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Differential Regulation of RasGAPs in Cancer

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Abstract

Ever since their discovery as cellular counterparts of viral oncogenes more than 25 years ago, much progress has been made in understanding the complex networks of signal transduction pathways activated by oncogenic Ras mutations in human cancers. The activity of Ras is regulated by nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), and much emphasis has been put into the biochemical and structural analysis of the Ras/GAP complex. The mechanisms by which GAPs catalyze Ras-GTP hydrolysis have been clarified and revealed that oncogenic Ras mutations confer resistance to GAPs and remain constitutively active. However, it is yet unclear how cells coordinate the large and divergent GAP protein family to promote Ras inactivation and ensure a certain biological response. Different domain arrangements in GAPs to create differential protein-protein and protein-lipid interactions are probably key factors determining the inactivation of the 3 Ras isoforms H-, K-, and N-Ras and their effector pathways. In recent years, *in vitro* as well as cell- and animal-based studies examining GAP activity, localization, interaction partners, and expression profiles have provided further insights into Ras inactivation and revealed characteristics of several GAPs to exert specific and distinct functions. This review aims to summarize knowledge on the cell biology of RasGAP proteins that potentially contributes to differential regulation of spatiotemporal Ras signaling.

Keywords: Ras/GAP assembly, NF1, p120GAP, GAP1, scaffolds

Introduction

Over the last 2 decades, substantial progress in the understanding of signal transduction at the molecular level has resulted in the identification of signaling cascades that are involved in the development of cancer. In particular, the complex network of signaling pathways initiated by activated cell surface receptors to control a wide variety of cellular processes, including proliferation, differentiation, and apoptosis, has received strong interest. H-, K-, and N-Ras were one of the first cellular proteins identified that link activated growth factor receptors with effector pathways that regulate cell growth and differentiation. Originally discovered as cellular counterparts of viral oncogenes, it soon became evident that mutations in codon 12, 13, and 61 result in constitutively active Ras that is resistant to downregulation.[1,2] Approximately 20% to 30% of human tumors express oncogenic Ras, with mutations being most common in adenocarcinomas of the pancreas (90%), colon (50%), lung (30%), thyroid tumors (50%), and myeloid leukemia (30%).[2,3] K-Ras mutations are most frequent (85%) and predominantly found in pancreatic, colon, and lung cancers. N-Ras (15%) and H-Ras (<1%) mutations are less common and mainly found in myeloid leukemia and kidney and bladder cancers, respectively. In addition, aberrant activation of wild-type Ras (hyperactive Ras) can occur through deregulation of growth factor receptors or, as outlined in more detail below, via inactivation of negative Ras regulators.[3-5] In this review, we will discuss the current models and views on Ras inactivation and how this might link to oncogenic activity of Ras isoforms and spatiotemporal Ras signaling.

Ras Inactivation

Ras proteins are predominantly localized at the inner leaflet of the plasma membrane and act as binary switches, cycling between an inactive Ras-GDP and an active Ras-GTP. GTP-bound Ras is able to bind and activate effector pathways, including the Raf/mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K)/Akt signaling cascades, which play central roles in cell growth and differentiation.[3-6] The activity of the 3 Ras isoforms is controlled by the ratio of bound GTP to GDP, and because Ras proteins have a very slow rate of GTP/GDP exchange and possess a very low intrinsic GTPase activity, these processes are regulated by GEFs and GAPs.[7,8] GEFs enhance the rate of GDP dissociation, whereas GAPs accelerate the intrinsic Ras-GTPase activity to promote Ras inactivation by several orders of magnitude. Biochemical and structural analyses of the purified Ras/GAP complex identified glutamine 61 of Ras and an arginine (arginine finger) provided by GAP as crucial to coordinate Ras-GTP hydrolysis.[9-11] Using the crystal structure of Ras complexed with a

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nonhydrolyzable GTP analog, the molecular mechanisms involved in GAP-mediated Ras-GTP hydrolysis were clarified, and we refer the reader to more detailed reports from others.[7-11] In brief, phosphoryl transfer by GAPs requires the proper orientation of a water molecule attacking the γ-phosphate opposite to the GDP and stabilization of the transition state.[7,8] The common oncogenic Ras mutation at glutamine 61 abolishes GAP-induced hydrolysis, and mutations at positions 12 and 13 sterically block the proper orientation of glutamine 61 and the arginine finger, which is essential to stabilize the transition state.[9-11] Interestingly, several members of the GAP1 family of RasGAPs display dual specificity to stimulate GTP hydrolysis of Ras as well as Rap1.[8,12-15] While the requirement for dual GTPase activity of GAPs awaits further confirmation in physiological settings, one can envisage that GAPs simultaneously regulating Ras and other small GTPases could integrate multiple signal transduction pathways.

Functional Differences of Ras Isoforms and Differential GAP Activity

H-, K-, and N-Ras are ubiquitously expressed and highly homologous, which was initially interpreted as an indication for redundancy within the Ras family. Yet, the association of different mutation rates of Ras isoforms in different cancers already gave fundamental clues of functional differences.[1-3] In addition, knockout mice revealed essential roles for K-Ras in development, while H- and N-Ras appeared dispensible for mouse embryogenesis.[16-19] Along these lines, defects in human development have recently been associated with Ras isoform–specific functions.[19-21] The different magnitude of oncogenic Ras isoform signaling further supports signal specificity of Ras isoforms. Although there is some controversy about the differential ability of Ras isoforms to activate downstream effectors, some studies suggested that H-Ras strongly activates the PI3K/Akt pathway, while K-Ras is a more potent activator of the Raf/MAPK pathway. Different potencies of Ras isoforms to induce cellular transformation and differential Ras isoform gene expression patterns seem to further contribute to establish differential Ras isoform signaling.[22,23]

Altogether, these findings implicate that Ras activation and inactivation must be fine tuned and tightly regulated. But how do cells coordinate the GAP protein family in space and time to mediate a certain biological response? Different domain arrangements in GAPs to create differential protein-protein and protein-lipid interactions are probably key factors determining the GTP/GDP ratio bound to H−, K−, and N-Ras.[14,24-30] Cell- and animal-based studies examining GAP activity, interaction partners, and expression profiles have provided further insights into Ras inactivation in normal and transformed cells. Mammalian proteins capable of functioning as GAPs for H-, K-, and N-Ras represent a large and divergent protein family and include p120GAP, neurofibromin (NF1), the GAP1 family, including GAP1^{IP4BP}, Ca^{2+}-promoted Ras inactivator (CAPRI), Ras GTPase activating-like protein RASAL, as well as the SynGAP family (DAB2IP, nGAP, SynGAP).[14,24-30] Some of those are expressed ubiquitously, while others show very restricted expression profiles, making it difficult to assign a member of the GAP family with Ras inactivation in a given cell/tissue. In addition, structural analysis indicated that NF1 and p120GAP bind Ras isoforms without any preference.[9-11]

Yet, an increasing number of studies suggest that differential GAP expression patterns and Ras/GAP assembly must be involved in cellular transformation. NF1, which is a tumor suppressor only in neuronal and myeloid cells,[25-28] is probably the best example for the tissue-specific transforming potential of an individual member of the GAP protein family. CAPRI confers tumor suppressor activity in mammary epithelial cells,[31] and RASAL is downregulated in tumors from the brain, skin, bladder, head, and neck as well as multiple cell lines from the nasopharynx, breast, lung, liver, and esophagus.[32] Recently, loss of RASAL activity was correlated with hyperactive Ras in the colon and hepatocellular carcinoma lacking oncogenic Ras.[33,34] In some cancers, diminution of RASAL is probably due to the downregulation of the transcription factor PITX1, which is required for RASAL mRNA expression.[35] Furthermore, expression of DAB2IP, a less well-characterized member of the SynGAP family, is often silenced in hepatocellular carcinoma.[36]

While earlier studies were not able to link p120GAP with tumor suppressor activity in human cancers, more recent work has associated p120GAP mutations with malfunctions in angiogenesis.[37] Moreover, we recently demonstrated that p120GAP-mediated inactivation of epidermal growth factor receptor (EGFR)–induced Ras/MAPK signaling is facilitated by annexin A6 (AnxA6), a member of the annexin protein family.[30,38] Loss of AnxA6 in EGFR-overexpressing and estrogen receptor (ER)–negative breast cancer cells (BCCs) is associated with elevated Ras, Raf, and MAPK activity, and *vice versa*, upregulation of AnxA6 strongly reduces cell growth of EGFR-overexpressing BCCs.[39,40]

Taken together, the potential tumor-suppressive function of each GAP is associated with elevated Ras activity but seems to be restricted to certain cell types, and it is tempting to speculate that specific GAPs control Ras activity in different tissues, but their inhibition is a general mechanism to provoke oncogenic activity and carcinogenesis via elevated wild-type Ras activity. In support of this hypothesis, the different domain arrangements in GAPs point at different functions and interactions.[24-30]

Subcellular Localization of Ras Signaling Complexes

In addition to complex expression profiles and different interaction partners determining the ability of the various GAP proteins to associate with H-, K-, or N-Ras, it has become evident that the

subcellular localization of Ras isoforms is another major determinant of signal output and biological response. This includes the different localization and lateral segregation of activated Ras isoforms in different microdomains of the plasma membrane but also Ras isoform–specific signaling from different endomembranes.[19,41-43] The stabilization of microdomains containing different Ras isoforms could then provide platforms for the differential recruitment of GAPs and other Ras regulators/effectors, leading to the formation of signal- and compartment-specific protein complexes.[19,41-43]

It would go beyond the scope of this review to summarize the literature on the microlocalization of Ras isoforms, and we refer the reader to more detailed reviews from others on this topic.[19,41-43] Most of the current knowledge on Ras isoform localization is based on ectopic expression of tagged Ras isoforms, either utilizing fluorescent proteins or short sequence motifs to target Ras isoforms to specific localizations at the cell surface or to endomembranes. Limited sensitivity of currently available technology is still impeding verification of the majority of these findings for endogenous H-, K-, and N-Ras. In summary, based on the Ras isoform overexpression studies, at the plasma membrane, H- and K-Ras are believed to be localized in largely non-overlapping microdomains (nanoclusters), and their distributions are modulated by GTP/GDP exchange, thus GEF and GAP activity. H-Ras-GDP is predominantly found in cholesterol-sensitive domains (lipid rafts/caveolae), whereas active H-Ras preferentially targets cholesterol-independent microdomains. Both active and inactive K-Ras is localized in cholesterol-independent nanoclusters, which are physically distinct from H-Ras-GTP–containing microdomains. N-Ras-GTP is mainly found in cholesterol-sensitive membrane domains. It was initially believed that Ras signaling occurs exclusively at the plasma membrane, but since the discovery of active Ras in the endoplasmic reticulum and Golgi complex,[43,44] all 3 Ras isoforms have been detected in endosomes[45-47] and mitochondria.[48-50] Thus, Ras isoforms cannot be considered stationary but translocate between cellular compartments. The presence of Ras in multiple cellular locations must have consequences for the regulation of Ras activation and inactivation. Indeed, several studies implicate differential participation of certain GEFs to promote Ras activation at the endoplasmic reticulum or the Golgi complex.[51-53]

Despite the increased knowledge on the mechanisms that target active H-, K-, and N-Ras into different microdomains at the plasma membrane and intracellular localizations, the recruitment of GAPs to specific Ras-containing microdomains is still poorly understood. Current models suggest that active Ras recruits regulators and effectors in small (<15 nm) signaling platforms.[19,41-43] Electron microscopy and single-particle tracking studies showed activated H- and K-Ras to become transiently immobile, possibly to recruit downstream effectors, such as Raf and MAPK.[41-43] Similarly, once the Ras binding domain of p120GAP is recruited to the membrane, it is transiently immobile to interact with H- and K-Ras-GTP.[54] Given that some full-length GAPs act differently compared to their GAP domains,[15] it is still to be determined if this mechanism also applies for full-length p120GAP or other GAPs.

The different localization of Ras isoforms is likely to create a microenvironment that enables differential recruitment of GAPs for Ras inactivation. As described in more detail below, the various protein domains and second messengers promoting membrane association of NF1, p120GAP, CAPRI, RASAL, GAP1^{m}, GAP1^{IP4BP}, and SynGAP probably reflect the need and adaptation to ensure efficient targeting of all Ras isoforms in any cellular location. All GAPs are characterized by a multiple domain structure,[24-30] and modules such as the src homology 2 (SH2), SH3, pleckstrin homology (PH), Ca^{2+}-dependent phospholipid-binding/conserved region 2 (CALB/C2), and the Bruton tyrosine kinase Cys-rich (BTK) domain probably enable targeting of any Ras isoform by at least one GAP independent of Ras isoform microdomain localization. But little is yet known how protein-protein and protein-lipid interactions and calcium (Ca^{2+}) coordinate the targeting of the GAP family members to different Ras isoforms in various locations in different cells and tissues.

In addition, targeting/scaffolding proteins are likely to stabilize Ras/GAP assembly directly/indirectly via interacting with GAPs and/or Ras or via membrane microdomain formation/stabilization. Several scaffolds for Ras isoforms and Raf and MAPK kinases have been identified, which is reviewed in detail elsewhere.[4,19,41,42,55,56] Scaffolds stabilizing protein-protein and protein-lipid interactions of GAPs via their SH2, SH3, PH, C2, and even GAP domains probably contribute to determine membrane targeting and Ras/GAP assembly in certain cellular locations. This includes proteins like 14-3-3, AnxA6, receptor for activated C kinase 1 (RACK1), syndecan 2 for p120GAP, and NF1, respectively. In addition, the differential interaction of GAPs with growth factor receptors, protein kinase C (PKC), but also regulators of actin remodeling, such as RhoGAPs and Rac/Rho GTPases, and other yet unknown proteins provides further opportunity for signal complex specificity.[14,24-30]

To date, experimental evidence for GAP activity is almost exclusively associated with Ras inactivation at the plasma membrane. NF1 has been found at the plasma membrane and multiple intracellular localizations and could thus be involved in Ras inactivation in various endomembranes.[57-59] The fact that p120GAP remains associated with EGFR in endosomes[60] is another indication for the involvement of GAPs in the regulation of spatiotemporal Ras signaling, as active H-Ras is internalized with EGFR after stimulation.[45] The targeting protein for p120GAP, AnxA6, is also found in endosomes and able to bind active H-Ras.[30,39,40] Altogether, this could ensure H-Ras signal termination both at the plasma membrane and in early endosomes upon EGFR activation.

Only few studies investigated the association of GAPs with cholesterol-rich membrane microdomains (lipid rafts), which are important for H- and N-Ras signaling.[19,41] For instance, NF1 binds to the scaffolding domain of caveolin, the major structural protein found in caveolae, a subtype of lipid rafts.[61] NF1 contains 4 potential caveolin-binding domains, and interestingly, missense mutations in NF1 patients occur with high frequency in 3 of the 4 putative caveolin-binding domains.[61] Similarly, SynGAP was found in lipid rafts from neuronal cells.[62] p120GAP complexed with scaffold proteins such as syndecan-2, RACK1, and AnxA6 probably fine tunes the association with rafts and nonrafts.[30,40,63-65] Future studies will have to clarify if GAP localizations associated with lipid rafts can be linked to H- and N-Ras inactivation at the cell surface.

Membrane Targeting and Regulation of GAP Activity

GAP-mediated inactivation of Ras requires the translocation of GAPs to the plasma membrane followed by Ras/GAP complex formation and stimulation of Ras-GTPase activity. The coordinated recruitment of the various GAPs to H-, K-, and N-Ras in a given cell is still unclear. Yet, the dynamics of targeted membrane association and release into the cytosol upon Ras inactivation provides an opportunity to reversibly target specific GAP proteins to different Ras isoforms in various locations within the cells. In the following, we will not aim to give a detailed review of each GAP, as several comprehensive reviews covering NF1, p120GAP, and the GAP1 family have been published recently.[14,24-30] The aim of this section is to highlight aspects that could contribute to differential GAP membrane targeting and Ras/GAP assembly.

NF1

Loss of neurofibromin (NF1) is to date the only mutation for Ras-specific GAPs known to cause tumor predisposition disorders in humans.[26-28] NF1 encodes a 320-kD protein, and clinical manifestations of NF1 include multiple benign and malignant neurofibromas as well as gliomas, pheochromocytomas, and myeloid leukemias. NF1 is ubiquitously expressed but most abundant in neurons, Schwann cells, astrocytes, oligodendrocytes, and leukocytes.[26-28] The tumor suppressor activity of NF1 is believed to occur through its RasGAP domain, as loss of NF1 results in hyperactive Ras and increased activity of downstream effectors such as the Raf/MAPK and PI3K/Akt pathway.[27,66]

While NF1 reduces overall Ras activity and appears to interact with all 3 Ras isoforms,[9-11] the ability of NF1 to downregulate each Ras isoform in cells and tissues is not fully characterized. Neurological deficits in NF1 knockout mice can be rescued by K-Ras depletion and pharmacological inhibition of Ras using farnesyl transferase inhibitors (FTIs) or lovastatin, which interferes with Ras prenylation, suggesting that NF1 can target all Ras isoforms.[27] NF1 appears to be localized in multiple cellular locations,[57-59] but relatively little is known how NF1 localization and activity are regulated. Besides the central RasGAP domain, the remaining sequence contains an N-terminal cystein/proline-rich domain (CSRD) and a C-terminal domain (CTD) with a nuclear localization signal[57,67] but lacks the domains common to the other GAPs. Relevant for NF1 membrane targeting is most likely a bipartite phospholipid-binding Sec14-PH module adjacent to the RasGAP domain.[68] The biological role of the Sec14 domain is still unclear, but its yeast homolog Sec14p is implicated in protein and lipid trafficking,[69] and Sec14-like domains have been found in a number of mammalian lipid-binding proteins.[70] In recent follow-up studies, several glycerophospholipids, in particular phosphatidylethanolamine and phosphatidylglycerol, were found to interact with the Sec14 domain.[70] Thus, the Sec14 module could facilitate NF1 interaction with glycerophospholipids in certain compartments to selectively regulate distinct Ras signaling pathways.

Several other proteins are likely to impact on NF1 localization. Phosphorylation sites within the CT domain are constitutively phosphorylated by protein kinase A (PKA).[71,72] The phosphorylation of these serine and threonine residues is important for interaction of NF1 with 14-3-3 proteins, which negatively regulates the RasGAP activity of NF1.[72] In addition, protein kinase C (PKC) has also been shown to phosphorylate NF1.[73,74] In response to EGF, PKCα-mediated phosphorylation of NF1 increases its RasGAP activity and also its interaction with actin.[73] Altogether, this might point at NF1 targeting K-Ras at the plasma membrane, which requires an intact actin cytoskeleton for signaling.[75] Further implicating protein kinases in NF1 localization and activity, NF1 can be degraded via the proteosome with growth factors that stimulate both G protein–coupled receptors and receptor tyrosine kinases.[76] Recently, Phan *et al*. identified the ETEA/UBXD8 protein to interact and promote NF1 ubiquitination and degradation, which would ultimately regulate the amount of NF1 at the cell surface.[77] It is unknown if these findings are linked to earlier studies reporting that NF1 phosphorylation inhibits its lysosomal degradation.[78] Interestingly, ETEA/UBXD8 does not interact with p120GAP,[77] the other ubiquitously expressed RasGAP, indicative of this interaction contributing to differential and spatiotemporal Ras signaling.

In this context, it should be noted that the localization of protein kinases such as PKC and PKA is regulated by scaffold proteins, including RACKs, A kinase anchoring proteins (AKAPs), and annexins,[79-83] all of which facilitate the complex and spatiotemporal targeting of PKC and PKA to unique subcellular localization to be part of specialized signaling complexes. The complex interplay of protein kinases with their specific scaffolds could therefore determine NF1 and, as outlined below, p120GAP activity and localization.

p120GAP

The modular structure of p120GAP is probably the basis for its multiple interaction partners and functions that go beyond Ras inactivation, which has been

summarized in detail.[26,29,30,37] p120GAP is ubiquitously expressed and has been implicated in the downregulation of EGF, platelet-derived growth factor (PDGF), insulin, colony-stimulating factor, and ephrin receptors. In addition, the N-terminal region of p120GAP exerts effector functions that appear independent of its GAP activity.[26,29] Here, we want to focus on mechanisms that are involved in Ras/GAP assembly and Ras inactivation. The modular structure of p120GAP includes an SH3 domain flanked by 2 SH2 domains, followed by a PH, CALB/C2, and the GAP domain. The coordinated interaction of these domains with other proteins, including protein kinases, adaptors, and scaffolding proteins, probably organizes p120GAP membrane recruitment and Ras/GAP assembly.

SH2 domains facilitate the binding of p120GAP to activated EGF, PDGF, and other receptor tyrosine kinases, such as insulin receptors.[29,30,84-86] Several nonreceptor tyrosine kinases, including Src, also interact with the SH2 domains of p120GAP.[87,88] Dok-1 ($p62^{dok}$) is an adaptor that binds to the p120GAP SH2 domain[89] and was initially identified as a tyrosine-phosphorylated, 62-kDa protein associated with p120GAP in chronic myelogenous leukemia (CML) and in v-Abl–transformed B cells.[90,91] It later turned out to be the prototype of the Dok adaptor protein family, which plays key roles in the inhibition of cell proliferation and Ras/MAPK signaling triggered by diverse stimuli through receptor and nonreceptor kinases.[89]

As mentioned above, mutational defects in p120GAP cause abnormal angiogenic remodeling in capillary malformation–arteriovenous malformation (CM-AVM) that cannot be compensated by other RasGAPs.[37] These observations underscore a prominent and specific role for p120GAP in Ras signaling pathways regulating the actin cytoskeleton. In support of this, multiple binding partners of p120GAP as well as results from the p120GAP knockout mice provide links to actin remodeling. For instance, the SH2 domains bind p190-RhoGAP and focal adhesion kinase (FAK), which both regulate the GTPase activity of Rho GTPases.[92-94] Furthermore, the SH3 domain in p120GAP, which is unusual because of its inability to interact with proline-rich motifs, binds the calpain subunit Capns1 and has been implicated in Rho-mediated cytoskeletal rearrangements.[95,96] Altogether, this could indicate that SH2 and SH3 domains recruiting p120GAP to specific plasma membrane microdomains involved in actin remodeling, where coordination of Ras and Rho regulated signaling pathways, for example, for the formation of stress fibers, focal adhesions, establishment of cell polarity, and cell migration, are essential.[92-96]

Little is known about the PH domain in p120GAP, which generally facilitates binding to phosphoinositides in membranes or in some cases proteins.[97] Some evidence suggests that the PH domain can interfere with Ras/GAP assembly.[98] In addition, interaction of RACK1 with the PH domain of p120GAP has been described.[64,65] RACK1 is a scaffold for PKCα, which in earlier studies was shown to regulate p120GAP activity in T lymphocytes,[99] but also might target p120GAP to cholesterol-rich membrane domains, such as caveolae, in other settings.[100]

By extending the previous work from Davis *et al*.,[101] we demonstrated that AnxA6 binds to the CALB/C2 domain of p120GAP to facilitate the Ca^{2+}-dependent recruitment of p120GAP to inactivate H-Ras at the plasma membrane upon EGFR activation.[30,39,40] For a more comprehensive description of the AnxA6/p120GAP complex and its involvement in Ras inactivation upon EGFR activation, we refer the reader to previous reviews from our laboratories.[30,40,102] As mentioned above, AnxA6/p120GAP inhibiting H-Ras might also be operational in early endosomes.[30,40-45,60,102] Reduced growth of EGFR-overexpressing A431 cells ectopically expressing AnxA6 in mouse xenografts supports tumor suppressor activity of the AnxA6/p120GAP complex.[103] Further emphasizing AnxA6 to create signal specificity, overexpression of p120GAP, but not CAPRI, inhibits Ras/MAPK activity in AnxA6-expressing BCCs.[39] It remains to be determined if AnxA6-mediated inhibition of H-Ras via membrane recruitment of p120GAP is linked to earlier reports identifying several fatty acids, including arachidonic acid, and their metabolites, eicosanoids, as potent inhibitors of p120GAP and NF1.[104,105] Recent studies from our laboratory revealed decreased cytoplasmic phospholipase A_2 ($cPLA_2$) activity in cells expressing elevated levels of AnxA6,[106] and it is tempting to speculate that the reduced ability of $cPLA_2$ to cleave arachidonic acid from phospholipids in certain membrane microdomains creates a microenvironment that potentiates and stabilizes p120GAP membrane association and H-Ras/p120GAP assembly.

We have also demonstrated that AnxA6 stimulates the Ca^{2+}-inducible involvement of PKCα in the regulation of H-Ras and possibly EGFR signal transduction pathways.[40,102,107] On the other hand, Agell *et al*. identified PKC-mediated and calmodulin (CaM)–dependent phosphorylation of K-Ras to modulate K-Ras activity and function.[108,109] In these studies, it was proposed that microdomains of nonphosphorylated K-Ras would be more accessible to p120GAP, consequently followed by K-Ras inactivation, whereas segregation of phosphorylated K-Ras into other microdomains would recruit a different set of effectors. Hence, interplay of RACK1, AnxA6, and CaM might coordinate the involvement of PKCα in the regulation of p120GAP localization and activity for H- and K-Ras.

Together with the ability of AnxA6 to modulate caveolae formation and induce actin rearrangements at the plasma membrane,[110-112] it is evident that the multiple features that determine AnxA6 localization and protein-protein interaction, together with the SH2, SH3, and PH domains within p120GAP, create a unique combination of targeting signals

to direct p120GAP into specific microdomains in multiple locations.

The GAP1 Family

The mammalian GAP1 family consists of GAP1^{m}, GAP1^{IP4BP}, CAPRI, and RASAL, and their role in the regulation of Ras inactivation has been reviewed in detail by Cullen and coworkers.[14,24] In this section, we will briefly summarize previous findings and highlight recent data that suggest the dual Ras and Rap1 GAP activity of the GAP1 family contributing to spatiotemporal Ras signaling.

GAP1^{m} and GAP1^{IB4BP}

The GAP1 family is characterized by a conserved domain structure with 2 N-terminal C2 domains, followed by the central GAP domain and C-terminal PH domain that contains a BTK motif. Membrane recruitment of GAP1^{m} and GAP1^{IB4BP} is facilitated by their PH domains, which are well established to act as binding sites for phosphoinositides and proteins but are generally believed to confer little specificity and low affinity.[97] However, the PH domains of GAP1^{m} and GAP1^{IB4BP} bind phosphatidylinositol 3,4,5-triphosphate (PIP_3) with high affinity, and GAP1^{IB4BP} also displays affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2).[113] Sequence variations in their PH domains seem to allow selective interaction with particular phosphoinositide head groups.[113-116] Cytosolic GAP1^{m} undergoes rapid membrane translocation upon elevation in plasma membrane PIP_3 levels because of activation of cell surface receptors that couple to class I PI3K. Given the potential role of PI3K in signaling events in lipid rafts, these mechanisms could be involved in N-Ras inactivation and/or the lateral movement of active H-Ras out of lipid rafts.

In contrast to GAP1^{m}, GAP1^{IB4BP} is constitutively localized at the plasma membrane probably because of the high levels of PIP_2 that are constantly found at the inner leaflet of the plasma membrane. Little is known about the regulation of the RasGTPase activity of GAP1^{IB4BP}. Recently, one study identified the involvement of GAP1^{IB4BP} in the dopamine D2 receptor–mediated inhibitory regulation of endocrine functions in pituitary cells via the Ras/MAPK pathway.[117]

However, the ability of GAP1^{IB4BP} to function as a Rap1 GTPase-activating protein indicates that depending on the cellular microenvironment, GAP1 GAPs might target Rap1 as well as Ras GTPases.[12-15] Indeed, all GAP1 family members except GAP1^{m} and even SynGAP can stimulate Rap1-GTP hydrolysis.[12-15,118] At first, these unexpected findings appeared difficult to interprete, as GAPs specific for Ras and Rap1 act differently. Unlike Ras, Rap1 does not possess a glutamine at position 61, and RapGAPs do not employ a catalytic arginine residue (arginine finger) but provide an asparagine (asparagine thumb) to stimulate GTP hydrolysis.[12-15] Interestingly, the RasGAP domain of GAP1^{IP4BP} is sufficient to drive the hydrolysis of Rap1-GTP,[13] and recent studies suggest that the GAP domain of GAP1^{IP4BP} and RASAL can undergo conformational changes that enable them to interact with either Ras or Rap1.[15] Hence, both Ras and Rap1 GTPases might compete for GAP1^{IP4BP} and RASAL recruitment in cells, and the local concentration of Ras and Rap1 in any microenvironment might determine the biological activity of these GAPs.

CAPRI and RASAL

CAPRI and RASAL both contain a pair of CALB/C2 domains with a full set of 5 Ca^{2+}-coordinating acidic residues that, upon Ca^{2+} increase, mediate the rapid recruitment of cytosolic CAPRI and RASAL to the membrane to inhibit Ras and MAPK.[14,119,120] Most strikingly, CAPRI and RASAL respond to distinct temporal aspects of the Ca^{2+} signal. Whereas CAPRI is constantly associated with the membrane in response to Ca^{2+}, RASAL senses the frequency of Ca^{2+} oscillations by undergoing synchronous and repetitive oscillatory associations with the plasma membrane.[121-123] Consistent with CAPRI promoting Ras GTPase activity, Fcγ receptor (FcγR) activation in macrophages from CAPRI-deficient mice is associated with strong Erk activation.[124] However, the impaired immune response after bacterial infection of these mice is probably due to the uncoupling of FcγR-mediated phagocytosis from Rac1 and Cdc42 activation. Rac1 and Cdc42 are both Ras-related GTPases that are necessary for actin reorganizations during the formation of the phagocytic cup. Interestingly, CAPRI constitutively interacts with both Rac and Cdc42 via its RasGAP domain and appears to recruit both GTPases during FcγR-mediated phagocytosis.[124] This scaffolding function of CAPRI might be similar to the one of IQGAP, which also stabilizes active Rac and Cdc42.[125] The ability of CAPRI, and possibly RASAL, to act as a scaffold, together with their dual GAP specificity towards Ras and Rap1, is likely to compete with their involvement in Ca^{2+}-mediated Ras inactivation.

Differential Expression of GAPs in Cancer

Several recent studies have analyzed the expression patterns of various RasGAPs in different cancers, altogether supporting a model of the GAP protein family differentially contributing to tumorigenesis in different organs. Two independent RNA interference studies originally identified RASAL and CAPRI as potential tumor suppressors in cell culture models. While CAPRI reduced transformation in mammary epithelial cells,[31] Kolfschoten *et al*. used a transformation model that only requires Ras for transformation and identified RASAL to possibly inhibit cell growth in prostate, bladder, and colon cancer. In these studies, downregulation of RASAL was achieved through transcriptional control driven by PITX1.[35] These findings increased interest to examine the potential role of RASAL in oncogenesis, which is highly expressed in the thyroid and adrenal medulla but only found in residual amounts in the brain, spinal

cord, and trachea.[126] Jin *et al*. provided strong evidence that expression of RASAL, but not CAPRI or PITX1, is silenced through CpG methylation in multiple tumors, including the brain, skin, bladder, head, and neck. Importantly, ectopic expression of RASAL suppressed the malignant phenotype of tumor cells with silenced RASAL.[32]

Until recently, a role for RasGAPs in colorectal cancers (CRC) was unknown, but recent studies identified RASAL as being significantly downregulated in all CRC lines that express wild-type K-Ras.[34] Knockdown and overexpression experiments support a role of RASAL as a negative regulator of wild-type K-Ras in CRC cells. Primary CRC tumors revealed a correlation between RASAL levels and CRC disease progression, implicating a prognostic role for RASAL in colon cancer.[34] Interestingly, reduced RASAL levels were predominantly observed in tumors of the distal colon and rectum, indicating that particular cells defined by their tissue location are particularly vulnerable to RASAL depletion. Similar to the studies described above, methylation of the RASAL gene in several CRC lines was observed, which further emphasizes epigenetic silencing of RASAL contributing to tumorigenesis.[34]

Aberrant activation of wild-type Ras and its downstream effector pathways is also common in human hepatocellular carcinomas (HCC). In a large collection of human HCC, Calvisi *et al*. identified that in HCC with hyperactive wild-type Ras, but lacking Ras mutations, either RASAL, DAB2IP, NF1, or the RASAL inducer PTX1 was downregulated.[33] Strikingly, all HCCs exhibited promoter methylation of RASAL, DAB2IBP, or NF1, indicating that silencing of GAP mRNA expression can contribute to oncogenic events leading to increased wild-type Ras activity in HCC. Interestingly, elevated levels of several other GAPs did not appear to compensate for the loss of RASAL, DAB2IP, or NF1 nor reduce elevated Ras activity and cell growth in these liver cancers.[33] Further highlighting the involvement of RASAL in liver carcinogenesis, PITX1 is frequently silenced by promoter hypermethylation.[127] Low DAB2IP and PTX1 levels in HCC subclasses characterized by poor survival could indicate an involvement in tumor aggressiveness.

Similarly, epigenetic silencing of DAB2IP in HCC has been reported by others.[36] DAB2IP, which is a RasGAP associated with the disabled gene family member DAB2, is also silenced in prostate, breast, lung, and gastrointestinal tumors through aberrant promoter CpG methylation.[128-132] Furthermore, 2 genome-wide studies identified DAB2IP as a putative tumor suppressor in aggressive prostate adenocarcinoma.[133] Unfortunately, little is known about the regulation of DAB2IP *in vivo* and correlation to Ras activity in the cancer samples analyzed, but together with the observations described below, epigenetic silencing of the various GAPs implicates a general theme that requires further investigation.

It is evident that the concept of differential expression patterns and epigenetic silencing conferring specificity for Ras/GAP assembly also extends to the various scaffolds and kinases targeting p120GAP. In this context, we just want to highlight AnxA6, the membrane-targeting protein for p120GAP. Although AnxA6 is often viewed as a ubiquitous and abundant protein, it is not expressed in epithelial cells of the small intestine, kidney (including the parathyroid gland), and colon, which have low to undetectable amounts of AnxA6.[30,40] In several tumors and cancer models, loss of AnxA6 correlates with elevated Ras activity and tumor progression, which has been reviewed in detail.[30,40,102] Perhaps the best examples are EGFR-overexpressing and ER-negative BCCs and A431 skin carcinoma cells with low or undetectable amounts of AnxA6, respectively, probably because of promoter methylation.[39,134] In addition, loss of large regions of chromosome 5q (5q31-q35), which carries the AnxA6 locus, is associated with ER-negative tumors carrying ErbB2 gene amplifications as well as myelodysplastic syndrome and risk of transformation to acute myelogenous leukemia (AML).[39,40,135] Thus, AnxA6 might display tumor suppressor activity only in the context of certain genetic lesions (e.g., EGFR levels) or susceptible cell types.

Conclusion

Even though it has been known for almost 2 decades that Ras mutations contribute to tumorigenesis in a large number of human cancers, the identification of strategies to selectively inhibit oncogenic Ras has remained one of the major challenges in cancer therapeutics. In addition, in a large number of cancers, Ras signaling is often upregulated because of an increased coupling to overexpressed or deregulated growth factor receptors. As pointed out above, the differential and cell type–specific activity and expression patterns of GAPs will modulate the contribution of each GAP in Ras inactivation. In addition, a complex cellular machinery of scaffold and adaptor proteins facilitates the targeting, assembly, and stabilization of Ras/GAP complexes in specific subcellular compartments. It is this cellular machinery that creates a cell-specific and locally restricted microenvironment to channel signaling information arriving from the extracellular milieu to the right location, thereby providing an efficient and accurate biological response. Mapping the association and composition of GAPs in Ras isoform–containing signaling modules at the plasma membrane and endomembranes in space and time will not only provide a better understanding of spatiotemporal Ras signaling but also hopefully identify new targets and therapeutic approaches aiming to downregulate Ras-GTP levels in cancer.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

1. Barbacid M. Ras genes. Ann Rev Biochem. 1987;56:799-827.
2. Bos JL. Ras oncogenes in human cancer: a review. Cancer Res. 1989;49:4682-9.
3. Downward J. Targeting ras signalling pathways in cancer therapy. Nat Rev Cancer. 2003;3:11-22.
4. Grewal T, Tebar F, Pol A, Enrich C. Involvement of targeting and scaffolding proteins in the regulation of the EGFR/Ras/MAPK pathway in oncogenesis. Curr Signal Trans Ther. 2006;1:147-67.
5. Buday L, Downward J. Many faces of Ras activation. Biochim Biophys Acta. 2008;1786:178-87.
6. Wellbrock C, Karasarides M, Marais R. The Raf proteins take centre stage. Nat Rev Mol Cell Biol. 2004;5:875-85.
7. Vetter IR, Wittinghofer A. The guanine-nucleotide-binding switch in three dimensions. Science. 2001;294:1299-304.
8. Bos JL, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. Cell. 2007;129:865-77.
9. Scheffzek K, Ahmadian MR, Kabsch W*, et al*. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. Science. 1997;277:333-8.
10. Ahmadian MR, Hoffmann U, Goody RS, Wittinghofer A. Individual rate constants for the interaction of Ras proteins with GTPase-activating proteins determined by fluorescence spectroscopy. Biochemistry. 1997;36:4535-41.
11. Ahmadian MR, Kiel C, Stege P, Scheffzek K. Structural fingerprints of the Ras-GTPase activating proteins neurofibromin and p120GAP. J Mol Biol. 2003;329:699-710.
12. Kupzig S, Deaconescu D, Bouyoucef D*, et al*. GAP1 family members constitute bifunctional Ras and Rap GTPase-activating proteins. J Biol Chem. 2006;281:9891-900.
13. Kupzig S, Bouyoucef-Cherchalli D, Yarwood S, Sessions R, Cullen PJ. The ability of GAP1^{IP4BP} to function as a Rap1 GTPase-activating protein (GAP) requires its Ras GAP-related domain and an arginine finger rather than an asparagine thumb. Mol Cell Biol. 2009;29:3929-40.
14. Yarwood S, Bouyoucef-Cherchalli D, Cullen PJ, Kupzig S. The GAP1 family of GTPase-activating proteins: spatial and temporal regulators of small GTPase signalling. Biochem Soc Trans. 2006;34:846-50.
15. Sot B, Koetting C, Deaconescu D, Suveyzdis Y, Gerwert K, Wittinghofer A. Unravelling the mechanism of dual-specificity GAPs. EMBO J. 2010;29:1205-14.
16. Koera K, Nakamura K, Nakao K*, et al*. K-ras is essential for the development of the mouse embryo. Oncogene. 1997;15:1151-9.
17. Esteban LM, Vicario-Abejon C, Fernandez-Salguero P*, et al*. Targeted genomic disruption of H-ras and N-ras, individually or in combination, reveals the dispensability of both loci for mouse growth and development. Mol Cell Biol. 2001;21:1444-52.
18. Plowman SJ, Williamson DJ, O'Sullivan MJ*, et al*. While K-ras is essential for mouse development, expression of the K-ras 4A splice variant is dispensable. Mol Cell Biol. 2003;23:9245-50.
19. Omerovic J, Laude AJ, Prior IA. Ras proteins: paradigms for compartmentalised and isoform-specific signalling. Cell Mol Life Sci. 2007;64:2575-89.
20. Schubbert S, Zenker M, Rowe SL*, et al*. Germline KRAS mutations cause Noonan syndrome. Nat Genet. 2006;38:331-6.
21. Kratz CP, Niemeyer CM, Zenker M. An unexpected new role of mutant Ras: perturbation of human embryonic development. J Mol Med. 2007;85:223-31.
22. Yan J, Roy S, Apolloni A, Lane A, Hancock JF. Ras isoforms vary in their ability to activate Raf-1 and phosphoinositide 3-kinase. J Biol Chem. 1998;273:24052-6.
23. Walsh AB, Bar-Sagi D. Differential activation of the Rac pathway by Ha-Ras and K-Ras. J Biol Chem. 2001;76:15609-15.
24. Cullen PJ, Lockyer PJ. Integration of calcium and ras signalling. Nat Rev Mol Cell Biol. 2002;3:339-48.
25. Donovan S, Shannon KM, Bollag G. GTPase activating proteins: critical regulators of intracellular signaling. Biochim Biophys Acta. 2001;87510:1-23.
26. Bernards A, Settleman J. GAPs in growth factor signalling. Growth Factors. 2005;23:143-9.
27. Le LQ, Parada LF. Tumor microenvironment and neurofibromatosis type I: connecting the GAPs. Oncogene. 2007;26:4609-16.
28. Dasgupta B, Gutmann DH. Neurofibromatosis 1: closing the GAP between mice and men. Curr Opin Gen Dev. 2003;13:20-7.
29. Pamonsinlapatham P, Hadj-Slimane R, Lepelletier Y*, et al*. P120-Ras GTPase activating protein (RasGAP): a multi-interacting protein in downstream signaling. Biochimie. 2009;91:320-8.
30. Grewal T, Enrich C. Molecular mechanisms involved in Ras inactivation: the annexin A6-p120GAP complex. Bioessays. 2008;28:1211-20.
31. Westbrook TF, Martin ES, Schlabach MR*, et al*. A genetic screen for candidate tumor suppressors identifies REST. Cell. 2005;121:837-48.
32. Jin H, Wang X, Ying Y*, et al*. Epigenetic silencing of a Ca2+-regulated Ras GTPase-activating protein RASAL defines a new mechanism of Ras activation in human cancers. Proc Natl Acad Sci U S A. 2007;104:12353-8.
33. Calvisi DF, Ladu S, Conner EA*, et al*. Inactivation of Ras GTPase-activating proteins promotes unrestrained activity of wild-type Ras in human liver cancer. J Hepatol. 2011;54:311-9.
34. Ohta M, Seto M, Ijichi H*, et al*. Decreased expression of the Ras-GTPase activating protein RASAL1 is associated with colorectal tumor progression. Gastroenterology. 2009;136:206-16.
35. Kolfschoten IG, van Leeuwen B, Berns K*, et al*. A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. Cell. 2005;121:849-58.
36. Qiu GH, Xie H, Wheelhouse N*, et al*. Differential expression of hDAB2IPA and hDAB2IPB in normal tissues and promoter methylation of hDAB2IPA in hepatocellular carcinoma. J Hepatol. 2007;46:655-63.
37. Boon LM, Mulliken JB, Vikkula M. RASA1: variable phenotype with capillary and arteriovenous malformations. Curr Opin Genet Dev. 2005;15:265-9.
38. Grewal T, Evans R, Rentero C*, et al*. Annexin A6 stimulates the membrane recruitment of p120GAP to modulate Ras and Raf-1 activity. Oncogene. 2005;24:5809-20.
39. de Muga SV, Timpson P, Cubells L*, et al*. Annexin A6 inhibits Ras signalling in EGF receptor overexpressing breast cancer cells. Oncogene. 2009;28:363-77.
40. Enrich C, Rentero C, de Muga SV*, et al*. Annexin A6-linking Ca(2+) signaling with cholesterol transport. Biochim Biophys Acta. Epub 2010 Oct 1.
41. Henis YI, Hancock JF, Prior IA. Ras acylation, compartmentalization and signaling nanoclusters. Mol Membr Biol. 2009;26:80-92.
42. Calvo F, Agudo-Ibáñez L, Crespo P. The Ras-Erk pathway: understanding site-specific signaling provides hope of new anti-tumor therapies. Bioessays. 2010;32:412-21.
43. Fehrenbacher N, Bar-Sagi D, Philips M. Ras/MAPK signaling from endomembranes. Mol Oncol. 2009;3:297-307.
44. Choy E, Chiu VK, Silletti J*, et al*. Endomembrane trafficking of Ras: the CAAX motif targets proteins to the ER and Golgi. Cell. 1999;98:69-80.
45. Roy S, Wyse B, Hancock JF. H-Ras signaling and K-Ras signaling are differentially dependent on endocytosis. Mol Cell Biol. 2002;22:5128-40.
46. Gomez GA, Daniotti JL. H-Ras dynamically interacts with recycling endosomes in CHO-K1 cells: involvement of Rab5 and Rab11 in the trafficking of H-Ras to this pericentriolar endocytic compartment. J Biol Chem. 2005;280:34997-5010.
47. Lu A, Tebar F, Alvarez-Moya B*, et al*. A clathrin-dependent pathway leads to KRas signaling on late endosomes en route to lysosomes. J Cell Biol. 2009;184:863-79.
48. Rebollo A, Perez-Sala D, Martinez AC. Bcl-2 differentially targets K-, N-, and H-Ras to mitochondria in IL-2 supplemented or deprived cells: implications in prevention of apoptosis. Oncogene. 1999;18:4930-9.
49. Wolfman JC, Wolfman A. Endogenous c-N-Ras provides a steady-state anti-apoptotic signal. J Biol Chem. 2000;275:19315-23.
50. Matallanas D, Arozarena I, Berciano MT*, et al*. Differences in the inhibitory specificities of H-Ras, K-Ras and N-Ras (N17) dominant

negative mutants are related to their membrane microlocalization. J Biol Chem. 2003;278: 4572-81.
51. Caloca MJ, Zugaza JL, Bustelo XR. Exchange factors of the RasGRP family mediate Ras activation in the Golgi. J Biol Chem. 2003;278: 33465-73.
52. Bivona TG, Perez De Castro I, Ahearn IM, *et al*. Phospholipase Cgamma activates Ras on the Golgi apparatus by means of RasGRP1. Nature. 2003;424:694-8.
53. Arozarena I, Matallanas D, Berciano MT, *et al*. Activation of H-Ras in the endoplasmic reticulum by the RasGRF family guanine nucleotide exchange factors. Mol Cell Biol. 2004;24:1516-30.
54. Murakoshi H, Iino R, Kobayashi T, *et al*. Single molecule imaging analysis of Ras activation in living cells. Proc Natl Acad Sci U S A. 2004;101:7317-22.
55. Kolch W, Calder M, Gilbert D. When kinases meet mathematics: the systems biology of MAPK signalling. FEBS Lett. 2005;579:1891-5.
56. Raabe T, Rapp UR. Ras signaling: PP2A puts Ksr and Raf in the right place. Curr Biol. 2003;13:R635-7.
57. Li C, Cheng Y, Gutmann DA, Mangoura D. Differential localization of neurofibromatosis (NF1) gene product, neurofibromin, with the F-actin or microtubule cytoskeleton during differentiation of telencephalic neurons. Dev Brain Res. 2001;130:231-48.
58. Roudebush M, Slabe T, Sundaram V, Hoppel CL, Golubic M, Stacey DW. Neurofibromin colocalizes with mitochondria in cultured cells. Exp Cell Res. 1997;236:161-72.
59. Nordlund M, Gu X, Shipley MT, Ratner N. Neurofibromin is enriched in the endoplasmic reticulum of CNS neurons. J Neurosci. 1993;13:1588-600.
60. Wang Z, Tung PS, Moran MF. Association of p120 ras GAP with endocytic components and colocalization with epidermal growth factor (EGF) receptor in response to EGF stimulation. Cell Growth Differ. 1996;7:123-33.
61. Boyanapalli M, Lahoud OB, Mesian L, *et al*. Neurofibromin binds to caveolin-1 and regulates ras, FAK, and Akt. Biochem Biophys Res Commun. 2006;340:1200-8.
62. Delint-Ramirez I, Fernández E, Bayés A, Kicsi E, Komiyama NH, Grant SG. In vivo composition of NMDA receptor signaling complexes differs between membrane subdomains and is modulated by PSD-95 and PSD-93. J Neurosci. 2010;30:8162-70.
63. Huang JW, Chen CL, Chuang NN. P120-GAP associated with syndecan-2 to function as an active switch signal for Src upon transformation with oncogenic ras. Biochem Biophys Res Commun. 2005;329:855-62.
64. Koehler JA, Moran MF. Regulation of extracellular signal-regulated kinase activity by p120 RasGAP does not involve its pleckstrin homology or calcium-dependent lipid binding domains but does require these domains to regulate cell proliferation. Cell Growth Differ. 2001;12:551-61.
65. Koehler JA, Moran MF. RACK1, a protein kinase C scaffolding protein, interacts with the PH domain of p120GAP. Biochem Biophys Res Commun. 2001;283:888-95.
66. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007;7:295-308.
67. Vandenbroucke I, Van Oostveldt P, Coene E, De Paepe A, Messiaen L. Neurofibromin is actively transported to the nucleus. FEBS Lett. 2004;560:98-102.
68. D'Angelo I, Welti S, Bonneau F, Scheffzek K. A novel bipartite phospholipid-binding module in the neurofibromatosis type 1 protein. EMBO Rep. 2006;7:174-9.
69. Mousley CJ, Tyeryar KR, Ryan MM, Bankaitis VA. Sec14p-like proteins regulate phosphoinositide homeostasis and intracellular protein and lipid trafficking in yeast. Biochem Soc Trans. 2006;34:346-50.
70. Welti S, Fraterman S, D'Angelo I, Wilm M, Scheffzek K. The Sec14 homology module of neurofibromin binds cellular glycerophospholipids: mass spectroscopy and structure of a lipid complex. J Mol Med. 2007;366:551-62.
71. Izawa I, Tamaki N, Saya H. Phosphorylation of neurofibromatosis type 1 gene product (neurofibromin) by cAMP-dependent protein kinase. FEBS Lett. 1996;382:53-9.
72. Feng L, Yunoue S, Tokuo H, *et al*. PKA phosphorylation and 14-3-3 interaction regulate the function of the neurofibromatosis type 1 tumor suppressor neurofibromin. FEBS Lett. 2004;557:275-82.
73. Mangoura D, Sun Y, Li C, *et al*. Phosphorylation of neurofibromin by PKC is a possible molecular switch in EGF receptor signaling in neural cells. Oncogene. 2006;25:735-45.
74. Leondaritis G, Petrikkos L, Mangoura D. Regulation of the Ras-GTPase activating protein neurofibromin by C-tail phosphorylation: implications for protein kinase C/Ras/extracellular signal-regulated kinase 1/2 pathway signaling and neuronal differentiation. J Neurochem. 2009;109:573-83.
75. Plowman SJ, Muncke C, Parton RG, Hancock JF. H-ras, K-ras, and inner plasma membrane raft proteins operate in nanoclusters with differential dependence on the actin cytoskeleton. Proc Natl Acad Sci U S A. 2005;102:15500-5.
76. Cichowski K, Santiago S, Jardim M, Johnson BW, Jacks T. Dynamic regulation of the Ras pathway via proteolysis of the NF1 tumor suppressor. Genes Dev. 2003;17:449-54.
77. Phan VT, Ding VW, Li F, Chalkley RJ, Burlingame A, McCormick F. The RasGAP proteins Ira2 and neurofibromin are negatively regulated by Gpb1 in yeast and ETEA in humans. Mol Cell Biol. 2010;30:2264-79.
78. Kaufmann D, Junge I, Bartelt B, Lattke H, Müller R. On the lysosomal degradation of neurofibromin and its phosphorylation in cultured melanocytes. Biol Chem. 1999;380:1071-8.
79. Steinberg SF. Structural basis of protein kinase C isoform function. Physiol Rev. 2008;88:1341-78.
80. Kheifets V, Mochly-Rosen D. Insight into intra- and inter-molecular interactions of PKC: design of specific modulators of kinase function. Pharmacol Res. 2007;55:467-76.
81. Kirschner LS, Yin Z, Jones GN, Mahoney E. Mouse models of altered protein kinase A signaling. Endocr Relat Cancer. 2009;16:773-93.
82. Taylor SS, Kim C, Cheng CY, Brown SH, Wu J, Kannan N. Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design. Biochim Biophys Acta. 2008;1784:16-26.
83. Mulay V, Wood P, Rentero C, Enrich C, Grewal T. Signal transduction pathways provide opportunities to enhance HDL and apoAI-dependent reverse cholesterol transport. Curr Pharm Biotech. In press.
84. Cooper JA, Kashishian A. In vivo binding properties of SH2 domains from GTPase-activating protein and phosphatidylinositol 3-kinase. Mol Cell Biol. 1993;3:1737-45.
85. Kazlauskas A, Ellis C, Pawson T, Cooper JA. Binding of GAP to activated PDGF receptors. Science. 1990;247:1578-81.
86. Jones RB, Gordus A, Krall JA, MacBeath G. A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature. 2006;439:168-74.
87. Moran MF, Polakis P, McCormick F, Pawson T, Ellis C. Protein-tyrosine kinases regulate the phosphorylation, protein interactions, subcellular distribution, and activity of p21ras GTPase-activating protein. Mol Cell Biol. 1991;11:1804-12.
88. Ellis C, Measday V, Moran MF. Phosphorylation-dependent complexes of p120 Ras-specific GTPase-activating protein with p62 and p190. Methods Enzymol. 1995;255:179-92.
89. Zhao M, Janas JA, Niki M, Pandolfi PP, Van Aelst L. Dok-1 independently attenuates Ras/mitogen-activated protein kinase and Src/c-myc pathways to inhibit platelet-derived growth factor-induced mitogenesis. Mol Cell Biol. 2006;26:2479-89.
90. Carpino N, Wisniewski D, Strife A, *et al*. p62(dok): a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. Cell. 1997;88:197-204.
91. Yamanashi Y, Baltimore D. Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. Cell. 1997;88:205-11.
92. van der Geer P, Henkemeyer M, Jacks T, Pawson T. 1997. Aberrant Ras regulation and reduced p190 tyrosine phosphorylation in cells lacking p120-Gap. Mol Cell Biol. 1997;17:1840-7.
93. Kulkarni SV, Gish G, van der Geer P, Henkemeyer M, Pawson T. Role of p120 Ras-GAP in directed cell movement. J Cell Biol. 2000;149:457-70.
94. Endo M, Yamashita T. Inactivation of Ras by p120GAP via focal adhesion kinase dephosphorylation mediates RGMa-induced growth cone collapse. J Neurosci. 2009;29:6649-62.
95. Pamonsinlapatham P, Gril B, Dufour S, *et al*. Capns1, a new binding partner of RasGAP-SH3 domain in K-Ras(V12) oncogenic cells: modulation of cell survival and migration. Cell Signal. 2008;20:2119-26.
96. Leblanc V, Tocque B, Delumeau I. Ras-GAP controls Rho-mediated cytoskeletal reorganization through its SH3 domain. Mol Cell Biol. 1998;18:5567-78.
97. Lemmon MA. Pleckstrin homology domains: two halves make a hole? Cell. 2005;120:574-6.
98. Drugan JK, Rogers-Graham K, Gilmer T, Campbell S, Clark GJ. The Ras/p120 GTPase-activating protein (GAP) interaction is regulated by the p120 GAP pleckstrin homology domain. J Biol Chem. 2000;275:35021-7.
99. Downward J, Graves JD, Warne PH, Rayter S, Cantrell DA. Stimulation of p21ras upon T-cell activation. Nature. 1990;346:719-23.

100. Mineo C, Ying YS, Chapline C, Jaken S, Anderson RG. Targeting of protein kinase Calpha to caveolae. J Cell Biol. 1998;141:601-10.
101. Davis AJ, Butt JT, Walker JH, Moss SE, Gawler DJ. The Ca2+-dependent lipid binding domain of p120GAP mediates protein-protein interactions with Ca2+-dependent membrane-binding proteins: evidence for a direct interaction between annexin 6 and p120GAP. J Biol Chem. 1996;271:24333-6.
102. Grewal T, Enrich C. Annexins: modulators of EGF receptor signalling and trafficking. Cell Signal. 2009;21:847-58.
103. Theobald J, Hanby A, Patel K, Moss SE. Annexin VI has tumour-suppressor activity in human A431 squamous epithelial carcinoma cells. Br J Cancer. 1995;71:786-8.
104. Sermon BA, Eccleston JF, Skinner RH, Lowe PN. Mechanism of inhibition by arachidonic acid of the catalytic activity of Ras GTPase-activating proteins. J Biol Chem. 1996;271:1566-72.
105. Han JW, McCormick F, Macara IG. Regulation of Ras-GAP and the neurofibromatosis-1 gene product by eicosanoids. Science. 1991;252:576-9.
106. Cubells L, de Muga SV, Tebar F*, et al*. Annexin A6-induced inhibition of cytoplasmic phospholipase A2 is linked to caveolin-1 export from the Golgi. J Biol Chem. 2008;283:10174-83.
107. Rentero C, Evans R, Wood P*, et al*. Inhibition of H-Ras and MAPK is compensated by PKC-dependent pathways in annexin A6 expressing cells. Cell Signal. 2006;18:1006-16.
108. Villalonga P, López-Alcalá C, Chiloeches A*, et al*. Calmodulin prevents activation of Ras by PKC in 3T3 fibroblasts. J Biol Chem. 2002;277:37929-35.
109. Alvarez-Moya B, López-Alcalá C, Drosten M, Bachs O, Agell N. K-Ras4B phosphorylation at Ser181 is inhibited by calmodulin and modulates K-Ras activity and function. Oncogene. 2010;29:5911-22.
110. Cubells L, de Muga SV, Tebar F*, et al*. Annexin A6-induced alterations in cholesterol transport and caveolin export from the Golgi complex. Traffic. 2007;8:1568-89.
111. Monastyrskaya K, Babiychuk EB, Hostettler A, Wood P, Grewal T, Draeger A. Plasma membrane-associated annexin A6 reduces Ca2+ entry by stabilizing the cortical actin cytoskeleton. J Biol Chem. 2009;284:17227-42.
112. Grewal T, Koese M, Rentero C, Enrich C. Annexin A6-regulator of the EGFR/Ras signalling pathway and cholesterol homeostasis. Int J Biochem Cell Biol. 2010;42:580-4.
113. Cozier GE, Lockyer PJ, Reynolds JS*, et al*. GAP1IP4BP contains a novel group I pleckstrin homology domain that directs constitutive plasma membrane association. J Biol Chem. 2000;275:28261-8.
114. Lockyer PJ, Bottomley JR, Reynolds JS*, et al*. Distinct subcellular localisations of the putative inositol 1,3,4,5-tetrakisphosphate receptors GAP1IP4BP and GAP1m result from the GAP1IP4BP PH domain directing plasma membrane targeting. Curr Biol. 1997;7:1007-10.
115. Lockyer PJ, Wennstroem S, Kupzig S*, et al*. Identification of the ras GTPase-activating protein GAP1(m) as a phosphatidylinositol-3,4,5-trisphosphate-binding protein in vivo. Curr Biol. 1999;9:265-8.
116. Cozier GE, Bouyoucef D, Cullen PJ. Engineering the phosphoinositide-binding profile of a class I pleckstrin homology domain. J Biol Chem. 2003;278:39489-96.
117. Nafisi H, Banihashemi B, Daigle M, Albert PR. GAP1(IP4BP)/RASA3 mediates Galphai-induced inhibition of mitogen-activated protein kinase. J Biol Chem. 2008;283:35908-17.
118. Krapivinsky G, Medina I, Krapivinsky L, Gapon S, Clapham DE. SynGAP-MUPP1-CaMKII synaptic complexes regulate p38 MAP kinase activity and NMDA receptor-dependent synaptic AMPA receptor potentiation. Neuron. 2004;43:563-74.
119. Cook SJ, Lockyer PJ. Recent advances in Ca(2+)-dependent Ras regulation and cell proliferation. Cell Calcium. 2006;39:101-12.
120. Cullen PJ. Decoding complex Ca2+ signals through the modulation of Ras signaling. Curr Opin Cell Biol. 2006;18:157-61.
121. Lockyer PJ, Kupzig S, Cullen PJ. CAPRI regulates Ca(2+)-dependent inactivation of the Ras-MAPK pathway. Curr Biol. 2001;11:981-6.
122. Walker SA, Kupzig S, Bouyoucef D, Davies LC, Tsuboi T. Identification of a Ras GTPase-activating protein regulated by receptor-mediated Ca2+-oscillations. EMBO J. 2004;23:1749-60.
123. Lio Q, Walker SA, Gao DC*, et al*. CAPRI and RASAL impose different modes of information processing on Ras due to contrasting temporal filtering of Ca2+. J Cell Biol. 2005;170:183-90.
124. Zhang J, Guo J, Dzhagalov I, He Y-W. An essential function for the Ca2+-promoted Ras activator in Fcγ-receptor-mediated phagocytosis. Nat Immunol. 2005;6:911-9.
125. Briggs MW, Sacks DB. IQGAP proteins are integral components of cytoskeletal regulation. EMBO Rep. 2003;4:571-4.
126. Allen M, Chu S, Brill S*, et al*. Restricted tissue expression pattern of a novel human rasGAP-related gene and its murine ortholog. Gene. 1998;218:17-25.
127. Gao W, Kondo Y, Shen L*, et al*. Variable DNA methylation patterns associated with progression of disease in hepatocellular carcinomas. Carcinogenesis. 2008;29:1901-10.
128. Chen H, Tooka S, Gazdar AF, Hsieh JT. Epigenetic regulation of a novel tumor suppressor gene (hDAB2IP) in prostate cancer cell lines. J Biol Chem. 2003;278:3121-30.
129. Dote H, Tooka S, Tsukuda K*, et al*. Aberrant promoter methylation in human DAB2 interactive protein (hDAB2IP) gene in breast cancer. Clin Cancer Res. 2004;10:2082-99.
130. Yano M, Toyooka S, Tsukuda K*, et al*. Aberrant promoter methylation of human DAB2 interactive protein (hDAB2IP) gene in lung cancers. Int J Cancer. 2005;113:59-66.
131. Dote H, Tooka S, Tsukuda K*, et al*. Aberrant promoter methylation in human DAB2 interactive protein (hDAB2IP) gene in gastrointestinal tumour. Br J Cancer. 2005;92:1117-25.
132. Chen H, Tu SW, Hsieh JT. Down-regulation of human DAB2IP gene expression mediated by polycomb Ezh2 complex and histone deacetylase in prostate cancer. J Biol Chem. 2005;280:22437-44.
133. Duggan D, Zheng SL, Knowlton M*, et al*. Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. J Natl Cancer Inst. 2007;46:655-63.
134. Smythe E, Smith PD, Jacob SM, Theobald J, Moss SE. Endocytosis occurs independently of annexin VI in human A431 cells. J Cell Biol. 1994;124:301-6.
135. Boultwood J, Fidler C, Strickson AJ*, et al*. Narrowing and genomic annotation of the commonly deleted region of the 5q- syndrome. Blood. 2002;99:4638-41.