Interaction of G-protein-coupled receptors with synaptic scaffolding proteins

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Abstract

The calcium-independent receptors for latrotoxin (CIRL1–CIRL3) constitute a family of seven-transmembrane receptors with an unusually large N-terminal extracellular domain which comprises several motifs usually found in cell adhesion molecules. By yeast two-hybrid screening, we have identified the intracellular C-termini of CIRL1 and CIRL2 as interaction partners of the PDZ domain of the proline-rich synapse-associated protein (ProSAP)/somatostatin receptor-interacting protein (SSTRIP) family of postsynaptic proteins (SSTRIP, ProSAP1 and ProSAP2, also known as Shank1–Shank3 respectively). Overlay assays indicate that the ProSAP1/Shank2 PDZ domain in particular interacts strongly with the C-terminus of CIRL1 and CIRL2. Co-immunoprecipitation of ProSAP1 and CIRL1 (but not CIRL2) from rat brain extracts indicates that this interaction also occurs in vivo in rat brain. The known postsynaptic localization of ProSAP1, as well as our observation that CIRL1 (but not CIRL2) is enriched in postsynaptic density preparations from the rat brain, suggests that CIRL1 is localized pre- as well as post-synaptically in the central nervous system.

Key words: CIRL, latrotoxin, PDZ domain, postsynaptic density.

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Neurotransmitter receptors are often attached to intracellularly associated proteins via a complex network of protein interactions [1,2]. The purpose of these interactions is believed to be severalfold: (i) the targeting of receptors to their sites of action (e.g., pre- or post-synaptic specializations), (ii) anchoring of receptors to the actin-based cytoskeleton, and (iii) physical association of receptors with elements of the signal transduction machinery [3]. The last of these appears to be required for rapid and efficient signal transduction. We have addressed this issue by searching for proteins interacting with C-terminal intracellular regions of the somatostatin neuropeptide receptors (SSTR1–SSTR5). One group of proteins, comprising SSTRIP (also known as shank1 or synamon), ProSAPl (also CortBPl or shank2) and ProSAP2/shank3, was shown to interact with the C-terminus of SSTR2 via the single PDZ domain common to all ProSAP/shank forms [4]. The ProSAP/shank PDZ domain can also bind to a postsynaptic protein termed SAPAP [SAP90/PSD95 (postsynaptic density 95)-associated protein]/GKAP (guanylate kinase-associated protein), which links ProSAP/shank to the N-methyl-D-aspartate (NMDA) receptor complex in excitatory synapses [5-7].

In order to identify other targets of the ProSAP/shank PDZ domain, we used this domain as a bait in a yeast two-hybrid screen. In this screen, the PDZ domain of ProSAPl/shank2 yielded several clones, each containing the C-terminus of CIRL1; a similar screen with the PDZ domain of SSTRIP/shank1 identified the human homologue of CIRL2, as well as several clones containing brain angiogenesis inhibitor 2 (BAI2) protein, as specifically interacting proteins [8]. Similarly, Tobaben et al. [9] identified ProSAP/shank proteins as targets for the C-terminus of CIRL1 in a similar yeast two-hybrid screen. CIRL1–CIRL3, as well as the BAI proteins BAI1–BAI3, are members of a subfamily within the larger family of G-protein-coupled receptors [10,11], the members of which consist of a rather large, heavily glycosylated N-terminal domain and a C-terminal part which includes the seven-transmembrane regions as well as an intracellular domain that mediates the interaction with the ProSAPl/shank PDZ domains. In CIRLs the N-terminal extracellular region is proteolytically cleaved from the rest of the protein, resulting in a two-subunit receptor [10].

The CIRL/BAI–ProSAP/shank interaction was verified by overlay assays using isolated fusion proteins of the C-terminus of the receptor proteins as well as the PDZ domains of ProSAP/shank proteins. This analysis indicated that the PDZ domain of ProSAPl/shank2 has the highest affinity for the CIRL1 and CIRL2 C-terminus, followed by the PDZ domain of SSTRIP/shank1. The BAI protein, on the other hand, did not bind to ProSAP/shank PDZ domains in this assay. On comparing sequences of C-terminal ligands of ProSAP/shank PDZ domains, it becomes obvious that, although ProSAP/shank ligands conform to the general PDZ recognition motif S/T-X-Φ-COO (where Φ is large and hydrophobic; see [12]), ProSAP/shank prefers leucine or isoleucine over valine in the C-terminal position, which is preferentially found, for example, in binding partners of the PDZ domain of PSD95/SAP90

Figure 1

C-terminal sequences of several proteins linked to PDZ domains

<table>
<thead>
<tr>
<th>protein</th>
<th>C-terminal sequence</th>
<th>target</th>
</tr>
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<tbody>
<tr>
<td>CIRL1</td>
<td>G Q M G L V T S L *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>CIRL2</td>
<td>G Q M Q L V T S L *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>mGluR1</td>
<td>D Y K Q S S T L *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>SAPAP/GKAP</td>
<td>Y I P E A Q T R L *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>SSTR2</td>
<td>L N G D L Q T S I *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>CIRL3</td>
<td>G P A H L V T S L *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>BAI-2</td>
<td>P D G D F Q T E V *</td>
<td>ProSAP/shank PDZ</td>
</tr>
<tr>
<td>NHEAR2</td>
<td>K L S S I E S D V *</td>
<td>PSD95/PDE1,2</td>
</tr>
<tr>
<td>SHAKEr/Kv1.4</td>
<td>N A K A V E T D V *</td>
<td>PSD95/PDE1,2</td>
</tr>
<tr>
<td>CRIPT</td>
<td>T K N Y K Q T S V *</td>
<td>PSD95/PDE3</td>
</tr>
</tbody>
</table>
proteins such as the NMDA receptor 2A subunit [1] (see Figure 1).

ProSAP/shank proteins are constituents of the postsynaptic density of excitatory synapses [4–7], whereas latrotoxin receptors (CIRL1, as well as the non-related protein neurexin) mediate the presynaptic effect of latrotoxin on neurotransmitter release [10]. A Western blot analysis of rat brain membrane fractions showed, however, that CIRL1 (but not CIRL2) is strongly enriched in the postsynaptic density fraction [8,9], indicating that CIRL1 is localized both pre- and postsynaptically.

In agreement with the enrichment of both ProSAP/shank proteins and CIRL1 in the postsynaptic density, CIRL1 could be co-immunoprecipitated with ProSAP1/shank2 from solubilized rat brain membranes, demonstrating that the interaction between the two proteins occurs in vivo [8]. CIRL2, on the other hand, was not detected in the ProSAP1 precipitate, suggesting that, although the sequence requirement at the C-terminus of CIRL2 is fulfilled, this receptor is not targeted into a ProSAP1-containing complex.

CIRL proteins — a family of unconventional G-protein-coupled receptors

CIRL proteins were originally identified based on their ability to bind α-latrotoxin [10], and their role in toxin-stimulated transmitter release has been most intensely investigated. Here the function of CIRLs appears to be restricted to recruiting the toxin to presynaptic sites of transmitter release; once the toxin has reached its site of action, it inserts into the plasma membrane to form an ion channel, leading to uncontrolled release of transmitter from synaptic vesicles [13–15]. CIRL expression sensitizes cells to the actions of latrotoxin; the extracellular region of CIRL1 is sufficient for this effect, whereas the transmembrane and intracellular parts are not required [13,14]. These data therefore indicate that coupling of CIRL to intracellular signalling pathways is not involved in latrotoxin action. More recently, CIRL1-deficient mice have been generated by homologous recombination [16]. Whereas these mice show an altered responsiveness towards α-latrotoxin and have helped to elucidate a complex interplay between neurexin (the calcium-dependent receptor for this toxin) and CIRLs, they have provided no evidence so far with respect to the physiological function of CIRL-like proteins [16].

CIRLs and the related proteins HE6, BA11–BA13 and CD97 fulfill the structural criteria for G-protein-coupled receptors; association with the G-protein Ga, has been demonstrated for CIRL1 in transfected COS cells [17], suggesting that these proteins indeed function in G-protein-mediated signalling. A further analysis of the intracellular signal transduction pathways activated by CIRL proteins has not been presented due to the lack of a physiological activator of CIRL proteins. In the case of the more distantly related family member CD97, the cell surface molecule CD55 was identified as a binding protein for the large extracellular region [18]. Interaction is mediated by epidermal growth factor-like and short consensus repeat domains of CD97 and CD55 respectively [19], a pattern not uncommon for cell surface proteins or cell adhesion molecules. However, it has not been demonstrated if binding of CD55 induces a G-protein-mediated signal on the side of CD97. If any conclusions may be drawn from this for putative ligands of CIRLs, it appears possible that these proteins are also activated by cellular ligands such as adhesion molecules, rather than by soluble, secreted ligands which activate most known G-protein-coupled receptors. This view is supported by the presence of lectin-like and olfactomedin-like domains in the N-terminal, extracellular portion of CIRL [10]. Thus the presence of CIRL on both pre- and post-synaptic membranes would be of relevance to a putative function in the formation of contacts between cells. Interestingly, expression of CIRL isoforms in the central nervous system, as detected by in situ hybridization, is highly regulated, with CIRL3 appearing very early in adult life in many brain regions, followed by CIRL2 and later by CIRL1, which appears to be the adult form of CIRL protein [8]. Thus the three CIRL proteins may not be functionally redundant, but might replace each other during brain development.

Role of shank/ProSAP proteins in organizing membrane-associated signalling complexes

Numerous interaction partners have been identified for the ProSAP/shank PDZ domain, linking ProSAP/shank either directly or indirectly to the intracellular domains of cell surface receptors. ProSAP/shank proteins contain several additional protein interaction motifs, including a set of six ankyrin repeats, a Src homology 3 (SH3) domain, a long proline-rich region and a C-
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terminal sterile α-motif (SAM) domain (Figure 2). Within the proline-rich region, one segment has been shown to bind to the enabled (vasodilator-stimulated phosphoprotein) homology (EVH) domain of homer proteins [20]. Homer itself can dimerize via a coiled coil motif; the second homer molecule in this dimer can then bind to the intracellular region of metabotropic glutamate receptors, again via the EVH domain of homer [21]. Thus, in the postsynaptic density of a glutamatergic synapse in the central nervous system, ProSAP/shank proteins connect different classes of glutamate receptor (i.e. metabotropic and NMDA-type receptors) via a complex network of protein interactions. Consistent with this notion, NMDA receptor subunits, PSD95/SAP90, SAPAP/GKAP, ProSAP/shank proteins, homer and metabotropic glutamate receptors (as well as numerous other postsynaptic proteins) can be co-purified from rodent brain as members of a large NMDA-receptor-associated signalling complex [3].

Two further domain interactions of the ProSAP/shank family suggest a role for ProSAP/shank proteins in anchoring membrane receptor complexes to the actin-based cytoskeleton. Cortactin, an actin binding protein which has also been identified as a major substrate of the Src tyrosine kinase, is attached to a proline-rich motif in ProSAP1/shank2 (and possibly also the other family members) via its SH3 domain [22]. Furthermore, the ankyrin repeats of SSTRIP/shank1 and ProSAP2/shank3 bind to spectrin repeat 21 of α-fodrin [23]. α-Fodrin associates with β-fodrin to form a tetrameric complex, linking numerous membrane-associated proteins to microfilaments. The prominent presence of α-fodrin in the postsynaptic density of glutamatergic synapses [24] and the co-localization of ProSAP/shank proteins with fodrin in synapses [23] demonstrates that the shank–fodrin interaction occurs in synapses and serves to anchor ProSAP/shank–receptor complexes in the postsynaptic compartment (Figure 2).

Taken together, these findings implicate ProSAP/shank proteins as a major part of the interface between membrane receptors and the cytoskeleton. Overexpression of shank1 in hippocampal neurons induces morphological changes in dendritic spines, suggesting that ProSAP/shank proteins indeed play a major role in the structural organization of synaptic junctions [25]. The expression of some ProSAP/shank forms in non-neuronal tissues such as lung and pancreas [26,27] raises the question of whether ProSAP/shank proteins fulfil a similar role in the organization of membrane-associated signalling complexes in other cell types. Interestingly, we have observed localization of cortactin, ProSAP/shank and CIRL proteins in focal adhesion sites of a breast cancer epithelial cell line (M. Soltau and H.-J. Kreienkamp, unpublished work), supporting the
idea that the ProSAP/shank-CIRL interaction may indeed not be restricted to neuronal tissues.

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References

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Development and potential of non-peptide antagonists for calcitonin-gene-related peptide (CGRP) receptors: evidence for CGRP receptor heterogeneity

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Abstract
Calcitonin-gene-related peptide (CGRP) is a 37-amino-acid vasodilatory peptide, of which two isoforms, αCGRP and βCGRP, have been described. The use of C-terminal fragments of CGRP peptide, such as human αCGRP-(8–37), has led to the pharmacological subdivision of CGRP receptors into CGRP-1 [high potency for binding of human αCGRP-(8–37)] and CGRP-2 (lower potency) receptors. We have recently developed BIBN4096BS, the first non-peptide CGRP antagonist, which has sub-nanomolar affinity for primate CGRP receptors. The use of BIBN4096BS has led to the discovery of further functional CGRP receptor heterogeneity in rat tissues. To further exploit BIBN4096BS as a pharmacological tool, we used BIBN4096BS in