Prenylation of Ras Facilitates hSOS1-Promoted Nucleotide Exchange, upon Ras Binding to the Regulatory Site†

Markos Pechlivanis, Rieke Ringel, Boriana Popkirova, and Juergen Kuhlmann*

Department of Structural Biology, Max-Planck-Institut für molekulare Physiologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany

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ABSTRACT: The oncoprotein Ras is anchored in lipid membranes due to its C-terminal lipid modification. The ubiquitously expressed Ras nucleotide exchange-factor hSOS1 promotes nucleotide exchange and thus Ras activation. This reaction is enhanced by a positive feedback loop whereby activated Ras binds to an allosteric site of SOS to enhance GEF activity. Here we present biochemical data showing that prenylation of both active site bound and allosterically bound N-Ras is required for efficient hSOS1-promoted nucleotide exchange. Our results indicate that prenyl sensitivity of the allosteric feedback-activation is mediated by the PH domain of hSOS1. Farnesylation of Ras thereby allows hSOS1 to bind even GDP-loaded allosteric regulator to maintain basal hSOS1-activity.

Ras proteins are key regulators of cell-differentiation, proliferation, and apoptosis. To fulfill their biological functions Ras proteins have to be posttranslationally modified. These modification steps include farnesylation of the conserved cysteine of the C-terminal CaaX-motif, subsequent proteolytic cleavage of the last three amino acids (−aaX), carboxymethylesterification of the farnesyl-cysteine, and in the case of N- and H-Ras one or two additional palmitoylation events, respectively (*1*).

As a molecular switch Ras exists in two conformational states, an inactive GDP-loaded and an active GTP-complexed conformation. The intrinsic nucleotide exchange reaction of GDP for GTP is slow (k_{diss} 10^{-5} s^{-1}) (*2*) and thus has to be catalyzed by nucleotide exchange factors (GEF[1]). The human son of sevenless 1 protein (hSOS1) is a ubiquitously expressed GEF for H-Ras, N-Ras, and K-Ras, consisting of multiple domains of distinct function (Figure 1) (*3*−*5*).

More than one decade ago Porfiri et al. showed that prenylation of K- and H-Ras is necessary and sufficient for an efficient hSOS1-promoted nucleotide exchange of Ras (*4*). Intriguingly it was shown that the observed effect is less dependent on the structure of the lipid moiety, but more on its length (*6*). In 2003 Margarit et al. identified a positive

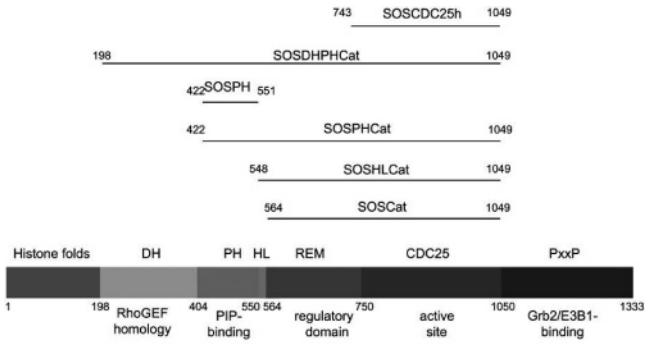

FIGURE 1: Domain structure of hSOS1 and constructs used in this study. DH: Dbl homology. PH: pleckstrin homology. HL: helical linker. REM: Ras exchanger motif. CDC25: cdc25 homology. PxxP: Proline-rich region. Numbers indicate domain boundaries.

feedback mechanism of the catalytical fragment of hSOS1 (SOS^{Cat}; aa 564−1049), in which GTP-loaded Ras binds to a second, distal site of the catalytical fragment (also referred to as the allosteric site) and stimulates nucleotide exchange of Ras bound at the active site (*7*). In the inactivated state the allosteric site of hSOS1 is blocked by an intramolecular interaction of hSOS1 DH domain keeping hSOS1 in an autoinhibited state (*8*). Since it is known that farnesylation of Ras is required for an efficient hSOS1-promoted nucleotide exchange (*4*), we wanted to investigate the exact role of this modification in the case of Ras bound to the active and Ras bound to the allosteric site of hSOS1. Particularly we addressed the ability of prenylated Ras to release the N-terminal autoinhibition.

Our data suggest that at least *in vitro* Ras proteins bound to both binding sites on hSOS1 need to be prenylated to allow for partial reversion of the GEF's autoinhibition. Therefore the PH domain of hSOS1 seems to function as a sensor domain in detecting the prenylation status of Ras bound to the distal site of hSOS1.

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* To whom correspondence should be addressed. Phone: 0049-231-1332104. Fax: 0049-231-1331435. E-mail: juergen.kuhlmann@mpi-dortmund.mpg.de.

[1] Abbreviations: C8-PIP3, (1,2-dioctanoyl-*sn*-glycero-3-[phosphoinositol-3,4,5-trisphosphate]); DH domain, Dbl homology domain; DTE, dithioerythritol; GEF, guanine nucleotide exchange factor; GppNHp, guanosine-5′-(*β*,*γ*-imidotriphosphate); hSOS1, human son of sevenless 1 protein; ITC, isothermal titration calorimetry; mGDP, 2′,3′-bis-*O*-(methylanthraniloyl)guanosine diphosphate, mixture of 2′/3′-isomers; PH domain, pleckstrin homology domain; PIP_3, (1,2-dioleoyl-*sn*-glycero-3-phosphoinositol-3,4,5-trisphosphate); Ras*GDP, Ras protein complexed with guanosine diphosphate; SDS, sodium dodecyl sulfate.

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EXPERIMENTAL PROCEDURES

Cloning, Expression, and Purification. All hSOS1 fragments were amplified by PCR with Pfu-Polymerase and cloned into pProExHTb expression vectors (Invitrogen) using the *Bam*HI and *Xho*I site (SOS^{CDC25h}, aa 743−1049; SOS^{Cat}, aa 564−1049; SOS^{PH}, aa 422−551; SOS^{HLCat}, aa 548−1049; SOS^{PHCat}, aa 422−1049). Introduction of the W729E mutation into the SOS^{Cat}-cDNA sequence was done with the Stratagene Quikchange Mutagenesis Kit. CE sequencing confirmed correct DNA sequences and fragment boundaries. The DHPHCat fragment was a kind gift from H. Sondermann (cloned into the pProExHTb vector using *Nco*I and *Hin*dIII restriction sites, respectively). Expression and purification of the hSOS1 fragments was performed essentially as described by Boriack-Sjodin et al., 1998 (*33*); Margarit et al. 2003, Sondermann et al. 2004 and Chen et al. 1997 (*7*−*9*).

N-Ras wt fl. (full length) cDNA sequence was cloned into a ptac expression vector (Tucker et al.) (*10*). Introduction of the Y64A mutation into N-Ras wt fl. cDNA sequence was achieved by using the Stratagene Quikchange Mutagenesis Kit and confirmed by CE-DNA sequencing. Expression and purification of N-Ras fl. wt and N-RasY64A fl. proteins was done essentially as described by Tucker et al., 1986 (*10*).

The rat FTase clone was a gift from Dr. J. D. Scholten, and recombinant FTase was prepared essentially as published by Zimmerman et al., 1998 (*11*).

Proteins were characterized by SDS−PAGE and ESI-MS (Supporting Information, Figure S1).

In Vitro Farnesylation of N-Ras Proteins. In vitro farnesylation of N-Ras wt fl. and N-RasY64A fl. proteins was performed by incubation of 100 μM N-Ras, 6 μM rat FTase, and 200 μM farnesylpyrophosphate (FPP) in 30 mM Tris pH 7.8, 20 mM KCl, 1 mM $MgCl_2$, 20 μM $ZnCl_2$, 5 mM DTE, and 50 μM GDP for 3 h at 30 °C (volume: 5 mL). Addition of another 1 μmol of FPP to the reaction mixture was repeated three times every 30 min for the first 90 min. Separation of farnesylated N-Ras from non-farnesylated N-Ras and rat FTase was achieved by TritonX114 extraction (*12*). Purity of *in vitro* farnesylated N-Ras was verified by SDS−PAGE and MALDI-TOF analysis (PerSeptive Biosystems Voyager-DE Pro). For further details see Supporting Information.

Nucleotide Loading of N-Ras. Loading of non-farnesylated N-Ras with a fluorescently labeled GDP analogue (mant-GDP) was done by incubating 2.5 mg of N-Ras (300 μM) in buffer containing 20 mM Tris pH 7.4, 200 mM $(NH_4)_2SO_4$, 40 mM EDTA, and a 40-fold molar excess of mGDP for 90 min on ice. Passing the reaction mixture over a 5 mL HiTrap desalting column (Amersham) pre-equilibrated with 20 mM Hepes pH 7.4, 5 mM $MgCl_2$, and 150 mM NaCl removed excess nucleotide. mGDP loading efficiency was determined by HPLC (C18). Approximately 90−95% of applied Ras was loaded with mant-nucleotide following this procedure. Farnesylated N-Ras*mGDP was generated by loading N-Ras with mGDP prior to farnesylation.

Loading of processed and nonprocessed N-RasY64A with nonhydrolyzable GTP-analogue GppNHp was done by incubating 5 mg of N-RasY64A ± Far (600 μM) in buffer containing 20 mM Tris pH 7.4, 200 mM NH_4SO_4, 100 μM $ZnCl_2$, 30 units of alkaline phosphatase (Roche), and a 5-fold molar excess of GppNHp for 16 h at 4 °C. Passing the reaction mixture over a 5 mL HiTrap desalting column (Amersham) pre-equilibrated with 20 mM Hepes pH 7.4, 5 mM $MgCl_2$, and 150 mM NaCl removed excess nucleotide. Nucleotide loading was analyzed by HPLC (C18); in general 95% of N-RasY64A was loaded with GppNHp.

Nucleotide Exchange Assay. Unless otherwise noted all experiments were conducted at 20 °C in measuring buffer (20 mM Hepes pH 7.4, 5 mM $MgCl_2$, 150 mM NaCl). Nucleotide exchange reactions were started by addition of 240 μM GDP (final concentration).

Measurements were performed in Jobin Yvon Fluoromax I or II fluorescence spectrometers in 1 mL quartz cuvettes. The mant-fluorophore was excited at 366 nm, and emission was collected at 450 nm. Measurements were conducted for at least 1500 s.

Rate constants were determined by monoexponential curve fitting ($y = y_o + a\,e^{-kt}$) using the program Sigma-Plot (Systat Software).

Isothermal Titration Calorimetry (ITC). Experiments were performed in a Microcal VP-ITC instrument (cell volume: 1.42 mL) at 20 °C. Prior to ITC measurements all samples were passed over 5 mL HiTrap desalting columns (Amersham) pre-equilibrated with measuring buffer (20 mM Hepes pH 7.4, 5 mM $MgCl_2$, 150 mM NaCl [300 mM NaCl]) to avoid buffer effects. Concentrations of processed and nonprocessed N-RasY64A*GppNHp or hSOS1 fragments in the measuring cell ranged from 10 to 50 μM. Concentrations of nonprocessed Ras or hSOS1 fragments in the syringe were 80−450 μM. hSOS1 fragments were injected stepwise (8 μL) into N-Ras solution in the cell or vice versa. Spacing of 240 s between each injection sufficed in general for reaching a stable baseline.

As controls SOS^{PHCat} (140 μM), SOS^{Cat} (250 μM), or N-RasY64A (250 μM) were injected into measuring buffer to exclude dimerization/monomerization effects.

Data analysis was done with the software supplied by the manufacturer (Origin, Microcal LLC ITC).

RESULTS

Release of Autoinhibition of $hSOS1^{DHPHCat}$ Fragment. Autoinhibition of hSOS1 is mediated by an intramolecular interaction of the hSOS1-DH domain with its allosteric site, located at the REM-CDC25 homology domain interface. The autoinhibited DHPHCat fragment (see Figure 1) is insensitive to allosteric activation by non-prenylated Ras. In addition the lower basal activity of this fragment indicates that binding of Ras*GDP to the distal site of hSOS1 is required for an initial activation of hSOS1 (*8*).

Supporting evidence for such a mechanism comes from the findings that blocking the allosteric site by site-directed mutagenesis (SOS:W729E mutant) also reduced basal catalytic activity of the non-autoinhibited catalytic fragment of hSOS1 (SOS^{Cat}; see Figure 1). A crystal structure of a ternary Ras:$hSOS1^{Cat}$:Ras*GDP complex, in which GDP-loaded Ras was bound to the distal site of the catalytic fragment of hSOS1, supported the idea that Ras*GDP binding to the allosteric site is required for maintaining a basal level of hSOS1 activity (*8*).

To investigate the contributions of prenylation of Ras bound to the distal (allosterically bound) and proximal site

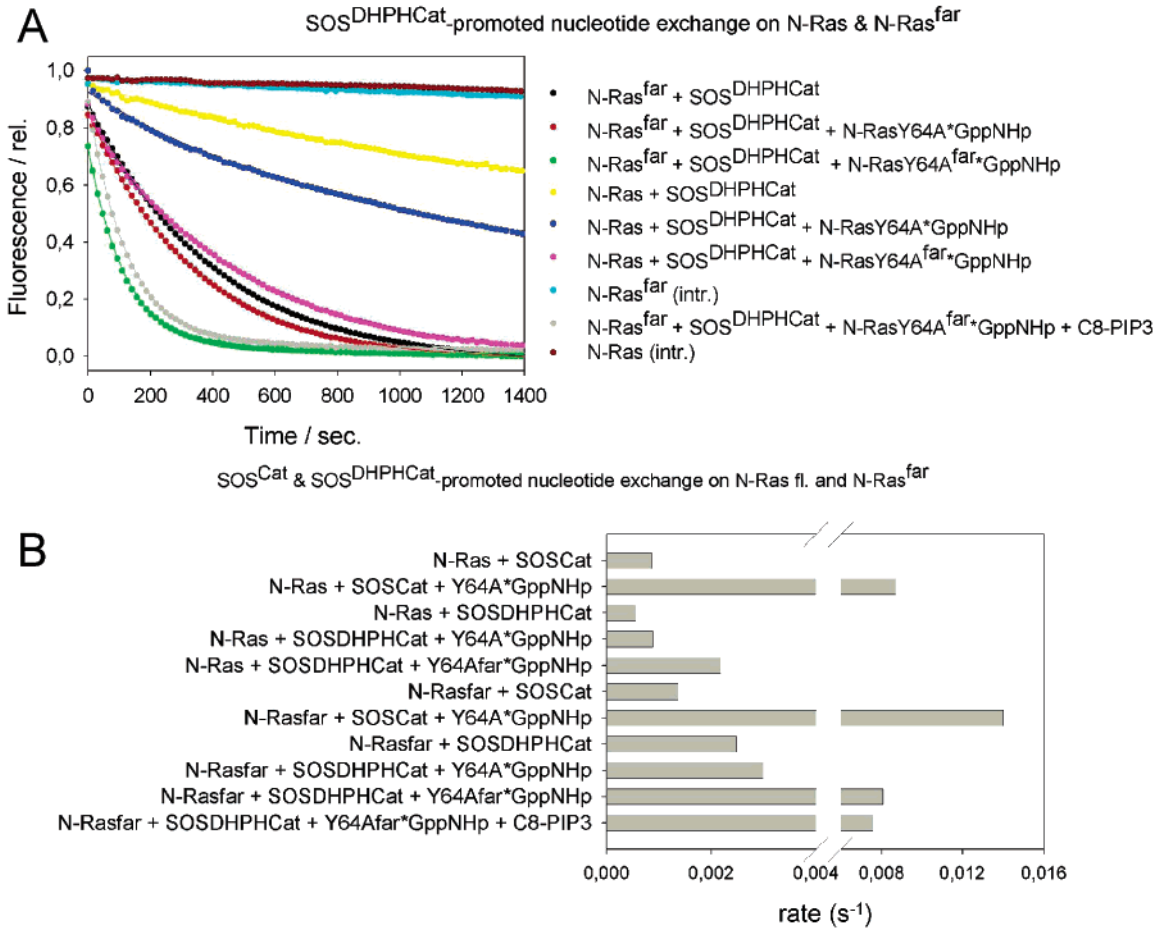


FIGURE 2: Effect of Ras prenylation on SOS^{DHPHCat}-mediated nucleotide exchange on mantGDP-loaded N-Ras proteins. (A) Normalized fluorescence traces after addition of GDP to a final concentration of 240 μM [N-Ras*mGDP (500 nM); SOS^{DHPHCat} (500 nM); N-RasY64A (1000 nM)]. (B) Observed rate constants from monoexponential curve fitting. For clarity, only curves for SOS^{DHPHCat}-promoted nucleotide exchange are shown.

(active/catalytical site bound) of hSOS1, we compared the ability of *in vitro* farnesylated N-Ras proteins and their non-prenylated counterparts to activate the hSOS1^{DHPHCat} fragment by releasing the autoinhibition of the GEF.

The nucleotide exchange activity of the hSOS1^{DHPHCat} fragment on prenylated and non-prenylated N-Ras was analyzed by monitoring the decrease in fluorescence intensity of a mant (methylanthranoylic acid)-labeled GDP analogue after release from N-Ras (*13*).

We found that even in the absence of allosteric regulation by N-RasY64A*GppNHp the basal activity of the DHPHCat fragment was higher for prenylated N-Ras than for non-prenylated N-Ras. To investigate the role of allosteric regulation we used a Ras-mutant (N-RasY64A) that is incapable of binding to the active site of hSOS1 (*14*) (here, referred to as the allosteric regulator). Addition of an active GppNHp-loaded non-prenylated allosteric regulator to either farnesylated or non-farnesylated mGDP-loaded N-Ras had no substantial effect on nucleotide exchange promoted by the SOS^{DHPHCat} fragment. In contrast, addition of *in vitro* farnesylated GppNHp-loaded allosteric regulator could significantly increase nucleotide exchange (Figure 2).

Since it was reported that products of PI(3)-kinase can bind to the hSOS1-PH domain and that binding of phosphoinositoles to the PH domain of hSOS1 modulates interdomain interactions (*15*), we tested for a possible effect of dioctanoyl phosphatidylinositol-3,4,5-trisphosphate (C8-PIP$_3$) a water-soluble form of PInsP$_3$ (*16*) on the nucleotide exchange reaction. Here, concentrations up to 10 μM C8-PIP$_3$ did not significantly alter allosteric stimulated nucleotide exchange on farnesylated Ras promoted by the SOS^{DHPHCat} fragment. Furthermore, addition of C8-PIP$_3$ to the hSOS1^{DHPHCat} fragment in the absence of N-RasY64A*GppNHp could also not stimulate SOS^{DHPHCat}-catalyzed nucleotide exchange on non-farnesylated N-Ras (data not shown).

Interestingly, lipid vesicles doped with 1% of a membrane bound form of PIP$_3$ (1,2-dioleoyl-*sn*-glycero-3-phosphoinositol-3,4,5-trisphosphate) revealed a costimulatory effect in the presence of allosteric regulator. While GEF experiments with either SOS^{DHPHCat} alone or supplemented with pure POPC or POPC/PIP$_3$ vesicles resulted in the same (basal) catalytic acitity of the SOS construct, combination with both non-farnesylated and farnesylated N-RasY64A*GppNHp enhanced the effect of the allosteric regulator becoming manifest in an increased nucleotide exchange rate (Supporting Information S2).

Dissecting the Prenyl Sensitivity of Distal Bound Ras. Since prenylation of the GppNHp-loaded allosteric regulator was essential to accelerate the nucleotide exchange activity of the autoinhibited DHPHCat fragment, we wanted to narrow down the site of hSOS1 that is responsible for the prenyl sensitivity of the allosteric feedback activation.

Interestingly, when comparing kinetic data of the non-autoinhibited catalytic fragment of hSOS1 (SOS^{Cat}) and the

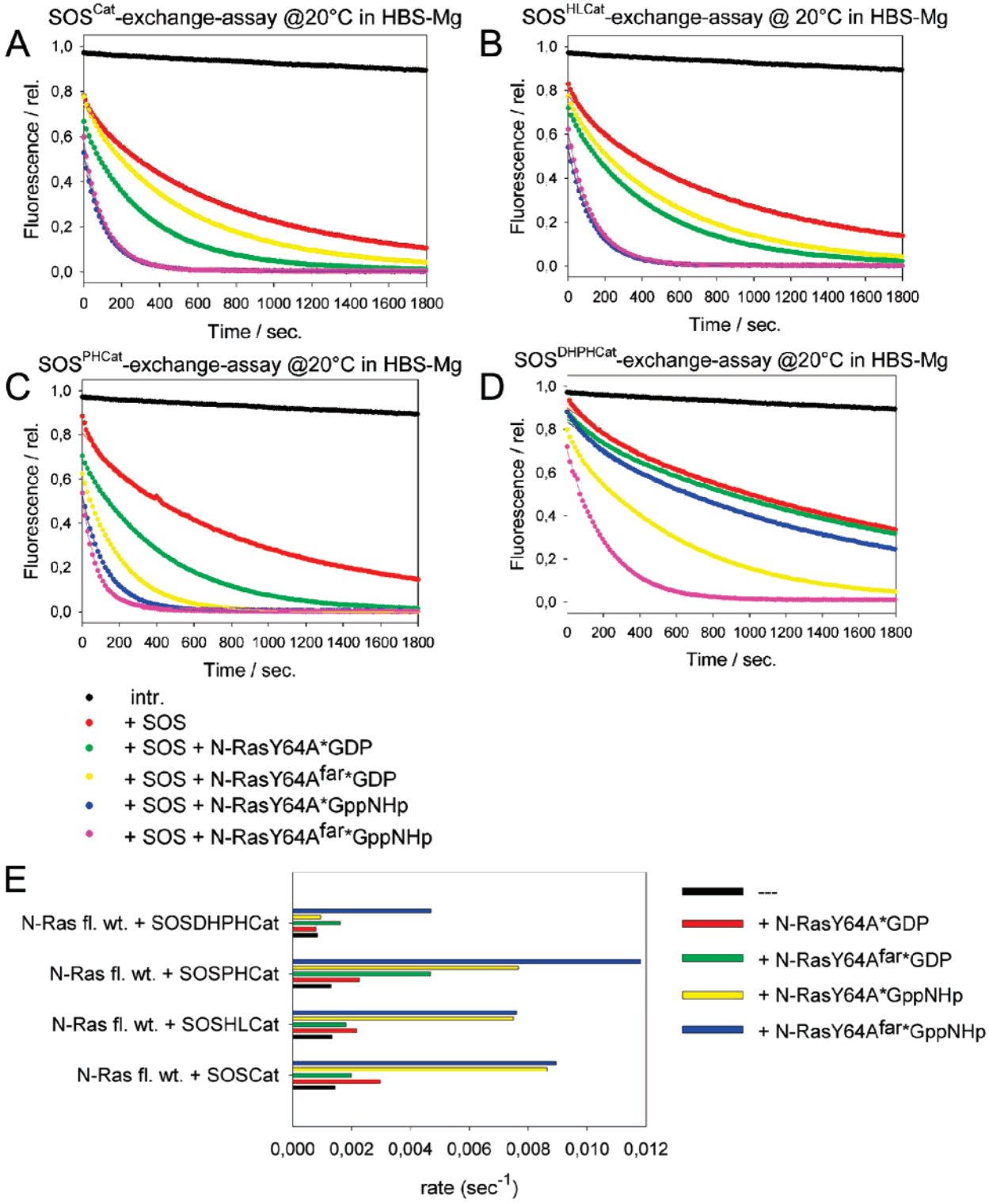
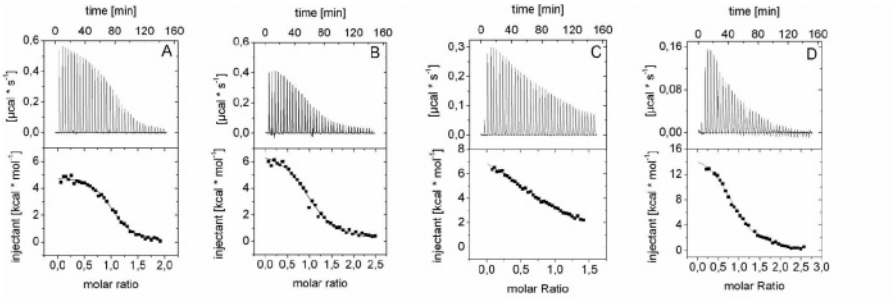


FIGURE 3: hSOS1 fragment (1 μM) promoted nucleotide exchange assay of mantGDP-loaded N-Ras wt fl. (1 μM) in absence or presence of prenylated and non-prenylated allosteric regulator (0.5 μM) (A, SOS^{Cat}; B, SOS^{HLCat}; C, SOS^{PHCat}; D, $SOS^{DHPHCat}$). (E) Observed rate constants from monoexponential curve fitting. Note different color codes of figures A−D and E.

autoinhibited DHPHCat fragment, we found that only the DHPHCat fragment senses the farnesylation status of the GppNHp-loaded allosteric regulator. Correspondingly, only if RasY64A*GDP is prenylated is it capable of accelerating $SOS^{DHPHCat}$-promoted nucleotide exchange above the basal level. In contrast, the catalytic fragment of hSOS1 alone does not respond to the prenylation status of allosterically bound Ras. Our results indicate a preference of $SOS^{DHPHCat}$ for prenylated allosteric regulators and also show that farnesylated GDP-loaded allosteric regulator is necessary for basal activation of hSOS1.

To narrow down structural elements in hSOS1 that account for the observed prenyl sensitivity, we used two fragments of hSOS1 that either comprise the PH domain and the catalytic region without the DH domain (SOS^{PHCat}) or a fragment consisting of the catalytic region with an N-terminal



K_D	N-RasY64A*GppNHp	N-RasY64Afar*GppNHp
SOSCat (150 mM NaCl)	**1.9 µM [A]** **2.0 µM***	**2.4 µM [B]**
SOSPHCat (150 mM NaCl)	**6.7 µM**	**0.31 µM** **0.35 µM**
SOSPHCat (300 mM NaCl)	**18.1 µM [C]**	**0.70 µM [D]** **0.83 µM**

FIGURE 4: ITC binding measurements with farnesylated or non-farnesylated GppNHp-loaded allosteric regulator (N-RasY64A ± Far*GppNHp) and hSOS1 fragments. Characters in brackets indicate corresponding measurements.

extension comprising the helical-linker region of hSOS1 that spans between the PH domain and the REM domain (SOS^{HLCat}). Whereas SOS^{HLCat} exhibits the same pattern as SOS^{Cat} regarding prenyl sensitivity of allosteric regulation, SOS^{PHCat}, which is resting in a non-autoinhibited state, behaves as the autoinhibited $SOS^{DHPHCat}$ fragment, i.e., has a preference for prenylated allosteric regulators (Figure 3). Since the remaining structural differences between SOS^{HLCat} and SOS^{PHCat} reside in the hSOS1-PH domain, the data suggest that the PH domain is responsible for the prenyl sensitivity of the feedback regulation.

To verify this finding we determined the affinity of GppNHp-loaded prenylated and non-prenylated N-RasY64A for SOS^{Cat} and SOS^{PHCat}, respectively.

Isothermal titration calorimetry confirmed the preference of the PHCat fragment of hSOS1 for prenylated allosteric regulators (~20-fold higher affinity compared to the non-farnesylated Ras protein). In contrast, the SOS^{Cat} fragment showed no stronger affinity for prenylated allosteric regulators. These results confirm that the PH domain supports the binding of the prenylated allosteric regulator to hSOS1 (Figure 4).

The data so far suggested a direct binding of prenylated allosteric regulators to the PH domain of hSOS1, but not of non-prenylated allosteric regulators. Thus we wondered if the isolated PH domain of hSOS1 could specifically compete for allosterically bound farnesylated GppNHp-loaded N-RasY64A in a SOS^{PHCat}-promoted nucleotide exchange assay.

However, in competition experiments with the isolated PH domain of SOS we could not observe a specific inhibition of the effect of the prenylated allosteric regulator on hSOS1 activity (Supporting Information S3, S4). Thus the promoting function of the PH domain in the SOS^{PHCat} fragment seems to be mediated indirectly.

Contributions of Prenylation of Ras Bound to the Active Site of hSOS1. The binding of allosteric Ras to the distal site of hSOS1 was previously shown to enhance the affinity of Ras for hSOS1 at the active site (*8*); thus we wondered if blocking the allosteric site might also influence hSOS1 preference for prenylated Ras at the active site. Using a mutant of hSOS1 that is incapable of binding prenylated and non-prenylated Ras at the distal site (SOS^{Cat}:W729E) (*8*), we found that blocking the distal site reduces nucleotide exchange on both farnesylated and non-farnesylated N-Ras but retains preference for prenylated N-Ras (Figure 5A).

When using the isolated CDC25 homology region of hSOS1 (SOS^{CDC25h}) that lacks the REM domain, we found that even the CDC25 homology domain of hSOS1 alone still has a preference for prenylated N-Ras [1.5-fold at the indicated concentrations] (Figure 5B). These observations lead to the conclusion that recognition of the prenylation status of N-Ras bound at the active site of hSOS1 is independent of allosteric regulation and thus contributes to the observed accelerated hSOS1-promoted nucleotide exchange on farnesylated Ras proteins.

DISCUSSION

Prenylation of Ras has been shown to modulate its function for a set of interaction partners including phosphatidylinositol(3)-kinase γ, members of the galectin family (galectin-1/3), PDEδ, and others (*17*−*21*).

Already in 1994 prenyl sensitivity was reported for the hSOS1 protein (*4*). During the past decade interesting new insights emerged concerning the interaction of Ras and hSOS1, i.e., the allosteric feedback activation and the DH domain mediated autoinhibition of hSOS1 (*7*, *8*), but for none of these latter findings has the potential connection to prenyl sensitivity been studied so far. Our data now indicate that prenylation of both Ras bound at the active site as well as distal site bound Ras are required for an efficient hSOS1-promoted nucleotide exchange.

The finding that the autoinhibited DHPHCat fragment of hSOS1 shows a clear preference for prenylated proteins and even requires prenylated Ras to overcome its autoinhibition supports scenarios in which autoinhibition of hSOS1 can only be released when hSOS1 is at the membrane to interact with processed Ras. Interestingly Rubio and colleagues described a very similar property of processed Ras in respect to its interaction with PI(3)-kinase γ. Whereas unprocessed Ras binds to PI(3)-kinase γ only in the GTP-loaded conformation, farnesylation of Ras allows Ras to bind to PI(3)-kinase γ even when GDP-loaded, albeit to a lesser extent compared to processed GTP-loaded Ras (*21*). The PH domain of hSOS1 was previously shown to bind to phosphoinositides (*9*, *15*, *22*−*24*), but Chen et al. also found that these phosphoinositide binding properties are not required for a serum stimulated membrane translocation (*9*).

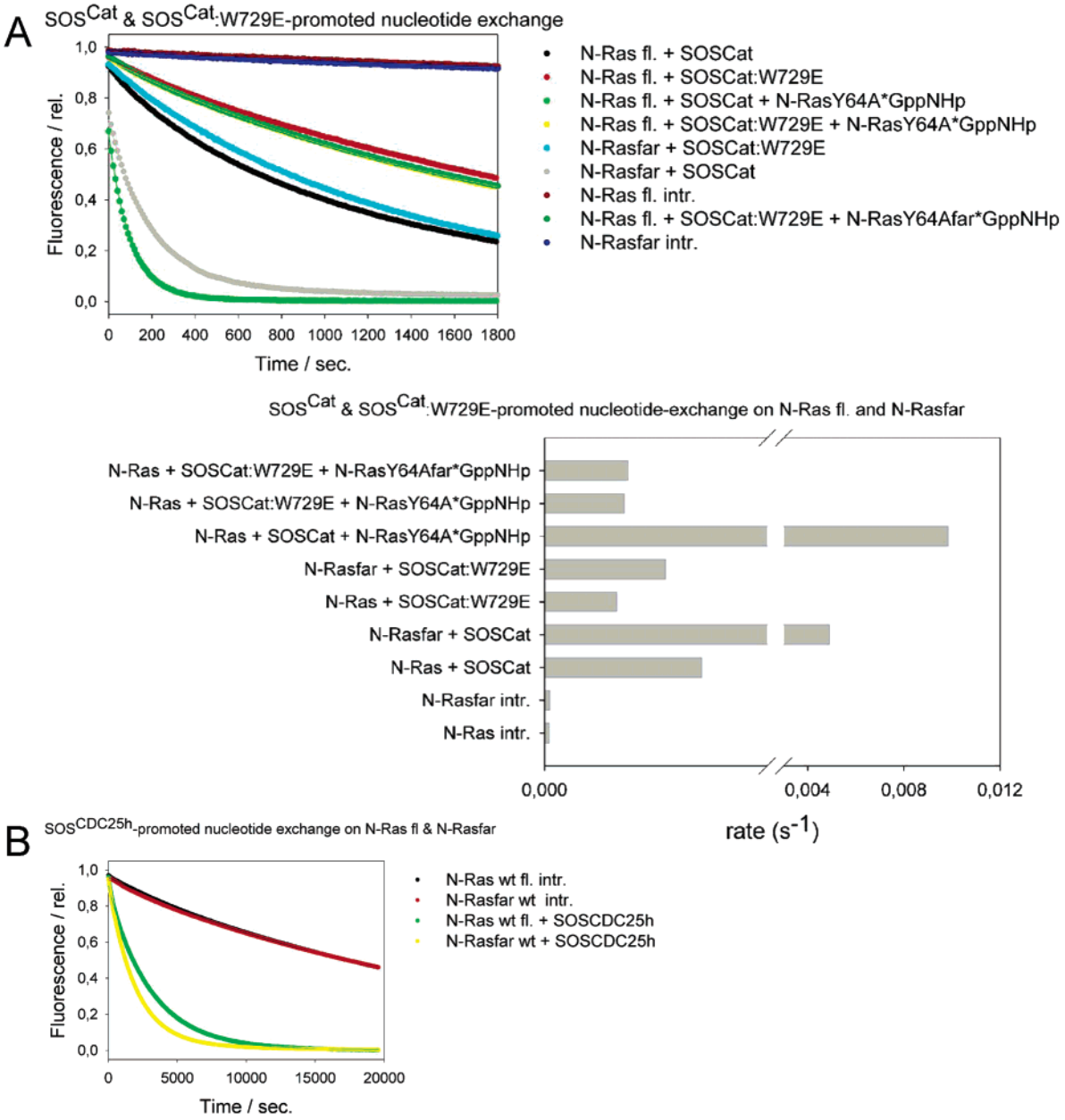


FIGURE 5: The active site of hSOS1 shows higher exchange activity for prenylated Ras independent of allosteric regulation. (A) SOS^{Cat}:W729E-promoted nucleotide exchange of mGDP-loaded N-Ras and farnesylated N-Ras in presence or absence of prenylated and non-prenylated allosteric regulator [1 μM Ras; 1 μM SOS^{Cat}; 1 μM RasY64A]. (B) SOS^{CDC25h}-promoted nucleotide exchange of mGDP-loaded N-Ras fl. wt and farnesylated N-Ras wt [1 μM Ras; 65 μM SOS^{CDC25h}].

In our experiments the phosphoinositol motif per se did not contribute for a release of the autoinhibition of $SOS^{DHPHCat}$, as demonstrated with the soluble form of PIP_3. In contrast, vesicle bound PIP_3 did enhance the function of allosteric regulator, indicating a concerted action of Ras and PIP_3 in a membranous context. Thus our data suggest that the PH domain is a sensor for allosteric Ras that in addition might open the PH domain for interaction with membrane localized PIP_3.

Although such a regulation via its PH domain was hitherto unknown for hSOS1, it is not uncommon for nucleotide exchange factors (*25*−*29*). For example, Rossman et al. (2002) found the PH domain of the RhoGEF Dbs to assist in stabilizing the interaction of DH domain of Dbs and CDC42 by generating additional amino acid contacts to CDC42 (*27*, *28*).

At first it was assumed that the PH domain of hSOS1 supports the binding of Rac1 to the hSOS1-Dbl homology domain. However, Nimnual et al. observed that only the isolated DH domain of hSOS1 is active with respect to nucleotide exchange on Rac *in vivo* and the tandem hSOS1-DHPH domain is not, except when coexpressed with Ras (*30*). Furthermore, by comparing the crystal structures of Dbs-DHPH fragment and hSOS1-DHPH fragment (*27*, *31*) Rossman et al. (2002) found that the orientation of the hSOS1-PH domain to the DH domain of hSOS1 is different from the one observed for Dbs. Thus it is likely that the PH domain is not subject to stabilization of Rac1 bound to hSOS1-DH domain.

In agreement with literature findings, our data suggest different functions for the PH domains in Dbs and hSOS1

and support the role of the hSOS1-PH domain in modulating Ras binding rather than Rac1 binding.

Since the isolated PH domain of hSOS1 could not specifically compete for allosterically bound farnesylated GppNHp-loaded N-RasY64A in a SOS^{PHCat}-promoted nucleotide exchange, the mechanism by which the PH domain of hSOS1 supports binding of prenylated Ras at the distal site seems to be mediated indirectly. Presumably the PH domain functions only in conjunction with other structural elements of hSOS1, e.g., the helical linker region spanning between the PH domain and the REM domain, and influences structural elements in the rest of the hSOS1 protein.

Summarizing, it could be shown that both prenylation of distal bound Ras and Ras bound to the active site are required for an efficient hSOS1-promoted nucleotide exchange and partial autoinhibition release. Moreover we found that both prenylation effects function independently and that the PH domain of hSOS1 assists in stabilizing allosterically bound prenylated Ras.

The question how isoprenylation of Ras does enhance its regulatory activity in the SOS-catalyzed nucleotide exchange reaction has to be addressed in more detail. While a direct contribution of the farnesyl moiety for binding of SOS has to be analyzed, e.g., by utilizing synthetic lipopeptides mimicking the processed N-Ras C-terminus, there are strong indications that isoprenylation might induce relevant changes in the overall structure of the Ras protein, thereby enhancing the binding affinity for hSOS1 (*32*). Besides such biochemical and structural investigations on the direct function of the farnesyl group on protein−protein interactions, further research will focus on hSOS1-promoted nucleotide exchange on Ras located at artificial membranes.

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SUPPORTING INFORMATION AVAILABLE

Additional details on cloning, expression, and purification of hSOS1 fragments and N-Ras proteins, in vitro farnesylation and purification of farnesylated N-Ras proteins, preparation of SUV, and $SOS^{DHPHCat}$-promoted nucleotide exchange are shown in Figures S1−S4 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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