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Mammalian Son of Sevenless Guanine Nucleotide Exchange Factors: Old Concepts and New Perspectives

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Abstract

The Son of Sevenless (Sos) factors were originally discovered 2 decades ago as specialized Ras activators in signaling pathways controlling the process of R7 cell development in the eye of *Drosophila melanogaster*. The 2 known members of the mammalian Sos family (Sos1 and Sos2) code for ubiquitously expressed, highly homologous (69% overall) proteins involved in coupling signals originated by cell surface receptor tyrosine kinases (RTKs) to downstream, Ras-dependent mitogenic signaling pathways. Mechanistically, the Sos proteins function as enzymatic factors interacting with Ras proteins in response to upstream stimuli to promote guanine nucleotide exchange (GDP/GTP) and subsequent formation of the active Ras-GTP complex. In this review, we summarize current knowledge on structural, regulatory, and functional aspects of the Sos family, focusing on specific aspects of Sos biology such as structure-function relationship, crosstalk with different signaling pathways, and *in vivo* functional significance as deduced from phenotypic characterization of Sos knockout mice and human genetic syndromes caused by germline *hSos1* mutations.

Keywords: Ras, Ras-GEF, Sos, RTK, signaling

Introduction

Ras proteins are key regulators of signal transduction pathways connecting the activation of multiple, distinct cell surface receptors to the control of cellular proliferation, differentiation, senescence, or death.[1] The mammalian genome contains 3 *ras* genes encoding highly related 21-kDa small GTPases termed H-Ras, N-Ras, and K-Ras, with 2 isoforms, K-Ras4A and K-Ras4B, generated from alternative splicing of the *kras* gene. The normal Ras proteins are continuously cycling between inactive (Ras-GDP) and active (Ras-GTP) conformations. Molecular activation triggered by different extracellular stimuli increases intracellular Ras-GTP levels and elicits a conformational change, enabling these GTPases to interact with downstream effector proteins.[2] The kinetics of cellular Ras activation is modulated through different negative and positive regulators. The negative regulators include GTPase-activating proteins (Ras-GAPs), able to enhance the intrinsic Ras-GTPase activity, thus eliciting rapid hydrolysis of bound GTP.[3] The positive regulators correspond to the guanine nucleotide exchange factors (Ras-GEFs) that stimulate release of bound GDP and exchange for GTP, thus yielding accumulation of the active Ras-GTP complex in response to upstream stimuli.[4,5] In mammalian cells, 3 main Ras-GEF families have been identified: Sos, Ras-GRF, and Ras-GRP.[5,6] The Sos proteins are ubiquitously expressed[7] and participate in signaling downstream of receptor tyrosine kinases (RTKs).[8] In contrast, the Ras-GRF proteins are involved in Ca^{2+} influx/calmodulin-dependent activation of Ras[9] and are mainly expressed in the central nervous system.[10-12] Finally, the Ras-GRP proteins are expressed in hematopoietic cells and activate Ras downstream of nonreceptor tyrosine kinases.[13-15] All these mammalian Ras-GEFs display multidomain, modular protein structures (Fig. 1) including in all cases 2 shared, well-conserved domains with high mechanistic relevance: the Ras exchange motif (REM), involved in the stabilization of binding to Ras, and the CDC25 homology domain (CDC25H), containing the catalytic region originally identified as the essential functional domain in the *Saccharomyces cerevisiae* Cdc25 protein.[6]

We focused this review on describing the current "state of the art" about the mammalian Sos proteins and their functional role(s) in various biological processes. We will describe regulatory mechanisms modulating biological activity of these Ras exchange factors, revising and updating data on a variety of aspects relative to Sos physiological

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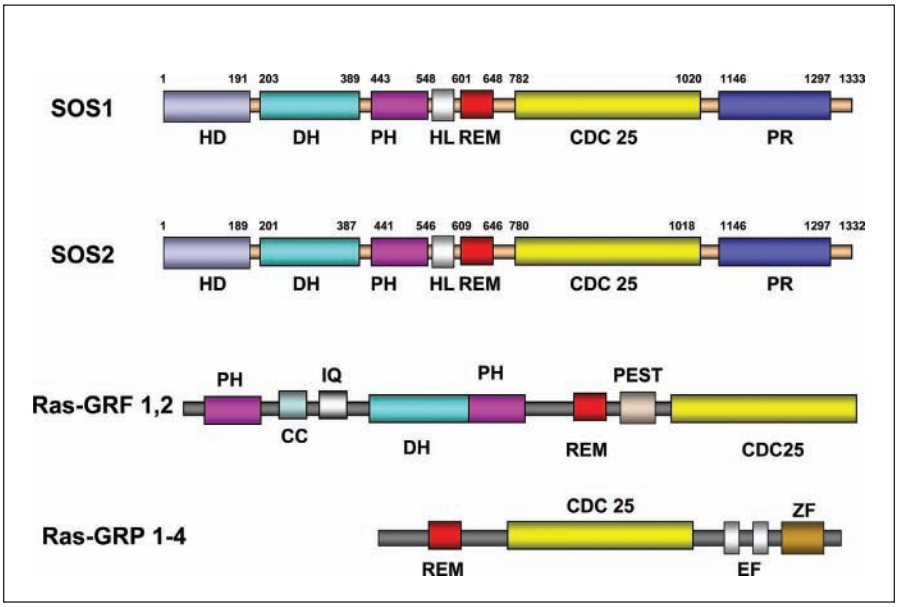

Figure 1. Mammalian Ras-GEF families. Schematics of the mammalian Sos1 and Sos2 proteins and comparison with other Ras-GEF families (Ras-GRF and Ras-GRP/CalDAG GEF). Residue numbers depicting the approximate position of the modular domains correspond to the human Sos1 and Sos2 sequences. DH = Dbl homology; PH = pleckstrin homology; REM = Ras exchanger motif; CDC25-H = CDC25 homology (catalytic GEF domain); IQ = calmodulin-binding domain; PEST = PEST-like region; HF = histone folding motif; PR = proline-rich region (containing the SH3-binding domains); EF = Ca^{2+} binding domain; ZF = zinc finger domain (diacylglycerol-binding domain).

activity, such as structure-function relationship among domains, crosstalk with different signaling pathways, and *in vivo* functional significance of the Sos proteins as deduced from analysis of the phenotypes of Sos knockout mice and of human genetic syndromes caused by germline mutations in SOS genes.

The Sos Family

About 2 decades ago, the study of signaling pathways driving development of the composed eye of *Drosophila melanogaster* uncovered dSos as a key signal modulator acting as an inducer of GDP/GTP exchange on dRas.[16] Homologs of dSos were later found from *Caenorhabditis elegans*[17] to mammalian cells,[18,19] indicating that the components of the Ras/ERK cascade are well conserved at the molecular level throughout metazoan evolution. Whereas flies and worms bear a single *sos* gene, the mammalian Sos family encompasses 2 different genes, located on different chromosomes (human SOS1 and SOS2 genes located to 2p22-p16 and 14q21-q22, respectively).[20] The mammalian Sos1 and Sos2 proteins share approximately 70% of overall homology (with lower level of sequence identity towards their carboxyl-terminal regions),[18,19] are ubiquitously expressed,[7] and function by promoting Ras activation downstream of a wide variety of receptors including RTK, cytokine, and G protein–coupled receptors.[5] Northern and Western analyses have identified a variety of distinct Sos isoforms that are differentially expressed in various tissues and/or developmental stages and may also show different biological potency.[7,18,21,22] Regardless of their high degree of structural and sequence homology, early studies showed that Sos1 and Sos2 are quite different in their relevance at the functional and developmental levels. For example, mSos1 appears to be much more active biologically than mSos2 because specific ubiquitination and degradation of mSos2 by the 26S proteasome significantly shorten its half-life in comparison to mSos1.[23] In addition, mSos1 has been reported to support both short-term and long-term activation of the Ras-ERK pathway, whereas mSos2 appears to support only the short-term activation.[24] Analysis of different *in vivo* animal models also highlights the differing functional significance of Sos1 and Sos2. Interestingly, loss-of-function *dsos* alleles are recessive lethal,[25] whereas mice lacking the *msos2* gene are viable,[26] and *msos1* homozygous null mice die in midgestation because of impaired development of trophoblastic layers of the placenta and heart defects.[24,27]

Structural Aspects of Sos Proteins

As with the other Ras-GEFs, the primary structure of the mammalian Sos proteins is composed of a defined, linear sequence of functionally distinct modular domains (Fig. 1). In this review, we will focus our attention on 3 segregated regions of the primary structure of Sos proteins because of the significance and structural-functional implications of the different protein domains contained in each of them.

REM and CDC25H Domains

The REM and the CDC25H domains (homologous to the CDC25 Ras activator protein in yeast) cover approximately 40% of the primary sequence of Sos proteins and map next to each other between the helical linker (HL) and the proline-rich motif (Fig. 1). Both domains are strictly necessary for proper nucleotide exchange activity,[28] as the *in vivo* effective GEF activity of Sos proteins can only become fully expressed after productive molecular interaction of these domains with their target Ras proteins.[29]

Amino-Terminal Region of Sos

The N-terminal region spans about the first 600 amino acids of Sos proteins and contains the histone-like domain (HD),

the Dbl homology domain (DH), the pleckstrin domain (PH), and the HL (Fig. 1). This particular region concentrates most of the naturally occurring germline mutations described so far in Sos proteins. The exact functional role of the amino-terminal region of Sos is not yet fully understood, and many regulatory questions concerning the domains within this region remain unsolved.

Some reports suggest that the N-terminal region is implicated in positive regulation of Sos function by securing the plasma membrane localization of Sos1 proteins near their potential targets.[30-32] Consistent with this notion, the PH domain of Sos is known to bind to phosphoinositol phosphates,[33,34] with 5-fold higher affinity for PI-3,4,5-P_3 than for PI-4,5-P_2.[35] However, it has also been reported that the stable membrane association of myristoylated Sos constructs devoid of the amino-terminal region is not sufficient for Sos to be biologically active.[36]

In contrast, other reports have proposed a negative regulatory role for the Sos N-terminal region, which would exert negative allosteric control on the activity of the Sos catalytic center (REM and CDC25H domains), directly interacting with the Ras targets.[37,38] In support of this idea, the available structural data indicate that only upon interaction of the REM and CDC25 domains with Ras-GDP, the Sos proteins can acquire their fully activated conformational state.[29,39] In addition, functional analysis of various Sos truncated mutants has also demonstrated that the amino-terminal region is absolutely necessary for the biological activity of hSos1.[40] The HD region (residues 1-191) appears to play an essential regulatory role in this particular functional context. This domain contains 2 tandem histone folds and is located next to the N-terminal end of the DH domain (Fig. 1).[40,41] HD is reported to exert negative control over the functional activity of the whole hSos1 protein. In the basal state, HD is supposed to bind to the PH domain, thus blocking the association of the DH-PH unit with its specific, unknown downstream targets.[40] Structural analyses have confirmed that the HD stabilizes the autoinhibited conformation of Sos proteins and that this domain and the DH-PH unit are conformationally coupled.[42]

Carboxyl-Terminal Region of Sos

The carboxyl-terminal region of Sos is a proline-rich domain that contains specific sequence motifs showing binding affinity for the SH3 domains of Grb2.[19,43,44] This region adopts a left-handed polyproline type II helix conformation.[45-47] Of relevance to this particular region, 2 distinct hSos1 isoforms (termed Isf I and Isf II) have been described in human tissues that differ by the presence in Isf II of a 15–amino acid sequence located next to the first proline-rich motif.[21] Both isoforms showed clearly different Grb2-binding affinity.[21,22] Functional studies showed that Isf II exhibits higher Ras-GEF activity than Isf I and is also more potent than Isf I to induce the transforming phenotype in transfected NIH3T3 cells.[22] Besides the 4 canonical proline-rich Grb2-binding motifs (Grb2-BM) responsible for interaction with Grb2 (PΨΨPPR),[48-50] a number of other putative domains containing the SH3 minimal-binding site (SH3-MBS) (ΨPXΨP)[51] have also been identified in the C-terminal region of hSos1. Interestingly, the Isf II–specific 15–amino acid sequence contains one of the SH3-MBS that is responsible for the increased Grb2-binding affinity of this isoform in comparison to Isf I.[52]

Reports from different laboratories suggest that the carboxyl-terminal region of hSos1 acts to downregulate the global biological activity of the complete hSos1 molecule. Thus, deletion of the carboxyl-terminal region of Sos1 results in significant increases of overall Sos1 Ras-GEF activity both *in vitro* and *in vivo*, therefore leading to enhanced Ras activation levels.[22,37,52,53] Consistent with this model, a mutation causing a premature stop codon in the *hSos1* gene has been described in cases of a rare, autosomal dominant form of gingival overgrowth (see later section); this mutation abolishes the proline-rich domain and generates a truncated hSos1 protein, showing gain of function in comparison to its wild-type counterpart.[54] The proline-rich, carboxyl-terminal region of Sos also contains several potential phosphorylation sites for p90 RSK-2,[55] and such phosphorylation events may provide an additional mechanism for negative feedback modulation of the pathway of Ras activation by Sos GEFs. Finally, Grb2 function may account not only for recruitment of hSos1 to the plasma membrane but also for negative regulation of the intrinsic Ras-GEF activity of hSos1. This negative modulatory effect of Grb2 is dependent on both its SH3 and SH2 domains and seems to be linked to its capacity to bind to the first and second Grb2-BM of hSos1.[56] Indeed, knockdown of Grb2 levels by means of siRNA results in increased Ras-GEF activity of a hSos1 construct constitutively targeted to the plasma membrane.[56] This dual Grb2 regulatory role involving 1) displacing hSos1 to the plasma membrane upon RTK stimulation and 2) negatively regulating the Ras-GEF activity of hSos1 under basal conditions is physiologically relevant. On one side, Grb2 can limit the access of hSos1 to its substrate (Ras proteins at the plasma membrane) only upon cell mitogenic activations. On the other side, the negative regulation of hSos1 by Grb2 reduces the stimulus-independent Ras activation (in distinct endomembrane cell compartments) through unspecific hSos1 locations because of its overexpression.[56]

Current Model of Sos Function

A generally accepted model indicates that recruitment of Sos to the plasma membrane via formation of a complex with Grb2 is the basic mechanism ultimately responsible for activation of the mature, membrane-bound Ras

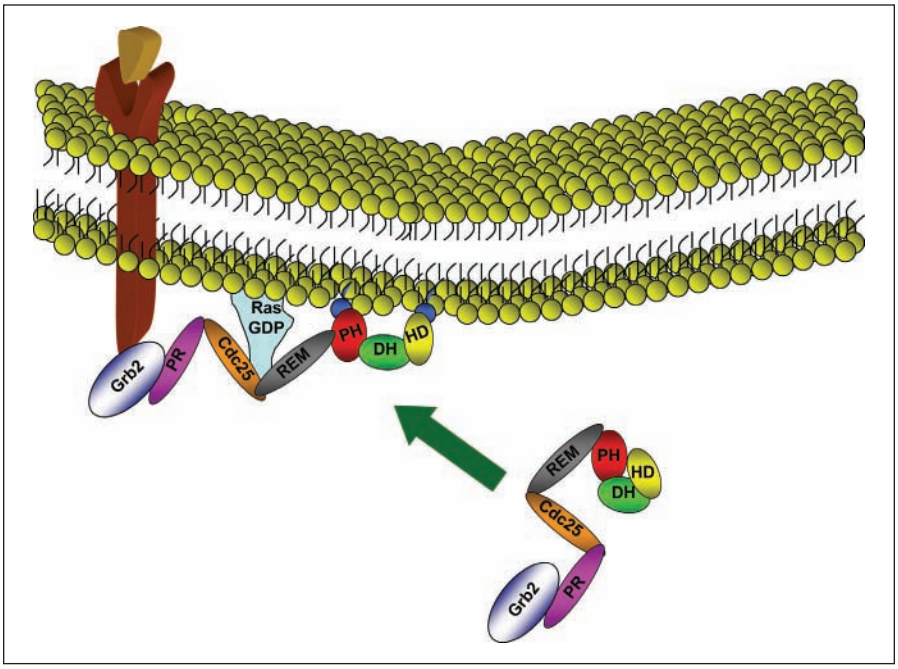

Figure 2. Regulatory mechanisms of Sos1 Ras-GEF activity. Under resting conditions, different molecular interactions may prevent Sos activation. In the cytosol, Grb2 elicits negative regulation, and the DH-PH domain blocks the allosteric binding site for Ras. Upon growth factor stimulation, Sos1 is recruited by Grb2 to the plasma membrane with subsequent release of autoinhibition. Once Sos is activated, the interaction of the REM domain with the switch 2 region of Ras mediates the anchoring to Ras-GDP, whereas the interaction of the CDC25H domain with the switch 1 region of Ras leads to GDP dissociation.

proteins.[57-59] Under basal resting conditions, the Ras-GEF activity of Sos proteins may be kept in check through various intramolecular interactions such as the C-terminal–dependent, negative regulation exerted through Grb2 action[56] or the allosteric autoinhibition of GEF activity mediated by the amino-terminal region of Sos[37,38] (Fig. 2). In this situation, initially, the DH-PH domain would block the allosteric binding site for Ras,[29] and later on, the second and necessary step for Sos activation, recruitment to the plasma membrane and subsequent release of autoinhibition, would happen only after growth factor stimulation of their specific TyrK receptors (Fig. 2). Such a process would involve 1) growth factor–induced generation of phosphatidic acid via phospholipase D2; 2) recruitment of Sos to the plasma membrane through the PH domain[60]; and 3) HD binding to phosphatidic acid, thus promoting Sos activation in the plasma membrane[61] (Fig. 2). Once Sos becomes activated, 4) the interaction of the REM domain with the Ras switch 2 region mediates the anchoring to Ras-GDP, whereas 5) the interaction of the CDC25 domain (2 β-sheets) with the Ras switch 1 region leads to disruption of the nucleotide-binding site and GDP dissociation.[62]

With regards to RTK signaling in particular, recruitment of Sos (complexed with Grb2) to the tyrosine-phosphorylated receptor(s) located at the plasma membrane is considered to be the crucial step for the onset of Ras activation.[8] Sos proteins are known to be able to induce GDP/GTP exchange on all 3 Ras isoforms, in the hierarchy H-Ras > N-Ras > K-Ras.[63] They are also reportedly able to effectively induce GDP/GTP exchange on H-Ras at the plasma membrane and the endoplasmic reticulum (although with a more restrictive pattern than Ras-GRF proteins), but not on Golgi-associated H-Ras.[64]

The Sos GEFs can also be involved in Rac activation.[65-68] The Sos-Rac1 connection appears to be mediated by the generation of a complex between Sos and the scaffold proteins Eps8 and E3b1-Abi-1.[66,67] Consequently, Sos can be engaged in dual interactions, each one leading to the activation of a different biological response. Indeed, the Sos-Grb2 complex is disrupted upon RTK activation,[55,69,70] whereas the Sos-E3b1-Eps8 complex is stable.[69] In addition, growth factor–elicited Ras activation is transient, whereas Rac activation is sustained[69] and downregulated by the interaction of the Sos DH domain with LC3, a microtubule-associated small protein.[71]

Sos and Human Disease

Sos1 and Cancer

Despite years of extensive investigation, with the Ras-Raf-MEK-ERK cascade being probably the most studied signaling pathway, there is no solid experimental evidence of any human disease associated with somatic mutations in *SOS* genes. Although the aberrant expression of many downstream and upstream components of the Ras/ERK pathway is clearly associated with carcinogenic processes, very few reports suggest a possible role of SOS genes in human proliferative pathologies. Indeed, screening studies indicate that SOS1 mutations are uncommon in human tumors.[72] On the other hand, recent reports have described the enhanced expression of Sos1 in prostate cancer epithelial cells of African American men[73] or the integrity of the Sos1/EPS8/ABI1 tricomplex as a determinant of ovarian cancer metastasis.[74] In summary, it is clearly apparent that the most likely mechanism by which Sos proteins may be implicated in cancer entails their aberrant activation by growth factor–induced RTKs or GPCRs.

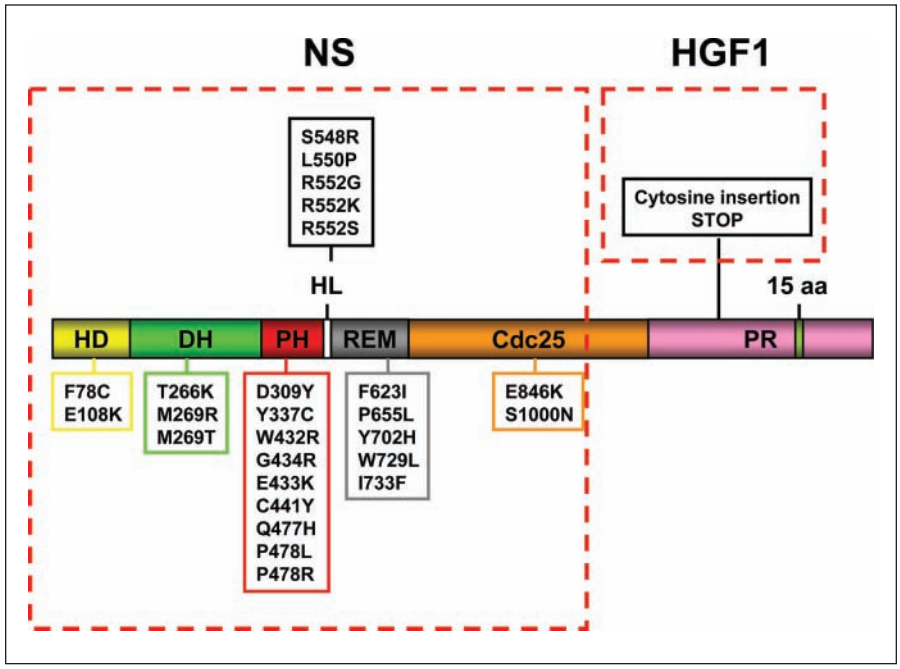

Figure 3. Human Sos1 residues altered in Noonan syndrome (NS) and hereditary gingival fibromatosis type 1 (HGF1). Reported positions of residues mutated in NS and HGF1 are highlighted. Position of the 15–amino acid stretch specific for human Sos1 Isf II (15 aa) is also indicated.

SOS1 in Human Genetic Syndromes

In opposition to cancer, recent reports have documented the possible participation of *SOS1* genes in at least 2 different human hereditary syndromes.

Noonan syndrome. Noonan syndrome (NS) is an autosomal dominant, genetically heterogeneous disorder that affects approximately 1 per 1,000 to 2,500 newborns and is characterized by postnatal reduced growth, hypertelorism, low-set posterior rotated ears with a thickened helix, pulmonary valve stenosis, and hypertrophic cardiomyopathy.[75,76] Gain-of-function, germline mutations in PTPN11, KRAS, MEK1, SHOC2, B-RAF, and RAF1 have been identified in approximately 60% of NS patients,[76-80] and *SOS1* mutations are also associated with NS. Indeed, reports from 2 independent laboratories have identified germline, gain-of-function *SOS1* mutations in approximately 13% to 20% of individuals with NS.[76,81] Notably, all these mutations introduce amino acid substitutions, resulting in hypermorphs of hSos1 and, therefore, increased Ras activation. This behavior is probably consistent with the localization of most of the mutations in the DH, PH, and REM domains implicated in autoinhibition of hSos1 Ras-GEF activity (Fig. 3). In addition, the NS carriers of *SOS1* mutations display a distinctive phenotype with high prevalence of ectodermic abnormalities but normal development.[76,82] It may be relevant to emphasize that *SOS1* mutations have not been observed in various other NS-related disorders such as cardiofaciocutaneous syndrome (CFCS), Costello syndrome, or Leopard syndrome, which share most of their phenotypical abnormalities with NS. Of note, the first mouse model for NS caused by *msos1* mutation has been recently described.[68] In heterozygosis, this knockin mouse model showed some of the typical NS phenotypes, whereas in homozygosis, it displayed a severe NS condition. It has also been described that NS patients are at an elevated risk of developing hematological malignancies, especially juvenile myelomonocytic leukemia (JMML).[83] Other reports point to higher risk for solid tumors such as neuroblastomas[84,85] and embryonic rhabdomyosarcomas.[86,87] In addition, benign tumor-like giant cell lesions (GCLs) affecting the jaws and other bones and soft tissues have been observed in patients with clinical features of NS.[88,89] However, it has not been described, until now, that NS patients with *SOS1* mutations showed increased risk of cancer compared to the general population.

Hereditary gingival fibromatosis type 1. Hereditary gingival fibromatosis type 1 (HGF1) is a rare autosomal genetic condition characterized by benign gingival overgrowth and caused by an insertion mutation (a single cytosine) in exon 21 of the *SOS1* gene[54] (Fig. 3). The insertion causes a frameshift mutation, generating a truncated hSos1 protein devoid of the proline-rich motifs required for Grb2 binding and the 5 p90 RSK-2 phosphorylation sites[54] and yielding gain-of-function properties consistent with the increased ERK signaling that drives enhanced gingival fibroblast proliferation.[90] Intriguingly, the effects promoted by the HGF1-specific *SOS1* mutation do not match those promoted by the NS gain-of-function hSos1 mutants. A transgenic mouse construct involving a comparable Sos1 chimera displays phenotypic skin hypertrophy.[91]

Concluding Remarks and Future Directions

Despite the considerable progress of knowledge derived from the vast amount of Sos-related literature generated during past years, many relevant areas concerning the specific biological properties of the Sos family members still remain to be clarified in the future. A potential growth area is the identification of additional, new Sos protein–interacting reagents or drugs. As proven for many other components of

signaling pathways, progress on our current understanding of the biology and properties of the members of the Sos GEF family would be greatly helped by the availability of specific drugs or reagents able to interact specifically with Sos proteins. Interestingly, many specific drugs interacting with most other components of Ras signaling pathways (including RTK-blocking agents, FT inhibitors, MEK, ERK, or Raf inhibitors, etc.) have been developed in the past, but still no specific Sos-reacting drugs are available for use in the laboratory. The fact that Sos proteins have not been implicated in human disease until recently justifies in part the absence of studies on Sos proteins as potential drug targets. However, the observation that SOS1 mutations in NS patients give rise to hyperactive SOS1 gene products has prompted an excellent avenue for search and design of hSos1 activity–blocking agents. Another approach could be modification of pre-existing drugs. A potential candidate could be UCS15A, an antibiotic produced by *Streptomyces* species, described as a non–tyrosine kinase Src signaling inhibitor[92] that reduces *in vivo* SH3-mediated protein-protein interactions in a widespread manner.[93] UCS15A and some of their synthetic analogs have been shown to efficiently disrupt Grb2-Sos complexes[94] and also to inhibit breast cancer invasion and metastasis.[95]

The canonical mechanisms of Ras activation by Sos GEFs as a result of stimulation of classic plasma membrane tyrosine kinase receptors by external stimuli have been extensively analyzed in the past. However, some recent reports have identified additional external signals capable of triggering Ras activation without interacting with classic RTK surface receptors. Signaling molecules such as nitric oxide (NO) or the cyclopentenone prostaglandins (CyPG) PGA_1 and 15d-PGJ_2 have been reported to trigger cellular Ras activation in a process including direct modification of specific cysteine residues of the C-terminal Ras region.[96-99] In the current paradigm, upon RTK or GPCR activation, Sos proteins are recruited to the plasma membrane via formation of a complex with Grb2 adaptor proteins.[5,57-59] However, a still unanswered question is the mechanism of the possible participation of Sos in the process of CyPG-mediated Ras activation. At first glance, a mechanism similar to the GEF-less model previously proposed for NO[100] could be postulated. However, a dominant-negative hSos1 construct is able to block the activation of H-Ras induced by 15d-PGJ_2 or PGA_1 (J.L. Oliva *et al.*, unpublished data).

Another potential area for future Sos research is suggested by reports indicating that very common pollutants directly affect Sos1 expression. A highly persistent environmental contaminant, the carcinogenic dioxin 2,3,7,8-tetrachlorodibenzodioxin (TCDD), is known to bind with high affinity to the Aryl hydrocarbon receptor (AhR),[101] a transcription factor able to complex with the AhR nuclear translocator (ARNT) to form heterodimer complexes that bind to specific xenobiotic responsive elements (XREs), thus inducing specific target genes related to detoxification functions.[102] Interestingly, a recent report demonstrated that AhR binds to, and activates, the SOS1 gene promotor, increasing gene expression and inducing a dioxin-dependent Ras-GTP state and ERK activation.[103]

Finally, a complete clarification of the functional specificity, or redundancy, of the mammalian Sos1 and Sos2 isoforms is a very important issue for research in future years. Despite their high degree of sequence identity and the rather parallel gene expression patterns of mammalian Sos1 and Sos2, it is clear that Sos2 cannot compensate the embryonic lethality caused by the loss of Sos1 in *msos1* knockout mice.[24] This observation suggests unique functional roles for each of these proteins in mammals and raises a number of still unanswered questions concerning the *in vivo* function(s) of Sos1 versus Sos2.

An interesting possibility is that the divergent C-terminal regions of Sos1 and Sos2 proteins may be responsible, at least in part, for the functional differences observed between them. Sequence homology between Sos1 and Sos2 is much lower within the proline-rich domain than within any other domains and may help to explain reported functional differences between Sos1 and Sos2, including the observation that mSos1 is able to sustain both short-term and long-term activation of cellular Ras/ERK pathways, whereas mSos2 is only able to sustain short-term activation of this pathway.[24,27] The different C-terminal regions of Sos1 and Sos2 also provide a rationale to explain the different half-lives of Sos1 and Sos2 proteins,[23] as they are likely to contribute to the different levels of intracellular protein stability and ubiquitination shown by these 2 distinct Sos proteins. Furthermore, the complete elucidation of the structure and regulation of the promoter regions of the *SOS1* and *SOS2* loci, as well as the characterization of putative epigenetic modifications affecting these loci, are further challenges whose solution will also be helpful to improve understanding of the biological differences between Sos1 and Sos2.

Understanding why only *SOS1* mutations (but not their homologous *SOS2* mutations) have been found in association with human pathologies is also a timely, appealing question. Noticeably, most of the hSos1 amino acids susceptible to mutation in NS are also present at the corresponding sequence position of hSos2 proteins, yet no report of any such possible *SOS2* mutations still exists. The hypothesis that these equivalent *SOS2* mutations may be lethal could be tested by generating knockin mouse models harboring *msos2* mutations equivalent to those already reported for *SOS1* in human NS.

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Declaration of Conflicting Interests

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