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Proteins with SH2 and SH3 Domains Couple Receptor Tyrosine Kinases to Intracellular Signalling Pathways

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Proteins with SH2 and SH3 domains couple receptor tyrosine kinases to intracellular signalling pathways

TONY PAWSON[1,2], PAUL OLIVIER[1,2], MARIA ROZAKIS-ADCOCK[1], JANE MCGLADE[1] AND MARK HENKEMEYER[1]

[1]*Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada*

[2]*Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada*

SUMMARY

The targets of receptor protein-tyrosine kinases are characterized by Src homology 2 (SH2) domains, that mediate specific interactions with receptor autophosphorylation sites. SH2-mediated interactions are important for the activation of biochemical signalling pathways in cells stimulated with growth factors. A distinct protein module, the SH3 domain, is frequently found in polypeptides that contain SH2 domains, and is also implicated in controlling protein–protein interactions in signal transduction. Evidence suggesting that SH2 and SH3 domains act synergistically in stimulation of the Ras pathway is discussed.

1. INTRODUCTION

Many of the polypeptide hormones that regulate the growth, differentiation and metabolism of mammalian cells exert their effects through cell-surface receptors with cytoplasmic protein-tyrosine kinase domains (Cantley *et al.* 1991; Pawson *et al.* 1990). Binding of the appropriate growth factor to a receptor tyrosine kinase causes the receptor to cluster in the membrane, and subsequently to autophosphorylate by an intermolecular reaction (Ullrich *et al.* 1990). Receptor autophosphorylation serves as a molecular switch, to induce the association of cytoplasmic signalling proteins with the activated receptor. Such receptor-binding proteins include phospholipase C (PLC)-γ1, Ras GTPase activating protein (GAP), phosphatidylinositol (PI) 3′-kinase, Src-like cytoplasmic protein-tyrosine kinases, and the Syp/SHPTP2 phosphotyrosine phosphatase (Koch *et al.* 1991) (figure 1).

These proteins all contain one or two copies of a protein module, the SH2 domain, that directly recognizes phosphotyrosine-containing sites, and is responsible for their association with autophosphorylated receptors (Anderson *et al.* 1990; Matsuda *et al.* 1990; Moran *et al.*1990). Recent structural data indicate that SH2 domains have a conserved binding pocket for phosphotyrosine, and also possess a pocket for the amino acid three residues C-terminal to the phosphotyrosine (the +3 position) (Waksman *et al.* 1992; Waksman *et al.* 1993; Eck *et al.* 1993). This latter pocket is lined by variable residues, and hence allows different SH2 domains to bind preferentially to distinct residues at the +3 site. SH2 domains also have specific contact sites on their surface for the residues at the +1 and +2 positions. Hence, a minimal SH2-binding site is composed of a phosphorylated tyrosine and the three following residues. Phosphorylation of the binding site serves as the signal to induce SH2 association, while the C-terminal residues provide for specific recognition of the relevant SH2 domain (Songyang *et al.* 1993) (figure 2). Hence, the ability of any receptor tyrosine kinase to interact with SH2-containing targets depends on the sequence context of its autophosphorylation sites.

In the β receptor for platelet-derived growth factor, for example, distinct receptor autophosphorylation sites are responsible for the binding of different SH2-containing signalling proteins (figure 2). Ablation of any one site, by substitution of the autophosphorylated tyrosine with phenylalanine, incapacitates binding to a specific SH2-containing protein without affecting binding to others (Fantl *et al.* 1992; Kashishian *et al.* 1992; Kazlauskas *et al.* 1992; Rönnstrand *et al.* 1992). Hence, substitution of Tyr1021 within the tail of the receptor eliminates binding to PLC-γ1, without compromising binding to GAP or PI 3′-kinase. Concomitantly, the receptor loses its ability to stimulate hydrolysis of PI-4,5-P_2 to the second messengers diacylglycerol and inositoltrisphosphate. The selective binding of PLC-γ1 to the autophosphorylated tail of the PDGF-receptor is therefore crucial for its activation in response to PDGF.

Biochemical and genetic evidence suggests that the biological effects of many receptor tyrosine kinases require activation of the Ras signalling pathway (Mulcahy *et al.* 1985; Smith *et al.* 1986; Szeberenyi *et al.* 1990; Thomas *et al.* 1992; Wood *et al.* 1992). The

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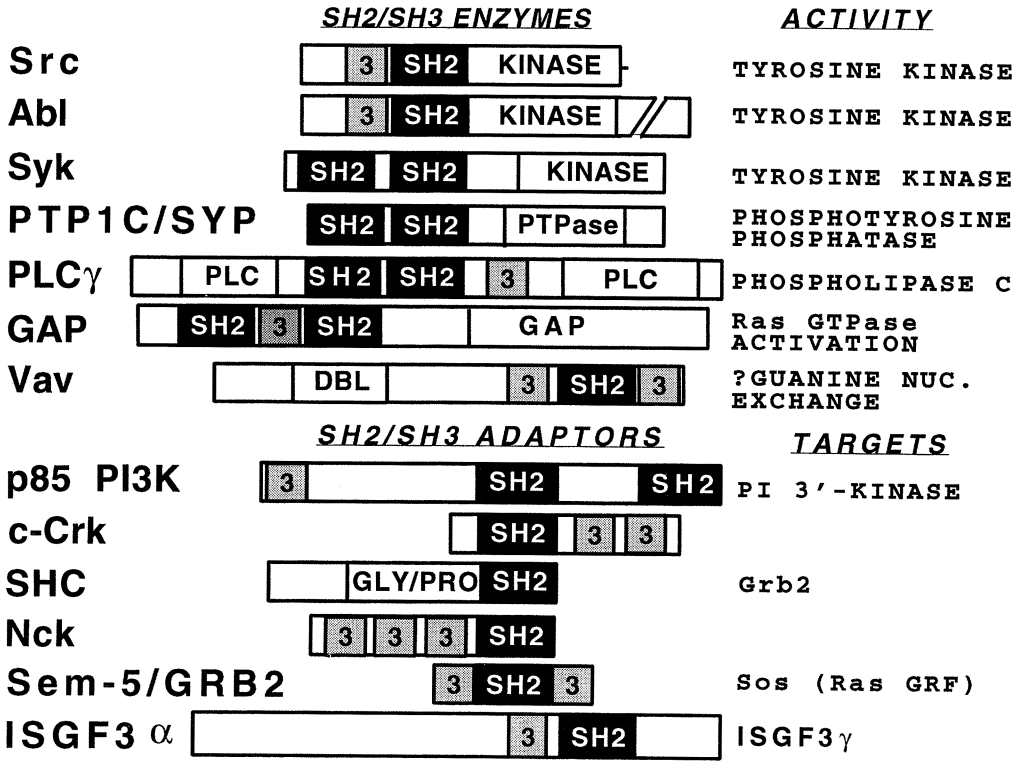

Figure 1. Structures of cytoplasmic signalling proteins with SH2 and SH3 domains. Proteins are divided into polypeptides with catalytic sequences and SH2/SH3 domains within the same chain, and adaptor proteins that apparently couple to downstream signalling subunits. PTPase = phosphotyrosine phosphatase; GLY/PRO-glycine/proline-rich region. DBL = region of homogy to the Dbl protein.

Ras GTPase is inactive in the GDP-bound state, and is activated by exchange of GDP for GTP under the control of a guanine nucleotide releasing protein (GNRP) (Bourne *et al.* 1991). Once in the GTP-bound form Ras delivers downstream signals that modify cell morphology and growth. Although the identities of the immediate targets of Ras.GTP remain uncertain, it is evident the GTP-bound Ras interacts with GAPs that stimulate Ras GTPase activity, and hence serve as negative regulators (Nori *et al.* 1991; DeClue *et al.* 1991). It is also possible that GAPs function as Ras targets (Cales *et al.* 1988). Here, we discuss the mechanism by which tyrosine kinases activate SH2-containing proteins, and in particular the means by which they couple to Ras.

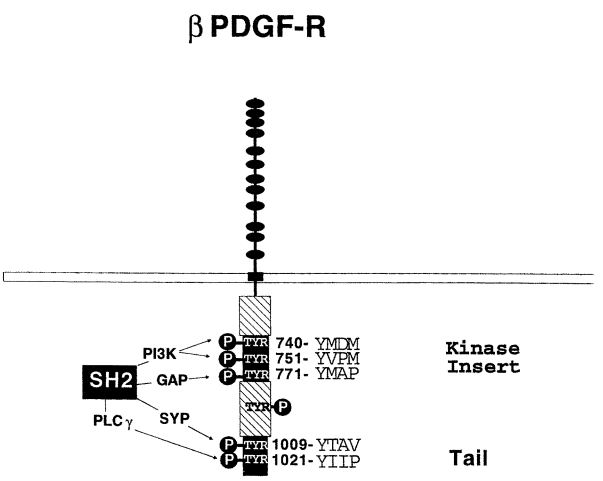

Figure 2. SH2-binding sites on the human βPDGF-receptor. Autophosphorylation sites, numbered according to the phosphorylated tyrosine, and the SH2 domains to which they bind are depicted. Adjacent to each phosphorylation site is the sequence immediately following the phosphorylated tyrosine. The non-catalytic kinase insert, and C-terminal tail of the receptor are shown in black; kinase domain sequences are striped. Solid oval symbols = cysteine residues of the extracellular domain. P13K = PI 3′-kinase. SYP = SH2-containing phosphotyrosine phosphatase (Feng *et al.* 1993; Kazlauskas *et al.* 1993).

2. REGULATION OF SIGNAL TRANSDUCTION BY SH2 AND SH3 DOMAINS

(*a*) *SH2/SH3 adaptor proteins*

The cytoplasmic signalling proteins detailed above generally contain enzymic domains and SH2 domains within the same polypeptide chain. Most of these proteins also possess a distinct motif of approximately 50 amino acids, termed the SH3 domain, which like the SH2 domain is apparently involved in protein–protein interactions during signal transduction (Musacchio *et al.* 1992; Cicchetti *et al.* 1992) (figure 1). PI 3′-kinase, in contrast, is composed of an 85 kDa (p85) receptor-binding subunit which contains SH2 and SH3 domains, and a distinct 110 kDa catalytic subunit (p110) (Hiles *et al.* 1992). p85 therefore appears to function as a molecular adaptor that couples receptor tyrosine kinases to the p110 enzymic subunit (McGlade *et al.* 1992). A number of proteins

with SH2 and SH3 domains contain no intrinsic catalytic sequences (Crk, Nck, Sem-5/Grb2), and may therefore serve as adaptors to link receptor tyrosine kinases to downstream targets that themselves lack SH2 domains (Pawson & Gish 1992) (figure 1).

It is of interest that these proteins are comprised almost exclusively of SH2 and SH3 domains. If their SH2 domains direct interactions with autophosphorylated receptors, or other phosphotyrosine-containing proteins, it seems likely that their SH3 domains engage the proposed downstream targets. Recent evidence suggests that SH3 domains recognize a proline-rich motif, and might therefore act in concert with SH2 domains to link tyrosine kinases to targets with such proline-rich SH3-binding sites (Ren *et al.* 1993).

(*b*) *Activation of intracellular signalling pathways*

The binding of a cytoplasmic SH2-containing signalling protein to an activated receptor could modulate its activity in several different ways. Firstly, stimulation of mammalian cells with growth factors such as PDGF or epidermal growth factor (EGF) results in the translocation of PLC-γ1 and GAP from the cytosol to the plasma membrane (Molloy *et al.* 1989; Todderud *et al.* 1990). A similar observation has been made for the *Drosophila* homologue of Sem-5/Grb2 (termed Drk), which associates with the membrane of cells in the eye imaginal disc by virtue of its SH2 domain, presumably reflecting an association of the SH2 domain with activated receptor tyrosine kinases (Olivier *et al.* 1993). Receptor autophosphorylation may therefore provide a means of recruiting SH2-containing signalling proteins to the membrane. Because the substrates of these proteins are themselves located at the membrane (i.e. phospholipids in the case of PLC-γ1, or Ras.GTP in the case of GAP) this inducible association with the membrane may represent a control point. As noted above, binding of PLC-γ1 to receptors appears crucial for stimulation of PLC activity, even under circumstances where PLC-γ1 becomes phosphorylated (Valius *et al.* 1993; Mohammadi *et al.* 1992; Vega *et al.* 1992).

Receptor-bound signalling proteins are, in many cases, very good substrates for tyrosine phosphorylation by the receptor's tyrosine kinase domain. For PLC-γ1, the presence of a single SH2 domain lowers the Km for phosphorylation by the EGF-receptor, and two SH2 domains act synergistically to increase the efficiency of PLC-γ1 phosphorylation by the receptor (Rotin *et al.* 1992). This is likely to have physiological significance, since mutagenesis studies indicate that phosphorylation of Tyr783 in PLC-γ1 is important for its activation by the PDGF-receptor (Kim *et al.* 1991). The physical complex formed between a receptor tyrosine kinase and an SH2-containing signalling protein may therefore identify the latter as a preferential substrate for phosphorylation, and thereby contribute to its activation.

A third possible mode of activation is that the mere binding of the SH2 domains of a signalling protein to phosphotyrosine-containing sites might modify the activity of the associated catalytic domain. A prototype for this scheme involves the activation of PI 3′-kinase by the insulin-receptor. Autophosphorylation of the insulin-receptor stimulates its tyrosine kinase activity, but does not induce the marked binding of SH2-containing signalling proteins to the activated receptor. Rather, the principal receptor substrate is a large protein, IRS-1, with multiple potential tyrosine phosphorylation sites (Sun *et al.* 1991). These IRS-1 tyrosine phosphorylation sites are located within sequences that are reminiscent of SH2-binding sites for proteins such as PI 3′-kinase and Grb2. Indeed, tyrosine phosphorylation of IRS-1 induces its association with the p85 SH2 domains, and hence with PI 3′-kinase (Lavan *et al.* 1992). These results present a conundrum, however, as there is no evidence for tyrosine phosphorylation of PI 3′-kinase in insulin-stimulated cells. A possible explanation for this puzzle is that binding of the SH2 domains of PI 3′-kinase to phosphorylated sites on IRS-1 directly stimulates PI 3′-kinase activity. Data supporting this notion have recently been reported (Backer *et al.* 1992).

(*c*) *Coupling tyrosine kinases to Ras*

A variety of data indicate that activation of Ras is crucial for the induction of DNA synthesis by mitogenic tyrosine kinases (Mulcahy *et al.* 1985; Smith *et al.* 1986). Furthermore, Ras is necessary for neurite outgrowth in PC12 neuronal cells stimulated with nerve growth factor (Thomas *et al.* 1992; Wood *et al.* 1992), which activates the Trk tyrosine kinase, and for developmental events induced in the *Drosophila* compound eye by the Sevenless and DER receptor tyrosine kinases (Simon *et al.* 1991). These results beg the question as to how tyrosine kinases communicate with Ras.

In principle, Ras could be activated either by stimulating exchange of GDP for GTP, or by inhibiting GAP activity. In mammalian cells stimulated by growth factors, it appears that Ras is converted to the GTP-bound form primarily by induction of a GNRP, which promotes GDP–GTP exchange (Zhang *et al.* 1992; Medema *et al.* 1993; Buday & Downward 1993). These results suggest that tyrosine kinases activate a Ras-GNRP. Clues as to the series of protein-protein interactions that lead to stimulation of Ras guanine nucleotide exchange have recently been garnered from the genetic analysis of signal transduction in invertebrate organisms.

In the nematode worm *Caenorhabditis elegans*, differentiation of vulval precursor cells requires an inductive signal from the anchor cell, which triggers a signalling cascade within the vulval progenitor (Horvitz *et al.* 1991). Genetic evidence indicates that a receptor tyrosine kinase, Let-23, transmits the signal through a Ras protein encoded by the let-60 gene. Upstream of Let-60, and most probably downstream of Let-23, is the product of the *sem-5* gene, which contains a central SH2 domain flanked by two SH3 domains (Clark *et al.* 1992) (figure 1). A plausible explanation for the role of the Sem-5 protein is that it binds to the activated Let-23 receptor tyrosine kinase

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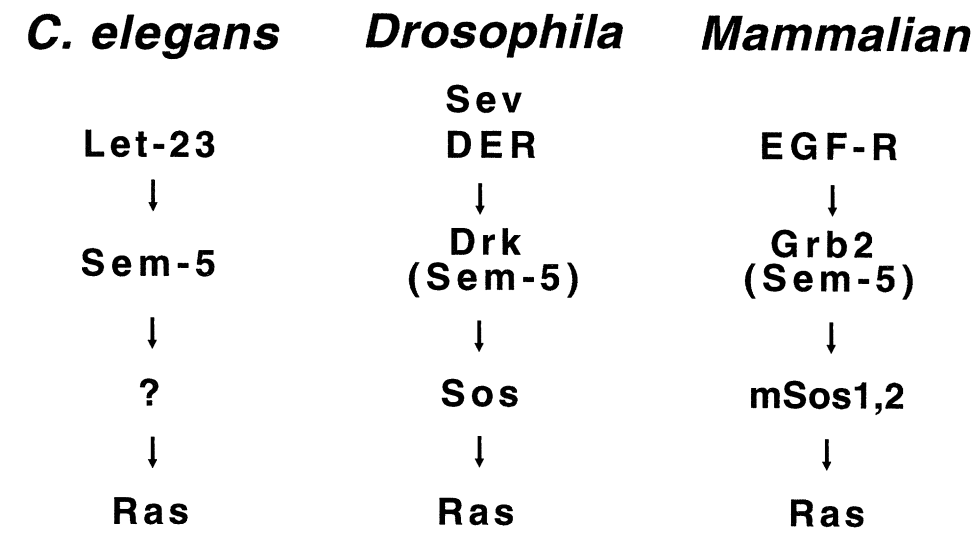

Figure 3. A comparison of signalling pathways involved in Ras activation in *C. elegans*, *Drosophila* and mammalian cells. See text for details.

through its SH2 domain, and to a downstream target involved in control of Ras guanine nucleotide exchange through its SH3 domains. Results obtained with the *Drosophila* homologue of Sem-5 (termed Drk for 'Downstream of receptor kinases') suggest that this target is the product of the *Son-of-sevenless (Sos)* gene (Olivier *et al.* 1993). Sos has a central domain related to the CDC25 protein of *Saccharomyces cerevisiae*, which acts as a Ras-GNRP (Simon *et al.* 1991; Rogge *et al.* 1991). In addition, Sos has a long C-terminal tail which is rich in proline residues, and contains several potential SH3-binding sites. *In vitro*, the Drk protein binds to the C-terminal tail of Sos. Drk also binds through its SH2 domain to activated receptor tyrosine kinases, including an activated version of the Sevenless receptor (Olivier *et al.* 1993). These results imply that in *Drosophila* Drk may function to link tyrosine kinases to Sos, which in turn can potentially activate Ras.

The components of this signalling pathway are also found in vertebrates. A mammalian homologue of Sem-5, Grb2, is apparently involved in Ras activation in mouse fibroblasts (Lowenstein *et al.* 1992); indeed cDNAs for both human Grb2 and *Drosophila* Drk can suppress the effects of *sem-5* mutations on vulval development in *C. elegans* (M. Stern, personal communication). Hence the Grb2, Drk and Sem-5 proteins are functional, as well as structural homologues. Furthermore, two mammalian cDNAs have been isolated whose products (mSos1 and 2) are remarkably similar to the *Drosophila Sos* gene product (Bowtell *et al.* 1992). These observations raise the possibility, outlined in figure 3, that the pathway through which tyrosine

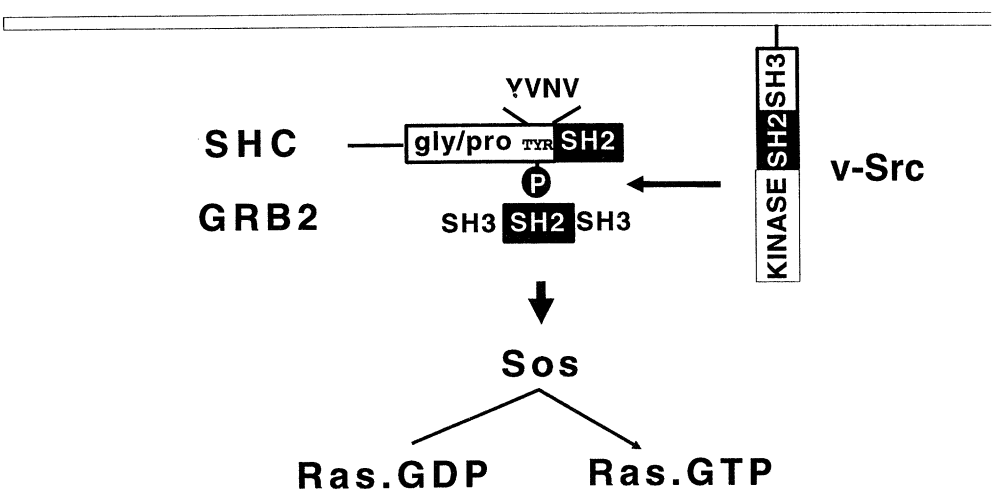

Figure 4. Formation of a Shc-Grb2 complex. Shc proteins are highly phosphorylated on tyrosine in cells transformed by the v-Src cytoplasmic tyrosine kinase. The sequence of the apparent Shc phosphorylation site ($Y^{317}VNV$) is a phosphorylation-dependent binding site for the Grb2 SH2 domain. Grb2 can potentially stimulate Ras through its interactions with the Sos GNRP. Hence, one function of Shc may be to couple v-Src to Grb2, and hence to the Ras pathway.

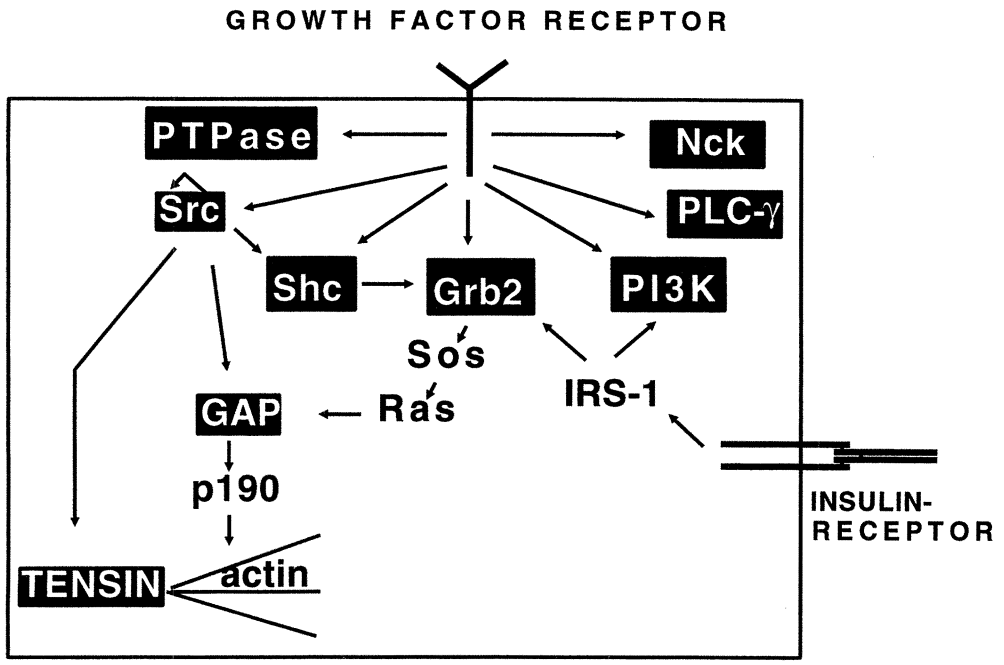

Figure 5. SH2 and SH3 domains mediate a complex series of protein-protein interactions in signal transduction. Proteins with SH2/SH3 domains are indicated by black boxes. See text for details. The c-Src cytoplasmic tyrosine kinase is negatively regulated by an interaction between its SH2 domain and its own phosphatyrosine-containing tail (Liu *et al.* 1993).However, dephosphorylation of Tyr527 within the tail frees the SH2 domain to interact with other phosphotyrosine-containing proteins.

kinases control Ras has been conserved throughout metazoan evolution.

(*d*) *Phosphotyrosine-containing Shc proteins bind Grb2*

Pelicci *et al.* (1992) have identified a novel human gene, *SHC*, that encodes at least three overlapping proteins, each with a C-terminal SH2 domain and a more N-terminal glycine/proline-rich region. Tyrosine phosphorylation of Shc proteins, which is a common event in response to a wide range of polypeptide hormones, induces their association with the SH2 domain of Grb2 (McGlade *et al.* 1992; Rozakis-Adcock *et al.* 1992). The predicted Shc phosphorylation site at Tyr317 lies within a sequence context (YVNV) which fits well with the binding specificity of the Sem-5/Grb2 Sem-5 SH2 domain (Songyang *et al.* 1993). Hence it seems likely that Shc phosphorylation provides an alternative mode of coupling to Ras. Consistent with this view, overexpression of Shc proteins induced transformation in NIH 3T3 mouse fibroblasts, and Ras-dependent neurite outgrowth in PC12 cells (Pelicci *et al.* 1992; Rozakis-Adcock *et al.* 1992). The precise function of the Shc-Grb2 complex is not yet clear. Because Grb2 is not itself a substrate for phosphorylation, but appears to be controlled by interaction of its SH2 domain with phosphotyrosine-containing proteins, one possible role is to couple tyrosine kinases which lack intrinsic Grb2-binding sites to the Ras pathway (figure 4). It is of interest that in some cell types Grb2 forms multiple complexes; for example in EGF-stimulated cells Grb2 binds to both the EGF-receptor and to Shc. These results indicate that Grb2 is regulated in a complex fashion.

3. CONCLUSION

The activation of receptor tyrosine kinases stimulates a complex network of protein-proteins interactions, regulated by SH2 and SH3 domains (figure 5). Tyrosine phosphorylation acts as the trigger for these events, by inducing the association of SH2 domains of cytoplasmic signalling proteins with receptors or other phosphotyrosine-containing proteins. In addition to binding receptors, SH2 domains direct interactions with cytoplasmic proteins, as typified by the binding of Grb2 to phosphorylated Shc polypeptides. SH3 domains apparently extend the ability of signalling proteins to complex with one another. A detailed understanding of the specific interactions mediated by SH2 and SH3 domains will define the intracellular events that initiate cellular responses to growth factors.

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