How is SOS activated? Let us count the ways

Greg M Findlay & Tony Pawson

New work shows that activation of the Ras guanine nucleotide exchange factor SOS is dependent upon the membrane density of phosphatidylinositol-4,5-bisphosphate (PIP_2) and GTP-bound Ras. These signals synergize to release the autoinhibitory DH-PH domain, while the histone domain fine-tunes SOS activation in response to PIP_2.

External signals stimulate pathways that control cellular functions such as proliferation, migration, survival and metabolism. Such signaling events therefore have profound biological consequences, and their aberrant activation can induce malignant transformation or developmental defects. To complicate matters, normal cells are potentially beset by multiple stimuli in the form of soluble hormones, extracellular matrix cues and adhesion proteins on the surface of adjacent cells, among others. Thus, cells must not only prevent the adventitious firing of key signaling pathways in the absence of an appropriate stimulus, but they must also integrate numerous external signals and respond decisively once an appropriate threshold has been crossed.

Recent work on the cytoplasmic proteins that couple cell-surface receptors to intracellular response pathways is starting to reveal the molecular basis for these complex control mechanisms. Frequently, these polypeptides have several domains with binding or catalytic functions and are maintained in an inactive state through inhibitory intramolecular interactions. Their multidomain organization allows them to respond to a range of activating inputs, including tyrosine phosphorylation, the modification of phospholipids and the activation of small GTPases, which function cooperatively to relieve the autoinhibited state. This idea is brought home by an elegant series of experiments from Kuriyan and colleagues[1] on SOS, a guanine nucleotide exchange factor (GEF) that activates Ras by inducing it to exchange GDP for GTP; this causes a conformational switch that allows Ras to engage downstream targets such as the Raf serine-threonine protein kinase.

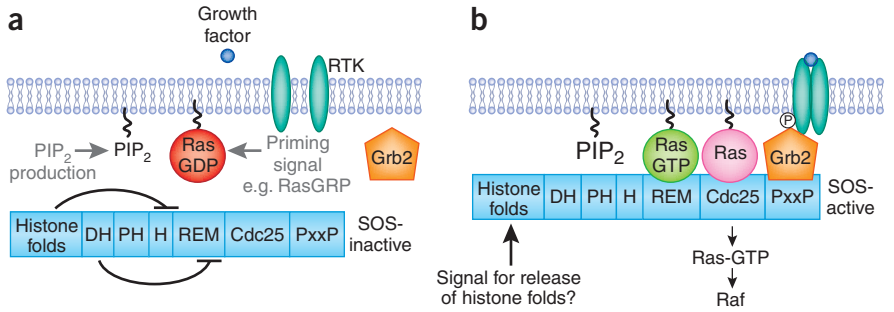

Figure 1 Model for activation of SOS at the membrane. (**a**) In the absence of stimulatory cues, SOS is maintained in an inactive conformation by the autoinhibitory function of the DH-PH domain and histone folds. (**b**) Activation of SOS occurs through the cooperative effects of membrane-localization signals and allosteric control. Substrate Ras is shown in pink. See text for details. Cdc25, Cdc25 homology domain; DH,Dbl homology domain; Histone folds, N-terminal histone-like domain; PH, Pleckstrin homology domain; PxxP, proline-rich region; REM, Ras exchanger motif; RTK, receptor tyrosine kinase.

Membrane-based control of SOS activity

The domain organization of SOS includes a histone-fold domain, a Dbl Homology (DH)-Pleckstrin Homology (PH) domain cassette, a helical linker, a Ras exchanger motif (REM), a Cdc25 domain with GEF activity and a proline-rich C-terminal region that links SOS to activated receptor tyrosine kinases through the Grb2 SH2-SH3 adaptor (**Fig. 1**). Although attention has historically focused on Grb2-mediated recruitment to receptor tyrosine kinases as a means of localizing SOS to the plasma membrane, recent work has revealed increasing complexities in SOS regulation. A structural framework for understanding these new facets of SOS control has emerged primarily from studies by the Kuriyan and Bar-Sagi laboratories.

A completely unexpected finding was that SOS activity is markedly potentiated by binding of a nonsubstrate Ras molecule to a region spanning the regulatory REM and the catalytic Cdc25 domain[2]. Binding of Ras to this allosteric site shifts the Cdc25 domain to a more active conformation and increases the affinity of the catalytic site for substrate Ras, thus stimulating nucleotide exchange[3]. As GTP-bound Ras is a more potent allosteric activator than the GDP-bound form, this provides a positive feedback loop to amplify the effects of Ras activation. Recruitment of 'allosteric' Ras is controlled by the DH-PH module, which in the autoinhibited state masks the allosteric Ras binding site through an intramolecular interaction[3]. Therefore, allosteric Ras fails to activate wild-type SOS in the presence of autoinhibitory elements, either *in vitro*[3] or in quiescent cells[4], raising the question of how upstream signals relieve these

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autoinhibitory interactions. Now, Gureasko *et al.* reveal new mechanisms by which SOS localization to membranes stimulates guanine nucleotide release from Ras, and they argue that the local density of priming activators such as allosteric Ras-GTP and PIP_2 are crucial for activation[1]. The word 'dramatic' is much abused in the scientific literature, but can fairly be applied to the effect on SOS of linking Ras to a phospholipid membrane; this increases the exchange activity of a polypeptide containing the REM and Cdc25 domains of SOS by 500-fold, as compared to Ras in solution.

Molecular mechanisms for release of DH-PH module

Since the demonstration that the DH-PH domain inhibits SOS activation by masking the allosteric Ras site[3], the means by which signaling events can release the DH-PH module has been an outstanding question. Gureasko *et al.*[1] demonstrate that high concentrations of PIP_2 and allosteric Ras on membranes can fully activate a truncated SOS protein, indicating release of the DH-PH domain. In this scheme, concentration of SOS on the membrane by a PH domain–PIP_2 interaction allows high densities of Ras to drive the release of the DH-PH domain, thus opening the allosteric site. This scenario seems the most plausible, as release of the DH-PH module before PIP_2 engagement would bring SOS to the membrane as a result of the affinity of the allosteric site for Ras-GDP, thus bypassing the influence of PIP_2 on SOS activity. An important corollary to these findings is that PIP_2 concentration may have a determining influence on SOS tethering to membranes, allowing Ras to modulate SOS activity; the proline-rich C-terminal region is also likely to have a role in stabilizing SOS at membranes, as discussed below.

Autoinhibitory regulation by the SOS histone domain

Some patients with Noonan syndrome, a developmental disorder that includes heart malformation, have a gain-of-function *SOS1* mutation that causes a substitution in the PH-REM helical linker region (R552G) and abolishes the packing of the histone domain against the PH-REM linker[5,6]. Although the R552G mutation increases SOS activity in cell-based assays[5,6], it has no effect on *in vitro* SOS activity when Ras is in solution or anchored to lipid vesicles composed only of phosphatidylcholine or phosphatidylserine. However, both the R552G substitution or deletion of the histone domain strongly increase the activity of SOS proteins when Ras is attached to vesicles containing PIP_2. Surprisingly, given these data, the histone domain does not interfere with the binding of PIP_2 to the PH domain. The authors suggest that the histone domain constrains SOS activation by preventing its ability to simultaneously bind PIP_2 and Ras. Whether release of the inhibitory histone domain normally requires outside help is an open question. One possibility is that conserved basic patches on the surface of the histone folds may form contacts with negatively charged membrane components[7], leading to an alteration in the histone domain configuration. Upon release of the histone domain, the PIP_2-associated PH domain might correctly orient SOS in the plane of the membrane, thus allowing Ras to more efficiently burrow into the allosteric site. Further analysis is required to resolve these issues.

SOS compartmentalization, clustering and activation

The findings of Gureasko *et al.* indicate that activation of SOS at the membrane is highly dependent upon the density of PIP_2 and allosteric Ras. Given its high affinity for allosteric Ras-GTP[3], SOS may be concentrated and activated at recently reported membrane microdomains of intense Ras activation[8], although the precise nature of these Ras clusters remains to be established. SOS may also be clustered at specific membrane sites through the association of C-terminal proline-rich motifs with the SH3 domains of the SH2-SH3 adaptor Grb2 (refs. 9–11). The presence of multiple binding sites for the Grb2 SH2 domain in tyrosine-phosphorylated receptors and docking proteins, and for the Grb2 SH3 domains in the SOS C-terminal tail, can allow the formation of multipoint interactions and concentrated oligomeric signaling complexes important for Ras-dependent signal transduction[12]. It will be interesting to determine the relative importance of each of these devices for clustering SOS at the membrane in a physiological setting and to ask whether Grb2-dependent, PIP_2-dependent and allosteric Ras–dependent activation collaborate in different signaling systems (**Fig. 1b**). One possibility is that the various options for membrane localization available to SOS may allow different modes of activation in different contexts. Many interesting mechanistic questions remain, such as the nature of the priming mechanisms that generate Ras-GTP to engage the allosteric site in SOS. In T cells, Weiss and colleagues have recently shown that a distinct Ras GEF, Ras guanine nucleotide–releasing protein (RasGRP), generates Ras-GTP that in turn primes SOS activation, potentially allowing T cells to respond to low levels of signaling[13].

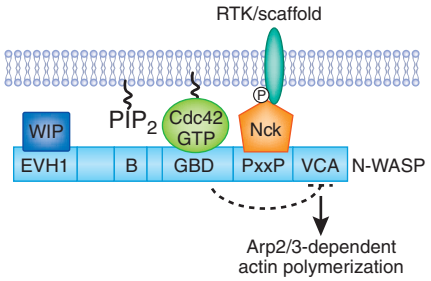

Figure 2 Regulation of Arp2/3 by N-WASP. The mechanisms by which SOS integrates multiple signals at the membrane bears striking resemblance to those controlling N-WASP, an Arp2/3-dependent regulator of actin polymerization. See text for details. B, basic region; EVH1, Ena-VASP homology 1 domain; GBD, GTPase binding domain; VCA, Verprolin homology, Cofilin homology and acidic domain–containing region; WIP, WASP-interacting protein.

Does the recruitment of SOS to receptor tyrosine kinases through Grb2 provide a priming burst of Ras-GTP or does it stabilize activated SOS at a specific membrane site, and how does the C-terminal region contribute to autoinhibition[14,15]? How does phosphatidic acid, a recently described ligand for the SOS PH domain[16], exert its stimulatory effect, and are there as yet unknown activating ligands, for example, for the histone-fold domain?

A more general principle?

As Gureasko *et al.* point out, SOS shows remarkable parallels to neural Wiskott-Aldrich syndrome protein (N-WASP), a multidomain protein that induces branching actin polymerization through its interaction with the actin-related protein complex Arp2/3 via a C-terminal VCA region (**Fig. 2**). In the autoinhibited state, the Arp2/3 binding site is occluded through an intramolecular interaction with the GTPase binding domain (GBD), but this autoinhibitory conformation is disrupted by GTP-bound Cdc42, which engages the GBD and liberates the VCA region to engage Arp2/3 (ref. 17). However, N-WASP membrane localization and activity are also controlled by a basic region, which binds membrane phospholipids and senses PIP_2 density, and by a lengthy proline-rich sequence, which couples N-WASP to phosphotyrosine sites through SH2-SH3 adaptors such as Nck and Grb2, as well as an N-terminal EVH1 domain that recruits the regulatory WIP protein[18,19]. Although the details are different, SOS and N-WASP have apparently evolved a similar set of regulatory devices to prevent inappropriate activation on the one hand, and to allow responses to diverse signaling inputs on the other.

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Evil versus 'eph-ective' use of ephrin-B2

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Crystal structures of the Nipah and Hendra virus attachment protein complexed with ephrin-B2 shed light on the apparent paradox of ephrin-B2's flexibility for binding multiple receptors. Surprisingly, the switch from the use of glycan-based to protein-based receptors seems to have evolved independently from other protein-receptor–using paramyxoviruses such as the measles virus.

Nipah and Hendra viruses, a new genus of paramyxoviruses, can cause fatal encephalitis and are classified as 'Priority Pathogens' because of their extreme pathogenicity and potential for bioterrorism. The receptors for henipaviruses include ephrin-B2 (EFNB2) and ephrin-B3 (EFNB3)[1–3], highly conserved receptor tyrosine kinases that have crucial roles in many developmental and oncogenic processes[4]. The use of EFNB2 and EFNB3 accounts for the observed cellular tropism of henipaviruses and their broad cross-species zoonotic transmission, which is highly unusual for paramyxoviruses. The crystal structure of the henipavirus attachment glycoproteins bound to EFNB2 sheds light on how henipaviruses evolved to use protein-based receptors with such high affinity while maintaining the common architecture of paramyxoviral attachment proteins with hemagglutinin and neuraminidase activity[5].

Paramyxovirus entry is mediated by distinct fusion (F) and attachment (G) virus envelope glycoproteins, with a complex choreography of F and G interactions that result in virus–cell membrane fusion[6]. The initial cell attachment involves receptor recognition and binding by the viral attachment protein. The attachment proteins of many paramyxoviruses have hemagglutinin and neuraminidase activity, and they have long been known to have a common architecture: a six-bladed β-propeller fold[6]. Some paramyxoviruses such as the measles virus retain their hemagglutinin activity and six-bladed β-propeller fold despite using protein receptors[7,8]. The henipavirus G proteins (HNV-G) have neither hemagglutinin nor neuraminidase activity and are still predicted

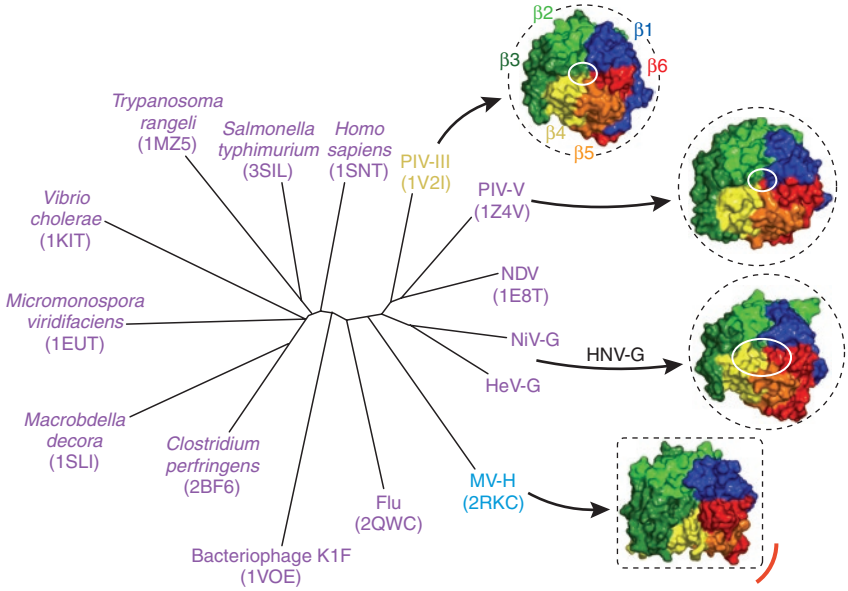

Figure 1 Structural phylogeny of six-bladed β-propellers. Note the distinct separation between viral and nonviral proteins. Surface representations of the paramyxoviral attachment proteins are shown. Unexpectedly, the globular head domain of henipavirus G (HNV-G) is clearly distinct from the cuboidal head domain of measles virus H (MV-H), another paramyxovirus that also uses a protein receptor. Dotted lines trace the globular versus cuboidal shape of the head domain, respectively. Additionally, the receptor binding region of HNV-G is in the center of the six-bladed β-propeller (circled in white) and is situated at the 'top' of the head domain, more or less already oriented toward the incoming receptor. This is similar to, although much larger than, the sialic acid binding region of the attachment protein of parainfluenza viruses. In contrast, in MV-H the receptor binding region has been mapped more toward the 'sides' of the cuboidal head domain (the red arc exemplifies the SLAM binding region). Structural evidence suggests that the MV-H head domain is tilted more toward the horizontal plane so as to orient the receptor binding sites and sides upward toward the incoming receptor on the target cell[8]. Thus, the closer structural homology of HNV-G to sialic acid binding attachment proteins suggests that the switch to using a protein-based receptor has evolved more than once during paramyxoviral evolution.

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