Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2009 | 108 | 1–10 doi: 10.1111/j.1471-4159.2008.05726.x

REVIEW

Developmental roles for Homer: more than just a pretty scaffold

Lisa Foa* and Robert Gasperini†

*Laboratory of Molecular Neurobiology, School of Medicine, University of Tasmania, Hobart, Australia
†Laboratory of Molecular Neurobiology, Menzies Research Institute, University of Tasmania, Hobart, Australia

Abstract

Homer proteins are best known as scaffold proteins at the post-synaptic density where they facilitate synaptic signalling and are thought to be required for learning and memory. Evidence implicating Homer proteins in the development of the nervous system is also steadily accumulating. Homer is highly conserved and is expressed at key developmental time points in the nervous system of several species. Homer regulates intracellular calcium homeostasis, clustering and trafficking of receptors and proteins at the cytosolic surface of the plasma membrane, transcription and translation, and cytoskeletal organization. Each of these functions has obvious potential to regulate neuronal development, and indeed Homer is implicated in several pathologies associated with the developing nervous system. Current data justify more critical experimental approaches to the role of Homer in the developing nervous system and related neurological disorders.

Keywords: Homer, mental retardation, mGluR, neuronal development, synaptic signalling.

J. Neurochem. (2009) **108**, 1–10.

The Homer family of scaffolding proteins are crucial components of many intracellular signalling cascades in excitable and non-excitable cells. Homer function underpins a variety of neuronal processes, ranging from calcium homeostasis to synaptic plasticity associated with learning and memory in the mature brain. Such processes are also crucial for the normal development of neuronal architecture in the embryonic brain.

An evolving theme from our understanding of Homer function is that there is a 'dose-dependent' effect of Homer isoforms on cellular function. That is, the cellular effects attributable to Homer are dependent upon the balance of expression levels between Homer isoforms (Foa *et al.* 2001; Van Keuren-Jensen and Cline 2006; Ary *et al.* 2007; Kammermeier 2008). Aberrant Homer signalling has been associated with several developmentally related neurological syndromes, including neuropathic pain, addiction, epilepsy, schizophrenia, Fragile X syndrome and Alzheimer's disease (AD) (for reviews see de Bartolomeis and Iasevoli 2003; Szumlinski *et al.* 2006, 2008). While Homer structure (Shiraishi-Yamaguchi and Furuichi 2007) and function (Worley *et al.* 2007) have been well reviewed, the specific topic of Homer function during neuronal development has not been examined. This review will focus on what is known about the expression, structure and function of Homer proteins in the developing nervous system and the implications of these studies for the function of the mature nervous system and for neurological disease.

Homer protein structure

The family of mammalian Homer proteins comprises splice variants from three *homer* genes (*homer 1, 2 and 3*). There

Received July 10, 2008; revised manuscript received September 19, 2008; accepted September 25, 2008.

Address correspondence and reprint requests to Dr Lisa Foa, Laboratory of Molecular Neurobiology, School of Medicine, University of Tasmania, Hobart 7001, Australia. E-mail: lisa.foa@utas.edu.au

Abbreviations used: AD, Alzheimer's disease; AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate; APP, amyloid precursor protein; BACE, Beta side App cleaving enzyme; CaMKII, calcium/calmodulin dependent protein kinase II; CC, coiled-coil; EVH1, enabled/vasodilator-stimulated phospho-protein homology domain; IP_3, inositol 1,4,5-triphosphate; IP_3R, IP_3 receptors; LTD, long-term depression; mGluR, metabotropic glutamate receptor; MRX, X-linked metal retardation; NFAT, nuclear factor of activated T cells; NMDAR, NMDA receptor; PAX6, *paired hox gene 6*; PI3, phosphoinositide 3; PSD, post-synpatic density; RyR, ryanodine receptors; TRPC, transient receptor potential canonical.

are multiple isoform products from these genes: Homer 1a–h, Homer 2a–d, and 10 isoforms of Homer 3 (Xiao *et al.* 1998; Soloviev *et al.* 2000; Saito *et al.* 2002; Klugmann *et al.* 2005). Homer proteins are well known for their role as adaptor molecules that enhance signalling at the post-synaptic density (PSD) by altering receptor trafficking and clustering, Ca^{2+} flux and actin organization. Homer proteins carry out these functions via an N-terminal enabled/vasodilator-stimulated phosphoprotein homology domain (EVH1) and C-terminal coiled-coil (CC) domain (Fig. 1). The evolutionarily conserved EVH1 domain defines the Homer family. Through the EVH1, Homer binds synaptic signalling ligands, such as the metabotropic glutamate receptor (mGluR), the inositol tri-phosphate (IP_3R) and ryanodine receptors (RyR) (Kato *et al.* 1998; Tu *et al.* 1998), anchoring proteins such as shank (Tu *et al.* 1999), channels such as transient receptor potential canonical channels (TRPC) (Yuan *et al.* 2003), transcription factors such as nuclear factor of activated T cells (NFAT) and PAX6 (*paired hox gene 6*) (Cooper and Hanson 2005; Stiber *et al.* 2005; Huang *et al.* 2008) and GTPases (Rong *et al.* 2003; Govek *et al.* 2004). Constitutively expressed 'long-form' Homer proteins possess a CC domain, which mediates homophilic and/or heterophilic interactions with other members of the Homer protein family. Until relatively recently, a clear understanding of how the physical coupling of Homer proteins to their ligands occurs had been elusive. Recent elegant biophysical and biochemical experiments by Hayashi *et al.* (2006) suggest that native long-form Homers exist as tetrameric hubs with CC domains aligned in a parallel fashion. This tetramerization exposes four EVH1 domains in a spatially optimized configuration for ligand binding and and is required for efficient localization to dendritic spines. These data suggest that Homer tetramers and antiparallel Shank homodimers may form multidimension scaffolding lattices upon which ligands for both proteins can be efficiently assembled (Hayashi *et al.* 2006).

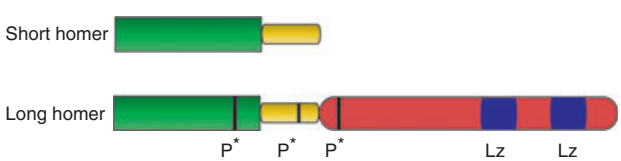

Fig. 1 Schematic representation of the principal functional motifs of Homer proteins. All members of the Homer protein family are defined by their highly-conserved (60–70% homology) N-terminal Ena-Vasp-Homology (EVH1) domain (green). Through the EVH1 domain, long-form Homer proteins bind and couple other synaptic signalling elements such as membrane bound receptors (mGluR1-5, TRPC1, IP3R and RyR) and scaffolding partners (Shank1, Shank 3, Dynamin and oligophrenin) through a specific proline-rich ligand motif (–PxxF–). Long-form Homers possess a C-terminal coiled-coil (CC) domain (20–30% homology, through which they multimerize via two leucine-zipper motifs (blue) with other long form Homer proteins. Short form Homer proteins, Homer 1a and Ania-3, contain a conserved EVH1 domain, but lack the CC domain, preventing multimerization with other Homer proteins and are effective negative regulators of long-form Homer multimerization. Putative phosphorylation sites (P*) based on the demonstrated *in vivo* phosphorylation of serine residues (120, 159 and 176) of Homer 3 (Mizutani *et al.* 2008).

In addition to the long forms of Homer, there are two activity-inducible 'short' forms of Homer, Homer 1a (Vesl-1s) and Ania-3 that have been demonstrated in rodents and *Xenopus* (Brakeman *et al.* 1997; Kato *et al.* 1997; Berke *et al.* 1998; Foa *et al.* 2005). These isoforms possess a fully functional EVH1 domain, but lack a CC domain. Homer 1a and Ania-3 are thought to act as naturally occurring dominant negatives that function by uncoupling long-form Homer-ligand interactions resulting in molecular rearrangements at the membrane (Kato *et al.* 1998; Tu *et al.* 1998; Sala *et al.* 2003; Kammermeier and Worley 2007). Expression of the short form Homers is comparatively low in the absence of synaptic activity. However, an increase in synaptic activity can cause premature exon-to-intron conversion, activating a transcript termination in the large central intron, thereby converting most Homer1b/c transcription to Homer1a (Bottai *et al.* 2002). This regulation of short-form Homer represents a novel mechanism of fine-tuning synaptic activity.

While we have learnt much about the regulation of Homer by activity, and the function of Homer via its EVH1 and CC domains, evidence that phosphorylation is also important for Homer function is emerging. Phosphorylation of Homer proteins was first suggested by Shiraishi *et al.* (2003a), who demonstrated by 2-D gel analysis of cell lysates, that glutamate induction of post-translational Homer 2 modifications requires phosphatase activity (Shiraishi *et al.* 2003b). Subsequently, it was confirmed that Homer 2 is indeed phosphorylated, and significantly its phosphorylation state is reduced in Fragile X knockout mice (Giuffrida *et al.* 2005). Recently, Homer 3 was shown to be phosphorylated by calcium/calmodulin-dependent protein kinase II (CaMKII). Phosphorylation of Homer 3 by CaMKII within 2 min of synaptic activity uncouples mGluR-Homer 3-IP_3R complexes (Mizutani *et al.* 2008). Such uncoupling mimics the action of short isoforms, providing a rapid mechanism that negatively regulates neuronal activity, as opposed to activity induction of Homer 1a/Ania-3, which occurs after 30 min (Brakeman *et al.* 1997). The conservation of the probable CaMKII phosphorylation site amongst most Homer proteins suggests that this is a consistent mechanism for regulating Homer function (Mizutani *et al.* 2008).

Expression of Homer isoforms during development

The likely necessity of Homer function during development is revealed by the significant neuronal and behavioural deficits that occur in *homer 1* and *homer 2* null mice (Kalivas *et al.* 2004; Lominac *et al.* 2005; Szumlinski *et al.* 2005; Jaubert *et al.* 2007). Furthermore, Homer proteins are highly

conserved across species, from human, rat and mouse, to *Xenopus* and *Drosophila* (Xiao *et al.* 1998; Foa *et al.* 2001; Diagana *et al.* 2002) where there is extensive expression of Homer proteins in the developing nervous system.

Homer expression during mammalian development

As would be expected from an integral PSD protein, there is widespread Homer expression in the developing rodent CNS. Long-form Homer proteins (1b/c, 2a/b and 3a/b) are expressed in skeletal muscle, heart, liver, spleen, lung and kidney, although Homer expression in these non-neuronal tissues is low in comparison to the CNS (Shiraishi *et al.* 2004). Homer 1b/c and Homer 2 isoforms follow relatively parallel patterns of expression in the olfactory bulb, cortex, hippocampus, corpus striatum and thalamus. Homer 3 expression is restricted to the cerebellum and hippocampus, where it is developmentally regulated (Shiraishi *et al.* 2004). Differential expression of the three *homer* genes is also striking in the hippocampus, where Homer 1 and Homer 2 are expressed in both excitatory and inhibitory neurons of CA1-CA2 and Homer 3 expression is restricted to excitatory cells of CA2-CA3 (Shiraishi *et al.* 2004). These patterns of differential Homer expression suggest distinct developmental and synaptogenic roles in the postnatal mouse brain. A potential isoform specific role in developing hippocampal neurons is in clustering PSD proteins prior to the onset of synaptogenesis. At this early stage of development, Homer 2 complexes with the NMDA receptor (NMDAR) subunit, NR2B, and PSD95 in developing hippocampal neurons *in vitro*. This clustering is commensurate with dendritic development and precedes synaptogenesis (Shiraishi *et al.* 2003a).

The role of the activity-induced isoforms during development is unclear. The expression of Homer 1a compared with the long-form Homer proteins is relatively low during mammalian development, as would be predicted, given that Homer1a expression is regulated by synaptic activity (Brakeman *et al.* 1997; Kato *et al.* 1997; Xiao *et al.* 1998). Given its dominant negative role, Homer 1a over-expression would be predicted to impede normal development. Accordingly, transgenic mice over-expressing Homer 1a in the striatum develop significant defects in motor coordination and motor learning with increased levels of fear associated behaviour or anxiety (Tappe and Kuner 2006). Similar defects are also observed in *homer 1* and *2* knockout mice. (Szumlinski *et al.* 2005; Jaubert *et al.* 2007). This data support the concept that the differential expression of Homer isoforms within the cell is crucial for normal function (Foa *et al.* 2001; Van Keuren-Jensen and Cline 2006; Ary *et al.* 2007).

Homer expression during *Drosophila* development

To date, only one *homer* gene has been demonstrated in the *Drosophila* genome. *Drosophila homer* (*dhomer1*) conforms to the stereotypic long-form Homer, displaying 73% homology to mouse Homer 1b/c at the EVH1 domain but only 23% at the CC domain (Xiao *et al.* 1998; Diagana *et al.* 2002). Conserved residues in the C-terminus, however, predict that *dhomer1* possesses equivalent multimerization propensities as the mammalian long-form Homers. A ubiquitous neuronal localization of *dhomer1* RNA at embryonic stage 12 precedes high levels of Homer protein in both the CNS and PNS in stage 16 embryos. Homer is predominantly located within the neuropil, closely associated with the synaptic marker, synaptotagmin, suggesting a pre-synaptic localization. Over-expression of *homer*-myc-green fluorescent protein (GFP) constructs, however, results in Homer-myc localization primarily in dendrites as opposed to axons of motor neurons (Diagana *et al.* 2002). In addition to its synaptic localization, Homer is prominently associated with proteins at or on the endoplasmic reticulum, an association that requires a functional EVH1 domain (Diagana *et al.* 2002). In *Drosophila* mutants lacking functional Homer protein, there are no gross anatomical defects in nervous system development. However, there are subtle functional defects such as behavioural plasticity deficits in the courtship learning paradigm, which may be related to their hyperactive locomotor activity, and potentially in hyperactive olfactory sensation (although other sensory modalities such as the visual system appear to be unaffected) (Diagana *et al.* 2002). These results confirm the high degree of conservation of Homer proteins, but point to potentially divergent pre-synaptic and post-synaptic developmental roles (discussed later).

Homer expression during *Xenopus* development

The long-form Homer homolog in *Xenopus* (*xhomer 1b*) is widely expressed in differentiated neurons, glia and muscle, in patterns that suggest Homer functions in synaptogenesis and circuit formation (Foa *et al.* 2005). Homer is expressed in the *Xenopus* embryonic spinal cord at a time when developing spinal circuits are spontaneously active. This developmental regulation of Homer is a consistent theme that appears in sensory development across several vertebrate species (Gasperini and Foa 2004; Shiraishi *et al.* 2004; Foa *et al.* 2005). In the *Xenopus* optic system, Homer expression correlates with synaptogenesis and early retinal responses to light. In the olfactory system, Homer expression parallels the maturation of sensory neurons in the larva, persisting through adulthood, as has been observed in the mouse olfactory system (Shiraishi *et al.* 2004). In the tadpole brain, early Homer expression is prominent in neuropil areas of the tectum and tegmentum, persisting until metamorphosis (Foa *et al.* 2005).

Analysis of the developing *Xenopus* brain by western blot shows steadily increasing levels of long-form Homer, with a plateau of expression at metamorphosis, with a putative short-form *Xenopus* Homer isoform remaining relatively

constant. Significantly, expression of *Xenopus* short-form Homer is up-regulated by kainate-induced seizures. This is the only non-mammalian example of such induction demonstrated so far, although the exact nature of the induced 'Homer 1a-like' protein has not been fully described (Foa *et al.* 2005).

Homer expression during zebrafish development

Developmental expression of a zebrafish (*Danio rerio*) Homer1b/c homologue is correlated with the development of sensory systems, as has been observed in mouse and *Xenopus* neuronal development (Gasperini and Foa 2004; Shiraishi *et al.* 2004; Foa *et al.* 2005). Significantly, maximal Homer 1b/c expression is coincident with crucial behavioural milestones in larval development, such as eye movements, predatory feeding behaviour and a generalized increase in motor activities (Gasperini and Foa 2004). In the developing olfactory placode, Homer is prominently expressed in the apical portions of sensory olfactory neurons occurring concurrently with the onset of locomotor behaviours involved in predation and feeding. This expression pattern is conserved until adulthood where prominent Homer immunostaining can be demonstrated in the adult olfactory neuroepithelium.

Studies focusing on rodent, *Xenopus* and *Danio rerio* development suggest that Homer is necessary for the development of neural circuitry that is ultimately responsible for the survival of the organism in the environment. It will be instructional to continue to explore in detail at the cellular level, Homer's role in the developing mammalian nervous system, where alternative splicing events have led to a variety of Homer isoforms. Future over-expression studies in the *homer* knockout mice may determine whether specific isoforms function in key areas of the brain potentially responsible for the development of higher cognitive functioning.

'Pre'- and post-synaptic Homer function

In the adult brain, Homer proteins regulate neuronal function and development by mediating post-synaptic signalling. Homer's coupling and clustering of receptors results in functional linkages between cell-surface receptors and their downstream effectors. At the PSD, Homer interacts with other scaffold proteins such as Shank and PSD-95 to regulate receptor trafficking and clustering (Roche *et al.* 1999; Tadokoro *et al.* 1999; Ango *et al.* 2000; Ciruela *et al.* 2000; Sala *et al.* 2001; Ango *et al.* 2002; Usui *et al.* 2003), the actin cytoskeleton (Shiraishi *et al.* 1999; Sala *et al.* 2001; Govek *et al.* 2004; Lu *et al.* 2007) and dendritic remodelling (Sala *et al.* 2001) to enhance the distribution and signalling of class 1 mGlu, NMDA and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors.

Perhaps, the most intensively studied Homer-receptor interaction is the functional regulation of class 1 mGluRs 1α and 5, henceforth referred to as mGluRs (Tu *et al.* 1998; Xiao *et al.* 1998; Roche *et al.* 1999). mGluRs are G protein-coupled receptors that activate phospholipase C to hydrolyse phosphatidylinositol 4,5-bisphosphate to form diacylglycerol, which subsequently activates protein kinase C (PKC) and inositol 1,4,5-triphosphate (IP_3). IP_3 activates IP_3 receptors IP_3R, releasing calcium from intracellular stores. Long-form Homer protein dimers couple mGluR and IP_3Rs via EVH1 domains, while Homer 1a competes with constitutively expressed long-form Homer dimers to uncouple the Homer-mGluR complexes, effectively attenuating glutamate-induced intracellular calcium release (Tu *et al.* 1998; Kammermeier *et al.* 2000; Kammermeier and Worley 2007; Kammermeier 2008). Recently, class 1 mGluR have been shown to also signal via phosphoinositide 3 kinase (PI3 kinase). Homer 1c and Homer 2a were shown to couple mGluR1 in a complex with long-form PI3 kinase enhancer and PI3 kinase, thereby enabling mGluR1-induced apoptosis (Rong *et al.* 2003). Regulation of apoptosis represents a further mechanism by which mGluR-Homer signalling may contribute to the formation and refinement of neuronal networks. With so many functions attributed to mGluR-Homer signalling, it is likely that specificity depends on the context of the cell, such as environment, activity and developmental stage (Fig. 2).

At excitatory synapses, changes in AMPA receptor transmission underpin the maintenance of synaptic strength. Homer1b and Shank cooperate to induce maturation of dendritic spines and synapses (Sala *et al.* 2001). Conversely, over-expression of Homer1a negatively regulates dendritic spine size and density and decreases the expression of PSD proteins, such as Shank, PSD-95 and the AMPA and NMDAR subunits GluR2 and NR1, respectively (Sala *et al.* 2003). Indeed, over-expression of Homer1a was found to reduce the amplitude of AMPA and NMDAR-mediated excitatory post-synaptic currents (Sala *et al.* 2003). Similarly, mGluR modulation of AMPA receptor plasticity *in vivo* is dependent on the relative ratio of Homer 1a to Homer 1b, which is regulated by synaptic activity (Van Keuren-Jensen and Cline 2006). More recently, Homer 1b/c was shown to interact with dynamin-3 to couple the endocytic zone to the post-synaptic zone. Disruption of this coupling (for example by Homer 1a) resulted in a decrease in synaptic AMPA receptors and an increase in NMDA-only, or 'silent' synapses (Lu *et al.* 2007). These studies suggest that the strength of a synapse is reflected in the activation history and by the consequent expression of activity-related proteins such as Homer 1a.

While Homer is considered a post-synaptic protein, several pieces of early data hint at a pre-synaptic role. Homer 1a increased targeting of mGluR5 to axons (Ango *et al.* 2000) and Shank, a closely related scaffold protein that co-operates with Homer, increased the size of the pre-synaptic vesicle

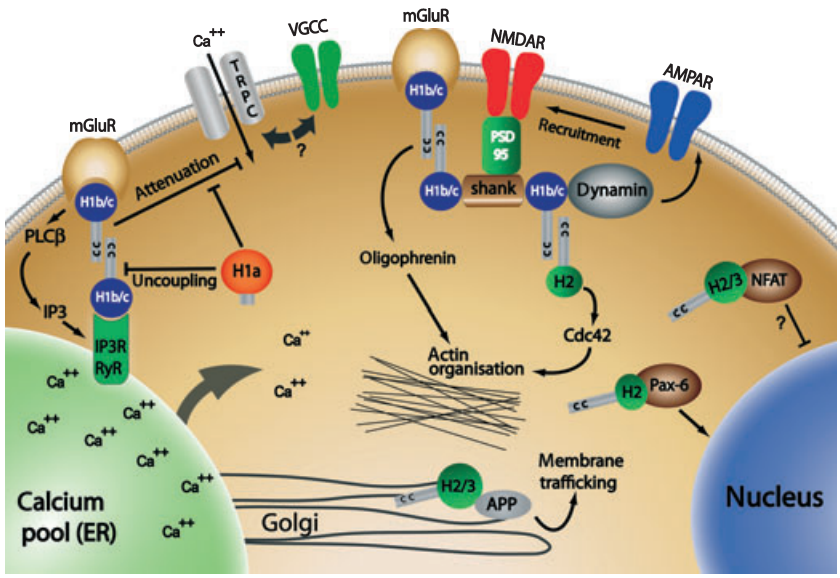

Fig. 2 A simplified graphical representation illustrating the important cell signalling and transduction interaction of Homer proteins. All of these molecular relationships have been implicated in developmental mechanisms that shape the function of the mature nervous system. Homer proteins function in four crucial areas of neural cell biology: control of intracellular calcium homeostasis: Constitutively expressed Homer 1b/c multimers facilitate the coupling of mGluR's to key signalling elements of the cell calcium pool (e.g. endoplasmic reticulum) such as IP3 and ryanodine receptors. In non-neuronal cells, Homer 1b/c also attenuates the gating and activity of non-specific cationic TRPC channels at the cell membrane. Activity-induced Homers (e.g. Homer1a or Ania-3) decouple long-form Homer multimers, negatively regulating mGluR mediated calcium release, while potentially having the reverse effect on TRPC channels. Clustering and trafficking of receptors and proteins at the cytosolic surface of the plasma membrane: At the synapse, Homer 1b/c regulates the activity of mGluR's and in cooperation with other important synaptic proteins, Shank and Dynamin-3, orchestrates the spatial organisation and function of Key synaptic molecules, including the NMDAR and AMPAR. Cytoskeletal stabilisation and organisation: Homer 2 binds members of the Rho family of small GTPases such as Cdc42 to functionally couple actin reorganistion and receptor function at the synapse. Genomic function: Homer 3 can interact with the transcription factor, pax-6, and in non-neuronal cells Homer 2 and 3 regulate the nuclear translocation of NFAT. These transcriptional mechanisms would suggest a role for Homer in the differentiation and maturation of excitable cells, further implicating Homer as an important regulator of nervous system development.

pool (Sala *et al.* 2001). More recent data suggesting post-synaptic activity induces changes to clustering of pre- and post-synaptic proteins such as synaptotagmin and PSD95, respectively, and that these changes are abolished with *homer 1a* knockdown, suggesting that homer may mediate pre-synaptic structure by regulating retrograde signalling (Inoue *et al.* 2007).

A putative pre-synaptic developmental role for Homer has been suggested from *in vivo* work in the tadpole visual system. Over-expression of Homer isoforms in wild-type *Xenopus* tectal cells results in significant axon path-finding errors in both commissure recognition and tectal targeting (Foa *et al.* 2001). Over-expression of Homer1a and 1c causes growth cones to ignore choice points, inappropriately cross the midline, and fail to recognize target cells. Mutant and wild-type Homers both cause growth cone errors indicating that both the EVH1 and CC domain of Homer 1c are necessary for accurate axon path-finding (Foa *et al.* 2001). A potential mechanism by which Homer functions in axon path-finding may be by regulating cytosolic calcium influx through a Homer-TRPC interaction (Yuan *et al.* 2003). TRPC signalling is an important component of calcium signalling that regulates growth cone turning towards brain-derived neurotrophic factor and netrin in cerebellar granule cells and *Xenopus* spinal neurons (Li *et al.* 2005; Wang and Poo 2005). A complete understanding of Homer function in neuronal growth cones has yet to be elucidated; however, *in vivo* over-expression studies demonstrate that altering the balance of Homer isoform expression levels causes gross path-finding errors and that Homer multimerization and/or scaffolding contributes to growth cone guidance (Foa *et al.* 2001).

Homer and calcium homeostasis

It is now widely accepted that the role of Homer at the PSD is to not only act as a facilitator of PSD signalling, but also as a key calcium regulatory protein. This topic is the subject of a recent, excellent review (Worley *et al.* 2007) and hence will only be mentioned briefly here. Homer proteins couple key calcium signalling proteins to the PSD, in particular the IP_3 and RyRs (Tu *et al.* 1998) as well as the presumptive store-operated non-specific cation channels, TRPCs (Yuan *et al.*

2003; Kim *et al.* 2006). Homer may also regulate basal cytosolic calcium via its interaction with the plasma membrane calcium reuptake pump (Sgambato-Faure *et al.* 2006; Kurnellas *et al.* 2007). In pancreatic acinar cells, Homer 3 differs from Homer 1 and 2 in that it does not function in calcium signalling (Shin *et al.* 2003). Whether such diversity of Homer function exists in neuronal cells, remains to be determined. It would be informative to determine the role of Homer 3 in the developing cerebellum, where it is the predominant Homer isoform (Shiraishi *et al.* 2004). Each of the above interactions highlights the considerable potential for Homer interactions to influence neuronal calcium signalling.

Homer and transcriptional regulation

Homer function may have significant influence on neuronal development through its role in regulating protein synthesis. Homer 3 interacts with the transcription factor, PAX6 in brain extracts, and it has been suggested that the cytoplasmic form of PAX6 is sequestered to the PSD via Homer 3 binding (Cooper and Hanson 2005). In this context, Homer 3 would facilitate communication between synaptic signalling at the dendrite and the nucleus. The exact nature of this interaction is unknown as the C-terminal peptide of PAX6 used in the yeast-2 hybrid screen for interacting proteins lacks the Homer binding motif, PxxF (Tu *et al.* 1998). If Homer 3 is able to regulate PAX6 function, even via an indirect interaction, Homer could have a profound role in development, as PAX6 is required for normal brain development (Mastick *et al.* 1997).

A detailed analysis of Homer's role in transcriptional regulation has been conducted in immune and muscle cells, in which Homer binds and regulates another important transcription factor, NFAT. In T cells, Homer 2 and Homer 3 compete with calcineurin for NFAT binding, preventing calcineurin dephosphorylation of NFAT, and subsequent NFAT translocation to the nucleus, acting as a negative regulator of NFAT transcription (Huang *et al.* 2008). In an effort to understand Homer 3-NFAT binding, Huang *et al.* (2008) solved the crystal structure of Homer 3 and identified the probable NFAT binding region within the EVH1 domain. The binding site is distinct from the region that binds proteins such as the mGluR and IP_3R, suggesting that despite being a defining feature of the Homer protein family, not all Homer EVH1 domains are the same. It is not known whether Homer 2 possesses the same binding site, however, the alanine residues required for NFAT binding are unique to Homer 2 and Homer 3 and absent from Homer 1 (Xiao *et al.* 1998). Interestingly, Homer 2 expression levels are up-regulated after T cell activation (Diehn *et al.* 2002), suggesting that expression of the long-form Homer is not purely constitutive, but an active player in the dynamic regulation of T cell protein synthesis. It will be important to determine whether Homer 2 and/or 3 have similar roles in neurons.

In muscle, Homer 2 has been shown to interact with NFAT to regulate myoblast differentiation (Stiber *et al.* 2005). Homer 2 interacts with IP_3R and RyRs in immature and mature myocytes to regulate calcium release from intracellular stores and subsequent NFAT translocation to the nucleus. Homer 1a was shown to regulate Homer 2 function in these cells. Hence, by regulating NFAT translocation, Homer 2 may promote muscle differentiation and maturation. This role for Homer 2 in gene regulation within muscle fibres has far reaching developmental and clinical implications as Homer 2 has been linked with muscle atrophy associated with Duchenne muscular dystrophy (Fisher *et al.* 2005).

Implications for the developmental roles of Homer in pathological conditions

While this review is primarily focused on the role of Homer in development, it would not be complete without a discussion of Homer's role in certain pathological states. Some neurological disorders can be better understood from a developmental perspective as they involve disruption of mechanisms that underlie synaptic plasticity in the mature nervous system. We will focus on diseases that are primarily developmental in origin, the mental retardation syndromes and Fragile X, and conditions in which developmental-like processes involved in synaptic plasticity may be important such as neuropathic pain and Alzheimer's disease.

Mental retardation syndromes

Homer has not been shown to directly regulate transcription or translation in neuronal cells, but such a role is hinted at in an inherited mental retardation syndrome. Fragile X is one of the most common causes of inherited intellectual disabilities. In *Fmr1* knockout mice, there is an absence of fragile X mental retardation protein. Activation of mGluR5 in hippocampal CA1 neurons triggers long-term depression (LTD) that is dependent on local post-synaptic translation (Huber *et al.* 2000). This mGluR5-activated LTD was subsequently shown to be negatively regulated by fragile X mental retardation protein, that is, LTD was enhanced in *Fmr1* knockout mice (Huber *et al.* 2002). A reduced level of mGluR5-Homer 1b/c co-immunoprecipitation in *Fmr1* knockout mice suggested a role for Homer 1 in mGluR-mediated LTD (Giuffrida *et al.* 2005). Recent work has found that Homer is necessary for mGluR-LTD-mediated translation (Ronesi and Huber 2008). A peptide that mimics the action of Homer 1a was used to disrupt mGluR5 endogenous long-form Homer interactions. In doing so, mGluR-induced LTD in *Fmr1* knockout mice is not affected, but mGluR-dependent translation is reduced, suggesting that the interaction between mGluR and Homer is to mediate translation and not LTD (Ronesi and Huber 2008). The identity of the endogenous Homer isoform necessary for local neuronal translation remains to be determined. While

Homer 2 and 3 are already implicated in transcription in non-neuronal cells, Homer 1 is clearly implicated in neuronal translation (Giuffrida *et al.* 2005). mGluR-induced translation directly affects dendritic spine maturation, which is dysfunctional in Fragile X, placing mGluR (and therefore Homer) signalling at the nexus of Fragile X mental retardation syndrome, suggesting that mGluRs are realistic targets for therapeutics against Fragile X (Bear *et al.* 2004).

Homer is implicated in the inherited intellectual disability, X-linked metal retardation (MRX). In MRX, there is a significant reduction of oligophrenin-1, a protein that normally displays ubiquitious pre- and post-synaptic expression throughout the brain. Oligophrenin-1 is a Rho-GTPase activating protein that negatively regulates RhoA. Knockdown of oligophrenin-1 significantly reduces spine length without affecting dendritic spine density (Govek *et al.* 2004). This is in contrast with Fragile X where there is an increase in spine density (Nimchinsky *et al.* 2001). Homer 1b/c couples mGluRs in the PSD with oligophrenin-1, thereby providing a mechanism for synaptic plasticity mechanisms to directly interact to regulate the actin cytoskeleton and spine morphology (Govek *et al.* 2004). Perturbing mGluR-Homer mediated translation, as in Fragile X or mGluR-Homer interactions with oligophrenin, as in MRX clearly has significant impacts on intellectual development.

Homer and neuropathic pain

Activity-dependent plasticity underpins connectivity of neuronal networks during nervous system development and may form the cellular basis of learning and memory (reviewed in Sheng and Kim 2002). Activity-dependent plasticity is also thought to underpin the development of neuropathic pain (Ji and Woolf 2001). Homer has been implicated in the signalling cascade that regulates neuropathic pain (Guo *et al.* 2004). Inflammation-induced hyperexcitability within the dorsal horn of the spinal cord causes activity-induced synaptic plasticity, resulting in hyperalgesia (Guo *et al.* 2004). Inflammation-mediated activation of Class1 mGluR and subsequent IP_3-mediated calcium release and protein kinase C activation induces Src phosphorylation of the NR2B, enhancing calcium flux through the NMDAR, thus causing hyperexcitability (Guo *et al.* 2004). Given that long-form Homer regulates intracellular mGluR-IP_3R-mediated calcium release (Yuan *et al.* 2003), it would seem likely that long-form Homer is part of the signalling cascade responsible for NR2B phosphorylation and hence hyperalgesia in the dorsal horn of the spinal cord, a hypothesis confirmed in recent work (Miyabe *et al.* 2006; Tappe *et al.* 2006).

Both long- and short Homer isoforms are implicated in neuropathic pain. Homer 1 proteins are expressed throughout the mammalian spinal cord, with basal levels of Homer 1a expression comparatively low compared with Homer 1b/c (Tappe *et al.* 2006). However, in a manner similar to that of plasticity associated with learning and memory, inflammatory and pain stimuli increase Homer1a expression in the short term, within hours after the injury in the dorsal horn of the spinal cord (Miyabe *et al.* 2006; Tappe *et al.* 2006). As in earlier studies (Guo *et al.* 2004), activation of the NMDARs is required for Homer 1a activation. Homer 1a inhibits phosphorylation of extracellular signal-regulated protein kinases (ERK1/2), thus reducing ERK 1/2 mediated transcription, and spine density on proximal dendrites, resulting in a decrease in nociceptive activity within the spinal cord (Tappe *et al.* 2006). Conversely, while Homer 1a expression may be increased in the short term, there is a slow increase in expression levels of Homer 1b/c and Shank 1a within the PSD of dorsal horn neurons, over days to weeks post-injury (Miletic *et al.* 2005). Short- and long-term responses to pain stimuli are both NMDA-dependent and represent 'promising therapeutic targets' in the treatment of chronic inflammatory pain (Tappe *et al.* 2006).

Homer in Alzheimer's disease

Alzheimer's disease is principally caused by the build-up of β-amyloid protein in the brain (reviewed in Small 2008). This accumulation results in a programmed series of neurodegenerative events affecting regions of the brain involved with higher order functioning such as learning and memory. Increasingly, it is now understood that AD is a disease of synaptic dysfunction that underlies cognitive decline (Selkoe 2002). Indeed, Braak and Braak (1996) have proposed that neurodegeneration in AD is the inverse recapitulation of brain development, suggesting that developmental factors may play an important role in susceptibility to neurodegeneration (Braak and Braak 1996).

The role of Homer in neurodegeneration is unclear, however, recent work implicates both long- and short-form Homer. In an animal model for AD, the amyloid precursor protein + presenilin-1 (APP + PS1) transgenic mouse, there is reduced mRNA for several memory and learning-related proteins, including Homer 1a (Dickey *et al.* 2003). These changes are found in brain regions displaying amyloid accumulation, and coincide with an onset of cognition impairment, suggesting changes in synaptic function (Dickey *et al.* 2003). Given that Homer1a is induced by synaptic activity, it is perhaps not surprising that there is a reduction in mRNA in circumstances of synaptic and memory degradation. Perhaps, a more interesting question is the role of the constitutively expressed *homer* genes during this disease progression. Recently, Homer 2 and Homer 3 expression was shown to reduce trafficking of APP to the membrane surface in human embryonic kidney cells (Parisiadou *et al.* 2008). In the same study, Homer 2 and Homer 3 were found to reduce the glycosylation and maturation of APP and the APP cleavage enzyme, Beta site APP cleaving enzyme (BACE1). A functional Homer 2 or Homer 3 EVH1 domain is required, but suspiciously, not the C-terminal of the APP protein. It is thought that Homer may interact with the YENPTY motif in

the C-terminal of APP. However, this seems unlikely as the interaction persists after the removal of the protein motif. Furthermore, the Homer binding motif PxxF is not found in APP or BACE. It is therefore unlikely that this is a direct interaction between Homer and APP or BACE and more likely that Homer is an intermediary (Parisiadou *et al.* 2008). Even if it is an indirect interaction, Homer 2 and Homer 3 appear to be required for normal APP development and function, and may inhibit β-amyloid production, potentially reducing amyloid plaque formation and deposition (Parisiadou *et al.* 2008). Concordantly, amyloid deposition and disease onset are associated with a decrease in Homer 1a levels (Dickey *et al.* 2003). The result of crossing AD transgenic mice with *homer* knockout mice could prove to be an informative experiment (Parisiadou *et al.* 2008) and at the least, the addition of Homer proteins to the signalling cascade thought to underpin AD may provide a new focus in the search for successful treatments for this disease.

Conclusion

While *homer* knockout mice are viable, they do exhibit significant neuronal and behavioural deficits (Kalivas *et al.* 2004; Lominac *et al.* 2005; Szumlinski *et al.* 2005; Jaubert *et al.* 2007), highlighting the importance of Homer proteins during neuronal development. It is now apparent that there is some segregation, although not absolute, of duties between the *homer* genes. Homer 2 and Homer 3 clearly interact with transcription factors to regulate nuclear function, while Homer 1 appears to predominantly function within the PSD to facilitate Ca^{2+} signalling, regulate synaptic strength and dendritic morphology. Given these functions, it is no surprise that Homer is implicated in some devastating conditions, such as Fragile X and MRX, neuropathic pain and AD. The hope is that studies into the cellular mechanisms of Homer will provide clues for realistic therapeutic targets.

Acknowledgements

The authors would like to thank Prof David Small and Dr Adele Holloway and members of the Foa lab for critically reviewing this manuscript. LF is funded by the NH & MRC, Grant no.: #512801.

References

Ango F., Pin J.-P., Tu J. C., Xiao B., Worley P. F., Bockaert J. and Fagni L. (2000) Dendritic and axonal targeting of type 5 metabotropic glutamate receptor is regulated by Homer1 proteins and neuronal excitation. *J. Neurosci.* **20**, 8710–9716.

Ango F., Robbe D., Tu J. C., Xiao B., Worley P. F., Pin J.-P., Bockaert J. and Fagni L. (2002) Homer-dependent cell surface expression of metabotropic glutamate receptor type 5 in neurons. *Mol. Cell. Neurosci.* **20**, 323–329.

Ary A. W., Aguilar V. R., Szumlinski K. K. and Kippin T. E. (2007) Prenatal stress alters limbo-corticostriatal Homer protein expression. *Synapse* **61**, 938–941.

de Bartolomeis A. and Iasevoli F. (2003) The Homer family and the signal transduction system at glutamatergic postsynaptic density: potential role in behavior and pharmacotherapy. *Psychopharm. Bull.* **37**, 51–83.

Bear M. F., Huber K. M. and Warren S. T. (2004) The mGluR theory of fragile X mental retardation. *Trends Neurosci.* **27**, 370–377.

Berke J. D., Paletzki R. F., Aronson G. J., Hyman S. E. and Gerfen C. R. (1998) A complex program of striatal gene expression induced by dopaminergic stimulation. *J. Neurosci.* **18**, 5301–5310.

Bottai D., Guzowski J., Schwarz M., Kang S., Xiao B., Lanahan A., Worley P. F. and Seeburg P. (2002) Synaptic activity-induced conversion of intronic to exonic sequence in Homer 1 immediate early gene expression. *J. Neurosci.* **22**, 167–175.

Braak H. and Braak E. (1996) Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. *Acta Neuropathol.* **92**, 197–201.

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.

Ciruela F., Soloviev M. M., Chan W.-Y. and McIlhinney R. A. J. (2000) Homer-1c/vesl-1L modulates the cell surface targeting of metabotropic glutamate receptor type 1α: evidence for an anchoring function. *Mol. Cell. Neurosci.* **15**, 36–50.

Cooper S. T. and Hanson I. M. (2005) A screen for proteins that interact with Pax-6: C-terminal mutations disrupt interaction with Homer3, DNCL1 and TRIM11. *BMC Gen.* **6**, 43–51.

Diagana T. T., Thomas U., Prokopenko S. N., Xioao B., Worley P. F. and Thomas J. B. (2002) Mutation of *Drosophila* homer disrupts control of locomotor activity and behavioral plasticity. *J. Neurosci.* **22**, 428–436.

Dickey C. A., Loring J. F., Montgomery J., Gordon M. N., Eastman P. S. and Morgan D. (2003) Selectively reduced expression of synaptic plasticity-related genes in amyloid precursor protein + presenilin-1 transgenic mice. *J. Neurosci.* **23**, 5219–5226.

Diehn M., Alizadeh A. A., Rando O. J., Liu C. H., Stankunas K., Botstein D., Crabtree G. R. and Brown P. O. (2002) Genomic expression programs and the integration of the CD28 costimulatory signal in T cell activation. *Proc. Natl Acad. Sci.* **99**, 11796–11801.

Fisher I., Abraham D., Bouri K., Hoffmann E. P., Muntoni F. and Morgan J. (2005) Prednisolone-induced changes in dystrophic skeletal muscle. *FASEB J.* **19**, 834–856.

Foa L., Rajan I., Hass K., Wu G.-Y., Brakeman P., Worley P. F. and Cline H. (2001) The scaffold protein, Homer1b/c, regulates axon pathfinding in the central nervous system in vivo. *Nat. Neurosci.* **4**, 499–506.

Foa L., Jensen K., Rajan I., Gasperini R., Bronson K., Worley P. F., Tu J. C. and Cline H. T. (2005) Homer expression in the *Xenopus* tadpole nervous system. *J. Comp. Neurol.* **487**, 42–53.

Gasperini R. and Foa L. (2004) Homer 1b/c expression correlates with zebrafish olfactory system development. *J. Neurocytol.* **33**, 671–680.

Giuffrida R., Musumeci S., D'Antoni S., Bonaccorso C. M., Giuffrida-Stella A. M., Oostra B. A. and Catania M. V. (2005) A reduced number of metabotropic glutamate subtype 5 receptors are associated with constitutive Homer proteins in a mouse model of Fragile X syndrome. *J. Neurosci.* **25**, 8908–8916.

Govek E. E., Newey S. E., Akerman C. J., Cross J. R., Van der Veken L. and Van Aelst L. (2004) The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nat. Neurosci.* **7**, 364–372.

Guo W., Wei F., Zou S. *et al.* (2004) Group I metabotropic glutamate receptor NMDA receptor coupling and signaling cascade mediate

spinal dorsal horn NMDA receptor 2B tyrosine phosphorylation associated with inflammatory hyperalgesia. *J. Neurosci.* **24**, 9161–9173.

Hayashi M. K., Ames H. M. and Hayashi Y. (2006) Tetrameric hub structure of postsynaptic scaffolding protein Homer. *J. Neurosci.* **26**, 8492–8501.

Huang G. N., Huso D. L., Bouyain S. *et al.* (2008) NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer proteins. *Science* **319**, 476–481.

Huber K. M., Kayser M. S. and Bear M. F. (2000) Role for rapid dendritic protein synthesis in hippocampal mGlur-dependent long-term depression. *Science* **288**, 1254–1256.

Huber K. M., Gallagher S. M., Warren S. T. and Bear M. F. (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl Acad. Sci.* **99**, 7746–7750.

Inoue Y., Udo H., Inokuchi K. and Sugiyama H. (2007) Homer1a regulates the activity-induced remodeling of synaptic structures in cultured hippocampal neurons. *Neuroscience* **150**, 841–852.

Jaubert P. J., Golub M. S., Lo Y. Y. *et al.* (2007) Complex, multimodal behavioral profile of the Homer1 knockout mouse. *Genes Brain Behav.* **6**, 141–154.

Ji R. R. and Woolf C. J. (2001) Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiol. Dis.* **8**, 1–10.

Kalivas P. W., Szumlinski K. K. and Worley P. F. (2004) Homer2 gene deletion in mice produces a phenotype similar to chronic cocaine treated rats. *Neurotox. Res.* **6**, 385–387.

Kammermeier P. J. (2008) Endogenous homer proteins regulate metabotropic glutamate receptor signaling in neurons. *J. Neurosci.* **28**, 8560–8567.

Kammermeier P. J. and Worley P. F. (2007) Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effectors. *Proc. Natl Acad. Sci.* **104**, 6055–6060.

Kammermeier P. J., Xiao B., Tu J. C., Worley P. F. and Ikeda S. R. (2000) Homer proteins regulate coupling of group I metabotropic glutamate receptors to N-type calcium and M-type potassium channels. *J. Neurosci.* **20**, 7238–7245.

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.

Kato A., Ozawa F., Saitoh Y., Fukazawa Y., Sugiyama H. and Inokuchi K. (1998) Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. *J. Biol. Chem.* **273**, 23969–23975.

Kim J. Y., Zeng W., Kiselyov K., Yuan J. P., Dehoff M. H., Mikoshiba K., Worley P. F. and Muallem S. (2006) Homer1 mediates store- and IP_3Rs-dependent translocation and retrieval of TRPC3 to the plasma membrane. *J. Biol. Chem.* **281**, 32540–32549.

Klugmann M., Symes C. W., Leichtlein C. B., Klaussner B. K., Dunning J., Fong D., Young D. and During M. J. (2005) AAV-mediated hippocampal expression of short and long Homer 1 proteins differentially affects cognition and seizure activity in adult rats *Mol. Cell. Neurosci.* **28**, 347–360.

Kurnellas M. P., Lee A. K., Li H., Deng L., Ehrlich D. J. and Elkabesa S. (2007) Molecular alterations in the cerebellum of the plasma membrane calcium ATPase 2 (PMCA2)-null mouse indicate abnormalities in Purkinje neurons. *Mol. Cell. Neurosci.* **34**, 178–188.

Li Y., Jia Y.-C., Cui K., Li N., Zheng A.-Y., Wang Y.-z. and Yuan X.-b. (2005) Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. *Nature* **434**, 894–898.

Lominac K. D., Oleson E. B., Pava M. *et al.* (2005) Distinct roles for different Homer1 isoforms in behaviors and associated prefrontal cortex function. *J. Neurosci.* **25**, 11586–11594.

Lu J., Helton T. D., Blanpied T. A., Ra′ cz B., Newpher T. M., Weinberg R. J. and Ehlers M. D. (2007) Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. *Neuron* **55**, 874–889.

Mastick G. S., Davis N. M., Andrew G. L. and Easter S. S. (1997) Pax-6 functions in boundary formation and axon guidance in the embryonic mouse forebrain. *Development* **124**, 1985–1997.

Miletic G., Miyabe T., Gebhardt K. J. and Miletic V. (2005) Increased levels of Homer1b/c and Shank1a in the post-synaptic density of spinal dorsal horn neurons are associated with neuropathic pain in rats. *Neurosci. Lett.* **386**, 189–193.

Miyabe T., Miletic G. and Miletic V. (2006) Loose ligation of the sciatic nerve in rats elicits transient up-regulation of Homer1a gene expression in the spinal dorsal horn. *Neurosci. Lett.* **398**, 296–299.

Mizutani A., Kuroda Y., Futatsugi A., Furuichi T. and Mikoshiba K. (2008) Phosphorylation of Homer3 by calcium/calmodulin-dependent kinase II regulates a coupling state of its target molecules in Purkinje cells. *J. Neurosci.* **28**, 5369–5382.

Nimchinsky E. A., Oberlander A. M. and Svoboda K. (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. *J. Neurosci.* **21**, 5139–5146.

Parisiadou L., Bethani I., Michaki V., Krousti K., Rapti G. and Efthimiopoulos S. (2008) Homer 2 and Homer 3 interact with amyloid precursor protein and inhibit Abeta production. *Neurobiol. Dis.* **30**, 353–364.

Roche K. W., Tu J. C., Petralia R. S., Xiao B., Wenthold R. J. and Worley P. F. (1999) Homer 1b regulates the trafficking of group I metabotropic glutamate receptors. *J. Biol. Chem.* **274**, 25953–25957.

Ronesi J. A. and Huber K. M. (2008) Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. *J. Neurosci.* **28**, 543–547.

Rong R., Ahn J.-Y., Huang H. *et al.* (2003) PI3 kinase enhancer-Homer complex couples mGluR1 to PI3 kinase, preventing neuronal apoptosis. *Nat. Neurosci.* **6**, 1153–1161.

Saito H., Kimura M., Inanobe A., Ohe T. and Kurachi Y. (2002) An N-terminal sequence specific for a novel Homer1 isoform controls trafficking of group I metabotropic glutamate receptor in mammalian cells. *Biochem. Biophys. Res. Commun.* **296**, 523–529.

Sala C., Valentin P., Wilson N. R., Passafaro M., Liu G. and Sheng M. (2001) Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* **31**, 115–130.

Sala C., Futai K., Yamamoto K., Worley P. F., Hayashi Y. and Sheng M. (2003) Inhibition of dendritic spine morphogenesis and synaptic transmission by activity-inducible protein Homer1a. *J. Neurosci.* **23**, 6327–6337.

Selkoe D. J. (2002) Alzheimer's disease is a synaptic failure. *Science* **298**, 789–791.

Sgambato-Faure V., Xiong Y., Berke J. D., Hyman S. E. and Strehler E. E. (2006) The Homer-1 protein Ania-3 interacts with the plasma membrane calcium pump. *Biochem. Biophys. Res. Commun.* **343**, 630–637.

Sheng M. and Kim M. J. (2002) Postsynaptic signaling and plasticity mechanisms. *Science* **298**, 776–780.

Shin D. M., Dehoff M., Luo X., Kang S. H., Tu J. C., Nayak S. K., Ross E. M., Worley P. F. and Muallem S. (2003) Homer 2 tunes G protein-coupled receptors stimulus intensity by regulating RGS proteins and PLCbeta GAP activities. *J. Cell Biol.* **162**, 293–303.

Shiraishi Y., Mizutani A., Bito H., Fujisawa K., Narumiya S., Mikoshiba K. and Furuichi T. (1999) Cupidin, an isoform of Homer/Vesl,

interacts with the actin cytoskeleton and activated rho family small GTPases and is expressed in developing mouse cerebellar granule cells. *J. Neurosci.* **19**, 8389–8400.

Shiraishi Y., Mizutani A., Mikoshiba K. and Furuichi T. (2003a) Coincidence in dendritic clustering and synaptic targeting of homer proteins and NMDA receptor complex proteins NR2B and PSD95 during development of cultured hippocampal neurons. *Mol. Cell. Neurosci.* **22**, 188–201.

Shiraishi Y., Mizutani A., Yuasa S., Mikoshiba K. and Furuichi T. (2003b) Glutamate-induced declustering of post-synaptic adaptor protein Cupidin (Homer 2/vesl-2) in cultured cerebellar granule cells. *J. Neurochem.* **87**, 364–376.

Shiraishi Y., Mizutani A., Yuasa S., Mikoshiba K. and Furuichi T. (2004) Differential expression of Homer family proteins in the developing mouse brain. *J. Comp. Neurol.* **473**, 582–599.

Shiraishi-Yamaguchi Y. and Furuichi T. (2007) The Homer family proteins. *Gen. Biol.* **8**, 206.

Small D. H. (2008) Network dysfunction in Alzheimer's disease: does synaptic scaling drive disease progression? *Trends Mol. Med.* **14**, 103–108.

Soloviev M. M., Ciruela F., Chan W.-Y. and McIlhinney R. A. J. (2000) Molecular characterisation of two structurally distinct groups of human Homers, generated by extensive alternative splicing. *J. Mol. Biol.* **295**, 1185–1200.

Stiber J. A., Tabatabaei N., Hawkins A. F., Hawke T., Worley P. F., Williams R. S. and Rosenberg P. (2005) Homer modulates NFAT-dependent signaling during muscle differentiation. *Dev. Biol.* **287**, 213–224.

Szumlinski K. K., Lominac K. D., Kleschen M. J., Oleson E. B., Dehoff M. H., Schwartz M. K., Seeburg P. H., Worley P. F. and Kalivas P. W. (2005) Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia. *Genes Brain Behav.* **4**, 273–288.

Szumlinski K. K., Kalivas P. W. and Worley P. F. (2006) Homer proteins: implications for neuropsychiatric disorders. *Curr. Opin. Neurobiol.* **16**, 251–257.

Szumlinski K. K., Ary A. W. and Lominac K. D. (2008) Homers regulate drug-induced neuroplasticity: implications for addiction . *Biochem. Pharmacol.* **75**, 112–133I.

Tadokoro S., Tachibana T., Imanaka T., Nishida W. and Sobue K. (1999) Involvement of unique leucine-zipper motif of PSD-Zip45 (Homer 1c/vesl-1L) in group 1 metabotropic glutamate receptor clustering. *Proc. Natl Acad. Sci.* **96**, 13801–13806.

Tappe A. and Kuner R. (2006) Regulation of motor performance and striatal function by synaptic scaffolding proteins of the Homer1 family. *Proc. Natl Acad. Sci.* **103**, 774–779.

Tappe A., Klugmann M., Luo C., Hirlinger D., Agarwal N., Benrath J., Ehrengruber M. U., During M. J. and Kuner R. (2006) Synaptic scaffolding protein Homer1a protects against chronic inflammatory pain. *Nat. Med.* **12**, 677–681.

Tu J. C., Xiao B., Yuan J. P., Lanahan A. A., Leoffert K., Li M., Linden D. J. 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.

Tu J. C., Xiao B., Naisbitt S. *et al.* (1999) Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **23**, 583–592.

Usui S., Konno D., Hori K., Maruoka H., Okabe S., Fjikado T., Tano Y. and Sobue K. (2003) Synaptic targeting of PSD-Zip45 (Homer1c) and its involvement in the synaptic accumulation of F-actin. *J. Biol. Chem.* **278**, 10619–10628.

Van Keuren-Jensen K. and Cline H. T. (2006) Visual experience regulates metabotropic glutamate receptor-mediated plasticity of AMPA receptor synaptic transmission by homer1a induction. *J. Neurosci.* **26**, 7575–7580.

Wang G. X. and Poo M.-M. (2005) Requirement of TRPC channels in netrin-1-induced chemotropic turning of nerve growth cones. *Nature* **434**, 898–904.

Worley P. F., Zeng W., Huang G. N., Kim J. Y., Shin D. M., Kim M. S., Yuan J. P., Kiselyov K. and Muallem S. (2007) Homer proteins in Ca^{2+} signaling by excitable and non-excitable cells. *Cell Calcium* **42**, 363–371.

Xiao B., Tu J. C., Petralia R. S. *et al.* (1998) Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* **21**, 707–716.

Yuan J. P., Kiselyov K., Shin D. M. *et al.* (2003) Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. *Cell* **114**, 777–789.

