

Homer/Vesl Proteins and Their Roles in CNS Neurons

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Abstract

Since their initial discovery in 1997, Homer/Vesl proteins have become increasingly investigated as putative regulators of receptor and ion-channel function in the central nervous system. Within a relatively brief period, numerous research reports have described manifold effects of Homer proteins, including the modulation of the trafficking of type I metabotropic glutamate receptors (mGluRs), axonal pathfinding, mGluR coupling to calcium and potassium channels, agonist-independent mGluR activity, ryanodine receptor regulation, locomotor activity, and behavioral plasticity. This review summarizes our current knowledge on the induction, expression, and structure of the various forms of Homer proteins, as well as their roles in neuronal function. In addition, we provide an outlook on novel developments with regard to the involvement of Homer-1a in hippocampal synaptic function.

Index Entries: hippocampus; immediate early gene; inositol (1,4,5)-trisphosphate receptor; metabotropic glutamate receptor; ryanodine receptor; Shank protein; synaptic transmission.

Introduction

Homer/Vesl (V_{ASP}/E_{na}-related protein induced during seizure and LTP) proteins localize to the molecular scaffold at postsynaptic den-

sities of excitatory synapses in the mammalian brain (1–4). Postsynaptic targeting of Homer proteins presumably occurs via their binding to proline-rich sequences that are present in type I metabotropic glutamate receptors (mGluRs), inositol (1,4,5)-trisphosphate receptors (IP₃Rs), ryanodine receptors (RyRs) type 1 and 2, C-type transient receptor potential (TRPC) channels, Shank proteins, and dynamin 3 (see below). It is therefore possible that Homer proteins regulate the number of neurotransmitter receptors in the

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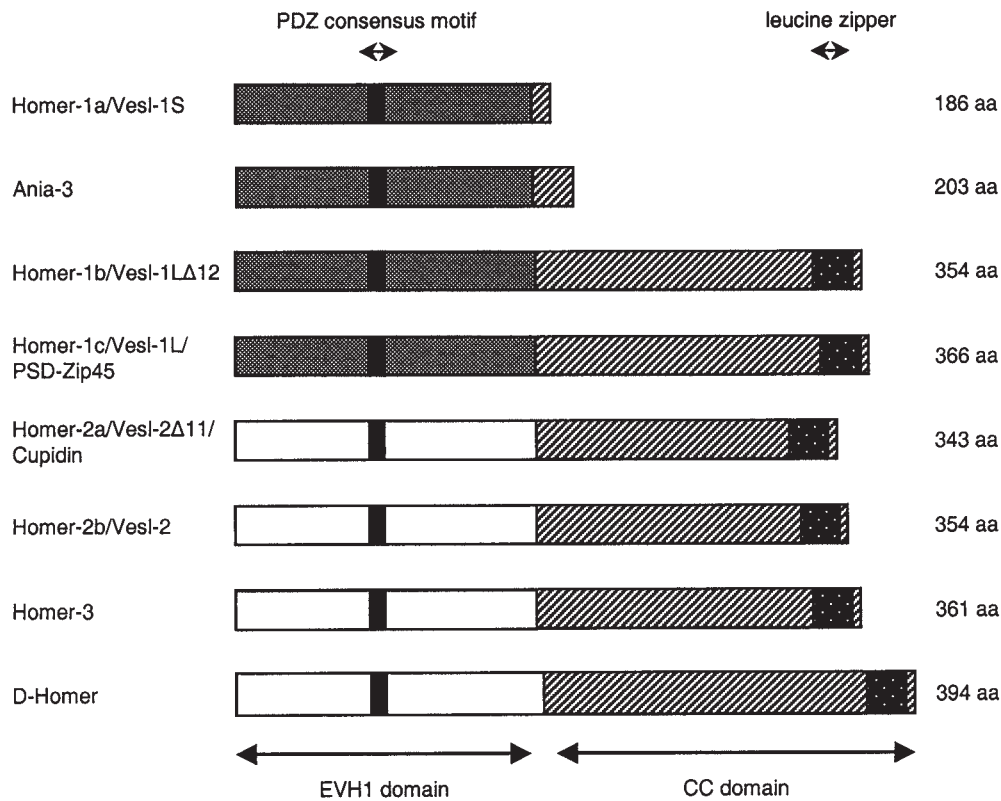


Fig. 1. Schematic representation of the Homer protein family members. Based on their number of amino acid residues, Homer proteins can be grouped into short (around 200 aa) and long (>300 aa) forms. All Homer proteins contain an EVH1-like domain, including a single PDZ consensus motif (black), in the N-terminal via which type I mGluRs (mGluR1a/5) are bound. Except for the two short forms Homer-1a and Ania-3, all Homer proteins possess at their C-terminus a predicted coiled-coil (CC) domain that includes several leucine zipper motifs.

postsynaptic membrane and/or their functional coupling to intracellular signal transduction cascades, thereby modulating synaptic strength. In this review, we first characterize the Homer proteins as well as their interactions with known binding partners, then summarize the published data on functional effects of Homer proteins, and finally discuss the putative involvement of Homer proteins in hippocampal synaptic transmission.

The Homer Family

Several Homer family members have been identified in mammals, amphibians, and insects.

A total of 12 Homer cDNAs have been cloned from rat, mouse (5,6), *Drosophila* (7), and human brains (6). Proteins encoded by these cDNAs share a similar structure and are splice variants of three independent mammalian genes (*homer-1*, 2, and 3) and of one *Drosophila* gene (*D-homer*).

Alternative splicing of the *homer-1* gene leads to four isoforms, i.e., Homer-1a/Vesl-1S, Ania-3/Vesl-1M, Homer-1b/Vesl-1Δ12, and Homer-1c/Vesl-1L/PSD-Zip45 (Fig. 1). Based on their number of amino acid residues, Homer proteins can be grouped into short (around 200 aa) and long (>300 aa) forms, e.g., Homer-1a and Ania-3 vs. Homer-1b/c. The first discovered member of the family was Homer-1a, identified

Table 1
Stimuli That Induce Homer-1a mRNA

Tissue	Treatment	Refs.
Hippocampus	kainate injection tetanic stimuli synaptogenesis electroconvulsive seizure epileptic seizure	8,9,11,56
Striatum	quinpirole	10
Hypothalamic suprachiasmatic nucleus	light pituitary adenylate cyclase activating polypeptide glutamate	57,58
Hypothalamus, neocortex	exploration of a novel environment	59
Caudate putamen, nucleus accumbens	haloperidol clozapine olanzapine	60,61
Cultured hippocampal and cortical neurons	phorbol esters proteasome inhibitors	2,62,63
Cultured cerebellar granule cells	TEA + 4-AP NMDA kainate BDNF glutamate	29,64

Expression of Homer-1a, the short splice isoform of the *homer-1* gene, is induced by numerous stimuli in several areas of the brain including the hippocampus, the hypothalamus, the striatum, the nucleus accumbens, the neocortex, and the cerebellum. Upregulation of Homer-1a expression was found both in vitro and in vivo (adapted from ref. 65).

as an immediate-early gene (IEG) in neurons from seizure-stimulated rat hippocampus (8,9). *In situ* hybridization showed that Homer-1a is a neuron-specific splice isoform that is rapidly and transiently upregulated by synaptic activity (8). Table 1 summarizes the known stimuli that cause neuronal Homer-1a expression both in vitro and in vivo. Similar to Homer-1a, Ania-3 is an activity-dependent and neuron-specific form, induced by dopamine-dependent signals and seizures in rodent striatum, forebrain, and dentate gyrus of the hippocampus (10,11). Both Homer-1a and Ania-3 are upregulated within minutes upon neuronal stimulation (8,9,11). In contrast to Homer-1a and Ania-3, Homer-1b and Homer-1c are constitutively expressed splice isoforms without any activity-dependent regulation; they occur in both neuronal and

non-neuronal cells (5,6,11,12). Two splice isoforms of the *homer-2* gene have been described so far (1,5,6). Homer-2a/Vesl-2 Δ 11/cupidin and Homer-2b/Vesl-2 were cloned based on their sequence similarity with Homer-1a (5,6) and exhibit a developmental increase in post-natal day 7 mouse cerebellum (1). Similar to Homer-1b/c, Homer-2a and Homer-2b are constitutively expressed in both neurons and other cells, and no modulation of their expression levels in mature neurons has been reported.

For the *homer-3* gene, only one product has been described (6). Again, it was cloned on the basis of sequence similarity with Homer-1a, and no activity-dependent modulation of its expression levels was found.

The mammalian brain constitutively expresses high mRNA levels for Homer-1b/c,

2, and 3 (13). For example, long forms of Homer proteins are found in the postsynaptic elements of cerebellar granule cells, hippocampal neurons, and retinal ganglion cells (1–4,14). High levels of these mRNAs and proteins also occur in mouse heart and skeletal muscle, and in the diaphragm (13,15).

Homer Protein Structure

Homer proteins belong to a wider family of PDZ domain-containing proteins (16,17). The N-terminal 110 amino acid residues of Homer proteins share sequence and secondary structure similarity with (a) the Ena/VASP homology domain 1 (EVH1 domain) of the Enabled protein of *Drosophila* (Ena) (18), (b) the vasodilator-stimulated phosphoprotein (VASP) (19,20), and (c) the Ran-binding protein family (21). All these proteins have been implicated in binding to proline-rich domains of various cytoskeletal proteins. Although the EVH1 domains of the different Homer family members share 70–90% sequence identity, they are not more than 35% identical to the EVH1 domains found in other proteins such as Ena or VASP. Crystal structure analyses as well as interaction studies showed that the peptide motif PPxxF that is bound by Homer-type EVH1 domains differs from the proline-rich motifs interacting with other EVH1 domains (22,23). So far, several partners have been described that bind Homer proteins, including type I mGluRs, IP₃Rs, RyRs, TRPC channels, Shank/ProSAP scaffolding proteins, and dynamin 3 (see below).

Except for Homer-1a and Ania-3, the two short isoforms of the *homer-1* gene, all long Homer isoforms contain a C-terminal domain with a predicted coiled-coil (CC) structure (Fig. 1). The Homer CC domain is necessary for the formation of Homer dimers and tetramers (5,6). Furthermore, it includes one or two typical leucine zipper motifs that support further multimerization of at least some isoforms (1,5,24). Homer-1c diverges from Homer-1b by a 12-amino acid residue insertion between the N-terminus and the CC domain (6).

The N-terminal 110 amino acid residues of *Drosophila* Homer, which contain the EVH1 domain, are 73% identical with rodent Homer-1 proteins, while the C-terminal region exhibits only 25% identity with Homer-1b. Nevertheless, the fly Homer protein, compared to vertebrate Homer forms, shares common amino acid residues within the CC domain and the two putative leucine zippers, allowing the multimerization of *Drosophila* Homer proteins.

Homer Protein-Binding Partners

Type I Metabotropic Glutamate Receptors (mGluRs)

Yeast two-hybrid and coimmunoprecipitation assays revealed that all Homer proteins bind to the cytoplasmic tails of mGluR1a and mGluR5 (termed group or type I mGluRs) both in vitro and in vivo (1,5,6,8,12). Deletion analysis has identified a proline-rich Homer ligand consensus sequence (PPxxF) approx 50 amino acid residues from the C-terminus of type I mGluRs that is crucial for Homer binding (23). This finding provides an interesting clue to the function of Homer proteins as type I mGluRs couple to phospholipase C and activate hydrolysis of phosphoinositides, thereby generating inositol trisphosphate (IP₃) and diacylglycerol.

Several ectopic expression studies have provided evidence for an alteration in the cellular distribution of mGluRs induced by Homer proteins, as listed in Table 2. Depending on the cell line used (HeLa, HEK 293, COS-7, and Ptk2 cells), various results have been obtained. In summary, diffuse plasma membrane localization of mGluR1a/5 occurred when the receptor was expressed alone. Cotransfection of Homer-1a had no effect on the distribution of mGluR1a/5 (24–26, see also 27), while coexpression of Homer-1b/c either clustered the receptor at the plasma membrane (24,28) or decreased its surface expression by retarding mGluR1a/5 in the endoplasmic reticulum (25,28, see also 26). The effect of Homer-1b on

Table 2
Effect of Homer-1 Proteins on Type I mGluR Trafficking in Cotransfection Systems

Cell type	Homer-1a		Homer-1b		Homer-1c		Refs.
	mGluR1a	mGluR5	mGluR1a	mGluR5	mGluR1a	mGluR5	
HeLa	no effect	no effect	retention in ER	retention in ER	n.d.	n.d.	25
HEK 293	clustering at PM	n.d.	n.d.	n.d.	clustering at PM	decrease at PM	27,28
	no effect	n.d.	n.d.	n.d.	no effect	n.d.	26
COS-7	no effect	no effect	n.d.	n.d.	clustering	clustering	24
Ptk2	n.d.	n.d.	n.d.	n.d.	n.d.	clustering at PM	48
Cerebellar granule cells	n.d.	targeting to dendrites and axons	n.d.	targeting to dendrites	n.d.	targeting to dendrites	29
	n.d.	PM surface expression	nd.	intracellular retention	n.d.	n.d.	33
Cortical neurons	n.d.	n.d.	n.d.	n.d.	targeting to dendrites	n.d.	28
Hippocampal neurons	n.d.	n.d.	n.d.	n.d.	n.d.	clustering at PM	48

The table summarizes the varying results obtained with the coexpression of Homer-1 proteins and type I mGluRs in cell lines and cultures of dissociated neurons. Abbreviations: ER, endoplasmic reticulum; n.d., not determined; PM, plasma membrane.

mGluR5 trafficking was abolished by point mutations in the Homer binding site of mGluR5 (i.e., PPSPF), which demonstrates that Homer-1b affects mGluR5 localization via a direct protein–protein interaction (25).

Similar to cell lines, mGluR5 also localizes to the cell bodies of cerebellar granule cells when transfected alone (29). However, when coexpressed with Homer-1b/c, mGluR5 redistributed to dendrites (colocalizing with the presynaptic marker synaptophysin), whereas it was detected in both dendrites and axons when cotransfected with Homer-1a. Upregulation of endogenous Homer-1a (through depolarizing the neurons with a combination of ionotropic glutamate receptor agonists or with potassium-channel blockers) also redistributes mGluR5 into neurites (29). In support of these data,

another study found Homer-1c to increase the transport of mGluR1a into dendrites from cultured cortical neurons (28). Taken together, these results suggest that Homer proteins play an important role in the trafficking and targeting of type I mGluRs into dendrites and/or axons via a so-far-unknown mechanism.

Inositol (1,4,5)-Trisphosphate Receptors (IP₃Rs)

In hippocampal neurons, IP₃Rs are restricted to dendritic shafts (30), whereas in cerebellar Purkinje cells they occur in spines, associating with the spine apparatus (31). Homer-1, 2, and 3, by contrast, are enriched in the cytosol at the lateral margin of the postsynaptic density (PSD) (6).

In cerebellar Purkinje cells, where IP₃Rs and Homer proteins are highly enriched, an IP₃R antibody specifically coimmunoprecipitates the long Homer forms (Homer-1b/c, 2a/b, and 3) and mGluR1a (22). As IP₃Rs are not known to directly bind to mGluR1a, it has been concluded that Homer proteins bridge IP₃Rs and mGluRs. This interaction is particularly important because IP₃Rs are downstream effectors of type I mGluRs and act as calcium-release channels in the endoplasmic reticulum (32). The connection between mGluRs and IP₃Rs, via Homer proteins, involves close proximity of both receptors, which may be a key feature of the postsynaptic mGluR-dependent signal transduction. A study of mGluR-induced calcium transients in Purkinje cells from primary cerebellar cultures showed that overexpression of Homer-1a, as compared to transfection with Homer-1b, decreased the amplitude and increased the latency of mGluR-evoked Ca²⁺ responses (6). However, although the decay phase of the Ca²⁺ response was slower in neurons transfected with Homer-1a, the total Ca²⁺ flux was similar to the one in cells overexpressing Homer-1b. Another study in cerebellar granule cells yielded conflicting results (33) in that cotransfection of mGluR5 with Homer-1a increased both the amplitude and latency of agonist-induced responses. The apparent contradiction may result from the different techniques used to detect intracellular Ca²⁺. While the former study analyzed the free intracellular Ca²⁺ concentration with fura-2 imaging, the latter one measured in a more indirect manner the activity of BK-type K⁺ channels, which are activated by cytosolic Ca²⁺ increases. In addition, the first report, using quisqualate as an agonist, stimulated endogenous mGluR1/5 in Purkinje cells, while the second study, applying DHPG, activated ectopically expressed mGluR5 in cerebellar granule cells. In the latter case, the effect of Homer-1a was abolished by cotransfection with Homer-1b (33).

Shank/ProSAP Proteins

It has been shown with immunogold electron microscopic analysis that Shank, an NMDA

receptor scaffolding protein, and Homer proteins colocalize at the PSD (34). In addition, when Homer-1b and Shank-1 were coexpressed in COS-7 cells and cell extracts immunoprecipitated with Shank antibodies, Homer-1b protein could be detected in the precipitates (34). The Homer-Shank interaction was confirmed in vivo by coimmunoprecipitation experiments using rat brain samples. As opposed to Homer proteins, the AMPA receptor scaffolding protein GRIP did not coprecipitate with anti-Shank antibodies, which confirms the specificity of the Homer-Shank interaction.

Experiments in cell cultures revealed that Homer-1a/b/c proteins also interact with other components of the NMDA receptor/PSD-95 complex (34). Homer-1b and PSD-95 coclustered only upon coexpression of Shank and guanylate kinase-associated protein (GKAP). These results indicate that Shank and GKAP participate in the formation of a quaternary protein complex including Shank, GKAP, PSD-95, and Homer proteins. In support of this suggestion, coexpression of Homer, Shank-1, and mGluR5 resulted in prominent coclustering of mGluR5 with Shank-1 in COS-7 cells, while cotransfection of Shank-1 with mGluR5 alone did not cluster the receptor. Also, the colocalization of mGluR5 and Shank-1 depended on the ability of Homer proteins to bind the receptor, because it was abolished by a point mutation in the Homer binding site of mGluR5. In summary, both Homer and Shank proteins are required to cluster mGluR5. By physically linking components of the NMDA and mGluR signaling pathways, the Homer-Shank interaction may contribute to glutamate-receptor cross-talk.

Ryanodine Receptor Type 1 (RyR1)

Homer expression has also been detected in skeletal and cardiac muscle (13,15). Crystallographic analysis of the EVH1 domain of Homer-1 revealed its specific association with the putative Homer binding sequence present in RyR1 (23). The physical interaction between RyR1 and Homer-1c/2/3 proteins was con-

firmed by coimmunoprecipitation experiments using junctional membrane vesicles from the sarcoplasmic reticulum of skeletal muscle. Moreover, by analyzing macroscopic Ca^{2+} efflux and ryanodine binding, it was revealed that the interaction of Homer-1c with RyR1 significantly increased the Ca^{2+} responsiveness of RyR1 at the single-channel level, whereas mutating the Homer-ligand sequence of RyR1 reduced excitation-contraction coupling of myotubes (15,35). Importantly, Homer-1a alone had no effect on RyR1 activity, but it dose-dependently decreased the effects of Homer-1c on RyR1 (36). In conclusion, Homer proteins modulate the function of the target protein RyR1 in skeletal muscle.

Ryanodine Receptor Type 2 (RyR2)

Another type of calcium-activated calcium channel, RyR2, physically interacts with Homer-1c (37). In contrast to RyR1, Homer-1c decreased the function of RyR2 as it reduced both the cyclic ADP ribose- and the caffeine-induced Ca^{2+} release mediated by RyR2 from cardiac sarcoplasmic reticulum microsomes (37). The reduction in RyR2 activity results from a decrease in the open probability of the RyR2 channel. Although Homer-1a alone had no significant effect on RyR2 function, it reversed the Homer-1c effects. These findings, together with the data on RyR1 described above, demonstrate that varying RyR isoforms are differentially regulated by Homer proteins to control muscle function.

C-Type Transient Receptor Potential (TRPC) Channels

A recent paper reports that Homer proteins bind TRPC channels via their EVH1 domain (38). TRPC channels are nonspecific cation channels activated by G protein-coupled receptors and/or depletion of intracellular calcium stores, e.g., via IP_3 receptors (39). Through two distinct binding sites in the TRPC N- and C-termini Homer-1 and 3 proteins bind TRPC1 and 5, but Homer-1 pro-

teins also bind TRPC2, whereas Homer-3 proteins additionally bind TRPC 4 (38). The interaction with Homer proteins is critical for regulating TRPC channel activity because the agonist-stimulated activity of overexpressed TRPC1 in HEK 293 cells (detected with electrophysiological and intracellular Ca^{2+} imaging techniques) was mimicked in the absence of agonists by both (a) a TRPC1 mutant with impaired Homer binding site, and (b) cotransfection of wild-type TRPC1 with Homer-1a, which is unable to multimerize (38). These results indicate that long forms of Homer proteins physically associate TRPC1 channels with IP_3 receptors into a signal-responsive TRPC channel complex, while the IEG Homer-1a blocks this interaction. Interestingly, the interaction between Homer-3 and TRPC1 was dissociated by thapsigargin, which depletes intracellular Ca^{2+} stores (38). The following model for cellular calcium homeostasis was proposed: Depletion of intracellular calcium stores activates TRPC channels by relieving their block by long Homer forms, thus causing extracellular Ca^{2+} influx. When intracellular Ca^{2+} stores are refilled, long Homer proteins associate with TRPC proteins again to diminish TRPC channel activity back to resting state.

Dynamin 3

Dynamin proteins, formed by a family of three different genes, constitute mechanoenzymes with GTPase activity that are involved in vesicle trafficking (40). After the original proposal of an interaction between dynamin 3 and Homer proteins (22), it was recently shown that dynamin 3 is present in dendritic spines of hippocampal neurons where it forms, in contrast to dynamin 1, a complex with Homer proteins and mGluR5 (41). However, coexpression of an epitope-tagged mGluR5 with a putative dominant-negative form of dynamin 3 (containing a mutation in the GTPase domain) did not affect mGluR5 internalization, indicating that dynamin 3 is not involved in mGluR5 trafficking (41).

Cdc42

The C-terminal portion of Homer-2a has been shown to interact with the GTP-bound, active form of Cdc42, a member of the Rho family of monomeric G proteins (1). HeLa cells expressing a constitutively active Cdc42 variant lead to filopodia-like structures, while coexpression of Homer-2a with Cdc42 diminished these structures (1). Although it cannot be ruled out that sequestering of active Cdc42 by Homer-2a suppressed filopodia formation, the data may suggest that Homer-2a affects cytoskeletal dynamics via the recruitment of active Cdc42.

Syntaxin 13

By using a yeast two-hybrid screening with the C-terminal region of Homer-1c as bait, syntaxin 13 has been identified as a Homer-1c binding partner (42). Both Homer-1c and syntaxin 13 possess at their C-terminus CC domains via which the interaction is proposed. syntaxin 13 binding is selective for Homer-1c, as it does not occur with Homer-2. Because Syntaxin 13 is a typical component of recycling endosomes, Homer-1c might affect the trafficking of, e.g., mGluRs that are linked to Homer proteins (see Fig. 2).

Functional Effects of Homer Proteins

Coupling of mGluRs to Calcium and Potassium Channels

In superior cervical ganglion neurons, group I mGluRs can regulate N-type calcium currents in three ways: (a) a voltage-dependent mechanism, (b) a voltage-independent pathway (43), and (c) a $G_{\beta\gamma}$ -mediated pathway (44). In this preparation, it was demonstrated that overexpression of the long Homer forms rather than Homer-1a reduces mGluR-mediated inhibition of N-type calcium currents (43). In addition, coexpression of Homer-1a counteracted the reduction of calcium current modulation by Homer-

1b/1c/2/3, suggesting a dominant-negative role for the IEG Homer form. The specificity of this regulatory role for type I mGluRs was demonstrated by the fact that neither Homer-3 nor Homer-1a affected the voltage-dependent inhibition of N-type calcium currents by mGluR2.

The same study also showed that long Homer forms (Homer-1b/c/2/3) reduce mGluR1a signaling to M-type potassium channels without altering the voltage-dependence of glutamate-induced calcium current inhibition (43). As it is known that activating group I mGluRs inhibits M-type potassium currents via a G-protein-dependent mechanism (45), it appears that the long Homer forms inhibit signal transduction from mGluR1a/5 to G-proteins, thus regulating both N-type calcium and M-type potassium channels. Homer-1a, in contrast, acts as a dominant-negative competitor interfering with the action of the long Homer forms.

The effect of Homer protein binding on the activity of type I mGluRs was shown in cultured cerebellar granule cells by measuring the activity of Ca^{2+} -activated BK channels (46). In this test system, both overexpression of exogenous Homer-1a and induction of endogenous Homer-1a increased BK channel activity even in the presence of competitive mGluR1a/5 antagonists. Similar data were obtained by suppressing endogenous Homer-3 with antisense oligonucleotides and by disrupting the mGluR-Homer interaction. Based on these results, it was concluded that the long forms of Homer proteins suppress agonist-independent activity of mGluRs, while the IEG form Homer-1a increases this activity (46).

Regulation of Axonal Pathfinding

Homer proteins may also play a role in axon guidance as *Xenopus* optic tectal neurons overexpressing normal Homer-1a formed aberrant axon projections (47). In contrast to wild-type Homer-1a, overexpression of a Homer-1a point mutant (Homer-1aW24A) that does not bind Homer ligands caused no such abnormal axonal projections. Interestingly, similar to

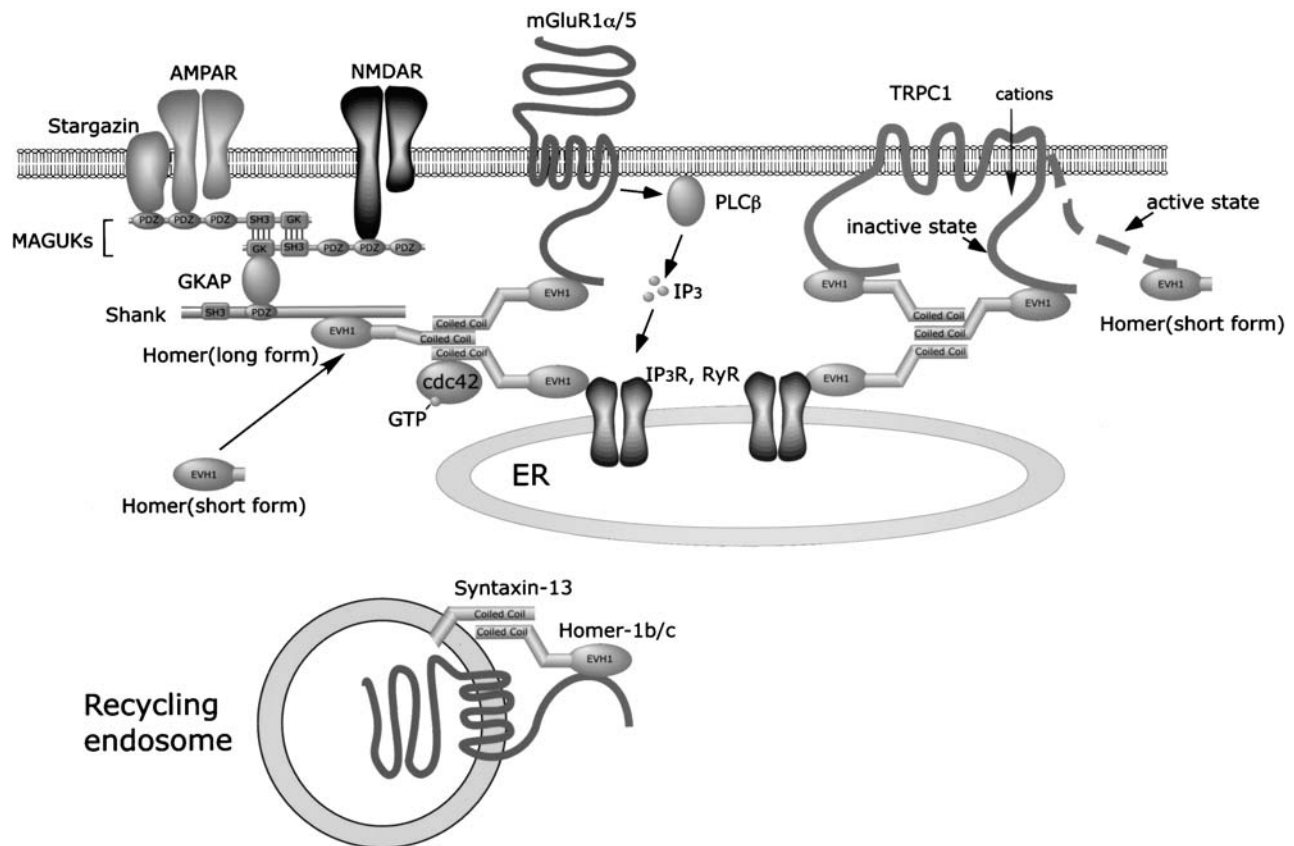


Fig. 2. Schematic representation of the postsynaptic protein interactions that involve Homer proteins. The EVH1 domain of Homer proteins binds mGluR1 α /5 (which in turn couple to phospholipase C; PLC), intracellular calcium channels (IP₃R, RyRs), and PSD proteins (Shank/ProSAP) that interact with ionotropic glutamate receptors. CC domains in the C-terminal region of the long Homer forms (Homer-1b/c, Homer-2a/b, and Homer-3) permit homo- and heteromultimerization. In contrast to the long Homer forms, the inducible Homer proteins Homer-1a and Ania-3 lack CC domains. These short Homer isoforms supposedly disrupt protein complexes that are mediated by constitutively expressed long Homer proteins. MAGUKs are membrane-associated PDZ proteins that contain guanylate kinase-like domains (PSD95, PSD93, SAP97, and SAP102). Long forms of Homer proteins bind TRPC1 channels and suppress their activity. When Homer-1a is upregulated, it replaces the constitutively expressed Homer proteins from TRPC1 channels and thus removes the block of constitutive activity. The C-terminal region of Homer-2 has been reported to associate with active Cdc42, while Homer-1c has been described to bind to Syntaxin 13 from recycling endosomes.

wild-type Homer-1a, overexpression of Homer-1c and Homer-1cW24A (which has the same point mutation as Homer-1aW24A but can dimerize with endogenous Homer-1b/c) also leads to axonal pathfinding errors. Homer-1a/c expression levels within the axonal growth cone thus appear to be critical

for effective signal transduction related to the correct targeting of axons.

Lateral Movement of mGluR5

One study examined the lateral movement of exogenously expressed, epitope-tagged mGluR5

in both primary hippocampal neurons and Ptk2 cells (48). The movement of mGluR5 was divided into two phases: (a) a diffusing phase in which mGluR5 moves quite freely, and (b) a confined phase in which mGluR5 is more trapped. While mGluR5 mobility increased with receptor activation, it decreased by coexpressing Homer-1b. In addition, the surface distribution of exogenous mGluR5 was uniform in both primary neurons and Ptk2 cells transfected with mGluR5 alone, whereas it was patchy in cells cotransfected with mGluR5 and Homer-1b. These results are compatible with the previously described effects of Homer proteins on the cell surface clustering of mGluR1a/5 (see above) and show that mGluR5 binding into larger protein complexes results in slower lateral receptor diffusion.

Animal Behavior

To establish the function of Homer proteins in *Drosophila* locomotor activity and behavioral plasticity, mutant flies were generated (*homer*^{R102}) in which the first two exons and half of the third exon of the *homer* gene were removed (7). A role for Homer in behavioral plasticity was verified by evaluating the performance of *homer*^{R102}-mutant males in a courtship-conditioning assay, an associative learning paradigm in *Drosophila* (49). Male flies react to olfactory, visual, and tactile signals with a complex and strong courtship toward females, and this behavior can be conditioned by previous experience. As compared with wild-type flies, *homer*^{R102} mutants were lacking behavioral plasticity and failed to form and/or retain the conditioning by the nonreceptive mated female (7). In addition, *homer*^{R102}-mutant flies exhibited higher levels of spontaneous locomotor activity. It thus appears that Homer, at least in *Drosophila*, is important in the development and function of neuronal networks underlying locomotion and behavioral plasticity.

Homer proteins have also been implicated in cocaine-induced behavioral plasticity in rats (50). Activation of mGluRs by dialyzing the group I mGluR agonist DHPG into the medial

nucleus accumbens augments both the extracellular glutamate concentration and locomotor activity. In this system, repeated cocaine administration increased group I mGluR-induced glutamate release and locomotor activity in parallel with a reduction in Homer-1b/c protein.

Spine Maturation and Synaptic Function

Many of the regulatory proteins involved in the dynamic morphological rearrangement of spines are embedded within the PSD. Using quantitative morphometric analysis of spines in cultured hippocampal neurons, Shank overexpression was shown to promote the development and enlargement of spine heads (3). These changes did not occur when a Shank mutant was used that does not bind Homer-1b protein any more. Moreover, overexpression of this Shank mutant decreased the amount of endogenous Homer-1b in spines, and reduced the number of dendritic spines (3). While Homer-1b overexpression in the absence of Shank had no detectable effect on spine morphology, coexpression of both Shank and Homer-1b caused a more pronounced spine growth and increased the frequency of miniature excitatory postsynaptic currents (3). These data suggest that an unknown retrograde signal triggers the maturation of presynaptic function.

To study the synaptic function of Homer-1a and Homer-1c in hippocampal neurons, we used Semliki Forest virus vectors (51,52) to upregulate these proteins. We found that overexpression of Homer-1a enhanced AMPA receptor function and clustering, whereas it affected neither NMDA receptor function and clustering nor presynaptic glutamate release (53). In contrast to Homer-1a, Homer-1c overexpression did not alter synaptic transmission. While Homer-1a resulted in a greater AMPA-to-NMDA ratio in mEPSCs (recorded in the presence of tetrodotoxin), the NMDA component remained constant. In addition, the increase in AMPA-to-NMDA ratio caused by Homer-1a was higher for evoked EPSCs than for mEPSCs. We therefore proposed in

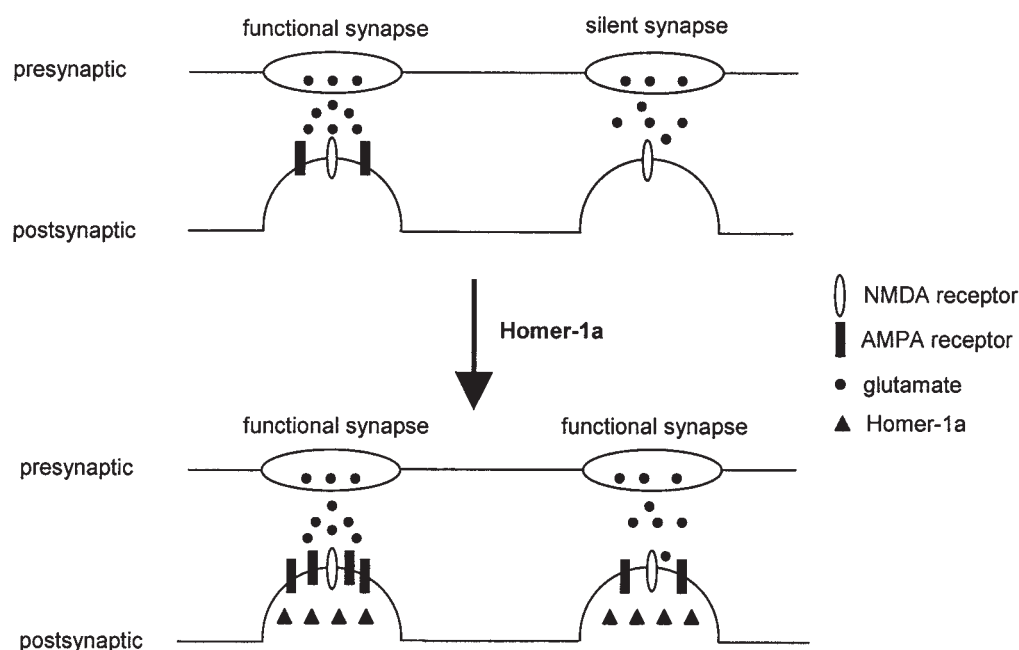


Fig. 3. Working model for the effect of Homer-1a on hippocampal synaptic transmission. Presynaptic release of glutamate into the synaptic cleft can activate two kinds of synapses: (a) functional synapses, i.e., synapses containing both NMDA and AMPA receptors (open and closed symbols, respectively), and (b) "silent synapses" that contain NMDA receptors but lack functional AMPA receptors. Overexpression of Homer-1a (triangles) induces an increase in the synaptic transmission without changing the glutamate release (circles). Our experiments lead to the conclusion that more AMPA receptors are expressed in functional synapses, but also in non-functional ones, which represents a conversion of silent synapses into functional ones.

our previous study (53) that Homer-1a promotes AMPA receptor insertion at both functional synapses (containing AMPA and NMDA receptors) and previously silent synapses (expressing only NMDA receptors; Fig. 3).

In contrast to our data, Sala et al. recently reported Homer-1a to inhibit rather than enhance hippocampal synaptic transmission (54). In their study, Homer-1a overexpression caused shrinkage of dendritic spines and a decrease in both AMPA and NMDA receptor currents. The apparent contradiction between these and our data may result from different experimental protocols used. While we examined hippocampal slices prepared from postnatal day 6 (P6) rats and cultured for 2–3 wk

(in the roller-tube configuration), Sala et al. employed slices from P8 rats at 4–6 d in culture (stationary on membranes). For dissociated hippocampal neurons, we used 3–4-wk-old cultures and Sala et al. 12-d-old cultures. Our experiments were thus conducted with more mature cultures, which might explain the discrepancy of the data. In addition, we employed conventional Semliki Forest virus vectors for gene transfer and analyzed the neurons at 1–2 d after transduction, whereas Sala et al. applied Ca^{2+} phosphate precipitation for dissociated neurons and a biolistic gene gun for slices and examined them at 4–5 and 2 d posttransfection, respectively (note that under physiological conditions Homer-1a protein is induced within few

hours of neuronal activation). Differences in transgene expression levels thus obtained with varying transfection techniques could also affect the experimental outcome. In any case, a positive feed-forward role, rather than a negative feedback function of Homer-1a in hippocampal synaptic transmission, seems to be a more straightforward explanation for Homer-1a being induced with protocols causing hippocampal long-term potentiation. In addition, our results agree with a recent report showing in the nucleus accumbens that downregulation of Homer-1b/c with antisense oligonucleotides specifically decreases the protein level of the AMPA receptor subunit GluR1 (55).

Conclusion

A rapidly increasing number of investigations provide data that support a role for Homer proteins in synaptic plasticity. The functions of Homer proteins result from their particular structural properties that permit them to interact with membrane receptors, such as mGluRs, but also with intracellular receptors, including Shank, IP₃Rs, and RyRs. It thus appears that Homer proteins not only are important in receptor scaffolding, but also modulate receptor activity. Intracellular Ca²⁺ is implicated in several mechanisms that lead to the potentiation of synapses, and Homer proteins have been shown to control intracellular Ca²⁺ homeostasis by regulating type I mGluRs, IP₃Rs, RyRs, TRPC channels, and voltage-gated Ca²⁺ channel activity. It is therefore probable that Homer proteins are also involved in hippocampal synaptic plasticity.

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