The Human Glycine Receptor Subunit α3

GLRA3 GENE STRUCTURE, CHROMOSOMAL LOCALIZATION, AND FUNCTIONAL CHARACTERIZATION OF ALTERNATIVE TRANSCRIPTS*

(Received for publication, March 5, 1998)

Zeljko Nikolic‡, Bodo Laube§, Ruthild G. Weber¶, Peter Lichter‡, Petra Kioschis¶, Annemarie Poustka, Cornel Mülhardt¶, and Cord-Michael Becker‡**

From the ‡Institut für Biochemie, Universität Erlangen-Nürnberg, Fahrstrasse 17, D-91054 Erlangen, Germany, the §Max-Planck-Institut für Hirnforschung, Deutscherstrasse 46, D-60528 Frankfurt, Germany, the ¶Abteilung Organisation Komplexer Genome and the §§Abteilung Molekulare Genomanalyse, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

The neuronal glycine receptor is a ligand-gated chloride channel composed of ligand binding α and structural β polypeptides. Homology screening of a human fetal brain cDNA library resulted in the identification of two alternative splice variants of the glycine receptor α3 subunit. The amino acid sequence predicted for the α3L variant was largely identical to the corresponding rat subunit. In contrast, the novel splice variant α3K lacked the coding sequence for 15 amino acids located within the cytoplasmic loop connecting transmembrane spanning region 3 (TM3) and TM4. Using P1 artificial chromosome (PAC) clones, the structure of the GLRA3 gene was elucidated and its locus assigned to human chromosomal bands 4q33-q34 by fluorescence in situ hybridization. Two transcripts of 2.4 and 9 kilobases, corresponding to α3L and α3K, respectively, were identified and found to be widely distributed throughout the human central nervous system. Structural analysis of the GLRA3 gene revealed that the α3K transcript resulted from a complex splice event where excision of the novel exon 8A comprising the alternative sequence of 45 base pairs coincides with the persistence of a large intronic sequence in the 3′-untranslated region. Functional expression in HEK 293 cells of α3L and α3K subunits resulted in the formation of glycine-gated chloride channels that differed significantly in desensitization behavior, thus defining the cytoplasmic loop as an important determinant of channel inactivation kinetics.

Glycine serves as a major inhibitory neurotransmitter throughout the mammalian central nervous system (1). The strychnine-sensitive glycine receptor (GlyR)1 is a pentameric assembly of ligand binding α and structural β subunits displaying significant sequence homology to nicotinic acetylcholine receptor (2), γ-aminobutyric acid receptor type A (GABA_A), and serotonin receptor type 3 (5-HT_3) subunits (1). As members of a superfamily of ligand-gated ion channels, these polypeptides share topological features including a large N-terminal extracellular domain followed by four transmembrane spanning regions (TM1-TM4). While the N-terminal domain carries structural determinants essential for agonist and antagonist binding (3), TM2 is thought to form the inner wall of the chloride channel (4).

The glycine receptor α3 subunit of rodent central nervous system exists in different subtypes (α1-α4) encoded by distinct genes (1). In the murine genome, the corresponding loci have been localized on chromosomes 11 (α1), X (α2, α4), and 8 (α3), respectively (5–8). Further diversity is achieved by alternative splicing of primary transcripts encoding the α1 and α2 subunits (9, 10). In the human, highly homologous α1 and α2 subunits have been identified by cDNA cloning (11) and assigned to the chromosomal regions 5q31.3 (12, 13) and Xp21.2–22.1 (11), respectively. In both, man and mouse mutant lines, mutations of GlyR subunit genes result in hereditary motor disorders characterized by exaggerated startle responses and increased muscle tone. Pathological alleles of the Glra1 gene are associated with the murine phenotypes oscillator (spd<sup>α</sup>) and spasmodic (spd) (5, 14–16). A mutant allele of Glrb has been found to underly the molecular pathology of the spastic mouse (spa), where the intronic insertion of a LINE-1 transposable element results in aberrant splicing of Glrb primary transcripts (17, 18). Resembling the situation in the mouse, a variety of GLRA1 mutant alleles have been shown to cause the human neurological disorder hyperekplexia or startle disease (12, 13). In contrast, mutations of the human GLRB gene in hyperekplexia have not yet been reported (19). By analogy, the gene encoding the GlyR α3 subunit has to be considered a candidate gene for human and murine neurological disorders.

Here we describe the cloning and characterization of two splice variants of the human α3 subunit and the corresponding GLRA3 gene which was mapped to the chromosomal region 4q33-q34. Functional expression in HEK 293 cells of GlyR α3L and GlyR α3K resulted in the formation of glycine-gated chloride channels that significantly differed in desensitization behavior.

EXPERIMENTAL PROCEDURES

Isolation of GlyR α3 cDNA Clones—A human fetal brain library (CLONTECH, Heidelberg, Germany) was used for isolation of cDNA contig, group of overlapping clones; FITC, fluorescein isothiocyanate; DAPI, 4,6-diamidino-2-phenylindol-dihydrochloride.
clones. After plating of ca. 9 × 10^6 plaque-forming units, screening was performed under intermediate stringency conditions using a complex cDNA probe which covered the whole open reading frames of rat α1, α2, α3 and human α and c2 CDAs. Hybridization was carried out at 52 °C in 1× ETDATA, 0.5× NaHPO4/NaH2PO4, 7% SDS (w/v) and 100 μg/ml denatured salmon sperm DNA for 2× SSC and 1% SDS at 52 °C for 20 min. Of the positive clones, a randomly selected sample was further analyzed by Southern blot hybridization. To this end, human α1- and α2-specific cDNA probes were generated. The α1 probe amplified by PCR corresponded to nucleotide positions 30 to 92, while an α2-specific probe was generated by EcoRI and PvuII restriction digestion of an α2 cDNA clone and covered nucleotides 1162–1262 encoding the cytoplasmic loop between TM3 and TM4. Hybridization and washing conditions were as given above, except that the temperature was 65 °C. Two of the resulting clones, p7 (3 kb) and p12 (1 kb), that hybridized to neither the α1 nor the α2 probe were characterized further. All other DNA manipulations were according to standard procedures (20). DNA sequencing was performed on the ABI PRISM 377 automated DNA sequencer.

Isolation and Characterization of Genomic PAC Clones—Genomic PAC clones were obtained by screening spotted artificial chromosome (PAC) clones were obtained by screening spotted clones, a randomly selected sample was further analyzed by Southern blot hybridization. To this end, human α1- and α2-specific cDNA probes were generated. The α1 probe amplified by PCR corresponded to nucleotide positions 30 to 92, while an α2-specific probe was generated by EcoRI and PvuII restriction digestion of an α2 cDNA clone and covered nucleotides 1162–1262 encoding the cytoplasmic loop between TM3 and TM4. Hybridization and washing conditions were as given above, except that the temperature was 65 °C. Two of the resulting clones, p7 (3 kb) and p12 (1 kb), that hybridized to neither the α1 nor the α2 probe were characterized further. All other DNA manipulations were according to standard procedures (20). DNA sequencing was performed on the ABI PRISM 377 automated DNA sequencer.

Northern Blot Analysis—Regional expression of the GlyR α3 subunit was analyzed using prefabricated RNA blots of human central nervous system tissues (human brain MT2 blot II; CLONTECH, Heidelberg, Germany). Two probes covering those nucleotide positions encoding the cytoplasmic loop between TM3 and TM4 were used for isofrom selective detection of both mRNA variants, α3L and α3K. Among the probes used, oligonucleotide hasa3-ISH-ins (5′-CCATATGCTGAGAAGAAGG- TAAAATCCTCAGTGCAAAAGGCT-3′) was 5′-end labeled with polynucleotide kinase (Promega, Mannheim, Germany) using [γ-32P]ATP, while the oligonucleotide hasa3-A10-exp (ACAGTGATCCCCCAGAGACTTAATCTTG) was annealed into the multiple cloning site of the expression vector pRK5. The cDNA clone p12, corresponding to GlyR α3L, lacks the 5′-end of the open reading frame. Thus, two PCR were performed for amplification of a complete GlyR α3 open reading frame. Nucleotide positions 8 to 1186 were amplified from clone p7 using the primers hasa3-S1-Exp and hasa3-A10–1167 (CTTTCGCTTGTAGACATGCT). The sequence harboring the alternatively spliced part of the GlyR α3L cDNA was amplified employing oligonucleotides hasa3-S8–999 (TTTTCGACACTTGGAG) and hasa3-A10–1167 (CTTTCGCTTGTAGACATGCT). The sequencing reaction was performed on an ABI PRISM 377 automated DNA sequencer.

Electrophysiological Recording—Transfected cells were viewed under an inverted microscope (Axiovert 35, Zeiss, Jena, Germany) and continuous perfusion (1 ml min^-1) of the extracellular bath solution containing: 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 11 mM EGTA, 10 mM HEPES (pH 7.2 with NaOH). Membrane currents were obtained from cells using an EPC-9 amplifier (HEKA Elektronik, Lambrecht, Germany) linked to an ATI STAC computer controlled by HEKA software. The membrane potential was clamped at 70 mV in all experiments and agonist-induced whole-cell currents were sampled at 20 Hz. Electrodes were filled with a borosilicate glass capillaries (Hilgenberg, Malsfeld, Germany) with a Zeitz DMZ Universal Puller (Zeitz Instruments, Augsburg, Germany) to yield tip resistance of 2–4 megohm. Pipettes were filled with a solution containing: 120 mM CsCl, 20 mM tetraethylammonium chloride, 1 mM CaCl2, 2 mM MgCl2, 11 mM EGTA, and 10 mM HEPES (pH 7.2). Series resistances after whole-cell formation were compensated for 50–90%. Superfusion was performed with a DAD-12 (Adams and List, Westbury, NY) drug application system. Dose-response curves of agonist-induced peak currents were normalized to the maximum value and data were fitted with the sigmoidal Hill equation using the Levenberg-Marquardt algorithm. Results are expressed as means ± standard deviation. Double exponential curves were fitted to the desensitizing phase of the GlyR responses.

RESULTS
cDNA Cloning and Characterization of GlyR α3 Transcripts—To identify novel variants of the ligand binding GlyR subunit from human central nervous system, a fetal brain cDNA library was screened using a complex probe composed of various human and rodent α subunit cDNAs (α1–α3). From a random sample comprising 20 out of >100 hybridizing clones, transcripts of the human GlyR α1 and α2 subunit genes were excluded by Southern blot hybridization to α1 and α2 cDNA fragments. While none of the clones hybridized to the α1 fragment, 18 clones were recognized by the α2-specific probe. This is consistent with a prevalence of the α2 subunit in the human fetal central nervous system, resembling the situation in the rodent (23, 24). The nonhybridizing clones p7 and p12 were found to contain inserts of 3069 and 940 base pairs, respectively. As revealed by DNA sequencing, clone p12 was highly homologous to the rat GlyR α3 subunit cDNA (25) representing a 3′ partial clone that lacked a 5′ segment of 733 nucleotides as counted from the translation start (Fig. 1). In contrast, clone p7 contained a complete open reading frame as well as large parts
and 3' untranslated regions (UTRs). Compared with clone p7 and the rat a3 subunit cDNA, clone p7 lacked a stretch of 45 bp corresponding to nucleotides 1072–1116 of the coding sequence (Fig. 1), indicative of alternative splicing. This predicts a loss of 15 amino acids positioned within the cytoplasmic loop connecting TM3 and TM4 of the subunit polypeptide, hence referred to as GlyR a3K. Moreover, the sequences of both clones differ within the 3' UTR starting with nucleotide position 1072 to 1117 to compensate for the deletion by alternate splicing of exon 8A in these transcripts. Sequences of the GlyR a3L cDNA contained within clone p12 are given below the corresponding nucleotides of clone p7. Amino acids deviant from the rat polypeptide are written in bold characters. The sequence of the neuron restricted silencer element is underlined in the 5' UTR, and the putative signal peptidase cleavage sites are marked by arrows. Proposed transmembrane regions are shown in shaded boxes. Exon boundaries are marked by vertical bars. A putative polyadenylation site is underlined in the 3' UTR.
GLRA3 Gene and Transcripts

![Fig. 2. Northern blot detection of GlyR α3 transcripts in central nervous system regions.](Image)

**Table I**

| Exon no. consensus | From nt | Acceptor nagG | Exon size | Donor AGtga/gagt | To nt |
|--------------------|---------|---------------|-----------|------------------|------|
| E1                 | 1       | tga/TGG       | 71        | CA/Gtggt        | 71   |
| E2                 | 72      | tga/TGTT      | 128       | AAGttaat        | 199  |
| E3                 | 200     | tca/GCC       | 68        | ATGtagg         | 267  |
| E4                 | 268     | tca/GAT       | 224       | AAGttagg        | 491  |
| E5                 | 492     | tca/ATT       | 83        | GCTagtaat       | 574  |
| E6                 | 575     | tca/TGG       | 138       | CA/Gtaggg       | 712  |
| E7                 | 713     | tta/GAA       | 215       | AAGtagtag       | 927  |
| E8                 | 928     | tca/GTT       | 144       | AAGttaga        | 1071 |
| E9A                | 1072    | acag/ACA      | 45        | ATGtaatna        | 1116 |
| E9b                | 1117    | acag/GAT      | 276       | AACCA/gta        | 1392 |

**GLRA3 Gene**—A human genomic PAC library (27) was screened using the radiolabeled 3-kb insert of cDNA clone p7. Based on the cDNA sequences, ten positive PAC clones were characterized further by PCR and Southern hybridization. Four clones proved to be overlapping and were used for the determination of the GLRA3 gene structure by sequencing the exon-intron boundaries and flanking intronic sequences. The coding sequence was found to be distributed over ten exons (exons 1–9; Fig. 1 and Table I), while exon 10 was completely located in the 3′-UTR. The sequences of the exon-intron boundaries (Table I) largely match the consensus sequences determined for mammalian splice sites (28). A comparison showed that the exon-intron anatomy of GLRA3 resembled those of the human and murine α1, α2, and α4 genes (8, 12, 29). Positions of exon-intron boundaries proved to be highly conserved, and gene structure homology was highest with GLRA1 sharing the same lengths for exons 1–8. The alternatively spliced stretch of 45 bp constitutes a separate novel exon, referred to as exon 9A. The putative splice site located in the 3′-UTR of the α3 transcripts was analyzed by sequencing the corresponding genomic region contained in PAC clone ZP5.1. As the genomic sequences obtained were completely identical to the 3′-UTR within clone p7, the sequence starting from nucleotide 1648 in clone p7 is thought to represent the unspliced intron 9. To exclude that the persistence of intron 9, as found in clone p7, was due to a splice artifact, the expressed sequence tags (EST) data base of the GenBank™ was searched using the 3′-UTR sequence of this clone. Indeed, two independent cDNA clones (AA283885, AA488804) were identified that contained the identical 3′-UTRs covering the exon 9/intron 9 transition sequence.

The chromosomal localization of GLRA3 was determined by FISH to normal human metaphase chromosomes using the two independent PAC clones ZP3.2 and ZP5.1 detected by an FITC-labeled antibody against digoxigenin. From each of the two experiments, at least 40 metaphase cells were analyzed microscopically and 15 metaphase cells with particularly extended chromosomes were selected for digital image analysis. The FITC images, revealing the probe signals, were overlaid with the corresponding images of DAPI-banded chromosomes. Both PAC clones mapped to the same chromosomal bands allowing the assignment of GLRA3 to 4q33-q34 (Fig. 3). No additional signals were found in other regions of the human genome for any of the PAC clones tested.

**GLRA3 Allelic Variants and the Human Hypertonic Motor Disorder, Hyperekplexia**—The human neurological disorder, hyperekplexia, is caused by mutations of the GLRA1 gene in a large number of cases. In the majority of cases, however, no association could be found with allelic variants of GLRA1 (12). We therefore investigated the role of GLRA3 as a candidate gene of hereditary hyperekplexia. DNA samples from 14 patients previously excluded to carry GLRA1 coding mutations,
were subjected to SSCP screening. In analogy to GLRA1 allelic variants, where all amino acid substitutions identified are restricted to TM2 and its flanking polypeptide segments, we focused on the corresponding exons 6, 7, and 8 (12, 30–32). In 4 out of 14 patients, an SSCP polymorphism was identified in amplimers from exon 7. Direct sequencing revealed that this polymorphism was due to a silent C to T exchange in the wobble position of codon T292. Polymorphism frequencies did not significantly differ between a sample of normal probands and affected individuals (data not shown).

**Functional Expression of GlyR α3 Variants in HEK 293 Cells**—The physiological properties of glycine receptor channels encoded by both α3 splice variants were analyzed by patch-clamp recording from transfected HEK 293 cells. Upon expression of the GlyR α3L and α3K cDNA constructs, superfusion with glycine elicited current responses characterized by maximal membrane currents (I_{max}) with mean amplitudes of 3988 ± 1169 pA and 3690 ± 737 pA, respectively (Fig. 4A, Table II). Half-maximal responses (EC_{50}) were observed at glycine concentrations of 54 ± 12 μM (n = 7) and 64 ± 14 μM (n = 5) for GlyR α3L and GlyR α3K, respectively (Fig. 4B, Table Iic).

When symmetrical chloride concentrations (145 mM) were used, analysis of the I-V relationships of glycine-induced currents produced no significant differences in reversal potentials of about 0 mV (data not shown). With both variants, the glycine currents induced proved to be sensitive to strychnine, but revealed no detectable differences in the strychnine-affinity (data not shown). To unravel differential efficacies of glycineergic agonists, the relative sizes of maximum currents induced by glycine, β-alanine, and taurine were compared for both GlyR α3 splice variants. No differences in the efficacies of β-alanine (10 mM) and taurine (10 mM) were observed as compared with glycine (1 mM, data not shown).

Glycine receptor currents show a pronounced desensitization behavior. Using the agonist glycine at near saturating concentrations (100 μM), the time courses of current desensitization were investigated for both α3 splice variants. As depicted in Fig. 4C for representative responses, application of glycine (10 μM) to cells expressing these splice variants revealed distinct desensitization kinetics. In most of the experiments, both variants exhibited time courses of desensitization that fitted a double exponential decay, where the relative amplitude of the second component consistently accounted for less than 20% of the first one. Receptors expressed from α3L constructs desensitized more slowly and declined to a lesser extent than α3K receptor channels (Table II).

**DISCUSSION**

Here, we present the structure and chromosomal localization of the human GlyR α3 subunit gene (GLRA3), and its splice variants GlyR α3L and GlyR α3K. As revealed by functional expression, these variants give rise to glycine-gated chloride channels differing in desensitization kinetics.

Molecular cloning led to the identification of the human GlyR α3 subunit that was found to exist in two splice variants differing in sizes of the polypeptides encoded. The amino acid sequences of both variants were highly homologous to the previously characterized rodent polypeptide (25), indicating that the gene family of glycine receptor subunits is conserved during phylogeny (6, 8, 25). While subunit variant α3L represented the homologue of the rat α3 transcript previously described (25), a 45-bp segment was deleted from the novel transcript α3K. When compared with the GLRA3 gene structure, it became apparent that this alternative segment reflected a distinct exon, termed exon 8A, which codes for 15 amino acids situated within the putative cytoplasmic loop connecting TM3 and TM4 of the mature polypeptide.

Both transcripts exhibited further divergence. The 3’-UTR of transcript α3K represented a continuous copy of the corresponding genomic region. In contrast, a diverging sequence was contained in the 3’-UTR of α3L, indicative of a further splice event. The difference in α3 transcript sizes (2.4 versus 9 kb) as observed by Northern analysis is most likely explained by the persistence of large intronic sequences within the 3’-UTR of the α3K mRNA. This analysis also showed that the inclusion of exon 8A is linked to the exclusion of these large 3’ sequences (>6 kb) from the α3L transcript, generating an inverted repeat of 31 bp. A sequence motif overlapping the boundary between exons 6 and 7 is invertedly repeated by a stretch of nucleotides contained within the segment of the 3’-UTR unique for variant α3L. As inverted repeats are capable to form stem structures of RNA loops, these α3 transcript variants are likely to differ substantially in mRNA secondary structures. Thus, inclusion of 3’ intronic sequences may lead to an altered mRNA folding, suggesting the formation of variant-specific mRNA secondary structures. The functional importance of these mRNA structures is not understood. A search for specific RNA consensus sequences revealed, however, that a cluster of three pentanucleotides occurred in the 5’-UTR, reminiscent of the consensus sequence (UCAU(N)_{0–2}U) recognized by the RNA binding protein Nova-1 (33). Binding of Nova-1 has been demonstrated for primary transcripts encoding the human GlyR α2 subunit (33), suggesting that the motif analyzed here may indeed serve a similar function. Taken together, it may be tempting to speculate that splicing regulates the formation of long stem-loop RNA structures, thus exposing determinants for RNA-protein interaction.

The GlyR α3 variants generated by alternative splicing differ within the cytoplasmic loop between TM3 and TM4. A similar heterogeneity exists for the splice variants of the GlyR α1 subunit (9) and the 5-HT_{6} receptor (34), where usage of an alternate acceptor splice site for exon 9 results in the insertion of eight and six amino acids, respectively, within a homologous position. These structural characteristics of variants α3K and α3L coincide with distinct desensitization behaviors of the re
GLRA3 Gene and Transcripts

Electrophysiological properties of GlyR α3K and GlyR α3L

| GlyR α3K   | GlyR α3L   |
|------------|------------|
| EC_{50}    | 64 ± 14 μM (n = 5) | 54 ± 12 μM (n = 7) |
| Hill coefficient (n_{H}) | 2.5 ± 0.2 (n = 5) | 2.8 ± 0.3 (n = 7) |
| I_{max}    | 3690 ± 737 pA | 3988 ± 1169 pA |
| τ_{1}      | 1.2 ± 0.6 s (n = 17) | 4.6 ± 1.4 s (n = 24) |
| F(1)       | 79 ± 13% | 52 ± 11% |

In contrast to the functional expression of GlyR α3 splice variants in HEK 293 cells, no physiological differences were apparent upon heterologous expression of the GlyR α1 and 5-HT_{3} receptor splice variants in Xenopus laevis oocytes (9, 34). It should be noted, however, that the phosphorylation background is high in Xenopus laevis oocytes due to a high basal level of protein kinase A activity, thus potentially masking functional differences (37). It remains to be shown whether the differences in GlyR α3 desensitization kinetics can be attributed to changes in phosphorylation status or to alterations in receptor architecture due to inclusion of the additional peptide sequence.

Analysis of the GLRA3 gene structure revealed a high degree of homology to the previously characterized glycine receptor subunit genes in mouse and man (8, 12, 19, 29). The alternatively spliced exon 8A, however, represents a structural element unique among glycine receptor genes. Mapping of the GLRA3 gene locus revealed its chromosomal localization in the vicinity of the GLRB gene, which was assigned to the human chromosomal band 4q31.3 (19, 40). This chromosomal region is linked by synteny homology to a region on mouse chromosome 8, where the murine Glra3 gene is situated (7). While no obvious correlations to currently known disease loci exist, the hu-
man gene GLRA3 nevertheless remains a major candidate gene for hypertonic and convulsive disorders.

Acknowledgments—We thank G. Hebel-Klebsch, A. Heister, and R. Facke-Kühnhauser for technical assistance, H. Betz, N. Milani, and C. Kling for support, and H.-G. Breitinger and T. Bonk for a critical reading of the manuscript.

REFERENCES

1. Becker, C.-M. (1995) Neuron Scientist 1, 130–141
2. Grenningloh, G., Bienen, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D., and Betz, H. (1987) Nature 328, 215–220
3. Sontheimer, H., Becker, C.-M., Fritchett, D. B., Schofield, P. R., Grenningloh, G., Kettenmann, H., Betz, H., and Seeburg, P. H. (1989) Neuron 2, 1491–1497
4. Vandenberg, R. J., Handford, C. A., and Schofield, P. R. (1992) Neuron 9, 491–496
5. Ryan, S. G., Buckwalter, M. S., Lynch, J. W., Handford, C. A., Segura, L., Shiang, R., Wasmuth, J. J., Camper, S. A., Schofield, P., and O’Connell, P. (1994) Nat. Genet. 7, 131–135
6. Derry, J. M., and Barnard, P. J. (1991) Annu. Rev. Biochem. 60, 585–619
7. King, M. F., Suh, D., and Seldin, M. F. (1994) Mamm. Genome 5, 831–832
8. Matzenbach, B., Mauel, Y., Sotelo, R., Courtier, B., Arner, P., Guenet, J. L., and Betz, H. (1994) J. Biol. Chem. 269, 2067–2072
9. Malosio, M.-L., Grenningloh, G., Kuhse, J., Schmieden, V., Schmitt, B., Prior, P., and Betz, H. (1991) J. Biol. Chem. 266, 2048–2053
10. Kuhse, J., Kuryatov, A., Mauel, Y., Malosio, M.-L., Schmieden, V., and Betz, H. (1991) FEBS Lett. 283, 73–77
11. Grenningloh, G., Schmieden, V., Schofield, P. R., Seeburg, P. H., Siddique, T., Mohandas, T. K., Becker, C.-M., and Betz, H. (1990) EMBO J. 9, 771–776
12. Shiang, R., Ryan, S. G., Zhu, Y. Z., Kiousis, P., Poustka, A., and Becker, C.-M. (1994) EMBO J. 13, 22317–22320
13. Buckwalter, M. S., Cook, S. A., Davison, M. T., White, W. F., and Camper, S. A. (1994) Hum. Mol. Genet. 3, 2025–2030
14. Saul, B., Schmieden, V., Kling, C., Mulhardt, C., Gass, P., Kuhse, J., and Becker, C.-M. (1994) FEBS Lett. 350, 71–76
15. Kling, C., Koch, M., Saul, B., and Becker, C.-M. (1997) Neuroscience 78, 411–417
16. Kuhse, J., Kuryatov, A., Malosio, M.-L., and Betz, H. (1990) EMBO J. 9, 22317–22320
17. Milani, N., Malosio, C., Weber, R. G., Lichter, P., Kiousis, P., Poustka, A., and Becker C.-M. (1996) Genomics, in press
18. Sambrook, J., Fritchett, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Lichter, P., Tang, C. J., Call, K., Hermansson, G., Evans, G. A., Housman, D., and Ward, D. C. (1990) Science 247, 64–69
20. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
21. Malosio, M.-L., Marquez-Pouey, B., Kuhse, J., and Betz, H. (1991) EMBO J. 10, 2401–2409
22. Becker, C.-M., Hoch, W., and Betz, H. (1988) EMBO J. 7, 3717–3726
23. Kuhse, J., Schmieden, V., and Betz, H. (1990) J. Biol. Chem. 265, 22317–22320
24. Schoenherr, C. J., and Anderson, D. J. (1995) Science 267, 1360–1363
25. Ioannou, P. A., Amemiya, C. T., Garnes, J., Kroisel, P. M., Shizuya, H., Chen, C., Baizer, M. A., and de Jong, P. J. (1994) Nat. Genet. 6, 84–89
26. Breathnach, R., and Chambon, P. (1981) Annu. Rev. Biochem. 50, 349–383
27. Monani, U., and Burghes, A. H. (1996) Genome Res. 6, 1200–1206
28. Rees, M. I., Andrew, M., Jawad, S., and Owen, M. J. (1994) Hum. Mol. Genet. 3, 2175–2179
29. Shiang, R., Ryan, S. G., Zhu, Y. Z., Fielder, T. J., Allen, R. J., Fryer, A., Yamashita, S., O’Connell, P., and Wasmuth, J. J. (1996) Ann. NeuroL 38, 85–91
30. Milani, N., Dalpra, L., Del Prete, A., Zanini, R., and Larizza, L. (1996) Am. J. Hum. Genet. 58, 420–422
31. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
32. Milani, N., Dalpra, L., Del Prete, A., Zanini, R., and Larizza, L. (1996) Am. J. Hum. Genet. 58, 420–422
33. Buckanovich, R. J., and Darnell, R. B. (1997) Mol. Cell. Biol. 17, 3194–3201
34. Uetz, P., Abdelatty, F., Villarroel, A., Rappold, G., Weiss, B., and Koenen, M. (1994) FEBS Lett. 339, 302–306
35. Pinna, L. A. (1990) Biochim. Biophys. Acta 1054, 267–284
36. Huganir, R. L., Delcour, A. H., Greengard, P., and Hess, G. P. (1986) Nature 321, 774–776
37. Hoffman, P. W., Ravindran, A., and Huganir, R. L. (1994) J. Neurosci. 14, 4185–4195
38. Vaello, M. L., Ruiz-Gomez, A., Lema, J., and Mayor, F., Jr. (1994) J. Biol. Chem. 269, 2062–2068
39. Ruiz-Gomez, A., Vaello, M. L., Valdivieso, F., and Mayor, F., Jr. (1991) J. Biol. Chem. 266, 559–566
40. Handford, C. A., Lynch, J. W., Baker, E., Webb, G. C., Ford, J. H., Sutherland, G. R., and Schofield, P. R. (1996) Mol. Brain. Res. 35, 211–219