Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7154–7158, July 1994
Neurobiology

Transmembrane topology of two kainate receptor subunits revealed by N-glycosylation

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Communicated by Robert H. Wasserman, April 5, 1994 (received for review January 19, 1994)

ABSTRACT **Glutamate receptors are the primary excitatory neurotransmitter receptors in vertebrate brain and are of critical importance to a wide variety of neurological processes. Recent reports suggest that ionotropic glutamate receptors may have a unique transmembrane topology not shared by other ligand-gated ion channels. We report here the cloning of cDNAs from goldfish brain encoding two homologous kainate receptors with protein molecular masses of 41 kDa. Using a cell-free translation/translocation system, we show that (*i*) a portion of these receptors previously thought to be a large intracellular loop is actually located extracellularly and (*ii*) the putative second transmembrane region of the receptor thought to line the ion channel may not be a true membrane-spanning domain. An alternative model for the transmembrane topology of kainate receptors is proposed that could potentially serve as a framework for future detailed study of the structure of this important class of neurotransmitter receptors.**

The ionotropic glutamate receptors have been subdivided into α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors and *N*-methyl-D-aspartate (NMDA) receptors according to their selective agonists (1), and recent advances indicate a high degree of molecular and functional diversity (2–4). Ionotropic glutamate receptors have generally been thought to belong to the superfamily of ligand-gated ion channels, which consists of nicotinic acetylcholine receptors, γ-aminobutyric acid receptors, glycine receptors, and a subtype of serotonin receptors. The proposed structural features of this family are four transmembrane domains (TMs), with both the N and C termini located extracellularly and a large cytoplasmic loop between TM III and TM IV (see Fig. 4*A*). However, important differences exist between ionotropic glutamate receptors and other ligand-gated ion channels: (*i*) the overall sequence identity is <20%, (*ii*) ionotropic glutamate receptor subunits are generally twice the size of the others (100 kDa vs. 50–60 kDa), and (*iii*) some well-conserved sequence patterns are missing from ionotropic glutamate receptors, such as a proline in TM I and two precisely conserved cysteine residues in the N-terminal extracellular domain. Therefore, the relationship between ionotropic glutamate receptors and other ligand-gated ion channels is fairly remote. Little biochemical evidence is available to support a four-TM model for ionotropic glutamate receptors. Recently, the C-terminal tail of a subtype (NMDAR1) of the NMDA receptor was shown, using protein kinase C phosphorylation, to be located intracellularly (5). This is consistent with an earlier observation that antibodies raised against the C-terminal peptide of a rat brain AMPA/kainate receptor stain the cytoplasmic side of the membrane (6). These findings suggest that ionotropic glutamate receptors may have a unique topology.

The brains of nonmammalian vertebrates have a much greater density of high-affinity kainate receptors than those of mammals (7–9). Kainate binding proteins with molecular masses of 50 kDa cloned from frog (10) and chicken (11) brains exhibit significant sequence homologies with the C-terminal portions of the much larger 100-kDa rat brain AMPA/kainate receptors (12). Although ion-channel activity has yet to be demonstrated for these nonmammalian kainate receptors, they have been implicated in the process of synapse formation in the avian brain (13). The sequence homologies and similar hydropathy plots suggest that their secondary and tertiary structures will be similar to the C-terminal portion of the AMPA/kainate receptors. Therefore, insights gained from the study of the 50-kDa kainate receptors should also shed light on the transmembrane orientation of the 100-kDa ionotropic glutamate receptors.

Two kainate receptor proteins of 41 and 45 kDa have been purified from goldfish brain (14, 15). Treatment with *N*-glycosidase F indicated that the 45-kDa protein is N-glycosylated but that the 41-kDa protein apparently is not (15). A 17-amino acid sequence was obtained from the 41-kDa protein that has significant homology with a conserved segment of the cloned frog and chicken kainate binding proteins and the 100-kDa mammalian AMPA/kainate receptors (15). We report here the cloning of the cDNAs for two proteins corresponding to the previously purified 41- and 45-kDa proteins.* N-glycosylation of these two cloned goldfish brain kainate receptors (GFKARs) was explored using a rabbit reticulocyte lysate *in vitro* translation/translocation system, supplemented with canine pancreatic microsomal membranes. This system has been used widely for detailed study of the topology of membrane proteins (16), and N-linked glycosylation can be used as a direct indication of the lumenal location of a nascent peptide segment. Our results suggest a model for the transmembrane topology of kainate receptors that may have general implications for the structure of the ionotropic glutamate receptor family.

MATERIALS AND METHODS

Materials. Goldfish (*Carassius auratus*) were purchased from Grassyfork Fisheries (Martinsville, IN). A goldfish brain cDNA library was constructed in the pcDNAII vector by oligo(dT) priming (Invitrogen). L-[^{35}S]Methionine (1100 Ci/mmol; 1 Ci = 37 GBq) was purchased from Amersham.

Cloning and Characterization of GFKAR Subunits α and β. A pair of degenerate oligonucleotides was synthesized based (*i*) on a portion of the known 17-amino acid sequence from the 41-kDa polypeptide coding for the amino acid sequence VTTILEDP (15) and from the conserved segments among cloned frog (10) and chicken (11) kainate binding proteins and (*ii*) on the sequence WW(F,V,L)F(T,S)(I,L) from 100-kDa ionotropic AMPA/kainate receptors from rat brain and *Drosophila* (17–19). Two homologous 600-bp fragments were PCR-

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; endo H, endo-β-*N*-acetylglucosaminidase H; NMDA, *N*-methyl-D-aspartate; TM, transmembrane domain; GFKAR, goldfish brain kainate receptor.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U08017 and U08016).

amplified from the goldfish brain cDNA library and were used subsequently as probes to screen 10^6 colonies of the same library. After three rounds of high-stringency screening, 35 full-length or partial clones were obtained for GFKARα (45-kDa form) and six were found for GFKARβ (41-kDa form). Nucleotide sequences were determined using the dideoxynucleotide chain-termination method (Sequenase DNA sequencing kit; United States Biochemical).

***In Vitro* Translation/Translocation.** Promega's TnT-coupled rabbit reticulocyte lysate system was used. The transcription/translation mixtures (25 μl) contain reticulocyte lysate, SP6 RNA polymerase, RNasin, [^{35}S]methionine (20 μCi), amino acid supplement lacking methionine, and the appropriate cDNA clone in the vector, pcDNAII, which has the SP6 promoter at the 5′ end. The mixture was incubated for 1 h at 30°C with or without pancreatic microsomes. For endo-β-*N*-acetylglucosaminidase H (endo H) treatment, a 2-μl sample was boiled for 2 min with an equal volume of 0.3 M sodium citrate (pH 5.5) containing 1% SDS, and the mixture (10 μl) was incubated with 2×10^{-3} units of endo H for 4 h at 37°C.

Expression of Truncated GFKARα Proteins. GFKARα cDNA in the vector pcDM8 was digested with restriction endonucleases *Sty* I, *Bst*NI, and *Mvn* I. The linearized plasmids containing truncated GFKARα cDNA were then used for transcription/translation. The mixture was incubated at 30°C for 45 min, and puromycin was then added to 200 μM for 15 min to discharge the nascent polypeptide chains from the ribosomes (20). Samples were analyzed by polyacrylamide/SDS gel electrophoresis on 12% gels.

Construction of Mutant GFKARα Proteins. A point mutation (Asn^{307} → Asp) in GFKARα was introduced by PCR. The antisense oligonucleotide primer 5′-GGAGCACGTGT-GAGGTCACAATATCTG-3′ and SP6 primer were used to amplify a 1.2-kb DNA fragment from GFKARα cDNA. The pcDNA II vector containing GFKARα cDNA was digested with *Bam*HI and then partially digested with *Afl* III so that a fraction of the *Afl* III site in pcDNAII was not cut. The intact pcDNAII vector with the C-terminal half of GFKARα cDNA was ligated with the PCR fragment, generating the Asn^{307} → Asp mutation.

To construct the $\beta\alpha$ chimera, GFKARβ cDNA in pcDNAII vector was digested with *Sty* I and *Xho* I. Intact pcDNAII vector, with the N-terminal half of GFKARβ cDNA, was recovered. A sense oligonucleotide primer 5′-GGCCATG-GAAGAAGGTTTCCGCAGG-3′ and T7 primer were used to PCR-amplify the C-terminal half of GFKARα cDNA. The 0.9-kb PCR fragment was digested with *Sty* I and *Xho* I and ligated into the pcDNAII vector containing the N-terminal half of GFKARβ cDNA.

To construct the deletion of TM II of GFKARα, cDNA in the pcDNAII vector was digested with *Sty* I and then partially digested with *Xmn* I. The 5-kb fragment containing the entire vector and most of the GFKARα cDNA was recovered and ligated with a linker (5′-CTGGTCCTCACCC-3′/3′-GAC-CAGGAGTGGGGTTC-5′), generating a deletion of 19 amino acids (FTLSHSFWYTMGAMTLQGA), which constitutes the proposed TM II. Mutants were confirmed by DNA sequencing.

RESULTS

Cloning and Characterization of Two Kainate Receptors. We isolated two types of cDNAs: GFKARα and GFKARβ (Fig. 1*A*). Both predicted mature polypeptides have 439 residues, with molecular masses of ≈48 kDa and a sequence identity of 55%. Previously, two distinct kainate receptor proteins of 41 and 45 kDa were purified (15) from goldfish brain. A 17-amino acid sequence obtained from the 41-kDa protein is found at the N terminus of GFKARβ. Antibodies raised to hypervariable regions of the two proteins confirmed that GFKARβ encodes the 41-kDa polypeptide and GFKARα encodes the 45-kDa polypeptide. Both subtypes can be transiently expressed in COS cells. GFKARβ exhibits an equilibrium dissociation constant of 30 nM for [^{3}H]kainate, which is in agreement with the binding constant measured in goldfish brain membrane preparations (15, 23). The affinity of kainate for GFKARα is too low to detect accurately.

Comparison of the hydropathy plots for GFKARα and GFKARβ with frog (10) and chicken (11) kainate binding proteins and the C-terminal half of cloned 100-kDa AMPA/kainate receptors reveals a high degree of similarity. Although hydropathy plots show three strong hydrophobic segments corresponding to TM I, TM III, and TM IV, the segment corresponding to TM II of GFKARα is weakly hydrophobic (Fig. 1*B*). Closely associated with the assignment of TMs is the identification of functional sites among the potential N-glycosylation sites (Asn-Xaa-Ser/Thr; ref. 24) of a transmembrane glycoprotein. The chicken (11) and frog (10) kainate binding proteins show one and two potential glycosylation sites, respectively, between the signal sequence and TM I. These sites are absent in GFKARα and GFKARβ. Instead, all four consensus N-glycosylation sites (Asn^{212}, Asn^{307}, Asn^{333}, and Asn^{340}) of GFKARα are located between TM III and TM IV (Fig. 1*A*). GFKARβ has only one consensus site (Asn^{332}), which is homologous to Asn^{333} of GFKARα. If GFKARα and GFKARβ have four TMs with an extracellular N terminus, these sites would be cytoplasmic and the protein would not be glycosylated.

Synthesis and Glycosylation of the Native and Mutant GFKARα *in Vitro*. In the absence of microsomal membranes, a 41-kDa protein is translated using both cDNAs (Fig. 2*A*). In the presence of microsomes, the 41-kDa GFKARα is converted into two species with higher apparent molecular masses (lane 2). The transfer of each high-mannose precursor oligosaccharide would result in an increase of ≈3 kDa in the apparent molecular mass of the translated protein (25), and the two species seen in Fig. 2*A* (lane 2) probably represent the attachment of one and two oligosaccharide chains. Treatment with endo H, which removes N-linked oligosaccharide chains, converts these two larger species back to the 41-kDa form (lane 3). For GFKARβ, addition of microsomal membranes did not result in any larger species (lane 5). These results are consistent with previous observations that the 45-kDa polypeptide but not the 41-kDa protein is N-glycosylated *in vivo* (15).

To locate the attachment sites of oligosaccharide chains, three truncated forms of GFKARα were generated using restriction enzymes (*Sty* I, *Bst*NI, and *Mvn* I), and the N-terminal portion of the protein was translated using the *in vitro* system (Fig. 2*B*). The GFKARα–*Sty* I translation product of 18 kDa is converted to a 16-kDa species when microsomes are present during translation (lane 1 vs. lane 2) due to N-terminal signal peptide cleavage. No effect of endo H was observed (lane 3), suggesting that the truncated protein was not glycosylated. The size of the GFKARα–*Bst*NI translation product (30 kDa) is unchanged upon the addition of microsomal membranes and endo H (lanes 4–6). These results indicate that glycosylation does not occur on the N-terminal extracellular domain and that Asn^{212} is not a functional glycosylation site. The GFKARα–*Mvn* I product (33 kDa) is converted to a higher molecular mass (36 kDa) in the presence of microsomes, and this effect is reversed by endo H treatment (lanes 7–9). Therefore, a functional N-glycosylation site must lie between the *Bst*NI and *Mvn* I recognition sites, and Asn^{307} is the only candidate.

The Asn^{307} → Asp mutation (α[N307D]) removes this potential N-glycosylation site from GFKARα and reduces the apparent molecular mass of the protein translated in the presence of microsomal membranes by an amount consistent with the removal of one oligosaccharide moiety (Fig. 2*C*, lane

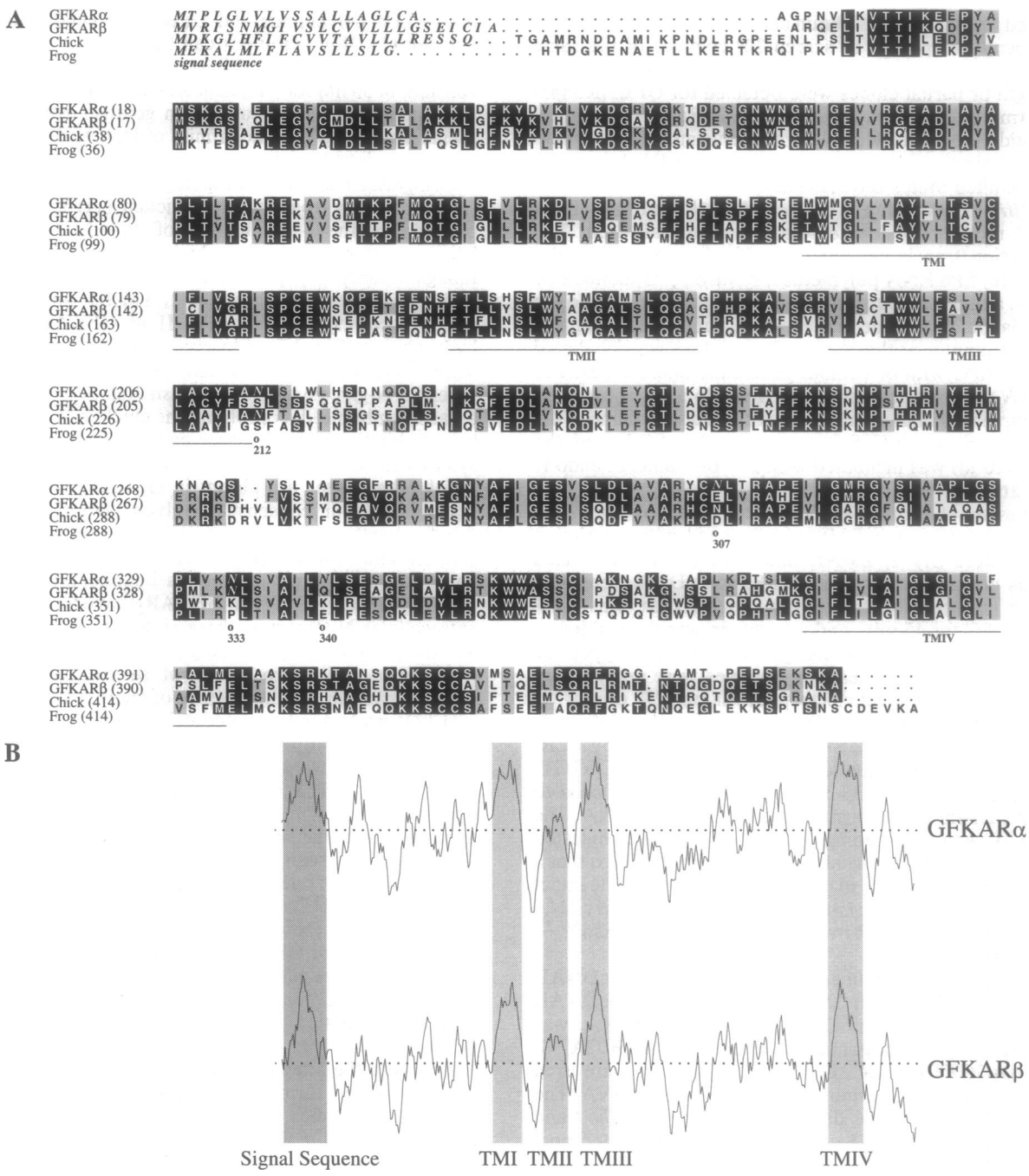

FIG. 1. (*A*) Alignment of the deduced amino acid sequences of GFKARα and GFKARβ with the published sequences of kainate binding proteins from frog (10) and chicken (11) brain. The protein sequences are numbered starting with their predicted mature N termini (21), with the signal sequences in italic type. The putative TMs, corresponding to those proposed for frog and chicken kainate binding proteins (10, 11), are underlined; amino acids identical to goldfish proteins are shown as white letters on a black background, and conservative amino acid changes are shown with a gray background. Potential N-linked glycosylation sites on GFKARα and GFKARβ are labeled with an o and numbered with the GFKARα sequence. (*B*) Hydropathy profiles of GFKARα and GFKARβ computed according to the Kyte and Doolittle method (22), with a window size of 19.

4). Nevertheless, an additional oligosaccharide moiety remains, indicating that the protein is also glycosylated at a second site (presumably either Asn^{333} or Asn^{340}). These results confirm that Asn^{307} in GFKARα is a functional N-glycosylation site and that at least a portion of the segment between TM III and TM IV is extracellular.

Synthesis and Glycosylation of $\beta\alpha$ Chimera. GFKARβ has one potential N-glycosylation site (Asn^{333}), but it is not glycosylated. Asn^{333} could be located cytoplasmically or could be extracellular but not functional. To distinguish these two possibilities, a $\beta\alpha$ chimera was constructed at a *Sty* I site with the N-terminal half from GFKARβ and the C-terminal half from GFKARα (Fig. 3*A*). The chimera contains the functional Asn^{307} of GFKARα and probably another functional site (either Asn^{333} or Asn^{340}). The transmembrane location of Asn^{307} and the remaining C-terminal part should depend on the N-terminal part of GFKARβ. The 41-kDa translation product of the $\beta\alpha$ chimera is converted to two higher molecular mass species in the presence of microsomal membranes (Fig. 3*A*, lane 2) and is sensitive to endo H. This indicates that the N-terminal part of GFKARβ, before Asn^{307}, has the same transmembrane topology as GFKARα. Thus, Asn^{333} of GFKARβ is probably located extracellularly but is not functional.

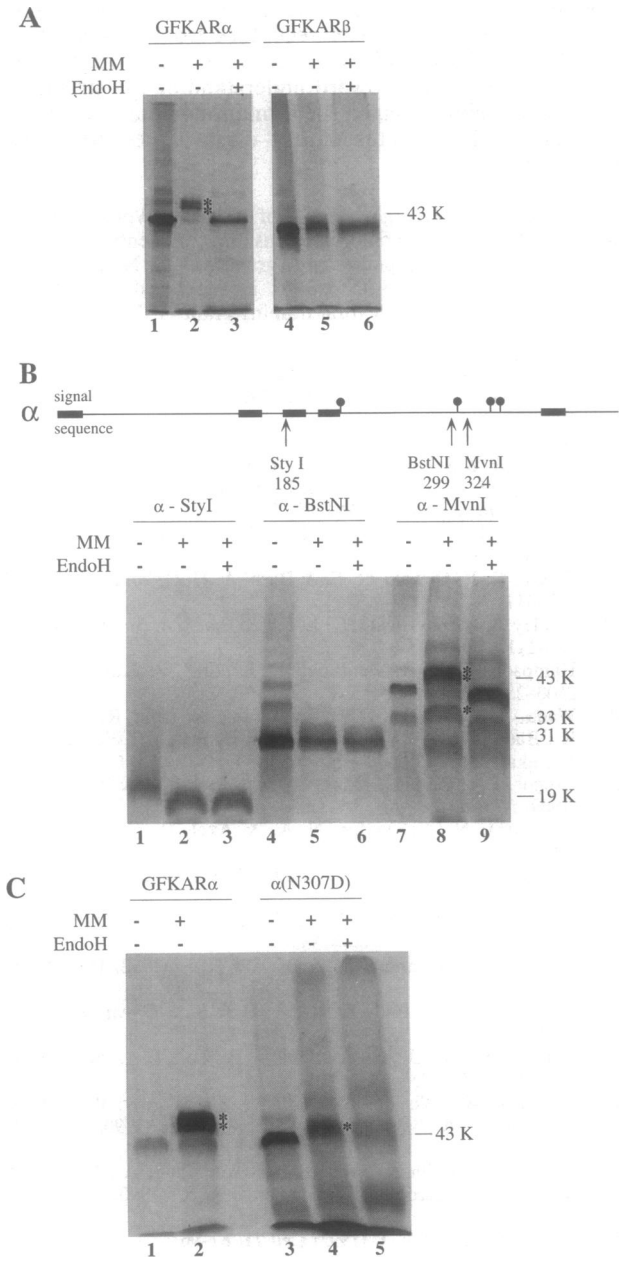

FIG. 2. (*A*) *In vitro* translation of GFKARα and GFKARβ with (+) and without (−) the addition of microsomal membranes (MM) and endo H. The positions of glycosylated proteins are indicated by asterisks. (*B*) Expression of three truncated forms of GFKARα by translation run-off, with (+) and without (−) the addition of MM and endo H. Note that for truncation using *Mvn* I, both the full-length (41–43 kDa) and truncated (33–36 kDa) forms are expressed. (*C*) *In vitro* translation of native and mutant [α(N307D)] GFKARα.

Synthesis and Glycosylation of $\alpha\Delta$TMII Mutant. If at least a portion of the protein between TM III and TM IV is extracellular, then either an additional membrane-spanning region is present or one of the first three proposed membrane spanning regions does not traverse the membrane. Since TM II is the least hydrophobic, this segment was deleted from native GFKARα, and the mutated protein ($\alpha\Delta$TMII) was expressed in the *in vitro* system. The translation product in the absence of microsomal membranes was slightly smaller than the native protein (Fig. 3*B*, lane 4), but upon addition of microsomal membranes, the product was glycosylated (lane 5). Deletion of a true TM would place the glycosylation sites on the cytoplasmic side of the membrane. Since the resulting protein is still glycosylated, TM II probably does not span the membrane.

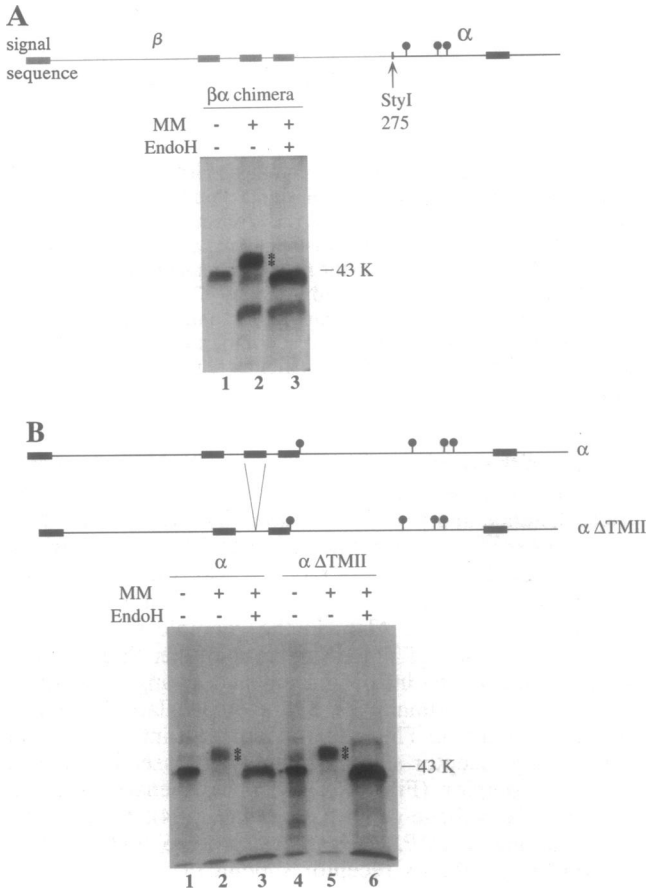

FIG. 3. (*A*) *In vitro* translation and glycosylation of the $\beta\alpha$ chimera. Schematic representation of the $\beta\alpha$ chimera cDNA that indicates the position of the restriction site used for construction of this chimeric cDNA. The numbers represent the positions of amino acids in the mature protein. Consensus sites for glycosylation are indicated by ♀ . (*B*) *In vitro* translation and translocation of the $\alpha\Delta$TMII mutant.

DISCUSSION

Asn^{307} of GFKARα is a functional oligosaccharide attachment site, and consequently, the sequence surrounding Asn^{307} is likely to be located extracellularly. Also, TM II does not seem to span the membrane in the initial translation/translocation steps. These conclusions contradict the original model of four TMs (10, 11). The simplest explanation for the data is that the mature polypeptide has three TMs (Fig. 4*B*). In the coupled translation/translocation process, insertion into the membrane is in a sequential fashion from the N to C terminus (25). The signal sequence would place the N terminus extracellularly, the sequence between TM I and TM III would remain cytoplasmic, the sequence between TM III and TM IV would be translocated extracellularly, and the C-terminal portion after TM IV would be cytoplasmic.

This model raises several testable points. (*i*) The ligand binding domain might be formed by the extracellular N-terminal domain and the segment between TM III and TM IV. A two-domain glutamate binding site has been suggested based on sequence similarity to a bacterial glutamine transporter (12). In addition, the alternatively spliced "flip" and "flop" modules of AMPA receptors have recently been suggested to be on the extracellular side of the membrane (28). (*ii*) This model (Fig. 4*B*) does not preclude the involvement of TM II in the formation of the channel pore, as has been suggested from a large number of site-directed mutagenesis experiments on AMPA/kainate receptors from rat brain (2–4). By assuming the four-TM model (Fig. 4*A*), the

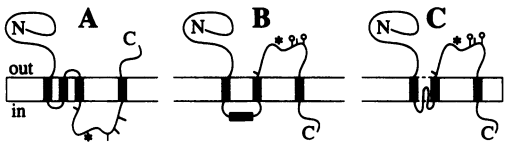

FIG. 4. (*A*) Transmembrane topology according to the originally proposed model of frog and chicken kainate binding proteins (10, 11). (*B* and *C*) Alternative models of the transmembrane topology of GFKARα and GFKARβ. TM II is depicted as either a cytoplasmic segment (*B*) or as inserted into the membrane-spanning portion of the protein due to receptor folding and assembly (*C*). Consensus sites for N-linked glycosylation are indicated by |, and functional sites are indicated by ♀. The position corresponding to Ser^{684}, which is the amino acid implicated in the functional regulation of GluR6 by protein kinase A [refs. 26 and 27; note that Ser^{666} may also be involved (27)], is indicated by an asterisk.

position of the site for Mg^{2+} blockade on the NMDA receptor from electrophysiological recordings of the equivalent electrical distance and the site identified by site-directed mutagenesis (Asn^{598}; ref. 29) are not consistent (30). In this model, Asn^{598} would be close to the external surface, whereas electrical measurements of the site place it close to the internal surface (31). Also, in the genomic sequence, the C-terminal portion of TM II (unlike the other three putative TMs) is split by an intron, an unusual arrangement for an intact structural domain (32, 33). We speculate that TM II, although not a true TM, could insert itself into the channel pore during receptor subunit folding and assembly into the oligomer complex (Fig. 4*C*). A similar scenario has been proposed for voltage-gated K^+ channels (34). Finally, antibody staining of AMPA/kainate receptors (6) and phosphorylation of an NMDA receptor subunit (5, 35) support the prediction of a cytoplasmic C terminus.

Until now, the only available biochemical data concerning the transmembrane topology of the 50-kDa kainate receptors is the protein kinase A-mediated phosphorylation of the 49-kDa chicken kainate binding protein (36). Two possible phosphorylation sites (e.g., Ser^{357} and Ser^{385}) exist between TM III and TM IV and seem to be phosphorylated *in vitro*. However, *in vitro* phosphorylation of the purified chicken kainate receptor in solution cannot directly address the location of these two serine residues. The fact that kainate binding and the attachment of a phosphate group seem to be mutually exclusive may indicate a competitive interaction, suggesting overlap of the kainate binding and the consensus protein kinase A site. On the other hand, the glutamate receptor subunit GluR6, expressed in cultured cells, was reported to be phosphorylated by cAMP-dependent protein kinase on Ser^{684}, which is contained in the loop between TM III and TM IV (refs. 26 and 27; position 272 using GFKARα numbering). A potential explanation for both these findings and our N-glycosylation results would be the five-TM model proposed by Seeburg (28). This would place an additional TM between the phosphorylation site in GluR6 and the N-glycosylation site of GFKARα. This region, which consists of $\approx$30 amino acids in both the 50-kDa kainate receptors and the 100-kDa AMPA/kainate receptors, is relatively hydrophilic and contains scattered charged amino acids. Therefore, this region does not seem to be a likely candidate for a TM. Distinguishing between the three- and five-TM models will require additional experimental work.

The difference in transmembrane topology and lack of sequence homology between the ionotropic glutamate receptor family and other ligand-gated ion channels may suggest that the glutamate receptor family arose from a different ancestral gene. The fact that the two families are both ligand-gated ion channels may be a case of functional convergence rather than evolution from a common ancestor. Clearly, the correct assignment of the transmembrane topology of the 50-kDa kainate receptors from nonmammalian vertebrates and the 100-kDa ionotropic glutamate receptors will be a crucial step toward understanding the structure of the ligand binding domain, the formation of the channel pore, and the regulatory sites of this class of neurotransmitter receptors.

We thank Prof. George Hess, Prof. Gregory Weiland, and Ms. Joanna Feltham for helpful discussions and comments on the manuscript. This work was supported by a grant from the National Science Foundation (IBN-9309480). Z.G.W. is supported on a National Institutes of Health predoctoral training grant (T32-GM08210).

1. Monaghan, D. T., Bridges, R. J. & Cotman, C. W. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29**, 365–402.
2. Gasic, G. P. & Hollmann, M. (1992) *Annu. Rev. Physiol.* **54**, 507–536.
3. Nakanishi, S. (1992) *Science* **258**, 597–603.
4. Wisen, W. & Seeburg, P. (1993) *Curr. Opin. Neurobiol.* **3**, 291–298.
5. Tingley, W. G., Roche, K. W., Thompson, A. K. & Huganir, R. L. (1993) *Nature (London)* **364**, 70–73.
6. Petralia, R. S. & Wenthold, R. J. (1992) *J. Comp. Neurol.* **318**, 329–354.
7. Ziegra, C. J., Oswald, R. E. & Bass, A. H. (1990) *Brain Res.* **527**, 308–317.
8. Henley, J. M. & Oswald, R. E. (1988) *Biochim. Biophys. Acta* **937**, 102–111.
9. Hampson, D. R. & Wenthold, R. J. (1988) *J. Biol. Chem.* **263**, 2500–2505.
10. Wada, K., Dechesne, C. J., Shimasaki, S., King, R. G., Kusano, K., Buonanno, A., Hampson, D. R., Banner, C., Wenthold, R. J. & Nakatani, Y. (1989) *Nature (London)* **342**, 684–689.
11. Gregor, P., Mano, I., Maoz, I., McKeown, M. & Teichberg, V. (1989) *Nature (London)* **342**, 689–692.
12. Nakanishi, N., Schneider, N. A. & Axel, R. (1990) *Neuron* **5**, 569–581.
13. Kimura, N., Kurosawa, N., Kondo, K. & Tsukada, Y. (1993) *Mol. Brain Res.* **17**, 351–355.
14. Ziegra, C. J., Willard, J. M. & Oswald, R. E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4134–4138.
15. Ziegra, C. J., Willard, J. M. & Oswald, R. E. (1992) *Mol. Pharmacol.* **42**, 203–209.
16. Chavez, R. A. & Hall, Z. W. (1991) *J. Biol. Chem.* **266**, 15532–15538.
17. Schuster, C. M., Ultsch, A., Schloss, P., Cox, J. A., Schmitt, B. & Betz, H. (1991) *Science* **254**, 112–114.
18. Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T. A., Sakmann, B. & Seeburg, P. H. (1990) *Science* **249**, 556–560.
19. Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. & Heinemann, S. (1990) *Science* **249**, 1033–1037.
20. Ooi, C. E. & Weiss, J. (1992) *Cell* **71**, 87–96.
21. von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4691.
22. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
23. Willard, J. M., Ziegra, C. J. & Oswald, R. E. (1991) *J. Biol. Chem.* **266**, 10196–10200.
24. Hart, G. W., Brew, K., Grant, G. A., Bradshaw, R. A. & Lennarz, W. J. (1979) *J. Biol. Chem.* **254**, 9747–9753.
25. Wessels, H. P. & Spiess, M. (1988) *Cell* **55**, 61–70.
26. Raymond, L. A., Blackstone, C. D. & Huganir, R. L. (1993) *Nature (London)* **361**, 637–641.
27. Wang, L. Y., Taverna, F. A., Huang, X. P., MacDonald, J. F. & Hampson, D. R. (1993) *Science* **259**, 1173–1175.
28. Seeburg, P. H. (1993) *Trends Neurosci.* **16**, 359–365.
29. Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J. P., Günther, W., Seeburg, P. H. & Sakmann, B. (1992) *Science* **257**, 1415–1419.
30. Imoto, K. (1993) *FEBS Lett.* **325**, 100–103.
31. Ascher, P. & Nowak, L. (1988) *J. Physiol. (London)* **339**, 247–267.
32. Sommer, B., Köhler, M., Sprengel, R. & Seeburg, P. H. (1991) *Cell* **67**, 11–19.
33. Gregor, P., Yan, X., Mano, I., Takemura, M., Teichberg, V. I. & Uhl, G. R. (1992) *Mol. Brain Res.* **16**, 179–186.
34. Pongs, O. (1992) *Trends Pharmacol. Sci.* **13**, 359–365.
35. Mori, H., Yamakura, T., Masaki, H. & Mishina, M. (1993) *NeuroReport* **4**, 519–522.
36. Ortega, A. & Teichberg, V. I. (1990) *J. Biol. Chem.* **265**, 21404–21406.