DOI: 10.1534/genetics.106.055822

The Adaptor Protein *soc-1*/Gab1 Modifies Growth Factor Receptor Output in *Caenorhabditis elegans*

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Manuscript received January 13, 2006
Accepted for publication March 6, 2006

ABSTRACT

Previous genetic analysis has shown that *dos/soc-1*/Gab1 functions positively in receptor tyrosine kinase (RTK)-stimulated Ras/Map kinase signaling through the recruitment of *csw/ptp-2*/Shp2. Using sensitized assays in *Caenorhabditis elegans* for *let-23*/Egfr and *daf-2*/InsR (insulin receptor-like) signaling, it is shown that *soc-1*/Gab1 inhibits phospholipase C-γ (PLCγ) and phosphatidylinositol 3′-kinase (PI3K)-mediated signaling. Furthermore, as well as stimulating Ras/Map kinase signaling, *soc-1*/Gab1 stimulates a poorly defined signaling pathway that represses class 2 *daf-2* phenotypes. In addition, it is shown that SOC-1 binds the C-terminal SH3 domain of SEM-5. This binding is likely to be functional as the *sem-5(n2195)*G201R mutation, which disrupts SOC-1 binding, behaves in a qualitatively similar manner to a *soc-1* null allele in all assays for *let-23*/Egfr and *daf-2*/InsR signaling that were examined. Further genetic analysis suggests that *ptp-2*/Shp2 mediates the negative function of *soc-1*/Gab1 in PI3K-mediated signaling, as well as the positive function in Ras/Map kinase signaling. Other effectors of *soc-1*/Gab1 are likely to inhibit PLCγ-mediated signaling and stimulate the poorly defined signaling pathway that represses class 2 *daf-2* phenotypes. Thus, the recruitment of *soc-1*/Gab1, and its effectors, into the RTK-signaling complex modifies the cellular response by enhancing Ras/Map kinase signaling while inhibiting PI3K and PLCγ-mediated signaling.

GROWTH factors commonly act through cell surface receptors with intrinsic tyrosine kinase activity to control a wide range of cellular activities including movement, differentiation, proliferation and survival. Receptor tyrosine kinases (RTKs) are activated by growth factor binding leading to the tyrosine phosphorylation of substrates. This may activate substrates directly or create specific binding sites for proteins containing Src homology-2 (SH2) domains. Recruitment of effector molecules leads to the activation of a small number of intracellular signaling cascades, including those mediated by Ras, phosphatidylinositol 3′-kinase (PI3K), and phospholipase C-γ (PLCγ) activation (Figure 1). How the specificity of the response is achieved is not completely understood, but it is believed that the context of the cell limits the possible responses to growth factor signaling and the magnitude and duration of RTK activation and, together with the relative degree of stimulation of particular intracellular signaling pathways, determines the specific response from this limited set (Marshall 1995; Schlessinger 2000). Distinct growth factors are differentiated by the relative abundance of their receptors upon the cell surface, coupled with the fact that distinct receptors activate intracellular signaling pathways differentially (Pawson and Scott 1997; Schlessinger 2000). The intracellular signaling pathways activated by a particular growth factor receptor are determined by the presence/abundance of effector binding sites on the receptor itself or on associated scaffolding/docking proteins (Pawson and Scott 1997). Scaffolding/docking proteins typically contain an N-terminal pleckstrin homology (PH) domain followed by a phosphotyrosine binding (PTB) domain and a C terminus containing multiple SH2 binding sites. The use of scaffolding/docking proteins broadly separates growth factor receptors into two classes. For example, the insulin-like growth factor-1 (IGF-1) receptor and the fibroblast growth factor (Fgf) receptor (Fgfr) primarily utilize scaffolding/docking adaptor proteins such as IRS1 (insulin receptor substrate 1) or FRS2 (Fgf receptor substrate 2) to mediate effector binding. By contrast, the epidermal growth factor receptor (Egfr) and platelet-derived growth factor receptor utilize tyrosines within C-terminal extensions as substrates onto which multi-protein signaling complexes are assembled (Pawson and Scott 1997; Schlessinger 2004). The Gab family of adaptor proteins is structurally similar to scaffolding/docking proteins but lacks a PTB domain and is utilized downstream from a broad range of growth factor receptors (reviewed in Liu and Rohrschneider 2002; Gu and Neel 2003; Nishida and Hirano 2003). Gab1 was

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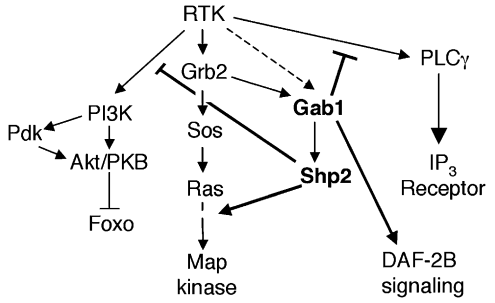

Figure 1.—Schematic showing the effect of Gab1 on Ras/Map kinase-mediated signaling as revealed by genetic studies. Additional effects on PLCγ-, PI3K-, and DAF-2B-mediated signaling are derived from this study (thick lines). Arrows represent a positive/activating interaction. Lines ending in a perpendicular represent a negative/inhibitory interaction. IP_3 receptor, inositol trisphosphate receptor. Dashed arrow shows that there is evidence that Gab1 can bind the Met RTK, but not other RTKs directly (Weidner *et al.* 1996).

initially identified as a Grb2-binding protein and contains SH2-binding sites for the regulatory subunits of PI3K, PLCγ, and Shp2 (Holgado-Madruga *et al.* 1996). Recruitment of Gab1 into the signaling complex usually occurs through its interaction with Grb2, although there is some evidence that Gab1 binding to the c-Met RTK may be more direct (Weidner *et al.* 1996). The interaction between Gab1 and Grb2 occurs between the C-terminal SH3 domain of Grb2 and an atypical proline motif within Gab1 (Lock *et al.* 2000; Feller *et al.* 2002). Recruitment of Gab1 is under control of a positive-feedback loop: Gab1 recruits PI3K, increasing phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] production, which in turn promotes further binding of Gab1 through its PH domain (Isakoff *et al.* 1998; Rodrigues *et al.* 2000). Thus the recruitment of Gab1 may be regulated by the extent of RTK signaling.

Genetic systems have proven to be important in demonstrating the function of signaling components. In Drosophila, the Gab1 homolog, *dos*, was identified as a positively acting signaling component located downstream of the *sev* RTK (Herbst *et al.* 1996; Raabe *et al.* 1996). More recently, the *Caenorhabditis elegans* Gab1 homolog, *soc-1*, was identified as a positive signaling component downstream of the Fgfr (Schutzman *et al.* 2001). Previous genetic assays for Gab1 function, using these model organisms, utilized signaling mediated by the RTK/Ras/Map kinase pathway. In this context, the essential function of Gab1 is to recruit Shp2, a dual SH2 domain containing protein tyrosine phosphatase (Figure 1; Herbst *et al.* 1999; Bausenwein *et al.* 2000; Schutzman *et al.* 2001). The role of Gab1 in RTK signaling mediated by intracellular pathways other than the Ras/Map kinase-signaling pathway has not been previously addressed in a genetic model system. Here the role of Gab1 in RTK signaling utilizing intracellular signaling pathways mediated by PI3K and PLCγ is reported, using assays for Egfr and insulin receptor-like signaling in *C. elegans*.

In *C. elegans*, there is a single Egfr encoded by *let-23*, which functions during development to mediate induction of several specializations of the hypodermis through activation of the conserved Ras/Map kinase pathway (Sternberg and Han 1998). In addition, *let-23* activity is required for hermaphrodite fertility. This adult function of *let-23*/Egfr is genetically separable from the developmental functions and is not mediated through Ras activation (Aroian *et al.* 1994; Jongeward *et al.* 1995; Lesa and Sternberg 1997). Instead, the *let-23*/Egfr fertility function is mediated through PLCγ signaling (Clandinin *et al.* 1998; Yin *et al.* 2004).

Similarly, there is a single insulin/IGF-1 receptor-like (InsR) molecule in *C. elegans*, encoded by the *daf-2* gene (Kimura *et al.* 1997). Mutations in *daf-2* were first identified in screens for animals that form dauers constitutively—the Daf-c phenotype (Riddle and Albert 1997). Dauers are stress resistant, diapausal, third stage larvae that normally develop in response to increased population density and starvation. There are two classes of *daf-2* mutations. Class 1 *daf-2* mutants form dauers constitutively, are intrinsically thermotolerant, and are long lived. Class 2 *daf-2* mutants display these phenotypes plus one or more additional phenotypes, which include embryonic/early larval arrest, reduced pharyngeal pumping, and late progeny production (Gems *et al.* 1998). Class 1 and class 2 *daf-2* alleles do not fall into a single allelic series, as class 1 alleles can be more severe for the Daf-c phenotype than many class 2 alleles. This suggests that the *daf-2* gene may have distinct functional elements, hypothesized as *daf-2A* and *daf-2B* (Gems *et al.* 1998). However, unlike the *let-23*/Egfr gene, functional elements of the *daf-2*/InsR gene are not genetically separable. Thus, class 1 phenotypes may result from loss of *daf-2A* activity whereas class 2 phenotypes result from loss of both *daf-2A* and *daf*-2B activities (Gems *et al.* 1998).

daf-2/InsR signaling is primarily mediated through *age-1*/PI3K, which produces PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2 (Morris *et al.* 1996). The production of PtdIns(3,4,5)P_3 stimulates PDK-1 and the AKT-1 and AKT-2 protein kinase B proteins, which in turn inactivate the FOXO transcription factor DAF-16 (Figure 1; (Ogg *et al.* 1997; Paradis and Ruvkun 1998; Paradis *et al.* 1999). In addition, *let-60*/Ras modulates *daf-2*/InsR signaling (Nanji *et al.* 2005).

Here it is shown that *soc-1*/Gab1 has positive and negative regulatory functions in both Egfr and insulin receptor-like signaling in *C. elegans*. Downstream of *let-23*/Egfr, *soc-1*/Gab1 functions positively during vulval induction, but negatively upon *let-23*/Egfr-mediated hermaphrodite fertility. Downstream of *daf-2*/InsR, *soc-1*/Gab1 functions to negatively regulate dauer formation, but contributes positively to *daf-2B* signaling: mutation of *soc-1* in a class 1 *daf-2* background causes class 2 phenotypes to be displayed.

Furthermore, it is shown that a C-terminal SH3 domain mutation in *sem-5*/Grb2 has a qualitatively similar effect as a null mutation in *soc-1*/Gab1 in all assays performed for Egfr and InsR signaling. This same mutation in *sem-5* blocks SOC-1 binding to SEM-5, consistent with a model where SOC-1/Gab1 recruitment into the signaling complex downstream of the Egfr and insulin receptor is dependent upon the C-terminal SH3 domain of SEM-5/Grb2. However, genetic analysis of *ptp-2*/Shp2 suggests that recruitment of PTP-2/Shp2 may not be the sole function of SOC-1/Gab1.

Together the data suggest that the recruitment of *soc-1*/Gab1 into the RTK-signaling complex regulates signaling output by potentiating Ras/Map kinase-mediated signaling while inhibiting PI3K- and PLCγ-mediated signaling. As Gab1 recruitment is itself regulated by the extent of signaling (Rodrigues *et al.* 2000), this provides a mechanism that could link the magnitude and duration of RTK signaling to distinct cellular responses.

MATERIALS AND METHODS

Strains and genetics: The N2 strain was used as the wild-type *C. elegans* strain. The following mutant strains were used in this study: DR1567 *daf-2(m577)*, CB1370 *daf-2(e1370)*, MT5547 *clr-1(e1745); soc-1(n1789)*, MT5998 *sem-5(n2195)*, WS841 *ptp-2(op194) unc-4(e120)/mln1[dpy-10(e128)]; him-5(e1490)*, HP36 *let-23(sy10) unc-4(e120)/mnC1[dpy-10 unc-52]; let-60(n1046gf)*, MT2124 *let-60(n1046gf)* (Beitel *et al.* 1990; Clark *et al.* 1992; Gems *et al.* 1998; Gutch *et al.* 1998; Hopper *et al.* 2000; Schutzman *et al.* 2001). *soc-1(n1789)* results in a G87STOP within the N-terminal PH domain and is defined as a null mutation of *soc-1* (Schutzman *et al.* 2001). The *ptp-2(op194)* null mutation deletes the entire phosphotyrosine phosphatase domain of *ptp-2* (Gutch *et al.* 1998). The following strains were generated for this study using standard techniques: HP25 *soc-1(n1789)*, HP26 *daf-2(m577); sem-5(n2195)*, HP27 *daf-2(e1370); sem-5(n2195)*, HP28 *daf-2(m577); soc-1(n1789)*, HP29 *daf-2(e1370); soc-1(n1789)*, HP30 *let-23(sy10) unc-4(e120)/mnC1[dpy-10 unc-52]; let-60(n1046gf); sem-5(n2195)*, HP31 *let-23(sy10) unc-4(e120)/mnC1[dpy-10 unc-52]; let-60(n1046gf); soc-1(n1789)*, HP32 *let-23(sy10) unc-4(e120)/mnC1[dpy-10 unc-52]; let-60(n1046gf); sos-1(n1031) unc-46(e177)*, HP33 *let-60(n1046gf); sem-5(n2195)*, HP34 *let-60(n1046gf); soc-1(n1789)*, HP35 *ptp-2(op194) unc-4(e120); let-60(n1046gf)*.

RNA-mediated interference experiments: *C. elegans* animals were reared on HT115 bacteria expressing *ptp-2* dsRNA to reduce *ptp-2* gene activity. *ptp-2* genomic DNA (nucleotides 67–2233 relative to start codon) was generated by PCR and inserted between the T7 promoters of the L4440 vector. HT115 bacteria were transformed with this *ptp-2*-containing vector and separately with L4440, which was used as a control. Expression of T7 RNA polymerase was induced in HT115 as described in Kamath *et al.* (2001). Animals were maintained on HT115 expressing dsRNA for at least two generations before assaying. Experimental (*ptp-2*) and control (L4440) worms were treated in exactly the same way except *ptp-2(RNAi)* worms were maintained on HT115 transformed with *ptp-2* inserted into L4440 (as above) whereas L4440*(RNAi)* worms were maintained on HT115 transformed with L4440 alone.

Vulval induction and fertility assays: Both assays were performed as previously described (Hopper *et al.* 2000). Briefly, animals were maintained at 20° and vulval induction scored at the early to mid-L4 stage. To control for possible temperature effects and the effect of starvation upon the *let-60(n1046gf)* phenotype (Battu *et al.* 2003), vulval induction assays were performed in parallel upon animals from well-fed plates incubated in the same location within the 20° incubator. For fertility assays, L4 animals were transferred to individual plates and fertility was defined as having more than two offspring.

Terminal arrest and dauer assays: Adult animals were placed at the test temperature on fresh plates for 4–6 hr to lay eggs. The eggs were then transferred to fresh plates, counted, and incubated at the test temperature. For the dauer assays, animals were scored after 66–72 hr. L3d is defined as having a dauer-like appearance but actively pumping pharynx. For the terminal arrest experiments, animals were scored at 72 hr. The stage of arrest was defined by direct observation by DIC of gonad development. L1 arrest was between the 4- and 10-cell gonad stage. L2 arrest was beyond this but before a large proliferation of gonad.

Late offspring and longevity assays: Animals were grown at 20° until the L4 stage of development. They were then picked to a fresh plate and placed at 25°. On each of the subsequent 8 days, they were moved to a fresh plate, as required. Control worms stopped producing offspring on days 4–5. On day 9, worms were placed individually on a plate to allow individual animals to be assayed for late progeny. These worms were moved to fresh plates again 10–14 days later. Animals dying as a result of bagging were censored from the longevity experiment. All worms dying before day 9 were censored from the late offspring experiment. Age at death was recorded to an accuracy of within 3 days. Death was defined as no longer being responsive to being poked repeatedly with a platinum wire. Survival analyses were performed using the Kaplan–Meier method upon censored data and *P*-values calculated for differences between survival curves using the log-rank test.

Yeast two-hybrid experiments: Fragments of *soc-1* cDNA encoding amino acids 179–268 and 286–357 were generated by PCR using primers to incorporate *Eco*RI and *Sma*I restriction sites in frame at each end, respectively. These fragments were cloned into pGBKT7 (CLONTECH, Palo Alto, CA) to create the bait vectors. Fragments of *sem-5* cDNA encoding amino acids 1–212 were also generated by PCR using primers to incorporate *Sma*I and *Pst*I restriction sites in frame at each end, respectively. The G201R mutation was introduced into the SEM-5 fragment using an extended reverse primer for the PCR. Fragments were cloned into pGAD424 (CLONTECH) to create the prey vectors. Yeast manipulations and two-hybrid experiments were performed as previously described (Hopper *et al.* 2000).

RESULTS

SOC-1 binds the C-terminal SH3 domain of SEM-5: Studies of Drosophila and of mammalian cell culture have demonstrated that the C-terminal SH3 domain of Grb2 binds to an atypical proline-rich motif within Gab1 and that this interaction is required for recruitment of Gab1 downstream of the Egfr (Lock *et al.* 2000; Feller *et al.* 2002). This atypical proline-rich motif is conserved in *soc-1*, the *C. elegans* Gab1 homolog (Figure 2A). The *C. elegans* gene, *sem-5*, encodes Grb2. To test whether the binding of the C-terminal SH3 of Grb2 to Gab1 is also conserved in the *C. elegans* proteins, the yeast two-hybrid assay was employed. In these assays, SEM-5 bound the portion of SOC-1 containing the conserved atypical

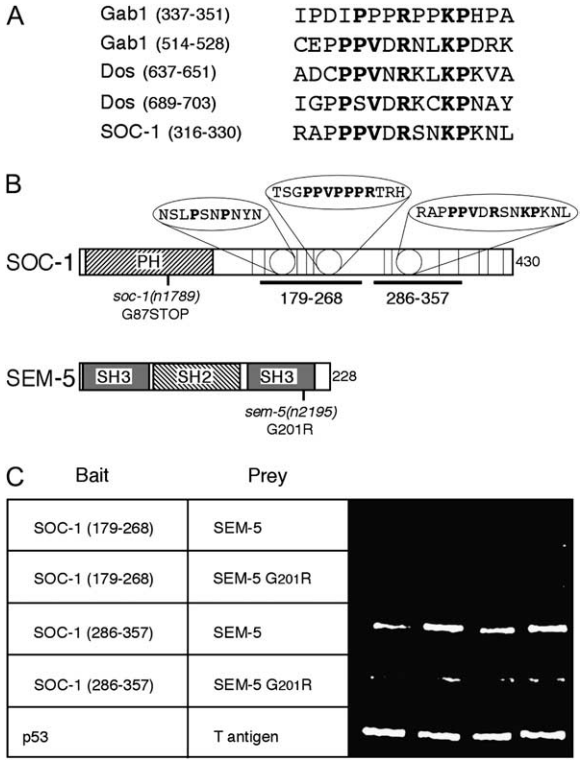

Figure 2.—The C-terminal SH3 domain of SEM-5/Grb2 binds an atypical proline-rich motif within SOC-1/Gab1. (A) Alignment of atypical proline-rich motifs of Gab1 and Dos with a similar sequence found in SOC-1. Gab1 (337–351) constitutes a weak Grb2-binding site, and Gab1(514–528) binds strongly (Lock *et al.* 2000). Both Dos sites shown contribute to Drk/Grb2 binding equally (Feller *et al.* 2002). Residues conserved in three of the four Gab1/Dos sites are in boldface type, as are the corresponding residues in SOC-1. (B) Domain structures of SOC-1 and SEM-5 showing location of mutations used in this study. Thick horizontal line shows regions of SOC-1 used in the yeast two-hybrid assay. Bubbles highlight proline-rich motifs. Vertical lines indicate positions of tyrosines. (C) Yeast two-hybrid analysis of the interaction between SEM-5 and SOC-1. Bait two-hybrid constructs were cloned into pGBKT7 and prey proteins were cloned into pGAD424 (CLONTECH) and two-hybrid analysis was performed as described previously (Hopper *et al.* 2000). SOC-1 (179–268) contains two PxxP motifs, most notably PPVPPPR at residues 236–242. The SEM-5 G201R construct bears the same mutation as found in the *sem-5(n2195)* allele.

proline-rich motif but not to a region containing the two PxxP motifs also found in SOC-1 (Figure 2C). This interaction failed to occur when the conserved glycine at position 201 in SEM-5 was substituted to arginine (Figure 2C). This substitution is within the C-terminal SH3 domain of SEM-5/Grb2 and corresponds to the *sem-5(n2195)* mutation (Clark *et al.* 1992). Thus the interaction of the C-terminal SH3 of *sem-5*/Grb2 with *soc-1*/Gab1 is conserved in *C. elegans*. If this interaction is functionally important, then the *sem-5(n2195)* mutation should behave similarly to a null mutation in *soc-1*, with the caveat that the C-terminal SH3 domain of *sem-5*/Grb2 may have additional functions.

***soc-1* acts positively during *let-23*/Egfr-mediated vulval induction:** *soc-1* was identified as a *s*uppressor *o*f the *c*lear phenotype, resulting from hyperactivity of the Fgfr/Ras/Map kinase pathway in *C. elegans* (Schutzman *et al.* 2001). The *sem-5(n2195)* mutation was also recovered in the same screen (Clark *et al.* 1992). The function of *soc-1* in this Fgfr assay is to recruit *ptp-2* (Schutzman *et al.* 2001). The function of *soc-1* in Egfr signaling in *C. elegans* has not been previously addressed. The vulva in *C. elegans* hermaphrodites is induced by an Egf signal produced from the gonadal anchor cell (Kornfeld 1997). A field of up to six cells, termed the vulva precursor cells (VPCs), can respond to this signal through the activation of an Egfr/Ras/Map kinase pathway. Normally three cells do so, but in animals carrying a weak gain-of-function Ras mutation, *let-60(n1046gf)*, additional cells also respond, producing pseudovulvae. Null mutations in *soc-1* and *ptp-2* and the *sem-5(n2195)* mutation cause no observable defect in vulval differentiation in an otherwise wild-type background. However, previous studies have shown that both a null mutation in *ptp-2* and *sem-5(n2195)*G201R weakly suppress the multivulva phenotype of *let-60(n1046gf)* mutant animals (Gutch *et al.* 1998; Hopper *et al.* 2000). Thus, if *soc-1* functions to recruit *ptp-2* during Ras/Map kinase signaling downstream of the Egfr, as it does downstream of the Fgfr, it is expected that *soc-1* will also weakly suppress the multi-vulva phenotype of *let-60(n1046gf)* animals.

To determine whether the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 function together in Egfr/Ras/Map kinase signaling, double-mutant animals carrying *let-60(n1046gf)* with *sem-5(n2195)*G201R or a null mutation in either *soc-1* or *ptp-2* were generated and subjected to the vulval induction assay in parallel. As expected, all three mutations weakly suppressed *let-60(n1046gf)* (Table 1). The suppression seen by the *soc-1(n1789)* null mutation is identical to the suppression seen by the *ptp-2(op194)* null mutation in this and a previous study (Gutch *et al.* 1998). This is consistent with findings from other genetic assays for Ras/Map signaling, which suggest that the function of *soc-1*/Gab1 is to recruit *ptp-2*/Shp2 (Herbst *et al.* 1999; Bausenwein *et al.* 2000; Schutzman *et al.* 2001). Suppression of the *let-60gf* multi-vulva phenotype was significantly stronger by *sem-5(n2195)*G201R than by *soc-1(n1789)* or *ptp-2(op194)*, suggesting that the C-terminal SH3 domain of *sem-5*/Grb2 has an additional function(s) other than recruiting *soc-1*/Gab1 (Fisher's exact test $P < 0.05$; Table 1). It has been reported that the C-terminal SH3 domain of Grb2 is also required for endocytosis of the Egfr in response to Egf (Wang and Moran 1996), which in turn is required for sustained Map kinase signaling (Vieira *et al.* 1996). In addition, the C-terminal SH3 domain of *sem-5*/Grb2 contributes to the binding of Sos, which in turn activates Ras (Figure 1; Sastry *et al.* 1995).

TABLE 1

***soc-1*/Gab1 suppresses the multi-vulva phenotype arising from a gain-of-function Ras mutation**

Genotype	Average induction[a]	% Muv[b]	*n*
+/+	3.0	0	Many
let-60(n1046gf)	3.9	80	45
*let-60(n1046gf); sem-5(n2195)*G201R	3.2	18	40
let-60(n1046gf); soc-1(n1789)	3.5	53	36
ptp-2(op194); let-60(n1046gf)	3.4	56	41

soc-1(n1789) and *ptp-2(op194)* are null mutations. *ptp-2* was linked to *unc-4(e120)*. Suppression of *let-60(gf)* by *sem-5(n2195)*, *soc-1(0)*, and *ptp-2(0)* was significant (Fisher's exact test $P < 0.05$). A significantly greater number of animals with more than three cells was induced in *let-60ras(gf); soc-1(0)* and *ptp-2(0); let-60ras(gf)* relative to *let-60ras(gf); sem-5(n2195)* animals (Fisher's exact test $P < 0.05$). There was no difference between *let-60ras(gf); soc-1(0)* and *ptp-2(0); let-60ras(gf)* (Fisher's exact test $P = 0.82$).

[a] Total number of VPCs adopting a vulval cell fate divided by the number of animals scored.

[b] Proportion of animals having more than three VPCs induced.

This, and previously published data, is consistent with a model in which the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 act in a genetic pathway to promote/sustain Ras/Map kinase signaling (Herbst *et al.* 1999; Bausenwein *et al.* 2000; Schutzman *et al.* 2001; Feller *et al.* 2002). As mutations in these genes in an otherwise wild-type background are mostly silent in Egfr and Fgfr assays, this pathway is not essential for Ras/Map kinase signaling *per se*, but rather acts to enhance its efficacy. To address whether this pathway also operates in RTK signaling not mediated by Ras/Map kinase, the function of these genes was assessed in assays for RTK signaling mediated by PLCγ and PI3K.

***soc-1* functions negatively in *let-23*/Egfr fertility:** In addition to the function of *let-23*/Egfr in mediating inductive signaling during development in *C. elegans*, *let-23* activity is required for hermaphrodite fertility. The focus of *let-23*/Egfr signaling for the fertility function is in the contractile tissue of the spermatheca and/or gonadal sheath cells (Clandinin *et al.* 1998; Yin *et al.* 2004). Unlike inductive signaling that utilizes the Ras/Map kinase pathway, the fertility function of *let-23* is mediated through PLCγ and IP_3 production (Clandinin *et al.* 1998; Yin *et al.* 2004). Thus, a Ras gain-of-function mutation, *let-60(n1046*gf*)*, suppresses the defect in inductive signaling but not the sterility of animals homozygous for a non-null Egfr mutation, *let-23(sy10)*.

To determine the function of *soc-1*/Gab1 in PLCγ-mediated signaling, an assay for *let-23*/Egfr hermaphrodite fertility was used. In the following experiments, it was necessary to have the *let-60(n1046gf)* mutation present to recover viable animals. Although reducing or increasing Ras activity has no effect upon the fertility function of *let-23*/Egfr, a null mutation in *sem-5*/Grb2 has previously been shown to suppress the sterile Egfr mutation, *let-23(sy10)* (Hopper *et al.* 2000). To test whether the inhibitory effect of *sem-5*/Grb2 is dependent upon the C-terminal SH3 domain, *let-23(sy10); let-60(n1046gf); sem-5(n2195)*G201R triple mutants were generated and assayed for fertility. It was found that *sem-5(n2195)*G201R suppressed *let-23(sy10)* sterility almost as well as the *sem-5* null mutation (Table 2). Thus, the inhibitory function of *sem-5*/Grb2 upon PLCγ signaling is largely mediated through its C-terminal SH3 domain. To test whether this is, in turn, mediated through *soc-1*/Gab1 recruitment, *let-23(sy10); let-60(n1046gf); soc-1(n1789)* animals were generated. Again, it was found that *soc-1*/Gab1 suppressed *let-23*/Egfr sterility (Table 2). However, the suppression of *let-23(sy10)* sterility by *soc-1(n1789)* was not as strong as by *sem-5(n2195)*G201R (Fisher's exact test $P < 0.05$; Table 2). This indicates that the inhibitory function of *sem-5*/Grb2 upon PLCγ signaling is only partly mediated through *soc-1*/Gab1. To test whether the recruitment of *sos-1*/Sos by the C-terminal SH3 domain of *sem-5*/Grb2 is also required for the inhibition of PLCγ signaling, *let-23(sy10); let-60(n1046gf); sos-1(s1031)* animals were generated. Despite *sos-1(s1031)* being a genetic null, no significant

TABLE 2

***soc-1*/Gab1 suppresses the *let-23*/Egfr sterility**

Genotype	% of fertile animals (*n*)
let-23(sy10); let-60(n1046gf)	0 (45)
let-23(sy10); let-60(n1046gf); sem-5(ay73)	55 (31)[a]
*let-23(sy10); let-60(n1046gf); sem-5(n2195)*G201R	33 (110)
let-23(sy10); let-60(n1046gf); soc-1(n1789)	18 (74)
let-23(sy10); let-60(n1046gf); ptp-2(RNAi)	0 (64)
let-23(sy10); let-60(n1046gf); sos-1(s1031)	3 (36)

unc-4(e120) was present in all strains and used for a marker of *let-23(sy10)*, which was balanced in the parental strain by *mnC1*. Animals carrying *sos-1(s1031)* also carried the tightly linked *unc-46(e177)* mutation. In both *let-23(sy10); let-60(gf); soc-1(n1789)* and *let-23(sy10); let-60(gf); ptp-2(RNAi)* animals, ~50% of vulvae ruptured. These ruptured animals were not censored from the assay, as one *let-23(sy10); let-60(n1046gf); soc-1(n1789)* animal whose vulva ruptured was scored as fertile.

[a] Data previously reported (Hopper *et al.* 2000).

TABLE 3

***soc-1*/Gab1 suppresses *daf-2* Daf-c**

	22.5°		24°	
Genotype	Dauers (%)	*n*	Dauers (%)	*n*
daf-2(m577)	9	461	66	888
*daf-2(m577); sem-5(n2195)*G201R	5	356	48	524
daf-2(m577); soc-1(n1789)	0	254	1	253
daf-2(m577); L4440[RNAi]	20	327	77	202
daf-2(m577); ptp-2[RNAi]	2	327	8	146
daf-2(e1370)	100	309	—	
*daf-2(e1370); sem-5(n2195)*G201R	100	160	—	
daf-2(e1370); soc-1(n1789)	73[a]	161	—	
daf-2(e1370); L4440[RNAi]	99[a]	203	—	
daf-2(e1370); ptp-2[RNAi]	1[b]	207	—	

Animals arresting development prior to L2 molt were excluded. —, not determined.
[a] All non-dauers were scored as L3d (dauer-like appearance, but exhibiting strong pharyngeal pumping).
[b] Non-dauers were scored as follows: L3d, 1%; L4s/adults, 98%.

suppression of *let-23*/Egfr sterility was observed (Table 2). This suggests an additional activity of the C-terminal SH3 domain of *sem-5*/Grb2 in the inhibition of PLCγ-mediated signaling other than in recruiting *soc-1*/Gab1 or Sos. This may be in the stimulation of endocytosis (Wang and Moran 1996), which is also expected to inhibit PLCγ activity (Vieira *et al.* 1996; Haugh *et al.* 1999; Haugh and Meyer 2002).

In genetic assays for *soc-1*/Gab1 that utilize the Ras/Map kinase pathway, the function of *soc-1*/Gab1 is to recruit *ptp-2*/Shp2 (Herbst *et al.* 1999; Bausenwein *et al.* 2000; Schutzman *et al.* 2001; this study). *ptp-2* maps very close to *let-23* on chromosome II and *ptp-2(op194)* animals, like *let-23(sy10)* animals, have a fertility defect, making construction of a *ptp-2(op194) let-23(sy10); let-60(n1046gf)* strain exceedingly difficult. Therefore, to address whether *ptp-2*/Shp2 also suppresses *let-23(sy10)* sterility, *let-23(sy10) unc-4(e120)/mnC1; let-60(n1046gf)* animals were reared upon HT115 bacteria expressing *ptp-2* dsRNA to reduce *ptp-2*/Shp2 gene function by RNA-mediated interference (RNAi) (Timmons and Fire 1998). After being reared for at least two generations upon these *ptp*-2 dsRNA-expressing bacteria, *let-23(sy10); let-60(n1046gf)* progeny remained sterile (Table 2). Although in these experiments there is no direct evidence that *ptp-2* gene function is reduced in *let-23(sy10); let-60(n1046gf)* animals, *daf-2* mutant animals reared on the same bacterial strain expressing *ptp-2* dsRNA did show an effect (see below). In addition, previous work has revealed the tissue in which the *let-23*/Egfr fertility pathway acts to be sensitive to RNAi (Yin *et al.* 2004). Together, this suggests that *ptp-2*/Shp2 may not be the downstream effector of *soc-1*/Gab1 that mediates inhibition of PLCγ-mediated signaling.

***soc-1* functions negatively in *daf-2*/InsR signaling during dauer formation:** Upon hatching, *C. elegans* larvae undergo four molts before becoming adults. Depending upon environmental conditions, L2 larvae either molt into the L3 stage or form dauers. The decision of which developmental pathway is used is made by L1 and L2 animals, depending upon temperature, nutritional status, and the concentration of dauer pheromone (Riddle and Albert 1997). *daf-2* encodes an insulin receptor-like molecule that senses the nutritional status of the worm to represses dauer formation and promote reproductive development through the activation of PI3K-mediated signaling (Morris *et al.* 1996; Kimura *et al.* 1997). Mutations in *daf-2* may result in a conditional or nonconditional constitive dauer formation (Daf-c) phenotype, depending upon the severity of the allele. To address the function of *soc-1*/Gab1 in insulin receptor-like-mediated signaling in *C. elegans*, two mutations in *daf-2*/InsR were used: *daf-2(m577)* and the stronger *daf-2(e1370)* allele, both of which are temperature sensitive for the Daf-c phenotype. Double-mutant animals combining either the *daf-2* allele with *sem-5(n2195)*G201R or a null mutation in *soc-1* were generated. A null mutation in *ptp-2* is semisterile and this sterility is enhanced by either *daf-2* mutation (not shown). Therefore the effects of *ptp-2* upon DAF-2 signaling were assayed upon animals that had been fed *ptp-2* dsRNA to reduce *ptp-2* gene activity by RNAi (Timmons and Fire 1998).

*sem-5(n2195)*G201R weakly suppressed *daf-2(m577)* Daf-c under sensitive conditions (Table 3). However, *sem-5(n2195)*G201R did not suppress the more severe *daf-2(e1370)* allele under any conditions tested. *soc-1(n1789)* almost completely suppressed *daf-2(m577)* Daf-c under the sensitive conditions used (Table 3). Again, however, there was very little suppression of *daf-2(e1370)*, with the non-dauers scored arresting as L3ds, having radial shrinkage typical of dauers, but exhibiting strong pharyngeal pumping. *daf-2(m577)* animals reared on bacteria expressing *ptp-2* dsRNA were also

suppressed for the Daf-c phenotype when compared to *daf-2(m577)* animals reared on control plates. Strikingly, however, *daf-2(e1370)* animals reared on bacteria expressing *ptp-2* dsRNA were also strongly suppressed for dauer formation at 22.5° (Table 3). Almost all nondauers on these plates, in contrast with the weak suppression seen by *soc-1(n1789)*, were fully suppressed, forming L4's and adults. To test the strength of suppression by *ptp-2* RNAi, *daf-2(e1370)* animals reared on bacteria expressing *ptp-2* dsRNA were assayed for dauer formation at 25°. At this temperature, 231/233 animals formed dauers and the others arrested earlier (one each as an embryo and at the early L1 stage), indicating no suppression.

It has recently been shown that the Daf-c phenotype of *daf-2(m577)* animals is also suppressed by a gain-of-function *let-60*/Ras mutation (Nanji *et al.* 2005), although this suppression is weaker than by *soc-1(n1789)* or by *ptp-2* RNAi (not shown). Thus, in the *daf-2* Daf-c assay, *let-60*/Ras functions positively and the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 function negatively. Therefore, the function of the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 downstream of DAF-2 in the dauer assay is likely to be distinct from the function in Ras/Map kinase signaling. As the repression of dauer formation by DAF-2 signaling is largely mediated through PtdIns(3,4,5)P_3 production, this suggests that the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 have an inhibitory activity upon PI3K-mediated signaling (Figure 1).

That *sem-5(n2195)*G201R is a weaker suppressor of *daf-2* Daf-c than a null mutation in *soc-1*/Gab1 may reflect that the C-terminal SH3 domain of *sem-5*/Grb2 has additional functions, which positively regulate DAF-2 signaling and act in opposition to the inhibitory effect of Gab1 recruitment. Since *let-60*/Ras is a weak positive modulator of DAF-2 signaling in the Daf-c assay (Nanji *et al.* 2005), these additional, positive functions are likely to be in the activation of Ras. Consistent with this, in the vulval induction assay for Egfr/Ras/Map kinase signaling, the C-terminal SH3 domain of *sem-5*/Grb2 has additional positive functions beyond recruiting Gab1 (Table 1). Thus, the degree of inhibition of PI3K-mediated signaling by *sem-5(n2195)*G201R is likely to be underestimated in the *daf-2* Daf-c assay due to the additional and opposing effect upon Ras activation.

In the *daf-2* Daf-c assay, *ptp-2* RNAi is a stronger suppressor than *soc-1*. This presumably indicates that *ptp-2*/Shp2 recruitment may be only partially dependent upon *soc-1*/Gab1 in insulin receptor-like signaling. An IRS homolog, IST-1, which is predicted to contain an SH2-binding site for *ptp-2*/Shp2, is known to function in DAF-2 signaling (Wolkow *et al.* 2002).

***soc-1* enhances *daf-2* longevity:** In addition to being Daf-c, mutations in *daf-2* also increase life span (Kenyon *et al.* 1993). To test the effects of *soc-1*/Gab1 on this *daf-2* increased life-span (Age) phenotype, life-span trials were performed upon adult worms raised at 20° and shifted to 25° at the L4 stage. Introduction of the *soc-1(n1789)* mutation did not affect the mortality rate of *daf-2(+)* or *daf-2(m577)* animals (Table 4). However, it did significantly affect the mortality rate of *daf-2(e1370)* animals: the median life span of *daf-2(e1370); soc-1(n1789)* was enhanced by 40% compared to *daf-2(e1370)* animals in two of two trials (Table 4). Thus, although *soc-1* suppresses *daf-2* Daf-c, it enhances the *daf-2* Age phenotype. In parallel experiments, *sem-5(n2195)*G201R reproducibly increased the life span of *daf-2(+)* animals, but not of *daf-2(m577)* or *daf-2(e1370)* animals (Table 4).

It has recently been shown that reducing Ras pathway signaling in *C. elegans* increases mortality of *daf-2* animals and that the weak gain-of-function Ras allele, *let-60(n1046gf)*, enhances maximum life span of *daf-2* animals (Nanji *et al.* 2005). Thus, as in the *daf-2* Daf-c assay, *let-60*/Ras and *soc-1*/Gab1 have opposing functions in a life-span assay. The failure of *sem-5(n2195)*G201R to enhance the *daf-2(e1370)* Age phenotype, which would be expected if *sem-5*/Grb2 acted upstream of *soc-1*/Gab1, may therefore be due to the opposing effect that reduced *let-60*/Ras activation would be expected to have upon *daf-2* mortality.

Similar experiments were performed with *daf-2* mutant animals fed on *ptp-2* dsRNA. In these experiments, reduction of *ptp-2* activity by RNAi increased *daf-2(+)* mortality. *daf-2(m577)* animals fed *ptp-2* dsRNA displayed enhanced early mortality, but in this case maximum life span was unaffected (Table 4). Feeding *daf-2(e1370)* animals *ptp-2* dsRNA had little effect on mortality (Table 4).

***soc-1* enhances *daf-2* early larval arrest:** As mutation of *soc-1*/Gab1 has opposing effects upon the constitutive dauer formation phenotype and the long-lived phenotype of *daf-2* animals, the interaction between *soc-1* and *daf-2* was addressed using other *daf-2* assays. *daf-2* mutations form two classes. Class 1 mutants form dauer larvae constitutively, are thermotolerant, and are long lived. Class 2 mutants also exhibit these traits plus one or more additional phenotypes, including embryonic/early larval arrest, reduced pharyngeal pumping, and late progeny production (Gems *et al.* 1998). It has been hypothesized that *daf-2* has two functional elements, *daf-2A* and *daf-2B*. Under this hypothesis, class 1 phenotypes would result from loss of *daf-2A* signaling and class 2 phenotypes from loss of *daf-2A* and *daf-2B* signaling (Gems *et al.* 1998). This does not preclude the possibility that *daf-2B* signaling affects class 1 phenotypes (Gems *et al.* 1998; see below). Thus, one possible explanation for the opposing effects of *soc-1*/Gab1 on *daf-2* signaling is that *soc-1*/Gab1 modulates *daf-2A* and *daf-2B* signaling distinctly. Therefore, the effect of *soc-1*/Gab1 upon class 2 *daf-2* phenotypes was addressed using the class 1 *daf-2(m577)* allele, which does not exhibit class 2

TABLE 4

The effect of *sem-5(n2195)*G201R, *soc-1(n1789)*, and feeding *ptp-2* dsRNA on aging

Genotype	Trial	Median life span	75% mortality[a]	Maximum life span	n^b	P^c
+/+	1	18	18	22	56 (6)	—
	2	17	19	25	71 (36)	—
sem-5(n2195)	1	19	21	25	83 (39)	0.0005
	2	18	22	31	77 (62)	0.006
soc-1(n1789)	1	15	18	25	44 (9)	0.5
	2	15	19	27	58 (56)	0.2
daf-2(m577)	1	32	38	44	36 (15)	—
	2	33	40	56	102 (59)	—
daf-2(m577); sem-5(n2195)	1	25	36	60	45 (42)	0.2
	2	40	49	66	80 (51)	0.0002
daf-2(m577); soc-1(n1789)	1	30	44	60	52 (41)	0.7
	2	32	40	57	84 (55)	0.6
daf-2(e1370)	1	38	44	56	24 (64)	—
	2	59	70	89	93 (98)	—
daf-2(e1370); sem-5(n2195)	1	38	44	74	54 (5)	0.6
	2	68	81	104	96 (16)	<0.0001
daf-2(e1370); soc-1(n1789)	1	53	71	107	81 (6)	0.0002
	2	87	98	115	117 (41)	<0.0001
L4440(RNAi)	—	21	25	28	37 (1)	—
ptp-2(RNAi)	—	13	15	17	44 (16)	<0.0001
daf-2(m577); L4440(RNAi)	—	35	37	42	52 (11)	—
daf-2(m577); ptp-2(RNAi)	—	23	35	44	62 (15)	0.007
daf-2(e1370); L4440	—	32	44	60	48 (58)	—
daf-2(e1370); ptp-2(RNAi)	—	37	42	61	46 (41)	0.6

Life span was measured at 25°.
[a] The time (in days) at which 75% of animals in the trial had died.
[b] Deaths scored (number of censored values).
[c] *P*, probability of being identical to the isogenic control strain (log-rank test on censored data).

phenotypes, and the weak class 2 allele *daf-2(e1370)*, which displays partially penetrant class 2 phenotypes.

Animals homozygous for the severe class 2 allele *daf-2(e979)*, when raised at 25°, arrest development either prior to hatching or early in the L1 stage (Vowels and Thomas 1992; Gems *et al.* 1998). *daf-2(e979)* arrested worms are typically at the four-cell gonad stage (L1), are nonpumping, and are nonvacuolated (Nanji *et al.* 2005). Similarly, ~3% of *daf-2(e1370)* animals raised at 25° arrest at this same stage (Table 5). This arrest is distinguishable from the rod-like L1 lethality phenotype produced by reduced Ras signaling, which is typified by a high degree of vacuolization. Moreover, the Ras lethality is irreversible, but the *daf-2* L1 arrest is largely recoverable by placing *daf-2* animals at 15°. The class 1 mutant, *daf-2(m577)*, does not arrest (Table 5). Also, control worms, singly mutant for a null mutation in *soc-1*/Gab1 or with the C-terminal SH3 domain mutation in *sem-5*/Grb2, do not exhibit the *daf-2* L1 arrest. However, a small proportion of *sem-5(n2195)*G201R animals suffered the rod-like L1 lethality phenotype typical of reduced Ras signaling at 25° and 2 of 143 *soc-1(n1789)* animals arrested development at the L2 stage (Table 5).

In addition to displaying a low frequency of rod-like dead L1's (Ras lethality), a small proportion of *daf-2(m577); sem-5(n2195)*G201R animals arrested development at the L1 stage at 25° in a manner typical of class 2 *daf-2* alleles (Table 5; 0/316 *vs.* 4/258: Fisher's exact test $P < 0.05$). Although this effect is small, it is also seen in *daf-2(m577); soc-1(n1789)* animals (17% of *daf-2(m577); soc-1(n1789)* animals arrest as nonvacuolated L1's; Table 5). Thus the addition of *sem-5(n2195)*G201R or *soc-1(n1789)* to *daf-2(m577)* causes this class 1 allele to display this class 2 phenotype. Consistent with this finding, both *sem-5(n2195)*G201R and *soc-1(n1789)* strongly enhanced the L1 arrest phenotype of *daf-2(e1370)* animals (Table 5). In contrast, rearing *daf-2(m577)* or *daf-2(e1370)* animals on bacteria expressing *ptp-2* dsRNA did not produce the L1 arrest phenotype (Table 5).

***soc-1* acts positively in *daf-2B* signaling:** The L1 arrest phenotype of *daf-2(e979)* is suppressed by the weak gain-of-function Ras allele, *let-60(n1046gf)* (Nanji *et al.* 2005). At 22.5°, *daf-2(m577)* enhanced the L1 lethality of *let-60 (n2021)* animals to 100%, all animals being vacuolated, precluding the test of whether reducing Ras signaling would enhance the *daf-2* early larval arrest phenotype

TABLE 5

***daf-2* L1 arrest is enhanced by *sem-5(n2195)*G201R and *soc-1* but not by feeding *ptp-2* dsRNA**

Genotype	Dead eggs (%)	L1 let (%)	L1 (%)	L2 (%)	*n*
+/+	6	0	0	0	102
*sem-5(n2195)*G201R	0.5	3	0	0	213
soc-1(n1789)	7	0	0	1	143
L4440(RNAi)	0	0	0	0	112
ptp-2(RNAi)	1	0	0	0	109
daf-2(m577)	3	0	0	0	316
*daf-2(m577); sem-5(n2195)*G201R	4	2	2	0.4	258
daf-2(m577); soc-1(n1789)	4	0	17	6	434
daf-2(m577); L4440(RNAi)	1	0	0	0	148
daf-2(m577); ptp-2(RNAi)	0	0	0	0	228
daf-2(e1370)	2	0	3	0	229
*daf-2(e1370); sem-5(n2195)*G201R	7	9	60	0	232
daf-2(e1370); soc-1(n1789)	9	0.3	55	2	351
daf-2(e1370); L4440(RNAi)	0	0	0	0	170
daf-2(e1370); ptp-2(RNAi)	0.4	0	0.4	0	232

Percentage of animals at each stage 72 hr post-egg laying (at 25°).

(Nanji *et al.* 2005). In addition, *let-60(n1046gf)* weakly suppresses the reduced pharyngeal pumping of *daf-2(e1370)* and reducing Ras signaling causes *daf-2(m577)* to feed less (Nanji *et al.* 2005). Thus, the finding that both *sem-5(n2195)*G201R and *soc-1(n1789)* enhance *daf-2* L1 arrest could reflect their positive role in Ras signaling. An alternative, but not mutually exclusive, possibility is that *soc-1*/Gab1 contributes positively to *daf-2B* signaling. To test this, the effect of *sem-5(n2195)* G201R and *soc-1(n1789)* upon class 2 *daf-2* signaling was determined using an assay for late progeny production. Increasing or decreasing Ras signaling in *daf-2* mutants has little effect upon this class 2-specific phenotype (Nanji *et al.* 2005).

Wild-type worms will cease producing offspring in the absence of mating within 5 days of adult life. However, class 2 *daf-2* mutant animals can produce a small number of progeny relatively late in life (Gems *et al.* 1998). Late progeny production in this assay is defined as producing offspring after 9 days of adult life in the absence of mating at 25° (Nanji *et al.* 2005). Control worms singly mutant for *sem-5(n2195)*G201R or *soc-1* did not produce late progeny. Neither did N2 worms reared on bacteria expressing *ptp-2* dsRNA. As expected for a class 1 allele, animals mutant for *daf-2(m577)* also did not produce late offspring. However, 49% of *daf-2(m577); sem-5(n2195)*G201R animals and 17% of *daf-2(m577); soc-1(n1789)* animals produced late progeny (Table 6). Thus, in this second assay for a class 2-specific phenotype, the addition of *sem-5(n2195)*G201R or *soc-1(n1789)* to *daf-2(m577)* causes these class 1 mutant animals to display class 2 phenotypes.

About two-thirds of the class 2 *daf-2(e1370)* mutant hermaphrodites produce late progeny at 25°. This proportion is not significantly affected by introducing *sem-5 (n2195)*G201R or *soc-1(n1789)* (Table 6). In addition, feeding *daf-2(m577)* or *daf-2(e1370)* animals *ptp-2* dsRNA did not produce or enhance late progeny production, respectively.

Thus both *sem-5(n2195)*G201R and *soc-1(n1789)* cause *daf-2(m577)* to display class 2 phenotypes and so convert a class 1 *daf-2* allele into a class 2 allele (Tables 5 and

TABLE 6

***sem-5(n2195)*G201R and *soc-1*, but not feeding *ptp-2* dsRNA, causes *daf-2(m577)* to display the class 2-specific late offspring phenotype**

Genotype	Animals producing late offspring (%)	*n*
+/+	0	87
*sem-5(n2195)*G201R	0	105
soc-1(n1789)	0	34
L4440 [RNAi]	0	34
ptp-2[RNAi]	0	29
daf-2(m577)	0	71
*daf-2(m577); sem-5(n2195)*G201R	49	76
daf-2(m577); soc-1(n1789)	17	88
daf-2(m577); L4440(RNAi)	0	64
daf-2(m577); ptp-2(RNAi)	0	63
daf-2(e1370)	65	60
*daf-2(e1370); sem-5(n2195)*G201R	64	67
daf-2(e1370); soc-1(n1789)	50	107
daf-2(e1370); L4440(RNAi)	72	54
daf-2(e1370); ptp-2(RNAi)	70	46

Late offspring are defined as appearing 9 days post-L4. Animals dying before day 9 were censored from assay.

Table 6). This suggests that both the C-terminal SH3 domain of *sem-5*/Grb2 and *soc-1*/Gab1 contribute positively to class 2 *daf-2* signaling. As feeding animals *ptp-2* dsRNA did not cause class 2 phenotypes in *daf-2(m577)* or enhance them in *daf-2(e1370)*, it is unlikely that *ptp-2*/Shp2 mediates this positive *daf-2* signaling.

DISCUSSION

Previous genetic studies have suggested that the multi-substrate adaptor protein SOC-1/Dos/Gab1 contributes positively to RTK signaling (Herbst *et al.* 1996; Raabe *et al.* 1996; Schutzman *et al.* 2001). However, the assays used in these studies were for RTK signaling mediated by the Ras/Map kinase pathway. In this study, using sensitized assays for Egfr and InsR signaling in *C. elegans*, we show that *soc-1*/Gab1 has both positive and negative effects upon RTK signaling. The opposing effects of *soc-1*/Gab1 upon RTK signaling using distinct assays is likely to reflect the relative contribution of the various intracellular signaling pathways activated by the RTK to the tissue-specific function that forms the basis of the assay (Figure 1). Thus, when RTK signaling achieves a particular function through activation of the Ras/Map kinase pathway, *soc-1*/Gab1 acts positively (Table 1; Herbst *et al.* 1996; Raabe *et al.* 1996; Schutzman *et al.* 2001). When Ras activation downstream of the RTK signaling is not essential for the function of RTK in a particular cell(s), then additional functions for *soc-1*/Gab1 may be observed. For example, *let-23*/Egfr activates PLCγ, leading to IP_3 production, which mediates its function in fertility (Clandinin *et al.* 1998; Yin *et al.* 2004). Ras activation downstream of the Egfr is not a requirement for fertility and *soc-1*/Gab1 acts negatively in this assay (Table 2). Likewise, although Ras signaling modulates *daf-2*/InsR signaling in *C. elegans*, it is not essential and *soc-1*/Gab1 has the opposite function to *let-60*/Ras in *daf-2*/InsR signaling of repressing dauer formation and promoting aging (this study and Nanji *et al.* 2005). Thus, *soc-1*/Gab1 has activities in addition to its function in Ras/Map kinase-mediated signaling. These activities are likely to be regulatory in nature as they are readily observed only in sensitized backgrounds.

The recruitment of *soc-1*/Gab1 into the RTK-signaling complex is likely to be mediated through its interaction with the C-terminal SH3 domain of *sem-5*/Grb2. This interaction maps to two atypical SH3-binding sites on Gab1 (Lock *et al.* 2000). Likewise, the Drosophila Gab1 homolog Dos has two atypical SH3-binding sites that bind Grb2 and these sites are necessary for Dos function in *sev* RTK and Egfr signaling (Feller *et al.* 2002). The *soc-1* gene is predicted to produce a protein containing a single atypical SH3-binding site matching the consensus from Gab1 and Dos (Figure 2A). Furthermore, the interaction between the Gab1 and the C-terminal SH3 domain of Grb2 is also likely to be conserved in *C. elegans* as the atypical SH3-binding site in SOC-1 can bind SEM-5, the *C. elegans* Grb2 homolog, in yeast two-hybrid experiments (Figure 2C). In addition, the introduction of the *sem-5(n2195)* mutation, which substitutes glycine at position 201 (corresponding to 203 in Grb2) for arginine within the C-terminal SH3 domain, abolishes this interaction with SOC-1. In all the genetic assays performed here, *sem-5(n2195)*G201R behaves qualitatively similarly to a null mutation in *soc-1*, although there are quantitative differences (see below). Together, this provides strong evidence that recruitment of *soc-1*/Gab1 downstream of the Egfr and InsR in *C. elegans* is dependent upon its interaction with the C-terminal SH3 domain of *sem-5*/Grb2.

The function of *soc-1*/Gab1 in Ras/Map kinase signaling is to recruit Shp2 (Herbst *et al.* 1999; Bausenwein *et al.* 2000; Schutzman *et al.* 2001). Shp2 contains two SH2 domains preceding a tyrosine phosphatase domain. Shp2 is not required for initial Map kinase activation, but is required for sustained Map kinase signaling (Hadari *et al.* 1998; Maroun *et al.* 2000; Zhang *et al.* 2002). Evidence that sustained Map kinase signaling is focused in endosomes is accumulating (Vieira *et al.* 1996; Kranenburg *et al.* 1999; Wu *et al.* 2001; Teis *et al.* 2002). Thus it is possible that the function of Shp2 is to establish a sustained Map kinase signaling complex within an endosomal compartment. In this process, the C-terminal SH3 domain of *sem-5*/Grb2 is likely to have three distinct activities. First, it weakly contributes to Sos binding and subsequent Ras activation (Sastry *et al.* 1995). Second, it recruits Gab1, which in turn binds Shp2. And third, it promotes endocytosis of the activated RTK, presumably through interaction with dynamin (Wang and Moran 1996). Thus, although *sem-5(n2195)*G201R and null mutations in *soc-1*/Gab1 and *ptp-2*/Shp2 all suppress a gain-of-function *let-60*/Ras allele, *sem-5(n2195)* G201R has the stronger effect (Table 1).

*sem-5(n2195)*G201R is a stronger suppressor of *let-23*/Egfr sterility than *soc-1*/Gab1, suggesting that *sem-5*/Grb2 has additional, negative functions in fertility other than binding *soc-1*/Gab1 (Table 2). In this case, it is unlikely that the function of binding Sos is important, as *sos-1* did not suppress *let-23*/Egfr sterility. Instead, the role of the C-terminal SH3 domain of *sem-5*/Grb2 in the binding of signaling attenuators such as *ark-1*, which inhibits the *let-23*/Egfr fertility function, may be important (Hopper *et al.* 2000). This may lead, in turn, to the stimulation of endocytosis of the activated RTK, a process known to involve the C-terminal SH3 domain of *sem-5*/Grb2 (Wang and Moran 1996). The fertility function of *let-23*/Egfr is mediated through PLCγ and IP_3 production (Clandinin *et al.* 1998; Yin *et al.* 2004). Blocking endocytosis has been shown to increase phosphorylation of PLCγ whose activity may be confined to the cell surface (Vieira *et al.* 1996; Haugh *et al.* 1999; Haugh and Meyer 2002).

The C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 also inhibit *daf-2*/InsR signaling during dauer formation (Table 3). In this case, *ptp-2*/Shp2 had the strongest effect. This is likely to be because downstream of the *daf-2*/InsR, in contrast with *let-23*/Egfr signaling, *ptp-2*/Shp2 is not completely dependent upon recruitment by *soc-1*/Gab1. The IRS homolog, IST-1, is predicted to contain an SH2-binding site for *ptp-2*/Shp2 (Wolkow *et al.* 2002). This activity of *soc-1*/Gab1 and *ptp-2*/Shp2 is distinct from that which promotes Ras/Map kinase signaling, as Ras signaling positively modulates *daf-2*/InsR signaling to repress dauer formation (Nanji *et al.* 2005), whereas *soc-1*/Gab1 and *ptp-2*/Shp2 activity negatively modulates *daf-2*/InsR signaling in the same assay. DAF-2 signaling is primarily mediated through AGE-1/PI3K, which produces PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2 to inhibit dauer formation and promote reproductive development (Morris *et al.* 1996). This suggests that the C-terminal SH3 domain of *sem-5*/Grb2, *soc-1*/Gab1, and *ptp-2*/Shp2 inhibits PI3K-mediated signaling. It may be that *ptp-2*/Shp2 leads to the establishment of a sustained Ras/Map kinase signaling complex and that this indirectly inhibits PI3K-mediated signaling by removing the receptor-signaling complex from a source of PIP_2, the precursor of PIP_3 (Haugh and Meyer 2002). Alternatively, the inhibitory effect of *ptp-2*/Shp2 may be more direct, through the dephosphorylation of PI3K-binding sites (Myers *et al.* 1998; Zhang *et al.* 2002).

In contrast to their inhibitory effect upon *daf-2*/InsR signaling during dauer formation, the C-terminal SH3 domain of *sem-5*/Grb2 and *soc-1*/Gab1 contributes positively to class 2 *daf-2*/InsR signaling. The *daf-2* gene has been proposed to contain distinct functional elements, hypothesized as *daf-2A* and *daf-2B*, with class 1 *daf-2* phenotypes arising from loss of *daf2A* signaling and with class 2 *daf-2* phenotypes arising from loss of both *daf-2A* and *daf-2B* signaling (Gems *et al.* 1998). The class 1 allele, *daf-2(m577)*, displays class 2 phenotypes when the C-terminal SH3 domain of *sem-5*/Grb2 or *soc-1*/Gab1 is mutated (Tables 5 and Table 6). Under the above hypothesis, this positive function of the C-terminal SH3 domain of *sem-5*/Grb2 and *soc-1*/Gab1 in *daf-2*/InsR signaling may be specific to *daf-2B* signaling.

*sem-5(n2195)*G201R was more potent than *soc-1*/Gab1 in causing the class 1 *daf-2(m577)* allele to display the class 2-specific phenotype of late progeny production (Fisher's exact test $P < 0.0001$; Table 6). This may reflect a role for Ras in *daf-2B* signaling, although reducing Ras signaling in *daf-2(m577)* does not produce an appreciable late offspring phenotype (Nanji *et al.* 2005). An alternative possibility is that the C-terminal SH3 domain of *sem-5*/Grb2 promotes endocytosis of *daf-2*/InsR as it does for the Egfr and this additional effect upon endocytosis produces the stronger late progeny phenotype.

The suppression of *daf-2* Daf-c and the enhancement of the *daf-2* L1 arrest and late offspring phenotypes suggests that *soc-1*/Gab1 modulates *daf-2A* and *daf-2B* signaling distinctly. It is hypothesized that *soc-1* negatively regulates *daf-2A* signaling and positively regulates *daf-2B* signaling. However, *soc-1*/Gab1 enhances the Age phenotype of *daf-2(e1370)* animals, a class 1 phenotype. If *soc-1* negatively regulates *daf-2A* signaling, this enhancement of the Age phenotype could be explained only if *daf-2B* signaling also contributes to class 1 signaling. There is evidence that this is the case from the comparison of the Daf-c phenotypes of *daf-2(e1369)* and *daf-2(e1370)*. *daf-2(e1369)* is a class 1 allele and *daf-2(e1370)* is a class 2 allele; therefore, *daf-2(e1370)* is more severely affected for *daf-2B* signaling than *daf-2(e1369)*. As *daf-2(e1369)* is more severe in terms of Daf-c than *daf-2(e1370)*, it follows that *daf-2(e1369)* is more severely affected for *daf-2A* signaling than *daf-2(e1370)* (Gems *et al.* 1998). *daf-18(e1375)* completely suppresses *daf-2(e1369)* Daf-c, but not *daf-2(e1370)* Daf-c (Nanji *et al.* 2005). This implication of this is twofold: *daf-2A* but not *daf-2B* signaling is strongly suppressed by *daf-18(e1375)* and a defect in *daf-2B* signaling in *daf-2(e1370)* contributes to its Daf-c phenotype (Nanji *et al.* 2005). The enhancement of the *daf-2(e1370)* Age phenotype by *soc-1(n1789)* might therefore be explained by the positive contribution that *soc-1*/Gab1 makes to *daf-2B* signaling.

Thus, the recruitment of *soc-1*/Gab1 by the C-terminal SH3 domain of *sem-5*/Grb2 into the RTK-signaling complex enhances Ras/Map kinase signaling and depresses PI3K- and PLCγ-mediated signaling. What is the function of this change in RTK signaling output? Recruitment of Gab1 into the RTK-signaling complex is regulated by the extent of signaling (Rodrigues *et al.* 2000). Subsequent Shp2 recruitment is required for sustained Map kinase signaling, which can result in distinct cellular responses to transient signaling (Marshall 1995). Different intracellular signaling pathways are regulated distinctly by *soc-1*/Gab1 recruitment. The regulated recruitment of *soc-1*/Gab1 therefore provides a mechanism whereby the magnitude and duration of RTK signaling can subtly alter the signaling output and produce distinct cellular responses.

I thank Anne Wooller for technical support and for performing the yeast two-hybrid experiments (presented in Figure 2C). Some strains in this study were gratefully received from the Caenorhabditis Genetics Center (funded by the National Institutes of Health) and David Gems (who provided DR1567). Thanks also to David Gems, Lindy Holden-Dye, Giovanni Lesa, and Vincent O'Connor for critical reading of the manuscript and discussions. This work was supported by the Wellcome Trust. The author was a Wellcome Trust Research Career Development Fellow (064988).

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Communicating editor: B. J. Meyer