MINIREVIEW

Effects of Vesl/Homer Proteins on Intracellular Signaling

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The clustering of signaling molecules at specialized cellular sites allows cells to effectively convert extracellular signals into intracellular signals and to produce a concerted functional output with specific temporal and spatial patterns. A prime example for these molecules and their effects on cellular signaling are the postsynaptic density proteins of the central nervous system. Recently, one group of these proteins, the Vesl/Homer protein family has received increased attention because of its unique molecular properties that allow both the clustering and functional modulation of a plethora of different binding proteins. Within multiprotein signaling complexes, Vesl/Homer proteins influence proteins as diverse as metabotropic glutamate receptors; transient receptor potential channels; intracellular calcium channels; the scaffolding protein, Shank; small GTPases; transcription factors; and cytoskeletal proteins. Furthermore, interaction with such functionally relevant proteins also links Vesl/Homer proteins indirectly to an even larger group of cellular effector proteins, putting the Vesl/Homer proteins at the crossroads of several critical intracellular signaling processes. In addition to the initial reports of Vesl/Homer protein expression in the central nervous system, members of this protein family have now been identified in other excitable cells in various muscle types and in a large number of nonexcitable cells. The widespread expression of Vesl/Homer proteins in different organs and their functional importance in cellular protein signaling complexes is further evidenced by their conservation in organisms from Drosophila to humans. Exp Biol Med 230:527–535, 2005

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Molecular Determinants of Vesl/Homer Proteins

Control of multiprotein signaling complexes at neuronal synapses regulates synaptic activity and plays a critical role in synaptic plasticity, learning, and memory (1–3). Scaffolding proteins are involved in maintaining the architecture of signaling complexes and can link plasma membrane receptors and channels to channels on intracellular membranes (4–10). Homer proteins are scaffolding molecules that facilitate the clustering of specific synaptic proteins and, in addition, modulate their activities at neuronal synapses (6, 7, 11–17). In mammals, the Homer family of proteins is found in many tissues but is best characterized in neurons. Although Homer proteins were originally characterized in neurons, their presence in other tissues suggests a role not exclusive to neuronal synapses.

Three Homer genes have been characterized in mammals, *Homer* 1, *Homer* 2, and *Homer* 3 (4, 6, 18–20). A short form of Homer 1, Homer 1a (Vesl-1S),was the first isoform discovered after induction of excitatory synaptic activity in cerebellar granule neurons, during induced convulsive seizure in rat hippocampus (4, 18, 20) and long-term potentiation (LTP) in hippocampal synapses (18, 20). The induction of Homer 1a by LTP in the dentate gyrus occurs in an *N*-methyl-D-aspartate (NMDA) receptor–mediated mechanism (20). Similar to other immediate early

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genes (IEGs), *Homer* 1a responds rapidly to neuronal activity (6). Unlike most IEGs that encode transcription factors, Homer 1a is unique because it functions at synapses (18). Other Homer isoforms, many of which are long isoforms, are constitutively expressed and likely exhibit different functions in the synapse than short forms (4, 6, 7).

All Homer isoforms contain an Ena/vasodilator-stimulated phosphoprotein homology-1 (EVH1) domain within the first 110 N-terminal amino acids, which is involved in protein-protein interactions (6, 18, 20, 21). This domain is related in sequence to the vasodilator-stimulated phosphoprotein (VASP) and PDZ (PSD/Discs/ZO-1 [epithelial tight junction protein]) domains. Homer EVH1 shares sequence similarity with VASP/Ena proteins (20). Members of the VASP family of proteins regulate actin dynamics (22) and are often localized to focal adhesions, actin stress fibers, and edges of lamellipodia (23). The Homer EVH1 domain exhibits the sequence RxxxxxGLGF found in PDZ proteins, which can regulate targeting of ion channels in many different cell types (24, 25). For example, PDZ proteins bind shaker-type K^+ channels (26, 27) and NMDA receptor subunits (NR2) and target them to the plasma membrane (26, 28). In *Drosophila*, Homer plays a role in anchoring the developmental protein Oskar to the posterior cortex of oocytes (29). Homer proteins may also play an important role in neuronal development by affecting axonal pathfinding in neurons (30).

All long Homer isoforms exhibit a C-terminal coiled-coil (CC) domain containing two leucine zipper motifs which mediate homo- and hetero-dimerization between Homer monomers (4, 13, 19). This dimerization is critical for their scaffolding function at synapses.

The EVH1 domain of Homer binds to Group I metabotropic glutamate receptors (mGluRs; Refs. 4, 7, 18), transient receptor potential canonical (TRPC) channels (8), dynamin III (31), Shank (11), syntaxin 13 (32), actin (15), inositol (1,4,5)-trisphosphate receptors (IP_3Rs; Refs. 7, 8), ryanodine receptors (RyR; Refs. 5, 9, 10), and members of the Rho family of small GTPases (15). Homer proteins link Group I mGluRs and TRPC channels in the plasma membrane to IP_3Rs in the membrane of the endoplasmic reticulum (ER) and alter the functional properties of these proteins (8). Homer binds to RyR and modulates its calcium signaling properties (5, 9, 10, 33). Homer proteins also aid in targeting Group I mGluRs to the plasma membrane (6, 12–14, 16).

Known Isoforms of Vesl/Homer Proteins

The genes encoding *Homer* 1, *Homer* 2, and *Homer* 3 in humans are found on chromosomes 5, 15, and 19, respectively (6). Among the three classes of Homer proteins, there are at least 15 different splice variants within the Homer protein family in mammals. Homer proteins share a high degree of sequence similarity with one another, with the greatest similarities located in the N-terminal 110 amino acid EVH1 domain. The C-terminal CC domain exhibits little sequence similarity between different Homer members.

Table 1 summarizes known Homer isoforms and their molecular mass. There are four Homer 1 isoforms (Homer 1a, Homer 1b, Homer 1c, and Homer 1d; Refs. 4, 6, 15, 34). Homer 1a shares the N-terminal 175 amino acids with longer Homer 1 isoforms but it does not contain a C-terminal CC domain. Because it lacks the CC domain, it does not homo- or hetero-dimerize and link proteins. Instead, the C-terminal tail consists of a unique 11–amino acid sequence (6), which is necessary for proteasome-mediated degradation (35). Homer 1a has six amino acid residues, which act as potential phosphorylation sites for protein kinase C and a C-terminal PEST (enriched in proline [P], glutamic acid [E], serine [S], and threonine [T]) sequence (20), which are ubiquitin proteasome–dependent degradation signals (35). Most Homer isoforms contain PEST sequences (18, 35), but only Homer 1a seems to be sensitive to proteasome-mediated degradation (35). Deletion of the unique 11–amino acid C-terminal tail of Homer 1a prevents ubiquitin-mediated proteolysis (35). Beneken *et al.* have resolved the crystal structure of Homer 1a, and Tanaka *et al.* have elucidated the secondary structure of Homer 1a using nuclear magnetic resonance spectroscopy (21, 36). These analyses have demonstrated that Homer 1a contains eight β-strands and two α-helices (21, 36). Homer 1b, 1c, and 1d are long Homer 1 isoforms that contain CC domains at their C-termini (4, 6, 19, 34). There are four Homer 2 splice variants, Homer 2a, 2b, 2c, and 2d. Homer 2a and 2b isoforms are long and Homer 2c and 2d are short. Homer 2c and Homer 2d are short splice variants lacking the CC domain. Homer 2b and Homer 2d contain PEST sequences, whereas Homer 2a and Homer 2c lack this sequence, representing a means for differential protein regulation (37, 38). The Homer 3 gene encodes 10 known splice variants, 8 are long (Homer $3A_{00}$,

Table 1. Known Homer Isoforms

Homer isoform	No. of amino acid residues	Molecular mass (kDa)	Reference
Homer 1a	186	28	(18)
Homer 1b	354	47	(6)
Homer 1c	366	47	(6)
Homer 1d	370	48	(34)
Homer 2a	343	47	(6)
Homer 2b	354	47	(6)
Homer 2c	171	29	(38)
Homer 2d	182	29	(38)
Homer $3a_{00}$	352	48	(38)
Homer $3a_{01}$	355	48	(38)
Homer $3a_{10}$	355	48	(38)
Homer $3a_{11}$	358	48	(38)
Homer $3b_{00}$	316	45	(38)
Homer $3b_{01}$	319	45	(38)
Homer $3b_{10}$	319	45	(38)
Homer $3b_{11}$	322	45	(38)
Homer 3c	145	16	(38)
Homer 3d	121	14	(38)

$3A_{01}$, $3A_{10}$, $3A_{11}$, $3B_{00}$, $3B_{01}$, $3B_{10}$, and $3B_{11}$) and 2 are short (Homer 3C and Homer 3D; Ref. 38).

Tissue Localization of Vesl/Homer Protein Isoforms

Homer transcripts are expressed in many different tissues, including the brain, retina, cardiac muscle, skeletal muscle, smooth muscle, liver, kidneys, spleen, testis, thymus, placenta, and intestine (6, 39, 40). Table 2 summarizes the tissues of the central nervous that express Homer isoforms.

Homer isoforms are present in similar quantities in the brain (6, 19, 39, 40) and are expressed in the cortex, hippocampus, and cerebellum (6, 19, 39, 40). In the cortex, they are found in the superficial and deep layers (6). All isoforms are enriched in cerebellar Purkinje neurons (6, 39). Homer 1b/c is present in the internal granule layer, with light labeling in the molecular layer (39). In the hippocampus, Homer 1b/c is localized to the CA1, CA2, CA3, dentate gyrus, and subiculum (39). Homer 3a/b labeling is high in CA3, intermediate in CA2, and low in other hippocampal layers (39). In the olfactory bulb, Homer 1b/c is highly expressed throughout development (39). Homer 1c is expressed in the retina and was detected in postsynaptic elements of glutamatergic ribbon synapses in the inner and outer plexiform layers (41, 42). In the suprachiasmatic nucleus, Homer 1 mRNA expression is regulated by light phase shifts in the dark/light cycle (43).

Homer 2a/b protein is expressed in the cortex, hippocampus, and cerebellum (6). *Homer* 2 mRNA is expressed predominantly in the thalamus, olfactory bulb, and hippocampal primary neurons, and, to a lesser degree, in the cortex (6). Like Homer 1b/c, Homer 2a/b is also present in the internal granule layer of the cerebellum, with light labeling in the molecular layer (39). In the hippocampus, Homer 2a/b exhibits intense labeling in the CA1 and CA2 layers, intermediate labeling in the subiculum, and weak labeling in the CA3 and dentate gyrus (39). In the olfactory bulb, Homer 2a/b is highly expressed throughout development (39).

In mice, *Homer* 3 mRNA is expressed predominantly in the brain (40). Homer 3 expression is highest in the cerebellum and hippocampus with low label intensity in the cortex (6, 39). *Homer* 3 mRNA is constitutively expressed in the hippocampus, striatum, and cerebral cortex and is expressed at higher levels than *Homer* 1 in the cerebellum (6, 19). In the cerebellum, Homer 3a/b is localized to the molecular layer and to the Purkinje cell layer (39). In the hippocampus, Homer 3a/b labeling is high in CA3, intermediate in CA2, and low in other hippocampal layers (39). In the olfactory bulb, Homer 3a/b was expressed only early in development (39).

Homer proteins have been localized to muscle tissue (6, 9, 10, 40, 44). All Homer proteins are expressed in skeletal

Table 2. Neuronal Localization of Homer Isoforms[a]

	Cortex	Hippocampus	Cerebellum	Striatum	Thalamus	Olfactory bulb	Retina	Midbrain, pons, inferior colliculus, medulla oblongata
Homer 1a[b]	T (6, 19)[c]	T (6, 19)	—	T (6, 19) P (39)	—	T (19)	—	—
Homer 1b/c	T (6, 19) P (6, 39)	T (19) P (6, 39)	T (19) P (6, 39)	T (6, 19)	P (39)	T (6, 19) P (39)	T (41, 42)	P (39)
Homer 2a/b	T (6, 39) P (6)	T (6) P (6, 39)	P (6, 39)	P (39)	T (6) P (39)	T (6) P (39)	—	P (39)
Homer 3a/b	P (6)	T (6) P (6, 39)	T (6) P (6, 39)	—	—	P (39)[d]	—	—

[a] References in parentheses.
[b] Induction of Homer 1a.
[c] T, transcript; P, protein.
[d] Expressed at very low levels only early in development.

Table 3. Nonneuronal Localization of Homer Proteins[a]

Homer	Skeletal muscle	Cardiac muscle	Smooth muscle	Kidney	Liver	Spleen	Intestine	Lung	Thymus	Ovary	Testis
1b/c	T (40) P (39)[b]	P (6, 39) T (40)	T (40)	P (6)	P (6)	—	—	—	—	P (39)	P (39)
2a/b	P (6), T (40)	P (6, 39) T (40)	T (40)	—	P (6, 39)	P (39)	P (6)	—	—	—	—
3	T (40)	T (40)	T (40)	P (39)	—	P (39)	—	P (6, 39)	P (6)	P (39)	—

[a] References in parentheses.
[b] T, transcript; P, protein.

muscle and the heart, and Homer 3 is also expressed in diaphragm smooth muscle (6, 40).

Homer proteins are also expressed in nonexcitable tissues, such as kidney, liver, intestine, lung, and thymus (6, 39). The differential distribution of Homer isoforms in tissues other than the nervous system is summarized in Table 3.

Subcellular Localization of Vesl/Homer Proteins

All Homer isoforms colocalize with Group I mGluRs in the postsynaptic density of excitatory synapses in many regions of the brain (6, 18, 19, 41). The subcellular localization of Homer proteins is predominantly in the soma and apical dendrites (6, 18, 41). In subcellular fractionation studies, all Homer isoforms are localized to the crude nuclear pellet, synaptosomal pellet, microsomal pellet, and PSD fraction (6, 19). Unlike Homer 1 and Homer 3, Homer 2a/b is present in the soluble fraction and synaptic vesicle fraction (6). This suggests a role for Homer 2 in receptor trafficking functions. In the retina, Homer 1c immunoreactive puncta were observed in postsynaptic structures of bipolar cell dyads, particularly in the dendrites of ganglion cells postsynaptic to bipolar cells (41). In the murine myoblast cell line, C2C12, Homer 1a was localized primarily to the cytoplasm, whereas Homer 1c was localized in a punctate ER pattern with some labeling around nuclei (44).

Interaction of Vesl/Homer Proteins with Other Proteins

All Homer isoforms bind to proteins containing a proline-rich motif (PPxxFr) through their N-terminal EVH1 domain, which is required for protein–protein interaction. All long Homer isoforms can form homo- or heteromultimers with themselves and other Homer family members, respectively, through the C-terminal CC domain containing leucine zipper motifs. This dimerization is critical for the scaffolding function of Homer proteins at synapses. Homer proteins not only link receptors on the plasma membrane to intracellular signaling complex and cytoskeleton proteins, but also bind to multiple cytosolic proteins involved in cellular signaling. Homer proteins play important roles in numerous neuronal processes including neurotransmission and synaptic plasticity. Table 4 summarizes binding partners of Homer proteins based on localization of the binding partners, and the functional relevance of these interactions for intracellular signaling will be discussed next.

Interaction of Vesl/Homer Proteins with Plasma Membrane Proteins

Group I mGluRs. Experiments using yeast two-hybrid and co-immunoprecipitation assays demonstrated that all Homer isoforms bind to C-termini of Group I mGluR1 and 5 via an EVH1 domain at the N-terminus (4, 6, 18). This allows Homer proteins to functionally influence Group I mGluR–mediated G-protein signaling through phospholipase C and the generation of IP_3 and diacylglycerol. Homer proteins regulate the trafficking and clustering of mGluRs in various cell types. HEK-293 cells co-transfected with *Homer* 1a showed increased translocation of mGluR1α to the plasma membrane (12). In cerebellar granule cells, Ango and colleagues showed that when *mGluR5* is co-transfected with *Homer* 1b/c, translocation of mGluR5 from the soma to the dendrites is observed (50), whereas co-transfection with *Homer* 1a results in the appearance of mGluR5 in dendrites and axons. Co-transfection of *Homer* 1b/c with *mGluR5* triggered intracellular retention and clustering of the receptor at synaptic sites (51, 52). Similarly, co-transfection of *mGluR5* with *Homer* 1b in HeLa cells caused the ER retention of mGluR5, whereas HeLa cells co-expressing mGluR5 and Homer 1a showed normal transport of mGluR5s to the plasma membrane (14). If the Homer binding sites are disrupted, *mGluR5* is not retained in the ER, suggesting that the mGluR5–Homer 1b protein–protein interaction affects the trafficking of mGluR5 (14). Homer 1a increases the surface expression of mGluR1α in various cell types, including HEK-293 cells and cerebellar Purkinje neurons (53, 54). Tadokoro and colleagues showed in COS-7 cells that Homer 1c triggers clustering of mGluRs *via* a C-terminal leucine-zipper motif (13). It has been also shown in hippocampal neurons that Homer 1b, another long form of Homer 1, induces clustering of mGluR5 to the plasma membrane (55). Similarly, Ciruela and colleagues reported that Homer 1c also increases the clustering of mGluR1α (16). Rat superior cervical ganglion neurons co-expressing mGluR5 and Homer 2b showed cell-surface clustering of the receptors (17). It has been suggested that dynamin III, which is known to be involved in endocytosis, might play a crucial role in

Table 4. Protein Binding Partners of Homer Proteins Categorized Based on Subcellular Distribution of the Binding Partners

Localization	Binding partner	Homer	Detection method	Function	Reference
Plasma membrane	mGluR1α	1a, 1c, 2a/b, 2b, 3	Co-IP, Y2H[a]	Clustering and trafficking, activity modulation, coupling to signaling complex	(4, 6, 18)
	mGluR5	1a, 1c, 2a, 2b, 3	Co-IP, Y2H	Clustering and trafficking, activity modulation, coupling to signaling complex	(4, 6, 18)
	TRPC1, 2, 4, 5, 1/5	1, 3	Co-IP	Coupling to signaling complex	(8)
Intracellular membrane	IP_3R type I	1b/c, 2a/b, 3	Co-IP	Modulation of Ca^{2+} signaling	(7)
	RyR type 1 and 2	1a, 1c, 2, 3	Co-IP	Activity modulation, modulation of Ca^{2+} signaling	(5, 9, 10)
Cytosol/plasma membrane associated	Actin	2a/b	Co-IP	Coupling to signaling complex	(15)
	Dynamin III	1, 2a/b	Co-IP	Endocytosis, trafficking	(7, 31)
	Cdc42, Rho family small GTPase	2a/b	Co-IP	Coupling to signaling complex	(15)
	PI3 kinase enhancer	1c, 2a	Co-IP	Apoptosis	(45)
	PLC-β	2	Co-IP	Coupling to signaling complex	(46)
	Shank	1a, 1b, 1c, 2a/b, 3	Co-IP	Coupling to signaling complex	(11, 42)
	S8 ATPase	3	Y2H	Protein degradation	(47)
	Syntaxin 13	1c	Y2H	Trafficking	(32)
	C/EBP	3	Co-IP	Gene expression	(48)
	Other Homer proteins	1b, 1c, 2b, 3	Co-IP	Trafficking, coupling to signaling complex, generation of homo- or hetero-multimers	(4, 6, 49)

[a] Co-IP, co-immunoprecipitation; Y2H, yeast two-hybrid.

trafficking of mGluRs mediated by Homer because dynamin III has been shown to interact with Homer (7, 54).

In addition to the effect of Homer protein on the clustering and trafficking of mGluRs, it has been demonstrated that Homer proteins modulate the functional activity of mGluRs. In rat superior cervical ganglion neurons, co-transfection of *Homer* 2b decreases mGluR1α-mediated current modulation of N-type Ca^{2+} and M-type K^+ channels, which is restored by co-expression of Homer 1a (17). Ango and colleagues showed that Homer 3 proteins have inhibitory effects on mGluR1α function by demonstrating that knockdown of Homer 3 results in constitutively active mGluR1α (56). Homer 1a directly competes with Homer 3 in this functional effect on mGluR1α activity (56). Co-transfection of *Homer* 1a with *mGluR5* enhanced the amplitude and latency of the agonist-induced response of the receptor, whereas co-transfection with *Homer* 1b abolished the agonist response of the receptor because of Homer 1b–mediated cytosolic retention of mGluR5 (52). Minami and colleagues reported that activity-induced Homer 1a protein modulates mGluR1 activity, leading to an increase in mGluR1-mediated Ca^{2+} responses and inward currents (54). They also showed that an inhibition of mitogen-activated protein kinase (MAPK) eliminates the changes in mGluR1 function, suggesting cross talk between mGluRs-Homer signaling and the MAPK signaling pathway. Abe and colleagues showed that HEK-293 cells co-expressing Homer 1c and mGluR1α display increased mGluR1α-mediated intracellular Ca^{2+} transients (53). In contrast, Homer 1b with a C-terminal deletion constituting a functional equivalent of Homer 1a induces mGluR-evoked Ca^{2+} responses with a decreased amplitude and slower Ca^{2+} decay phase (7).

Taken together, these reports suggest a mechanism by which neurons at rest expressing constitutively long forms of Homer proteins lead to an intracellular pool of mGluR5 at synaptic sites, resulting in an inhibitory effect on receptor function. When Homer 1a expression is upregulated by neuronal activity, it triggers functional targeting of mGluRs to the synaptic membrane, increasing the high fidelity of synaptic transmission through clustering, targeting, and functional activity of neurotransmitter receptors by synaptic density proteins.

In addition to the functional clustering activity, Homer proteins link mGluRs to the MAPK pathway (54) and to extracellular signal–regulated proteins kinase 1 and 2 (ERK1/2) signaling cascades (57), suggesting novel Homer-dependent signaling pathways in addition to the conventional second-messenger pathways triggered by mGluR activation.

TRPC Channels. The TRPC protein family comprises nonspecific cation channels (58). It is thought that

TRPC participates in capacitive calcium entry to replenish intracellular Ca^{2+} stores (59). It has been shown that TRPC channels directly interact with intracellular Ca^{2+} channels, namely IP_3Rs, inducing channel opening (60, 61). The TRPC family members have proline-rich consensus sequences at the C-terminus to bind Homer proteins, mediating a direct protein-protein interaction (8). Homer 1c binds to TRPC1, C2, and C5, whereas Homer 3b binds to TRPC1, C4, and C5. Homer 2 also binds to TRPC1 (8). The Homer EVH1 domains are required for the interaction with TRPC1 (8). In addition, TRPC1 contains a second Homer protein–binding site in the N-terminus, which contributes to association of Homer and TRPC1 in a cooperative manner (8). In TRPC1 mutants lacking Homer protein binding sites, spontaneous TRPC channel activation was found (8). Similarly, co-expression of a dominant-negative form of Homer, Homer 1a, also increased basal TRPC channel activity (8). In TRPC mutants with disrupted Homer binding sites, diminished interaction between TRPC and IP_3R was found, suggesting a contribution of Homer proteins to the association of these channels (8). Association of TRPC1 with Homer 3 was reduced by thapsigargin-induced Ca^{2+} store depletion, indicating that intracellular Ca^{2+} store filling regulates a functional protein complex of TRPC1, Homer, and IP_3R (8). Taken together, these data suggest a regulatory mechanism mediated by Homer proteins whereby depletion of intracellular Ca^{2+} stores dissociates inhibitory long Homer forms, leading to activation of TRPC channels followed by extracellular Ca^{2+} influx. Once Ca^{2+} stores are replenished, long Homer proteins re-associate with and subsequently inactivate TRPC channels. Homer 1a modulates this interaction to introduce synaptic activity–dependent regulation to the system.

Interaction of Vesl/Homer Proteins with Intracellular Proteins

IP_3R. Homer proteins bind to the C-terminal tail of IP_3R type 1, as demonstrated by co-immunoprecipitation (7). Cerebellar Purkinje cell cultures were used to demonstrate linking of IP_3R and mGluR1a by Homer 1b/c and Homer 3 (7). The physical link between mGluR and IP_3R could serve a functional optimization of calcium signaling (7). Introduction of an IEG-like form of Homer 1b (C-terminal deletion mutant) into Purkinje cells and examination of quisqualate-mediated calcium transients led to a decrease in the amplitude of calcium transients and an increase in the latency. Blockage of mGluR activity by (+)-α-methyl-(4-carboxyphenyl)-glycine attenuates this effect (7). In addition to linking Group I mGluRs with IP_3Rs, Homer proteins also link TRPC channels (TRPC1, 2, 4, 5, and 1/5) to IP_3Rs. Intracellular Ca^{2+}-store depletion decreases the interaction between the TRPC1 channel and IP_3R, demonstrating that Ca^{2+} can have an indirect role in regulating Homer function (8).

RyR. All three Homer isoforms can bind RyR1 in skeletal muscle through the Homer EVH1 N-terminal domain. The interaction can be blocked by a competing EVH1 peptide (5). Homer 1c couples to RyR1 and regulates gating in response to caffeine, Ca^{2+} and depolarization by increasing open probability of RyR and it increases the initial release rate of Ca^{2+} in response (5, 10, 33). This action is dependent upon the N-terminal EVH1 domain because the W24A mutation within the EVH1 domain of Homer attenuates this action (5). The presence of the Homer CC domain leads to an increase in agonist activation (33). Homer 3 also increases the gating properties of RyR1. Both short and long Homer isoforms bind to and exhibit effects on RyR1 activity. Addition of Homer EVH1 domain (or intact Homer proteins) to RyR1 in bilayer lipid membrane systems results in increased channel-open probability in single-channel electrophysiological recordings (5, 10).

Addition of Homer 1a to Ca^{2+}-loaded microsomes reduces the stimulatory effect of Homer 1c on RyR1 in a dose-dependent manner (10). This suggests that Homer 1a competes with Homer 1c for the RyR1 Homer binding site (10). The presence of Homer EVH1 increases ryanodine binding slightly and Homer 1a has no significant effect (10), whereas full-length Homer 1c or glutathione-S-transferase-Homer 1c can greatly increase ryanodine binding (10, 33). This suggests that Homer dimerization/multimerization is critical for functionally relevant effects on the activity of RyR1. In intact skeletal muscle fibers expressing predominantly RyR1, Homer 1c increases Ca^{2+} spark frequency (33), suggesting that the mechanism of RyR1 activation by Homer 1c is by making the RyR1 more sensitive to activating stimuli (10, 33).

Unlike its action on RyR1, Homer 1c decreases RyR2 activity, as shown by Ca^{2+} imaging and release studies (9). Homer 1c decreases the open probability of the channel, as demonstrated in single-channel electrophysiological studies (9). The presence of Homer 1a reduces the inhibitory effect of Homer 1c on the RyR2 channel in a dose-dependent manner, suggesting competition between the two Homer isoforms for RyR2 binding and the importance of the CC domain for Homer function (9). The differential regulation of RyR isoforms by Homer 1c demonstrates how Homer proteins can regulate Ca^{2+} homeostasis.

Other Protein Interactions. Dynamin III is involved in endocytosis and localizes to dendritic spines in dissociated rat hippocampal neurons (31). Homer 1 and Homer 2 bind to dynamin III through their EVH1 domain, and deletion of this domain abolishes this interaction (31). Dynamin III also interacts with mGluR5, suggesting that dynamin III may be recruited to mGluR5 at the postsynaptic density through Homer proteins (31).

Homer can bind the scaffolding protein Shank through its EVH1 domain and C-terminal leucine zipper motifs (62). Overexpression of Homer 1c EVH1 domain or C-terminal leucine-zipper motif mutants in cultured hippocampal neurons exhibit reduced synaptic localization of Shank (62). Overexpression of Homer 1c also increases the synaptic localization of actin (62). Depolymerization of actin by lantruculin A reduces the localization of Homer 1c,

Shank, and actin to synapses (62). This demonstrates that Homer 1c or the Homer 1c-Shank complex facilitates the accumulation of F-actin in synapses (62). Homer 2a also binds to actin through its N-terminal domain, suggesting that it may link mGluR1α to the cytoskeleton (15).

Homer 1c also binds the SNARE (soluble *N*-ethylmaleimide–sensitive attachment protein receptor) protein syntaxin 13 (32). Co-expression of both Homer 1c and syntaxin 13 in cultured COS-7 cells results in colocalization in intracellular vesicular structures (32), indicating a potential role for Homer 1c in endosomal trafficking.

Homer 2a (Cupidin) interacts with RhoA, Rac1, and Cdc42, which are all members of the Rho family of small GTPases (15). These interactions are dependent on the presence of C-terminal sequences in Homer 2a and upon GTP binding (15).

The C-terminus of Homer 3 binds to the N-terminus of the S8 ATPase, which is a component of the 19S regulator of the 26S proteasome (47). This interaction facilitates targeting of mGluR1α from the ER to the 26S proteasome by an ubiquitin-independent mechanism (47).

Homer 3 also interacts with the transcription factor C/enhancer-binding protein β in Jurkat T cells through its N-teminal EVH1 domain (48). Overexpression of Homer 3 or an EVH1 domain peptide is translocated to the nucleus and decreases the transcriptional activation of serum response element (SRE) in response to various stimuli (48).

General Mechanisms Underlying the Interaction of Vesl/Homer Proteins with Other Proteins—Antagonism of Long and Short Isoforms

Many functions of the long Homer isoforms are regulated in a dominant-negative fashion by the short Homer isoforms. The short Homer isoforms compete with the long isoforms for Homer binding sites on all proteins functioning as Homer ligands. For example, the short isoform Homer 1a induces increased plasma membrane expression of Group I mGluRs, whereas the long isoform Homer 1b causes retention of mGluR5 in the ER (14). Similarly, Homer 1a reduces the stimulatory effect of Homer 1c on RyR1 while reducing the inhibitory effect of Homer 1c on RyR2 (9, 10). This exemplifies the potential involvement of the inducible short Homer isoforms in the regulation of intracellular signaling and trafficking processes controlled by ubiquitously expressed long Homer isoforms (63). Such processes also indicate a general stimulus-dependent mechanism using the molecular determinants of Vesl/Homer proteins to regulate intracellular signaling pathways.

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