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AUTOINHIBITION OF SOS BY INTRAMOLECULAR INTERACTIONS

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1. ABSTRACT

Sos proteins function as activators of Ras signaling by catalyzing guanine nucleotide exchange on Ras. Sos regulation was initially thought to be accomplished primarily through its growth factor-dependent recruitment to the plasma membrane. More recent data has indicated that while membrane association is an indispensable means of Sos regulation, additional mechanisms involving intramolecular interactions function to control Sos activity towards Ras. This review will examine the experimental evidence for Sos intramolecular interactions and their contribution to Sos regulation.

2. INTRODUCTION

Ras proteins are small plasma membrane associated GTPases that function as molecular switches in signal transduction pathways controlling cell proliferation and differentiation (1, 2). In quiescent cells, Ras is predominantly GDP bound and inactive. Growth factor stimulation promotes the conversion of Ras-GDP to Ras-GTP through a reaction that is catalyzed by guanine nucleotide exchange factors (GEFs) (3). In the context of receptor tyrosine kinases (RTKs) this process is dependent on the Son of sevenless (Sos) GEF (4, 5). Sos was originally identified in *Drosophila* by its ability to couple the sevenless RTK to Ras (6). Human cells contain two *Sos* (*hSos*) genes, *Sos1* and *Sos2*, encoding highly related ubiquitously expressed proteins (7). Loss of *Sos1* results in embryonic lethality in mice, indicating that it is an essential gene for mammalian development (8).

3. SOS DOMAIN STRUCTURE

Full length human Sos1 (hereafter referred to as Sos), which has a molecular mass of ~150kd, is composed of multiple functional domains (figure 1).

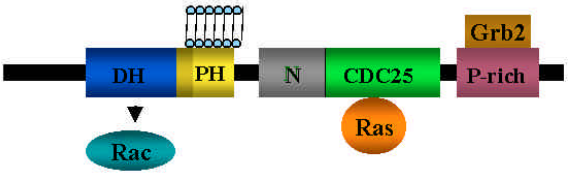

Figure 1. Schematic diagram of the domain architecture of human Sos1. In the N-terminal region, the Dbl homology (DH) domain (residues 198-404) is shown in *blue* and the Pleckstrin homology (PH) domain (residues 442-550) is shown in *yellow* with the connective linker shown in *dark yellow*. In the central catalytic region, the CDC25 homology domain (residues 752-1044) is shown in *green* and the N domain (residues 600-741) is shown in *gray*. In the C-terminal region, the segment including the proline rich motifs is shown in *magenta*.

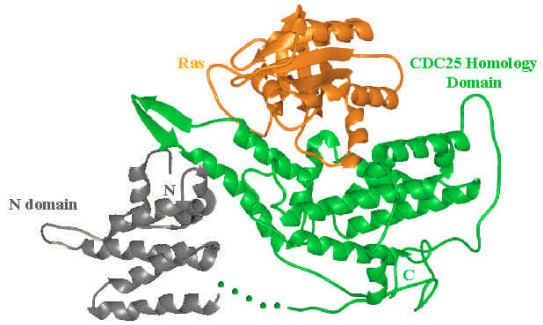

Figure 2. Ribbon diagram of Ha-Ras in complex with the human Sos1 catalytic domain. Ha-Ras (residues 1-166) is shown in *orange*. The CDC25 homology domain (residues 752-1044) is shown in *green* and the N domain (residues 600-741) is shown in *gray*. The N and C termini are indicated with N and C, respectively. The image was created from Protein Data Bank 1BKD code using the Midas ribbonjr command. The CDC25 homology domain directly interacts with Ras to induce the structural changes required for guanine nucleotide exchange. The N domain does not contact Ras but plays a critical role in stabilizing the CDC25 homology domain.

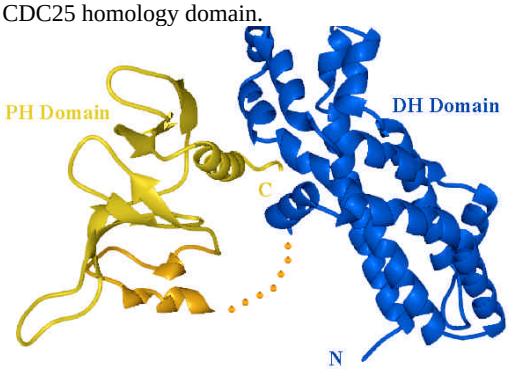

Figure 3. Ribbon diagram of the Dbl Homology (DH) and Pleckstrin Homology (PH) domains of human Sos1. The DH domain (residues 198-404) is shown in *blue* and the PH domain (residues 442-550) is shown in *yellow* with the connective linker shown in *dark yellow*. The N and C termini are indicated with N and C, respectively. The image was created from Protein Data Bank code 1DBH using the Midas ribbonjr command.

3.1. The Catalytic Core

The catalytic domain of Sos (Sos-Cat) spans a central region of approximately 450 amino acids (residues 600-1047). This domain is necessary and sufficient to catalyze guanine nucleotide exchange on Ras and displays sequence similarity to the catalytic domain of the *Saccharomyces cerevisiae* Ras exchanger CDC25 (3, 9, 10). The crystal structure of Ras complexed with the Sos-Cat reveals that Sos-Cat is composed of two discrete functional regions connected by a linker (11) (figure 2). The C-terminal segment of Sos-Cat, the CDC25 homology domain (residues 752-1044), interacts directly with Ras. Binding of the CDC25 homology domain to Ras induces the structural changes necessary for Sos catalyzed nucleotide dissociation. The N-terminal segment of Sos-Cat, the N domain (residues 600-741), contains a conserved Ras Exchange Motif (REM, residues 600-646) (3). The N domain does not contact Ras directly, but appears to stabilize structural features of the CDC25 homology domain critical for functional interaction with Ras (11). Accordingly, disruption of critical contacts between the N domain and the CDC25 homology domain results in the reduction of Sos catalytic activity *in vitro* (12).

3.2. The N-terminal Region

The N-terminus of Sos (Residues 1-550) contains a Dbl Homology (DH) domain (residues 198-404) followed by a Pleckstrin Homology (PH) domain (residues 442-550) connected by a linker segment (residues 404-442). DH domains, which are invariably followed by PH domains, function as exchange factors for members of the Rho family of GTPases (13). The Sos-DH domain has been implicated in the activation of Rac (14). PH domains, many of which interact with specific phospholipids, are often involved in intermolecular interactions and membrane targeting (15). The PH domain of Sos has been shown to bind phosphotidyl inositol derivatives and participate in Sos membrane localization (16-21). The crystal structure of the DH-PH domains of Sos reveals that the putative GTPase binding site of the DH domain is in proximity to the interface between the DH and PH domains (22) (figure 3). The relative orientations of the PH domain and DH domain suggests that ligation of the PH domain might regulate the accessibility of the DH domain to the GTPase (22).

3.3. The C-terminal Region

The C-terminal region of Sos (residues 1048-1333) contains multiple proline rich sequences and phosphorylation sites. The proline rich sequences mediate interactions with multiple Src homology 3 (SH3) domain containing proteins. These include brain specific proteins involved in endocytosis such as Intersectin and Amphiphysin (23, 24) as well as adapter proteins composed solely of Src Homology 2 (SH2) and SH3 domains such as Crk, Nck and Grb2 (25). The best characterized ligand for the proline rich sequence of Sos is the adapter molecule Grb2. Grb2 consists of two SH3 domains bracketing a single SH2 domain (26). The N-terminal SH3 domain of Grb2 binds preferentially to one of the proline rich motifs in the Sos C-terminus (27, 28), resulting in a constitutive cytoplasmic complex between Sos and Grb2 (29). It has

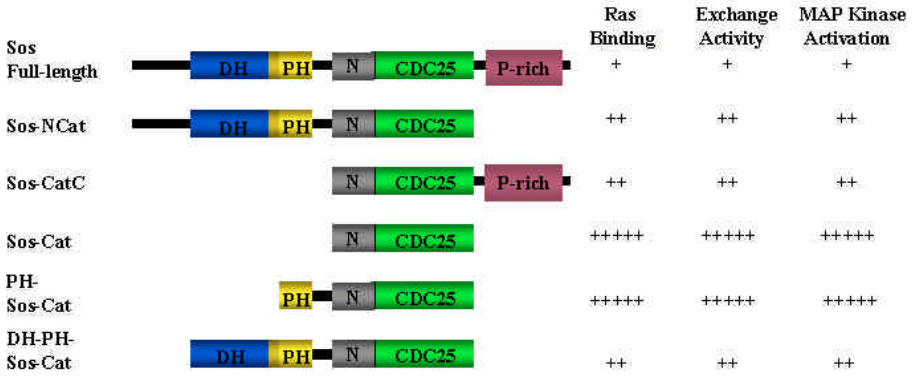

Figure 4. Truncation mutants of Sos used to analyze the role of intramolecular interactions in the regulation of Sos activity. The left column indicates the abbreviations used in the text to describe each Sos fragment. The central column indicates the regions included in each fragment. The three right columns indicate the relative abilities of each Sos fragment to bind to Ras (*in vivo* or *in vitro*), to exchange nucleotide on Ras (*in vivo* or *in vitro*), and to activate MAP kinase.

been proposed that C-terminal SH3 domain of Grb2 might either stabilize the interaction with Sos or interact with other proteins to coordinate multiple signaling activities (28). The SH2 domain of Grb2 has high affinity for specific phosphorylated tyrosine motifs and is responsible for the ligand dependent recruitment of the Sos-Grb2 complex to the activated EGF receptor at the plasma membrane (9, 26, 27, 29-32). Another mode of Sos recruitment involves SH2-dependent interaction of Grb2 with tyrosine phosphorylated docking molecules such as Shc, FRS-2, and IRS-1 (32-35).

4. EVIDENCE FOR SOS REGULATION BY INTRAMOLECULAR INTERACTIONS

Truncations of either the N- or C-terminal regions of Sos substantially enhance the ability of Sos to catalyze nucleotide exchange on Ras and activate the Ras signal transduction pathways (36-38). These findings indicate that the catalytic output of Sos might be regulated in part through intramolecular interactions. Much of the work on the role of intramolecular interactions in the regulation of Sos activity has been preformed using various Sos truncation mutants. A schematic representation of these mutants is provided in figure 4.

5. EVIDENCE FOR A SUPPRESSIVE ROLE OF THE C-TERMINAL REGION

The initial evidence for regions outside of the catalytic domain influencing Sos activity is derived from studies by Aronheim et al in which Sos constructs containing membrane targeting sequences were analyzed. Sos constructs with C-terminal truncations (Sos-NCat) were more effective activators of Ras signaling than full length Sos in both transient transfection assays in mammalian cells and in yeast complementation assays (39) (figure 4). These observations suggest that the C-terminal region exerts an autoinhibitory effect on the catalytic activity of Sos. Consistent with this interpretation, it has been shown that full length murine Sos was unable to transform NIH3T3 cells but murine Sos missing its C-terminal region (Sos-NCat) was transforming (36). Furthermore, it has been demonstrated that C-terminally truncated Sos constructs are better exchangers of Ras *in vivo* and more effective stimulators of the Ras-Mitogen Activated Protein (MAP) kinase pathway than full length Sos constructs (37, 38) (figure 4). Taken together, these studies indicate that the C-terminus of Sos plays a suppressive role its catalytic activity.

6. POSSIBLE MECHANISMS OF AUTOINHIBITION BY THE C-TERMINAL REGION

6.1. Regulation of Ras-Sos binding

A critical step in the guanine nucleotide exchange reaction is the formation of a stable complex between Ras and Sos (40). Thus, one possible mechanism by which the C-terminal region interferes with Sos activity could be through a reduction in the ability to interact with Ras. In support of this possibility, the catalytic domain of Sos alone (Sos-Cat) was shown to have a 50-fold increase in affinity for nucleotide free Ras as compared to full-length Sos. Likewise, addition of the C-terminus to Sos-Cat resulted in decreased Sos-Cat affinity for Ras (38) (figure 4). This indicates that the C-terminus can suppress Sos activity by decreasing its affinity for Ras. The molecular mechanisms underlying the effect of the C-terminus on the affinity of Sos for Ras are unknown. Possibilities include steric hindrance of the Ras binding site of Sos or allosteric effects on the conformation of the Sos catalytic region.

6.2. Regulation by Grb2 binding

Since the C-terminal region of Sos binds to Grb2, it is reasonable to postulate that Grb2 binding may influence the inhibition of Sos by the C-terminus. However, based on immunoprecipitation and reconstitution assays it has been shown that catalytic activity of Sos is not altered following interaction with Grb2 and the EGF receptor (29, 31). In addition, mutations in the C-terminal proline rich sequences which disrupt SH3 domain binding had no effect on the extent of Ras activation by Sos *in vitro*

(S-S. Yang and D. Bar-Sagi, unpublished observations). Hence, it appears that Grb2 binding to Sos does not impact on the suppressive activity of the C-terminal domain.

6.3. Regulation of membrane targeting

It was demonstrated that when over-expressed under serum starvation conditions, a large fraction of Sos-Cat is membrane localized and Ras signaling is activated (37). This finding raises the possibility that Sos-Cat has a membrane-targeting signal. Significantly, by extending the fragment to include the C-terminus (Sos-CatC), membrane localization was reduced along with Ras signaling indicating that the C-terminus may mask a membrane localization signal contained within Sos-Cat (37) (figure 4). While intriguing, this interpretation remains tentative until the specific sequence within Sos-Cat that confers membrane binding can be defined.

6.4. Regulation by phosphorylation

The C-terminus of Sos contains multiple phosphorylation sites which become rapidly phosphorylated upon growth factor stimulation (41). Phosphorylation is mediated predominantly by MAP kinases. Under certain experimental settings, MAP kinase phosphorylation results in the dissociation of the Sos-Grb2 complex (42-45). However, since Grb2 binding per se does not alter the intrinsic catalytic activity of Sos (see above, section 6.2.), the phosphorylation-dependent dissociation of Grb2 is not likely to influence the autoinhibitory effect of the C-terminal region. Nevertheless, the phosphorylation of the C-terminal domain of Sos does seem to contribute to autoinhibition by the C-terminal domain. This is indicated by our findings that a phosphorylation-deficient mutant of Sos displays an enhanced exchange activity compared to the wild type protein (S-S. Yang and D. Bar-Sagi, unpublished observations).

6.5. Regulation by interaction with other factors

The main approach by which C-terminally truncated Sos constructs have been analyzed has been through *in vivo* cell-based assays. This is due to the difficulty in producing stable recombinant Sos proteins containing the proline rich C-terminal domain. Therefore it is difficult to exclude the possibility that the autoinhibitory effect of the C-terminus is exerted in part through interaction with additional proteins. Obvious candidates would be SH3 domain containing proteins that have been shown to interact with Sos such as Vinexin, Abl1, intersectin (46-48).

7. EVIDENCE FOR A SUPPRESSIVE ROLE OF THE N-TERMINAL REGION

Truncation of the N-terminus of Sos (Sos-CatC) results in the upregulation of Sos activity indicating that the N-terminal region can exert an autoinhibitory effect on Sos-catalyzed Ras activation (38). In order to determine the domain within the N-terminal region that mediates its suppressive effect on Sos catalytic activity, our laboratory tested the guanine nucleotide exchange activity of bacterially expressed and purified N-terminal truncation mutants of Sos using *in vitro* exchange assays. By comparing the exchange activity of Sos-Cat constructs containing N-terminal extensions through the PH domain (PH-Sos-Cat) or the DH domain (DH-PH-Sos-Cat) we found that the autoinhibitory effect of the N-terminal region was dependent on the DH domain (figure 4). Similarly, it was found that the ability of PH-Sos-Cat constructs to stimulate MAP kinase activity was equivalent to that displayed by Sos-Cat but was higher than that of the DH-PH-Sos-Cat construct (37) (figure 4). Together these data suggest that the DH domain mediates intramolecular interactions that suppress the catalytic activity of Sos.

8. POSSIBLE MECHANISMS OF AUTOINHIBITION BY THE N-TERMINAL REGION

8.1. Regulation of Ras-Sos Binding

As mentioned above, the formation of a stable binary complex between Ras and Sos is a critical step in Sos catalyzed nucleotide exchange on Ras. The Sos-Cat fragment has a considerably higher affinity toward Ras compared with full length Sos. Addition of the N-terminus (Sos-NCat) resulted in decreased Sos-Cat affinity for Ras, indicating that N-terminal-dependent suppression of Sos might result from a decrease in Sos affinity for Ras (38) (figure 4). Using purified preparations of bacterially expressed Sos-Cat, PH-Sos-Cat and DH-PH–Sos-Cat we have determined that the interference of the N-terminal region with the binding of Ras to Sos *in vitro* is dependent on the DH domain (S-S. Yang and D. Bar-Sagi unpublished results) (figure 4). These observations indicate that that the autoinhibitory effect of the DH domain represents an intrinsic property of the N-terminal region of Sos.

8.2. DH-domain-dependent Intramolecular Interactions

Since the interacting surfaces between Ras and Sos are located within Sos-Cat, it is possible that the DH-domain exerts an inhibitory effect through allosteric interaction with Sos-Cat. In this context it is of interest to note that the electrostatic potential maps of the catalytic domain and DH-PH domains of Sos reveal a positively charged patch on the N domain of Sos-Cat and an acidic patch on the DH domain (figure 5). The acidic patch on the DH-domain is located opposite to the molecular surface presumed to be involved in its catalytic function (22). The positively charged area on the catalytic domain of Sos has a convex shape, whereas the negatively charged area on the DH domain is concave raising the possibility of electrostatically driven molecular interactions between the DH domain and the N domain of Sos. Since the conformation of the N domain is critical for the catalytic function of Sos (12), these interactions could be responsible for maintaining the autoinhibited form of Sos. Rigorous structural and functional analysis will be required to test this idea.

9. COOPERATIVE N- AND C- TERMINAL REGULATION OF SOS ACTIVITY

Although Sos truncation mutants lacking either the C- or N-terminal regions (Sos-NCat or Sos-CatC) were demonstrated to have increased activity compared to full-length Sos, a Sos construct lacking both termini (Sos-Cat)

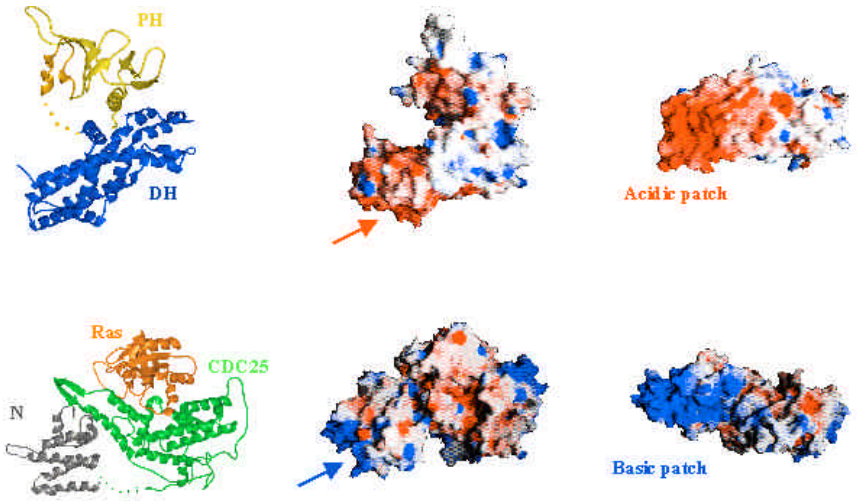

Figure 5. Surface representations of the DH-PH domains and the catalytic domain of human Sos1. The top row shows the DH-PH domain and the bottom row shows the catalytic domain. Solvent exposed basic patches are indicated in blue and solvent exposed acidic patches are indicated in red. The views on the right represent a 90 degree rotation with respect to the views in the center.

was a significantly more effective activator of Ras signaling (38). The increase in activity displayed by Sos-Cat in comparison to both Sos-NCat and Sos-CatC was in direct correlation with an increase in affinity for Ras (figure 4). These results suggest that although the N-terminal and C-terminal regions of Sos can function independently to suppress Sos activity, cooperativity between the two regions may determine the overall level of Sos activity.

10. CONCLUSIONS AND IMPLICATIONS

It is becoming increasingly evident that the activity of Sos can be constrained by intramolecular interactions. Therefore, earlier models for ligand-dependent activation of Ras through Sos need to be revised to include specific mechanisms for the release of Sos from its autoinhibited conformation. The molecular details of such mechanisms remain to be determined. Since it has been suggested that the relative orientation of the DH and PH domains of Sos can be altered by phospholipid binding to the PH domain (22, 49), it is enticing to speculate that the generation of specific phospholipids following receptor activation could serve as a mechanism to attenuate the autoinhibitory effect of the DH domain. Likewise, one could envision a scenario whereby signal-dependent post-translational modifications or protein-protein interactions within the C-terminal region would alter its conformation such that it is no longer positioned in the proper orientation to exert an autoinhibitory effect. It is of interest to note that even under conditions of maximal growth factor stimulation, the activity of full length Sos remains reduced compared to the activity of the catalytic domain under the same conditions (38). This may suggest that the role of the N- and C-terminal regions of Sos is to maintain Sos in a constitutively repressed form, thereby limiting levels of Ras activation. The functional justification for such a mechanism is based on the findings that differences in the intensity of Ras activation can lead to profound differences in biological responses (50-55).

12. ACKNOWLEDGEMENTS

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