The 'Gab' in signal transduction

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Tyrosine phosphorylation plays an important role in controlling cellular growth, differentiation and function. Abnormal regulation of tyrosine phosphorylation can result in human diseases such as cancer. A major challenge of signal transduction research is to determine how the initial activation of protein-tyrosine kinases (PTKs) by extracellular stimuli triggers multiple downstream signaling cascades, which ultimately elicit diverse cellular responses. Recent studies reveal that members of the Gab/Dos subfamily of scaffolding adaptor proteins (hereafter, 'Gab proteins') play a crucial role in transmitting key signals that control cell growth, differentiation and function from multiple receptors. Here, we review the structure, mechanism of action and function of these interesting molecules in normal biology and disease.

Much work over the past fifteen years has established a paradigm for signal transduction through tyrosine phosphorylation [1]. The receptors for most peptide growth factors and some hormones (termed 'receptor tyrosine kinases; RTKs') have intrinsic protein-tyrosine kinase (PTK) activity. Cytokines signal via receptors that associate with Janus family non-receptor PTKs, whereas multi-chain immune recognition receptors (MIRRs), such as the T- and B-cell antigen receptors (TCRs, BCRs, respectively) and Fc receptors (FcRs), utilize Src and Syk family PTKs. Activation results in receptor tyrosine phosphorylation, which creates docking sites for signal relay proteins containing Src-homology 2 (SH2) and phosphotyrosine-binding (PTB) domains. These proteins, in turn, fall into two general categories, enzymes or transcription factors (e.g. PLCγ, Stats) or adaptors (e.g. Grb2, Shc). Adaptors lack catalytic activity, but typically associate with one or more enzymes. For example, Grb2 associates with the GDP–GTP exchange factor Sos, whereas the p85 subunit of phosphoinositide 3-kinase (PI3K) associates with the catalytic p110 subunit. Specific protein-tyrosine phosphatases (PTPs) also play crucial roles in controlling the level of protein tyrosyl phosphorylation.

Many signaling pathways also utilize 'scaffolding adaptors'. Several families of scaffolding adaptors exist, including the IRS, FRS or p62dok-related families [1]. Scaffolding adaptors contain a membrane-targeting sequence [e.g. pleckstrin-homology (PH) domain or myristoylation site], binding sites for SH3 domain-containing proteins and multiple tyrosine phosphorylation sites that can recruit additional SH2 or PTB domain-containing molecules. In contrast to adaptors such as Grb2 and Shc, scaffolding adaptors can be targeted to specific membrane lipids. As scaffolding adaptors bind to multiple signal relay molecules (including adaptors and enzymes), they can assemble multimeric signaling complexes. In general, although relatively little is known about scaffolding adaptor function, these adaptors appear to serve as signal 'amplifiers'.

Gab proteins comprise a distinct family of scaffolding adaptors characterized by similar overall structural organization and ~40–50% sequence similarity (see below). Gab proteins are conserved from worms to mammals (Fig. 1) and include mammalian Gab1, Gab2 and Gab3, *Drosophila* Dos, and *Caenorhabditis elegans* Soc1. Gab1 (for *G*rb2-*a*ssociated *b*inder 1) was identified in a search for Grb2 SH3-domain binding proteins [2]. Gab2 was cloned as a binding protein and substrate of the SH2 domain-containing PTP Shp2 [3]. Gab3 was identified by sequence similarity to Gab1 and Gab2 [4]. Gab1 and Gab2 are expressed ubiquitously, but at relatively low levels in lymphoid tissue [2,3]. Gab3 also has a widespread expression pattern, although it is particularly highly expressed in lymphoid tissue [4]. Dos was identified as a potential substrate for Corkscrew (Csw), the *Drosophila* Shp2 ortholog [5], and independently in a screen for mutants that suppress the rough-eye phenotype caused by a hyper-activated *sevenless* allele [6]. Soc1 was found in a screen for suppressors of hyperactive Egl-15 (an FGF receptor ortholog) signaling [7]. Human chromosome 22 also contains a Gab-related sequence. A cDNA clone containing this sequence has been reported, but it has multiple upstream stop codons (GenBank accession number AK057252). Thus, this sequence might represent an expressed pseudogene, rather than a *bona fide* Gab protein.

Structure, recruitment and phosphorylation of Gab proteins

Although the overall sequence identity amongst Gab family members is only 40–50%, they share a similar topology (Fig. 1). Each contains an N-terminal PH domain, proline-rich motifs and multiple potential tyrosyl and seryl/threonyl phosphorylation sites. Each Gab protein also has unique structural motifs that allow it to signal downstream of specific receptors.

Recruitment of Gab proteins to receptors and/or membranes

Gab proteins can be recruited to activated receptors through direct or indirect mechanisms (Fig. 2). Direct

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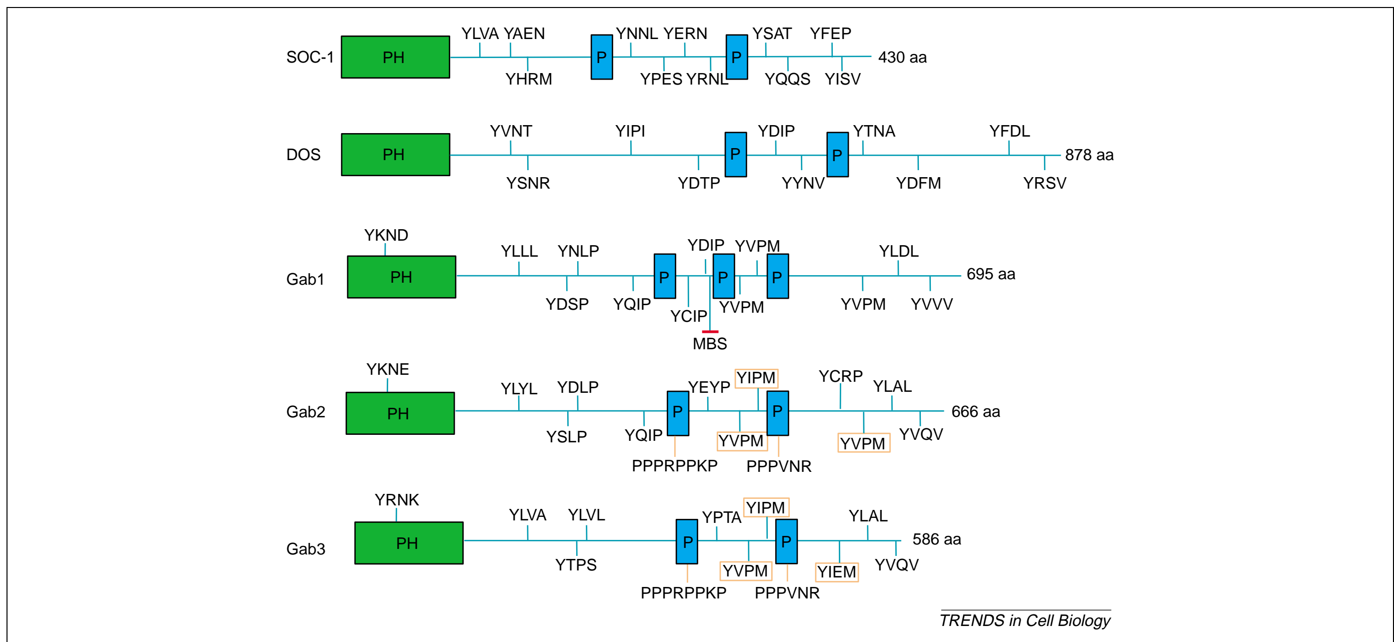

Fig. 1. Schematic structures of members of the Gab subfamily of scaffolding adaptors. Shown are the three mammalian Gab family members (Gab1–3) and their invertebrate orthologs, *Drosophila* Dos and *Caenorhabditis elegans* Soc-1. The positions of prolyl-rich domains (P) important for binding to Grb2 and possibly other SH3 domain-containing proteins (e.g. Gads for Gab3) are shown, as are potential tyrosyl (Y) phosphorylation sites. The Met-binding sequence (MBS) within the Met-binding domain (MBD) in Gab1 also is indicated. For further details, see text. Abbreviations: aa, amino acids; PH, pleckstrin-homology domain.

recruitment has been demonstrated only for interaction between Gab1 and c-Met (the receptor for hepatocyte growth factor) [8]. A region in Gab1 (amino acids 450–532), termed the Met-binding domain (MBD), interacts directly with tyrosyl-phosphorylated Met in yeast two-hybrid and *in vitro* protein overlay assays (Fig. 2a). The minimal amino acid sequence sufficient for direct interaction between Gab1 and Met, termed the MBS (Met-binding sequence), comprises 13 amino acids (487–499) and is absent in other Gab proteins [9,10]. Whether the MBS interacts directly with receptors other than Met remains unclear. As the MBD and MBS lacks obvious sequence similarity to other phosphotyrosyl peptide-binding modules (e.g. SH2 and PTB domains), structural studies of MBD–ligand interaction should be very interesting.

Most Gab protein–receptor interactions are mediated indirectly, via Grb2. Gab proteins contain several proline-rich motifs (Fig. 1), two of which can mediate binding of Gab1 or Gab2 to Grb2 [9,11]. One of these is a typical Grb2 SH3 binding site (PXXPXK/R), but the other (PXXXRXXKP) fails to conform to this motif. Grb2 also contains an SH2 domain, which targets the constitutive Grb–Gab complex to receptors containing Grb2 SH2 domain binding sites (YXNX) (Fig. 2a). Even for Met,

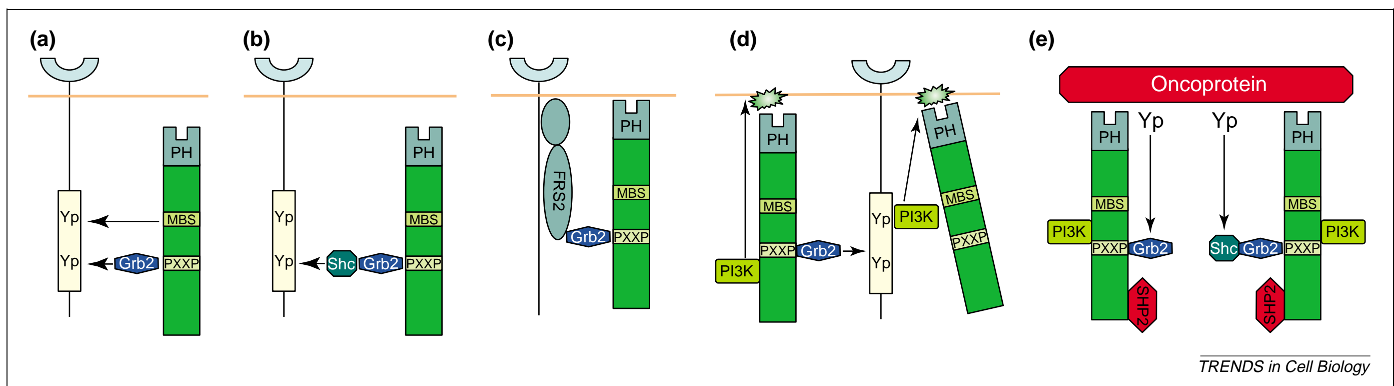

Fig. 2. Gab protein recruitment mechanisms. (a) Recruitment via MBS and Grb2–Gab1 complexes. Shown is a schematic of Gab1 recruitment to the HGF receptor (c-Met). Gab1 can bind directly to the HGF receptor (HGFR) through its Met-binding sequence (MBS), as well as indirectly, as part of a Grb2–Gab1 complex, to a Grb2 SH2 domain binding site in the HGFR. (b) Recruitment via Shc–Grb2–Gab2. Gab2 is recruited to IL3/GM-CSF/IL-5 receptor family members (which signal via the IL3 receptor β common chain) by means of a Shc–Grb2–Gab2 complex. (c) Recruitment via another scaffolding adaptor. In FGF receptor signaling, Gab1 is recruited to the receptor complex via Grb2 binding to the scaffolding adaptor FRS2. (d) Recruitment via Gab family PH domains. In some signaling pathways, binding of the Gab PH domain to appropriate phosphoinositides might either be required for initial recruitment to receptor complexes (e.g. BCR) or for sustained signaling (e.g. EGFR). In other signaling pathways, however, (e.g. βc signaling), the PH domain appears to be dispensable for recruitment to the receptor, although it is important for Gab protein function. The Gab1 PH domain has been shown to bind directly to 3-phosphoinositide lipids. The binding specificity of other Gab protein PH domains has not been reported, but sequence similarity to the Gab1 PH domain strongly suggests that they also bind to 3-phosphoinositides. (e) Recruitment of Gab proteins to oncoproteins. Gab proteins are phosphorylated in response to several oncogenic stimuli. The best understood are the recruitment of Gab1 to polyoma middle T as part of a Shc–Grb2–Gab1 complex (not shown), and the recruitment of Gab2 to Y177 of Bcr–Abl as part of a Grb2–Gab2 complex (illustrated here). Abbreviations: BCR, B-cell receptor; EGFR, epidermal growth factor receptor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; PH, pleckstrin-homology; PI3K, phosphoinositide 3-kinase; SH2, Src-homology 2; Yp, phosphotyrosine.

which can bind to Gab1 directly (see above), indirect recruitment of Gab1 via Grb2 is physiologically important as mutation of the Grb2 SH2 domain binding site in c-Met greatly diminishes its association with Gab1 [12,13].

The Grb2 SH3 binding sites are conserved among all mammalian Gab proteins, suggesting a common recruitment mechanism. Notably, Gads, a Grb2 relative expressed selectively in hematopoietic cells, also can bind to Gab3 [14]. For reasons that are currently unclear, Gads fails to bind to Gab2. Dos also contains two PXXXRXXKP motifs, either of which can mediate binding to the Drk (*Drosophila* Grb2 ortholog) C-terminal SH3 domain [15]. Dos carrying mutations in both of these sites fails to function in either the Sevenless or *Drosophila* EGF receptor (DER) signaling pathways [15]. By contrast, Soc1 lacks a PXXXRXXKP motif, and mutation of all putative SH3 domain binding motifs (i.e. PXXP sites) has no effect on its biological function [7]. Presumably, Soc1 is recruited to receptors through a mechanism distinct from that of other Gab proteins, and the requirement of Grb2 for Dos and Gab function evolved later during evolution. Whether the other (non-Grb2-binding) PXXP sequences in Gab proteins bind to other SH3 domain-containing proteins remains to be determined.

In some signaling pathways, Grb2–Gab2 complexes are recruited to receptors indirectly, via another tyrosyl-phosphorylated adaptor protein (Fig. 2b). Cytokine receptors that signal via the so-called 'β common chain (βc)' (IL-3/GM-CSF/IL-5) lack direct Grb2 binding sites in their cytoplasmic domains. Instead, βc contains a binding site (Y577) for the Shc PTB domain, and tyrosyl-phosphorylated Shc contains three Grb2 SH2 binding sites [16]. Mutation of the Shc binding site in βc eliminates most Gab2 tyrosyl phosphorylation. Moreover, a chimeric receptor in which Shc is fused directly to the βc cytoplasmic tail can promote Gab2 phosphorylation in the absence of other receptor tyrosyl residues [17]. These data strongly suggest that Gab2 recruitment to βc occurs predominantly via a Shc–Grb2–Gab2 complex [17]. Residual recruitment of Gab2 to βc lacking Y577 might occur via an analogous Shp2–Grb2–Gab2 complex [17]. Alternatively, Shc also might bind via its SH2 domain to this mutant receptor as the Shc SH2 domain can bind to Y612 in βc *in vitro* [18]. Recruitment of Gab1 and Gab2 to the thrombopoietin receptor [19] and the Ret RTK [20] also appears to involve a Shc–Grb2–Gab2 complex. Likewise, the IL-2 receptor (IL2R) lacks direct Grb2 SH2 domain binding sites in the cytoplasmic domains of its signaling chains (IL2Rβ and γ), and the activated IL2R evokes Gab2 tyrosyl phosphorylation [17,21]. Most likely, this also occurs through a Shc–Grb2–Gab2 complex [17].

In the FGF receptor pathway, Gab1 phosphorylation occurs via a more baroque mechanism involving an additional scaffolding adaptor, FRS2 (Fig. 2c). Upon receptor activation, FRS2 becomes tyrosine phosphorylated and binds to Grb2, which, in turn, recruits Gab1 [22,23]. Trk receptors (e.g. NGFR, NT-receptors) also evoke FRS2 and Gab1 phosphorylation, but it is unclear whether a similar mechanism is involved in Gab1 phosphorylation in these pathways. Notably, the NGFR also has a direct binding site for Shc [16], so Gab1 recruitment could occur via a Shc–Grb2–Gab1 complex.

Gab protein PH domains also might play a role in recruitment, at least to some receptors (Fig. 2d). These PH domains have sequence similarity to the Btk PH domain, especially in the loop between the β1 and β2 sheet, a region that determines the binding preference for phospholipids. The Btk PH domain strongly prefers the lipid products of PI3K, particularly phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [24]. Yeast complementation experiments [25] and binding studies show that the Gab1 and Dos PH domains also bind to PIP3 [26,27]. Although the specificities of the Gab2 and Gab3 PH domains have not been reported, their strong similarity to the Gab1 PH domain (particularly in the region crucial for determining specificity) makes it highly likely that they bind to PI3K lipid products.

The role of the PH domain in Gab recruitment is best understood for epidermal growth factor receptor (EGFR) signaling [26]. Gab1 is initially targeted to the EGFR via Grb2, whereupon it becomes tyrosyl phosphorylated and interacts with p85. This leads to activation of PI3K and the generation of 3-phosphoinositide lipids (see below). The Gab1 PH domain then binds to these lipids, resulting in Gab1 retention near the receptor and more sustained signaling (Fig. 2d). Consistent with this model, the PH domain is required for maximal EGF-induced Gab1 tyrosyl phosphorylation and activation of downstream effectors [26]. The Gab1 PH domain is even more important for recruitment in BCR signaling as a PH domain deletion mutant fails to become tyrosyl phosphorylated upon BCR engagement [28]. In other pathways, however, the PH domain appears to be dispensable for recruitment. For example, in Met signaling in MDCK cells, PH domain deletion has no effect on Gab1 tyrosyl phosphorylation, and a Gab2 PH domain mutant is tyrosyl phosphorylated normally in response to IL-3 stimulation of BaF3 cells [17].

Despite its variable requirement for Gab protein recruitment, the PH domain appears to be required for Gab protein function. Point mutations that disrupt folding of the Dos or Soc-1 PH domains behave as protein-nulls in genetic rescue experiments [7,29]. Surprisingly, however, the Dos mutation does not appear to affect Dos subcellular localization [29]. Likewise, a PH domain deletion mutant of Gab1 (ΔPH) is defective in EGFR signaling [26] and fails to support Met-induced morphogenesis (i.e. tubulogenesis) in MDCK cells [30]. In contrast to the Dos mutants, the inability of Gab1 ΔPH to function in EGFR and HGFR signaling correlates with its failure to translocate (in a PIP3-dependent manner; see above) to the plasma membrane [26,30]. Similarly, deletion of the Gab2 PH domain impairs the effects of Gab2 on TCR-evoked signaling [31].

Kinases that phosphorylate Gab proteins

Gab proteins undergo tyrosyl phosphorylation in response to diverse stimuli, including cytokines, growth factors and MIRR engagement. Although activated RTKs (or, in the case of cytokine receptors and MIRRs, activated receptor-associated PTKs) are required for Gab tyrosyl phosphorylation, the receptor (or the receptor-associated PTK) itself

does not appear to directly phosphorylate all Gab protein tyrosyl phosphorylation sites. Instead, intermediate PTKs are important for Gab phosphorylation in some settings. For example, Gab1 tyrosine phosphorylation induced by lysophosphatidic acid (LPA) is inhibited by the Src-family kinase (SFK) inhibitor PP1 [32], whereas a recent report suggests that Fyn is responsible for FcεRI-induced Gab2 tyrosine phosphorylation [33] in mast cells. In TCR signaling, ZAP-70 has been suggested to be the kinase responsible for Gab2 phosphorylation on the basis of co-transfections and experiments with a ZAP-70-deficient T-cell line [34]. SFK might contribute to Gab2 phosphorylation in response to CSF-1/MCSF, although the CSF-1 receptor (c-Fms) also directly phosphorylates Gab2 [35].

Serine/threonine kinases also phosphorylate Gab proteins and regulate their function. The mobility of Gab1 and Gab2 in SDS–PAGE is reduced markedly following most forms of stimulation. This shift can be inhibited partially by treatment with MEK or PI3K inhibitors [17]. Gab1, through its MBD domain, is found in a complex with activated Erk2 and can be phosphorylated by Erk2 *in vitro* [36]. Interestingly, Gab1 phosphorylation by Erk has opposite effects on Met [37] and EGFR [38] signaling. Upon Met stimulation, Erk phosphorylates Gab1 on Thr477, which lies adjacent to a Gab1 binding site (Tyr472) for p85 ($Y^{472}VPMT^{477}P$). Phosphorylation at Thr477 enhances p85 recruitment to Gab1. Since Gab1 regulates Erk activation (via Shp2; see below) and PI3K can potentiate Erk activation [39,40], Erk2 phosphorylation of Gab1 might result in a positive-feedback loop. By contrast, upon EGF stimulation, Erk2 phosphorylates Gab1 on unidentified Ser/Thr residues, and this correlates with inhibition of PI3K. It will be important to determine how Erk2 has such dramatically different effects on Gab1 function in these two signaling pathways. Most likely, Erk2 phosphorylates different sites in response to these two RTKs, but how this is regulated and whether Gab2 function is regulated by Erk phosphorylation remain unclear.

Recent work indicates that phosphorylation of Gab2 at Ser159 (human sequence) functions in a negative-feedback loop. Akt forms a constitutive complex with Gab2 and phosphorylates Ser159 upon ErbB2 stimulation of breast cancer cell lines [41]. Overexpression of Gab2 with Ser159 mutated to alanine potentiates ErbB2 phosphorylation and transforms NIH3T3 cells. How Ser159 phosphorylation on Gab2 inhibits Erb2 signaling remains to be determined, but it will be interesting to see whether a similar mechanism operates downstream of other receptors, as well as whether Ser159 mutations occur in, and contribute to, human breast cancer.

Signaling from Gab proteins

Gab proteins contain multiple tyrosine phosphorylation sites that, upon phosphorylation, recruit and activate key SH2-domain-containing signal relay molecules, most prominently Shp2 and PI3K. Several studies have established the crucial role of Gab proteins in mediating Shp2 and PI3K actions in a variety of cell signaling pathways (Fig. 3).

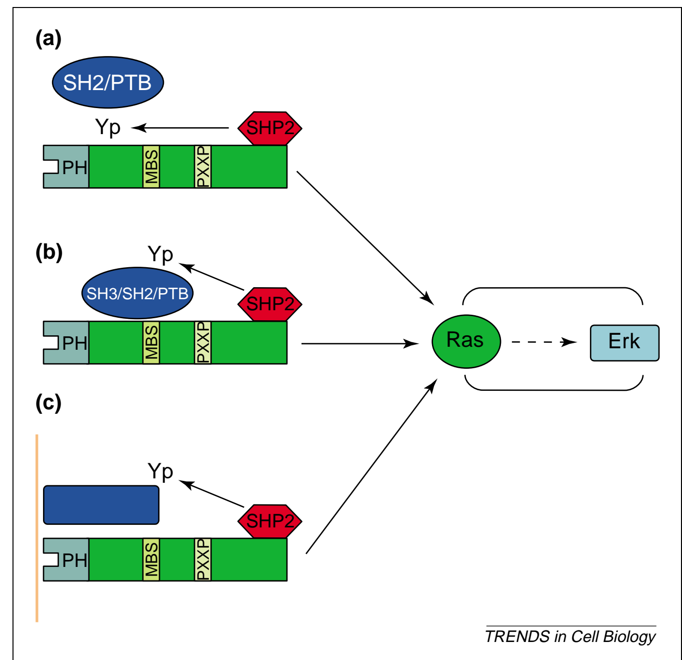

Fig. 3. Models for Gab–Shp2 regulation of the Ras–Erk pathway. Shown are three potential models for how Gab proteins, acting via Shp2, signal to Ras activation. In model (a), Shp2, bound to the Gab protein, regulates the phosphorylation of another Gab protein phosphorylation site (Yp). In model (b), Shp2 controls the phosphorylation of a Gab binding protein. In model (c), the main function of the Gab protein is to target Shp2 to the appropriate location of its key substrate, most likely a membrane compartment in view of the PH domain in Gab proteins. Most available evidence favors model (c). For further details, see text. Abbreviations: MBS, Met-binding sequence; PH, pleckstrin-homology domain; PTB, phosphotyrosine-binding domain.

Regulation of Shp2

All Gab proteins contain at least one conserved tyrosine motif I/V/LXYXXI/V/L (where X = any amino acid), capable of binding to Shp2 or its *Drosophila* or *C. elegans* orthologs [Corkscrew (Csw) and Ptp2, respectively]. Mammalian Gabs and Dos contain two SHP2/Csw binding sites; Soc-1 has only a single Ptp2 site. Shp2 (and its orthologs) has low basal activity owing to allosteric inhibition of its PTP domain by the N-terminal SH2 domain. Upon Shp2 binding to a Gab protein, basal inhibition is relieved, resulting in strong activation. Vertebrates express another SH2 domain-containing PTP, Shp1. The Shp1 and Shp2 SH2 domains recognize similar phosphotyrosyl peptides. However, Gab proteins preferentially interact with Shp2. Elucidating the reason why the mammalian Shps bind differentially to Gab proteins is an important goal of future research. Nevertheless, this differential binding ability helps explain the distinct biological functions of Shp1 and Shp2 [42].

Gab/Dos family proteins, acting via Shp2, are required for full Erk activation in many signaling pathways. Studies of chimeric receptors revealed that Gab1 mutants lacking Shp2 binding sites are unable to activate Erk or downstream transcriptional reporters [9]. Overexpression of such mutants also fails to potentiate EGF-evoked Erk activation [26,43,44]. Fibroblasts from $Gab1^{-/-}$ mice (see below) also are defective in Erk activation in response to multiple growth factors, as well as to the cytokine IL-6 [45,46]. Subsequent work showed that the Gab1–Shp2 complex acts at a step upstream of Ras [47,48]. These

findings are consistent with analogous studies of cells expressing dominant-negative mutants of Shp2 or Shp2-mutant fibroblasts [42,49,50] that exhibit an impaired Ras–Erk response to a variety of growth factors. Thus, Gab1, through binding and activation of Shp2, is crucial for normal levels of Ras activation. In most signaling pathways, Gab1–Shp2 complex formation is not *absolutely* essential for Ras–Erk activation; instead, initial Ras–Erk activation occurs, but peak and/or sustained activity is defective in Gab1$^{-/-}$ or Shp2 mutant cells. In this way, Gab–Shp2 complexes might serve as 'amplifiers' of initial Ras–Erk pathway activation.

Overexpression of mutant forms of Gab2 [51] also can impair Erk activation in response to some stimuli (e.g. CSF-1). Moreover, mast cells and macrophages from Gab2$^{-/-}$ mice have decreased Erk activation in response to SCF [52] or CSF-1 (H. Gu and B.G. Neel, unpublished), respectively. However, Gab2–Shp2 complexes appear to have an additional, distinct signaling role in response to other stimuli. For example, overexpression of Gab2 mutants that fail to bind to Shp2 blocks IL-3-evoked immediate-early gene activation without affecting Erk activation [3]. Thus Gab–Shp2 complexes appear to act at least twice (and presumably on distinct targets): once upstream of Ras, and another time downstream of, or parallel to, Erk activation. These biochemical studies are consistent with epistasis analysis of Csw function in Sevenless signaling [42].

Genetic analysis has established the essential role of the Dos–Csw and Soc-1–Ptp-2 interactions. Indeed, the *only* Dos tyrosyl residues required for Dos function in the Sevenless pathway are the two Csw binding sites [29,53]. Likewise, only the Ptp-2 binding site is essential for Soc-1 function in the Egl-15 pathway [7]. Importantly, in these systems, it has not been shown explicitly that Dos–Csw or Soc-1–Ptp-2 act upstream of Erk, although this can probably be inferred reasonably safely from studies in mammalian systems.

Three general models of Gab–Shp2 signaling to the Ras–Erk pathway can be envisioned (Fig. 3): (a) upon recruitment, Shp2 regulates the phosphorylation of another site(s) on a Gab protein, thereby controlling binding of an SH2 or PTB domain protein; (b) Shp2 regulates the phosphorylation of a protein that binds to the Gab protein in either a phosphotyrosine-dependent or –independent manner; or (c) the Gab protein could merely target Shp2 to where the substrate resides – presumably a membrane compartment.

Recent data strongly support the third model. As indicated above, only the Csw binding site on Dos and the Ptp-2 site on Soc-1 are essential for their respective functions, at least in the pathways analyzed. A Trk–Met–Gab1 chimera containing only the extreme Gab1 C-terminus (amino acids 411–695) subserves the function of Gab1 in Met-evoked branching morphogenesis in MDCK cells, and mutation of the Shp2 binding sites in Gab1 abrogates this function [9]. Shp2 binding to Gab1 also is required for branching morphogenesis in response to HGF stimulation [43]. A Gab1–Shp2 fusion protein can evoke growth-factor-independent Erk activation in 293 cells and potentiates activation in response to EGF [54]. Again, other Gab1 tyrosyl residues appear be dispensable as fusion of the Gab1 PH domain alone (or several other membrane-targeting sequences) to the Shp2 PTP domain has similar effects [48]. Ligation of the N-terminus of Src to Csw also produces a gain-of-function mutant [42]. The target(s) that Shp2 must dephosphorylate to mediate Ras (and, ultimately, Erk) activation remain unclear [42]; identifying this target(s) is crucial for our understanding of Gab protein function.

Although Gab proteins themselves probably are not what Shp2 must dephosphorylate to sustain Ras activation, they are Shp2 targets. Gab1 [54], Gab2 [55] and Dos [5] tyrosyl phosphorylation is increased in cells expressing 'substrate trapping' mutants of Shp2 or Csw, respectively. Most likely, Shp2-mediated dephosphorylation of Gab proteins functions in signal termination. For example, Shp2 dephosphorylates the p85 binding sites in Gab1 and negatively regulates the activation of PI3K–Akt by EGF [50]. Shp2 also might dephosphorylate its own binding sites, thereby releasing itself from the Gab protein and terminating signaling by Gab–Shp2 complexes.

Role in PI3K–Akt pathway

All Gab proteins have at least one potential binding site (YXXM) for the SH2 domain of p85. The function of these sites in Dos and Soc-1 (and whether these Gab proteins actually recruit PI3K *in vivo*) remains to be determined (see above). In mammalian systems, PI3K binding has been demonstrated for all Gab proteins, and binding depends on the presence of at least two of the three YXXM motifs in these proteins [17,56]. By binding p85, Gab proteins can provide a major route to PI3K pathway activation for receptors that lack p85 binding sites. For example, Gab2 is implicated in PI3K activation from the IL-3/GM-CSF, EGFR and FcεRI receptors [17,57,58], whereas Gab1 binding to p85 is suggested to be a route to PI3K pathway activation downstream of FGFRs [22] and the EGFR [26]. Gab proteins also recruit PI3K in response to stimulation of receptors that have p85 binding sites, such as the NGFR [56] and Ret [20], or receptor systems in which co-receptors also recruit PI3K, such as the BCR [28] and TCR [31,34]. In the latter systems, Gab–p85 probably serves to amplify receptor-evoked PI3K activity. The consequences of Gab-mediated PI3K activation can be different, depending on the cell type. Recruitment of PI3K to Gab2 is crucial for mast cell functions such as degranulation [58]. Conversely, Gab2–PI3K complexes appear to inhibit TCR-evoked IL-2 production [31,34].

Other signaling pathways from Gab proteins

Biochemical analyses and yeast two-hybrid screens have identified several other signaling molecules that can bind to Gab1 and/or Gab2, including PLCγ [2] Crk [59], CrkL [60,61] and Ship [62]. The functional significance of most of these interactions remains unclear. Y307/Y373/Y407 in Gab1 reportedly bind to PLCγ and are required for Met-induced branching morphogenesis [63]. As Shp2 binding to Gab1 also is required for this process (see above), PLCγ and Shp2 could act in concert. However, the ability of Trk–Met–Gab1 chimeras lacking their PLCγ

binding sites to evoke morphogenesis [9] would seem to be inconsistent with such a model.

Based on studies of a Gab1 mutant with an internal deletion of amino acids 242–410, it was suggested that Gab1 association with CrkL correlates with Met-evoked activation of the GTPase Rap1 [61]. However, this region contains potential binding sites for other SH2- and SH3-containing molecules such as PLCγ. It will be important to generate a mutant of Gab1 that cannot bind to CrkL and reassess the effect of this mutant on Rap1 activation. Gab1 association with Crk also is reported to correlate with the activation of JNK in response to Met [59]. Interestingly, Shp2-mutant fibroblasts have increased Jnk activation in response to some stimuli [49]. It will be interesting to determine whether Shp2 dephosphorylates the Crk binding sites in the Met signaling pathway.

Biological functions of Gab proteins

Invertebrate Gab proteins

Early genetic analyses indicated that Dos was an essential component of the Sevenless pathway [5,6], where it signals via Csw [29,53]. However, Dos also is required for signaling by other *Drosophila* RTKs such as DER (EGF receptor ortholog) and Torso (PDGF receptor ortholog) [6,64].

Soc-1 was cloned during a screen for *s*uppressors *o*f the *C*lear (Clr) phenotype caused by hyperactive Egl-15 [7]. *Egl-15* null worms arrest development during early larval stages, whereas *soc-1* null worms can develop to adulthood. *Soc-1* null worms exhibit a *Scrawny* body morphology, similar to the phenotype caused by hypomophic Egl-15 alleles. Thus, consistent with biochemical analyses in mammalian cells (see above), Soc-1 is required for only some downstream signaling from Egl-15. Interestingly, gene knockout studies in mice have shown that Gab1 and another scaffolding adaptor, Frs2, are required for FGF signaling (see below). Although *C. elegans* has an Frs2 ortholog, it has not been identified as a Soc gene, suggesting that the primordial signaling module probably is FGFR–Gab1 (Egl-15–Soc-1), whereas FRS2 functions in FGFR signaling developed later in evolution.

Physiologic function of Gab proteins in mammals

Recent studies of Gab1 and Gab2 knockout mice have begun to delineate distinct functions for individual Gab proteins. Reports have also emerged indicating that Gab proteins might play important roles in oncogenic transformation.

Gab1$^{-/-}$ mice die between embryonic day (E) 12.5–E18.5, with multiple defects in heart, placenta, liver, skin and muscle development [45,46]. As mice with mutations in EGFR, PDGFR, HGFR and gp130 have one or more of these defects, the Gab1$^{-/-}$ phenotype probably results from the combined effects of defective EGF, PDGF, HGF and gp130 signaling [45,46]. However, the abnormal placental development in Gab1$^{-/-}$ mice makes attributing specific Gab1$^{-/-}$ phenotypes to defects in other lineages somewhat risky. Experiments using aggregation chimeras to tetraploid embryos to bypass the placental defects or tissue-specific knockout mice should provide more definitive answers to these issues.

In contrast to these positive roles for Gab1, analyses of radiation chimeras reconstituted with Gab1$^{-/-}$ fetal liver cells show that Gab1 is a negative regulator of the thymus-independent antigen-2 (TI-2) response of marginal zone B cells [65]. This negative function of Gab1 depends upon its ability to associate with Shp2 [65] and is reminiscent of the inhibitory effects of Gab2 overexpression on TCR signaling in cultured cell lines [31,34].

In marked contrast, Gab2$^{-/-}$ mice are viable, generally healthy, and have an apparently normal life span. Gab2 was initially believed to be a key mediator of Shp2 function in hematopoietic cells. Nevertheless, steady-state hematopoiesis is largely normal in Gab2$^{-/-}$ mice [52,58], although they might have lower numbers of eosinophils and basophils (H. Gu and B.G. Neel, unpublished).

The most prominent defects in Gab2$^{-/-}$ mice are in the mast cell lineage [58]. Gab2$^{-/-}$ mice have severe defects in their response to passive allergic challenge, and their mast cells show impaired degranulation and cytokine gene expression in response to activation of FcεRI, the high-affinity IgE receptor. Defective activation of Gab2$^{-/-}$ mast cells is attributable to impaired PI3K activation, establishing an essential role for Gab2 in mediating PI3K activation in response to FcεRI ligation. Gab2$^{-/-}$ mice also show decreased mast cell numbers in various tissues, such as skin and stomach. Most likely, this reflects defective signaling downstream of the receptor tyrosine kinase Kit as Gab2$^{-/-}$ mast cells display decreased Kit-initiated signal transduction and mast cell proliferation [52].

Gab proteins in cancer

Genetic and biochemical studies have established a crucial role for Gab2 in Bcr–Abl transformation [66]. Similar to the mechanism of Gab protein recruitment to normal growth factor and cytokine receptors (see above), Gab2 is recruited via Grb2 to a Grb2 SH2 domain-binding site, Y177, in Bcr–Abl and subsequently recruits p85 and Shp2 (Fig. 2e). Gab2$^{-/-}$ cells expressing Bcr–Abl exhibit defective PI3K–Akt and Erk activation, which likely reflects the inability of Bcr–Abl to signal to p85 and Shp2, respectively, via Gab2. Very recent studies using retroviral gene transduction/bone marrow transplantation indicate that myeloid and lymphoid leukemogenesis are defective in Gab2$^{-/-}$ mice (G. Mohi, H. Gu, S. Li., R. Van Etten and B.G. Neel, unpublished). These studies provide a molecular explanation for the defective leukemogenic potential of Bcr–Abl Y177F mutants [67].

Efficient induction of endothelial tumors by polyomavirus middle T antigen (PymT) requires a ShcA binding site, Y250. This site directs formation of a ShcA–Grb2–Gab1 complex, and consequently Gab1 tyrosine phosphorylation and association with p85. Activation of PI3K is defective in PymT Y250F-expressing cells, which suggests that the above complex is responsible for PI3K activation by PymT [68], although there has not been a direct demonstration that Gab1 is required for PymMT oncogenesis. This use of a ShcA–Grb2–Gab1–p85 pathway by PymT resembles the activation of PI3K by means of a Shc–Grb2–Gab2–p85 pathway in βc cytokine receptor signaling (see above and [17]). Several other oncoproteins, including Tpr–Met [13,69], v-Sea [70] and gp55, the

oncogene of spleen-focus-forming virus (SFFV) [71], also might signal through Gab proteins. Interestingly, in v-Sea-expressing cells, only Gab2 becomes tyrosine phosphorylated and associated with p85 and Shp2, even though Gab1 is expressed these cells [70]. The role of Gab1 and Gab2 proteins in the above pathways requires further studies – in particular, the ability of these oncogenes to transform cells lacking Gab1 and/or Gab2 should be addressed.

Gab2 also might play a role in breast cancer. The gene encoding Gab2 is found on human chromosome 11q13.3–14.2 [72], and 11q13 amplification is found in 10–15% of breast cancer patients [73]. It will be interesting to see whether Gab2 amplification is a common feature of the 11q13 amplicon. Whether or not Gab2 is amplified in human breast cancer, it is overexpressed in a substantial fraction of breast cancer lines and primary tumors [74]. There is some correlation between Gab2 overexpression and estrogen receptor (ER) positivity in breast cancer cell lines, although this is not absolute. Although these data are provocative, it remains to be seen whether Gab2 overexpression plays a causal role in breast carcinogenesis. It also will be interesting to see whether breast cancers have mutations of the Akt phosphorylation site within Gab2 because, as discussed above, mutation of this site converts Gab2 to a transforming protein, at least in fibroblasts [41]. Crosses between Gab2$^{-/-}$ mice and mouse models of breast carcinogenesis and/or mammary-specific overexpression of Gab2 should help to resolve these questions.

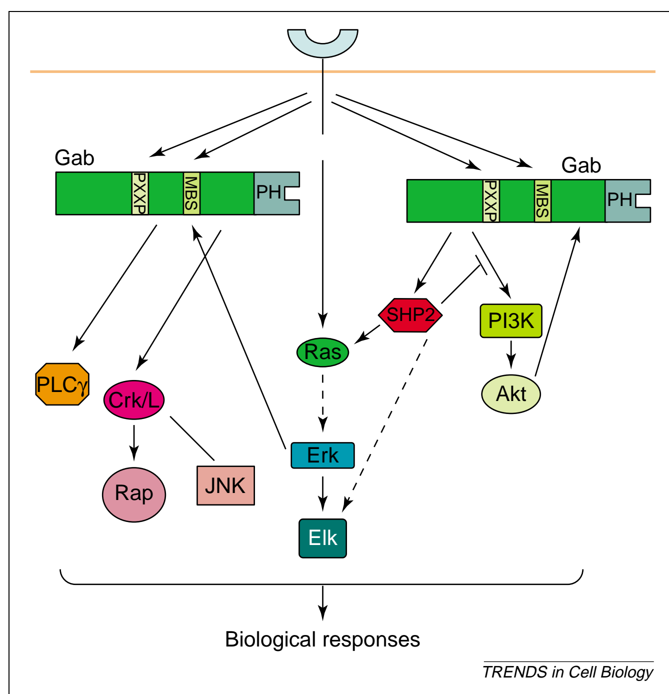

Fig. 4. Signal transduction from Gab proteins. Gab proteins are implicated in regulation of the Ras–Erk pathway through their ability to bind to and activate the SH2 domain-containing protein-tyrosine phosphatase Shp2, as well as in activation of the PI3K–Akt pathway, by virtue of their ability to bind to the p85 regulatory subunit of PI3K, thereby activating the associated p110 catalytic subunit. Various reports also have suggested that, by recruiting other signal relay proteins such as PLCγ and/or Crk/CrkL, Gab proteins can activate other signaling pathways, such as the JNK pathway. However, the generality of these associations, as well as their physiological relevance, remain to be determined. For details, see text. Abbreviations: MBS, Met-binding sequence; PH, pleckstrin-homology domain; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ.

Finally, a recent study implicates a PH domain-truncated form of Gab1 (Δ1–103) in an experimental model of cancer progression [75]. Overexpression of Gab1(Δ1–103) enhances (by three fold), whereas wild-type Gab1 inhibits, EGF-induced soft agar colony formation of pre-neoplastic Syrian hamster cells. Notably, however, this report stands in contrast to previous studies in which Gab1 overexpression enhanced growth factor responsiveness and tumorigenicity in NIH3T3 cells [2]. It will be important to carry out genetic analyses to assess the role of Gab1 in tumorigenesis.

Concluding remarks

In the few years since their discovery, it has become clear that Gab proteins play key roles in a variety of important cell signaling pathways (Fig. 4). The challenge for the future is to elucidate the detailed mechanism by which these molecules transmit signals. In particular, it will be important to delineate the direct targets of Shp2 downstream of Gab proteins and to determine which (if any) signal relay molecules besides Shp2 and PI3K are crucial for Gab signaling. It also will be important to establish which functions are unique to particular Gab family members, as opposed to redundant, and the extent to which Gab proteins have crucial functions in oncogenic signaling pathways. The availability of genetic models of Gab protein deficiency should allow for rapid progress on all of these questions.

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References

1 Schlessinger, J. (2000) Cell signaling by receptor tyrosine kinases. *Cell* 103, 211–225
2 Holgado-Madruga, M. *et al.* (1996) A Grb2-associated docking protein in EGF- and insulin-receptor signalling. *Nature* 379, 560–564
3 Gu, H. *et al.* (1998) Cloning of p97/Gab2, the major SHP-2 binding protein in hematopoietic cells, reveals a novel pathway for cytokine-induced gene activation. *Mol. Cell* 2, 729–740
4 Wolf, I. *et al.* (2002) Gab3, a new DOS/Gab family member, facilitates macrophage differentiation. *Mol. Cell. Biol.* 22, 231–244
5 Herbst, R. *et al.* (1996) Daughter of sevenless is a substrate of the phosphotyrosine phosphatase Corkscrew and functions during sevenless signaling. *Cell* 85, 899–909
6 Raabe, T. *et al.* (1996) DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in *Drosophila*. *Cell* 85, 911–920
7 Schutzman, J.L. *et al.* (2001) The *Caenorhabditis elegans* EGL-15 signaling pathway implicates a DOS-like multisubstrate adaptor protein in fibroblast growth factor signal transduction. *Mol. Cell. Biol.* 21, 8104–8116
8 Weidner, K.M. *et al.* (1996) Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature* 384, 173–176
9 Schaeper, U. *et al.* (2000) Coupling of Gab1 to c-Met, Grb2, and Shp2 mediates biological responses. *J. Cell Biol.* 149, 1419–1432
10 Lock, L.S. *et al.* (2002) Distinct recruitment and function of Gab1 and

Gab2 in Met receptor-mediated epithelial morphogenesis. *Mol. Biol. Cell* 13, 2132–2146

11 Lock, L.S. *et al.* (2000) Identification of an atypical Grb2 carboxyl-terminal SH3 domain binding site in Gab docking proteins reveals Grb2-dependent and -independent recruitment of Gab1 to receptor tyrosine kinases. *J. Biol. Chem.* 275, 31536–31545

12 Nguyen, L. *et al.* (1997) Association of the multisubstrate docking protein Gab1 with the hepatocyte growth factor receptor requires a functional Grb2 binding site involving tyrosine 1356. *J. Biol. Chem.* 272, 20811–20819

13 Bardelli, A. *et al.* (1997) Gab1 coupling to the HGF/Met receptor multifunctional docking site requires binding of Grb2 and correlates with the transforming potential. *Oncogene* 15, 3103–3111

14 Bourgin, C. *et al.* (2002) Induced expression and association of the Mona/Gads adaptor and Gab3 scaffolding protein during monocyte/macrophage differentiation. *Mol. Cell. Biol.* 22, 3744–3756

15 Feller, S.M. *et al.* (2002) SH3 domain-mediated binding of the Drk protein to Dos is an important step in signaling of *Drosophila* receptor tyrosine kinases. *Mech. Dev.* 116, 129–139

16 Ravichandran, K.S. (2001) Signaling via Shc family adaptor proteins. *Oncogene* 20, 6322–6330

17 Gu, H. *et al.* (2000) New role for Shc in activation of the phosphatidylinositol 3-kinase/Akt pathway. *Mol. Cell. Biol.* 20, 7109–7120

18 Bone, H. and Welham, M.J. (2000) Shc associates with the IL-3 receptor beta subunit, SHIP and Gab2 following IL-3 stimulation. Contribution of Shc PTB and SH2 domains. *Cell. Signal.* 12, 184–194

19 Bouscary, D. *et al.* (2001) Role of Gab proteins in phosphatidylinositol 3-kinase activation by thrombopoietin (Tpo). *Oncogene* 20, 2197–2204

20 Besset, V. *et al.* (2000) Signaling complexes and protein-protein interactions involved in the activation of the Ras and phosphatidylinositol 3-kinase pathways by the c-Ret receptor tyrosine kinase. *J. Biol. Chem.* 275, 39159–39166

21 Gadina, M. *et al.* (2000) The docking molecule Gab2 is induced by lymphocyte activation and is involved in signaling by Interleukin-2 and Interleukin-15 but not other common gamma chain-using cytokines. *J. Biol. Chem.* 275, 26959–26966

22 Ong, S.H. *et al.* (2001) Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6074–6079

23 Hadari, Y.R. *et al.* (2001) Critical role for the docking-protein FRS2alpha in FGF receptor-mediated signal transduction pathways. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8578–8583

24 Rameh, L.E. *et al.* (1997) A comparative analysis of the phosphoinositide binding specificity of pleckstrin homology domains. *J. Biol. Chem.* 272, 22059–22066

25 Isakoff, S.J. *et al.* (1998) Identification and analysis of PH domain-containing targets of phosphatidylinositol 3-kinase using a novel *in vivo* assay in yeast. *EMBO J.* 17, 5374–5387

26 Rodrigues, G.A. *et al.* (2000) A novel positive feedback loop mediated by the docking protein Gab1 and phosphatidylinositol 3-kinase in epidermal growth factor receptor signaling. *Mol. Cell. Biol.* 20, 1448–1459

27 Maroun, C.R. *et al.* (1999) A conserved inositol phospholipid binding site within the pleckstrin homology domain of the Gab1 docking protein is required for epithelial morphogenesis. *J. Biol. Chem.* 274, 31719–31726

28 Ingham, R.J. *et al.* (2001) The Gab1 docking protein links the b cell antigen receptor to the phosphatidylinositol 3-kinase/Akt signaling pathway and to the SHP2 tyrosine phosphatase. *J. Biol. Chem.* 276, 12257–12265

29 Bausenwein, B.S. *et al.* (2000) *In vivo* functional analysis of the daughter of sevenless protein in receptor tyrosine kinase signaling. *Mech. Dev.* 90, 205–215

30 Maroun, C.R. *et al.* (1999) The Gab1 PH domain is required for localization of Gab1 at sites of cell–cell contact and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol. Cell. Biol.* 19, 1784–1799

31 Pratt, J.C. *et al.* (2000) Cutting edge: gab2 mediates an inhibitory phosphatidylinositol 3′-kinase pathway in T cell antigen receptor signaling. *J. Immunol.* 165, 4158–4163

32 Daub, H. *et al.* (1997) Signal characteristics of G protein-transactivated EGF receptor. *EMBO J.* 16, 7032–7044

33 Parravicini, V. *et al.* (2002) Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. *Nat. Immunol.* 3, 741–748

34 Yamasaki, S. *et al.* (2001) Docking protein Gab2 is phosphorylated by ZAP-70 and negatively regulates T cell receptor signaling by recruitment of inhibitory molecules. *J. Biol. Chem.* 276, 45175–45183

35 Lee, A.W. and States, D.J. (2000) Both src-dependent and -independent mechanisms mediate phosphatidylinositol 3-kinase regulation of colony-stimulating factor 1-activated mitogen-activated protein kinases in myeloid progenitors. *Mol. Cell. Biol.* 20, 6779–6798

36 Roshan, B. *et al.* (1999) Activated ERK2 interacts with and phosphorylates the docking protein GAB1. *J. Biol. Chem.* 274, 36362–36368

37 Yu, C.F. *et al.* (2001) ERK regulates the hepatocyte growth factor-mediated interaction of Gab1 and the phosphatidylinositol 3-kinase. *J. Biol. Chem.* 276, 32552–32558

38 Yu, C.F. *et al.* (2002) ERK negatively regulates the epidermal growth factor-mediated interaction of Gab1 and the phosphatidylinositol 3-kinase. *J. Biol. Chem.* 277, 19382–19388

39 Duckworth, B.C. and Cantley, L.C. (1997) Conditional inhibition of the mitogen-activated protein kinase cascade by wortmannin. *J. Biol. Chem.* 272, 27665–27670

40 Grammer, T.C. and Blenis, J. (1997) Evidence for MEK-independent pathways regulating the prolonged activation of the ERK-MAP kinases. *Oncogene* 14, 1635–1642

41 Lynch, D.K. and Daly, R.J. (2002) PKB-mediated negative feedback tightly regulates mitogenic signalling via Gab2. *EMBO J.* 21, 72–82

42 Neel, B.G. *et al.* SH2 domain-containing protein-tyrosine phosphatases. *Handbook in Cell Signaling.* (in press)

43 Maroun, C.R. *et al.* (2000) The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol. Cell. Biol.* 20, 8513–8525

44 Cunnick, J.M. *et al.* (2000) Requirement of SHP2 binding to Grb2-associated binder-1 for mitogen-activated protein kinase activation in response to lysophosphatidic acid and epidermal growth factor. *J. Biol. Chem.* 275, 13842–13848

45 Itoh, M. *et al.* (2000) Role of Gab1 in heart, placenta, and skin development and growth factor- and cytokine-induced extracellular signal-regulated kinase mitogen-activated protein kinase activation. *Mol. Cell. Biol.* 20, 3695–3704

46 Sachs, M. *et al.* (2000) Essential role of Gab1 for signaling by the c-Met receptor *in vivo*. *J. Cell Biol.* 150, 1375–1384

47 Yart, A. *et al.* (2001) A critical role for phosphoinositide 3-kinase upstream of Gab1 and SHP2 in the activation of ras and mitogen-activated protein kinases by epidermal growth factor. *J. Biol. Chem.* 276, 8856–8864

48 Cunnick, J.M. *et al.* (2002) Regulation of the mitogen-activated protein kinase signaling pathway by SHP2. *J. Biol. Chem.* 277, 9498–9504

49 Feng, G.S. (1999) Shp-2 tyrosine phosphatase: signaling one cell or many. *Exp. Cell Res.* 253, 47–54

50 Zhang, S.Q. *et al.* (2002) Receptor-specific regulation of phosphatidylinositol 3′-kinase activation by the protein tyrosine phosphatase Shp2. *Mol. Cell. Biol.* 22, 4062–4072

51 Liu, Y. *et al.* (2001) Scaffolding protein Gab2 mediates differentiation signaling downstream of Fms receptor tyrosine kinase. *Mol. Cell. Biol.* 21, 3047–3056

52 Nishida, K. *et al.* (2002) Requirement of Gab2 for mast cell development and KitL/c-Kit signaling. *Blood* 99, 1866–1869

53 Herbst, R. *et al.* (1999) Recruitment of the protein tyrosine phosphatase CSW by DOS is an essential step during signaling by the Sevenless receptor tyrosine kinase. *EMBO J.* 18, 6950–6961

54 Cunnick, J.M. *et al.* (2001) Phosphotyrosines 627 and 659 of Gab1 constitute a bisphosphoryl tyrosine-based activation motif (BTAM) conferring binding and activation of SHP2. *J. Biol. Chem.* 276, 24380–24387

55 Gu, H. *et al.* (1997) Characterization of two SHP-2-associated binding proteins and potential substrates in hematopoietic cells. *J. Biol. Chem.* 272, 16421–16430

56 Holgado-Madruga, M. *et al.* (1997) Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell

survival by nerve growth factor. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12419–12424

57 Kong, M. *et al.* (2000) Epidermal growth factor-induced phosphatidylinositol 3-kinase activation and DNA synthesis. Identification of Grb2-associated binder 2 as the major mediator in rat hepatocytes. *J. Biol. Chem.* 275, 36035–36042

58 Gu, H. *et al.* (2001) Essential Role for Gab2 in the allergic response. *Nature* 412, 186–190

59 Garcia-Guzman, M. *et al.* (1999) Met-induced JNK activation is mediated by the adaptor protein Crk and correlates with the Gab1–Crk signaling complex formation. *Oncogene* 18, 7775–7786

60 Crouin, C. *et al.* (2001) A yeast two-hybrid study of human p97/Gab2 interactions with its SH2 domain-containing binding partners. *FEBS Lett.* 495, 148–153

61 Sakkab, D. *et al.* (2000) Signaling of hepatocyte growth factor/scatter factor (HGF) to the small GTPase Rap1 via the large docking protein Gab1 and the adaptor protein CRKL. *J. Biol. Chem.* 275, 10772–10778

62 Lecoq-Lafon, C. *et al.* (1999) Erythropoietin induces the tyrosine phosphorylation of GAB1 and its association with SHC, SHP2, SHIP, and phosphatidylinositol 3-kinase. *Blood* 93, 2578–2585

63 Gual, P. *et al.* (2000) Sustained recruitment of phospholipase C-gamma to Gab1 is required for HGF-induced branching tubulogenesis. *Oncogene* 19, 1509–1518

64 Johnson Hamlet, M.R. and Perkins, L.A. (2001) Analysis of corkscrew signaling in the *Drosophila* epidermal growth factor receptor pathway during myogenesis. *Genetics* 159, 1073–1087

65 Itoh, S. *et al.* (2002) Adaptor molecule Grb2-associated binder 1 is specifically expressed in marginal zone B cells and negatively regulates thymus-independent antigen-2 responses. *J. Immunol.* 168, 5110–5116

66 Sattler, M. *et al.* (2002) Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell.* 1, 479–492

67 Van Etten, R.A. (2001) Pathogenesis and treatment of Ph + leukemia: recent insights from mouse models. *Curr. Opin. Hematol.* 8, 224–230

68 Ong, S.H. *et al.* (2001) ShcA and Grb2 mediate polyoma middle T antigen-induced endothelial transformation and Gab1 tyrosine phosphorylation. *EMBO J.* 20, 6327–6336

69 Fixman, E.D. *et al.* (1997) Efficient cellular transformation by the Met oncoprotein requires a functional Grb2 binding site and correlates with phosphorylation of the Grb2-associated proteins, Cbl and Gab1. *J. Biol. Chem.* 272, 20167–20172

70 Agazie, Y. *et al.* (2002) Concomitant activation of the PI3K-Akt and the Ras-ERK signaling pathways is essential for transformation by the V-SEA tyrosine kinase oncogene. *Oncogene* 21, 697–707

71 Nishigaki, K. *et al.* (2000) Erythroid cells rendered erythropoietin independent by infection with Friend spleen focus-forming virus show constitutive activation of phosphatidylinositol 3-kinase and Akt kinase: involvement of insulin receptor substrate-related adaptor proteins. *J. Virol.* 74, 3037–3045

72 Yamada, K. *et al.* (2001) Comparative FISH mapping of Gab1 and Gab2 genes in human, mouse and rat. *Cytogenet. Cell Genet.* 94, 39–42

73 Schwab, M. (1998) Amplification of oncogenes in human cancer cells. *BioEssays* 20, 473–479

74 Daly, R.J. *et al.* (2002) The docking protein Gab2 is overexpressed and estrogen regulated in human breast cancer. *Oncogene* 21, 5175–5181

75 Kameda, H. *et al.* (2001) Expression of Gab1 lacking the pleckstrin homology domain is associated with neoplastic progression. *Mol. Cell. Biol.* 21, 6895–6905

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