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Guanylate kinase domains of the MAGUK family scaffold proteins as specific phospho-protein-binding modules

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Membrane-associated guanylate kinases (MAGUKs) are a large family of scaffold proteins that play essential roles in tissue developments, cell–cell communications, cell polarity control, and cellular signal transductions. Despite extensive studies over the past two decades, the functions of the signature guanylate kinase domain (GK) of MAGUKs are poorly understood. Here we show that the GK domain of DLG1/SAP97 binds to asymmetric cell division regulatory protein LGN in a phosphorylation-dependent manner. The structure of the DLG1 SH3-GK tandem in complex with a phospho-LGN peptide reveals that the GMP-binding site of GK has evolved into a specific pSer/pThr-binding pocket. Residues both N- and C-terminal to the pSer are also critical for the specific binding of the phospho-LGN peptide to GK. We further demonstrate that the previously reported GK domain-mediated interactions of DLGs with other targets, such as GKAP/DLGAP1/SAPAP1 and SPAR, are also phosphorylation dependent. Finally, we provide evidence that other MAGUK GKs also function as phospho-peptide-binding modules. The discovery of the phosphorylation-dependent MAGUK GK/target interactions indicates that MAGUK scaffold-mediated signalling complex organizations are dynamically regulated.

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Introduction

MAGUKs originally referred to a group of cell junction proteins composed of synaptic scaffold protein PSD-95 from mammals, DLG tumour suppressor from *Drosophila*, and tight junction protein ZO-1 from mammalian epithelia (Cho *et al*, 1992; Willott *et al*, 1993; Woods and Bryant, 1993). The family has since grown to encompass a large number of scaffold proteins that play critical roles in diverse cellular processes including inter-cellular connections, cell polarity development and maintenance, synaptic plasticity, and cell survival in multicellular eukaryotes (Funke *et al*, 2005; Velthuis *et al*, 2007; Mendoza *et al*, 2010). Despite of large differences in their lengths and amino acid sequences, every member of the MAGUK family proteins contains a catalytically inactive GK-like domain. All MAGUKs, with the exception of MAGI, share a common structural core consisting of an SH3 domain followed by a GK domain (termed as the SH3-GK tandem). Extensive structural and functional studies of a number of MAGUKs have revealed that the SH3 domain and the GK domain in a SH3-GK tandem interact with each other forming an integral structural unit (also referred to as the SH3-GK supramodule), which often has functions distinct from those of the isolated or simple summation of the SH3 and GK domains (Shin *et al*, 2000; McGee *et al*, 2001, 2004; Tavares *et al*, 2001; Chen *et al*, 2004; Takahashi *et al*, 2004; Lye *et al*, 2010).

Extensive studies in the past have provided numerous evidences regarding the critical cellular functions of the MAGUK GK domains. For example, *Drosophila* DLG is a tumour suppressor; mutations leading to truncations of a part or the entire GK domain of DLG lead to the tumour growth of fly imaginal discs and the eventual death of animals (Woods *et al*, 1996). A DLG truncation mutant lacking the C-terminal 43 amino acid residues of the GK domain (DLG^{1P20}) is defective in the neuro-precusor asymmetric cell divisions (BellaIche *et al*, 2001), most likely due to the mutation-induced disruption of the DLG/Pins interaction (Sans *et al*, 2005; Johnston *et al*, 2009). Mice homozygous for a *DLG1/SAP97* mutation, which lacks the SH3-GK tandem due to the truncation caused by the mutation, die perinatally with severe and multi-faceted developmental defects such as cleft palate (Caruana and Bernstein, 2001). The GK domain of the voltage-gated calcium channel β-subunit binds to a small peptide fragment from the α1-subunit, and the GK-mediated interaction with the α1-subunit plays an essential role in regulating the surface expression and gating of the channel (Chen *et al*, 2004; McGee *et al*, 2004; Takahashi *et al*, 2004; Buraei and Yang, 2010).

Despite of their well-established functional roles, little is known about the molecular basis governing the functions of MAGUK GK domains. It is puzzling that little progress has been made in understanding how the GK domains of DLG family MAGUKs function mechanistically, although the structure of the DLG4/PSD-95 SH3-GK tandem has been solved for ~10 years (McGee *et al*, 2001; Tavares *et al*, 2001) and many GK domain-binding proteins have been identified since the discovery of the DLG MAGUKs (see Kim *et al*, 1997; Brenman *et al*, 1998; Deguchi *et al*, 1998; Hanada *et al*, 2000; Pak *et al*,

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