the FEBS Journal

MINIREVIEW

Docking proteins

Tilman Brummer[1,2,3], Carsten Schmitz-Peiffer[4] and Roger J. Daly[5]

1 Centre for Biological Systems Analysis (ZBSA), Albert-Ludwigs-University of Freiburg, Germany
2 Institute for Biology III, Albert-Ludwigs-University of Freiburg, Germany
3 Centre for Biological Signalling Studies, Albert-Ludwigs-University of Freiburg, Germany
4 Diabetes and Obesity Program Garvan Institute of Medical Research, Sydney, Australia
5 Cancer Research Program, Garvan Institute of Medical Research, Sydney, Australia

Keywords
Dok; DOS; FRS2; Gab; IRS; protein modules; protein phosphorylation; SH2; signal transduction; tyrosine kinase

Correspondence
R. J. Daly, Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW 2010, Australia
Fax: +61 2 9295 8321
Tel: +61 2 9295 8333
E-mail: r.daly@garvan.org.au

(Received 19 May 2010, revised 1 August 2010, accepted 23 August 2010)

doi:10.1111/j.1742-4658.2010.07865.x

Docking proteins comprise a distinct category of intracellular, noncatalytic signalling protein, that function downstream of a variety of receptor and receptor-associated tyrosine kinases and regulate diverse physiological and pathological processes. The growth factor receptor bound 2-associated binder/Daughter of Sevenless, insulin receptor substrate, fibroblast growth factor receptor substrate 2 and downstream of tyrosine kinases protein families fall into this category. This minireview focuses on the structure, function and regulation of these proteins.

Overview

In a review on receptor tyrosine kinase (RTK) signalling published a decade ago [1], docking proteins were classified as signal transducers that exhibit a membrane targeting region at the N-terminus, and multiple tyrosine phosphorylation sites that function as binding sites for src homology (SH)2 domains of a variety of downstream effectors. Proteins that fell into this category were members of the growth factor receptor bound (Grb)2-associated binder (Gab)/Daughter of Sevenless (DOS), insulin receptor substrate (IRS), fibroblast growth factor (FGF) receptor substrate (FRS)2 and downstream of tyrosine kinases (Dok) families. Also, linker for activated T cells (LAT) was classified as a docking protein, although the presence of a transmembrane region raises the issue of whether LAT and other related proteins (e.g. non-T-cell activation linker [2]) should be classified separately. In this context, it is noteworthy that other transmembrane proteins containing tyrosine phosphorylation-dependent recruitment sites (e.g. those with immunoreceptor tyrosine-based activation motifs) are not routinely classified as docking proteins. Another protein that has

Abbreviations
Dok, downstream of tyrosine kinases; DOS, Daughter of Sevenless; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FRS, FGF receptor substrate; Gab, Grb2-associated binder; Grb, growth factor receptor bound; IGF, insulin-like growth factor; IL, interleukin; IRS, insulin receptor substrate; LAT, linker for activated T cells; PH, pleckstrin homology; PKC, protein kinase C; PTB, phosphotyrosine binding; PtdIns, phosphatidylinositol; RTK, receptor tyrosine kinase; SH, src homology.

been described as a docking protein in the literature is p130Cas [3]. This protein contains a large number of tyrosine phosphorylation sites that mediate effector recruitment, but exhibits an N-terminally located SH3 domain rather than a motif/domain for plasma membrane localization. However, the SH3 domain does target Cas to a specific subcellular location, in this case focal adhesions by virtue of its interaction with focal adhesion kinase [4].

How does one distinguish between a docking protein, on the one hand, and an adaptor protein, on the other hand? This is somewhat arbitrary, because in certain contexts docking proteins perform an adaptor function: they establish a direct or indirect linkage between an activated tyrosine kinase or another type of tyrosine-phosphorylated protein and SH2 or phosphotyrosine-binding (PTB) domain-containing effectors. In addition, particular proteins commonly referred to as adaptors (e.g. SLP65) [5] may exhibit characteristics of docking proteins, in possessing multiple tyrosine phosphorylation sites that mediate protein–protein interactions. In order to focus this minireview, we have chosen to define docking proteins as intracellular proteins that contain an N-terminal motif or domain for direct membrane association (e.g. a pleckstrin homology [PH] domain or myristoylation sequence) and a large number (> 5) of tyrosine phosphorylation sites for effector recruitment. Based on these criteria, we have classified members of the Gab/DOS, IRS, FRS2 and Dok families as 'classical' docking proteins (Fig. 1A). We accept that particular members of a docking protein family may not fulfil both these criteria (e.g. Dok-5 has only three tyrosine phosphorylation sites), however, each of the identified families contains one or more proteins that do. In addition, other proteins also mediate a 'docking' function (i.e. multivalent recruitment of SH2-domain containing effectors) (Fig. 1B), but do not fulfil our criteria in terms of structural characteristics or numbers of binding sites.

What are the functional characteristics of docking proteins? In general, these proteins are recruited to sites of tyrosine kinase activation by two broad mechanisms, the first involving interaction with the plasma membrane, and the second, protein–protein interactions. With regard to the first mechanism, plasma membrane localization may reflect myristoylation of the docking protein, as in the case of FRS2 proteins [6], or recruitment by PH domain-mediated binding to specific phospholipid second messengers, as exemplified by Gab proteins [7]. In terms of the second mechanism, a

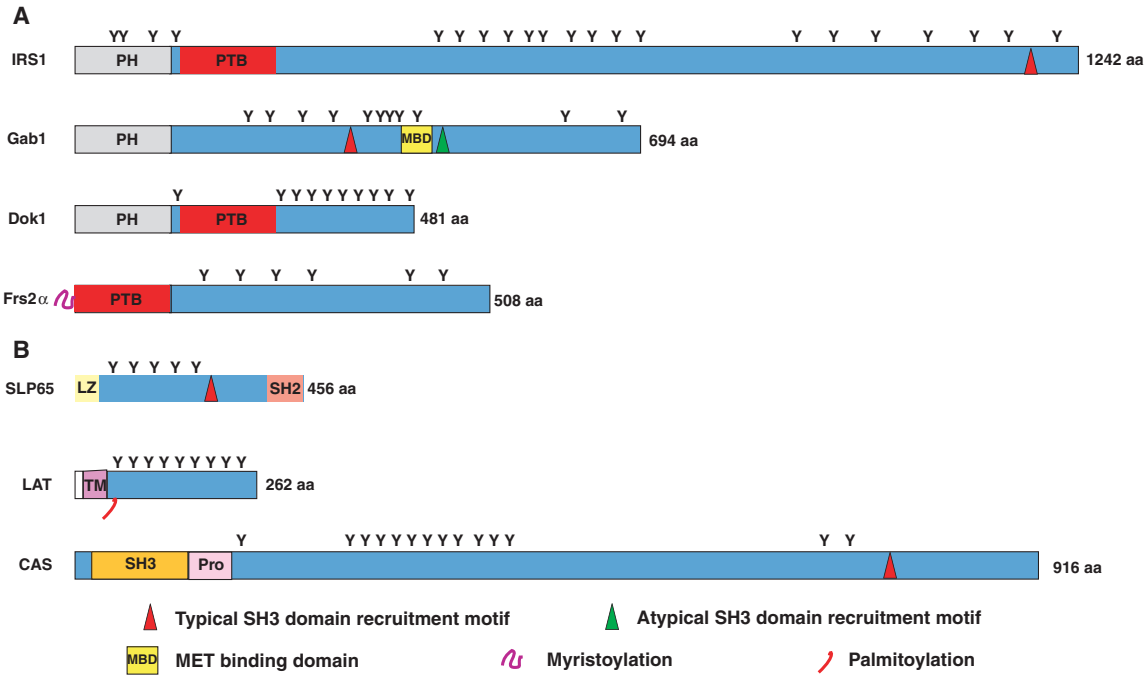

Fig. 1. Schematic representation of docking protein families. (A) Classical docking proteins. The founding members of the IRS, Gab, Dok and FRS2 families are shown. Binding motifs and fatty acid attachment sites mentioned in the text are indicated. Y, tyrosine phosphorylation site; Pro, proline-rich region; TM, transmembrane domain; LZ, leucine zipper domain; aa, length in amino acids. (B) Examples of other signalling proteins with similarities to docking proteins.

docking protein may bind a tyrosine-phosphorylated receptor either directly (e.g. via an interactive protein module, such as a PTB domain) or via one or more accessory adaptor proteins, such as Grb2. The docking protein is then phosphorylated on multiple tyrosine residues, leading to the recruitment of specific SH2 and/or PTB domain-containing proteins, determined by the sequence context of the phosphorylated tyrosine residue. This leads to the activation of one or more signalling pathways, or their modulation. Thus, docking proteins function as 'assembly platforms' for the activation, coordination and regulation of tyrosine kinase signalling events in specific subcellular compartments (Fig. 2). Finally, in addition to tyrosine phosphorylation, these proteins are also subject to serine/threonine phosphorylation, and this may mediate positive or negative effects on signal output, as well as cross-talk with other signalling systems [6,7]. In the following sections, we focus on individual families of docking proteins and review their structure, signalling and physiological functions, and regulation.

The Gab/DOS family

The Gab/DOS family currently contains five members that have been functionally characterized, which are Gab1–3 in vertebrates, DOS in *Drosophila* and Suppressor of Clear (SOC)-1 in *Caenorhabditis* [7]. These proteins contain a N-terminal PH domain, multiple tyrosine phosphorylation sites and canonical and/or atypical binding sites for the C-terminal SH3 domain of the adaptor protein Grb2. In addition, Gab1 contains a 16-amino acid motif that mediates direct binding to the activated kinase domain of c-Met [8]. The PH domain of Gab1 and Gab2 binds to phosphatidylinositol (PtdIns)3-kinase-generated PtdIns3,4,5P_3 and thereby recruits these docking proteins to the plasma membrane in the vicinity of activated receptors [9].

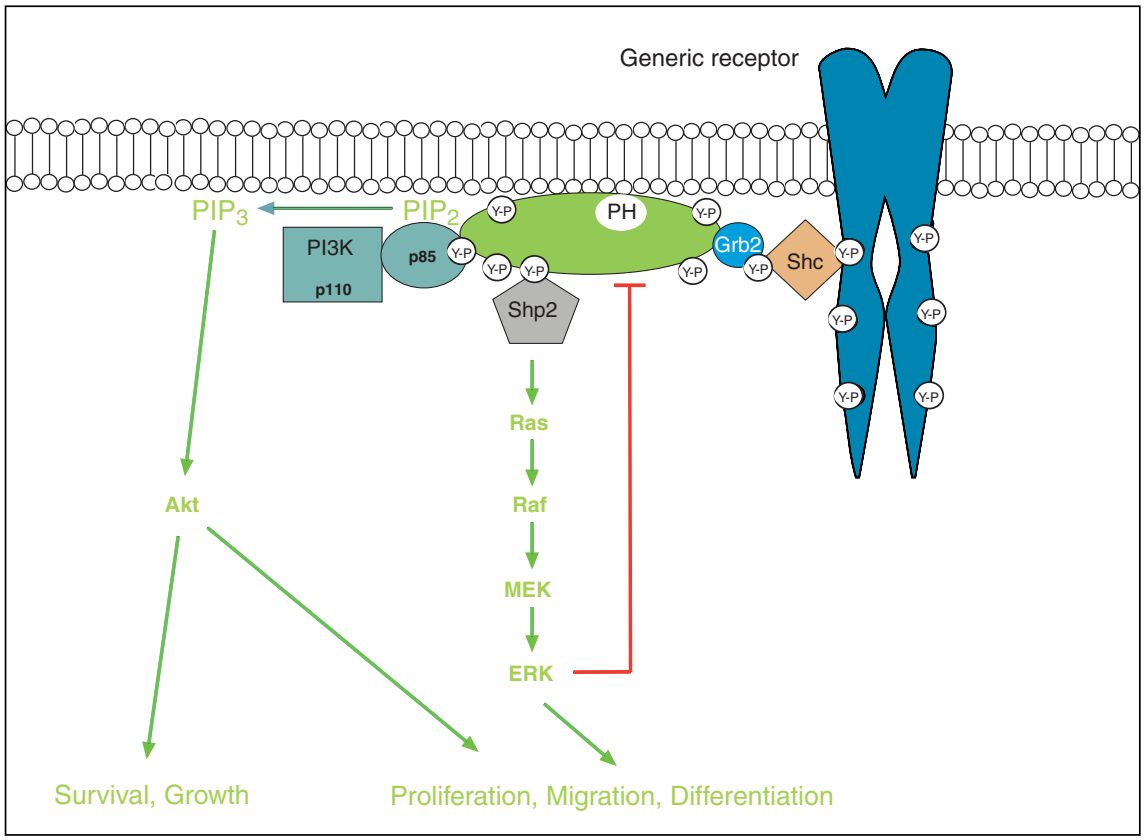

Fig. 2. Docking protein signalling. Summary of the functional characteristics of a docking protein: recruitment to the plasma membrane via an N-terminal domain/modification (in this case a PH domain); association with an activated receptor via protein–protein interactions (in this case, via the Grb2/Shc complex); phosphorylation on multiple tyrosine residues, leading to the recruitment of specific effectors via their SH2 domains and regulation of particular downstream pathways (in this case, recruitment of p85 and Shp2 are shown); negative feedback regulation of signalling mediated by serine/threonine phosphorylation of the docking protein by downstream kinases (in this case, Erk). Green arrows indicate activation, the red bar, inhibition. For details refer to text.

Although Gab1 can bind c-Met directly, it also interacts indirectly via Grb2 [10]. The physiological significance of the indirect recruitment mode was recently highlighted by generation of knockin mice expressing a Gab1 mutant defective in Grb2 binding. These mice exhibit an embryonic lethal phenotype and impaired placental, liver and craniofacial development [11]. Aside from Met, all other receptors that couple to Gab proteins do so via Grb2 [7].

Gab proteins are tyrosine phosphorylated following activation of diverse receptor types, including specific RTKs, B- and T-cell antigen receptors and β1-integrin, and depending on the cellular context, this may be mediated by the RTK itself, and/or kinases of the Src, Syk/ZAP-70 or JAK families [7]. Interestingly, a recent study reported that c-Src and c-Met exhibit contrasting selectivity towards particular Gab1 phosphorylation sites, and that phosphorylation of four tyrosine residues by c-Src contributes to hepatocyte growth factor-induced DNA synthesis [12]. Gab/DOS tyrosine phosphorylation leads to binding of specific SH2 domain-containing effectors, the best-characterized of which is the SH2 domain-containing protein tyrosine phosphatase Shp2. Signalling via this phosphatase leads to more sustained and/or increased activation of the Ras/Erk pathway [13–16]. In addition, it also promotes Rac or PtdIns3-kinase activation through mechanisms that are incompletely characterized [17–19]. In cell culture models, Gab/Shp2 coupling regulates diverse biological endpoints, ranging from cell migration to epithelial morphogenesis [14,17,19,20]. Knockin mice expressing a Gab1 mutant that cannot recruit Shp2 exhibit impaired muscle and placental development, highlighting the importance of this pathway *in vivo* [11]. In addition to Shp2, particular Gab proteins bind other SH2 domain-containing proteins, including the p85 subunit of PtdIns3-kinase, phospholipase Cγ isoforms, and the Crk and Nck adaptors [7,21]. In mice, Gab1 coupling to PtdIns3-kinase is required for eyelid closure during embryonic development and keratinocyte migration [11]. Furthermore, the impaired allergic responses of *Gab2* gene knockout mice reflect the requirement for Gab2 in activation of PtdIns3-kinase downstream of FcεRI [22]. Binding of the adaptor Crk enables Gab proteins to regulate the low molecular mass G proteins Rac and Rap, and in turn, processes such as cell motility, invasion and transformation [23–26].

Signalling by Gab/DOS proteins is also subject to negative regulation. Shp2 and its *Drosophila* orthologue Corkscrew are known to dephosphorylate specific tyrosine residues on Gab1 and DOS, respectively [7], and in the case of Gab1 this negatively regulates binding of the p85 subunit of PtdIns3-kinase [27]. In addition, Gab proteins are subject to negative feedback regulation by serine/threonine phosphorylation [7]. This has been studied in the greatest detail for Gab2. This docking protein is phosphorylated on S623 by Erk, which antagonizes Shp2 recruitment [28]. In addition, it is phosphorylated on S159 by Akt [29] and on S210 and T391 by uncharacterised kinases [30]. The potency of these negative regulatory events is highlighted by the transforming potential of Gab2 S159A and S210A/T391A mutants [29,30]. In the case of S210 and T391, phosphorylation of these sites leads to 14-3-3 protein binding, which uncouples Gab2 from the activated receptor complex [30].

The physiological roles of each of the mammalian Gab proteins have been interrogated by gene knockout studies in mice. Gab1 deficiency results in embryonic lethality because of defects in development of several tissues, including muscle [31,32]. This, in part, reflects the critical role of this docking protein in hepatocyte growth factor/Met signalling. By contrast, Gab2 and Gab3 gene knockout mice are viable. Whereas the latter do not exhibit a detectable phenotype [33], the former display impaired allergic responses [22], osteopetrosis (bone thickening, reflecting the role of receptor activator of NFκB/Gab2 signalling in osteoclast differentiation) [34] and abnormal haematopoiesis (reflecting important functions for Gab2 in cytokine signalling) [35]. Interestingly, Gab1/2 double knockouts exhibit cardiac insufficiency because of the role of both proteins in neuregulin-1β signalling [36].

In terms of the potential involvement of Gab proteins in human disease, *GAB2* allelic variation has been associated with Alzheimer's disease susceptibility [37]. Moreover, Gab2 is strongly implicated in several cancer types [7]. The *GAB2* gene is amplified and/or overexpressed in human gastric, ovarian and breast cancers, as well as in acute myeloid leukemia and metastatic melanoma [38–46]. Strong evidence that Gab2 plays a functional role in the development and/or progression of certain malignancies has been obtained from the use of transgenic and knockout mouse models. Such approaches have demonstrated that Gab2 is required for transformation of myeloid progenitors by the chronic myeloid leukemia-associated oncoprotein Bcr-Abl [47], and that Gab2 promotes erbB2-induced mammary tumour formation [39] and metastasis [48].

The FRS2 family

The FRS2 family has two mammalian members, FRS2α and FRS2β [6]. Both contain a consensus myristoylation sequence, and myristoylation of FRS2α is

required for plasma membrane localization of this docking protein [49]. This modification also targets FRS2 to cholesterol-rich plasma membrane microdomains termed lipid rafts, and in the case of RET signalling, this results in enrichment of the RET/FRS2 complex in these subdomains [50,51]. In addition, these proteins contain a PTB domain and six (FRS2α) or five (FRS2β) tyrosine phosphorylation sites. FRS2 proteins signal downstream of a more limited spectrum of cell-surface receptors or receptor complexes than the Gab family, being phosphorylated following activation of FGF receptors (FGFRs), the neurotrophin receptors TrkA and TrkB, RET and ALK [6]. However, the *Xenopus* orthologue xFRS2 associates with the Src family kinase Laloo and is required for mesoderm induction by this kinase [52,53], indicating that FRS2 proteins, like Gab proteins, may also act as substrates for particular cytoplasmic tyrosine kinases. The PTB domain of FRS2 proteins mediates direct interaction with certain tyrosine kinases, but exhibits an interesting target selectivity, binding to an unphosphorylated amino acid sequence in the juxtamembrane region of FGFR1, so that recruitment to this receptor is constitutive and FGF independent, whereas association with neurotrophin receptors or RET is mediated via phosphorylated NPXY motifs (X = any amino acid) and is dependent on receptor activation [54–56]. In addition, the PTB domain of FRS2β binds constitutively to the epidermal growth factor receptor (EGFR). However, FRS2 proteins are poor substrates for this receptor, and a FRS2β–Erk2 complex has been proposed to inhibit EGFR signalling [57].

FRS2α and FRS2β contain four and three tyrosine phosphorylation sites, respectively, that bind the SH2 domain of Grb2, and the remaining tyrosine phosphorylation sites on both proteins recruit Shp2 [6]. The combined action of Grb2, which binds the guanine nucleotide exchange factor for Ras, Sos, via the Grb2 N-terminal SH3 domain, and Shp2, leads to sustained activation of the Ras/Erk pathway [58]. However, Grb2 is a versatile adaptor given its size, and it also couples FRS2 proteins to Gab1 and hence PtdIns3-kinase signalling [59], as well as to the E3 ubiquitin ligase Cbl that promotes ubiquitylation of the FGFR and FRS2α and downregulation of the FGFR [60]. Other, less-well characterized binding partners for FRS2 proteins are Cks1 (a cell-cycle regulator) and Rnd1 (which antagonizes RhoA signalling) [61,62]. In addition, signalling via FRS2 is required for FGF-induced tyrosine phosphorylation of Sprouty 2, which is mediated by Src family kinases and represents a negative feedback mechanism for attenuation of Erk activation [63].

Whereas Gab2 is subject to negative regulation by phosphorylation on both serine and threonine residues [29,30], negative feedback regulation of FRS2α occurs predominantly on threonine residues [64]. FRS2α contains eight threonine residues within consensus motifs for phosphorylation by Erk, and a FRS2α mutant containing valine substitutions at these sites exhibits enhanced FGF-induced tyrosine phosphorylation and enhances mitogenic and motogenic responses to this growth factor [64]. Interestingly, threonine phosphorylation of FRS2α occurs in response to a variety of growth factors, including those that do not promote tyrosine phosphorylation of this docking protein, indicating that it allows for cross-modulation of cellular responses. Of note, FRS2β is not subject to this regulatory mechanism. Such specificity of negative feedback control processes within a docking protein family is also observed with Gab proteins, where signalling by Gab2, but not Gab1, is attenuated by 14-3-3 binding [30].

Gene knockout and knockin strategies have been utilized to determine the physiological role of FRS2α and its effector pathways. Ablation of FRS2α results in lethality by embryonic day 8, reflecting the critical role of particular FGFs in embryonic development [65]. Interestingly, mice expressing a form of FRS2α lacking the two Shp2 binding sites also exhibit severe phenotypic effects, including defective development of the eye and cerebral cortex, and suffer perinatal lethality [66]. The effects on eye development are associated with reduced Erk activation in the primordial eye. By contrast, ablation of the four Grb2 binding sites in FRS2α exerts relatively mild effects, with some of the corresponding knockin mice being viable and only exhibiting eyelid developmental defects [66].

Aberrant expression of FRS2 proteins has been detected in certain human malignancies. Amplification and overexpression of the FRS2α gene occurs in glioblastoma [67] and in a liposarcoma cell line [68]. By contrast, expression of FRS2β is downregulated in brain and lung cancer cell lines compared with normal controls, which may reflect its ability to negatively regulate signalling by the EGFR [57].

The IRS family

Six proteins have been assigned to the IRS family of docking proteins (IRS-1–6), based on the presence of N-terminal PH and PTB domains (Fig. 1) and on their tyrosine phosphorylation by insulin and insulin-like growth factor 1 receptors (IR and IGF-1R). IRS-1–4 also possess C-terminal regions responsible for the recruitment of specific SH2 domain proteins. By

contrast, the more distantly-related IRS-5 (Dok4) and IRS-6 (Dok5) have truncated C-termini [69] and are relatively weakly phosphorylated in response to insulin [70]. These are discussed with other Dok family proteins below.

IRS-1 is the prototype member of the family [71] and remains the best characterized. Deletion of either the PH domain or PTB domain of IRS-1 reduces its interaction with the IR [72]. The PH domain appears to exert two functions, localizing IRS proteins to the plasma membrane in either an insulin-inducible (IRS-1/2) or constitutive (IRS-3) fashion through binding to specific 3′-phosphorylated phosphoinositides [73], and promoting interaction with the IR [74]. The PTB domain of IRS-1 interacts with specific phosphorylated NPXY motifs, such as Y972 of the IR [75], further stabilizing the complex of docking protein and activated receptor. A kinase regulatory-loop binding domain has been identified only in IRS-2, containing two key tyrosine residues (Y624 and Y628) [76]. Although in their unphosphorylated form these residues were thought to stabilize the IR–IRS-2 complex, the kinase regulatory-loop binding domain has now been structurally defined as a disordered region which is phosphorylated by the receptor with a slow turnover rate, and most likely plays a novel inhibitory role [77]. Tyrosine phosphorylation of IRS proteins can also be catalysed by nonreceptor kinases. For example, interleukin (IL)-4 stimulation leads to phosphorylation of the IL-4Rα subunit on a NPXY motif homologous to that found in the IR and IGF-1R, binding of IRS-1 and IRS-2 to this phosphorylated motif via their PTB domains, and phosphorylation of these docking proteins by receptor-associated JAK kinases [78].

Upon association with activated receptors, IRS proteins become tyrosine-phosphorylated on multiple sites. IRS-1 has more than 20 potential tyrosine phosphorylation sites, almost all located C-terminal to the PH and PTB domains, and these include 9 within YXXM sequences that represent preferred binding sites for the SH2 domains of the p85 subunit of PtdIns3-kinase [79]. Consistent with the presence of the latter motifs, the most prominent and best-characterized signalling pathway downstream of IRS-1 is activation of PtdIns3-kinase. Maximal activation of this enzyme is promoted by occupancy of the two p85 SH2 domains by closely located IRS-1 YMXM motifs [80]. In turn, PtdIns3-kinase stimulates a variety of effectors including Akt, atypical protein kinase C (aPKC) enzymes and mTOR/S6K [81]. These effectors are mostly responsible for the effects of insulin and IGF-1 on glucose disposal, protein synthesis and lipid metabolism [81,82]. For example, Akt promotes plasma membrane translocation of the glucose transporter GLUT4 via phosphorylation of targets that include the Rab GTPase-activating protein AS160, and enhances glycogen synthesis via phosphorylation of glycogen synthase kinase 3 [81]. Other SH2 domain-containing binding partners of IRS-1 are Grb2, which promotes mitogenesis via the Ras/Erk pathway [83] and Nck, involved in reorganization of the actin cytoskeleton [84]. In addition, like Gab and FRS2 proteins, IRS-1 binds Shp2, and this phosphatase may play multiple roles in insulin and IGF-1 signalling. For example, whereas Shp2 mediates negative regulation through dephosphorylation of IRS-1 [85], ablation of Shp2 or inhibition of its signalling function causes defects in insulin action at the level of Akt and aPKCζ, and in subsequent glucose disposal [86,87].

IRS-1 and IRS-2 are widely expressed, whereas IRS-4 has a more limited tissue distribution, being expressed mainly in the pituitary and thyroid glands [88]. Although IRS-3 is expressed in rodent tissues [89], humans do not possess a functional *IRS-3* gene [90]. Although IRS-1 and IRS-2 have overlapping roles, distinct phenotypes are observed upon genetic ablation. IRS-1 deletion results in retarded growth and reduced glucose disposal by insulin target tissues such as skeletal muscle [91]. By contrast, IRS-2-deficient mice develop both defective insulin secretion and insulin resistance, because of additional defects in IGF-1R-dependent pancreatic β-cell development as well as on peripheral insulin action [92,93]. IRS-3-deficient mice exhibit no defects in growth, insulin signalling or glucose homeostasis [94]. There appear to be cell-type and tissue-specific differences in the coupling of IRS-1 and IRS-2 to glucose and lipid metabolism, which can be mediated by Akt2 but also by aPKCι/λ [95,96]. The role of IRS-1 and IRS-2 in signalling downstream of cytokine receptors such as IL-4R is to promote PtdIns3-kinase and Ras/Erk activation, leading to mitogenic effects in immune cells [78].

Serine phosphorylation of IRS proteins represents a normal inhibitory feedback mechanism that can be aberrantly induced in a chronic setting, leading to defective signalling and insulin resistance in peripheral tissues [97]. Under normal conditions, IRS-1 serine phosphorylation occurs subsequent to tyrosine phosphorylation, disrupting IRS-1–receptor and IRS-1–membrane interactions to reduce IRS-1 tyrosine phosphorylation and downstream signalling. Chronic insulin stimulation can lead to degradation of IRS-1, and a recent study reported that the CUL7 E3 ubiquitin ligase recognizes serine-phosphorylated forms of IRS-1 generated by mTOR/S6K and mediates polyubiquitylation of this docking protein, thereby targeting

it for proteasomal destruction [98]. The kinases responsible for negative feedback regulation of IRS-1 include the downstream effectors of insulin signalling S6K and aPKCζ. In addition, chronic conditions or treatments that lead to insulin resistance, such as lipid oversupply, inflammation and exposure to cellular stressors, lead to serine/threonine phosphorylation of IRS-1 and inhibition of downstream signalling. This can be mediated via the aforementioned kinases, as well as JNK [99], IKKβ [100], glycogen synthase kinase 3 [101] and several classical and novel PKC isoforms [102]. Numerous sites of serine phosphorylation have been identified, and the effects of their phosphorylation tend to correspond with their proximity to functional domains [97]. Thus S307, a key target for JNK-mediated phosphorylation, is close to the PTB domain and phosphorylation at this site reduces binding of IRS-1 with the IR [103], S24 phosphorylation by PKC reduces PH domain interaction with the plasma membrane [104], and serine phosphorylation close to C-terminal YXXM motifs reduces the recruitment of SH2-containing proteins such as p85α [105,106]. Fewer serine/threonine phosphorylation sites have been characterized on IRS-2, but there appear to be subtle differences. For example, aPKCζ fails to phosphorylate IRS-2, which may allow distinct functions to proceed [107]. Other post-translational mechanisms of IRS regulation include O-linked glycosylation [108,109] and acetylation [110,111].

Overexpression of one or more members of the IRS family relative to normal tissue has been detected in certain cancers, such as breast, hepatocellular, pancreatic and prostate cancer [112]. Hormone- and growth factor-dependent upregulation has been described, and this can be mediated by a variety of transcription factors depending on the IRS protein and context, including oestrogen and progesterone receptors, CREB, FOXO1, FOXO3a and AP1. Conversely, the tumour suppressor BRCA1 and also specific microRNAs can suppress IRS-1 expression [112]. Interestingly, use of gene knockout models has demonstrated a requirement for IRS-2 in metastatic progression of mouse mammary tumours [113].

The Dok family

The mammalian Dok protein family comprises seven members. Dok1–3 and Dok4–7 form two separate subfamilies, although Dok7 deviates considerably from the rest of its subfamily [114]. Dok proteins have also been identified in *Drosophila* [115]. The founding member of this family, Dok1, was originally cloned as an interaction partner of the oncogenic kinases v-Abl and Bcr-Abl [116,117]. All Dok proteins are characterized by an N-terminal PH domain followed by a PTB domain (Fig. 1). The importance of the PH domain for membrane recruitment has been demonstrated for all Dok family members [118–125]. By binding to specific phosphotyrosine residues within activated receptors, e.g. RTKs, the PTB domain also contributes to the membrane recruitment of Dok proteins. For example, the PTB domain of Dok2 promotes recruitment to the activated EGFR and efficient tyrosine phosphorylation of Dok2 [126]. The PH and PTB domains cooperate in the membrane recruitment of Dok4 [121]. The presence of two membrane recruitment mechanisms in Dok proteins highlights a recurring theme for classical docking proteins, as described elsewhere in this minireview. Interestingly, Dok1 and Dok2 form homo- and heterotypic oligomers in a manner dependent on their tyrosine phosphorylation and PTB domains [127]. A critical role is played by a phosphotyrosine residue located between the PH and PTB domain. The oligomerization of these Dok proteins is critical to their inhibitory functions in T cells [127] and NIH3T3 cells transformed by the gain-of-function c-Src^{Y527F} mutant [128]. Similarly, Dok3 also displays homotypic oligomerization, which relies on its PTB domain and the phosphorylation of Y140, which is also localized between the PH and PTB domains [120]. However, the functional relevance of Dok3 oligomerization remains to be elucidated. Dok1, -2, -3 and -7 contain multiple PXXP motifs, which represent putative recognition motifs for proteins with SH3 domains [129]. By contrast, Dok4 contains only one PXXP-motif, whereas Dok5/6 lack this recognition sequence (our own Scansite analysis) [130]. However, the functional importance of these PXXP-motifs remains an area for further studies. Similar to other docking proteins, the C-terminal regions of the Dok family contain multiple tyrosine phosphorylation sites, and Abl, Src- and Tec-family kinases are implicated in the phosphorylation of these residues [114].

Members of the Dok1–3 subfamily are predominantly expressed in haematopoietic cells where they act as negative regulators of tyrosine kinase signalling networks. Dok1 and Dok2 both exhibit a YxxP-motif that upon phosphorylation, recruits p120 Ras-GAP. In turn, this protein attenuates Ras/Erk-signalling by stimulating the GTPase activity of Ras ([114] and references therein). However, Dok3 lacks this YxxP-motif and instead inhibits activation of the JNK pathway and the function of the SLP-65/Btk/phospholipase Cγ2 signalling complex, which plays a pivotal role in B-cell development and function [120,131]. Dok3-deficient chicken DT40 B cells and murine B lymphocytes

display increased calcium mobilization as well as enhanced activation of NF-κB, JNK and p38 MAP kinase following B-cell receptor engagement [131]. The inhibitory function of Dok3 requires the recruitment of Grb2 via its SH2 domain [120] but the tyrosine phosphorylation and activation of an additional Dok3 binding partner, the inositol-phosphatase SHIP1, is also impaired upon Dok3 ablation [131]. Binding of Grb2 by Dok3, leading to sequestration of the Grb2/Sos complex away from Shc, also underpins the ability of Dok3 to inhibit Src-induced Ras/Erk activation [132]. By contrast to these negative regulatory functions, Nck is recruited to phosphorylated Y361 in Dok1 and promotes formation of filopodia during spreading of mouse embryonic fibroblasts [133].

Dok4–7 play mostly positive roles in nonhaematopoietic cells, in particular within the nervous system. A recent report identified Dok7 as an essential driver of neuromuscular synaptogenesis because of its PTB-dependent interaction with muscle-specific receptor kinase [124]. The physiological significance of this interaction is supported by the observation that loss-of-function or hypomorphic alleles of the human *DOK7* gene cause a limb girdle-type congenital myasthenic syndrome with malformations of neuromuscular synapses, which has been termed *DOK7* myasthenia [134–139]. The spectrum of gene alterations is diverse and includes exon skipping, missense and frameshift mutations that affect the PH and PTB domains and the C-terminal region [134].

Dok1, -3 and -4 are phosphorylated at serine and threonine residues (www.phosphosite.org), although the functional consequences of these events remain largely ill-defined. One exception is a recent study identifying Dok1 as an IKKβ substrate in response to γ-radiation or stimulation with pro-inflammatory cytokines. Phosphorylation of Dok1 takes place at S439, S443, S446 and S450, and mutation of these phospho-acceptor residues to alanine abrogated the inhibitory effect of this docking protein on the Ras/Erk pathway [140].

Although Dok1–3 have established roles as negative regulators of immune cell signalling, downregulated expression or function of these proteins has not been detected in autoimmune disorders or haematological malignancies. However, altered expression of these proteins might contribute to other pathologies. For example, high expression of Dok1–3 occurs in lung tissue, and disruption of the *Dok1-3* genes in mice results in the development of lung adenocarcinomas [141]. Furthermore, copy number loss and reduced expression of *DOK2* was demonstrated in human lung cancer, and Dok2 was shown to suppress the growth of lung cancer cells. These findings highlight *DOK2* as a novel tumour suppressor in human lung cancer. In addition, a recent publication has implicated Dok1 in development of obesity [142]. In this study, it was shown that *Dok1* mRNA and protein expression increased in white adipose tissue of mice fed a high-fat diet. Importantly, Dok1-deficient mice and murine embryonic fibroblasts derived from these animals showed a reduced diet-induced hypertrophy of adipose tissue and impaired adipogenic differentiation, respectively. The latter defect was correlated with increased Erk activity and inhibition of PPARγ by Erk-dependent phosphorylation.

Perspectives

The roles played by docking proteins within signalling networks are complex, involving signal transduction, localization, cross-talk and modulation. Consequently, it is likely that great gains will be made by application of 'systems level' approaches such as mathematical and computational modelling to the study of docking protein function. In addition, the use of 'knockin' mouse models will continue to provide important information regarding the physiological roles of particular protein domains, regulatory events and effector pathways. Finally, as cancer genome and SNP-association studies gather pace, it will be surprising if further links between docking proteins and human disease are not discovered.

Acknowledgements

TB is supported by the Emmy-Noether-Program of the German Research Foundation. CS-P and RJD are supported by the National Health and Medical Research Council of Australia.

References

1 Schlessinger J (2000) Cell signaling by receptor tyrosine kinases. *Cell* **103**, 211–225.
2 Fuller DM & Zhang W (2009) Regulation of lymphocyte development and activation by the LAT family of adapter proteins. *Immunol Rev* **232**, 72–83.
3 Hunter T (1998) The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease. *Phil Trans R Soc Lond B* **353**, 583–605.
4 Defilippi P, Di Stefano P & Cabodi S (2006) p130Cas: a versatile scaffold in signaling networks. *Trends Cell Biol* **16**, 257–263.
5 Koretzky GA, Abtahian F & Silverman MA (2006) SLP76 and SLP65: complex regulation of signalling in lymphocytes and beyond. *Nat Rev Immunol* **6**, 67–78.

6 Gotoh N (2008) Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins. *Cancer Sci* **99**, 1319–1325.

7 Wohrle FU, Daly RJ & Brummer T (2009) Function, regulation and pathological roles of the Gab/DOS docking proteins. *Cell Commun Signal* **7**, 22.

8 Lock LS, Frigault MM, Saucier C & Park M (2003) Grb2-independent recruitment of Gab1 requires the C-terminal lobe and structural integrity of the Met receptor kinase domain. *J Biol Chem* **278**, 30083–30090.

9 Rodrigues GA, Falasca M, Zhang Z, Ong SH & Schlessinger J (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.

10 Lock LS, Royal I, Naujokas MA & Park M (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.

11 Schaeper U, Vogel R, Chmielowiec J, Huelsken J, Rosario M & Birchmeier W (2007) Distinct requirements for Gab1 in Met and EGF receptor signaling *in vivo*. *Proc Natl Acad Sci USA* **104**, 15376–15381.

12 Chan PC, Sudhakar JN, Lai CC & Chen HC (2010) Differential phosphorylation of the docking protein Gab1 by c-Src and the hepatocyte growth factor receptor regulates different aspects of cell functions. *Oncogene* **29**, 698–710.

13 Cunnick JM, Dorsey JF, Munoz-Antonia T, Mei L & Wu J (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.

14 Maroun CR, Naujokas MA, Holgado-Madruga M, Wong AJ & Park M (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.

15 Cunnick JM, Meng S, Ren Y, Desponts C, Wang HG, Djeu JY & Wu J (2002) Regulation of the mitogen-activated protein kinase signaling pathway by SHP2. *J Biol Chem* **277**, 9498–9504.

16 Meng S, Chen Z, Munoz-Antonia T & Wu J (2005) Participation of both Gab1 and Gab2 in the activation of the ERK/MAPK pathway by epidermal growth factor. *Biochem J* **391**, 143–151.

17 Brummer T, Schramek D, Hayes VM, Bennett HL, Caldon CE, Musgrove EA & Daly RJ (2006) Increased proliferation and altered growth factor dependence of human mammary epithelial cells overexpressing the Gab2 docking protein. *J Biol Chem* **281**, 626–637.

18 Yu M, Luo J, Yang W, Wang Y, Mizuki M, Kanakura Y, Besmer P, Neel BG & Gu H (2006) The scaffolding adapter Gab2, via Shp-2, regulates kit-evoked mast cell proliferation by activating the Rac/JNK pathway. *J Biol Chem* **281**, 28615–28626.

19 Yu WM, Hawley TS, Hawley RG & Qu CK (2002) Role of the docking protein Gab2 in beta(1)-integrin signaling pathway-mediated hematopoietic cell adhesion and migration. *Blood* **99**, 2351–2359.

20 Kallin A, Demoulin JB, Nishida K, Hirano T, Ronnstrand L & Heldin CH (2004) Gab1 contributes to cytoskeletal reorganization and chemotaxis in response to platelet-derived growth factor. *J Biol Chem* **279**, 17897–17904.

21 Abella JV, Vaillancourt R, Frigault MM, Ponzo MG, Zuo D, Sangwan V, Larose L & Park M (2010) The Gab1 scaffold regulates RTK-dependent dorsal ruffle formation through the adaptor Nck. *J Cell Sci* **123**, 1306–1319.

22 Gu H, Saito K, Klaman LD, Shen J, Fleming T, Wang Y, Pratt JC, Lin G, Lim B, Kinet JP *et al.* (2001) Essential role for Gab2 in the allergic response. *Nature* **412**, 186–190.

23 Garcia-Guzman M, Dolfi F, Zeh K & Vuori K (1999) Met-induced JNK activation is mediated by the adapter protein Crk and correlates with the Gab1–Crk signaling complex formation. *Oncogene* **18**, 7775–7786.

24 Sakkab D, Lewitzky M, Posern G, Schaeper U, Sachs M, Birchmeier W & Feller SM (2000) Signaling of hepatocyte growth factor/scatter factor (HGF) to the small GTPase Rap1 via the large docking protein Gab1 and the adapter protein CRKL. *J Biol Chem* **275**, 10772–10778.

25 Watanabe T, Tsuda M, Makino Y, Ichihara S, Sawa H, Minami A, Mochizuki N, Nagashima K & Tanaka S (2006) Adaptor molecule Crk is required for sustained phosphorylation of Grb2-associated binder 1 and hepatocyte growth factor-induced cell motility of human synovial sarcoma cell lines. *Mol Cancer Res* **4**, 499–510.

26 Lamorte L, Rodrigues S, Naujokas M & Park M (2002) Crk synergizes with epidermal growth factor for epithelial invasion and morphogenesis and is required for the met morphogenic program. *J Biol Chem* **277**, 37904–37911.

27 Zhang SQ, Tsiaras WG, Araki T, Wen G, Minichiello L, Klein R & Neel BG (2002) Receptor-specific regulation of phosphatidylinositol 3′-kinase activation by the protein tyrosine phosphatase Shp2. *Mol Cell Biol* **22**, 4062–4072.

28 Arnaud M, Crouin C, Deon C, Loyaux D & Bertoglio J (2004) Phosphorylation of Grb2-associated binder 2 on serine 623 by ERK MAPK regulates its association with the phosphatase SHP-2 and decreases STAT5 activation. *J Immunol* **173**, 3962–3971.

29 Lynch DK & Daly RJ (2002) PKB-mediated negative feedback tightly regulates mitogenic signalling via Gab2. *EMBO J* **21**, 72–82.
30 Brummer T, Larance M, Herrera Abreu MT, Lyons RJ, Timpson P, Emmerich CH, Fleuren ED, Lehrbach GM, Schramek D, Guilhaus M *et al.* (2008) Phosphorylation-dependent binding of 14-3-3 terminates signalling by the Gab2 docking protein. *EMBO J* **27**, 2305–2316.
31 Sachs M, Brohmann H, Zechner D, Muller T, Hulsken J, Walther I, Schaeper U, Birchmeier C & Birchmeier W (2000) Essential role of Gab1 for signaling by the c-Met receptor *in vivo*. *J Cell Biol* **150**, 1375–1384.
32 Itoh M, Yoshida Y, Nishida K, Narimatsu M, Hibi M & Hirano T (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.
33 Seiffert M, Custodio JM, Wolf I, Harkey M, Liu Y, Blattman JN, Greenberg PD & Rohrschneider LR (2003) Gab3-deficient mice exhibit normal development and hematopoiesis and are immunocompetent. *Mol Cell Biol* **23**, 2415–2424.
34 Wada T, Nakashima T, Oliveira-dos-Santos AJ, Gasser J, Hara H, Schett G & Penninger JM (2005) The molecular scaffold Gab2 is a crucial component of RANK signaling and osteoclastogenesis. *Nat Med* **11**, 394–399.
35 Zhang Y, Diaz-Flores E, Li G, Wang Z, Kang Z, Haviernikova E, Rowe S, Qu CK, Tse W, Shannon KM *et al.* (2007) Abnormal hematopoiesis in Gab2 mutant mice. *Blood* **110**, 116–124.
36 Nakaoka Y, Nishida K, Narimatsu M, Kamiya A, Minami T, Sawa H, Okawa K, Fujio Y, Koyama T, Maeda M *et al.* (2007) Gab family proteins are essential for postnatal maintenance of cardiac function via neuregulin-1/ErbB signaling. *J Clin Invest* **117**, 1771–1781.
37 Reiman EM, Webster JA, Myers AJ, Hardy J, Dunckley T, Zismann VL, Joshipura KD, Pearson JV, Hu-Lince D, Huentelman MJ *et al.* (2007) GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. *Neuron* **54**, 713–720.
38 Daly RJ, Gu H, Parmar J, Malaney S, Lyons RJ, Kairouz R, Head DR, Henshall SM, Neel BG & Sutherland RL (2002) The docking protein Gab2 is overexpressed and estrogen regulated in human breast cancer. *Oncogene* **21**, 5175–5181.
39 Bentires-Alj M, Gil SG, Chan R, Wang ZC, Wang Y, Imanaka N, Harris LN, Richardson A, Neel BG & Gu H (2006) A role for the scaffolding adapter GAB2 in breast cancer. *Nat Med* **12**, 114–121.
40 Fleuren ED, O'Toole S, Millar EK, McNeil C, Lopez-Knowles E, Boulghourjian A, Croucher DR, Schramek D, Brummer T, Penninger JM *et al.* (2010) Overexpression of the oncogenic signal transducer Gab2 occurs early in breast cancer development. *Int J Cancer* **127**, 1486–1492.
41 Bocanegra M, Bergamaschi A, Kim YH, Miller MA, Rajput AB, Kao J, Langerod A, Han W, Noh DY, Jeffrey SS *et al.* (0000) Focal amplification and oncogene dependency of GAB2 in breast cancer. *Oncogene* **29**, 774–779.
42 Brown LA, Kalloger SE, Miller MA, Shih Ie M, McKinney SE, Santos JL, Swenerton K, Spellman PT, Gray J, Gilks CB *et al.* (2008) Amplification of 11q13 in ovarian carcinoma. *Genes Chromosomes Cancer* **47**, 481–489.
43 Lee SH, Jeong EG, Nam SW, Lee JY, Yoo NJ & Lee SH (2007) Increased expression of Gab2, a scaffolding adaptor of the tyrosine kinase signalling, in gastric carcinomas. *Pathology* **39**, 326–329.
44 Zatkova A, Schoch C, Speleman F, Poppe B, Mannhalter C, Fonatsch C & Wimmer K (2006) GAB2 is a novel target of 11q amplification in AML/MDS. *Gene Chromosome Cancer* **45**, 798–807.
45 Horst B, Gruvberger-Saal SK, Hopkins BD, Bordone L, Yang Y, Chernoff KA, Uzoma I, Schwipper V, Liebau J, Nowak NJ *et al.* (2009) Gab2-mediated signaling promotes melanoma metastasis. *Am J Pathol* **174**, 1524–1533.
46 Chernoff KA, Bordone L, Horst B, Simon K, Twadell W, Lee K, Cohen JA, Wang S, Silvers DN, Brunner G *et al.* (2009) GAB2 amplifications refine molecular classification of melanoma. *Clin Cancer Res* **15**, 4288–4291.
47 Sattler M, Mohi MG, Pride YB, Quinnan LR, Malouf NA, Podar K, Gesbert F, Iwasaki H, Li S, Van Etten RA *et al.* (2002) Critical role for Gab2 in transformation by BCR/ABL. *Cancer Cell* **1**, 479–492.
48 Ke Y, Wu D, Princen F, Nguyen T, Pang Y, Lesperance J, Muller WJ, Oshima RG & Feng GS (2007) Role of Gab2 in mammary tumorigenesis and metastasis. *Oncogene* **26**, 4951–4960.
49 Kouhara H, Hadari YR, Spivak-Kroizman T, Schilling J, Bar-Sagi D, Lax I & Schlessinger J (1997) A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* **89**, 693–702.
50 Ridyard MS & Robbins SM (2003) Fibroblast growth factor-2-induced signaling through lipid raft-associated fibroblast growth factor receptor substrate 2 (FRS2). *J Biol Chem* **278**, 13803–13809.
51 Lundgren TK, Luebke M, Stenqvist A & Ernfors P (2008) Differential membrane compartmentalization of Ret by PTB-adaptor engagement. *FEBS J* **275**, 2055–2066.
52 Kusakabe M, Masuyama N, Hanafusa H & Nishida E (2001) *Xenopus* FRS2 is involved in early embryogene-

sis in cooperation with the Src family kinase Laloo. *EMBO Report* **2**, 727–735.
53 Hama J, Xu H, Goldfarb M & Weinstein DC (2001) SNT-1/FRS2alpha physically interacts with Laloo and mediates mesoderm induction by fibroblast growth factor. *Mech Dev* **109**, 195–204.
54 Ong SH, Guy GR, Hadari YR, Laks S, Gotoh N, Schlessinger J & Lax I (2000) FRS2 proteins recruit intracellular signaling pathways by binding to diverse targets on fibroblast growth factor and nerve growth factor receptors. *Mol Cell Biol* **20**, 979–989.
55 Kurokawa K, Iwashita T, Murakami H, Hayashi H, Kawai K & Takahashi M (2001) Identification of SNT/FRS2 docking site on RET receptor tyrosine kinase and its role for signal transduction. *Oncogene* **20**, 1929–1938.
56 Dhalluin C, Yan KS, Plotnikova O, Lee KW, Zeng L, Kuti M, Mujtaba S, Goldfarb MP & Zhou MM (2000) Structural basis of SNT PTB domain interactions with distinct neurotrophic receptors. *Mol Cell* **6**, 921–929.
57 Huang L, Watanabe M, Chikamori M, Kido Y, Yamamoto T, Shibuya M, Gotoh N & Tsuchida N (2006) Unique role of SNT-2/FRS2beta/FRS3 docking/adaptor protein for negative regulation in EGF receptor tyrosine kinase signaling pathways. *Oncogene* **25**, 6457–6466.
58 Hadari YR, Gotoh N, Kouhara H, Lax I & Schlessinger J (2001) Critical role for the docking-protein FRS2 alpha in FGF receptor-mediated signal transduction pathways. *Proc Natl Acad Sci USA* **98**, 8578–8583.
59 Ong SH, Hadari YR, Gotoh N, Guy GR, Schlessinger J & Lax I (2001) Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins. *Proc Natl Acad Sci USA* **98**, 6074–6079.
60 Wong A, Lamothe B, Lee A, Schlessinger J & Lax I (2002) FRS2 alpha attenuates FGF receptor signaling by Grb2-mediated recruitment of the ubiquitin ligase Cbl. *Proc Natl Acad Sci USA* **99**, 6684–6689.
61 Zhang Y, Lin Y, Bowles C & Wang F (2004) Direct cell cycle regulation by the fibroblast growth factor receptor (FGFR) kinase through phosphorylation-dependent release of Cks1 from FGFR substrate 2. *J Biol Chem* **279**, 55348–55354.
62 Harada A, Katoh H & Negishi M (2005) Direct interaction of Rnd1 with FRS2 beta regulates Rnd1-induced down-regulation of RhoA activity and is involved in fibroblast growth factor-induced neurite outgrowth in PC12 cells. *J Biol Chem* **280**, 18418–18424.
63 Li X, Brunton VG, Burgar HR, Wheldon LM & Heath JK (2004) FRS2-dependent SRC activation is required for fibroblast growth factor receptor-induced phosphorylation of Sprouty and suppression of ERK activity. *J Cell Sci* **117**, 6007–6017.
64 Lax I, Wong A, Lamothe B, Lee A, Frost A, Hawes J & Schlessinger J (2002) The docking protein FRS2alpha controls a MAP kinase-mediated negative feedback mechanism for signaling by FGF receptors. *Mol Cell* **10**, 709–719.
65 Gotoh N, Manova K, Tanaka S, Murohashi M, Hadari Y, Lee A, Hamada Y, Hiroe T, Ito M, Kurihara T *et al.* (2005) The docking protein FRS2alpha is an essential component of multiple fibroblast growth factor responses during early mouse development. *Mol Cell Biol* **25**, 4105–4116.
66 Gotoh N, Ito M, Yamamoto S, Yoshino I, Song N, Wang Y, Lax I, Schlessinger J, Shibuya M & Lang RA (2004) Tyrosine phosphorylation sites on FRS2alpha responsible for Shp2 recruitment are critical for induction of lens and retina. *Proc Natl Acad Sci USA* **101**, 17144–17149.
67 Fischer U, Keller A, Leidinger P, Deutscher S, Heisel S, Urbschat S, Lenhof HP & Meese E (2008) A different view on DNA amplifications indicates frequent, highly complex, and stable amplicons on 12q13-21 in glioma. *Mol Cancer Res* **6**, 576–584.
68 Persson F, Olofsson A, Sjogren H, Chebbo N, Nilsson B, Stenman G & Aman P (2008) Characterization of the 12q amplicons by high-resolution, oligonucleotide array CGH and expression analyses of a novel liposarcoma cell line. *Cancer Lett* **260**, 37–47.
69 Cai D, Dhe-Paganon S, Melendez PA, Lee J & Shoelson SE (2003) Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. *J Biol Chem* **278**, 25323–25330.
70 Versteyhe S, Blanquart C, Hampe C, Mahmood S, Christeff N, De Meyts P, Gray S & Issad T (2010) Insulin receptor substrates-5 and -6 are poor substrates for the insulin receptor. *Mol Med Rep* **3**, 189–193.
71 White MF, Maron R & Kahn CR (1985) Insulin rapidly stimulates tyrosine phosphorylation of a M_r-185,000 protein in intact cells. *Nature* **318**, 183–186.
72 Yenush L, Makati KJ, Smith-Hall J, Ishibashi O, Myers MG Jr & White MF (1996) The pleckstrin homology domain is the principal link between the insulin receptor and IRS-1. *J Biol Chem* **271**, 24300–24306.
73 Razzini G, Ingrosso A, Brancaccio A, Sciacchitano S, Esposito DL & Falasca M (2000) Different subcellular localization and phosphoinositides binding of insulin receptor substrate protein pleckstrin homology domains. *Mol Endocrinol* **14**, 823–836.
74 Backer JM, Wjasow C & Zhang Y (1997) *In vitro* binding and phosphorylation of insulin receptor substrate 1 by the insulin receptor. Role of interactions mediated by the phosphotyrosine-binding domain and

the pleckstrin-homology domain. *Eur J Biochem* **245**, 91–96.
75 Eck MJ, Dhe-Paganon S, Trub T, Nolte RT & Shoelson SE (1996) Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. *Cell* **85**, 695–705.
76 Sawka-Verhelle D, Baron V, Mothe I, Filloux C, White MF & Van Obberghen E (1997) Tyr624 and Tyr628 in insulin receptor substrate-2 mediate its association with the insulin receptor. *J Biol Chem* **272**, 16414–16420.
77 Wu J, Tseng YD, Xu CF, Neubert TA, White MF & Hubbard SR (2008) Structural and biochemical characterization of the KRLB region in insulin receptor substrate-2. *Nat Struct Mol Biol* **15**, 251–258.
78 Nelms K, Keegan A, Zamorano J, Ryan J & Paul W (1999) The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* **17**, 701–738.
79 Sun XJ, Crimmins DL, Myers MG Jr, Miralpeix M & White MF (1993) Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. *Mol Cell Biol* **13**, 7418–7428.
80 Rordorf-Nikolic T, Van Horn DJ, Chen D, White MF & Backer JM (1995) Regulation of phosphatidylinositol 3′-kinase by tyrosyl phosphoproteins. Full activation requires occupancy of both SH2 domains in the 85-kDa regulatory subunit. *J Biol Chem* **270**, 3662–3666.
81 Taniguchi CM, Emanuelli B & Kahn CR (2006) Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* **7**, 85–96.
82 Saltiel AR & Kahn CR (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799–806.
83 Skolnik EY, Batzer A, Li N, Lee CH, Lowenstein E, Mohammadi M, Margolis B & Schlessinger J (1993) The function of GRB2 in linking the insulin receptor to Ras signaling pathways. *Science* **260**, 1953–1955.
84 Lee CH, Li W, Nishimura R, Zhou M, Batzer AG, Myers MG Jr, White MF, Schlessinger J & Skolnik EY (1993) Nck associates with the SH2 domain-docking protein IRS-1 in insulin-stimulated cells. *Proc Natl Acad Sci USA* **90**, 11713–11717.
85 Myers MG Jr, Mendez R, Shi P, Pierce JH, Rhoads R & White MF (1998) The COOH-terminal tyrosine phosphorylation sites on IRS-1 bind SHP-2 and negatively regulate insulin signaling. *J Biol Chem* **273**, 26908–26914.
86 Maegawa H, Hasegawa M, Sugai S, Obata T, Ugi S, Morino K, Egawa K, Fujita T, Sakamoto T, Nishio Y *et al.* (1999) Expression of a dominant negative SHP-2 in transgenic mice induces insulin resistance. *J Biol Chem* **274**, 30236–30243.
87 Princen F, Bard E, Sheikh F, Zhang SS, Wang J, Zago WM, Wu D, Trelles RD, Bailly-Maitre B, Kahn CR *et al.* (2009) Deletion of Shp2 tyrosine phosphatase in muscle leads to dilated cardiomyopathy, insulin resistance, and premature death. *Mol Cell Biol* **29**, 378–388.
88 Uchida T, Myers MG Jr & White MF (2000) IRS-4 mediates protein kinase B signaling during insulin stimulation without promoting antiapoptosis. *Mol Cell Biol* **20**, 126–138.
89 Sciacchitano S & Taylor SI (1997) Cloning, tissue expression, and chromosomal localization of the mouse IRS-3 gene. *Endocrinology* **138**, 4931–4940.
90 Bjornholm M, He AR, Attersand A, Lake S, Liu SC, Lienhard GE, Taylor S, Arner P & Zierath JR (2002) Absence of functional insulin receptor substrate-3 (IRS-3) gene in humans. *Diabetologia* **45**, 1697–1702.
91 Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S *et al.* (1994) Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* **372**, 182–186.
92 Withers DJ, Gutierrez JS, Towery H, Burks DJ, Ren JM, Previs S, Zhang Y, Bernal D, Pons S, Shulman GI *et al.* (1998) Disruption of IRS-2 causes type 2 diabetes in mice. *Nature* **391**, 900–904.
93 Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL & White MF (1999) Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. *Nat Genet* **23**, 32–40.
94 Liu SC, Wang Q, Lienhard GE & Keller SR (1999) Insulin receptor substrate 3 is not essential for growth or glucose homeostasis. *J Biol Chem* **274**, 18093–18099.
95 Bouzakri K, Zachrisson A, Al-Khalili L, Zhang BB, Koistinen HA, Krook A & Zierath JR (2006) siRNA-based gene silencing reveals specialized roles of IRS-1/Akt2 and IRS-2/Akt1 in glucose and lipid metabolism in human skeletal muscle. *Cell Metab* **4**, 89–96.
96 Farese RV, Sajan MP & Standaert ML (2005) Atypical protein kinase C in insulin action and insulin resistance. *Biochem Soc Trans* **33**, 350–353.
97 Boura-Halfon S & Zick Y (2009) Phosphorylation of IRS proteins, insulin action, and insulin resistance. *Am J Physiol* **296**, E581–591.
98 Xu X, Sarikas A, Dias-Santagata DC, Dolios G, Lafontant PJ, Tsai SC, Zhu W, Nakajima H, Nakajima HO, Field LJ *et al.* (2008) The CUL7 E3 ubiquitin ligase targets insulin receptor substrate 1 for ubiquitin-dependent degradation. *Mol Cell* **30**, 403–414.
99 Aguirre V, Uchida T, Yenush L, Davis R & White MF (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* **275**, 9047–9054.
100 Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ & Ye J (2002) Serine phosphorylation of

insulin receptor substrate 1 by inhibitor kappa B kinase complex. *J Biol Chem* **277**, 48115–48121.
101 Eldar-Finkelman H & Krebs EG (1997) Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc Natl Acad Sci USA* **94**, 9660–9664.
102 Schmitz-Peiffer C & Biden TJ (2008) Protein kinase C function in muscle, liver, and beta-cells and its therapeutic implications for type 2 diabetes. *Diabetes* **57**, 1774–1783.
103 Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE & White MF (2002) Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. *J Biol Chem* **277**, 1531–1537.
104 Greene MW, Ruhoff MS, Roth RA, Kim JA, Quon MJ & Krause JA (2006) PKCdelta-mediated IRS-1 Ser24 phosphorylation negatively regulates IRS-1 function. *Biochem Biophys Res Commun* **349**, 976–986.
105 Bouzakri K, Roques M, Gual P, Espinosa S, Guebre-Egziabher F, Riou JP, Laville M, Le Marchand-Brustel Y, Tanti JF & Vidal H (2003) Reduced activation of phosphatidylinositol-3 kinase and increased serine 636 phosphorylation of insulin receptor substrate-1 in primary culture of skeletal muscle cells from patients with type 2 diabetes. *Diabetes* **52**, 1319–1325.
106 Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J *et al.* (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* **431**, 200–205.
107 Lee S, Lynn EG, Kim JA & Quon MJ (2008) Protein kinase C-zeta phosphorylates insulin receptor substrate-1, -3, and -4 but not -2: isoform specific determinants of specificity in insulin signaling. *Endocrinology* **149**, 2451–2458.
108 Patti ME, Virkamaki A, Landaker EJ, Kahn CR & Yki-Jarvinen H (1999) Activation of the hexosamine pathway by glucosamine *in vivo* induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. *Diabetes* **48**, 1562–1571.
109 D'Alessandris C, Andreozzi F, Federici M, Cardellini M, Brunetti A, Ranalli M, Del Guerra S, Lauro D, Del Prato S, Marchetti P *et al.* (2004) Increased O-glycosylation of insulin signaling proteins results in their impaired activation and enhanced susceptibility to apoptosis in pancreatic beta-cells. *FASEB J* **18**, 959–961.
110 Kaiser C & James SR (2004) Acetylation of insulin receptor substrate-1 is permissive for tyrosine phosphorylation. *BMC Biol* **2**, 23.
111 Zhang J (2007) The direct involvement of SirT1 in insulin-induced insulin receptor substrate-2 tyrosine phosphorylation. *J Biol Chem* **282**, 34356–34364.
112 Mardilovich K, Pankratz SL & Shaw LM (2009) Expression and function of the insulin receptor substrate proteins in cancer. *Cell Commun Signal* **7**, 14.
113 Nagle JA, Ma Z, Byrne MA, White MF & Shaw LM (2004) Involvement of insulin receptor substrate 2 in mammary tumor metastasis. *Mol Cell Biol* **24**, 9726–9735.
114 Mashima R, Hishida Y, Tezuka T & Yamanashi Y (2009) The roles of Dok family adapters in immunoreceptor signaling. *Immunol Rev* **232**, 273–285.
115 Biswas R, Stein D & Stanley ER (2006) *Drosophila* Dok is required for embryonic dorsal closure. *Development* **133**, 217–227.
116 Yamanashi Y & Baltimore D (1997) Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. *Cell* **88**, 205–211.
117 Carpino N, Wisniewski D, Strife A, Marshak D, Kobayashi R, Stillman B & Clarkson B (1997) p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. *Cell* **88**, 197–204.
118 Noguchi T, Matozaki T, Inagaki K, Tsuda M, Fukunaga K, Kitamura Y, Kitamura T, Shii K, Yamanashi Y & Kasuga M (1999) Tyrosine phosphorylation of p62(Dok) induced by cell adhesion and insulin: possible role in cell migration. *EMBO J* **18**, 1748–1760.
119 Guittard G, Gerard A, Dupuis-Coronas S, Tronchere H, Mortier E, Favre C, Olive D, Zimmermann P, Payrastre B & Nunes JA (2009) Cutting edge: Dok-1 and Dok-2 adaptor molecules are regulated by phosphatidylinositol 5-phosphate production in T cells. *J Immunol* **182**, 3974–3978.
120 Stork B, Neumann K, Goldbeck I, Alers S, Kahne T, Naumann M, Engelke M & Wienands J (2007) Subcellular localization of Grb2 by the adaptor protein Dok-3 restricts the intensity of Ca^{2+} signaling in B cells. *EMBO J* **26**, 1140–1149.
121 Bedirian A, Baldwin C, Abe J, Takano T & Lemay S (2004) Pleckstrin homology and phosphotyrosine-binding domain-dependent membrane association and tyrosine phosphorylation of Dok-4, an inhibitory adapter molecule expressed in epithelial cells. *J Biol Chem* **279**, 19335–19349.
122 Gerard A, Ghiotto M, Fos C, Guittard G, Compagno D, Galy A, Lemay S, Olive D & Nunes JA (2009) Dok-4 is a novel negative regulator of T cell activation. *J Immunol* **182**, 7681–7689.
123 Fu G, Zhang F, Cao L, Xu ZZ, Chen YZ, Wang GY & He C (2008) Constitutive plasma membrane targeting and microdomain localization of Dok5 studied by single-molecule microscopy. *Biophys Chem* **136**, 13–18.
124 Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, Kubo S, Shiraishi H, Eguchi K, Motomura M *et al.* (2006) The muscle protein Dok-7 is essential

for neuromuscular synaptogenesis. *Science* **312**, 1802–1805.
125 Kurotsuchi A, Murakumo Y, Jijiwa M, Kurokawa K, Itoh Y, Kodama Y, Kato T, Enomoto A, Asai N, Terasaki H *et al.* (2010) Analysis of DOK-6 function in downstream signaling of RET in human neuroblastoma cells. *Cancer Sci* **101**, 1147–1155.
126 Jones N & Dumont DJ (1999) Recruitment of Dok-R to the EGF receptor through its PTB domain is required for attenuation of Erk MAP kinase activation. *Curr Biol* **9**, 1057–1060.
127 Boulay I, Nemorin JG & Duplay P (2005) Phosphotyrosine binding-mediated oligomerization of downstream of tyrosine kinase (Dok)-1 and Dok-2 is involved in CD2-induced Dok phosphorylation. *J Immunol* **175**, 4483–4489.
128 Songyang Z, Yamanashi Y, Liu D & Baltimore D (2001) Domain-dependent function of the rasGAP-binding protein p62Dok in cell signaling. *J Biol Chem* **276**, 2459–2465.
129 Di Cristofano A, Carpino N, Dunant N, Friedland G, Kobayashi R, Strife A, Wisniewski D, Clarkson B, Pandolfi PP & Resh MD (1998) Molecular cloning and characterization of p56dok-2 defines a new family of RasGAP-binding proteins. *J Biol Chem* **273**, 4827–4830.
130 Grimm J, Sachs M, Britsch S, Di Cesare S, Schwarz-Romond T, Alitalo K & Birchmeier W (2001) Novel p62dok family members, dok-4 and dok-5, are substrates of the c-Ret receptor tyrosine kinase and mediate neuronal differentiation. *J Cell Biol* **154**, 345–354.
131 Ng CH, Xu S & Lam KP (2007) Dok-3 plays a nonredundant role in negative regulation of B-cell activation. *Blood* **110**, 259–266.
132 Honma M, Higuchi O, Shirakata M, Yasuda T, Shibuya H, Lemura S, Natsume T & Yamanashi Y (2006) Dok-3 sequesters Grb2 and inhibits the Ras-Erk pathway downstream of protein-tyrosine kinases. *Genes Cells* **11**, 143–151.
133 Woodring PJ, Meisenhelder J, Johnson SA, Zhou GL, Field J, Shah K, Bladt F, Pawson T, Niki M, Pandolfi PP *et al.* (2004) c-Abl phosphorylates Dok1 to promote filopodia during cell spreading. *J Cell Biol* **165**, 493–503.
134 Ben Ammar A, Petit F, Alexandri N, Gaudon K, Bauche S, Rouche A, Gras D, Fournier E, Koenig J, Stojkovic T *et al.* (2009) Phenotype genotype analysis in 15 patients presenting a congenital myasthenic syndrome due to mutations in DOK7. *J Neurol* **257**, 754–766.
135 Hamuro J, Higuchi O, Okada K, Ueno M, Iemura S, Natsume T, Spearman H, Beeson D & Yamanashi Y (2008) Mutations causing DOK7 congenital myasthenia ablate functional motifs in Dok-7. *J Biol Chem* **283**, 5518–5524.
136 Muller JS, Jepson CD, Laval SH, Bushby K, Straub V & Lochmuller H (2010) Dok-7 promotes slow muscle integrity as well as neuromuscular junction formation in a zebrafish model of congenital myasthenic syndromes. *Human Mol Genet* **19**, 1726–1740.
137 Muller JS, Herczegfalvi A, Vilchez JJ, Colomer J, Bachinski LL, Mihaylova V, Santos M, Schara U, Deschauer M, Shevell M *et al.* (2007) Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes. *Brain* **130**, 1497–1506.
138 Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, Jayawant S, Yamanashi Y & Beeson D (2007) Clinical features of the DOK7 neuromuscular junction synaptopathy. *Brain* **130**, 1507–1515.
139 Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, Newsom-Davis J, Burke G, Fawcett P, Motomura M *et al.* (2006) Dok-7 mutations underlie a neuromuscular junction synaptopathy. *Science* **313**, 1975–1978.
140 Lee S, Andrieu C, Saltel F, Destaing O, Auclair J, Pouchkine V, Michelon J, Salaun B, Kobayashi R, Jurdic P *et al.* (2004) IkappaB kinase beta phosphorylates Dok1 serines in response to TNF, IL–1, or gamma radiation. *Proc Natl Acad Sci USA* **101**, 17416–17421.
141 Berger AH, Niki M, Morotti A, Taylor BS, Socci ND, Viale A, Brennan C, Szoke J, Motoi N, Rothman PB *et al.* (2010) Identification of DOK genes as lung tumor suppressors. *Nat Genet* **42**, 216–223.
142 Hosooka T, Noguchi T, Kotani K, Nakamura T, Sakaue H, Inoue H, Ogawa W, Tobimatsu K, Takazawa K, Sakai M *et al.* (2008) Dok1 mediates high-fat diet-induced adipocyte hypertrophy and obesity through modulation of PPAR-gamma phosphorylation. *Nat Med* **14**, 188–193.