Minireview

The gift of Gab

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Abstract Gab proteins, including mammalian Gab1, Gab2, Gab3, *Drosophila* DOS and *Caenorhabditis elegans* Soc1, comprise a growing family of scaffolding/docking molecules involved in multiple signaling pathways mediated by receptor tyrosine kinases (RTKs) and non-RTK receptors. This paper reviews the structure/function relationships of Gab proteins and their biological roles during normal growth, differentiation and development programs.

Key words: Gab; Tyrosine phosphorylation; Receptor tyrosine kinase; Signal transduction; Differentiation

1. Introduction

The *gab* genes, encoding mammalian Gab1, Gab2, Gab3, the *Drosophila* homolog DOS (Daughter Of Sevenless), and the *Caenorhabditis elegans* homolog Soc1 (Suppressor-Of Clear), define a family of scaffolding proteins closely related to insulin receptor substrates (IRS-1, IRS-2, IRS-3), fibroblast growth factor substrate (FRS2), linker of T cell (LAT) and downstream of kinase (Dok) (reviewed in [1–4]). They are so called scaffolding or docking proteins because of the presence of multiple functional motifs mediating interactions with other signaling molecules. Since the cloning of DOS and Gab1 in 1996, genetic and biochemical approaches have revealed a new family of genes involved in signaling events triggered by a variety of stimuli, including growth factors, cytokines, and T and B cell antigens. These studies also greatly enhanced our knowledge of the mechanisms by which growth factor and cytokine receptors transmit signals to downstream effectors that ultimately regulate cell growth and differentiation. This review will focus on the structure/function relationships of Gab family proteins and their biological roles in growth and differentiation pathways.

2. A family of proteins participating in multiple signaling pathways

Gab1 (Grb2-associated binder 1), the first of three mammalian *gab* genes cloned to date, was originally isolated as a Grb2-binding protein from a human glial tumor expression library and found to be tyrosine phosphorylated in response to epidermal growth factor (EGF) and insulin stimulations [5]. It was also identified independently as a Met-receptor interacting protein in a yeast two-hybrid screen and as the major tyrosine phosphorylated protein in cells transformed by the Tpr-Met oncogene [6,7]. Further studies demonstrated that Gab1 is also involved in a number of other signaling events mediated by the receptors of molecules such as interleukins (ILs), interferons (IFNs), erythropoietin (EPO), and thrombopoietin (TPO) (see Table 1).

Although the cDNA for *gab2* gene was cloned a few years later [8–10], a p97 or a p100 phosphoprotein associated with the protein tyrosine phosphatase SHP2 and the p85 subunit of phosphatidylinositol 3-kinase (PI3K) after IL3 or macrophage colony-stimulating factor (M-CSF) stimulation was recognized earlier [11,12]. This p97 or p100 protein later proved to be the *gab2* gene product [8,13]. The cloning of *gab3* cDNA, on the other hand, was achieved with the aid of a large sequencing project, and isolated based on sequence similarities to Gab1 [14]. Further biochemical and functional studies suggest that Gab3, like its relatives Gab1 and Gab2, is involved in several growth factor and cytokine signaling pathways (see Table 1).

While multiple Gab family members are found in mammalian genomes, DOS is the only identified homolog in *Drosophila*. DOS was cloned as a potential substrate of the protein tyrosine phosphatase Corkscrew (CSW, *Drosophila* homolog of mammalian SHP2) and a suppressor of a constitutively activated Sevenless receptor tyrosine kinase [15,16]. DOS is also required for signaling by other receptor tyrosine kinases (RTKs) such as Torso and DER (*Drosophila* homolog of mammalian EGF receptor), and is essential for normal development throughout the fly [15–18].

Recently, a DOS-like protein involved in fibroblast growth factor (FGF) signaling was found in *C. elegans* [19]. Soc1, identified via mutations that suppress the Clear phenotype resulting from a gain-of-function allele of *let-60 ras*, apparently is the *C. elegans* homolog of mammalian *gab* genes. Structure functional analyses of Soc1 indicate that this protein is also essential for development in the nematode, and acts together with PTP2 (*C. elegans* homolog of mammalian SHP2) to mediate a portion of the EGL-15 (*C. elegans* homolog of mammalian FGF receptor) signaling cascade [19].

3. Functional motifs mediating interactions with other signaling molecules

All Gab family proteins share a common architecture consisting of a highly conserved N-terminal Pleckstrin homology (PH) domain, a central proline-rich domain (PRD) and multi-

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ple tyrosines within potential binding motifs favored by various Src homology 2 (SH2) domain-containing proteins (Fig. 1). Mutagenesis and in vitro/in vivo binding assays have identified a number of molecules that interact with Gab family proteins. Functional analyses in various cell systems have revealed that these interactions are involved in numerous signal transduction pathways.

3.1. PH domain

The PH domain is the most conserved structural element among Gab family members. Several recent studies have shown that PH domains can recognize membrane components, most notably phosphoinositides, and therefore contribute to the membrane targeting of the protein (reviewed in [20]). Indeed, the PH domain of Gab1 binds specifically to phosphatidylinositol 3,4,5-triphosphate (PIP3), a product of PI3K [21–23]. Furthermore, the PH domain of Gab1 was shown to be required for subcellular localization of the protein to the areas of cell–cell contact [22]. Mutants of Gab1 with a deletion of the entire PH domain, or point mutations (W26A/C, R29A/C) at a conserved phospholipid-binding site, were unable to induce Met-dependent branching tubulogenesis in Madin–Darby canine kidney (MDCK) cells [22,24]. These results indicate that the PH domain of Gab1 is responsible for its membrane localization and its function in Met signaling as well. Consistent with findings in mammalian systems, PH domain mutants of *Drosophila* DOS fail to function in Sevenless signaling and to rescue the lethal phenotype of DOS loss-of-function mutant flies [17]. Similarly, a PH domain mutant of *C. elegans* Soc1 (W124A) is non-functional [19]. These data suggest that the function of Gab PH domains is conserved from nematodes to mammals. Interestingly, the PH domain of DOS can be replaced by the PH domain of Gab1, but not by a heterologous membrane anchor, a strategy used to activate Raf by localizing it to the membrane [17,18], suggesting that the PH domain might have additional roles in regulating intracellular signaling rather than acting simply as a localization module.

3.2. PRD

The central region of Gab proteins is rich in prolines and contains numerous PXXP motifs, signatures of the binding sites for Src homology 3 (SH3) domain-containing molecules [25]. In fact, all Gab proteins have been found to constitutively associate with the SH3 domain-containing protein Grb2 [5,8,14]. The binding sites for Grb2 have been mapped on Gab1: a canonical Grb2-binding site with PXXPXR motif and an atypical Grb2-binding site with a PX_3RX_2KP motif [26–28]. Both sites are conserved among all Gab members, as well as several other signaling proteins such as Slp76 and Sos [26–28].

The Met-binding site (MBS) of Gab1, a fragment responsible for the binding of phosphorylated Met receptor, is located within the PRD too (see Fig. 1). Using yeast two-hybrid analysis, it was found that 13 amino acids (GMQVPPPAHMGFR) within the initially identified Gab1 Met-binding domain account for direct association with Met receptor and this region is distinct from the Grb2-binding sites [26]. Interestingly, Gab1 appears to specifically bind to the Met receptor, but does not associate directly with a large number of other receptor tyrosine kinases tested (for example, EGF receptor) [6,27]. On the other hand, Gab2 and Gab3 lack an analogous MBS in their sequences and do no exhibit direct association with Met or other receptors. Therefore, how is Gab1 recruited to receptors other than Met? Also, what is the mechanism by which Gab2 and Gab3 are recruited? The most likely answer is that Gab proteins are recruited to the receptor complexes via an indirect mechanism. The Grb2 adapter protein is one of the candidates to mediate such an indirect mechanism. Grb2 associates with Gab proteins constitutively via its SH3 domains, and binds inducibly to specific phosphotyrosine(s) motifs in a number of receptor tyrosine

Table 1
Mammalian Gab proteins participate in multiple signaling pathways

Receptor		Ligand	Cell type	Gab protein phosphorylated?			Reference
				Gab1	Gab2	Gab3	
RTK	EGF receptor	EGF	A431	yes	yes	?	[5]
	Flt3	Flt3L	BaF3	?	yes	yes	[14]
	Fms	M-CSF	Bac1.2F5, FDFms	?	yes	yes	[8,11]
	insulin receptor	insulin	A431, HepG2	yes	?	?	[5,58]
	Kit	SCF	MO7E, FDFms	yes	yes	?	[9,11]
	Met	HGF	MDCK, A549	yes	?	?	[56,57]
	PDGF receptor	PDGF	NIH3T3	yes	?	?	[9]
	TrkA	NGF	PC12	yes	?	?	[39]
Non-RTK	B cell receptor	anti-IgM or F(ab′)2	Ramos, WEHI-231	yes	yes	?	[8,9,59]
	EPO receptor	EPO	HCD57, UT7	yes	yes	?	[60]
	G-CSF receptor	G-CSF	BAF-B03	?	yes	?	[9]
	GM-CSF receptor	GM-CSF	UT-7	?	yes	?	[11,60]
	gp130	IL-6	HepG2	yes	?	?	[61]
	IFN-α receptor	IFN-α	Hep3B	yes	?	?	[61]
	IFN-γ receptor	IFN-γ	Hep3B	yes	?	?	[61]
	IL-15 receptor	IL-15	T cell, NK3.3	?	yes	?	[62]
	IL-2 receptor	IL-2	Kit225, KT-3	?	yes	?	[8,9]
	IL-3 receptor	IL-3	TF-1, BaF3	yes	yes	yes	[8,9,14,61]
	Mpl	TPO	TF-1, UT-7, MK	yes	yes	?	[9,60,64]
	prolactin R	prolactin	HC11	?	yes	?	[63]
	T cell receptor	anti-CD3	Jurkat	?	yes	?	[8,9]

The references listed are representative, but not exclusive.

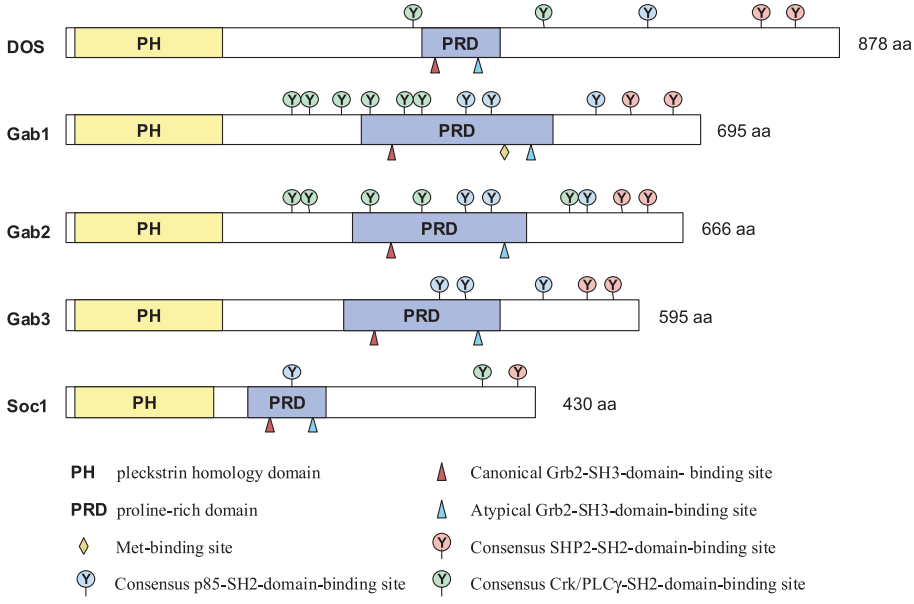

Fig. 1. Gab family proteins. Schematic domain structures of three mammalian Gab proteins (represented by mouse Gab1, 2 and 3), *Drosophila* DOS and *C. elegans* Soc1 are shown. All Gab family proteins consist of a N-terminal PH domain, a central PRD and multiple tyrosines (Y) within potential binding motifs favored by various SH2 domain-containing proteins (as indicated in the figure). The binding site for Grb2 and Met receptor is also indicated.

kinases, such as EGF receptor, Met receptor and Fms receptor. Therefore, Grb2 could carry its associated Gab proteins to the activated receptor complexes upon ligand stimulation. This hypothesis is supported by the fact that the association between Gab1 and EGF receptor is abolished by mutations either at the Grb2-binding site of Gab1 or that of EGF receptor [21–23,27]. Moreover, Gab1 fails to be tyrosine phosphorylated in response to EGF in fibroblasts isolated from mice expressing a null mutant of Grb2 [29], providing genetic evidence that Gab1 is recruited to EGF receptor through a Grb2-dependent mechanism. Interestingly, in IL2 and IL3 receptor signaling, the common β-chain of the receptors does not contain a binding site for Grb2, but does contain a binding site for SHC. Gab2 was found to be recruited to the receptor through a Gab2–Grb2–SHC pathway [30], a mechanism that is also Grb2-dependent but requires a fourth protein to achieve this goal. Apparently, in addition to Grb2, it is likely that other related proteins with SH2 and SH3 domains also might be capable of fulfilling such an adapter function.

Obviously, the direct and indirect mechanisms are not mutually exclusive. Gab1 can be recruited to Met receptor via both direct and indirect means, but to EGF receptor only through an indirect mechanism presumably. Would the difference in Gab1 recruitment account for the different biological activities of Met receptor versus EGF receptor? An interesting observation in the epithelial cell line MDCK is that stimulation with hepatocyte growth factor (HGF) induces prolonged tyrosine phosphorylation of Gab1 (>60 min) and promotes branching morphogenesis of the cells, whereas EGF stimulation only results in transient phosphorylation of Gab1 (15 min), and is unable to induce a morphogenic program [22]. Furthermore, the prolonged Gab1 phosphorylation in response to HGF also correlates with sustained activation of MAPK and Akt activities [31,32]. Although the key regulator responsible for these differences remains elusive, it is certainly worthwhile to investigate further the mechanism by which Gab proteins are recruited to various receptors and how these associations affect the downstream signaling events.

3.3. Multiple phosphotyrosines

Upon stimulation, Gab proteins undergo rapid tyrosine phosphorylation, creating a number of docking sites to mediate interactions with SH2 domain-containing proteins such as the tyrosine phosphatase SHP2 and the p85 subunit of PI3K. Association with these molecules was found to be critical for the function of Gab proteins in mediating intracellular signaling pathways from the receptors.

3.3.1. Gab–SHP2 interaction. One or two tyrosines located in the most C-terminal ends of the Gab family proteins fall within consensus binding motifs (YXXV/I/L) for SHP2, upon tyrosine phosphorylation (see Fig. 1). Indeed, all mammalian Gab proteins, as well as the *Drosophila* DOS and *C. elegans* Soc1, have been shown to bind SHP2 (or its homologs) [8,14,16,19,33–35], indicating that recruitment of SHP2 is a conserved feature that *gab* family genes retained from *C. elegans* to mammalian systems.

The functional significance of the Gab–SHP2 interaction has been extensively studied using mutants of Gab family proteins unable to bind SHP2 or to its homologs. Mutant DOS bearing a Y to F mutation at either of the two CSW-binding sites is unable to function during Sevenless signaling and fails to rescue the lethality associated with *dos* loss-of-function mutations [17,36]. The Gab1 mutant unable to bind SHP2 is defective in delivering a signal for Met-dependent morphogenesis and blocks MAPK activation by EGF and lysophosphatidic acid [26,34,35,37]; the Gab2 mutant unable to bind SHP2 blocked IL3-induced *c-fos* promoter activation and M-CSF-induced macrophage differentiation [8,13]. Similarly, mutant Soc1 unable to bind PTP2 fails to function in EGL15 signaling [19]. Moreover, mutations at tyrosines other than the SHP2-binding sites of DOS and Soc1 have no effect on the function of DOS or Soc1 [19,36]. These

data underscore the importance of Gab–SHP2 interaction and strongly suggest that the primary role of Gab proteins is to recruit SHP2 phosphatase.

How could the Gab–SHP2 interaction lead to activation of downstream signaling? Accumulated evidence indicates that Gab proteins can function to activate SHP2 phosphatase, which in turn activates MAPK signaling [8,13,26,34,35,37]. It was found recently that Y627 and Y659 of human Gab1 constitute a bisphosphoryl tyrosine-based activation motif that binds and activates SHP2 phosphatase [35]. In addition, a mutant DOS or Soc1 containing all tyrosines changed to phenylalanines, except the tyrosine(s) for SHP2-binding, is sufficient to mediate RTK signaling and to rescue the developmental lethality resulting from the loss-of-function mutations [19,36]. These results provide strong evidence that Gab proteins are physiological activators of SHP2 phosphatase. Association between the phosphotyrosine(s) of Gab proteins and the SH2 domains of SHP2 is an essential part of the mechanism that upregulates the phosphatase activity of SHP2, leading to activation of the MAPK cascade and subsequent biological responses.

A key question following this hypothesis is what the substrate(s) and downstream effector(s) are for SHP2 phosphatase. Gab2 and DOS were originally identified as the substrates of SHP2 since they were hyperphosphorylated in the cells expressing the substrate-trapping mutant of SHP2 (a catalytically inactive SHP2 mutant) [12,15]. In vitro phosphatase assays further supported that Gab proteins are substrates of SHP2 [9,12,35], although there is no evidence to date that links dephosphorylation of any phosphotyrosines on Gab proteins to the positive role of SHP2. Interestingly, it was found that an N-SH2 domain deletion mutant of SHP2, which is constitutively active but unable to bind Gab1, failed to rescue the defect of an SHP2-binding mutant of Gab1 in MAPK activation, whereas expression of a chimera with fusion of these two mutant proteins induced constitutive activation of MAPK activity [35]. These results suggest that physical association of Gab1 and SHP2 not only activates SHP2 phosphatase, but may also allow SHP2 to access its substrates. Such a substrate(s), if not Gab itself, would be present, most likely, in the complex of Gab–SHP2 proteins. One potential candidate that falls into this category is a p90 protein, which associates with Gab1 and is hyperphosphorylated in cells expressing an N-SH2 domain deletion mutant of SHP2 [38]. However, the identity of this p90 protein is currently unknown. Apparently, it remains a challenging job to identify the real substrate/effector(s) of SHP2, but this next step is crucial for understanding the mechanism by which SHP2 activates downstream signaling.

3.3.2. Gab–p85 interaction. Another well-defined binding partner of Gab proteins is the p85-subunit of PI3K. Three YXXM motifs (consensus binding site for the SH2 domain of p85) are present in all mammalian Gab proteins but only one such site was found in DOS or Soc1 (see Fig. 1). While the mutant of *Drosophila* DOS or *C. elegans* Soc1 ablating this site does not exhibit functional abnormalities [17,36], mutations at the p85-binding sites of mammalian Gab1 and Gab2 resulted in incapacitation of several signaling systems [30,39–41]. It has been shown that physical association between p85 and Gab1 or Gab2 is crucial in mediating the PI3K/Akt signaling pathway induced by a variety of stimuli [21,22,30,39–44]. Overexpression of Gab1 potentiates FGF-induced Akt activity, whereas overexpression of the p85-binding mutant of Gab1 results in decreased Akt activation [42]. Such a mutant is also unable to provide anti-apoptotic signals in response to nerve growth factor (NGF) stimulation [39]. Similarly, mutations at the p85-binding sites of Gab2 were found to impair the ability of IL-3 to activate Akt and to induce cell growth [30]. These results suggest that while the Gab–SHP2 interaction is important for MAP kinase activation, the Gab–p85 interaction plays a key role in activating the PI3 kinase/Akt pathway in mammalian cells. Moreover, since the activation of PI3K leads to the production of PIP3, which in turn can bind to the PH domain of Gab proteins and presumably promote further activation of PI3 kinases, a positive feedback loop could be formed to amplify the signals through the Gab proteins [21]. Such a positive feedback mechanism could be important for generating a specific biological response in certain signaling pathways.

3.3.3. Other interactions. In addition to the binding sites for SHP2 and p85, both Gab1 and Gab2 contain numerous YXXP motifs, potential binding sites for the SH2 domain of PLCγ or Crk family proteins [45]. Binding of Gab1 to Crk, CrkL and PLCγ have been reported [26,32,46–48]. Interestingly, it has been found that Gab1 associates with Crk in response to Met activation and this association correlates with anchorage-independent growth and JNK (c-Jun N-terminal kinase) activation in cells transformed by the Met receptor oncoprotein [46,47]. On the other hand, Gab2 has been found to interact with CrkL in yeast two-hybrid system and also in human T lymphocytes in response to IL2 [49,50], although the functional significance of the Gab2–CrkL association has not yet been addressed. Notably, potential Crk-binding sites are completely absent in Gab3 protein, implying a possible molecular mechanism for the functional differences among these family members.

4. Developmental requirement of mammalian Gabs

The presence of multiple *gab* genes in the mammalian genome suggests that the function of each Gab protein may be specialized or restricted in certain signaling pathways or tissues. Alternatively, these gene products may have functional redundancy and therefore the ability to compensate for defects of other co-expressed Gab proteins. By Northern blot and RT-PCR analyses, the expression patterns of the three mammalian *gab* genes have been extensively studied [5,6,8,14]. Gab1 appears to exhibit the most widespread expression and abundance. It is found in all tissues examined, including brain, heart, liver, lung kidney pancreas, spleen, thymus and uterus of the adult mouse, and expressed at an earlier stage (ES cell) during development [14]. Gab2 expression is relatively weak in most tissue samples as compared with Gab1. However, it is prominently expressed in a number of hematopoietic progenitor cell lines, such as BAF3, FDC-P1 [5,6,8,14]. The expression pattern of Gab3 is also restricted to the hematopoietic system [5,6,8,14]; it is expressed in spleen, thymus, and a number of hematopoietic cell lines, but very low in solid tissue samples. These results indicate that the three mammalian Gab genes have unique but overlapping expression patterns.

Consistent with early and widespread expression during development, a targeted disruption in Gab1 is embryonic lethal in mice [51,52]. These mice die between E13.5 and E18.5 and

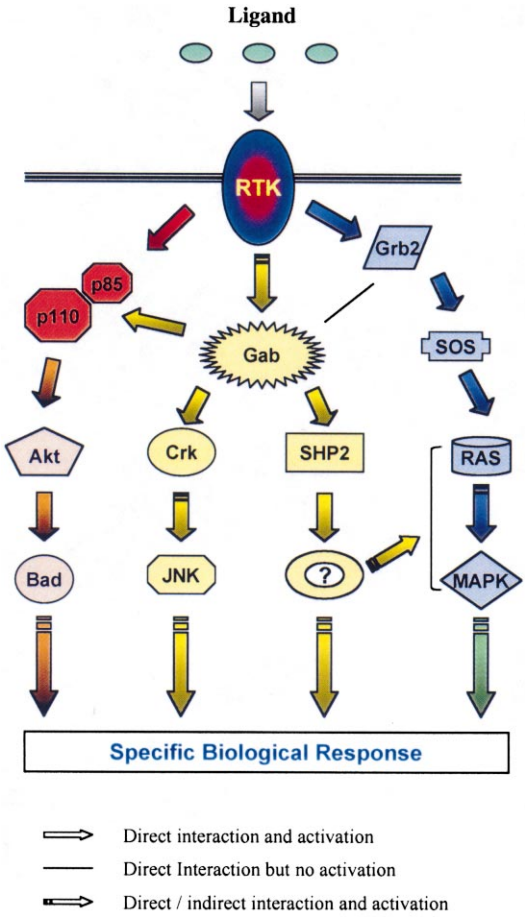

Fig. 2. A model of RTK signaling via Gab-dependent and -independent pathways. Gab proteins, recruited to the activated receptor complexes by direct and/or indirect means, are phosphorylated by receptor tyrosine kinases (or receptor-associated tyrosine kinases for non-RTK receptors). Phosphorylated Gab proteins bind to SHP2, p85 and Crk family proteins and induce subsequent activation of MAPK, PI3K and JNK pathway respectively. The total MAPK activities could result from the activation via a Gab–SHP2–? (an unknown protein) pathway (yellow arrow), or a Gab-independent classical Grb2–SOS–RAS pathway (blue arrows), or the blend of both (green arrow), depending on the receptor and the cell context. Similarly, the total PI3K activities could be contributed by the activation via Gab–p85 interaction (yellow arrow) or via direct association between the receptor and p85 (red arrow), or both (orange). The combination of the signals from both Gab-dependent and -independent pathways contribute to specific biological responses, which are crucial for the normal growth, differentiation and development programs.

display developmental defects in the heart, placenta and skin. Furthermore, Gab1$^{-/-}$ fibroblasts have markedly reduced MAPK activity in response to IL-6, EGF or PDGF stimulation [51], suggesting a role for Gab1 in mediating MAPK activity via multiple growth factor receptors.

Among Gab family members, Gab1 is unique in its ability to directly associate with the phosphorylated Met receptor. Interestingly, Gab1-deficient mice have a phenotype reminiscent of those mice harboring mutations in the HGF and c-Met genes. These mice die at similar embryonic stage as the Gab1$^{-/-}$ animals, due to a severe deficit in development of the placenta; they all display a reduced liver size and have defects in the migration of muscle precursor cells [52–55]. These results provide genetic evidence that Gab1 is essential for HGF-Met signaling, and Gab2 and/or Gab3 are unable to compensate for the function of Gab1 in vivo.

Mice with targeted disruption of the *gab2* gene have also been generated. Gab2 protein is quite abundant in hematopoietic cells and the functional importance of Gab2 in IL2, IL3 and M-CSF signaling pathways has been well established in tissue culture systems [8,13]. Intriguingly, no apparent defects in the development of hematopoietic lineages were observed in Gab2 knock-out mice [44]. These mice develop normally and are generally healthy. However, closer examination demonstrates that these mice have impaired allergic reactions. IgE receptor signaling is defective in bone marrow-derived mast cells from Gab2 $^{-/-}$ mice, due mainly to the inability to induce PI3K activation in these cells. Clearly, Gab2 has at least some distinct functions from Gab1 in vivo.

The lack of an apparent developmental phenotype of Gab2-deficient mice has raised a number of questions. First, are the Gab2 knock-out mice generated really Gab2 null mice? Although Gab2 targeting vector was constructed to delete the first exon of Gab2, presumably eliminating the gene product of *gab2*, a truncated protein, possibly arising from a cryptic promoter in the first intron of the Gab2 gene, was found in Gab2 $^{-/-}$ cells [44], suggesting that this truncated protein may partially compensate for full length Gab2. Second, could compensation by other Gab family members account for the lack of phenotype in the Gab2 knock-out animals? Since Gab1 is more abundant than Gab2 in most tissues and Gab3 is co-expressed with Gab2 in most hematopoietic cells, it is possible that the loss of Gab2 function can be compensated by other Gab proteins. Moreover, the genome sequence project has uncovered more Gab members in the mammalian genomes (unpublished observations). Therefore, continued efforts will be required to elucidate the in vivo function of each Gab member, and understand their individual roles in growth and differentiation signaling.

5. Concluding remarks

In vitro and in vivo studies of Gab family proteins during the last few years have revealed a novel mechanism that links RTK and non-TK receptors to several downstream signaling pathways. As shown in the proposed model (Fig. 2), Gab proteins, recruited to the activated receptor complexes by direct and/or indirect means, are phosphorylated by receptor tyrosine kinases (or receptor-associated tyrosine kinases for non-RTK receptors), and subsequently activate MAP kinase, PI3 kinase and JNK pathways via direct association with SHP2, p85 and Crk family proteins. The signals activated through Gab proteins and signals activated via Gab-independent pathways may merge to generate specific biological responses that are crucial for the normal growth, differentiation and development programs.

One central, enduring question regarding the signaling of receptor tyrosine kinases is: how is the specificity of each receptor tyrosine kinase generated? The discovery of Gab family genes provides new clues toward generating such specificity. First, different receptors might adopt different ways to recruit Gab proteins. Depending on the affinity between the receptor and Gab protein, different signaling kinetics could be generated, which could result in different biological responses. This is exemplified by the studies of Gab1 recruitment mechanisms and the different cellular responses to HGF and EGF

in MDCK cells [22,27,31,32]. Secondly, integration of signals generated by Gab protein and other activators could result in specific biological effects. Many receptors, such as Fms, directly associate with Grb2, p85, and other signaling molecules. While these proteins can facilitate the recruitment of Gab protein and result in subsequent activation of signaling events through Gab, they can also transmit signals independently. For example, binding of Grb2 to an activated receptor will trigger MAPK activation via a Grb2–SOS–RAS pathway. Although Gab proteins bind to Grb2 directly, mutational analyses in both Soc1 and DOS have shown that the MAPK activation from Gab proteins requires their associations with SHP2, but not Grb2 [17,36]. Therefore, signals via Gab–SHP2 and Grb2–SOS–RAS pathways could join together to activate MAPK pathway and result in specific biological response due to the additive or synergistic effects. A similar scenario may apply to Gab-dependent and -independent activation of PI3K and JNK pathways. Finally, the presence of three or more Gab members in mammalian cells provides further complexity and presumably specificity for RTK or non-RTK signaling. Although the functional differences among the three members remain ambiguous so far, there is clearly a structural basis for their disparities. Gab1 contains a unique MBS, which has been shown to be critical for Gab1-mediated branching morphogenesis in Met receptor signaling. On the other hand, Gab3 does not contain any potential Crk/CrkL-binding site, implicating its inability to mediate Crk-dependent JNK activation. Moreover, in a physiological cell context, a variety of receptors and more than one Gab protein might be co-expressed or expressed in a certain order, evidenced by the induction of Gab3 protein after activation of Fms receptor and Gab2 in FD-Fms cells [14]. Therefore, simultaneous or sequential activation of the receptors and Gab proteins could lead to qualitatively and quantitatively different signal output, which confers specific messages in the control of cell growth and differentiation during the development of multicellular organisms. These new molecular insights are but one fabulous gift we have received from the Gabs.

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