Glutamate receptors are not only abundant and important mediators of fast excitatory synaptic transmission in vertebrates, but they also serve a similar function in invertebrates such as Drosophila and the nematode Caenorhabditis elegans. In C. elegans, an animal with only 302 neurons, 10 different glutamate receptor subunits have been identified and cloned. To study the ion channel properties of these receptor subunits, we recorded glutamate-gated currents from Xenopus oocytes that expressed either C. elegans glutamate receptor subunits or chimeric rat/C. elegans glutamate receptor subunits. The chimeras were constructed between the C. elegans glutamate receptor pore domains and either the rat kainate receptor subunit GluR6, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunit GluR1, or the N-methyl-D-aspartate (NMDA) receptor subunit NMDAR1-1a. Although native subunits were nonfunctional, 9 of 10 ion pores were found to conduct current upon transplantation into rat receptor subunits. A provisional classification of the C. elegans glutamate receptor subunits was attempted based on functionality of the chimeras. C. elegans glutamate receptor ion pores, at a position homologous to a highly conserved site critical for ion permeation properties in vertebrate glutamate receptor pores, contain amino acids not found in vertebrate glutamate receptors. We show that the pore-constricting Q/R site, which in vertebrate receptors determines calcium permeability and rectification properties of the ion channel, in C. elegans can be occupied by other amino acids, including surprisingly, lysine and proline, without loss of these properties.

Glutamate serves as an important neurotransmitter mediating rapid excitatory synaptic transmission via a large and diverse number of postsynaptic ionotropic glutamate receptors in both vertebrate and invertebrate nervous systems. In vertebrates, distinct receptor subunits contribute to mature heteromeric receptors, which have properties that depend on the particular combination of receptor subunits (1). Functional receptors are classified according to their response to selective agonists. Thus, glutamate receptors are grouped into two major categories: non-NMDA receptors, which include α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors and kainate (KA) receptors, and N-methyl-D-aspartate (NMDA) receptors (1, 2). This pharmacological classification is supported by the analysis of amino acid sequence identities of receptor subunits (1).

Invertebrate nervous systems utilize multiple subtypes of glutamate receptor subunits. Analysis of the genomes of both Drosophila melanogaster (3) and Caenorhabditis elegans (4) by sequence similarity searches (BLAST) has revealed large families of genes comprising at least 10 genes encoding putative ionotropic glutamate receptor subunits. Three of these genes from C. elegans, glr-1 (5, 6), nmr-1 (7), and glr-2 (8), have been characterized in detail. glr-1 and glr-2 encode subunits most similar to mammalian non-NMDA receptor subunits, whereas nmr-1 encodes a subunit most similar to mammalian NMDA receptor subunits. Glutamate-gated, kainate-gated, and NMDA-gated currents could be recorded in vivo from a defined interneuron, and mutations in receptor subunits had specific effects on glutamate-gated currents (7, 8). For the remaining seven receptor subunits, partial cDNAs have been cloned (4).

All vertebrate ionotropic glutamate receptors presumably have the same topology, namely three transmembrane domains (TMD) A, B, and C (also called M1, M3, and M4, respectively), a pore-forming region (P-loop, also called M2) located between TMDs A and B, an extracellular N terminus, and an intracellular C terminus (Fig. 1). The ligand binding sites are made up of two interacting domains, S1 and S2, that flank the pore-forming region (9). Short stretches of amino acids (linkers A, B, and C) connect the S1 domain to TMD A and the S2 domain to TMDs B and C (Fig. 1). On the basis of sequence conservation, the C. elegans glutamate receptor subunits appear to have the same topological organization as vertebrate receptors (4).

In this study we show that the pore-forming domains of C. elegans glutamate receptors have conserved features and behave as functional ion permeation modules. Although the rules for receptor assembly and function are complex, the rules for pore function are more straightforward. We have cloned full-length cDNAs of the 7 remaining C. elegans glutamate receptors and expressed individual receptor subunits in Xenopus oocytes. We also constructed chimeras between three rat glutamate receptor subunits (GluR) (the kainate receptor subunit

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1 The abbreviations used are: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; CaR, calcium Ringer; ConA, concanavalin A; GLR, C. elegans glutamate receptor; GluR, glutamate receptor; MgR, magnesium Ringer; NFR, solution, normal frog Ringer’s solution; NMDA, N-methyl-D-aspartate; NMR, C. elegans NMDA receptor; PCS, pore cassette small; TMD, transmembrane domain.
GluR6, the AMPA receptor subunit GluR1, and the NMDA receptor subunit NMDAR1-1a) and the ion pores of all 10 C. elegans receptor subunits. These studies demonstrated a remarkable conservation of pore function and revealed unexpected insights into determinants of ion permeation.

EXPERIMENTAL PROCEDURES

Mutagenesis—The rat kainate receptor subunit GluR6(Q) and a non-desensitizing mutant of the rat AMPA receptor GluR1 (GluR1(L479F)) (10), as well as C. elegans subunits GLR1 to GLR8, NMR1 to NMR2 (4), were used for construction of glutamate receptor subunit chimeras. To enable transplantation of domains, identical restriction sites were introduced at homologous positions in all cDNAs by PCR-mediated site-directed mutagenesis using mutagenic primers as described previously (11). All mutated clones were sequenced across the PCR-amplified regions. We used two introduced restriction sites at homologous positions in all cDNAs of wild type GluR6, GluR1(L479F), and NMDAR1 (splice variant 1a) in some cases causing alterations of amino acids (see Fig. 1). The resulting constructs were named GluR6-PGLR1, GluR6-PGLR2, GluR6-PGLR3, GluR6-PGLR4, GluR6-PGLR5, GluR6-PGLR6, GluR6-PGLR7, GluR6-PGLR8, GluR6-PNMR1, GluR6-PNMR2, GluR1 (L479F)-PGLR1, GluR1(L479F)-PGLR2, GluR1(L479F)-PGLR3, GluR1(L479F)-PGLR4, GluR1(L479F)-PGLR5, GluR1(L479F)-PGLR6, GluR1(L479F)-PGLR7, GluR1(L479F)-PGLR8, GluR1(L479F)-PNMR1, GluR1(L479F)-PNMR2, and NMDAR1-PNMR1, respectively, where P stands for pore.

cRNA Synthesis—cRNA synthesis was performed as described earlier (11). Briefly, template DNA was linearized with a suitable restrict-
tion enzyme. cRNA was synthesized from 1 μg of linearized DNA using an *in vitro* transcription kit (Stratagene), with a modified protocol that employs 800 μM [γ-32P]UTP (Pharmacia) for capping and an extended reaction time of 3 h with T7 polymerase. Trace labeling was performed with [32P]UTP to allow calculation of yields and evaluation of transcript quality by agarose gel electrophoresis.

*Electrophysiological Measurements in Xenopus Oocytes—*Oocytes of stages V–VI were surgically removed from the ovaries of *C. elegans* as described elsewhere (13). Oocytes were injected with 10 ng of cRNA using a 10-μl Drummond (Broomall, PA) micropipette. Two-electrode voltage clamp recordings were performed 4–8 days after cRNA injection with a TurboTec 10CD amplifier (npi, Tamm, Germany). Glutamatergic agonists (300 μM) were prepared in normal frog Ringer’s solution (NFR) and were boiled in 40 mM NaCl, 1.5 MgCl2, 2.5 KCl, and 10 HEPES-NaOH, pH 7.2). Currents were filtered with 3 μs Cfilter and had resistances of ~0.5–1.5 megohms. Voltage electrodes were filled with 3 M KCl and had resistances of ~4 megohms. Oocytes were held at −70 mV, and agonists (kainate, glutamate, and AMPA) were applied by superfusion for 10 s at a flow rate of 10–14 μl/min. To minimize receptor desensitization, bath pretreatment of oocytes with cancavalin A (ConA, 10 μM for 8 min) preceded agonist application when recording from GluR6 mutants (13). Current-voltage relationships (I/V curves) were recorded in magnesium Ringer (MgR) (in mM, 115 NaCl, 1.5 MgCl2, 2.5 KCl, and 10 HEPES-NaOH, pH 7.2), employing 2-s ramps from −150 mV to +50 mV. Calcium permeability was analyzed in CaR (calcium Ringer, in mM, 115 NaCl, 1.5 CaCl2, 2.5 KCl, and 10 HEPES-CaOH2, pH 7.2). All agonists were prepared in the particular Ringer solution used in the experiment: measurements in NFR had agonists prepared in NFR, measurements in MgR had agonists prepared in MgR, and measurements in CaR had agonists prepared in CaR. To exclude contributions to the maximal amplitudes of endogenous calcium-activated chloride channels triggered by calcium influx through glutamate receptors, we did a series of control experiments and preinjected oocytes with 50 μl of 200 mM EGTA, pH 8.0, 20 min prior to recording agonist-activated currents. This injection achieved an intracellular concentration of ~20 mM EGTA, which assures efficient complexation of any incoming calcium before endogenous chloride channels can be activated (14).

**Surface Labeling of Cell Surfaces Using Biotinylated ConA—**To identify the fraction of receptor protein inserted in the plasma membrane, surface proteins were tagged with biotinylated ConA and isolated by streptavidin-Sepharose-mediated precipitation of the biotinyl-ConA-protein complex. Briefly, intact oocytes were incubated in 10 μM biotinyl-ConA (Sigma) for 30 min at room temperature. After five 10-min washes in NFR, intact oocytes were homogenized with a Teflon pestle in H buffer (20 μl/oocyte; 100 mM NaCl, 20 mM Tris-HCl, pH 7.4, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride plus a mixture of protease inhibitors (Complete™ tablets, Roche Applied Science)) and were kept at 4 °C for 1 h on a rotator. After centrifugation for 60 s at 16,000 × g, the supernatants were supplemented with 20 μl of washed streptavidin-Sepharose beads (Sigma) and incubated at 4 °C for 4 h on the rotator. The streptavidin-Sepharose beads were then pelleted by a 120-s spin at 1,600 × g and washed three times in H-buffer. The final pellets were boiled in 40 μl of SDS-PAGE loading buffer (0.8 M β-mercaptoethanol, 6% SDS, 20% glycerol, 25 mM Tris-HCl, pH 6.8, 0.1% bromphenol blue) for 10 min.

**Gel Electrophoresis and Western Blotting—**Proteins were separated on 20-cm SDS-polyacrylamide gels (11). Gels were blotted (11) onto Hybond enhanced chemiluminescence (ECL) nitrocellulose membranes (Advance MFS, Inc.). Following an established protocol (Bio-Rad, semi-dry electrophoretic transfer), an affinity-purified polyclonal GLR1 antibody (dilution 1:200) directed against a 22-amino acid fragment of an extracellular loop domain of GLR1 (4) was used along with a goat anti-rabbit antibody (dilution 1:5,000) conjugated with horseradish peroxidase to detect GLR1.

**Molecular Modeling—**The KcsA channel structure (15) was retrieved from the Protein Data Bank (1BLS). Three-dimensional structural models of the pore domains of glutamate receptors were constructed based on the crystal structure of the corresponding domain of KcsA. The glutamate receptor models GLR7 and GLR8 were generated by virtual mutagenesis of the individual residues leaving the backbone structures intact. The subunits of GluR6(Q), GluR6(R), GLR2, and GLR5 could not be modeled by direct homology to KcsA because of a deletion within the selective filter (see Fig. 6A, alignment). In these cases, the position and direction of the α-helical segment of the pore domain were fixed. The hydroxide bond between a nitrogen of Thr (67 in KcsA) of the pore helix and a side chain oxygen of Asp/Glu (80 in KcsA) were kept by maintaining these residues in almost identical position. This resulted in different conformations of the flexible selectivity filter in the models for GluR6(Q) and GluR6(R) compared with GLR2 and GLR5, which have a deletion in the selectivity filter. The δφ angles of these selectivity filters were adapted to favorable positions using Swiss PDB Viewer 3.7 (b2) (16). A 4-fold symmetry was then built, and the individual subunits were moved ~0.2 nm away from the central axis of the selectivity filter to increase the pore diameter to around 0.75–8 nm (the largest ion conducted by AMPA receptors is 0.75 nm in diameter (17)). All models were energy-optimized (Gromos96). Manipulations were performed using Swiss PDB Viewer 3.7 (b2) (16). The set of assumptions of an increased pore diameter, a maintained pore helix angle, and a fixed position of residues homologous to Asp-80 in KcsA was used previously for modeling of GLR1 (18). Because this approach neglects ion-water interactions, the remaining parts of the receptor and the lipids of the membrane, the proposed models have known limitations (18).

**RESULTS**

We individually expressed each of the ten native *C. elegans* glutamate receptor subunits (GLR1 through GLR8, and NMR1 to NMR2) in *Xenopus* oocytes and tested for GLU-, kainate-, or AMPA-evoked currents while voltage clamping the oocyte at −70 mV. NMR1 and NMR2 were additionally tested for GLU + glycine-activated currents. Surprisingly, we were unable to record any agonist-evoked currents from any of the *C. elegans* subunits. We also tested for one subunit, GLR1, in human embryonic kidney cells rather than *Xenopus* oocytes, to determine whether currents could be recorded. Again, we could not evoke currents, either from GLR1 alone or from GLR1 coexpressed with GLR2 in human embryonic kidney cells (data not shown). To test whether the apparent lack of agonist-evoked currents was simply the result of rapid desensitization kinetics, we applied agonist after preincubation with ConA (10 μM), or we coinjected agonist with cyclothiazide (100 μM). ConA and cyclothiazide are inhibitors of desensitization for kainate and AMPA receptors, respectively (19). We also varied the holding potential from −150 mV to +50 mV and applied agonists at a range of different membrane potentials. None of these manipulations...
C. elegans Glutamate Receptors

Current amplitudes of chimeras between C. elegans and rat GluR6 or rat GluR1(L479F) glutamate receptor subunits

Currents in response to either 300 µM glutamate or 300 µM kainate were recorded from Xenopus oocytes that expressed chimeric constructs. Currents mediated by GluR6 chimeras were measured after pretreatment of oocytes with ConA to minimize desensitization. For GluR6 chimeras relative currents were calculated by taking GluR6-PCS currents measured on oocytes from the same batch as 100%. GluR6-PCS shows no variations in expression levels. This prohibits direct comparison of absolute currents measured in different batches. Data represent means ± S.E.

Table I

<table>
<thead>
<tr>
<th>Clone</th>
<th>Absolute kainate current</th>
<th>Absolute Glu current</th>
<th>n</th>
<th>Relative kainate current</th>
<th>Relative Glu current</th>
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<tbody>
<tr>
<td>GluR6-PCS</td>
<td>11,436 ± 1,520</td>
<td>9,767 ± 1,247</td>
<td>15</td>
<td>100 ± 13</td>
<td>100 ± 13</td>
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<td>GluR6-PGLR1</td>
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<td>13</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>GluR6-PGLR2</td>
<td>5,543 ± 1,166</td>
<td>4,752 ± 1,177</td>
<td>20</td>
<td>121 ± 14</td>
<td>133 ± 11</td>
</tr>
<tr>
<td>GluR6-PGLR3</td>
<td>5,561 ± 949</td>
<td>4,388 ± 919</td>
<td>15</td>
<td>51 ± 8</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>GluR6-PGLR4</td>
<td>10,469 ± 2,596</td>
<td>6,068 ± 973</td>
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<td>90 ± 26</td>
<td>49 ± 7</td>
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<tr>
<td>GluR6-PGLR5</td>
<td>25 ± 7</td>
<td>13 ± 3</td>
<td>16</td>
<td>1.5 ± 0.6</td>
<td>1.1 ± 0.4</td>
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<tr>
<td>GluR6-PGLR6</td>
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<td>6</td>
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<td>28 ± 11</td>
<td>13</td>
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<tr>
<td>GluR6-PGLR8</td>
<td>93 ± 43</td>
<td>120 ± 47</td>
<td>16</td>
<td>11 ± 5</td>
<td>25 ± 10</td>
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Glutamate receptor subunits

The lack of currents at GluR6-PGLR2, GluR6-PGLR3, GluR6-PGLR4, GluR6-PGLR5, and GluR6-PGLR6, and the extremely low conductance of GluR6-PGLR7, may reflect ion pores that are nonfunctional, or of background, or in both. The current amplitudes observed varied from 5 nA (GluR6-PGLR6) to 10 µA (GluR6-PGLR4) (Table I and Fig. 3).

The pore-forming regions of glutamate receptor subunits are stretches of mostly hydrophobic amino acids that form hairpin loops (P-loops) that do not traverse the membrane (21). These regions contain amino acids that have been shown to control channel properties tightly in mammalian ionotropic glutamate receptors (1). In mammalian non-NMDA receptors, a conserved glutamine in the P-loop is a key determinant of permeation of calcium ions, single channel conductance, blockade by intracellular polyamines causing inward rectification, and permeation of anions (17, 22–24). In a subset of subunits, this conserved glutamine can be transcriptionally post-converted to an arginine by a process called RNA editing (25). Receptors that contain a subunit with an arginine at this position, named the Q/R site, have a significantly reduced calcium permeability (17, 22). Transgenic mice that express the nonedited form in place of the Q/R site, have a significantly reduced calcium permeability (17, 22). In C. elegans, there is no evidence for RNA editing at the Q/R site. However, five of the eight subunits predicted to represent non-NMDA receptor subunits contain the conserved glutamine...
in the P-loop. Three of the receptors have a basic amino acid (GLR5: lysine; GLR6: arginine, genome-encoded, not edited) or a proline (GLR7) substituted for the conserved glutamine. A proline does not occur naturally in any other glutamate receptor and, to our knowledge, has not been experimentally introduced into a glutamate receptor. We expected that the proline in GLR7 would disrupt channel conductance because most mutations at the Q/R site rendered the receptors nonfunctional.

**FIG. 3.** Glutamate-gated currents can be recorded from pore chimeras. **A**, non-NMDA receptor pore chimeras. Representative current traces measured in *Xenopus* oocytes in response to superfusion with 300 μM glutamate are shown. The swapped-in pore region is indicated by amino acid sequence shown below each trace. The Q/R editing site is italicized and underlined. **B**, NMDA receptor pore chimeras. Representative current records measured in *Xenopus* oocytes in response to superfusion with 300 μM glutamate (GLU) plus 10 μM glycine (GLY) are shown. Note the different amplitude scales of current traces. In some cases a current artifact was introduced when switching solutions, e.g. in GluR1(L479F)-PGLR6.
(27, 28). Surprisingly, however, GluR6-PGLR7 was functional, and we could record modest currents in response to application of either glutamate or kainate. The GLR5 pore with a lysine and the GLR6 pore with an arginine at the Q/R site were also functional when cloned into the GluR6 sequence background but not in the GluR1(L479F) sequence background (Fig. 3 and Table I). However, the current amplitudes of GluR6-PGLR6 were small (5 ± 2 nA) and only observed with kainate application.

None of the GluR6 or GluR1(L479F) chimeras containing the putative NMDA receptor subunit pores (NMR1 and NMR2) was functional (Table II and Fig. 2). Because the NMR subunits show higher homology to rat NMDA receptors (32–43%) than rat AMPA and kainate receptors (24–27% (4)), the ion pore domain of NMR1 was also transplanted into rat NMDAR1-1a, resulting in the chimera NMDAR1-PNMR1. Interestingly, this chimeric showed small currents after application of glutamate in the presence of glycine. Because only small currents can be recorded from *Xenopus* oocytes that express homomeric rat NMDAR1-1a, we coexpressed rat NMDAR1-1a with the rat NR2B subunit, a subunit combination that is known to functionally interact with rat NR2 subunit ion pore domains (NR2B, in our case) the same way as a rat NMDAR1 subunit. PNMR1 clearly is a functional ion pore domain, it does not substitute for a rat NR2 pore domain.

Because the Q/R site is highly conserved among the mammalian non-NMDA receptors, we were especially interested in analyzing the effect on ion pore properties of the amino acids lysine and proline present in GLR5 and GLR7, respectively. Therefore, current-voltage (I/V) curves were recorded from functional chimeras (Fig. 4). Chimeras containing the pores of GLR1, GLR2, GLR3, GLR4, and GLR8, all having a glutamine at the Q/R site, showed inwardly rectifying I/V curves (Fig. 4). This result is consistent with predictions based on channel properties of rat glutamate receptor subunits that are known to show inwardly rectifying I/V curves if a glutamine is present at the Q/R site (23). Interestingly, the GLR7 pore with a proline at the Q/R site reveals an almost perfectly linear I/V curve, whereas GluR6-PGLR5 with a lysine at the Q/R site shows inward and outward currents with limited inward rectification (Fig. 4). Unfortunately, we were not able to record an I/V curve for GluR6-PGLR6 because of its tiny current amplitudes. However, we predict that the GLR6 pore has a linear I/V curve as seen for mammalian rat glutamate receptors that have an arginine at this site.

Because of the central importance of the Q residue for calcium permeation in vertebrate glutamate receptors and the observation that some *C. elegans* subunits have unusual amino acids at the Q/R site, we investigated whether those *C. elegans* pores were calcium-permeable. We tested all functional GluR6 chimeras, for which current amplitudes were recorded in both NFR and CaR (80 mM Ca^{2+}), with 300 µM kainate applied to evoke currents. Interestingly, all chimeras showed currents not only in NFR but also in CaR, which contains calcium as the sole permeable cation (Table III and Fig. 5). Notably, the chimeras exhibited significant differences in their propensities to support calcium-mediated currents, as relative currents (the ratio of CaR current to NFR current) varied widely from 19.0 ± 2.6% for GluR6-PGLR7 to 454.5 ± 103.3% for GluR6-PGLR4 (Table III). The GLR2, GLR3, and GLR4 pores, all with a glutamine (Q) at the Q/R site, had large current amplitudes in CaR. However, a different result was observed for the GLR8 pore, which also contains a glutamine at the Q/R site. GluR6-PGLR8 showed only 30.8 ± 6.0% current in CaR compared with currents measured in NFR (Table III). GluR6-PGLR5, with a lysine at the Q/R site, displayed little differences in current amplitudes between both solutions, whereas GluR6-PGLR7, having a proline at the Q/R site, showed reduced current amplitudes in CaR (19.0%) compared with NFR (Table III and Fig. 5). However, this property of calcium permeability in the absence of rectification is unusual and so far has only been observed by Curutchet et al. (31), who studied a glutamate to histidine mutation at the Q/R site of GluR1. Thus, calcium permeability and rectification are properties of glutamate receptor channels that are not necessarily coupled and most probably have a different molecular basis. The pore of GLR1 unfortunately could not be tested in GluR6 because the construct GluR6-PGLR1 was not functional. However, utilizing the functional construct GluR1(L479F)-PGLR1 we confirmed that, as expected from the glutamate at the Q/R site, the GLR1 pore is also calcium-permeable, with currents in CaR larger than those in NFR (data not shown).

Although the existence of currents in CaR is positive proof for the calcium permeability of the GLR ion pores investigated,
an unknown percentage of these currents might be the result of contributions by calcium-activated chloride channels, which have long been known to be present endogenously in *Xenopus* oocytes (32). To obtain an estimate regarding the magnitude of that contribution, oocytes were preinjected with EGTA (for technical details, see “Experimental Procedures”) to buffer any calcium ions entering the cell, thereby preventing secondary activation of chloride channels. This procedure has previously been shown to be effective (14). We here confirmed in control experiments that the large currents of wild type GluR6(Q) (up to 10 μA in some oocytes), which, because of the contribution of calcium-activated chloride channels, have an almost linear I/V, were consistently and reliably rendered completely inwardly rectifying upon EGTA preinjection of oocytes (data not shown). The use of EGTA-preinjected oocytes indeed reduced the current amplitudes measured in CaR. However, that reduction was quite different for different chimeras (Table III). For example, although GluR6-PGLR2 showed little reduction of currents in CaR compared with NFR, relative currents were much reduced at GluR6-PGLR3. No currents could be measured for GluR6-PGLR5, GluR6-PGLR7, and GluR6-PGLR8 upon EGTA injection (Table III), as large leak currents prevented the reliable detection of the small currents associated with those chimeras (Table I).

The P-loop of glutamate receptors is inserted into the plasma membrane from the cytoplasmic side, with its N-terminal
α-helical part, the pore helix (Fig. 6A), pointed toward the inner cavity and the C-terminal selectivity filter arranged parallel to the central axis of the channel (Fig. 6B; 18, 33, 34). The tip of the pore loop defines the narrowest constriction of the channel. Because the recent identification and structural analysis of the glutamate-gated, potassium-conductive bacterial glutamate receptor GluR0 (35), it is generally believed that the P-loop of the glutamate receptors originated from potassium channel ancestors, as had been postulated earlier based on sequence homology considerations (36–38). For the potassium channel KcsA the crystal structure of the P-loop has been resolved (15) and can function as a template for generating models for glutamate receptor P-loops, for which no crystal structures are available so far.

The amino acid corresponding to the Q/R site of glutamate receptors in KcsA is Thr-75, which is involved in a complex network of hydrogen bonds, resulting in a stable structure built by the pore helix and the selectivity filter. The sterical arrangement of the residues Gly-77, Tyr-78, and Gly-79 within the pore is the structural basis of the selectivity filter of the KcsA potassium channel. The two Gly residues orient the backbone carbon atoms to the central axis of the selectivity filter. Gly-77 of KcsA is homologous to the Gly at position 2 (relative to the Q/R site) of glutamate receptors. The glycine at position 2 has been shown to be crucial for the narrow constriction of the pore in AMPA receptors (39). This glycine is also highly conserved among the C. elegans glutamate receptor subunits, with the sole exception of NMR2. The same is true for Drosophila glutamate receptor subunits (41). Similar to Drosophila, we found that none of the C. elegans wild type glutamate receptor subunits was functional in Xenopus oocytes. However, our Western blot data for GLR1 suggest that the reason for that nonfunctionality is not lack of receptor protein production or failure to insert C. elegans glutamate receptor subunits into the oocyte membrane. It appears likely that functional C. elegans glutamate receptors are heteromeric (8) and may also require other gene products for proper function.

Another possible explanation for the lack of function in oocytes is that C. elegans glutamate receptor subunits might require other agonists than mammalian glutamate receptors. However, electrophysiological recordings on C. elegans neurons have clearly demonstrated glutamate-, kainate-, and NMDA-gated currents (4, 8).

Ion pore transplants have long been used not only on glutamate receptors (11, 12) but also on potassium channels (42), and were shown to transfer faithfully those biophysical properties of the donor ion pore which are determined exclusively by the pore domain to the chimeric construct. Ion pore transplantation was even successful between a prokaryotic potassium channel and the eukaryotic Shaker channel (43). We now show that transplantation of C. elegans glutamate receptor P-loops into rat glutamate receptor subunits allows functional characterization of 9 of the 10 C. elegans ion pores. The pores of those 9 C. elegans subunits have electrophysiological characteristics similar to rat glutamate receptor subunit pores.

The failure to observe glutamate-gated currents in GluR6-PGLR6 might simply be explained by the fact that GluR de-
sensitization is slower with kainate (44). The small current amplitudes of GluR6-PGLR6, which is the only chimera carrying an arginine at the Q/R site, could also be explained by a recent observation by Greger et al. (45), who showed endoplasmic reticulum retention of rat GluR2(R) but not of GluR2(Q). Greger et al. suggest that the amino acid at the Q/R site controls endoplasmic reticulum exit.

Only two of the C. elegans receptor pores, PGLR2 and PGLR3, were functional in both rat GluR6 and rat GluR1 sequence environments. PNMR1 was functional only in the NMDAR1 subunit, in keeping with its prior tentative classification as a putative NMDA receptor subunit, based on the presence of an asparagine at the site homologous to the Q/R editing site in AMPA/kainate receptors (Fig. 1). Just one pore, PGLR1, was functional exclusively in the AMPA receptor GluR1 subunit, whereas PGLR4 through PGLR8 were only functional in the kainate receptor GluR6. One possible interpretation of these data is that NMR1 is more closely related to

**Fig. 5. Ca**\(^{2+}\) permeability of GluR6 chimeras. Representative current records of six chimeras measured in Xenopus oocytes in response to superfusion with 300 μM kainate are shown. The amino acid at the Q/R site is shown for all chimeras. All currents were measured after pretreatment of oocytes with ConA to minimize desensitization. For each oocyte, currents were recorded first in response to 300 μM kainate in NFR (first trace) and then in response to 300 μM kainate in 80 mM CaR (second trace). A third trace was recorded in 80 mM CaR after injection of oocytes with EGTA (traces labeled CaR, EGTA) but could only be obtained for three of the six chimeras.
...receptor family. They categorize our GLR1, GLR2, and GLR5 subunits (note different nomenclature of Sprengel receptor channel. They categorize our GLR1, GLR2, and GLR5 nature apparently characteristic for each subtype of glutamate receptor subunits and pointed out a conserved signature of the glutamate receptor family, with the notable exception of GLR8, which was reported to have the signature of AMPA receptors and the other non-NMDA-like subunits into the kainate receptor family. This interpretation rests on the assumption that the P-loop needs the proper structural environment to allow efficient gating of the ion channel in response to ligand binding. However, this argument fails to assign GLR2 and GLR3 unequivocally. Furthermore, it is partly inconsistent with a previous study (8), which showed that in vivo glr-1- and glr-2-dependent currents were gated by kainate and not by AMPA and that by electrophysiological criteria they appear like kainate receptor currents.

C. elegans glutamate receptor subunits were classified slightly differently in a study by Sprengel et al. (40). These authors compared sequences of the C-terminal part of the pore-forming region plus TMD B of rat, C. elegans, and Drosophila glutamate receptor subunits and pointed out a conserved signature apparently characteristic for each subtype of glutamate receptor channel. They categorize our GLR1, GLR2, and GLR5 subunits (note different nomenclature of Sprengel et al. (40) compared with Brockie et al. (4) into the group of AMPA receptors and the other non-NMDA-like subunits into the kainate receptor family, with the notable exception of GLR8, which was reported to have the signature of δ-receptors. Contrary to this interpretation, we show here that the pore of GLR8 can be transplanted without loss of function into rat GluR6. This points to a significant deviation of GLR8 pore behavior from that of rat δ-receptor subunit pores, which are nonfunctional when transplanted into GluR6 (11). This supports our classification and the classification by Brockie et al. (4), which put GLR8 in the kainate receptor family.

Our data clearly demonstrate significant calcium permeability of all GLR ion pores. Although we cannot exactly quantify the relative permeabilities of calcium versus sodium ions at the various GLR ion pores, our data allow us to state that pores containing a glutamine at the Q/R site are particularly permeable to calcium, just like vertebrate glutamate receptors. In addition, ion pores containing a lysine or a proline at the Q/R site, perhaps unexpectedly, also allow flux of calcium ions.

Our study shows conclusively that C. elegans glutamate receptor pores are capable of fluxing cations. In some subunits this is true despite the presence of unexpected amino acids at functionally critical positions in the ion pore domain which are highly conserved across vertebrate glutamate receptors. Therefore, the analysis of the various C. elegans pores allows the investigation of key amino acids in the pore regions that have been selected for by evolution.

Because crystal structures are not available for the pore-forming regions of glutamate receptors, systematic mutagenesis, substituted cysteine accessibility data, and functional comparison with other ion channels can be exploited to obtain an approximation of a possible three-dimensional structure of a glutamate receptor ion pore.

The pores of glutamate receptors and potassium channels share sequence homologies, suggesting a conserved secondary structure (18, 34, 36–38). Furthermore, the bacterial glutamate receptor GluR0 (35) is potassium-selective and bears a GYG motif in the putative selectivity filter domain, just like classical potassium channels. This suggests that the ion pores of glutamate receptors may share structural similarities with potassium channels (15). Structural motifs are often highly conserved, even when the amino acid sequences are somewhat variable. We created models of the pore of C. elegans subunits based on the solved structure of KcsA and compared them with rat GluR6 pore models to analyze the effect of substitutions of highly conserved amino acids at the Q/R site.

These models predict differences in flexibility of the region believed to constitute the selectivity filter. Such differences in flexibility should affect the permeation of large permeable molecules such as spermine and spermidine. In the case of Q variants of mammalian kainate receptors such as GluR6(Q), the permeation involves a voltage-dependent block resulting in rectification (35, 46). The R variants do not show this polyamine-mediated rectification. Most of the C. elegans glutamate receptor pore constructs exhibited rectification, with the exception of the GLR5 chimera where rectification is weak and the GLR7 chimera where it is absent. These experimental data...
correlate well with the proposed flexibility of the pore as derived from the intersubunit hydrogen bond networks of the residues at the Q/R sites in our homology models. GLR7 and GLR5 lack the intersubunit hydrogen bond, whereas GLR2 and GLR8, which show strong rectification, have them (Fig. 6). Although GluR6(R) harbors this intersubunit hydrogen bond, the longer side chain of the Arg compared with Gln (R/Q) presumably allows for more flexibility of the selectivity filter, resulting in the observed lack of rectification. Experimentally, we determined a reduced calcium permeability in GLR7 and GLR8. In our model we found a closer structural homology of these two pores with the potassium-selective KcsA channel. Those pores lack one amino acid in the selectivity filter compared with other C. elegans and mammalian AMPA/kainate receptors, possibly resulting in a weak partial selectivity for smaller ions.

Our data prove that C. elegans glutamate receptor ion pores are functional units that, despite differences in amino acids even at highly conserved positions, retain pore properties similar to those of mammalian glutamate receptor pores. The ion pore is obviously not a rigid structure, but rather a flexible unit that can function independently in different surroundings. It is an astonishing observation that in the course of evolution invertebrate and vertebrate glutamate receptors remained so closely related as to allow functional ion pore domain exchange between them.

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Functional Analysis of Caenorhabditis elegans Glutamate Receptor Subunits by Domain Transplantation
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