Neuron, Vol. 21, 707–716, October, 1998, Copyright ©1998 by Cell Press

Homer Regulates the Association of Group 1 Metabotropic Glutamate Receptors with Multivalent Complexes of Homer-Related, Synaptic Proteins

Bo Xiao,*§ Jian Cheng Tu,*§ Ronald S. Petralia,† Joseph P. Yuan,* Andrew Doan,* Christopher D. Breder,* Alicia Ruggiero,* Anthony A. Lanahan,* Robert J. Wenthold,† and Paul F. Worley*‡

*Department of Neuroscience
Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

†Laboratory of Neurochemistry
NIDCD/NIH
36 Convent Drive Msc 4162
Bethesda, Maryland 20892

Summary

Homer is a neuronal immediate early gene (IEG) that is enriched at excitatory synapses and binds group 1 metabotropic glutamate receptors (mGluRs). Here, we characterize a family of Homer-related proteins derived from three distinct genes. Like Homer IEG (now termed Homer 1a), all new members bind group 1 mGluRs. In contrast to Homer 1a, new members are constitutively expressed and encode a C-terminal coiled-coil (CC) domain that mediates self-multimerization. CC-Homers form natural complexes that cross-link mGluRs and are enriched at the postsynaptic density. Homer 1a does not multimerize and blocks the association of mGluRs with CC-Homer complexes. These observations support a model in which the dynamic expression of Homer 1a competes with constitutively expressed CC-Homers to modify synaptic mGluR properties.

Introduction

Homer was identified based on its rapid induction in rat hippocampal granule cell neurons following excitatory synaptic activity (Brakeman et al., 1997; Kato et al., 1997). As part of our analysis of Homer, we established that it is induced by synaptic mechanisms in association with long-term potentiation in the hippocampus (Brakeman et al., 1997). Homer is also dynamically responsive to natural neuronal activity in the developing postnatal visual cortex (Brakeman et al., 1997), to drugs that alter dopamine signaling in the striatum (Brakeman et al., 1997), and to visual stimuli that alter the circadian cycle (Park et al., 1997). Accordingly, the gene regulatory aspects of Homer are similar to other immediate early genes (IEGs) that are rapidly responsive to specific forms of natural and pathological activity, and they suggest a role for Homer in a broad range of activity-dependent neuronal responses.

In contrast to the more widely studied IEGs that encode transcription factors, Homer encodes a protein that appears to function directly at the synapse. This premise was based on the discovery that Homer specifically binds the C terminus of group 1 metabotropic glutamate receptors (mGluRs) (Brakeman et al., 1997). Moreover, Homer protein was found to localize at excitatory synapses (Brakeman et al., 1997). While its precise contribution to activity-dependent plasticity remains to be established, Homer provides precedent that IEGs may play a direct role in activity-dependent modulation of synaptic glutamate receptors.

In our initial report, we noted several Homer-related sequences in the EST databases. This suggested that Homer might be part of a family of evolutionarily conserved proteins. Here, we present a comprehensive picture of the family of Homer-related proteins and define their functional properties. These studies support a mechanism in which the Homer IEG acts as a natural "dominant negative," in dynamic competition with constitutively expressed Homer family members, to regulate synaptic metabotropic function.

Results

Homer Family Members

The original Homer sequence (Homer 1a) was used to screen cDNA libraries prepared from rat and mouse brain for related gene products. Homer 1a sequence was also used to search GenBank databases. Several related rodent, human, and *Drosophilia* sequences were identified, and their alignments are presented in Figure 1.

cDNAs that are most closely related to Homer 1a appear to represent alternative splice forms. This inference is based on nucleotide sequence identity of their 5′UTRs and the first 175 aa of the open reading frames (ORF). The presumptive novel splice variants, termed Homer 1b and 1c, are completely divergent from Homer 1a after aa 175 of the ORF and possess entirely distinct 3′ UTRs. Comparison at the point of sequence divergence indicates that Homer 1a encodes a unique 11 aa C terminus of the ORF and a ~5 kb 3′ UTR region. The unique 11 aa C-terminal sequence of Homer 1a does not possess a recognizable motif. Homer 1b and 1c substitute an additional 168/180 aa that is predicted to possess coiled-coil (CC) secondary structure (Lupas, 1996). While the 3′UTR sequence of Homer 1a includes multiple AUUUA repeats, which are implicated in destabilizing mRNAs of IEGs (Shaw and Kamen, 1986), the 3′UTR sequence of Homer 1b/c does not include this motif. The only difference between Homer 1b and 1c is the inclusion in Homer 1c of a 12 aa sequence insertion at aa position 177 between the conserved N terminus and the CC domain. Thus, Homer 1b/c appear to be formed by a splicing event that substitutes a relatively long and unique C terminus of the ORF and shorter 3′UTR sequence that lacks the characteristic IEG motif. Multiple independent isolates of rat and mouse Homer 1b and 1c were identified and sequenced to confirm their natural expression in brain. cDNAs of all reported sequences were confirmed to possess a poly A tail.

Further searches identified cDNA sequences that appear to represent two additional Homer genes, termed

‡To whom correspondence should be addressed (e-mail: pworley@bs.jhmi.edu).

§These authors contributed equally to this work.

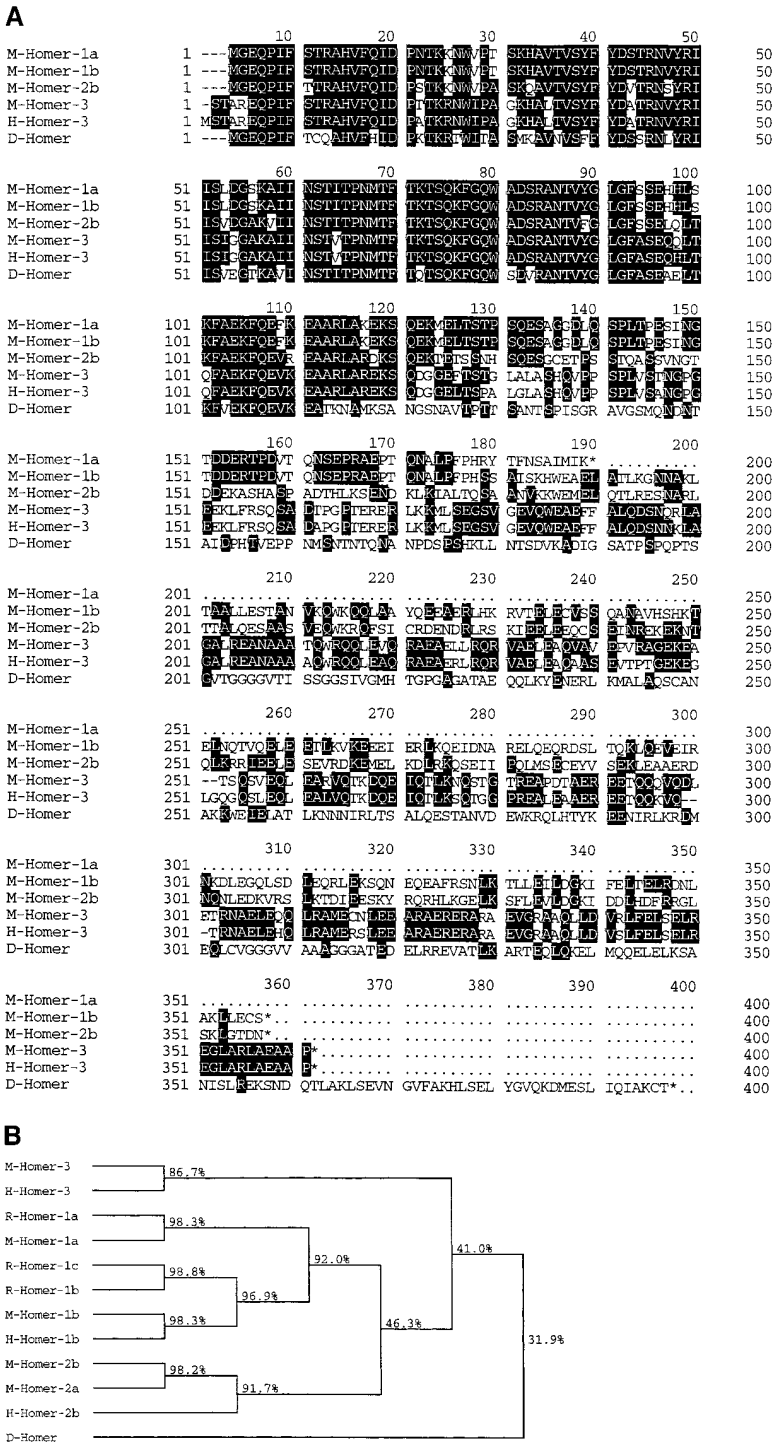

Figure 1. Homer Family Proteins

(A) Amino acid sequence alignment of mammalian Homer 1, 2, 3 proteins and *Drosophila* Homer homolog (R, rat; M, mouse; H, human; D, *Drosophila*) showing that the N-terminal ~120 aa is well conserved between Homer family members.

(B) Phylogenetic tree of all the Homer family members generated by using Higgins-Sharp algorithm (CLUSTAL4) on MacDNASIS.

Homer 2 and 3 (Figure 1). We report the sequence of two slice forms of Homer 2 and one Homer 3 cDNA sequence. The predicted size of the protein products and general domain structures are similar to Homer 1b/c. Like Homer 1b/c, each of the Homer 2 and Homer 3 predicted proteins contains an N-terminal ~120 aa that is highly similar to the N-terminal domain of Homer 1a. The degree of amino acid identity in these regions is 88% between Homer 1/Homer 2 and 86% between Homer 1/3. Many of the amino acid differences are conservative.

In contrast to the high degree of conservation in the N-terminal 120 aa, the C-terminal regions of Homer 2 and 3 are only ~22% identical to Homer 1b, but like Homer 1b/c they are predicted to possess a CC secondary structure (Lupas, 1996). The CC domains of all Homer family members exhibit significant homology (40%–45% aa similarity) to the CC regions of myosin heavy chain

(Strehler et al., 1986), kinesin heavy chain (Yang et al., 1989), and dynactin (Gill et al., 1991). The distinct splice forms of Homer 2, termed Homer 2a and 2b, are differentiated by an 11 aa insertion at position 131 in Homer 2b. Northern analysis of mouse forebrain indicates the size of Homer mRNAs to be as follows: Homer 1a, ~7 kb; Homer 1b/c, 4.8 and 5.5 kb; Homer 2a/b, 2 and 7 kb; and Homer 3, ~2.2 kb (data not shown; probes did not distinguish splice forms of Homer 1b/c or 2a/b). Human Homer 1, 2, and 3 are mapped to chromosomes 5, 15, and 19, respectively, by the Human Genome Project.

Drosophila Homer possesses the basic domain structure of mammalian Homers. The N terminus is highly homologous to that of mammalian Homer, and the C terminus is predicted to form a CC secondary structure. We have not detected a Homer 1a-like form in *Drosophilia*.

Homer 1a Expression in Brain Is Uniquely Dynamic

Rat Homer 1a was cloned based on its rapid upregulation in hippocampal granule cell neurons following maximum electroconvulsive seizure (MECS) (Brakeman et al., 1997). We therefore examined and compared the regulation of mouse Homer family members in brain following seizure. Radiolabelled riboprobes were prepared using unique sequence for Homer 1a, Homer 1b/c, Homer 2a/b, and Homer 3. Probes did not distinguish between the splice forms of Homer 1b and 1c or Homer 2a and 2b. Figure 2 illustrates the in situ hybridization pattern for each mRNA in the mouse brain of control mice and mice that were sacrificed 3 hr following MECS. The seizure-stimulated mouse was pretreated with the protein synthesis inhibitor cycloheximide to stabilize IEG mRNAs (Worley et al., 1990; Lanahan et al., 1997).

In situ studies illustrate the dramatic induction of Homer 1a in response to MECS. Comparisons of the anatomic distribution reveal that expression of Homer 1a in both the control and MECS-stimulated brains generally parallels that of Homer 1b/c with high-level expression in the hippocampus, striatum, and cortex. As previously described in rat, Homer 1a is induced in mouse brain following MECS. The magnitude of induction is estimated to be 8- to 10-fold by densitometry of autoradiographic images. By contrast, Homer 1b/c, Homer 2a/b, and Homer 3 mRNAs are not significantly upregulated after MECS. This differential regulation of Homer mRNAs was confirmed by Northern analysis (data not shown). Since Homer 1a and Homer 1b/c appear to represent products of the same gene, the uniquely dynamic expression of Homer 1a suggests that either the splicing, transcript termination, or turnover of the Homer 1a transcript is regulated by neuronal activity.

Expression of both Homer 1a and Homer 1 b/c in cortex is laminar with highest levels in the superficial and deep layers. By contrast, Homer 2a/b is discretely enriched in the thalamus, olfactory bulb, and principle neurons of the hippocampus, with lower levels in the cortex. Homer 3 mRNA is expressed at highest levels in the cerebellum and hippocampus. Thus, Homer genes appear to be expressed in the forebrain with distinct, yet overlapping patterns. The riboprobe activity and exposure times for each of the images were similar (2 days), suggesting approximately comparable levels of mRNA expression for each of the CC-Homer family members in brain.

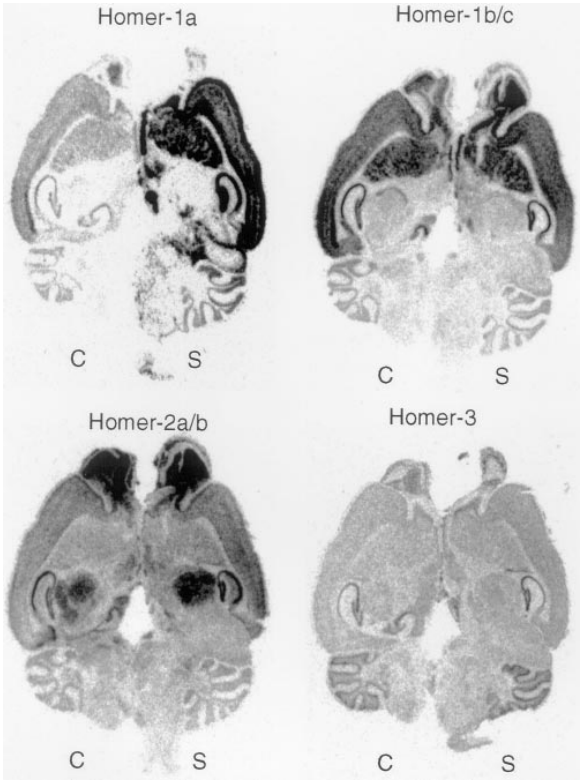

Figure 2. Expression and Regulation of Homer Family Members in Mouse Brain

In situ hybridization of MECS-stimulated mouse (S) and naive control mouse (C) brain. Adjacent sections reveal mRNA expression of Homer 1a, Homer 1b/c, Homer 2a/b, and Homer 3. Homer 1a expression in cortex, hippocampus, and striatum is induced by MECS. Homer 1b/c is expressed in cortex, hippocampus, and olfactory bulb. Homer 2 is expressed in olfactory bulb, hippocampus, and thalamus. Homer 3 is expressed in cerebellum and hippocampus. Note that only Homer 1a is strongly induced by MECS.

Homer Family Proteins Are Enriched in Brain Synaptic Fractions and Are Expressed in Select Peripheral Tissues

Rabbit polyclonal antibodies were generated against synthetic peptides derived from the unique C termini of Homer 1b/c, Homer 2a/b, and Homer 3 and used to examine the brain tissue expression of Homer family members (Figure 3A). Unpurified antibodies were first tested for their sensitivity and specificity in detecting heterologously expressed, full-length Homer proteins with N-terminal c-myc tags. Each Homer protein was selectively detected on Western blot by the appropriate Homer Ab in soluble extracts of transfected HEK293 cells. The myc-tagged Homer proteins migrated with an apparent molecular mass of 50 kDa. There was no cross-reactivity between antibodies for one Homer form and other family members. Affinity purification of the antisera did not enhance the quality of the reagents, and in all subsequent studies we used crude antisera.

We next examined immunoblot staining of SDS (2%)

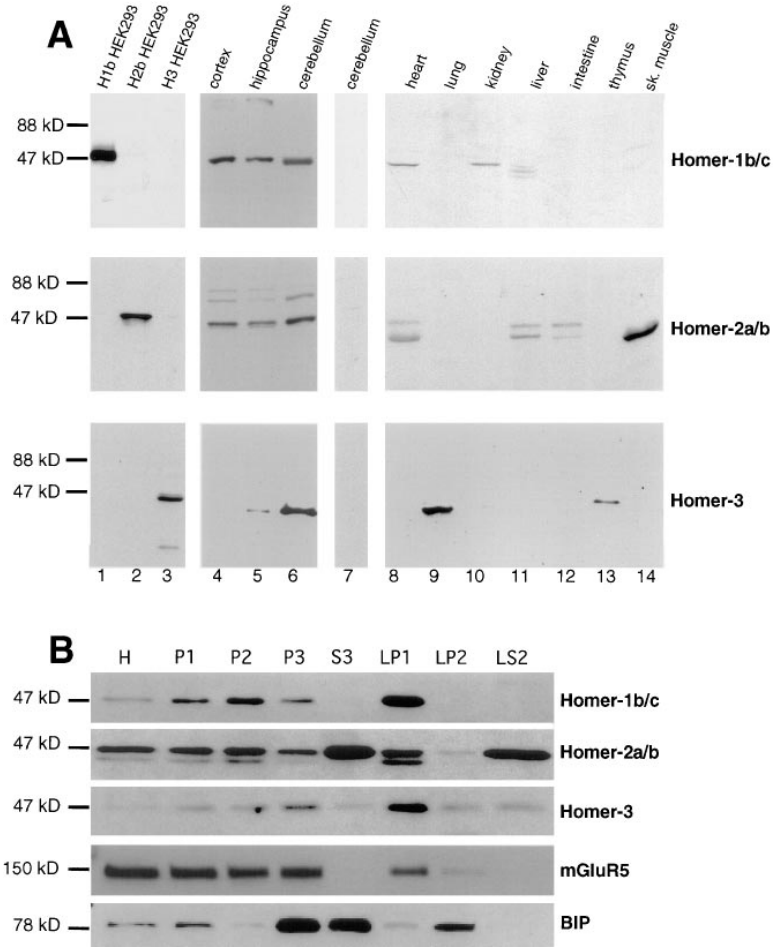

Figure 3. Tissue Expression of Homer Family Proteins

(A) Immunoblots with antibodies for Homer 1b/c, 2a/b, and 3. Lanes 1–3, HEK293 cell lysates transfected with Myc-tagged Homer 1b, Homer 2b, and Homer 3, respectively. Homer antibodies specifically recognize the appropriate fusion proteins. Lanes 4–6, cortex, hippocampus, and cerebellum extracts probed with indicated Homer Ab. Lane 7, cerebellum probed with Homer antibody preadsorbed with relevant peptide. Note that Homer 1b/c and Homer 2a/b are similarly expressed in different brain regions, while Homer 3 is enriched in the cerebellum. Homer family members are expressed in select peripheral tissues. Lane 8, heart; lane 9, lung; lane 10, kidney; lane 11, liver; lane 12, intestine; lane 13, thymus; and lane 14, skeleton muscle.

(B) Subcellular fractions from rat brain were prepared according to the method of Huttner et al. (1983), resolved by SDS-PAGE, transferred to nitrocellulose membranes, and blotted with antibodies specific for Homer 1, Homer 2, Homer 3, mGluR5, and BIP. Equal amounts of proteins were loaded on each lane (24 μg). Homer 1 and 3 were enriched in the PSD-enriched LP1 fraction, whereas Homer 2 was also present in the LP1 fraction but was most concentrated in the cytosol S3 and LS2 fractions. The blot was also probed with mGluR5, and BIP yielding bands at the appropriate indicated molecular masses.

extracts of various brain regions. Homer 1b/c Ab detected a single band of ~47 kDa in cortex, hippocampus, and cerebellum. These regions have similar levels of expression. The Homer 2a/b Ab also detected a single major band in each of cortex, hippocampus, and cerebellum. Less intense, higher apparent molecular mass bands were detected at ~60 and ~80 kDa. Homer 3 immunoblots showed low-level expression in cortex and hippocampus and intense staining of a single band in cerebellum (47 kDa). Immunostaining was completely blocked by preincubating the antibody with 10 μg/ml of the relevant peptide antigen.

Homer proteins are also expressed in peripheral tissues (Figure 3A). Homer 1b/c Ab detects a single 47 kDa band in detergent extracts of heart and kidney, and a complex of three bands, ranging from ~44–47 kDa, was detected in liver. The Homer 2a/b Ab detected staining in heart (~40 and 47 kDa), liver (42 and 47 kDa), intestine (42 and 47 kDa), and intense staining in skeletal muscle (~42 kDa). Homer 3 immunoreactivity was detected in lung and thymus.

To examine the subcellular distribution of Homer proteins, a biochemical fractionation of rat forebrain was performed and fractions were analyzed by Western blotting with Homer antibodies (Figure 3B). Fractions were also blotted for mGluR5, BIP, and synaptophysin to monitor anticipated enrichment of fractions. Homer 1b/c, 2a/b, and 3 were present in the crude nuclear pellet (P1), the medium spin crude synaptosomal pellet (P2), and the high-speed microsomal pellet (P3). BIP, a 78 kDa ER resident protein (Munro and Pelham, 1987), was enriched in both the P3 and the S3 fractions. While Homer 1b/c and Homer 3 were not abundant in the soluble (S3) fraction, Homer 2 was enriched in the S3 fraction. The P2 fraction was subfractionated after hypotonic lysis. The 25,000 × g pellet (LP1), which is enriched in PSDs (Huttner et al., 1983), showed enriched presence of mGluR5. The high-speed pellet (165,000 × g; LP2) showed the anticipated enrichment in the synaptic vesicle protein synaptophysin (data not shown). Each of the Homers was enriched in the LP1 fraction relative to LP2. The final soluble fraction (LS2) was uniquely enriched in Homer 2.

These observations demonstrate the region-specific expression of Homer family proteins in brain and indicate that Homer 1b/c and Homer 3 are enriched in the PSD fraction. Because Homer family proteins do not encode membrane spanning or highly hydrophobic domains, enrichment in the LP1 fraction suggests a tight association with other membrane-associated proteins. The relative solubility of Homer 2 is more consistent with the native biochemical character of the molecule.

These patterns of brain and tissue expression for each of the Homer proteins generally parallel the expression

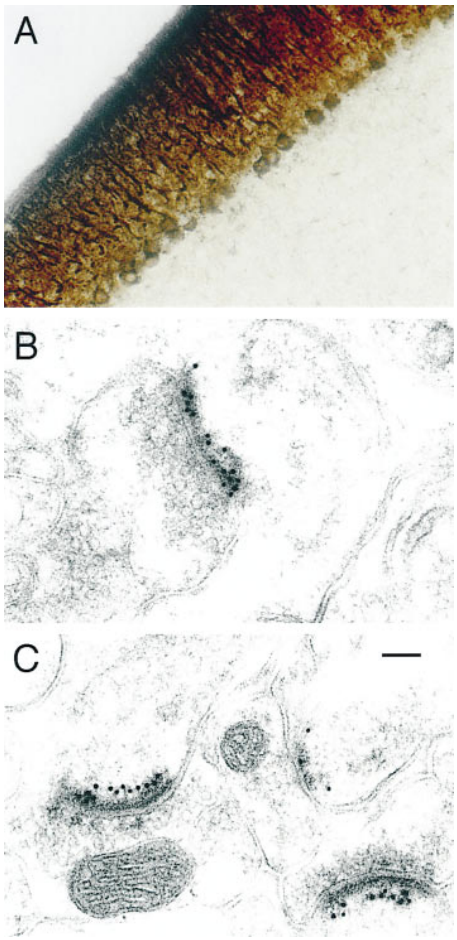

Figure 4. Immunolocalization of Homer 1b/c and Homer 3 in Rat Cerebellum

(A) Light microscopic localization of Homer 1b/c peroxidase staining. Magnification, 100×. Purkinje neuron cell bodies and dendrites are intensely stained.

(B and C) Immunogold localization of Homer 1b/c (B) and Homer 3 (C). Magnification, 55,000×; bar, 0.5 μm. Gold particles localize to the region of the postsynaptic density and subjacent cytoplasm.

of their mRNAs (Figure 2 and data not shown) and are consistent with reported EST sequences corresponding to Homer 1b/c, Homer 2a/b, and Homer 3 from various tissue sources, including brain, thymus, heart, testis, spleen, and placenta.

Homer Proteins Localize to the Postsynaptic Density

We performed immunohistochemistry to determine the cellular localization of Homer 1b/c, Homer 2a/b, and Homer 3 in rat brain. Light microscopic examinations indicated that all three Homer proteins are enriched in Purkinje neurons. Figure 4A demonstrates Homer 1b/c immunoreactivity as detected with peroxidase. Immunoreactivity is present in the cytoplasmic region of the soma and extends prominently into the dendritic arbor. The nucleus is not stained. Little or no staining is detected in the contiguous granule cell layer. A similar light microscopic pattern of cellular localization was detected for Homer 3 (data not shown). Homer 2 immunostaining in cerebellum also showed staining in Purkinje neurons but appeared technically less differentiated (data not shown).

To examine whether Homer family proteins are associated with synaptic structures, we performed immunogold EM of Purkinje neurons of the cerebellum. Homer 1b/c showed striking localization to the region of the postsynaptic spine. Gold particles are densely concentrated in the region of the PSD (Figure 4B). A very similar distribution is noted for Homer 3 immunoreactivity (Figure 4C). We note that rather than being concentrated directly over the PSD or the contiguous plasma membrane, the majority of the gold particles appear to be present in the cytoplasm immediately subjacent to these structures.

Homer Family Members Bind Group 1 mGluRs

We previously demonstrated that the N-terminal 131 aa of Homer 1a is sufficient to bind group 1 mGluRs (Brakeman et al., 1997). In view of the high degree of sequence conservation in this region of Homer family members, we examined the possibility that they would also bind group 1 receptors. GST fusion proteins were prepared of Homer 1a, Homer 1c, Homer 2b, Homer 3, and two N-terminal fragments of Homer 2. The GST fusion proteins were bound to glutathione agarose and assayed for binding to full-length mGluR5 or full-length mGluR1α expressed in HEK293 cells. Binding studies with mGluR5 are illustrated in Figure 5A. As reported previously, mGluR5 bound GST Homer 1a. mGluR5 also bound to all full-length Homer constructs and to a Homer 2 N-terminal fragment (aa 2–141) but not GST alone. While we did not measure binding affinity, the relative binding in these assays was comparable for each of the three Homers. A Homer 2 deletion mutant that includes only the N-terminal 92 aa (aa 2–92) did not bind mGluR5. These experiments demonstrate the functional conservation and approximate size of the mGluR binding domain of the Homer family. A GST fusion protein of *Drosophila* Homer also bound mGluR5 (data not shown). Identical binding specificities were found with mGluR1α (data not shown).

We next examined the possibility that the various Homer family members might naturally be associated with group 1 mGluRs in brain. For this analysis, we focused on the cerebellum, since all three Homer family members are expressed in this tissue (Figure 3). Detergent extracts of whole adult rat cerebellum were incubated with antibodies for Homer 1b/c, Homer 2a/b, or Homer 3, and immunoprecipitates were blotted with a mouse monoclonal antibody for mGluR1α (Figure 5B). Preimmune and peptide-blocked antisera were used as negative controls. mGluR1α coimmunoprecipitated with each of the Homer antibodies. The predominate band corresponded to the monomer form of mGluR1α (~150 kDa), but immunoprecipitates also contained lesser amounts of higher molecular weight proteins that are presumed multimers of mGluR1α. These observations indicate that all three Homers are involved in natural complexes with mGluR1α in brain.

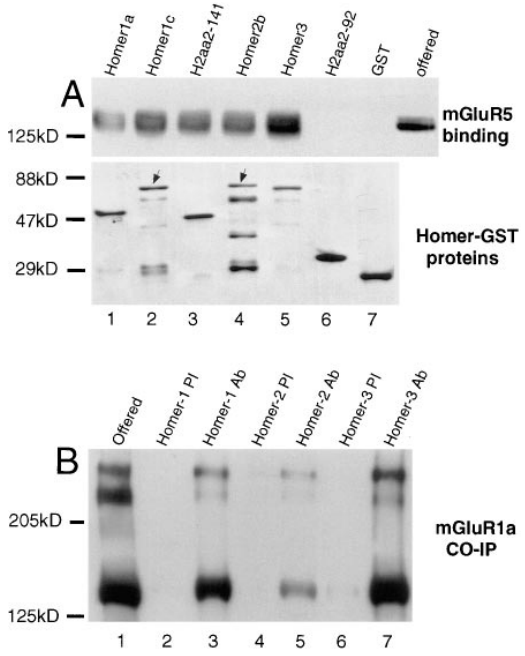

Figure 5. Homer Family Members Bind and Coimmunoprecipitate Group 1 mGluRs

(A) Upper panel: Homer family members bind full-length mGluR5 via N-terminal EVH domain. mGluR5 was expressed in HEK293 cells, and extracts were mixed with bead-linked Homer-GST fusion proteins. Bound proteins were eluted with SDS sample buffer and blotted with anti-mGluR5 C-terminal antibody.

Lower panel: Homer-GST fusion proteins used in each binding experiment probed with anti-GST antibody. Lane 1, Homer 1a; lane 2, Homer 1c; lane 3, Homer 2b; lane 4, Homer 3; lane 5, Homer 2N (aa 2–141); lane 6, Homer 2N (aa 2–92); lane 7, GST alone; lane 8, offered mGluR5 lysates.

(B) mGluR1α coimmunoprecipitates with Homer 1b/c, 2a/b, and 3. Rat cerebellum lysates were precipitated with Homer 1b/c, 2a/b, and 3 antiserum, with relevant preimmune serum as a negative control. The precipitates were separated on 8% SDS-PAGE gels, transferred to nitrocellulose membrane, and probed with anti-mGluR1α monoclonal antibody. Lane 1, offered rat cerebellum lysates; lane 2, preimmune for Homer 1 antibody; lane 3, anti-Homer 1b/c; lane 4, preimmune for Homer 2a/b; lane 5, anti-Homer 2a/b; lane 6, preimmune for Homer 3; and lane 7, anti-Homer 3.

The CC Domain of Homer Family Members Confers Specific Multimerization

The CC secondary structure is implicated in protein–protein interactions (Lupas, 1996). We therefore examined the possibility that this domain might confer the ability to form homo- or heteromultimers between Homer family members.

In the first set of experiments, we tested the ability of full-length, bacterially expressed GST fusion proteins of Homer to bind full-length myc-tagged Homer proteins expressed in HEK293 cells (Figure 6A). myc Homer 1c bound Homer 1b, Homer 2b, Homer 3, Homer 1b, and Homer 2b C-terminal CC domain, but not Homer 1a or Homer 2 N terminus. This is consistent with the notion that the CC domain is important in the interaction, since Homer 1a and Homer 2 N terminus do not encode the CC domain. To test the specificity of the CC domain interactions, we generated GST fusions of dynein IC-1a and dynein IC-2c. The CC domains of these proteins show modest sequence homology to Homer family CC domains and bind to the CC domain of dynactin (Gill et al., 1991). None of the myc-tagged Homer family members bound to either dynein IC-1a (Figure 6A) or dynein IC-2c (data not shown).

To determine whether Homer family members naturally form multimers in brain, we again examined immunoprecipitates of cerebellum (Figure 6B). Extracts immunoprecipitated with Homer 1b/c Ab contained Homer 3, while extracts immunoprecipitated with Homer 3 contained Homer 1b/c. While it is possible that these coimmunoprecipitated Homer family members are associated by means other than their CC domains, the fact that the N terminus of Homer is monovalent and cannot form extended concatomers supports a model of multimerization mediated by the CC domains. We did not detect Homer 2 multimerized with either Homer 1 or Homer 3 in these immunoprecipitation experiments.

Expression of Homer 1a Selectively Blocks Binding of Homer 1b/c to mGluR5 In Vivo

Homer 1a is unique within the family of Homer-related proteins in that it is dynamically regulated and lacks the CC domain. Accordingly, we hypothesized that the IEG form will bind to group 1 mGluRs and disrupt the formation of multivalent complexes of Homer and mGluR. To examine this hypothesis, we generated a transgenic mouse that expresses Homer 1a under the control of a modified Thy-1 promoter (Gordon et al., 1987), which drives neuron-specific expression in postnatal brain (Aigner et al., 1995). Transgenic mice expressed Homer 1a at high levels in cortex, hippocampus, cerebellum, and thalamus/brainstem relative to levels in wild-type littermate controls (Figure 7A). The pattern of Homer 1a transgene expression is consistent with the previously reported activity of this promoter (Gordon et al., 1987). As anticipated, antibodies for both Homer 1b/c and Homer 2a/b co-IPed mGluR5 from detergent extracts of wild-type forebrain (Figure 7B). By contrast, Homer 1b/c Ab did not co-IP mGluR5 from transgenic forebrain. mGluR5 was expressed at comparable levels in wild-type and transgenic mice. The effect of Homer 1a transgene expression was selective in that it did not disrupt the coimmunoprecipitation of Homer 3 with Homer 1b/c (Figure 7C). The latter observation is consistent with the notion that the Homer 1b/c–Homer 3 interaction is mediated by the CC domain and is predicted not to be altered by Homer 1a expression. Homer 1a was not part of the complex co-IPed with Homer 1b/c, suggesting that the CC is necessary for association with the complex (data not shown). The effect of the Homer 1a transgene in blocking the in vivo coupling of mGluR5 and Homer 1b/c was additionally selective in that Homer 2 Ab coimmunoprecipitated mGluR5 similarly from extracts of wild-type and transgenic mice. Thus, Homer 1a appears to selectively disrupt the interaction of Homer 1b/c with mGluR5, but not Homer 2 with mGluR5. Competition between Homer 1a and Homer 3 was assessed in the cerebellum (Figures 7D and 7E). Homer 3 co-IPs mGluR1α from detergent extracts of wild-type, but not transgenic, cerebellum. The association between Homer 1 and Homer 3 was not altered in the cerebellum of transgenic mouse. Identical results were obtained in three independent mouse lines that express

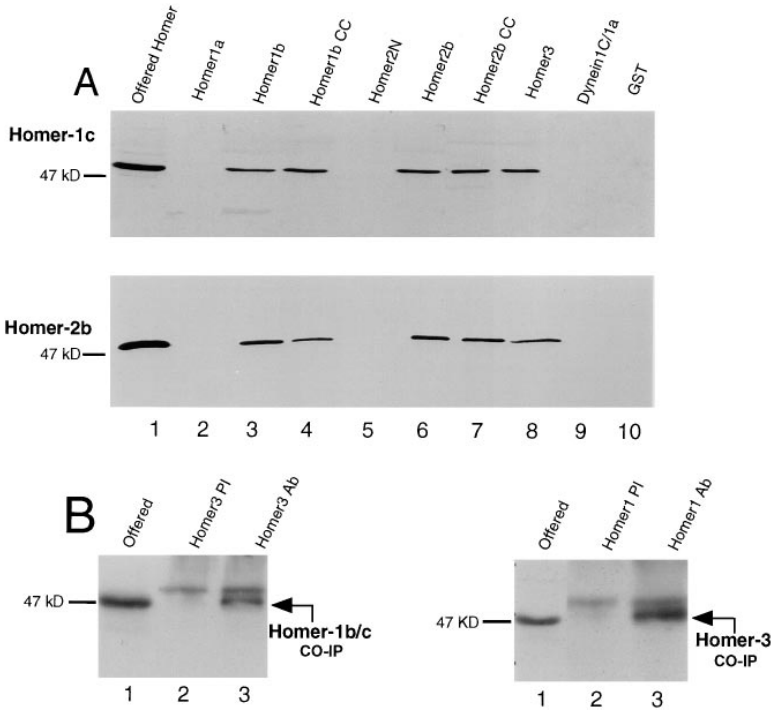

Figure 6. Homer Family Members Form Natural Multimers and Coimmunoprecipitate from Cerebellum

(A) In vitro binding assay. Upper panel: myc-tagged Homer 1c was expressed in HEK293 cells, and cell extracts were mixed with equal amounts of Homer-GST fusion proteins on beads and eluted with SDS sample buffer. Bound proteins were assayed by immunoblotting with anti-myc monoclonal antibody. Lane 1, offered HEK293 cell lysates transfected with myc-tagged Homer 1c; lane 2, Homer 1a GST; lane 3, Homer 1b GST; lane 4, Homer 1b CC (aa 175–354)GST; lane 5, Homer 2 N terminus (aa 2–141)GST; lane 6, Homer 2b GST; lane 7, Homer 2b CC (aa 121–354)GST; lane 8, Homer 3 GST; lane 9, Dynein 1C-1a GST; and lane 10, GST only. Lower panel: identical GST fusion proteins were mixed with myc-tagged Homer 2b. Lane 1 is the offered myc-Homer 2b cell lysate.

(B) Homer 1 and Homer 3 co-IP from cerebellum. Rat cerebellum lysates were precipitated with either Homer 3 or Homer 1 antibody, and with preimmune serum as control. The precipitates were analyzed by Western blot with Homer 1 or Homer 3 antibody detected with HRP-protein A. Left panel: lanes 1, offered rat cerebellum lysate probed with Homer 1 antibody; lane 2, immunoprecipitated with preimmune serum and probed with Homer 1 antibody; and lane 3, immunoprecipitated with Homer 3 antibody and probed with Homer 1 antibody. Right panel: lane 1, offered rat cerebellum lysate probed with Homer 3 antibody; lane 2, immunoprecipitated with preimmune serum and probed with Homer 3 antibody; and lane 3, immunoprecipitated with Homer 1 antibody and probed with Homer 3 antibody. The ~60 kDa band present in both the preimmune and immune lanes is the rabbit IgG heavy chain.

Homer 1a transgene. These observations support the hypothesis that Homer 1a functions as a natural dominant negative to selectively regulate association of group 1 mGluRs with multimeric complexes of Homer 1 and Homer 3.

Discussion

The present study describes a novel mechanism by which an IEG can regulate the function of signaling molecules at the excitatory synapse. Homer 1a is proposed to function as a natural dominant negative splice form that regulates the association of group 1 mGluRs with multivalent complexes of constitutively expressed, CC-containing forms of Homer 1 and 3. Since these CC-Homers are enriched at postsynaptic sites, it is hypothesized that Homer 1a expression will impact synaptic metabotropic glutamate function.

The present study provides several observations that support the dominant negative hypothesis for Homer 1a function. First, cloning studies demonstrate that Homer 1a represents a brain-enriched splice form of a family of closely related proteins. Homer family proteins possess a highly conserved N-terminal ~120 aa. The biochemical correlate of this conservation is that all Homer family proteins bind group 1 mGluRs. This appears to be a highly conserved core property of the Homer family. Previously, we demonstrated that an N-terminal 131 aa deletion mutant of Homer 1a is sufficient to bind group 1 mGluRs (Brakeman et al., 1997). This region of Homer was noted to include a RX_5GLGF sequence suggestive of a PDZ domain (Doyle et al., 1996). Subsequent to our report, others noted the stronger homology of Homer to the recently described EVH1 domain (Enabled/VASP homology) (Gertler et al., 1996; Kato et al., 1997; Ponting and Phillips, 1997). Proteins that encode the EVH1 domain include *Drosophila enabled* (termed Mena in mouse) (Gertler et al., 1996), yeast Bee1p (Li, 1997), vasodilator-stimulated phosphoprotein (VASP) (Haffner et al., 1995), and the Wiscott-Aldrich syndrome protein (WASP) (Symons et al., 1996). These proteins appear to function in the transduction of cell surface signals to the actin-based cytoskeleton.

Cloning studies further revealed the surprising finding that Homer 1a is actually the exception to the Homer family. Thus, Homer 1a lacks the characteristic CC multimerization motif present in all other Homer-related proteins. The CC domain is moderately divergent between different Homer genes but is conserved in predicted secondary structure from *Drosophila* to mammals. In vitro binding assays illustrate that the CC domains bind specifically to themselves and to the CC domains of other Homer family members. Consistent with a physiological role for the CC domain, we demonstrate the presence of natural heteromultimers of Homer 1b/c and 3 in cerebellum and forebrain. Thus, while each Homer molecule is monovalent with regard to binding group 1 mGluRs, the CC domain confers the functional capacity to cross-link group 1 mGluRs (or related Homer-interacting proteins). Homer 1a IEG lacks this ability to cross-link.

Homer 1a is uniquely dynamic in its expression, consistent with the notion that neurons regulate the cross-linking activity of constitutively expressed CC-Homers. Homer 1a mRNA is markedly enriched in brain but is also detected by RT-PCR in heart, skeletal muscle, and

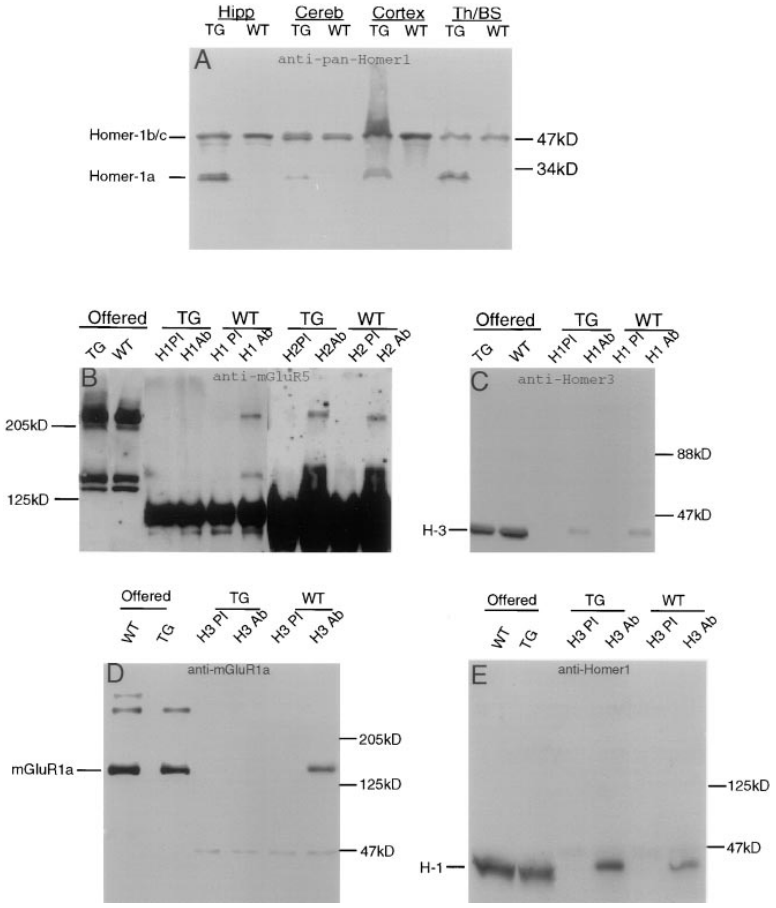

Figure 7. Homer 1a Blocks Association of Group 1 mGluRs with Specific CC-Homer Complexes

(A) Expression of Homer 1a in wild-type and transgenic mouse brain. Crude detergent (2% SDS) extracts were prepared from hippocampus (Hipp), cerebellum (Cereb), cortex, and combined thalamus and brainstem (Th/BS) and blotted (10 μg protein) with an antibody that recognizes all forms of Homer 1 (pan-Homer 1 Ab). Note the presence of the Homer 1a protein in each of the transgenic (TG) brain regions.

(B–E) Co-IP analysis of group 1 mGluRs and CC-Homers in Homer 1a transgenic mice. (B) Detergent (1% CHAPs) extracts of forebrain were precipitated with antibodies for Homer 1b/c (H1Ab) or Homer 2 (H2Ab) and blotted with antibodies for mGluR5. Control lanes use the preimmune serum (H1PI, H2PI). Offered samples show similar expression of mGluR5 monomer (~150 kDa) and dimer forms. Note co-IP of mGluR5 monomer and dimer forms from wild type, but not from transgenic forebrain. By contrast, mGluR5 co-IPs with Homer 2 from both wild-type and transgenic forebrain. The monomer band is partially obscured by the IgG heavy chain. (C) The blot from (B) was reprobed in with a Homer 3 Ab. Homer 3 is expressed and similarly co-IPed with H1b/c from wild-type and transgenic forebrain. (D) IP of cerebellum with Homer 3 Ab (H3Ab) demonstrates an association with mGluR1α in wild-type mouse that is blocked in the transgenic mouse. (E) The blot from (D) reprobed with Homer 1b/c Ab demonstrates that the Homer 3–Homer 1b/c association is not altered in the transgenic cerebellum.

liver (unpublished observation). This suggests that Homer 1a represents a general cellular mechanism to regulate CC-Homers.

In vivo studies also support the dominant negative hypothesis for the function of Homer 1a. Group 1 mGluRs are selectively uncoupled from Homer 1b/c and Homer 3 in forebrain and cerebellum of mice that express Homer 1a transgene. Homer 1a did not alter the presumed CC-dependent association of Homer 1b/c and Homer 3, nor did it block the association of Homer 2 with mGluR5. The block of association of group 1 mGluRs with Homer 1b/c and Homer 3 is in direct accordance with biochemically based predictions for a competitive interaction of Homer 1a with constitutively expressed family members. Failure to interrupt the association of mGluR5 with Homer 2 in the transgenic mouse was not anticipated based on biochemical analyses and suggests additional mechanisms that can confer this specificity.

Ultrastructural studies demonstrate a striking localization of both Homer 1b/c and Homer 3 to the PSD and provide strong support for the notion that Homer proteins function at the synapse. Biochemical fractionation studies also indicate an enrichment of Homer in the PSD fraction. The ultrastructural localization of Homer is similar to that of the mGluR1α receptor at Purkinje neuron synapses. mGluR1α is enriched at the plasma membrane at the periphery of the PSD and may also be associated with ER in the spine cytosol (Baude et al., 1993; Petralia et al., 1997). Homer is also present at the lateral margin of the PSD and appears be enriched at this site. Accordingly, the present ultrastructural localizations of CC-Homers are consistent with their hypothesized functional interaction with synaptic group 1 mGluRs.

The present study offers insight into the role of IEGs in synaptic plasticity. The present study indicates that CC-Homers may be viewed as bimodular adapter proteins that can bind group 1 mGluRs and either cross-link the receptor or couple the receptor to other proteins. In the accompanying paper, we demonstrate that CC-Homers couple mGluRs to the inositol trisphosphate receptor and appear to form a functional signaling complex (Tu et al., 1998 [this issue of *Neuron*]). Homer 1a modifies linkage of these receptors and may thereby produce long-term changes at synapses by altering signaling.

Experimental Procedures

Identification of Homer Family Members

We performed low-stringency screens of phage cDNA libraries and EST database searches to identify Homer family members. cDNA libraries were screened using rat Homer 1a coding region as a probe. Screens of mouse and rat brain cDNA libraries identified two isoforms (Homer 1b and Homer 1c) of Homer 1.

Searches of EST databases identified a mouse EST sequence

(ID#442801) that is about 73% homologous to a portion of 5′ coding region of Homer 1 cDNA sequence. Based on the EST, we used RT-PCR (forward: 5′-GAC AGC AGA GCC AAC ACC GTG-3′; reverse: 5′-GTC TGC AGC TCC ATC TCC CAC-3′) to amplify the corresponding region from various mouse tissues. The PCR products (~330 bp) consisted of two different sequences, one of which contained an additional insertion of 33 bp. We used a mixture of these two cDNA fragments as probes to screen an adult mouse brain cDNA library and cloned Homer 2. Out of 10^6 clones screened, five clones hybridized well to the probe. Sequence analysis of these clones indicated that they were partial cDNA clones representing two isoforms of Homer 2 gene and were identical to the isoforms amplified by RT-PCR. The 5′ region of Homer 2 was cloned using 5′-RACE technique. Total RNA from E14.5 mouse brain was reverse-transcribed using the above reverse primer. Another gene-specific primer (5′-CAC GGT GTT GGC TCT GCT GTC-3′) was used in the amplification of the 5′ region of Homer 2. The sequence authenticity of the 5′ RACE clones was further confirmed by sequencing a partial mouse EST clone #441857.

By searching the EST database, we were also be able to identify several human ESTs corresponding to mouse and rat Homer 1b and Homer 2a/b cDNA sequences. RT-PCR was used to clone the human Homer 1b and Homer 2a and 2b coding regions. A 5′ degenerate primer (5′-ATG GG(A/G/C) GA(A/G) CA(A/G) CC(T/C/G) AT(T/C) TTC-3′) was designed based on the N-terminal 7 aa (MGEQPIF) that is conserved among human EST clone #HCE003, mouse, rat, and *Drosophila* Homer homolog sequences. The 3′ primers (5′-GAG GGT AGC CAG TTC AGC CTC-3′) for human Homer 1 and human Homer 2 (5′-GTT GAT CTC ACT GCA TTG TTC-3′) were made from the sequences of human EST clones #562862 and #HIBAB15, respectively. We amplified human Homer 1b and Homer 2a/b from newborn human frontal cortex. The sequences of human Homer 1b and Homer 2a/b shown in Figure 1 were derived from sequencing several PCR clones and EST clones.

Human and mouse Homer 3 were identified by searching EST database, using Homer 1 and Homer 2 sequences. Two full-length human Homer 3 clones were identified (clone ID# 284002 and #38753) and sequenced. Numerous mouse Homer 3 were found, and one of them (clone ID# 1162828) contains almost full coding region. We also identified several *Drosophila* EST sequences exhibiting significant homology at the amino acid level to the N-terminal region of Homer family members. The sequence presented in Figure 1 is derived from clone #LD3829 (BDGF EST Project).

Expression Constructs

Mammalian expression constructs were made by cloning cDNA into SalI and NotI sites of pRK5 (Genentech), so that the cDNA was fused in-frame to an N-terminal c-Myc tag. GST-fusion constructs were made by cloning Homer cDNA into the SalI and NotI sites of pGEX4T-2 (Pharmacia). The full-length coding regions of mouse Homer 1b, rat Homer 1c, mouse Homer 2b, and human Homer 3 were engineered with SalI and NotI sites at the 5′ and 3′ ends by PCR using high-fidelity DNA polymerase *Pfu* (Stratagene). Various truncations of Homer 1b/c and Homer 2b coding regions were made by PCR with specific primers containing SalI and NotI sites. All the PCR-based constructs were sequenced to confirm the sequences and in-frame fusion.

In Situ Hybridization

Antisense and sense cRNA probes were generated from each mouse Homer plasmid by in vitro transcription in the presence of [^{35}S]UTP, as previously described (Lyford et al., 1995). Probe for Homer 1a (GenBank # AF093257) was derived from nucleotides 1342–2140, for Homer 1b/c (GenBank # AF093258) from nucleotides 785–1396, for Homer 2a/b (GenBank # AF093260 submission) from nucleotides 486–1561, and for Homer 3 (GenBank # AF093261) from nucleotides 371–2123. (Identical cDNA sequences were used for Northern analysis.) Probe (about 10^6 cpm in 75 μl hybridization buffer) was applied to each slide. Coverslipped slides were then incubated in humidified chambers overnight at 56°C. Following completion of wash steps, slides were air dried and exposed to Kodak Biomax MR film for 2–3 days.

Generation and Characterization of Homer Antisera

Synthetic C-terminal peptides of Homer 1, 2, or 3 were conjugated to thyroglobulin with glutaraldehyde and used to immunize rabbits according to previously published protocol (Martin et al., 1992). Peptide sequences used are as follows: Homer 1b/c, IFELTELRDNL AKLLECS; Homer 2a/b, GKIDDLHDFRRGLSKLGTDN; and Homer 3, RLFELSELREGLARLAEAA. Detergent (2% SDS) extracts from rat cortex, hippocampus, and cerebellum were separated on 8% SDS-PAGE gels and transferred to nitrocellulose membranes. Blot was probed with polyclonal anti-Homer sera. Specificity was tested by incubating the antiserum with 10 μg/ml of relevant peptide at room temperature for 1 hr prior to use. Rabbit polyclonal antiserum was also generated against the full-length GST-Homer 1a fusion protein, as described previously (Brakeman et al., 1997). This antiserum recognizes all Homer 1 isoforms.

In Vitro Binding

To examine the Homer and mGluR 1/5 interaction, HEK293 cells were transiently transfected (calcium phosphate) with full-length mGluR1α and mGluR5 constructs in pRK5 (Brakeman et al., 1997). Cell lysates were made 24–48 hr posttransfection. For examining the CC interaction of Homer family members, myc-tagged Homer 1c and Homer 2b were transfected into HEK293 cells and cell extracts were made 2–3 days posttransfection. Cell lysates were incubated with equivalent amounts of various Homer-GST fusion proteins at 4°C for 2 hr and washed with PBS and 1% Triton X-100. Proteins were eluted in 2% SDS sample buffer and separated on 8% or 12.5% SDS-PAGE gels and probed with appropriate antibody.

Coimmunoprecipitation

Rat or mouse brain tissues were sonicated (three times for 10 s each) in PBS (~200 mg/ml wet weight) containing 1% Triton X-100 or 1% CHAPs with protease inhibitors and centrifuged for 10 min at 15,000 g. Three microliters of Homer 1b/c, Homer 2a/b, or Homer 3 antiserum was added to 60 μl of tissue extract and incubated for 1.5 hr at 4°C, 60 μl of protein A Sepharose (Pharmacia) was added for an additional 1 hr, and then washed three times with PBS/Triton. Proteins were eluted in 2% SDS loading buffer. The samples were analyzed by gel electrophoresis and Western blot analysis. mGluR1α monoclonal antibody was obtained from PharMingen (San Diego, CA). Rabbit polyclonal mGluR5 Ab was a gift from Dr. Richard Huganir, John Hopkins School of Medicine.

Immunohistochemistry

For light microscopy, rats were deeply anesthetized with sevoflurane and perfused through the aorta with 250 ml of saline followed by 400 cc each of 4% paraformaldehyde in 0.1% phosphate buffer (pH 6.5) and 4% paraformaldehyde in 0.1% phosphate buffer (pH 8.5). The rat was allowed to postfix for 1 hr at room temperature and then prefused with 15% sucrose in 0.1% phosphate buffer (pH 7.4). The brain was removed and sectioned at 40 μm on a freezing sliding block microtome and collected in PBS. Tissue was stained with an immunoperoxidase technique, as follows. Brain sections were incubated in PBS containing 0.3% H_2O_2 and 0.25% Triton X-100 for 30 min and then washed three times for 5 min each in PBS. Sections were incubated in a buffer "PGT" containing 3% normal goat serum (Colorado Serum Co.) and 0.25% Triton X-100 in PBS for 1 hr and then transferred to the primary antiserum diluted 1:750 in the same PGT buffer. Sections were gently shaken for 48 hr at 4°C, washed four times for 5 min each in PBS and then incubated for 1 hr at room temperature in a goat anti-rabbit IgG conjugated to horseradish peroxidase (Biosource International) diluted 1:100 in PGT. Sections were washed four times for 5 min each in PBS and incubated for 6 min at room temperature in 0.05% diaminobenzidine dihydrochloride (DAB: Sigma) and 0.01% H_2O_2 in 0.1 M phosphate buffer. Sections were washed in PBS, mounted onto gelatin chrome-alum subbed slides, dehydrated in a series of graded ethanol, cleared in xylene, and coverslipped with DPX (BDH Limited).

For EM, we used a postembedding immunogold method as described previously (Wang et al., 1998) and modified from the method of Matsubara et al. (1996). Briefly, a male Sprague-Dawley rat was perfused with 4% paraformaldehyde plus 0.5% glutaraldehyde in

0.1 M phosphate buffer. Two hundred micrometer parasagittal sections of the rostral cerebellum (folia III-V) were cryoprotected in 30% glycerol and frozen in liquid propane in a Leica EM CPC. Frozen sections were immersed in 1.5% uranyl acetate in methanol at −90°C in a Leica AFS freeze-substitution instrument, infiltrated with Lowicryl HM 20 resin at -45°C, and polymerized with UV light. Thin sections were incubated in 0.1% sodium borohydride plus 50 mM glycine in Tris-buffered saline/0.1% Triton X-100 (TBST), followed by 10% normal goat serum (NGS) in TBST, primary antibody in 1% NGS/TBST, 10 nm immunogold (Amersham) in 1% NGS/TBST plus 0.5% polyethylene glycol, and finally staining in uranyl acetate and lead citrate. Primary antibodies were used at dilutions of 1:500 for Homer 1b and 1:100–1:400 for Homer 3.

Transgenic Mice

N-terminal myc-tagged full-length Homer 1a ORF was cloned into the expression vector pT2 (Gordon et al., 1987; Aigner et al., 1995). Transgenic mice were generated at the University of Alabama Transgenic Facility. Expression of the transgene protein was assayed by Western blot with rabbit polyclonal antisera that recognizes all Homer 1 isoforms (pan-Homer 1 Ab) and myc Ab.

Acknowledgments

We thank Dr. Y.-X. Wang for assistance in the immunogold immunocytochemistry, Maria Papapavlou for help with in situ hybridization, Patrick Burnett for help in preparation of subcellular fractions, Mitra Cowan of the JHH transgenic facility for assistance with animal breeding, and C.-Y. Li for genotyping transgenic mice. We also thank the University of Alabama Transgenic Facility. This work was supported by grants to P. W. from National Institute on Drug Abuse (DA10309, DA11742), National Institute of Mental Health (KO2 MH01152), National Institute on Aging (AG09219), and National Association for Research on Schizophrenia and Affective Disorders.

Received May 29, 1998; revised September 28, 1998.

References

Aigner, L., Arber, S., Kapfhammer, J.P., Laux, T., Schneider, C., Botteri, F., Brenner, H.R., and Caroni, P. (1995). Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell *83*, 269–278.

Baude, A., Nusser, Z., Roberts, J.D., Mulvihill, E., McIlhinney, R.A., and Somogyi, P. (1993). The metabotropic glutamate receptor (mGluR1α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron *11*, 771–787.

Brakeman, P.R., Lanahan, A.A., O'Brien, R., Roche, K., Barnes, C.A., Huganir, R.L., and Worley, P.F. (1997). Homer: a protein that selectively binds metabotropic glutamate receptors. Nature *386*, 284–288.

Doyle, D.A., Lee, A., Lewis, J., Kim, E., Sheng, M., and Mackinnon, R. (1996). Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by pdz. Cell *85*, 1067–1076.

Gertler, F.B., Niebuhr, K., Reinhard, M., Wehland, J., and Soriano, P. (1996). Mena, a relative of VASP and Drosophila Enabled, is implicated in the control of microfilament dynamics. Cell *87*, 227–239.

Gill, S.R., Schroer, T.A., Szilak, I., Steuer, E.R., Sheetz, M.P., and Cleveland, D.W. (1991). Dynactin, a conserved, ubiquitously expressed component of an activator of vesicle motility mediated by cytoplasmic dynein. J. Cell Biol. *115*, 1639–1650.

Gordon, J.W., Chesa, P.G., Nishimura, H., Rettig, W.J., Maccari, J.E., Endo, T., Seravalli, E., Seki, T., and Silver, J. (1987). Regulation of Thy-1 gene expression in transgenic mice. Cell *50*, 445–452.

Haffner, C., Jarchau, T., Reinhard, M., Hoppe, J., Lohmann, S.M., and Walter, U. (1995). Molecular cloning, structural analysis and functional expression of the proline-rich focal adhesion and microfilament-associated protein VASP. EMBO J. *14*, 19–27.

Huttner, W.B., Schiebler, W., Greengard, P., and De Camilli, P. (1983). Synapsin I (protein I), a nerve terminal-specific phosphoprotein: III. its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. J. Cell Biol. *96*, 1374–1388.

Kato, A., Ozawa, F., Saitoh, Y., Hirai, K., and Inokuchi, K. (1997). vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. FEBS Lett. *412*, 183–189.

Lanahan, A., Lyford, G., Stevenson, G.S., Worley, P.F., and Barnes, C.A. (1997). Selective alteration of long-term potentiation-induced transcriptional response in hippocampus of aged, memory-impaired rats. J. Neurosci. *17*, 2876–2885.

Li, R. (1997). Bee1, a yeast protein with homology to Wiscott-Aldrich syndrome protein, is critical for the assembly of cortical actin cytoskeleton. J. Cell Biol. *136*, 649–658.

Lupas, A. (1996). Coiled coils: new structures and new functions. Trends Biochem. Sci. *21*, 375–382.

Lyford, G., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Lanahan, A.A., and Worley, P.F. (1995). *Arc*, a growth factor and activity-regulated gene encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron *14*, 433–445.

Martin, L.J., Blackstone, C.D., Huganir, R.L., and Price, D.L. (1992). Cellular localization of a metabotropic glutamate receptor in rat brain. Neuron *9*, 259–270.

Matsubara, A., Laake, J.H., Davanger, S., Usami, S., and Ottersen, O.P. (1996). Organization of AMPA receptor subunits at a glutamate synapse: a quantitative immunogold analysis of hair cell synapses in the rat organ of Corti. J. Neurosci. *16*, 4457–4467.

Munro, S., and Pelham, H.R. (1987). A C-terminal signal prevents secretion of luminal ER proteins. Cell *48*, 899–907.

Park, H.T., Kang, E.K., and Bae, K.W. (1997). Light regulates Homer mRNA expression in the rat suprachiasmatic nucleus. Brain Res. Mol. Brain Res. *52*, 318–322.

Petralia, R.S., Wang, Y.X., Singh, S., Wu, C., Shi, L., Wei, J., and Wenthold, R.J. (1997). A monoclonal antibody shows discrete cellular and subcellular localizations of mGluR1α metabotropic glutamate receptors. J. Chem. Neuroanat. *13*, 77–93.

Ponting, C.P., and Phillips, C. (1997). Identification of Homer as a homologue of the Wiskott-Aldrich syndrome protein suggests a receptor-binding function for WH1 domains. J. Mol. Med. *75*, 769–771.

Shaw, G., and Kamen, R. (1986). A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degredation. Cell *46*, 659–667.

Strehler, E.E., Strehler-Page, M.A., Perriard, J.C., Periasamy, M., and Nadal-Ginard, B. (1986). Complete nucleotide and encoded amino acid sequence of a mammalian myosin heavy chain gene: evidence against intron-dependent evolution of the rod. J. Mol. Biol. *190*, 291–317.

Symons, M., Derry, J.M., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., and Abo, A. (1996). Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell *84*, 723–734.

Tu, J.C., Xiao, B., Yuan, J., Lanahan, A., Leoffert, K., Li, M., Linden, D., and Worley, P.F. (1998). Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. Neuron *21*, 717–726.

Wang, Y.X., Wenthold, R.J., Ottersen, O.P., and Petralia, R.S. (1998). Endbulb synapses in the anteroventral cochlear nucleus express a specific subset of AMPA-type glutamate receptor subunits. J. Neurosci. *18*, 1148–1160.

Worley, P.F., Cole, A.J., Saffen, D.W., and Baraban, J.M. (1990). Transcription factor regulation in brain: focus on activity and NMDA dependent regulation. In Molecular Mechanisms of Aging, K. Beyreuther and G. Schettler, eds. (Heidelberg: Springer-Verlag), pp. 62–76.

Yang, J.T., Laymon, R.A., and Goldstein, L.S. (1989). A three-domain structure of kinesin heavy chain revealed by DNA sequence and microtubule binding analyses. Cell *56*, 879–889.

GenBank Accession Numbers

The accession numbers for the sequences described in this paper are AF093257, AF093258, AF093259, AF093260, AF093261, AF093262, AF093263, AF093264, AF093265, AF093266, AF093267, and AF093268.