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Minireview

The GRB2/Sem-5 adaptor protein

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Abstract

GRB2/Sem-5 is a 25-kDa adaptor protein which contains a central Src homology type 2 (SH2) domain flanked by two Src homology type 3 (SH3) domains. GRB2/Sem-5 was first identified due to the essential role of the *sem-5* gene product in the vulval induction pathway in *Caenorhabditis elegans*. The SH2 domain of GRB2/Sem-5 binds to a number of tyrosine phosphorylated proteins, most notably the epidermal growth factor receptor, the insulin receptor substrate IRS-1 and another putative adaptor protein, Shc. The SH3 domains bind to Sos, a guanine nucleotide exchange factor for Ras proteins. GRB2/Sem-5 brings together Sos and tyrosine phosphoproteins into a complex and thereby may regulate the nucleotide exchange rate of Ras and hence its activation state.

Key words: Signal transduction; Adaptor protein; Receptor binding; SH2 and SH3 domains; Activation of RAS

1. Introduction

The low molecular weight GTP binding proteins of the Ras family have long been known to play a key role in the regulation of cellular growth and differentiation. They are biologically active when bound to GTP and inactive when bound to GDP. Their activation state in the cell is regulated by the opposing effects of two sets of proteins, the GTPase activating proteins (GAPs) which stimulate the intrinsic rate of hydrolysis of GTP on Ras, and the guanine nucleotide exchange factors that accelerate the replacement of bound GDP with fresh GTP from the cytosol (reviewed in [1,2]). Ras is stimulated in whole cells on exposure to a number of growth stimulatory factors. In certain cases these have been shown to activate Ras by suppressing GAP activity [3], but more commonly the activity of guanine nucleotide exchange factors is increased [4–6]. Investigation of the mechanisms by which growth factors control the activity of guanine nucleotide exchange factors towards Ras has lead to the identification of GRB2/Sem-5 as a key component of this pathway.

2. The role of Sem-5 in *C. elegans* vulval development

In the nematode worm the genetics of the development of the vulva has been mapped out in some detail. Vulval precursor cells carry the Let-23 receptor tyrosine kinase; when this interacts with its ligand, Lin-3, expressed on the surface of the anchor cell, a signal inducing differentiation to a vulval cell fate is transmitted into the vulval precursor cell [7]. A gene, *let-60*, lying downstream of *let-23* on this pathway encodes a worm homologue of Ras [8,9]. More recently, Sem-5 was identified as another component of this pathway, functioning upstream of Ras but downstream of Let-23 [10] (see Fig. 1).

From its sequence, Sem-5 appeared to be an 'adaptor' protein, one containing only domains capable of binding other proteins but not having any intrinsic enzymatic activity of its own. Sem-5 is made up of a central Src homology type 2 (SH2) domain, flanked by two Src homology type 3 (SH3) domains. This structure is reminiscent of Crk, a proto-oncogene product whose function is poorly understood [11]. SH2 domains bind to protein tyrosine phosphorylation sites [12], while SH3 domains bind to proline-rich motifs in proteins [13]. Mutations in Sem-5 that inhibited vulval development occurred in residues that were highly conserved in the SH2 and SH3 consensus sequences. Although the full significance of these observations was not immediately clear, it was obvious that Sem-5 was likely to function to couple a receptor tyrosine kinase to an activator of Ras, possibly a

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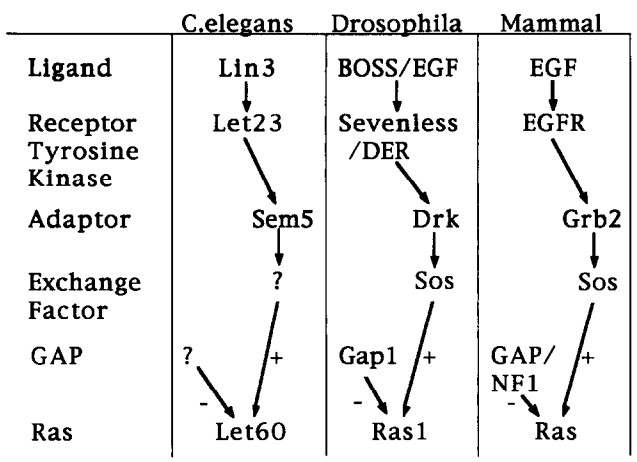

Fig. 1. Comparison of Ras regulatory pathways in different multicellar organisms. See text for details.

guanine nucleotide exchange factor. Since the data from this system was entirely genetic, it was impossible to know how direct these interactions might be.

3. GRB2 is a growth factor receptor binding protein

GRB2, the mammalian homologue of Sem-5, was cloned independently due to its ability to bind to tyrosine phosphorylated proteins through its SH2 domain. GRB2 clones were identified by screening a bacterial cDNA expression library with the tyrosine phosphorylated carboxy terminal region of the epidermal growth factor receptor [14]. The name, standing for growth factor receptor-bound protein 2 reflects the cloning protocol. The rat homologue of GRB2 was cloned independently using a consensus probe for SH2 sequences and was initially named ASH, for abundant Src homology protein [15]. Mouse GRB2 was subsequently cloned using a PCR method with probes based on the SH2 and SH3 domains of Vav [16].

The SH2 domain of GRB2 binds to the tyrosine autophosphorylation sites of activated EGF receptor [14], suggesting that the interaction of Sem-5 with Let-23 is direct. GRB2 has the same structure as Sem-5 with two SH3 domains and one SH2 domain in the order SH3-SH2-SH3. The N-terminal SH3 domain stretches from amino acids 5 to 55, the SH2 domain stretches from amino acids 60 to 158 and the C-terminal SH3 domain stretches from amino acids 164 to 214. The human and rat protein sequences are identical, with the mouse sequence differing at one position (M for V at 154). Human GRB2 shares 58% identity with *C. elegans* Sem-5. The sequences of GRB2 and Sem-5 are compared in Fig. 2.

4. The role of GRB2/Sem-5 in *Drosophila* eye development

During the development of the photoreceptor R7 cell in the fruit fly retina, Sevenless, a receptor tyrosine kinase on surface of the R7 precursor cell interacts with its ligand, Boss, on the apposed R? cell. This interaction generates a signal leading to development of the precursor cell to an R7 phenotype. The Ras1 protein functions downstream of Sevenless. Between Sevenless and Ras another component was identified which was named Sos, or Son of sevenless [17–19]. The Sos protein has homology to CDC25, a guanine nucleotide exchange factor for Ras from *Saccharomyces cerevisiae*, and is therefore likely to act as an exchange factor for Ras in the *Drosophila* eye.

The *Drosophila* homologue of GRB2/Sem-5, named Drk for 'downstream of receptor kinase', was subsequently cloned using exactly the same procedure as for human GRB2 [20]. It was then shown that the *drk* gene product functions downstream of the Sevenless receptor tyrosine kinase and upstream of Sos in R7 development (see Fig. 1). Simultaneously, an *Enhancer of sevenless* gene, whose product acted in the same way, was cloned by using P element insertion [21] and shown to encode the same GRB2/Sem-5 homologue. Human GRB2 shares 63% identity with *Drosophila* Drk. Sem-5 and Drk are 61% identical. This genetic evidence indicated that GRB2/Sem-5 couples a receptor tyrosine kinase to a guanine nucleotide exchange factor for Ras, although the directness of the interactions could not be predicted.

5. GRB2/Sem-5 links receptors and Sos

At the same time as work on the genetics of *Drosophila* Drk was suggesting that GRB2/Sem-5 was involved in linking receptor tyrosine kinases to exchange factors for Ras, a number of groups working on mammalian systems reported that GRB2/Sem-5 forms direct complexes with both activated growth factor receptors and the Sos exchange factors for Ras. The two murine homologues of Sos were cloned by homology to the *Drosophila* cDNA [22]. Subsequently, the human version of Sos1

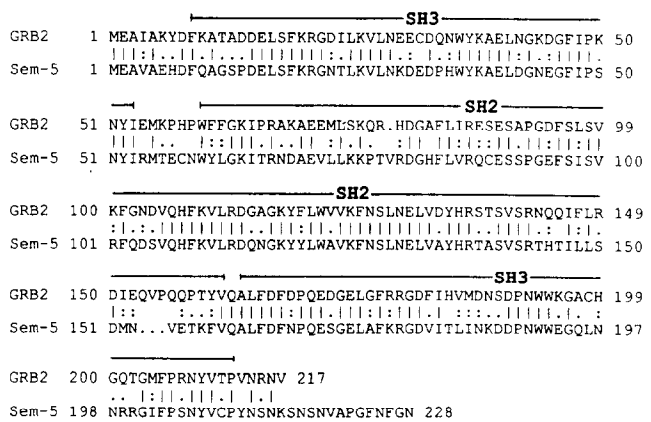

Fig. 2. Comparison of the protein sequences of GRB2 and Sem-5. The location of the SH3 and SH2 domains are shown above the sequence.

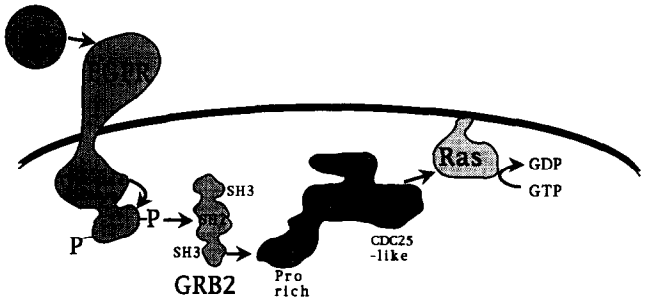

Fig. 3. A model for the regulation of Ras by EGF acting through GRB2 and Sos. See text for details.

was also cloned [23]. GRB2 can be found in a complex with Sos in mammalian cells [23–27]. In most cell types the association of Sos and GRB2 was found to be constitutive, although in Rat-1 fibroblasts it was shown to be inducible by EGF [24]. The interaction occurred between the SH3 domains of GRB2 and proline-rich sequences in the carboxy-terminal region of Sos [25,26].

At the same time, GRB2 interacted with the activated, autophosphorylated EGF receptor but not the inactive receptor. EGF treatment of cells therefore leads to formation of complexes of receptor, GRB2 and Sos at the plasma membrane (see Fig. 3). Given the background of genetic information from *C. elegans* and *Drosophila*, it appears very likely that formation of such a complex leads to activation of the rate of nucleotide exchange on Ras: this has not yet been definitively proved and could occur through a number of mechanisms. Two likely possibilities are by allosteric regulation of Sos on binding GRB2/EGF receptor or by translocation of Sos to the plasma membrane where both EGF receptor and Ras are located. By subcellular fractionation, both Sos and GRB2 are located in the cytosol of unstimulated cells but, in some cases, translocation to the particulate fraction has been observed upon EGF stimulation [24]. As yet there is no evidence for allosteric regulation of the activity of Sos by complex formation.

In addition to the activated EGF receptor, GRB2 has been shown to be bound in cell lysates to the tyrosine phosphorylated insulin receptor substrate, IRS-1 [28–31], the adaptor protein SHC [32], the chimeric oncogene product BCR-ABL [33] and the middle T antigen of polyoma virus. All of these proteins have in some way been linked to activation of Ras and all are, at least partly, localised to the particulate fraction. Binding of GRB2 to a number of other tyrosine phosphorylated proteins and peptides has been demonstrated in vitro, although the significance of these interactions is not always clear. It is very likely that binding can be forced to occur in vitro using high concentrations of bacterially expressed GRB2 fusion proteins and overexpressed tyrosine phosphorylated proteins or synthetic tyrosine phosphopeptides which does not reflect physiologically significant interaction in vivo.

6. Shc may provide another route to GRB2 activation

The adaptor protein Shc consists of one SH2 domain and a region of homology to collagen, but no SH3 domains [34]. Shc becomes phosphorylated on tyrosine in response to treatment of cells with a wide variety of growth stimuli, and also binds directly to the autophosphorylated EGF receptor through its SH2 domain. The SH2 domain of GRB2 will bind to tyrosine phosphorylated Shc [32]. Shc will act as an oncogene to cause transformation of fibroblasts when overexpressed, and will also induce neurite outgrowth in PC12 cells in a manner sensitive to inhibition by expression of dominant negative Ras mutants. Shc therefore acts in a manner consistent with it being an upstream activator of Ras. While the exact mechanism underlying these phenomena is unknown, it is possible that the interaction of GRB2/Sos with phosphorylated Shc leads to activation of nucleotide exchange on Ras in a similar way to the interaction with phosphorylated EGF receptor or IRS-1. In many signalling pathways leading to Ras activation, for example that regulated by NGF, GRB2 does not bind to any receptor, but inducible Shc/GRB2 interaction does occur [16]. Shc binding to GRB2 could act as an alternative route to activate Sos, again either through translocation to the membrane or through allosteric regulation (see Fig. 4). A major portion of Shc is particulate (J.D., unpublished observations); in addition it can bind to certain activated receptors such as the EGF receptor. It is not clear whether binding to the receptor, as opposed to phosphorylation by it, is essential for Shc function.

7. Characterisation of GRB2 in intact cells

The ability of GRB2 to form complexes with Sos and receptors suggests that it may be capable of promoting cellular growth when overexpressed. This proves to be the case only under certain circumstances, either when excess Ras is present [14] or when EGF receptors are overexpressed [35]. In the absence of overexpression of other components of the pathway, excess GRB2 can actually inhibit signalling to Ras (J.D., unpublished obser-

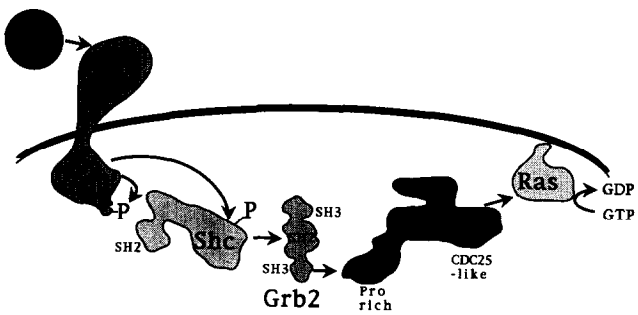

Fig. 4. A possible model for the involvement of Shc in GRB2 mediated signalling from NGF. See text for details.

vations); presumably this occurs through the blocking of Sos/GRB2 binding to tyrosine phosphoproteins by free GRB2. However, blocking GRB2 function by microinjection of antibodies against it into mammalian cells blocked the induction of S phase entry and the induction of membrane ruffling in response to PDGF and EGF [36].

The localisation of GRB2 in intact cells has been studied by immunofluorescence microscopy [37]. GST-GRB2 fusion protein was microinjected into fibroblasts and visualised using antibodies against GST. The fusion protein was found at membrane ruffles, a localisation which depended on the integrity of the SH3 domains. This could reflect binding of GRB2 to proteins important in the regulation of the actin cytoskeleton, as suggested by the results from microinjection of anti-GRB2 antibodies. Alternatively, the SH3 domains of GRB2 could bind to other proteins at the inner face of the plasma membrane: one possibility is dynamin, which has been shown to bind to GRB2 through its SH3 domains in vitro [38]. Dynamin is a GTPase, initially found in association with microtubules, which is associated with clathrin coated pits. When GRB2 binds to dynamin its GTPase activity is greatly accelerated. The significance of these observations to the function of GRB2 in the intact cell is not clear.

8. Conclusions

The identification and characterisation of mammalian GRB2 and its invertebrate homologues, Sem-5 in *C. elegans* and Drk in *Drosophila*, have brought us much closer to understanding the mechanisms by which the Ras proteins are regulated in response to extracellular mitogens. For the first time, all the components of the signalling pathways upstream of Ras appear to have been identified. The SH2 domain of GRB2 binds to tyrosine phosphorylated proteins, including receptor tyrosine kinases and the Shc protein, while the SH3 domains bind to the Ras guanine nucleotide exchange factor, Sos. The exact details of the mechanisms involved have yet to be fully understood, but it is clear that GRB2 plays a central role. GRB2 is only one of a growing number of SH2/SH3 containing adaptor proteins that have been identified. It is possible that others, such as Crk or Nck, could play similar roles in bringing together components of signalling pathways initiated by tyrosine phosphorylation.

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