

Short Sequence-Paper

Human glutamate receptor hGluR3 flip and flop isoforms: cloning and sequencing of the cDNAs and primary structure of the proteins

Vikarna Rampersad, Candace E. Elliott, Stephen L. Nutt, Robert L. Foldes,
Rajender K. Kamboj *

Allelix Biopharmaceuticals Inc., 6850 Goreway Drive, Mississauga, Ontario L4V 1V7, Canada

Received 7 June 1994

Abstract

Several cDNA clones encoding the human glutamate receptor subunit GluR3 flip and flop isoforms, were isolated from human hippocampus and fetal brain libraries. DNA sequence analysis revealed overlapping clones permitting the reconstruction of full-length *GluR3-flip* and *GluR3-flop* cDNAs. The *GluR3* cDNAs demonstrated an 94.1–94.7% nucleotide (nt) identity with the corresponding rat cDNAs. The nt sequence of the *GluR3* cDNAs would encode 894 amino acid proteins that have a 99.4% identity with the rat GluR3 isoforms. The human *GluR3* cDNAs predict an additional 6 amino acid in the N-terminal signal peptide as compared to the rat GluR3.

Keywords: Glutamate receptor; AMPA; Kainate; cDNA sequence; Amino acid sequence; RNA splicing; (Human)

The ionotropic glutamate receptors are heteromeric protein complexes with multiple subunits, each possessing transmembrane regions, and all arranged to form a ligand-gated ion channel. These receptors can be classified according to their selective agonists: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), 2-carboxy-4-(1-methylethenyl)-3-pyrrolidinacetic acid (kainate) or *N*-methyl-D-aspartate (NMDA) (see [1] for a recent review). The AMPA and kainate receptors are also referred to as non-NMDA receptors. One approach to understanding the properties of the AMPA

receptor involves the cloning and characterization of the genes encoding this receptor.

Recent molecular cloning studies have revealed a number of AMPA receptor subunits that can be classified into four families: GluR1 [2–9], GluR2 [3–6,9–11], GluR3 [3,4,6,10] and GluR4 [4,6,12]. Each of these families include flip and flop isoforms generated by alternative RNA splicing [6,13]. In addition, a carboxy-terminal splice variant has been observed in the case of GluR4 [12]. Further receptor subunit complexity is also generated by RNA editing [14–17]. Expression studies of the cloned proteins have shown that the functional properties of AMPA receptors depend on their subunit composition [1,3,5,6,10,11,15,18,19]. The GluR1–4 subunits and isoforms are differentially expressed in a cell-specific and developmentally-regulated fashion [1,4,6,12,15,20–25].

The non-NMDA receptors are of medical interest because of their postulated role in human central nervous system physiology and pathology [26]. In addition, several genetic diseases (including the oculocerebral renal syndrome of Lowe) have been mapped to the region of the X chromosome to which GluR3 has been localized [27]. Increasing evidence has emerged that brings into question the relevance of non-human mod-

Abbreviations: aa, amino acid(s); AMPA, (*RS*)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; bp, base pair(s); cDNA, DNA complementary to RNA; GluR, glutamate receptor; *GluR*, gene (DNA) encoding GluR; hGluR, human GluR; kainate, 2-carboxy-4-(1-methylethenyl)-3-pyrrolidinacetic acid; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; ORF, open reading frame; PCR, polymerase chain reaction; UTR, untranslated region(s); 50 \times Denhardt's reagent, 1% (w/v) Ficoll/1% (w/v) polyvinylpyrrolidone/1% (w/v) bovine serum albumin; 1 \times SSC, 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.6.

* Corresponding author. Fax: +1 (905) 6779595.

The nucleotide sequence data reported in this paper have been submitted to the EMBL/GenBank Data Libraries under the accession numbers U10301 and U10302.

els for the study of the human condition. In particular, it has become evident that amino acid substitutions between homologous proteins (e.g., species homologues) can lead to altered functionality [28–30]. For

these reasons, we have continued our studies regarding the molecular cloning and sequence analysis of the human non-NMDA receptor subunits in order to establish a framework for the further study of their pharmacology as it relates to molecular structure, and thereafter for rational drug design.

Two *hGluR3* cDNA clones (RKCH221 and RKCH521) were isolated by screening approx. $1 \cdot 10^6$ bacteriophage plaques of a human hippocampal cDNA library (Stratagene Cloning Systems, La Jolla, CA, USA) with a 1.1 kb *EcoRI* fragment constituting the 3' region of the *humEAA1* cDNA [31]. The *humEAA1* *EcoRI* fragment was labeled with [α - 32 P]dCTP (> 3000 Ci/mmol) using the Amersham Megaprime DNA labeling system. Positive plaques were identified on replica Hybond-N filters (Amersham) under the following low stringency hybridization conditions: $6 \times$ SSC/50% formamide/5 \times Denhardt's/0.5% SDS/100 μ g/ml denatured salmon sperm DNA. The hybridizations proceeded at 30°C overnight. The filters were washed with $2 \times$ SSC/0.5% SDS at 25°C for 5 min, followed by a 15 min wash at 50°C, and a final wash with $1 \times$ SSC/0.5% SDS for 15 min at 50°C. Three cDNA clones containing the 3' regions of *hGluR3* were isolated by screening the human hippocampus library (clone RKCSHG132) and a human fetal brain library (Stratagene) (clones RKCSFG34 and RKCSFG241) with a 32 P-labeled oligodeoxyribonucleotide (oligo) probe based on the nucleotide sequence of rat *GluR3* [4]: 5'-ACACTCAGAATTACGCTACATACAGAGAGGCTACAACGT. This hybridization proceeded at 42°C overnight under the following conditions: $6 \times$ SSC/25% formamide/5 \times Denhardt's/0.5% SDS/100 μ g/ml denatured salmon sperm DNA. The filters were exposed to Kodak XAR-5 film at -80°C overnight. Positive clones were plaque-purified and excised as phagemids (according to the supplier's specifications), to generate an insert carrying Bluescript-SK variant of the phagemid vector. Nucleotide sequence of denatured, double-stranded templates, was determined by the use of the dideoxy chain-termination reaction using Sequenase (U.S. Biochemical, Cleveland, OH, USA) and a series of strategically situated synthetic oligos. Sequence analysis and comparisons were performed with the GCG (Madison, WI, USA) Analysis Software Package and version 7.3 of the GenBank and European Molecular Biology Laboratory data banks [32].

Four overlapping cDNA clones, designated RKCH521, RKCH221, RKCSHG132 and RKCSFG34 containing cDNA inserts of about 1.6, 2.2, 1.2 and 2.3 kb, respectively, were characterized further. Two overlapping cDNA clones RKCH221 and RKCSFG34 encompassed a full-length *hGluR3-flip* cDNA. Sequence analysis indicated a putative ATG start codon together with 53 nt of 5' UTR, a 2682 nt open reading frame (ORF), and a 321 nt 3' UTR including the inframe

```

1      TGACGACTCCTGAGTTGCGCCCATGCTCTTGTCAGCTTCGTTTATAGGCGTAGCATGGCCA
-52    Q K K M G Q S V L R A V P P L V L G L L
4      GGCAGAAAGAAATGGGCAAGCGTGCTCCGGCGGCTCTTCTTTTATGCTGGGCTTT
24     G H S H G G P P N T I S I G G L F M R N
68     T G Q E H S A P R P A V Q L Y N T N Q N
128    ACACAGTCAGGAGCAGCGCTTTCCGCTTTGCGGTGAGTTATACACACCAACAGCA
64     T T E K P H T S H V D H L D S S N S
188    ACACCACCGAAGCCCTTCCATTGAAATTACGAGTACTGGATTCTCCCAATA
84     F S V T N A P F C S Q F S R G V Y A I P G
248    GTTTTTCGTTGACAAATGCTTCTGCTCCGAGTCTCGAGAGGGGTGATGCGATCTTTG
104    F Y D Q M S N T L T S F C G A L H T S
308    GATTCTATGACAGATGTCAATGAACCTTGACCTCTTCTGTGGGCGCTGACACAT
124    F V T P S F P T D A D V Q F V I Q M R P
368    CCTTTGTACGCTAGCTTCCCACTGACGAGATGTGAGTTTGTATCCAGATGCGCC
144    A L K G A I L S L L G H Y K W E K F V Y
428    CAGCCTTGAAGGCGCTATTCTGAGTCTTCTGGGTCTTACAGTGGGAGAAATGTTGT
164    L Y D T E R R G G P S I L Q A I M H A A V Q
488    ACCTCTATGACACAGAACGAGGATTTCTCTCTCCAGCGATTATGGAAGCAGCTGC
184    N M N Q V T S G V N I K G D V Q E F R
548    AAAACAACTGGCAAGTAAACAGCAAGGCTCTGGGAAACATAAAGAGCTCCAGAAATCA
204    R I E E M D R R Q E K R Y L I D C E V
608    GGCGCATCAATTGAAGATGGACAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
224    E N T T C I A L L G K H S R G Y
668    TCGAAGGATTAACAAATTTTGAAACAGTTGTGATCTTAGGAAACACTCAAGAGGTT
244    H L A N L G P D T I L L E R V M H G
728    ATCACTACATGCTGCTACCTGGGTTTACTGATTTTACTCGAAGAGTCAATGATG
264    G A N T G F Q L V N N E N P M V Q P
788    GGGAGGCAACATTAAGGTTTCCAGATGTCTCAACATGAAACCTTATGTTGAGCAGT
284    I Q R W V R G L T T R E P P E A K N A P L
848    TCATACAGCGCTGGGTGAGGCTGGAATGAAAGGAATTCCTGAGCGAAGATGCGCAC
304    K Y T S A L T H D A I L V I A E R Y
908    TAAAGTATACATCTGCAATGACACAGGCAATACTGCTATAGCAGAAGCTTCCGCT
324    L R R Q R V T V S R R R G S A G D C L A N
968    ACCTGAGGAGGAGCAGGAGTGTGTCGAGAGGAGGAGTGTGAGAGCTGCTTAGCA
344    P A V P W S Q G I D I E R A L K M V Q V
1028   ATCTGCTGTGCTGCTGAGTCAAGGAATGATATGTAGAGAGCTCTGAAATGCTGCAAG
364    Q G M T G N I Q P D T T Y G R R T N Y T I
1088   TACAAGGAATGACTGGAATATTTCAATTGACACTTATGAGCTAGGACAAATATACCA
384    D V Y E M K R Y V S G S R K A G Y W N B Y E
1148   TCGATGTGTGAAATGAAAGTCAAGTGGCTCTGAAAGCTGCTAGTGGAAAGTATG
404    R P V P P S I S N D S A S S B N R
1208   AAAGGTTTGTGCTTCTCAGATCAGCAATGACAGTGTGATCTCTGCAAGTATG
424    T I V V T T I L S R P Y V M Y K N H E
1268   GGACCATAGTAGTACTACATTTTGAATACCATATGATTAATGACAGAGAAACCATG
444    Q L E G N B R Y E G Y C V D L A V Y I A
1328   AGCAACTGGAAGGAAATGAAAGTATGAAAGCTATGTTGATAGCTAGCTTATGAAATAG
464    K H V R I K S I V G D G K Y G A R
1388   CCAACATGTAAAGATCAAAATGTCATCGTTGGTGAAGGAGTATGTTGATG
484    D P E T K I W N G M V G B L V Y A R A
1448   GGGATCCAGAGACTAAATATGGAAGCTGTTGGGGAACTTGCTATGGGAGCTG
504    I A V A P L T I T L V R E E V I D P S K
1508   ATATAGCTGTGCTCCTCACTATAAATTTGGTCCGTGAAGAGTCAATAGATTTTCA
524    P L M S L G I S I M I K K P Q K S K P P
1568   AGCCATTAATGAGCTGGGCTCTCAATGATGAAGAGCTCGAAGATCAAAACAG
544    V P S P L D L F S I W M C I V P A Y
1628   GCGTATTTCTATTTCTGGAATCCCTTGGCTTGAATCTGGAATGCTATGCTTGTCTT
564    I G V S V V L P L V S R P S P Y B W H L
1688   ACATTGGAGTCAGCTAGTTCTTTCTAGTCAGCAGGTTTCACTTATGAATGCGACT
584    E D N N B E P R D P S G P P D P P N E P
1748   TGGAAAGCAACATGAAGAACCTCGTGAAGCAAGTCTCTGATCTCCAAATGCAATG
604    G I F N S L S I G S L G A P M Q Q C G D I
1808   TTGGAATATTTAAAGCTTTTGTCTTGGTGGCTTTATGAGCAAGATGTTGATA
624    S P R S L S G R I V G V W M F P T L I
1868   TTCTCCAAAGTCACTCTCGGCGGCTATGTTGGAGGGGTTTGGTGGTCTTCTGACCTGA
644    I I S S Y T A N L A A P L T V E R M V S
1928   TCATAATTTCTTCTATCTGCAATCTGCTGCTTCTGACTGTGGAAGGATGGTTT
664    P I E S A E D L A K K T E I A Y G T L D
1988   CTCCTAGAGAGTGTGAAGACTTAGCTAAACAGACTGAATTTGATATGGAACCTGG
684    S G S T K E G F P R R S K I A V Y E K M W
2048   ACTCCGGTTCAACAAAGATTTTTCAGAAATCAAAATTTGCTGTGACGAGAAATGT
704    S Y M K S A A P P S V P T K T T A D G V A
2108   GGTCTTACATGAATCAGCGAGCCATCTGTGTTTACAAAACAGCAGCAGGAGTGG
724    R V R K S K G K P A F L L E S T M N B Y
2168   CCCGAGTGGAAAGTCCAAGGGAAGTTGCGCTTCTGCTGAGTCAACATGAATGAGT
744    I E Q R K P T S I G S K G N L D S K G
2228   ACATTGAGCAGAGAAACATGTGATACGATGAAGTTGGTGAATCTGGAATCCAAAG
764    Y G V A T P K G S A L G T P V N L A V L
2288   GCTATGGTGGCAACCCCTAAAGGCTCAGCATAGGAACGCTGTAAACCTTGCAATG
784    K L S E Q G I L D K L K N K W Y D K G
2348   TGAACCTCAGTGAACAGGCTCTTAGACAGCTGAAACAAATGGTGGTACGATAAGG
804    E C G A K D S I G S K K T S G L S S N
2408   GGGAAATGGAGCCAGGACTCCGGGATGAAGACAGACCGGCTCTGAGCTCAGCA
824    V A G V P X I L Y G G L G L A M H V A L
2468   ATGTGTGAGCGCTTTCTATATCTGTGCGAGGCTCGGCGCTGCGCATGATGTGGCTT
844    I E F C Y K S I E S K R N K L T N T
2528   TGATAGAAATCTGTACAAATCAGGGCAGAGTCCAAAGCATGAATCAAGAAACA
864    Q N F P P A P A T N T Q N Y A T Y R E G
2588   CCCAAATTTAAAGCTCTGCTGCTGCCAACCACTCAGAAATATGCTACATCAGAGAAG
884    Y N V Y G T E S V K I
2648   GCTACACGTTGATGGAACAGAGAGTGTAAAGTCTAGGATCCCTTCCCTCAGAGGCA
2708   TGTGATGAGAGGAATCACCGAAACCTGGCTCTCAAGGATCTGAGCCAGATTTTCA
2768   TCTCTTGGTGTGGGATGACAGGAATTTGTGATGGTGAATGACCTTTCAATAGGA
2828   AAAACGTGATTTTTTTTCTCTCAGTGCCTTATGGAACACTCTGAGACTCGGACATGC
2888   AAAACATCATGAAATCTTTTCTTGTCTTGTGTAAGAAAAATTAATTAATAAACAAC
2948   AAAAATGGACATGCATCAACCTTGTATGTAATATTTATATAGTTTTCATTA

```

Fig. 1. The nucleotide and deduced amino acid sequence of the *hGluR3-flip* cDNA.

	776	T	P	V	N	L	A	V	L	K	L	S	E	Q	G	I	L	D	792
flip	2325	A	A	C	G	C	T	G	T	A	A	C	T	T	G	C	A	G	2374
flop	2325	A	A	T	G	C	T	T	A	A	A	C	T	T	G	C	A	G	2374
	776	N	A	V	N	L	A	V	L	K	L	N	E	Q	G	L	L	D	792
		*	*									*			*				
	793	K	L	K	N	K	W	W	Y	D	K	G	E	C	G	A	K	808	
flip	2375	A	C	A	A	G	C	T	G	T	A	A	A	A	C	A	A	T	2424
flop	2375	A	C	A	A	T	G	A	A	A	C	A	A	T	G	G	T	G	2424
	793	K	L	K	N	K	W	W	Y	D	K	G	E	C	G	S	G	808	
																*	*		
	809	D	S	G	S	K												813	
flip	2425	G	A	C	C	G	G	A	G	T	A	A	G					2439	
flop	2425	G	G	C	G	T	G	A	C	T	C	C	A	A	G			2439	
	809	G	G	D	S	K												813	
		*	*	*															

Fig. 2. Comparison of the nucleotide and deduced amino acid sequence of the hGluR3-flip and hGluR3-flop cDNAs. Amino acid sequence differences are indicated (*).

stop codon (Fig. 1). The remaining two cDNA clones (RKCH521 and RKCSHG132) define the hGluR3-flop cDNA and differ from the hGluR3-flip cDNA in the region between nt 2327–2436 (Fig. 2). Comparison of the hGluR3-flip nt sequence with that of rat GluR3-flip [6,10], indicates a 94.1% identity. Comparison of the hGluR3-flop nt sequence with that of rat GluR3-flop [4] indicates a 94.7% identity. The 894-aa deduced sequence encoded by the hGluR3 cDNAs share a 99.4% identity (5 aa changes) with the rat homologues. These changes are summarized as follows (human → rat): Gly-154 → Ser; His-155 → Tyr; Ile-173 → Val; Ala-418 → Ser; Leu-525 → Phe. Interestingly all of these changes occur in the predicted extracellular N-terminal regions of the molecule [4]. The nt sequence reported by Boulter et al. [3] differs from those reported here and elsewhere [4,6,10] at 2 aa positions (human → rat[3]): Lys-523 → Asn; Pro-524 → Ala. In addition to these changes, the hGluR3 nt sequences predict an additional 6 aa in the putative N-terminal signal peptide, as compared to the rat homologues. The amino acid sequence conservation observed between human and rat GluR3 is comparable to that observed for NR1 [33], but greater than that observed for GluR1 [7,8], GluR2 [11], EAA1 [31] and EAA2 [34]. The significance of this higher degree of conservation is as yet unclear.

We are grateful for the expert assistance of Ms. Raya Kuperman for oligo synthesis. This work was part of a collaborative CNS Research Program between Allelix Biopharmaceuticals Inc., and Eli Lilly and Company (Indianapolis, IN, USA).

References

- [1] Hollman, M. and Heinemann, S. (1994) Annu. Rev. Neurosci. 17, 31–108.
- [2] Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W. and Heinemann, S. (1989) Nature 342, 643–648.
- [3] Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C. and Heinemann, S. (1990) Science 249, 1033–1037.
- [4] Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T.A., Sakmann, B. and Seeburg, P.H. (1990) Science 249, 556–560.
- [5] Sakimura, K., Bujo, H., Kushiya, E., Araki, K., Yamazaki, M., Yamazaki, M., Meguro, H., Warashina, A., Numa, S. and Mishina, M. (1990) FEBS Lett. 272, 73–80.
- [6] Sommer, B., Keinänen, K., Verdoorn, T.A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. and Seeburg, P.H. (1990) Science 249, 1580–1585.
- [7] Puckett, C., Gomez, C.M., Korenberg, J.R., Tung, H., Meier, T.J., Chen, X.N. and Hood, L. (1991) Proc. Natl. Acad. Sci. USA 88, 7557–7561.
- [8] Potier, M.-C., Spillantini, M.G. and Carter, N.P. (1992) DNA Seq. J. DNA Seq. Map. 2, 211–218.
- [9] Sun, W., Ferrer-Montiel, A.V., Schinder, A.F., McPherson, J.P., Evans, G.A. and Montal, M. (1992) Proc. Natl. Acad. Sci. USA 89, 1443–1447.
- [10] Nakanishi, N., Shneider, N.A. and Axel, R. (1990) Neuron 5, 569–581.
- [11] Sun, W., Ferrer-Montiel, A.V. and Montal, M. (1994) NeuroReport 5, 441–444.
- [12] Gallo, V., Upson, L.M., Hayes, W.P., Vyllicky, L. Jr., Winters, C.A. and Buonanno, A. (1992) J. Neurosci. 12, 1010–1023.
- [13] Monyer, H., Seeburg, P.H. and Wisden, W. (1991) Neuron 6, 799–810.
- [14] Sommer, B., Köhler, M., Sprengel, R. and Seeburg, P.H. (1991) Cell 67, 11–19.
- [15] Burnashev, N., Monyer, H., Seeburg, P.H. and Sakmann, B. (1992) Neuron 8, 189–198.
- [16] Higuchi, M., Single, F.N., Köhler, M., Sommer, B., Sprengel, R. and Seeburg, P.H. (1993) Cell 75, 1361–1370.
- [17] Cha, J.-H.J., Kinsman, S.L. and Johnston, M.V. (1994) Mol. Brain. Res. 22, 323–328.
- [18] Burnashev, N. (1993) Cell Physiol. Biochem. 3, 318–331.
- [19] Dildy-Mayfield, J.E. and Harris, R.A. (1994) J. Neurochem. 62, 1639–1642.
- [20] Pellegrini-Giampietro, D.E., Bennett, M.V.L. and Zukin, R.S. (1991) Proc. Natl. Acad. Sci. USA 88, 4157–4161.
- [21] Hunter, C., Petralia, R.S., Vu, T. and Wenthold, R.J. (1993) J. Neurosci. 13, 1932–1946.

- [22] Bochet, P., Audinat, E., Lambolez, B., Crépel, F., Rossier, J., Iino, M., Tsuzuki, K. and Ozawa, S. (1994) *Neuron* 12, 383–388.
- [23] Gallo, V., Wright, P. and McKinnon, R.D. (1994) *Glia* 10, 149–153.
- [24] García-Ladona, F.J., Palacios, J.M., Probst, A., Wieser, H.G. and Mengod, G. (1994) *Mol. Brain. Res.* 21, 75–84.
- [25] Patneau, D.K., Wright, P.W., Winters, C., Mayer, M.L. and Gallo, V. (1994) *Neuron* 12, 357–371.
- [26] Lipton, S.A. and Rosenberg, P.A. (1994) *N. Engl. J. Med.* 330, 613–622.
- [27] McNamara, J.O., Eubanks, J.H., McPherson, J.D., Wasmuth, J.J., Evans, G.A. and Heinemann, S.F. (1992) *J. Neurosci.* 12, 2555–2562.
- [28] Oksenberg, D., Marsters, S.A., O'Dowd, B.F., Jin, H., Havlik, S., Peroutka, S.J. and Ashkenazi, A. (1992) *Nature* 360, 161–163.
- [29] Hall, J.M., Caulfield, M.P., Watson, S.P. and Guard, S. (1993) *Trends Pharmacol. Sci.* 14, 376–383.
- [30] Johnson, M.P., Loncharich, R.J., Baez, M. and Nelson, D.L. (1994) *Mol. Pharmacol.* 45, 277–286.
- [31] Kamboj, R.K., Schoepp, D.D., Nutt, S., Shekter, L., Korczak, B., True, R.A., Rampersad, V., Zimmerman, D. and Wosnick, M. (1994) *J. Neurochem.* 62, 1–9.
- [32] Devereux, J., Haeberli, P. and Smithies, O. (1984) *Nucleic Acids Res.* 12, 387–395.
- [33] Foldes, R.L., Rampersad, V. and Kamboj, R.K. (1993) *Gene* 131, 293–298.
- [34] Kamboj, R.K., Schoepp, D.D., Nutt, S., Shekter, L., Korczak, B., True, R.A., Zimmerman, D.M. and Wosnick, M.A. (1992) *Mol. Pharmacol.* 42, 10–15.