Specificity in signaling pathways: assembly into multimolecular signaling complexes
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A critical issue in the field of signal transduction is how signaling molecules are organized into different pathways within the same cell. The importance of assembling signaling molecules into architecturally defined complexes is emerging as an essential cellular strategy to ensure specificity and selectivity of signaling. Scaffold proteins function as the pillars of these transduction complexes, bringing together a diversity of signaling components into defined ultramicrodomains of signaling.

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Abbreviations
Dlg discs large
PDZ post-synaptic density protein-95, Dlg, ZO-1
PH pleckstrin homology
PLC phospholipase C
PKC protein kinase C
PTB phosphotyrosine binding
SH src homology

Introduction
Every cell — whether an embryonic stem cell responding to a growth factor in the decision to differentiate into a muscle or epithelial cell, or a mature neuron that needs to respond differentially to synaptic inputs from its thousands of dendritic spines — is continuously faced with the task of transducing a vast array of external signals into appropriate intracellular responses. How is cross-talk between signaling cascades which share common molecular components avoided, and how are the pertinent downstream effectors and targets selected? In recent years, there has been increasing recognition that signaling events do not take place freely in the cytosol of the cell but, rather, occur in physically and functionally distinct signaling units or complexes. This signaling organization would prevent unwanted cross-talk while optimizing speed, selectivity and specificity.

Signaling complexes may be organized around scaffold/adaptor proteins containing multiple protein–protein binding/interaction motifs. Such binding motifs include SH2 and SH3 (src homology), PH (pleckstrin homology), PTB (phosphotyrosine binding), WW (W being tryptophan), and the more recently described PDZ (post-synaptic density protein-95, Dlg, ZO-1) domains [1*,2†]. In order to bring a unique assortment of signaling proteins together, adaptor proteins contain different numbers, varieties and combinations of these modular protein-binding domains. The discovery of molecular scaffolds assembling transduction complexes in a variety of systems from yeast to humans, and in processes as diverse as cell fate determination, synaptic plasticity, pheromone signaling and phototransduction illustrate their universal importance. In this review, we provide our perspective on the organization of transduction complexes (i.e. transducisomes) by focusing on scaffold proteins in general, and PDZ domain adaptors in particular.

Subcellular localization and assembly of signaling complexes is critical for signaling
In order for a neuron to function, ion channels and receptors must be correctly localized to specialized subcellular sites: at axon terminals to stimulate neurotransmitter release, at postsynaptic membranes to mediate postsynaptic potentials, and at nodes of Ranvier to conduct action potentials along axons. Similarly, the appropriate localization of receptors on epithelial cells is crucial to their physiology: membrane receptors are asymmetrically targeted either to apical or basolateral surfaces to receive and respond to specific signals. Genetic studies in several systems have demonstrated that scaffold proteins are essential for the localization of signaling proteins, and that proper localization of signaling complexes is critical to signaling.

In Drosophila photoreceptor neurons, signaling molecules are organized into transduction complexes by the photoreceptor protein INAD [3–5,6*,7**]. INAD is a multivalent adapter protein consisting of five different PDZ domains functioning as a modular scaffold, assembling several components of the phototransduction cascade, including the light-activated ion channels (TRP), the effector phospholipase C (PLC), and an eye-specific protein kinase C (PKC). Null inaD mutants display a complete loss of transduction complexes and a dramatic impairment in light responsiveness [7**]. Notably, mutations in individual PDZ domains prevent the recruitment of the specific target and produce a corresponding defect in signaling. Together, these results demonstrate the presence of a highly organized macromolecular unit of signaling, and show that it is not the mere presence of a signaling molecule that promotes effective signaling, but rather its location. The organization of this pathway into a transducisome also guarantees that molecules involved in activation (e.g. PLC) and regulation (e.g. PKC) would be ideally poised for rapid and specific responses, an important requirement in a process, like vision, that relies on high temporal resolution.
In the nematode *Caenorhabditis elegans*, the development of the vulva is dependent on a ras-signaling pathway mediated by the LET-23 tyrosine kinase receptor. *lin-7* and *lin-2* are two of the genes required for the induction of the vulva. LIN-7 is a PDZ-containing protein that interacts with LET-23 and is essential for localizing the receptor to cellular junctions [8]. Mutations in *lin-7* lead to mislocalization of the LET-23 receptor and a vulval less phenotype. *lin-2* encodes a member of the MAGUK family of cell junction proteins (see below; [9]) containing regions of similarity to CaM kinase II and membrane-associated guanylate kinase. Like LIN-7, LIN-2 is thought to be required for the localization of transduction components of the LET-23 pathway. Interestingly, *lin-2* transgenes lacking either kinase domain are still functional [10], consistent with the postulate that LIN-2 has a structural rather than an enzymatic function in vulval induction. These results illustrate another example of how subcellular localization plays a critical role in signaling.

A pivotal example of a scaffold protein critical for localizing signaling components is the *Drosophila* discs large (Dlg) protein. Dlg is one of the first identified members of the PDZ-domain protein family and is required for a number of processes including the formation of septate junctions (the equivalent of tight junctions in vertebrate epithelia) and synaptic complexes. Dlg and its mammalian homologues, PSD-95/SAP90, chapsyn-110/PSD-93, SAP102, and SAP97/Dlg contain, from the amino to carboxyl terminus, three PDZ domains, an SH3 domain, and a guanylate kinase domain (these are known collectively as a subfamily of MAGUKs: membrane-associated guanylate kinases) [11,12]. The PDZ domains of Dlg/PSD-95 proteins interact with specific targets and mediate their selective localization and clustering at the plasma membrane. Interestingly, different PDZ domains show selectivity for different targets, such as ion channels, cell-adhesion proteins, and signaling molecules. Indeed, mutations in *dlg* disrupts the localization as well as the clustering of Shaker K+ channels [13**, and the localization of the cell adhesion protein Fasciclin II at the neuromuscular junction [14**]. The modular nature of the interaction between Shaker/Fasciclin II and Dlg was demonstrated by the selectivity of these targets to the PDZ1-2 domains of Dlg and by showing that the last 11 amino acids of either Shaker or FasII are sufficient to target a foreign protein to the synapse in a Dlg-dependent manner. These results also open up the possibility of using PDZ-target interactions as a strategy to direct novel transduction components to the neuromuscular junction, as a means of generating dominant phenotypes, and a way of custom designing synapses (see below).

A typical neuron in the mammalian brain may have well over 1000 synapses, each tuned to a particular signal. A strategy to ensure that the correct machinery is localized at each synapse would be to assemble signaling complexes using distinct scaffold proteins. Indeed, the list of PDZ-containing proteins now include well over 100 members, many of which are localized at synapses and contain multiple PDZ motifs (4, 5, 6, 7 and even 11 PDZ domains in a single polypeptide chain; see [15]). The best studied PDZ family is PSD-95 and its homologs. The list of proteins known to interact with PDZ domains of the PSD-95 family include Kv1.4 and Kir 2.3 K+ channels [16,17], NMDA receptors [18], SynGAP [19], Ca2+ ATPase [20] and nNOS [21], Fasciclin II [14**,22**] and neuroligins [23**]. In several of these cases, the biochemical interactions have also been validated *in vivo*. For example, a PDZ domain in nNOS interacts with a PDZ in PSD-95, and homozygous mutant mice lacking the PSD-95 interaction domain produce nNOS that fails to associate with PSD-95 in brain [21]. Likewise, transfections of tissue culture cells with constructs containing wild-type or mutant PSD-95 and targets demonstrated that the interaction of PSD-95 with K+ channels and NMDA receptors is highly specific and essential for receptor clustering [18,24]. Although the physiological relevance of these proposed interactions has not been assessed *in vivo* (i.e. the natural cellular and organismal context), it is easy to infer the importance of the associations. For instance, the clustering of NMDA receptors in close proximity to nNOS would ensure that localized calcium changes efficiently modulate nNOS activity. Similarly, selective clustering of unique NMDA or different K+ channels at or near synapses could dramatically impact the nature of synaptic transmission.

The assembly of transduction complexes is not unique to multicellular organisms. In the yeast *Saccharomyces cerevisiae*, the mating response pathway is mediated by a G protein that activates a mitogen-activated MAP kinase cascade [25]. This kinase cascade is organized by the STE5 protein, a scaffold protein which tethers each of the kinases in this pathway into a macromolecular complex [26]. Null mutations in *ste5*, as well as mutations in the binding sites for any of the kinases [27–29], result in the loss of the pheromone mating response. The organization of this pathway into such a complex has important advantages for the organism, such as ensuring that activation of unrelated mitogen-activated pathways does not trigger a mating response.

**Are transduction complexes dynamic structures?**

A number of studies suggest that scaffolds may also function as dynamic structures. PKA phosphorylation of a carboxy-terminal serine residue in K+ channel Kir2.3 disrupts its association with PSD-95 [16], and amino-terminal palmitoylation of PSD-95 regulates its association with cell membranes and its interaction with K+ channel Kv1.4 [30**]. Recently, a novel neuronal protein, carboxy-terminal PDZ ligand of nNOS (CAPON), has been shown to bind to the PDZ domain of nNOS and compete *in vitro* and *in vivo* with the binding of PSD-95 to nNOS [31]. In more general terms, these types of regulatory strategies could be used during complex assembly to recognize different targets at different times or, after
assembly and localization, to modulate the output of the transduction pathway. Together, they add great versatility and flexibility to transduction assemblies.

The highly modular nature of scaffold/target interactions suggest that it should be possible to design molecules that either modulate or disrupt specific interactions. Taken one step further, we suggest that viruses and microorganisms might have evolved such molecules in their continued battle of securing the cell's signaling machinery. Although there are no clear examples of this yet, there are a couple of intriguing observations: Recently, the second PDZ domain of the human homologue of Dlg (hDlg) was shown to bind to the human papillomavirus E6 oncoprotein [32], and to an adenovirus E4 region oncoprotein [33]. Remarkably, the transforming activity of both oncoproteins was abolished when the hDlg-binding motif was eliminated. It would be of great interest to determine whether these oncoproteins are hijacking target binding sites in hDlg and, in doing so, coercing the transduction complexes into a gain-of-function phenotype or into a dominant-negative response.

**Anchoring of signaling complexes**

Scaffold proteins do not generally contain transmembrane domains, yet they are localized to the plasma membrane. Once targeted to their site, how are signaling complexes anchored to the membrane? Many PDZ-containing proteins have been found to be linked to integral membrane proteins—like ion channels and membrane receptors (see above)—whereas others have been found associated with components of the cytoskeleton. For example, the PDZ domain of the ALP protein binds directly to α-actinin-2 at the Z lines of myofibers [34], whereas the human erythrocyte p55 PDZ protein binds protein 4.1 [35]. Furthermore, some PSD-95 family members contain a conserved binding motif for band 4.1 [36], which has been implicated in linking membrane proteins with the actin/spectrin cytoskeleton [37], however, the biological significance of this motif has not been resolved. In mouse, the third PDZ domain of PSD-95 binds to the cytoplasmic tail of neuroli- gins, neuronal cell adhesion molecules involved in the formation of intercellular junctions [23*]. In a separate study [38], the third PDZ domain of PSD-95 was found to bind a novel presynaptic protein, CRIPT, which mediates the anchoring of PSD-95 to a tubulin-based cytoskeleton in heterologous cells.

**Scaffold interactions, and target–target interactions may promote formation of macromolecular networks**

Although the advantages of bringing signaling components into close proximity are significant (speed, specificity, regulation, etc.), we should also highlight the importance of organizing scaffold proteins into larger complexes. The assembly of large macromolecular complexes permits high concentrations of transduction molecules in small domains of signaling, an important requirement in a wide range of processes like synaptic transmission and phototransduction. Interestingly, the same biochemical events that promote assembly into larger complexes may also assist in the formation of signaling networks that encourage cross-talk and cross-regulation. This may underlie, and be particularly relevant to, well-defined pharmacological phenomena like cross-desensitization. Indeed, a number of recent reports suggest that individual scaffold proteins may interact with each other to form macromolecular complexes [17,39*]; for example, it has been shown that PSD-95 and chapsyn-110 form heteromultimers in vitro [17]. In the case of ion channels, it is easy to envision how assembly of a mature tetrameric or pentameric channel from individual subunits—each possibly carrying a scaffold protein and associated targets—would result in large macromolecular complexes.

**Concluding remarks**

We have explored the role of scaffold proteins and their importance in promoting speed, selectivity and specificity of signaling. We have also highlighted their potential role in segregating large numbers of signaling pathways in cells, as simple as a yeast and as complex as a central nervous system neuron with thousands of synapses. Finally, we suggest that manipulating scaffold–target interactions may have great utility as research tools.

Issues of localization, targeting and anchoring of complexes are just beginning to be explored. It will be interesting to see whether common themes will arise in how, when, and where signaling complexes are formed, and how they are targeted to subcellular domains. In some cases, the organization of signaling complexes probably plays a key role in the structural composition of specialized subcellular domains, like intercellular junctions or synaptic densities. It will be exciting to see how networks of different signaling complexes are formed and how they connect to their subcellular environment.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


A good review of PDZ containing proteins.


A good review for many of the protein binding domains.

This paper gives evidence defining the binding site in NORPA for INAD. The formation of transduction complexes is critical to signaling as demonstrated by the loss of light responsiveness in null mutants for INAD.


This paper gives evidence defining the binding site in NORPA for INAD. Mutants affecting NORPA's association with INAD show defects in signaling.


This study gives the first in vivo evidence that INAD functions as a scaffold to form transduction complexes in Drosophila photoreceptors. The formation of transduction complexes is critical to signaling as demonstrated by the loss of light responsiveness in null mutants for INAD.


This study gives the first in vivo evidence for the importance of a MAGUK protein in the clustering of channels at the synapse.


In vitro and in vivo experiments show that Facsinil II and Dlg interact. In addition, the study shows that the localization of Facsinil II to the NMJ is dependent on Dlg.


This study shows that PSD-95 interacts with neurotrophins, suggesting that MAGUKs may play an important role in the structure of intercellular junctions, like the synapse.


31. Biochemical and site-directed mutagenesis studies show that post-translational modification of PSD-95 may direct its targeting to membranes.


43. Deletion studies of PSD-95 in heterologous cells suggest that an amino-terminal sequence is responsible for multimerization.