Cell signalling: **MAGUK magic**

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Diverse extracellular signals are coupled at the plasma membrane to intracellular signal transduction pathways and the cytoskeleton. Members of a ubiquitous multidomain family of proteins, the MAGUK proteins, are emerging as common mediators of this coupling.

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Each cell's identity is specified by signals from neighboring cells. Incoming signals are transduced from specialized sites on the plasma membrane to specific intracellular signaling pathways and the cytoskeleton. The result is to induce changes in cytoskeletal structure and to regulate functions such as proliferation and differentiation. Members of a family of multidomain membrane proteins, called the membrane-associated guanylate kinase homologs (MAGUK proteins), are emerging as important in coupling the extracellular environment to intracellular signaling pathways and the cytoskeleton; they operate at cell surfaces as diverse as those at synapses and tight junctions. MAGUK proteins apparently arose early in the evolution of multicellular animals: their genes have been cloned from the simple metazoan species *Hydra*; mutants in *Drosophila* and *Caenorhabditis elegans* reveal a signalling function [1,2]; and mammalian forms define the location of transmembrane proteins around the cell cortex [3–6] and create links to the actin cytoskeleton ([7]; S.M. Marfatia, J.H. Morais-Cabrol, A.C. Kim and A.H. Chishti, personal communication).

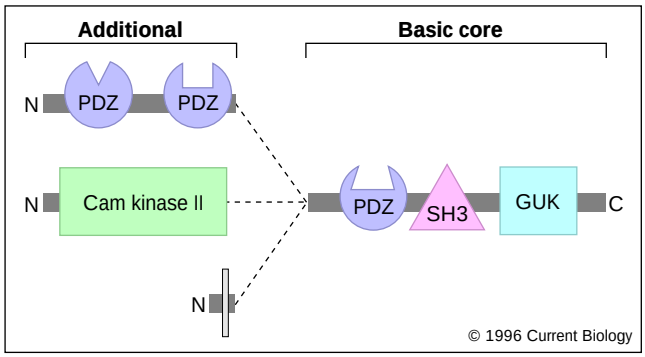
MAGUK proteins are defined by a basic core of three domains [1,6]: a Src homology 3 (SH3) domain; a domain with homology to the enzyme guanylate kinase (GUK), and a PDZ domain, named after the MAGUK family members PSD-95, a 95 kDa protein of the postsynaptic density; Dlg, the product of the *Drosophila lethal(1)discs-large-1* tumor suppressor gene; and ZO-1, a mammalian tight junctional protein (Fig. 1). On the basis of its role in other proteins, the SH3 domain is expected to bind a protein involved in signal transduction, the actin cytoskeleton, or both. Unfortunately, none of the binding partners has yet been identified. Likewise, the role of GUK domains is puzzling. They are homologous to the enzyme that catalyzes the ATP-dependent conversion of GMP to GDP, but by comparison with the authentic enzyme they seem to have diverged during evolution into subfamilies with varying nucleotide-binding capabilities. Some are predicted to bind both GMP and ATP (the red blood cell linker protein p55 and the *C. elegans* signalling protein Lin-2), some only GMP (the synapse-associated proteins SAP90 and SAP97, Dlg and its human homolog hDlg) and others neither GMP nor ATP (ZO-1 and ZO-2). This prediction has been confirmed for SAP90, which binds GMP but not ATP and thus lacks enzymatic activity [8]. If active as enzymes, are MAGUK proteins coupled to G-protein signalling pathways by a role in regulating local nucleotide levels? Alternatively, are nucleotides used as allosteric effectors of a protein-binding activity, as in other GTP-binding proteins?

Another evolutionary elaboration on the basic MAGUK core is evident in their variable amino-terminal sequences (Fig. 1). A large branch of the tree includes proteins that have two additional PDZ domains; proteins on a smaller branch have a domain with homology to Ca^{2+}–calmodulin (Cam) protein kinase II. Roles for the Cam kinase II and GUK domains have not yet been defined, and insight is further obscured by the observation these domains are not required for rescue of the *lin-2* mutant phenotype in *C. elegans* [2].

Evidence for a signaling function in invertebrates

The first member of the MAGUK family to be characterized genetically was Dlg, the product of the *Drosophila lethal(1)discslarge-1* tumor suppressor gene [1]. The Dlg protein is located on the cytoplasmic surface of septate junctions. These, like vertebrate tight junctions, form a continuous intercellular contact around the apical edge of

Figure 1



The basic core of a MAGUK protein consists of a PDZ, an SH3 and a GUK domain (see text). Some MAGUKs have additional domains at the amino terminus, such as two PDZ domains or a domain with homology to Ca^{2+}–calmodulin kinase II.

polarized epithelial cells. In *dlg* null mutants, septate junctions fail to form between cells lining imaginal discs in the embryo. Absence of junctions is associated with defective cell–cell adhesion, a loss of cell polarity, and unregulated proliferation. In mutants with 'weaker' alleles, morphologically normal junctions form but cell proliferation proceeds unchecked. Lethal tumors develop, perhaps because of a defect in growth-inhibiting signals normally propagated at the junction [9]. This separation of phenotypes suggests both a structural role for Dlg in junction formation and a requirement for Dlg in signalling.

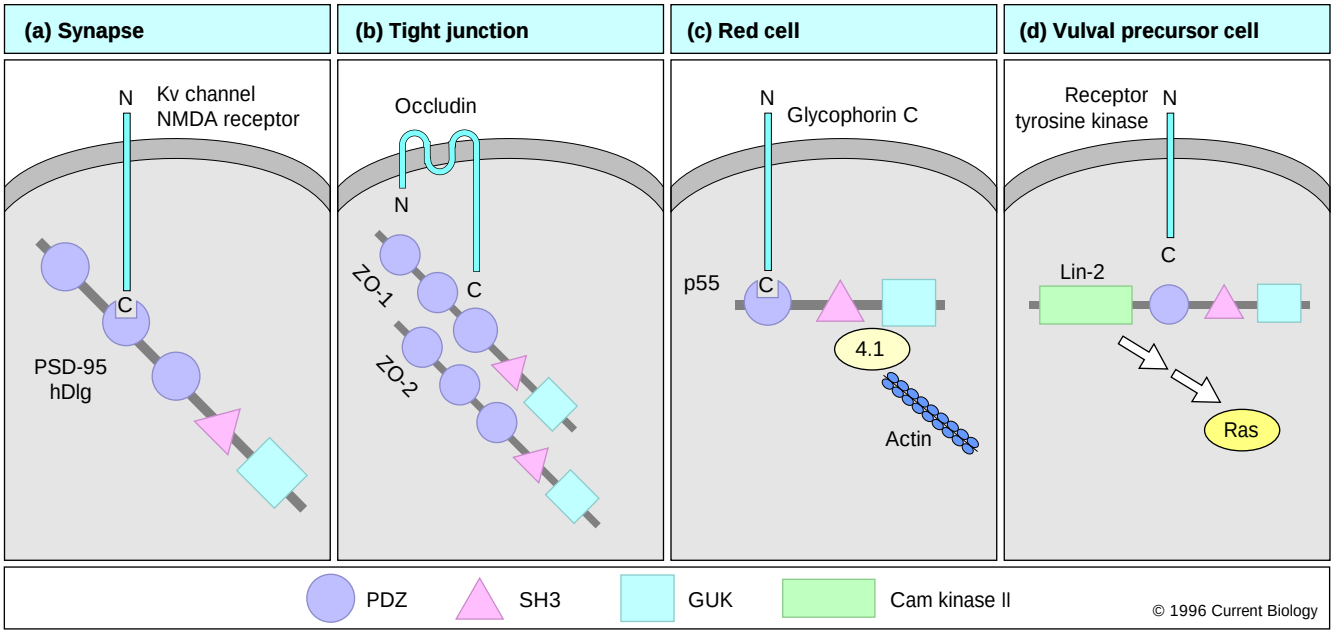
Further evidence of a role for MAGUK proteins in signal propagation at the membrane is provided by work on Lin-2, a component of the vulval cell induction pathway in *C. elegans* (Fig. 2) [2]. The vulva is formed from precursor cells whose fate is induced by activating a pathway made up of a receptor tyrosine kinase, Ras, and MAP (mitogen activated) kinase. On the basis of genetic evidence, *lin-2* functions in precursor cells to permit activation by the receptor *let-23* of the downstream cascade. Where does the Lin-2 protein fit on the signalling pathway? It's cellular location remains unknown. Unlike Dlg, however, it is probably not a component of intercellular junctions, because junctions appear morphologically normal in loss-of-function *lin-2* mutants. Alternative models being tested include a role for Lin-2 in localizing receptors to the apical cell surface or organizing the downstream signalling components [2]. The genetic studies imply that receptor tyrosine kinase signalling requires a higher level of cortical membrane organization than had previously been appreciated.

PDZ domains bind to transmembrane proteins

The 80–100 residue PDZ domains are also found in membrane-associated proteins other than MAGUK proteins [6]. Their emerging function is to bind the extreme carboxy-terminal cytoplasmic tail of transmembrane proteins in a sequence-specific fashion (Fig. 2.). Studies *in vitro* have demonstrated that both the voltage-gated K^+-channel (K_v channel) [10] and the *N*-methyl-D-aspartate (NMDA) receptor R2B subunit [11] bind in a binary fashion to PSD-95 through its second PDZ domain. The functional significance of linking synaptic ion channels to a MAGUK protein is unclear. One function may be in inducing synaptic structure. When PSD-95 and the K_v channel are separately transfected and expressed in cultured fibroblasts, their distributions are diffuse. When coexpressed, the two proteins cluster into spots on the cell surface reminiscent of synapses, suggesting they have the ability to crosslink or to recruit additional proteins that crosslink the complex [10].

PDZ domains appear to fall into subclasses defined by their binding specificities. Mutational analysis suggests that the minimum motif on the K_v channel for binding to the second PDZ domain of either PSD-95 or hDlg is the short peptide sequence Thr–Asp–Val [10]; these two

Figure 2



Some MAGUK proteins function to localize transmembrane proteins to specific sites (PSD-95, ZO-1); others provide links to the actin cytoskeleton (p55), or are required for coupling transmembrane signals to intracellular pathways (Lin-2).

PDZs are 88% identical in sequence. A similar motif, Ser–Asp–Val, is used on the NMDA R2B receptor subunit [11]. A more divergent PDZ domain within p55 appears to bind glycophorin C via a related motif, Tyr–Phe–Ile (S.M. Marfatia, J.H. Morais-Cabrol, A.C. Kim and A.H. Chishti, personal communication). Inspection of the databank reveals that many transmembrane proteins — including the cystic fibrosis transmembrane regulator, Na^+ channels and the β adrenergic receptor — contain a carboxy-terminal motif expected to bind the second PDZs of PSD-95 and hDlg. Outside the MAGUK family, a Ser–Leu–Val motif on the carboxyl terminus of Fas, the apoptosis receptor, appears to be required for binding a PDZ domain of the FAP-1 protein tyrosine phosphatase [12]. These observations should stimulate investigation into what could be a remarkably general and simple mechanism to couple transmembrane proteins to the cortical cytoskeleton.

In some cases, MAGUK proteins may localize transmembrane proteins using a PDZ-independent mechanism. For example, the ZO-1 and ZO-2 junctional proteins are bound to the cytoplasmic domain of occludin, a transmembrane protein at the tight junction (Fig. 2). Occludin proteins form linear polymers in the membrane and, through intercellular contacts, create the paracellular barrier. When occludin is transfected and expressed in cultured epithelial cells without its ZO-1-binding domain it fails to localize to tight junctions, suggesting that ZO-1 is part of a cortical scaffold required for the organization of occludin [13].

MAGUK proteins and the actin cytoskeleton

The mechanism of association between MAGUK proteins and the cortical actin cytoskeleton is not well defined and may vary between cell types. Studies *in vitro* revealed that both p55 and hDlg bind directly to protein 4.1 [7]. This actin-binding protein is one member of a superfamily of membrane-associated tyrosine kinase substrates that includes ezrin, radixin, moesin, talin and merlin, the product of the neurofibromatosis type 2 (*NF2*) tumor suppressor gene. In the red blood cell, p55 links the cytoplasmic tail of glycophorin C to the 4.1–fodrin–actin network (Fig. 2). An attractive speculation is that different MAGUK proteins may be coupled to the cortical cytoskeleton through different members of the protein 4.1 family. This link is apparently optional, however, because alternative RNA splicing generates an isoform of hDlg without the 4.1 binding site [7]. ZO-1 is reported to bind to fodrin *in vitro*, although the binding site and generality of this interaction for other MAGUK proteins is unknown [4].

A general mechanism for coupling at the membrane?

Can we build a tentative model for MAGUK protein function based on the available limited but provocative pieces of the puzzle? Encoded within their common PDZ–SH3–GUK domain core, all MAGUK proteins are likely to share some basic integrative function in coordinating signals at the cell cortex — perhaps the ability to link transmembrane proteins to cytosolic proteins while interacting with G-protein cascades. In addition, the possibility for enormous combinatorial diversity among these proteins is suggested by the variable expression of their other domains and the disparate properties of the GUK domains. The presence of multiple PDZ domains within one protein provides a scaffold for the recruitment of several proteins. Further defining the shared and diverse properties of MAGUK proteins will provide significant insights into the interdependence of signaling pathways and the cytoskeleton in transducing extracellular signals at the cortical membrane interface.

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