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Current Opinion in Structural Biology

Mechanisms of MAGUK-mediated cellular junctional complex organization

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Membrane-associated guanylate kinases (MAGUKs) are a family of scaffold proteins that are enriched in cellular junctions and essential for tissue development and homeostasis. Mutations of MAGUKs are linked to many human diseases including cancers, psychiatric disorders, and intellectual disabilities. MAGUKs share a common PDZ-SH3-GK tandem domain organization at the C-terminal end. In this review, we summarize the mechanistic basis governing target recognition and regulations of this binding by the PDZ-SH3-GK tandem of various MAGUKs. We also discuss recent discoveries showing unique folding features of MAGUK PDZ-SH3-GK tandems that facilitate ligand-induced oligomerization of MAGUKs and phase transition of MAGUK-assembled synaptic signaling complexes.

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Current Opinion in Structural Biology 2018, **48**:6–15

This review comes from a themed issue on **Folding and binding**

Edited by **Stefano Gianni** and **Amedeo Caflisch**

http://dx.doi.org/10.1016/j.sbi.2017.08.006

Introduction

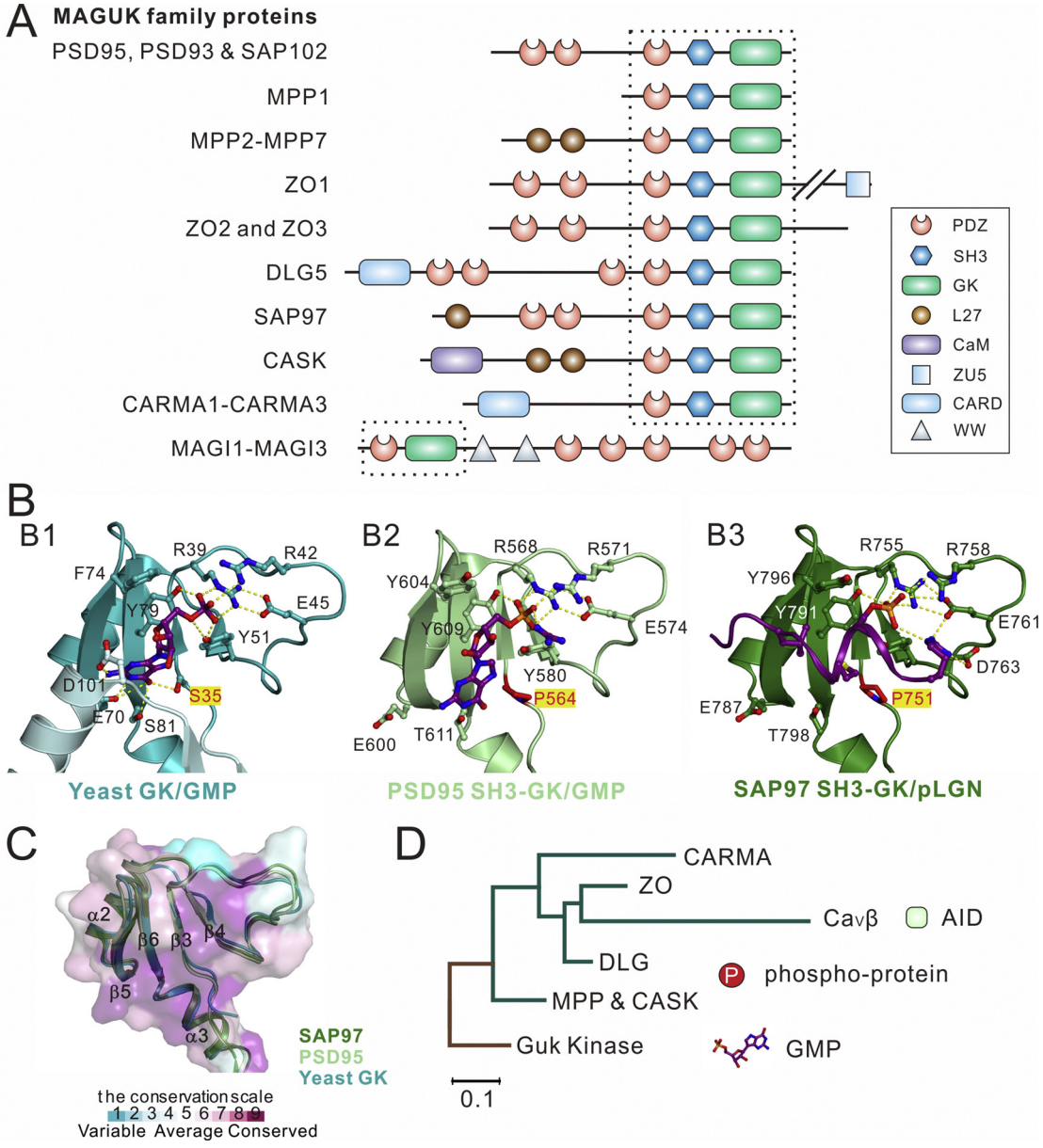
Membrane-associated guanylate kinases (MAGUKs), a group of scaffold proteins highly enriched at many different forms of cell–cell junctions, play critical roles in various cellular processes including cell polarity establishment and maintenance, cell adhesions and intercellular signal transductions [1–3]. Mammals contain several distinct MAGUK subfamilies including Discs larges (DLGs), calcium/calmodulin-dependent protein kinase (CASK), palmitoylated membrane proteins (MPPs), zonula occludens (ZOs), caspase recruitment domain-containing MAGUKs (CARMAs), voltage-gated calcium channel β subunit ($Ca_v\beta$) and membrane-associated guanylate kinase inverted (MAGI), each with distinct cellular functions (Figure 1a). For example, ZO-1, the first MAGUK member identified, mediates the formation of epithelial tight junctions and connects junctional proteins to skeletal components [4]. Malfunction of ZO-1 is associated with multiple cancers [5,6]. MPPs are required for cell polarity establishment in diverse tissues [7–9]. DLG, CASK, and MAGI families of MAGUKs are highly enriched in neuronal synapses and play critical roles in synaptic development, maintenance, and plasticity [3,10–14]. Mutations in these *MAGUKs* are associated with brain disorders including autism spectrum disorders (ASDs), X-linked mental retardation, intellectual disability, and schizophrenia [15–18]. CARMA is essential for T lymphocyte activation and proliferation via NF-κB signaling [19]. $Ca_v\beta$, an auxiliary subunit of the channel, modulates the calcium channel surface expression and activities [20].

As scaffold proteins, MAGUKs contain a series of protein–protein interaction modules including PDZ, SH3, GK, L27, ZU5, CARD and WW domains (Figure 1a). PDZ domain is one of the most prevalent protein–protein interaction modules in the multicellular eukaryotic proteomes and shared by all MAGUKs. It interacts with its targets through recognizing a short stretch of amino acids at the C-termini of its target proteins, although some PDZ domains can also bind to lipids. The structures and functions of individual PDZ domains or PDZ domain tandems have been extensively reviewed in the past [10,21,22•]. L27 domains locate in the N-terminal regions of several MAGUKs including CASK, SAP97 and MPP2-7 (Figure 1a). L27 domains can form highly specific heterotetramers, thereby organizing several sets of protein complexes vital for cell polarity [23–27]. The CaM kinase-like domain of CASK can function both as a protein–protein interaction module and a kinase capable of phosphorylating specific substrates such as Neurexins [28,29]. CASK is the only known MAGUK member that possesses enzymatic activity. Except for MAGI, all other MAGUKs share a common domain organization feature: each member contains a PDZ-SH3-GK tandem at its C-terminal end (Figure 1a). This brief review focuses on the structure and target bindings of the PDZ-SH3-GK tandem of MAGUKs.

Evolution of MAGUK GK target recognition

Each MAGUK contains a GK domain, which is evolved from the ancient enzyme guanylate kinase catalysing ATP-dependent GMP to GDP conversion. MAGUK GKs and yeast guanylate kinase share ~40% amino acid sequence identity and thus very similar overall 3D

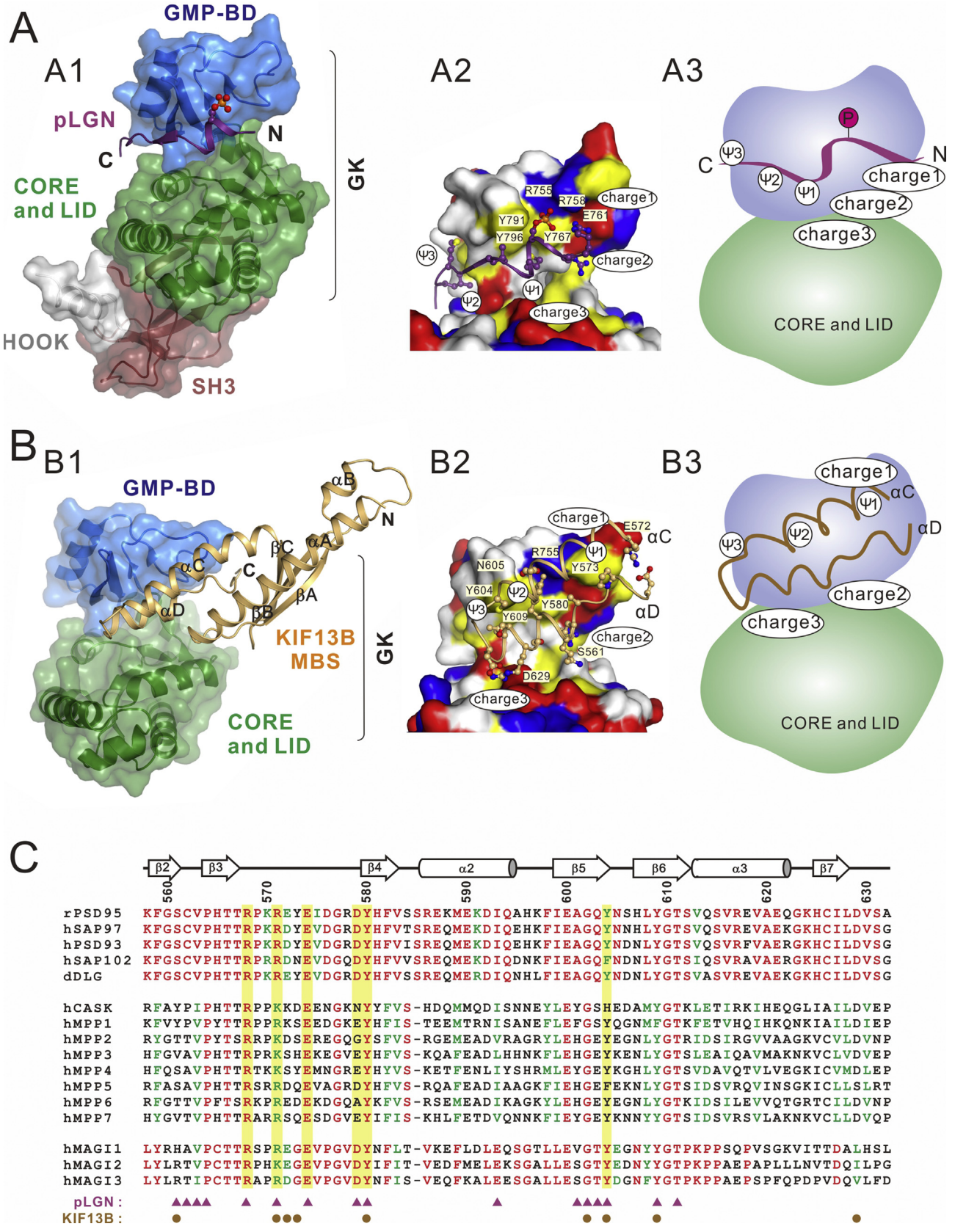
Figure 1



Domain organization of MAGUKs and evolution of GK. **(a)** Schematic diagrams of domain architecture of MAGUK family proteins. **(b)** Comparison of the structures of the yeast guanylate kinase/GMP complex (PDB code: 1EX7) (b1), the PSD95 SH3-GK/GMP complex (PDB code: 1JXM) (b2), the SAP97 SH3-GK/pLGN complex (PDB code: 3UAT) (b3). The binding sites responsible for the phosphate group binding are evolutionarily conserved between the GK enzyme and the MAGUK GKs. The guanine ring binding site of yeast GK has evolved to be able to bind to hydrophobic amino acid residues of MAGUK GK binding proteins. **(c)** Overlay of the backbone structures of SAP97 GK, PSD95 GK and yeast guanylate kinase domain, showing that the GK domain folding is retained throughout the evolution. The amino acid sequence conservation of all GK domains in guanylate kinase and MAGUK GKs are mapped on the surface of the SAP97 GK structure. **(d)** Reduced phylogeny of proteins containing GK domains derived the maximum likelihood sequence method [35,38,39]. The scale bar indicates number of substitutions per site. Cartoon diagram on the right shows the binding targets of each GK subfamily. Guanylate kinase binds to GMP; MPP, CASK and DLG subfamily MAGUK GKs can bind to phosphorylated proteins; and $Ca_v\beta$ can bind to the AID peptide of $Ca_v\alpha1$. Whether ZO1 and CARMA GKs can bind to proteins are unknown.

structures [30•,31•]. However, MAGUK GKs have lost the nucleotide kinase activity and evolved into specific protein–protein interaction modules [32,33••,34••,35]. Comparison of the structures of GMP-bound yeast guanylate kinase, GMP-bound PSD95 (aka DLG4) GK and phosphorylated LGN (pLGN)-bound SAP97 (aka DLG1) rationalizes why MAGUK GKs loose the kinase activity during the evolution (Figure 1b) [30•,31•,33••,36,37].

Figure 2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β3
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α2
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rPSD95 KFGSCVPHTTRPKREYEIDGRDYHFVSSREKMEKDIQAHKFIEAGQYNSHLYGTSVQSVREVAEQGKHCILDVSA
hSAP97 KFGSCVPHTTRPKRDYEVDGRDYHFVTSREQMEKDIQEHKFIEAGQYNNHLYGTSVQSVREVAEKGKHCILDVSG
hPSD93 KFGSCVPHTTRPKRDYEVDGRDYHFVISREQMEKDIQEHKFIEAGQYNDNLYGTSVQSVRFVAERGKHCILDVSG
hSAP102 KFGSCVPHTTRPRRDNEVDGQDYHFVVSREQMEKDIQDNKFIEAGQFNDNLYGTSIQSVRAVAERGKHCILDVSG
dDLG KFGSCVPHTTRPKREYEVDGRDYHFVSSREQMERDIQNHLFIEAGQYNDNLYGTSVASVREVAEKGKHCILDVSG
hCASK RFAYPIPHTTRPPKKDEENGKNYYFVS-HDQMMQDISNNEYLEYGSHEDAMYGTKLETIRKIHEQGLIAILDVEP
hMPP1 KFVYPVPYTTRPPRKSEEDGKEYHFIS-TEEMTRNISANEFLEFGSYQGNMFGTKFETVHQIHKQNKIAILDIEP
hMPP2 RYGTTVPYTSRRPKDSEREGQGYSFVS-RGEMEADVRAGRYLEHGEYEGNLYGTRIDSIRGVVAAGKVCVLDVNP
hMPP3 HFGVAVPHTTRPRKSHEKEGVEYHFVS-KQAFEADLHHNKFLEHGEYKENLYGTSLEAIQAVMAKNKVCLVDVEP
hMPP4 HFQSAVPHTTRTKKSYEMNGREYHYVS-KETFENLIYSHRMLEYGEYKGHLYGTSVDAVQTVLVEGKICVMDLEP
hMPP5 RFASAVPHTTRSRRDQEVAGRDYHFVS-RQAFEADIAAGKFIEHGEFEKNLYGTSIDSVRQVINSGKICLLSLRT
hMPP6 RFGTTVPFTSRKPREDEKDGQAYKFVS-RSEMEADIKAGKYLEHGEYEGNLYGTKIDSILEVVQTGRTCILDVNP
hMPP7 HYGVTVPHTTRARRSQESDGVEYIFIS-KHLFETDVQNNKFIEYGEYKNNYYGTSIDSVRSVLAKNKVCLLDVQP
hMAGI1 LYRHAVPCTTRSPREGEVPGVDYNFLT-VKEFLDLEQSGTLLEVGTYEGNYYGTPKPPSQPVSGKVITTDALHSL
hMAGI2 LYLRTVPCTTRPHKEGEVPGVDYIFIT-VEDFMELEKSGALLESGTYEDNYYGTPKPPAEPAPLLLNVTDQILPG
hMAGI3 LYLRTIPCTTRAPRDGEVPGVDYNFIS-VEQFKALEESGALLESGTYDGNFYGTPKPPAEPSPFQPDPVDQVLFD
pLGN :
KIF13B :

In yeast guanylate kinase, GMP inserts into a groove formed by the GMP binding domain and the CORE domain of the enzyme. The GMP/kinase interface can be divided into two parts: the GMP phosphate binding pocket involving Arg39, Arg42, Glu45, Tyr51 and Tyr79; and the bulky guanine ring binding pocket including three critical residues Ser35, Glu70 and Asp101 (Figure 1b1). During evolution, the residues in the phosphate group-binding pocket of MAGUKs are retained (Figure 1b,c). However, residues in the guanine binding site have changed and the domain has very weak or even no GMP binding capacity. In particular, Ser35 located in the link region between the GMP binding site and the CORE domain has evolved into a Pro (Pro564 in PSD95 and Pro751 in SAP97; highlighted in Figure 1b). This Ser to Pro mutation disrupts the hydrogen bond between GMP and GK domain, and further prevents the GMP binding-induced closure between the GMP binding domain and the CORE domain, a step essential for the enzyme activity of guanylate kinases [34••]. Phylogenetic analysis of MAGUK GKs indicates that MPPs and CASK are more ancient subfamilies and are evolutionarily close to the DLG subfamily [35,38,39]. These subfamilies of MAGUK GK domains all contain essential residues for binding phosphorylated target proteins (Figure 1d). In contrast, GK domains of $Ca_v\beta$, CARMAs and ZOs are more distant to GKs from DLG, MPP and CASK, and they lack several key residues for phosphorylated protein binding [33••,35,40]. Interestingly, $Ca_v\beta$ has been found to bind to a peptide segment from $Ca_v\alpha1$ using its ATP binding site instead of the GMP site [41,42].

A series of structures of MAGUK GK/target complexes solved in the past few years reveal the target recognition mechanisms of MAGUK GKs. The structure of SAP97 SH3-GK in complex with pLGN provides the first atomic picture showing that MAGUK GK can function as a highly specific, phosphorylation-dependent protein recognition module [33••,43]. In the complex structure, the pLGN peptide folds into a one-turn α-helix followed by a β-strand, occupying the elongated groove formed by the GMP binding domain and the helical CORE domain of GK (Figure 2a). The phosphate group of pLGN extensively interacts with residues from the GMP binding site. The phosphorylated Ser is absolutely required for pLGN to bind to SAP97 SH3-GK, as substitutions of pSer with Ala or Glu essentially eliminated the binding [33••]. The C-terminal half of pLGN contacts a hydrophobic groove on the GK domain formed by three hydrophobic sites ψ1, ψ2 and ψ3 (Figure 2a). Additional polar interactions further enhance the binding between pLGN and SAP97 SH3-GK (Figure 2a). Amino acid residues mediating phosphate group binding are highly conserved among GKs of DLG, CASK, MPP and MAGI MAGUKs (Figure 2c), suggesting that all these GKs can bind to their target proteins in a phosphorylation-dependent manner. Biochemical and structural characterizations of the interactions of PSD95 GK with its targets including LGL and SAPAP (aka GKAP) showed that PSD95 GK indeed binds to these targets in a phosphorylation-dependent manner [33••,43].

MAGUK GKs can also bind to unphosphorylated targets. Microtubule-associated protein 1A (MAP1A) is the first such MAGUK GK binding partner [44], though the binding is rather weak and the structural basis governing the interaction has recently been elucidated [45]. The binding of KIF13B (aka GAKIN) to PSD95 GK does not require KIF13B to be phosphorylated. A small domain from the C-terminal tail of KIF13B dubbed as MBS (MAGUK Binding Stalk) binds to PSD95 GK with very strong affinity (K_d ~70 nM) [46••]. The structure of PSD95 GK in complex with KIF13B MBS reveals the molecular basis of such phosphorylation-independent interaction (Figure 2b). Instead of a liner phosphorylated peptide, the GK-bound KIF13B MBS forms a well-folded domain composed of four α-helices and three β-strands and binds to the same groove as the pLGN peptide does on GK (Figure 2b1). A number of polar residues from the end of αC and throughout αD of KIF13B MBS form extensive hydrogen-bonding and charge–charge interactions with the polar and charged residues from PSD95 GK (Figure 2b2) [46••]. Several hydrophobic residues from αD of MBS insert into the PSD95 GK hydrophobic pockets and enhance the binding affinity (Figure 2b2, b3). The key residues responsible for KIF13B MBS binding are highly conserved in GKs of DLGs and MPPs, and these MAGUKs are also capable of binding to KIF13B [46••]. In contrast, only a few residue changes in the CASK and MAGI GKs render their GK incapable of binding to KIF13B MBS (Figure 2c) [46••].

Phosphorylation-dependent and phosphorylation-independent target binding modes of MAGUK GK domains. **(a)** Structure analysis of phosphorylation-dependent target bindings of MAGUK GKs. (a1) Combined ribbon and surface representation showing the structure of SAP97 SH3-GK in complex with pLGN (PDB code: 3UAT). (a2) and (a3) Surface representation and cartoon diagram showing the detailed interaction interface between SAP97 GK and pLGN. The hydrophobic residues are colored in yellow, the positively charged residues are in blue, and the negatively charged residues are in red. The rest are colored in white. The pLGN peptide is colored in purple. **(b)** Structural analysis of phosphorylation-independent target binding revealed by the PSD95 GK/KIF13B MBS complex. (b1) Combined ribbon and surface representation showing the structure of PSD95 GK in complex with KIF13B MBS (PDB code: 5B64). (b2) and (b3) Surface representation and cartoon diagram show the interaction interface between PSD95 GK and KIF13B MBS. The GK is colored with the same scheme as in panel A. **(c)** Structure-based sequence alignment of the GMP binding subdomains MAGUK GKs. Key residues involved in binding to pLGN and KIF13B MBS are indicated with purple triangles and brown dots respectively. Residues coordinating the phosphate group of pLGN are highlighted in yellow. Highly conserved and conserved residues are colored in red and green, respectively.

The MAGUK PDZ-SH3-GK supramodule

The PDZ-SH3-GK (PSG) tandem architecture is evolutionarily conserved from *choanoflagelleta*, the unicellular ancestor of metazoans, to human (Figure 1a) [39]. Accumulating evidences suggest that MAGUK PSG tandems form structural and functional supramodules with distinct target binding properties than individual or simple sum of each isolated domains.

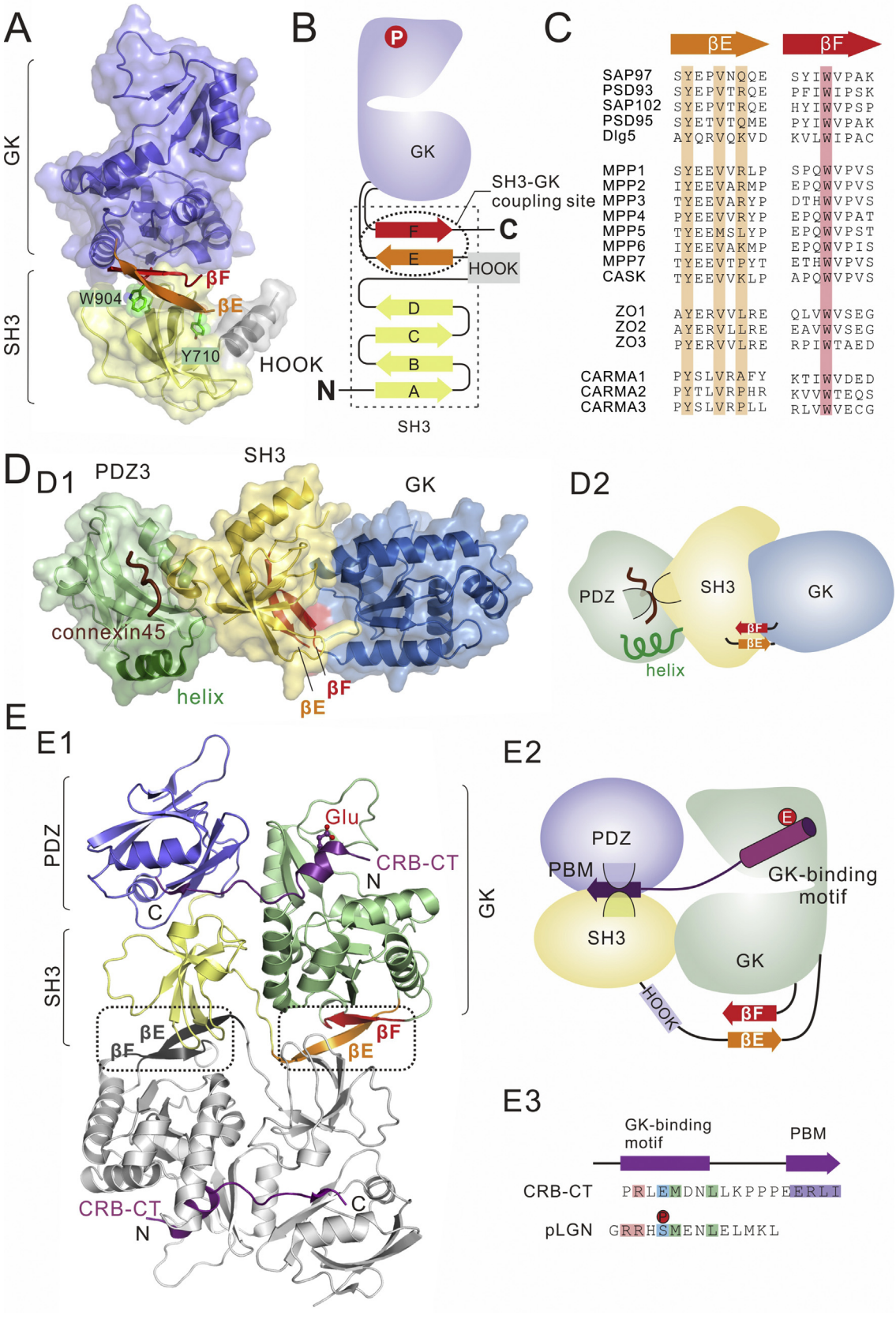
The SH3-GK tandem of DLG GKs is known to form an integral structural supramodule many years ago [30•,31•,33••]. An ~10-residue fragment immediate following the C-terminal end of GK forms a β-strand (βF) and folds back to pair with βE of SH3, completing the folding of the SH3 domain (Figure 3a,b). Tyr710 from βE inserts into the folding core and Trp904 from βF packs extensively with the hydrophobic surface of the SH3 domain (Figure 3a, residues are named following the human SAP97 sequence). A long flexible sequence known as the HOOK region proceeding βE blocks the canonical target binding site of SH3, implying that SH3 domains of MAGUKs are not likely to bind to canonical proline-rich sequences (Figure 3a,b) [31•]. The key residues mediating βE–βF pairing (i.e. the split SH3 domain folding) are highly conserved in MAGUKs (highlighted in Figure 3c), strongly suggesting that formation of the SH3-GK supramodule is a common structural feature of MAGUKs. This analysis is supported by the crystal structures of the SH3-GK tandems of PSD95, SAP97, ZO1 and $Ca_v\beta$ [30•,31•,40,41]. Formation of the SH3-GK supramodule is crucial for cellular functions of MAGUKs. A *Drosophila* DLG mutant lacking the last 14 residues (dlg^{sw}), which would disrupt the SH3-GK tandem formation but retain the GK fold intact, loses its tumor suppressive activity [47]. A missense mutation (W919R) of CASK GK (corresponding to Trp904 of SAP97) has been linked to X-linked mental retardation in humans [48,49].

A number of examples emerged in the last few years have demonstrated that the PDZ domain can further couple with the SH3-GK tandem in several MAGUKs, forming a PSG supramodule [40,50,51•,52•]. The crystal structure of ZO1 PSG in complex with its PDZ-binding peptide (i.e. connexin45 tail) shows that the PDZ domain directly contacts with the SH3 domain forming an integrated PSG structural unit with a rod-like shape (Figure 3d and [40]). The coupling interface is mediated by an α-helix between the PDZ and SH3 domains together with a number of residues from αB and the βB/βC loop of PDZ. The PDZ/SH3 coupling extends the canonical PDZ ligand binding groove, providing a structural explanation to the enhanced ligand binding by the ZO1 PSG supramodule (Figure 3d2). The PSG supramodule is also observed in the crystal structure of PALS1 (aka MPP5) in complex with the Crumbs C-terminal tail (CRB-CT) [53••]. The PALS1 and Crumbs interaction is critical in cell growth control and cell polarity maintenance in diverse tissues [54–57]. The PALS1 PSG tandem assembles into an integral structural unit binding to CRB-CT with an affinity ~100-fold higher than the isolated PDZ domain [53••]. Structural analysis revealed the detailed mechanism of this PSG supramodule-mediated CRB-CT binding [53••]. Although the isolated PSG is a monomer in solution, PSG forms a symmetrical domain-swapped homodimer in the PALS1-PSG/CRB-CT complex crystals (Figure 3e1). In the domain-swapped PSG dimer, the two split SH3 domains swap their βE–βF unit, and the flexible HOOK sequence proceeding βE presumably allows the domain swapping to occur. All three domains of PALS1 PSG are involved in binding to CRB-CT (Figure 3e1). Direct coupling between PDZ and SH3 forms a clasp to lock Glu2143 at the −3 position of CRB-CT (Figure 3e2). The residues forming the PDZ-SH3 clasp are unique among MPPs, partly explaining the specific binding between PALS1 and Crumbs. An upstream 8-residue fragment in CRB-CT mimics the phospho-peptide binding mode and binds to PALS1 GK with a Glu occupying the pSer binding site of the GK domain (Figure 3e). CASK PSG may adopt a similar mechanism in binding to the Neurexin tails, as its PSG also binds to the Neurexin tail with a much higher affinity than its isolated PDZ domain [53••].

High-order protein complex assembly and phase transition of MAGUKs

In excitatory synapse, endogenous NMDA receptors can be assembled into a supramolecular complex up to ~1.5 MDa, in which both PSD95 and PSD93 are within the complex and stabilize each other to facilitate the mega-complex formation [58••]. A recent study from our lab demonstrated that high-order protein complex assemblies mediated by MAGUK PSG could be important in the supramolecular complex formation [59••]. Earlier analysis showed that, in the apo form of PSD95 PSG, the PDZ domain is highly flexible and only transiently interacts with SH3-GK (Figure 4a) [51•,52•]. Our recent study demonstrated that the binding of the SynGAP tail to PDZ3 of PSD95 requires a long α-helix extension C-terminal to the PDZ domain (Figure 4b) [59••]. Importantly, binding of SynGAP to PSG can induce the PSG supramodule dimerization or even oligomerization, possibly via allosteric coupling between PDZ and SH3-GK by the extended α-helix (Figure 4b). The PSD95 PSG supramodule binds to the SynGAP tail with a significantly higher affinity than the extended PDZ3 alone does, further demonstrating the unique target binding property afforded by the PSG supramodule. Interestingly, SynGAP forms a parallel coiled-coil trimer. Although the formation of the SynGAP trimer does not further enhance its binding affinity to PSD95 PSG, it does promote formation of the PSD95/SynGAP complex oligomers. Most notably, the PSD95/SynGAP complex assembly can undergo a liquid-liquid phase separation (LLPS). The phase transition of the

Figure 3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SAP97 SYEPVNQQE SYIWVPAK
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Dlg5 AYQRVQKVD KVLWIPAC
MPP1 SYEEVVRLP SPQWVPVS
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ZO3 PYERVVLRE RPIWTAED
CARMA1 PYSLVRAFY KTIWVDED
CARMA2 PYTLVRPHR KVVWTEQS
CARMA3 PYSLVRPLL RLVWVECG
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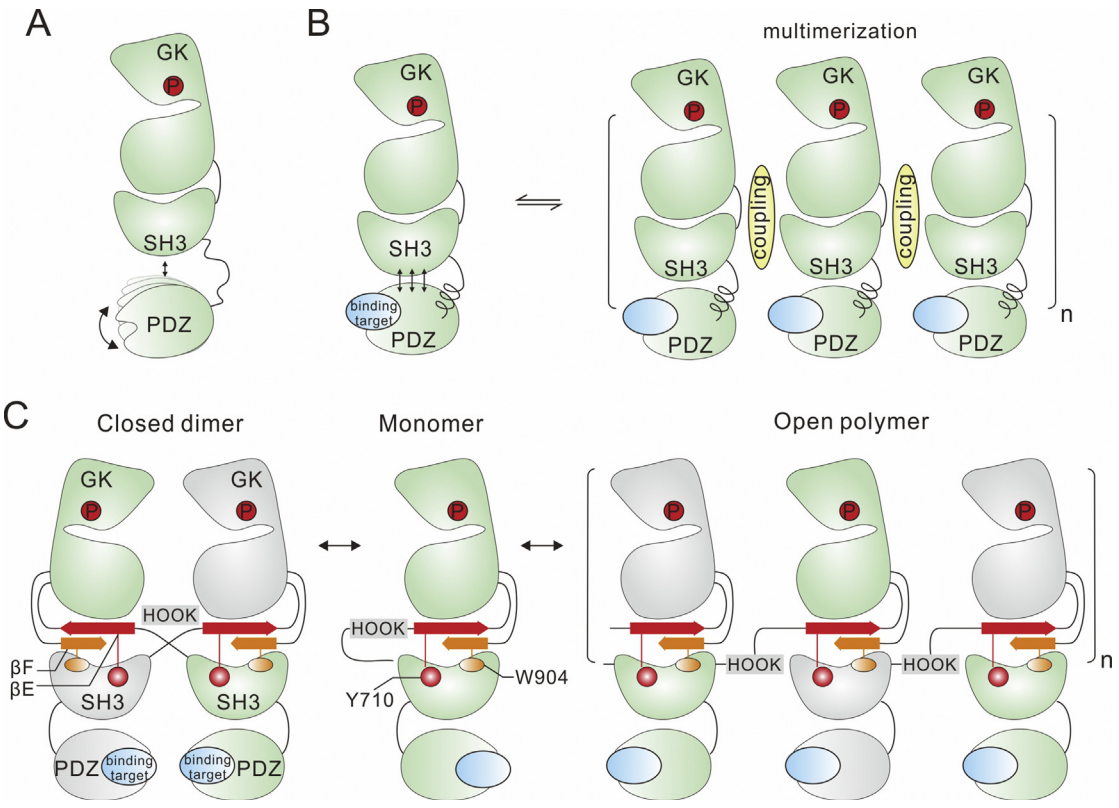
Figure 4



Ligand binding-induced formation of oligomeric MAGUK assemblies. **(a)** Schematic model showing the weak coupling between the PDZ domain and the SH3-GK domain in the apo form of PSD95 PSG. PDZ domain is dynamic and linked to the SH3-GK tandem with a flexible linker. **(b)** Schematic model showing that ligand binding to the PDZ domain can strengthen the domain coupling between PDZ and SH3-GK in PSD95 PSG, which can promote PSG oligomerization. **(c)** Schematic model depicting domain swapping-mediated MAGUK PSG multimerization. Intramolecular β-sheet formation between βE and βF allows PSG to form a monomer. Intermolecular pairing between βE and βF can result into a closed dimer or open polymer assemblies of MAGUK PSGs.

PSD95/SynGAP complex provides a possible mechanism for submicron-sized, membraneless structure organizations such as the postsynaptic densities and possibly other MAGUK-organized supramolecular structures such as tight and adherens junctions in epithelial cells [59••]. The detailed molecular mechanism of PSD95 PSG oligomerization is largely unknown. But the ligand binding-induced domain-swapped dimer of PALS1 PSG revealed by the PALS1/Crumbs complex structure points to a possible mechanism underlying the high-order assembly of MAGUKs (Figure 4c). Since all MAGUKs contain a split SH3 domain mediated by the βE–βF pairing and a long and flexible HOOK region, it is tempting to speculate that other members of MAGUKs may also form homo-oligomers or even hetero-oligomers among paralogs within the same subfamilies via intermolecular SH3-GK coupling (Figure 4c)

(Figure 3 Legend) Formation of the PDZ-SH3-GK supramodules in MAGUKs. **(a)** The molecular basis governing the SH3-GK coupling in MAGUKs. The surface diagram of the SAP97 SH3-GK tandem (PDB code: 3UAT) showing that βE from the SH3 domain and βF located C-terminal to the GK domain 'stitch' the SH3-GK domain together into an integral structural unit. **(b)** Schematic diagram showing the βE–βF coupling-mediated split SH3 folding in the MAGUK SH3-GK tandems. Note that the phosphorylated target binding site in GK is far away from βE and βF. **(c)** Sequence alignment of βE and βF showing that the βE–βF-mediated coupling of SH3 and GK domains is a common property of MAGUKs. **(d)** Formation of the PSG supramodule in ZO1. (d1) and (d2) Surface representation and cartoon diagram, respectively, showing the detailed interaction between ZO1 PDZ3-SH3-GK and the connexin45 tail peptide (PDB code: 3SHW). ZO1 PDZ domain contains a C-terminal helix extension coupling with the SH3-GK tandem. The formation of the ZO1 PSG supramodule enhances ZO1's binding to connexin45. **(e)** Formation of the PALS1 PSG supramodule and its implication in CRB-CT binding. (e1) Ribbon diagram showing the domain-swapped dimer assembly of PALS1 PSG in complex with CRB-CT (PDB code: 4WSI). The Glu residue occupying the phosphorylated Ser binding pocket of the GK domain is shown in the stick-and-ball model. (e2) Schematic diagram of the PALS1-PSG/CRB-CT complex structure. (e3) The structure-based sequence alignment of CRB-CT and phosphorylated LGN (pLGN) showing that the amino-terminal helical region of CRB-CT resembles the helical region of pLGN that binds to the SAP97 GK domain as dipicted in Figure 2a. The highlighted residues in the alignment are directly involved in the binding to the respective GK domains.

Conclusions and perspectives

Biochemical and structural studies in the last few years have significantly expanded our understanding of the molecular bases governing the folding, target binding and regulation of MAGUKs. The complex structures of SAP97/pLGN and PSD95/KIF13B complexes illustrate how the GK domain of MAGUKs can function as a diverse protein binding module. The PALS1/Crumbs complex structure and biochemical studies of the PSD95/SynGAP interaction indicate that the PSG supramodule of MAGUKs can undergo target binding-induced dimerization/oligomerization. The ligand binding-induced multimerization of the MAGUK PSG supramodules is 'encoded' in their unique folding and domain assembling properties. However, many questions concerning MAGUKs remain to be addressed. For example, different DLG MAGUKs have been shown to have distinct cognitive functions [60]. The molecular mechanisms underlying the diverse functions of DLG MAGUKs are not clear. It remains to be tested whether different paralogs of MAGUKs can form hetero-oligomers and if so, what the functional consequences for the hetero-MAGUK assembly would be. Also, the observation of MAGUK-mediated phase transition immediately raises questions such as whether such phase transition indeed occurs under physiological settings, how such phase transition might be regulated, and whether other MAGUK binding partners may modulate the phase transition. Since the PDZ, SH3 and GK domains in MAGUKs are structurally coupled to each other, it will be important to ask whether the target binding sites on the PSG supramodule are allosterically coupled with each other (e.g. a ligand binding to the GK domain may affect PDZ ligand binding and/or the PSG higher order assembly and vice versa) (Figure 4b).

Acknowledgements

This work in MZ's lab has been supported by grants from RGC of Hong Kong (664113, 16103614, and AoE-M09-12), a 973 program grant from the Minister of Science and Technology of China (2014CB910204), and a grant from the Asia Fund for Cancer Research. MZ is a Kerry Holdings Professor in Science and a Senior Fellow of IAS at HKUST.

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