How to Grb2 a Gab

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In this issue of *Structure*, Harkiolaki et al. use crystallography, peptide arrays and isothermal calorimetry to provide a detailed insight into the interaction between the C-terminal SH3 domain of adaptor protein Grb2 bound to the docking protein Gab2.

Since its discovery in 1992, growth factor receptor bound protein 2 (Grb2) has become the prime textbook example for an adaptor-type signaling protein (Lewitzky et al., 2001). Grb2 is composed of a central SH2 domain flanked on each side by a SH3 domain (Lewitzky et al., 2001). Upon ligand binding, many cell surface receptors, such as those for particular growth factors, become tyrosine phosphorylated, which provides binding sites for the SH2 domain of Grb2 (Pawson, 2007). The latter then uses its two SH3 domains to recruit additional proteins to the activated receptor. For example, Grb2 binds to proline-rich stretches in the Ras-guanine nucleotide exchange factor SOS via its N-terminal SH3 domain, while it uses its C-terminal SH3 domain to bind to two proline-rich sequences within Grb2-associated binder (Gab) proteins. These docking proteins contain a multitude of protein-protein interaction motifs and play an important role in the transduction, amplification and compartmentalization of extracellular signals received by a variety of receptor types. (Figure 1) (Gu and Neel, 2003). There are at least three Gab paralogues in vertebrates (Gab1-3) and Gab-like proteins are also found in the invertebrates *Drosophila* and *Caenorhabditis,* where they are represented by *daughter-of-sevenless* (DOS) and *suppressor-of-clear* 1 (SOC1), respectively. Importantly, recent analyses have revealed important roles for Gab2 in allergic responses and various cancer types (Harkiolaki et al., 2009; Sattler et al., 2002; Brummer et al., 2008).

Gab proteins need to be phosphorylated on particular tyrosine residues in order to recruit effector proteins with SH2 domains such as the protein tyrosine phosphatase Shp2 and the regulatory subunit of PI3K, p85 (Gu and Neel, 2003). These phosphorylation events usually take place at the plasma membrane. But how are Gab proteins recruited to the plasma membrane in the first place? One mechanism is based on recruitment to phosphatidyl-inositol-3,4,5-phosphate enriched membrane patches via the N-terminal pleckstrin homology (PH) domain, a hallmark of all Gab proteins. The second mechanism involves their interaction with Grb2, which either by itself or in conjunction with Shc adaptor proteins, establishes a "bridge" between Gab and the activated receptor (Figure 1) (see Brummer, et al. [2008] for review). Formation of this "bridge" appears necessary for the sustained tyrosine phosphorylation of Gab proteins and hence efficient coupling to their effectors (Brummer et al., 2008; Gu and Neel, 2003; Sattler et al., 2002; Schaeper et al., 2007).

Despite this well-documented role of the Grb/Gab interaction in physiology and disease, its molecular details are relatively ill-defined. Harkiolaki et al. (2009) now provide novel insights into this crucial protein-protein interaction. Using a library of permutated Gab2 derived peptides, the authors identify that the Grb2 binding sites, termed Gab2a and Gab2b, contain the core consensus motif RXXK. As the former site contains a canonical PXXP motif for SH3 binding while the latter does not, these motifs have been referred to in the Gab signaling field as typical and atypical Grb2 binding sites, respectively (Lock et al., 2000). Importantly, although each binding site contains a core RXXK motif, the individual modes of peptide binding differ. In the Grb2 C-SH3/Gab2a structure, a polyproline type II helix is used to dock onto the Grb2 SH3 domain, and the R and K of the core motif adopt a staggered conformation. Several hydrophobic interactions occur and the RXXK arginine forms hydrogen bonds with a key glutamate residue in Grb2, denoted $Glu16_{Grb2}$. In contrast, when Gab2b binds, it adopts an extended conformation, the R and K are parallel with respect to each other, and the binding mode is more mixed in terms of the relative contributions of hydrophilic and hydrophobic interactions. Common to both Gab2a and Gab2b binding modes is hydrogen-bonding to $Glu16_{Grb2}$. The demonstration of alternative binding modes for an individual SH3 domain when docking on to different binding sites on the same protein provides an elegant demonstration of the flexibility of particular SH3 domains with regard to target recognition. This concept is extended further by Harkiolaki et al. (2009) in their demonstration that the Gab2a peptide can tolerate substitution of the RXXK arginine by a leucine. In addition, they demonstrate that a complex between the C-terminal SH3 domain of the Grb2-related protein Mona/Gads and a binding site on a putative phosphatase HD-PTP exhibits a similar interaction mode to Grb2 C-SH3/Gab2a, but here the core binding motif is RXXXXK. These findings highlight the difficulty of

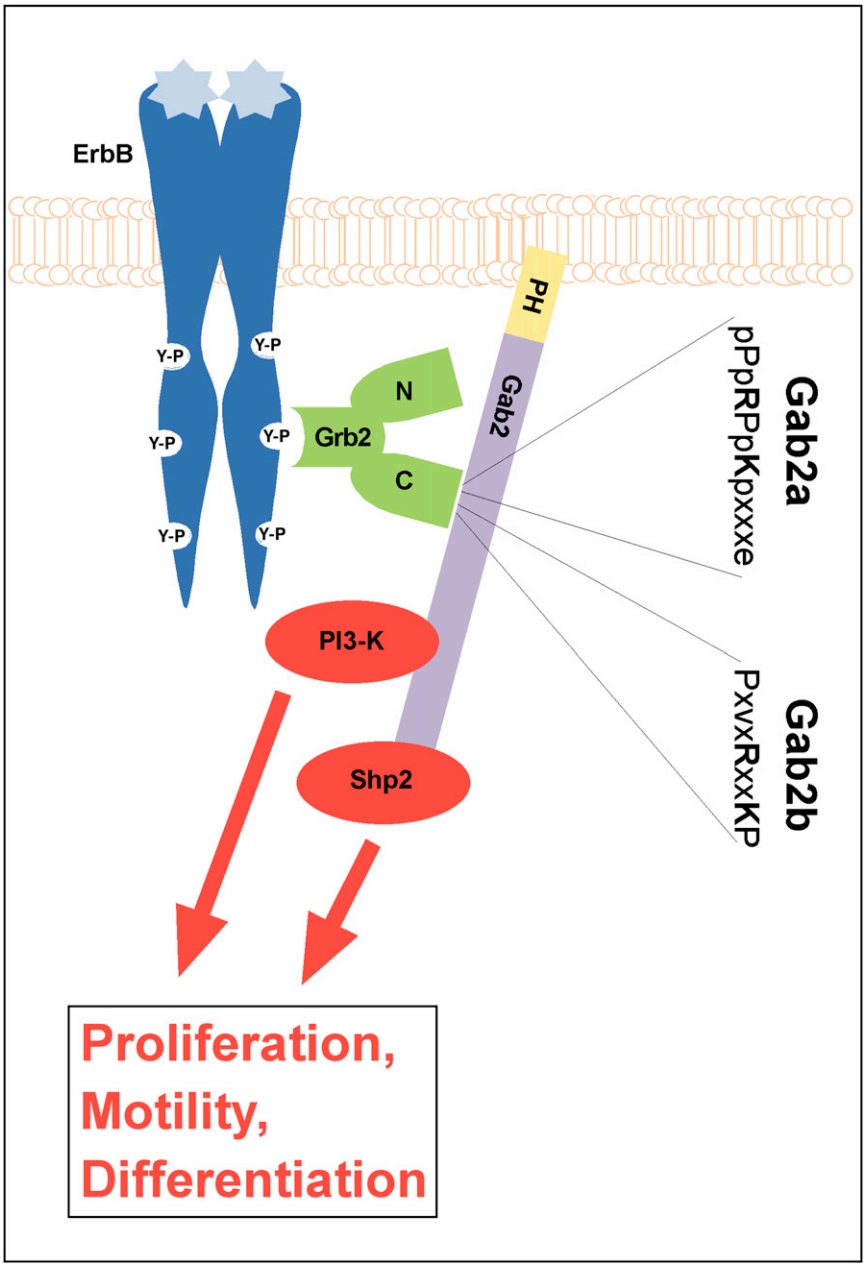

Figure 1. The Adapter Protein Grb2 Connects Tyrosine Phosphorylated Receptors to Gab Docking Proteins

Activated growth factor receptors, such as those of the ErbB/EGF receptor family, utilize the adaptor protein Grb2 to recruit docking proteins of the Gab family. The Grb2 binding motifs in Gab2 were characterized by Harkiolaki et al. (2009) and are designated as Gab2a and Gab2b (capital letters: restricted positions; lowercase letters: moderately restricted positions; x: marginally or non-restricted positions).

predicting binding sites for particular SH3 domains by standard sequence-based search techniques, and suggest that many more SH3 domain binding sites remain to be discovered in particular proteomes.

Interestingly, this study reports that the occurrence of at least two Grb2 binding motifs is also a feature of putative Gab proteins found in the sea anemone *Nematostella*, a basal metazoan, and in the sea squirt *Ciona*, a basal chordate. Consequently, these data suggest that Gab proteins were already a component of the early metazoan signaling toolbox. In addition, they indicate that caution should be applied when drawing conclusions based on comparison between the amino acid sequences of vertebrates and the popular model organisms *Drosophila* and *Caenorhabditis*, which are evolutionarily distant but not necessarily "primitive" in comparison to vertebrates. For example, the SOC1 protein contains only the atypical Grb2 binding site and both functional Grb2 binding sites in DOS resemble the atypical binding motif (Feller et al., 2002). Thus, one could conclude that the typical Grb2 binding motif first appeared with the emergence of vertebrates. However, Harkiolaki et al. (2009) demonstrate that the typical Grb2 binding motif is not only present in *Ciona* and therefore predates the rise of vertebrates, but already occurs in the Gab protein of *Nematostella*. Therefore, it is tempting to speculate that ancestral Gab proteins not only contained a PH domain, but also at least two distinct Grb2 binding motifs. This insight also invites the question as to whether there is a qualitative difference between the typical and atypical Grb2 binding motifs in terms of signaling output. In a previous publication, Feller et al. (2002) could show that both sites in DOS contribute to the binding of the Grb2 ortholog Drk. However, as both SH3 domain binding motifs in DOS resemble the atypical Grb2 binding site, this study does not provide an insight into the function of the typical Grb2 binding site in vertebrate Gab proteins. Although Harkiolaki et al. (2009) demonstrate that the atypical Grb2 binding site displays a 10-fold higher affinity toward the C-terminal SH3 domain of Grb2 than the typical one, it will be important to address the functional role of both sites in vivo.

Another aspect that needs to be addressed in the future is the series of events that lead to the Grb2/Gab2 interaction and its termination (e.g., by 14-3-3 proteins) (Brummer et al., 2008). For example, time course experiments have revealed that the Grb2/Gab ratio is increased by extracellular signals (Brummer et al., 2008; Gu et al., 1998). Although this increase could reflect indirect recruitment of the adaptor into the Gab signalosome, these observations might also reflect a conformational change of Gab that facilitates Grb2 binding, sequential use of the typical and atypical binding motifs, or use of one and then both of these sides. These possibilities are not mutually exclusive. Finally, the insights into the

Gab2/Grb2 interaction provided by this study might lead to the development of small-molecule inhibitors targeting this protein-protein interaction, which is known to play a key role in various malignant and inflammatory diseases.

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Adhesion Dance with Raver

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The localization of mRNAs in subcellular compartments is an efficient way to spatially restrict gene expression. Crystal structures of raver1-vinculin reported by Izard and coworkers now suggest a possible mechanism for mRNA localization during the assembly of focal adhesions.

Adhesion of cells to the extracellular matrix (ECM) is an essential process in multicellular organisms and is involved in some of the most prevalent human diseases, such as inflammation, thrombosis, cancer and infection. Several proteins play key roles in focal adhesion and tie the cell's actin cytoskeleton to the ECM. As shown in Figure 1, signaling across the plasma membrane triggers binding of intracellular anchor proteins such as talin, α-actinin and vinculin to the cytoplasmic integrin domain. Although vinculin does not bind integrins directly, it is essential for focal adhesion assembly as it provides a binding platform for α-actinin, talin, and the cytoskeleton (reviewed in Ziegler et al. [2008]).

Adhesion sites are differentiated in a tissue-specific manner and depend on the assembly of protein complexes at the sites of focal adhesion junctions, thereby allowing functional and architectural diversity. The assembly of focal adhesion proteins can be regulated at the level of transcription and splicing as well as mRNA localization and spatially restricted translation. An important factor interconnecting these processes is a widely expressed 80 kDa protein raver1 (Huttelmaier et al., 2001).

Raver1 shuttles between the nucleus and the cytoplasm and redistributes to the cytoplasm during cell differentiation. It contains three N-terminal RNA recognition motif (RRM) domains (Figure 1) and multiple conserved peptide motifs in the C terminus that mediate binding to the polypyrimidine tract binding protein (PTB) (Rideau et al., 2006). Raver1 has been identified as a co-repressor of PTB in tissue-specific alternative splicing of the pre-mRNA of the actin-binding protein α-tropomyosin (Gromak et al., 2003). It has been linked to focal adhesion proteins such as α-actinin and vinculin/metavinculin (Huttelmaier et al., 2001) and to the regulation of neuronal plasticity (Lahmann et al., 2008). However, molecular details for the role of raver1 in focal adhesion assembly have so far been elusive.

In the current issue of *Structure*, Lee et al. (2009) report crystal structures of the N-terminal region of human raver1 (comprising RRM1 or RRM1-3) bound to the vinculin tail (Vt). Only RRM1 of raver1 contacts the five-helical bundle of the Vt domain. Notably, the authors demonstrate that full-length vinculin is not able to bind raver1. This indicates that the interaction requires activation of full-length vinculin by talin or α-actinin binding, as the raver1 binding site in the Vt domain is partially occluded by an intramolecular interaction of the N-terminal "head" (Vh) and Vt domains. Activation leads to a conformational change in full-length vinculin that enables raver1 binding by the Vt domain. In the isolated Vt domain the raver1 binding site is freely accessible. Mutations of single residues in raver1 RRM1 that disrupt vinculin binding in vitro are shown to impair co-localization of raver1 and vinculin in cells.