZ318 (*Pmyo-3*::HA::GLR-1::GFP); *akEx570*, pYZ147; *akEx569*, pDM930 (*Pmyo-3*::GFP::GLR-1::HA) + pDM796 (*Pmyo-3*::STG-1) + pYZ146 (*Pmyo-3*::SOL-1); *akEx566*, pDM860 (*Pmyo-3*::STG-1::YFP) + pDM914 (*Pmyo-3*::GLR-1::CFP); *akEx565*, pDM861 (*Pmyo-3*::GFP::STG-1), *akEx584*, pDM743; *akEx573*, pYZ318 + pYZ146; *akEx567*, pYZ318 + pDM796; *akEx568*, pYZ318 + pDM796 + pYZ146; *akEx648*, pYZ150 + pDM796 + pDM1036 (*Pmyo-3*::SOL-1(G323H)::GFP). All constructs containing the *myo-3* promoter included 1.8kb of *myo-3* sequence (26). We isolated the full-length *Drosophila sol1* cDNA by PCR amplification from *Drosophila melanogaster* first-strand cDNAs (GenBank accession no. DQ015970). Analysis of predicted proteins was facilitated by the ExPASy suite of programs and CLUSTALW (27).

Additional Plasmid Constructs. The oocyte expression plasmids were as follows: pDM657, *glr-1*; pDM350, *C. elegans sol-1*; pDM654, *stg-1*; pDM862, *glr-1(A687T)*; pDM858, *glr-1(Q552Y)*; pDM863, *glr-1(Q552Y; A687T)*; pDM940, *Drosophila sol1*.

Immunolabeling and Microscopy. Immunolabeling in live worms followed previously developed methods (5, 28). In brief, Alexa Fluor 594-conjugated rabbit anti-GFP or anti-HA polyclonal sera (Molecular Probes) was diluted (1:200) in worm injection buffer and injected into the pseudocoelom of transgenic worms. All labeling experiments were repeated at least four times. Immunolabeling of primary cultured muscle cells was achieved by fixing cells for 30 min in 3% formaldehyde in egg buffer (118 mM NaCl/48 mM KCl/2 mM $CaCl_2$/2 mM $MgCl_2$/24 mM Hepes, 334 mOsm). After blocking with 3% milk for 1 h, cells were labeled by using affinity-purified mouse anti-HA primary antibody (1:500) (monoclonal cell 12CA5, University of Utah Antibody Core Facility) followed by Alexa Fluor 594-conjugated donkey anti-mouse secondary antibody (1:450) (Molecular Probes). Images were acquired by using a Zeiss compound microscope with a Roper CoolSnap camera or using confocal microscopy with a Zeiss LSM 510.

Primary Cultures of *C. elegans* Muscle Cells. Muscle cells were cultured as described (20) from transgenic worms that expressed various combinations of proteins under the regulation of the *myo-3* muscle-specific promoter. GFP was used as a marker to identify muscle cells from other cell types.

Electrophysiological Studies. We made electrophysiological recordings of ligand-gated currents from body wall muscles using standard patch–clamp technology as described (29). All electrophysiological experiments were repeated three to eight times. *Xenopus* oocyte recordings were carried out by using standard two-electrode voltage clamp as described (30). To reduce receptor desensitization, oocytes were pretreated for 8 min with a 10 μM concentration of the lectin Con-A, as described (30). Rapid perfusion experiments followed published protocols (18, 19). Drug and control solution were delivered by theta tube mounted on a piezoelectric manipulator (Burleigh). The rate of solution exchange was measured as the change in open-tip potential. Statistical significance was determined by using the standard Student's *t* test. Error bars represent the SEM.

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