The Tyrosine Kinase Pyk2 Regulates Arf1 Activity by Phosphorylation and Inhibition of the Arf-GTPase-activating Protein ASAP1*

Received for publication, March 5, 2003, and in revised form, May 8, 2003
Published, JBC Papers in Press, May 27, 2003, DOI 10.1074/jbc.M302278200

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Pyk2 and FAK1 comprise a distinct family of non-receptor protein tyrosine kinases that are involved in transmission of extracellular signals to intracellular kinase cascades and regulation of diverse cellular responses such as adhesion, proliferation, differentiation, and apoptosis (1). Pyk2 and FAK share about 45% amino acid identity and a similar domain structure: a unique N terminus containing a FERM (band 4.1/ezrin/radixin/moesin) domain, a central protein tyrosine kinase domain, two proline-rich sequences, and an FAT (focal adhesion targeting) domain in the C terminus. FAK is found in almost all tissues, whereas Pyk2 is mainly expressed in neuronal and hematopoietic cells. Whereas FAK is localized to focal adhesion sites in adherent cells, Pyk2 is rather diffused throughout the cytoplasm and concentrated in perinuclear regions (2). Both kinases can be regulated by integrins, growth factor receptors, and G-protein-coupled receptors. However, Pyk2 is unique in the way that its activation is triggered by intracellular calcium elevation and/or protein kinase C (3). Together with Src that binds to autophosphorylated tyrosine 402 of Pyk2, it can function upstream of small G-proteins, such as Ras, Rac, and Rho, linking different transmembrane receptors with mitogen-activated protein kinase cascades (1, 3, 4).

ADP-ribosylation factor (Arf) proteins constitute another family of small GTPases that were originally identified as co-factors required for the cholera toxin-catalyzed ADP-ribosylation of Gs subunits (5). Arf1, the best characterized mammalian Arf, recruits coat proteins to membranes of the Golgi apparatus and has been implicated in intra-Golgi and Golgi-to-endoplasmic reticulum transport, endosome function, and synaptic vesicle formation (6–8). However, Arf GTPases are also involved in recruitment of paxillin to focal adhesions and remodeling of the cytoskeleton as cells change shape or move (9). Arfs are distinct from other small GTPases in their strictly GTP-dependent recruitment to membranes and in the virtual absence of any intrinsic GTPase activity. Therefore, guanine-nucleotide exchange factors and GTPase-activating proteins (GAPs) are necessary for the completion of the full GTP-GDP cycle of Arfs (10, 11).

A number of Arf-GAPs have been identified of which some are co-regulated by acidic lipids and phosphoinositides. One of those Arf-GAPs is ASAP1 (Arf-GAP containing SH3, ankyrin repeats and PH domain) that binds to Src and displays phospholipid-dependent activity toward Arf1 and Arf5. Upon binding, Src can phosphorylate ASAP1, but the functional consequence of this phosphorylation has remained unclear (12). More recently, FAK has also been shown to interact with ASAP1, and an involvement of ASAP1 in cell spreading and focal adhesion localization of FAK as well as paxillin has been suggested (13). However, FAK does not phosphorylate ASAP1 nor modulate its activity. Pyk2 can also interact with an Arf-GAP called PAPs (Pyk2 C-terminal associated protein), but again the functional consequence of this interaction has remained elusive (14).

We have identified ASAP1 as a new binding partner of Pyk2.

Proline-rich tyrosine kinase 2 (Pyk2), a non-receptor tyrosine kinase structurally related to focal adhesion kinase, has been implicated in the regulation of mitogen-activated protein kinase cascades and ion channels, the induction of apoptosis, and in the modulation of the cytoskeleton. In order to understand how Pyk2 signaling mediates these diverse cellular functions, we performed a yeast two-hybrid screening using the C-terminal part of Pyk2 that contains potential protein-protein interaction sites as bait. A prominent binder of Pyk2 identified by this method was the Arf-GTPase-activating protein ASAP1. Pyk2-ASAP1 interaction was confirmed in pull-down as well as in co-immunoprecipitation experiments, and contact sites were mapped to the proline-rich regions of Pyk2 and the SH3 domain of ASAP1. Pyk2 directly phosphorylates ASAP1 on tyrosine residues in vitro and increases ASAP1 tyrosine phosphorylation when co-expressed in HEK293T cells. Phosphorylation of tyrosine 308 and 782 affects the phosphoinositide binding profile of ASAP1, and fluorimetric Arf-GTPase assays with purified proteins revealed an inhibition of ASAP1 GTPase-activating protein activity by Pyk2-mediated tyrosine phosphorylation. We therefore provide evidence for a functional interaction between Pyk2 and ASAP1 and a regulation of ASAP1 and hence Arf1 activity by Pyk2-mediated tyrosine phosphorylation.

* This work was supported by grants from the Deutsche Forschungsgemeinschaft (to A. K.-L. and A. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: FAK, focal adhesion kinase; GAPs, GTPase-activating proteins; FAT, focal adhesion targeting; PH, pleckstrin homology; SH, Src homology; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; GTPases, guanosine 5′-O-(thio)triphosphate; aa, amino acids; DMEM, Dulbecco’s modified Eagle’s medium; PMA, phorbol 12-myristate 13-acetate; GST, glutathione S-transferase; PS, phosphatidylinositol; PI, phosphatidylinositol; Arf, ADP-ribosylation factor; MEF, mouse embryonic fibroblasts.
and mapped the interaction to the SH3 domain of ASAP1 and the proline-rich regions of Pyk2. Furthermore, we could demonstrate a direct phosphorylation of ASAP1 by Pyk2 and a subsequent regulation of the ASAP1 GTPase activity. We therefore suggest a role of Pyk2 in regulating Arf function and potentially vesicular trafficking. Our results provide a first molecular mechanism how tyrosine kinases can modulate signaling of small Arf GTPases.

**EXPERIMENTAL PROCEDURES**

**Materials**—[γ-<sup>32</sup>P]ATP (110 TBq/mmol), [γ<sup>32</sup>P]orthophosphate (360 MBq/mg), and GST-Sepharose were from Amersham Biosciences; phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>) was from Calbiochem, and other lipids were from Avanti Polar Lipids; Bradykinin was from Bachem; aprotinin (TrasyloL<sup>®</sup>) was from Bayer; PMA was from Calbiochem; lipid arrays were from Echelon Research Laboratories; Pefabloc<sup>®</sup>, Schleicher & Schuell; Epidermal growth factor, GTP, GTP<sup>s</sup>, anti-hemagglutinin (a-hemagglutinin) monoclonal antibody (sc-11539), anti-phosphotyrosine (PY99, sc-7020) monoclonal antibody, and phatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>) was from Calbiochem, and 3T3-L1 adipocytes were from ICN, Merck, and Sigma.

**In Vitro Binding Assays**—GST fusion proteins were expressed in *E. coli* DH<sub>5</sub>a and purified using glutathione-Sepharose beads according to the manufacturer’s instructions (Amersham Biosciences). Equal amounts of GST fusion proteins or GST alone (about 5 μg) were incubated with 200–μl cell lysates from transfected HEK293T cells (100–200 μg) at 4°C for 3 h on a rotating platform. Beads were washed three times with lysis buffer, and associated proteins were released with SDS sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 25 mM dithiothreitol, 30% glycerol, 0.02% bromphenol blue) and 5 min of incubation at 98°C. Samples were subjected to SDS-PAGE and analyzed by Western blotting with a Pyk2-specific polycional antibody (antibody 684 (3)) or an anti-FLAG monoclonal antibody to detect FLAG-tagged ASAP1 constructs.

**Immunoprecipitation and Western Blotting**—Equal amounts of lysates (100–200 μg) were subjected to immunoprecipitation at 4°C for 3 h on a rotating platform by using anti-Pyk2 (antibody 598 (3)) or anti-FLAG antibodies. Immune complexes were isolated using protein A-Sepharose or primary antibodies pre-coupled to agarose beads. Beads were washed three times with lysis buffer, and proteins were released with SDS sample buffer and 5 min of incubation at 98°C. Samples were subjected to SDS-PAGE and analyzed by Western blotting with indicated antibodies.

**Two-dimensional Phosphopeptide Mapping**—FLAG-tagged ASAP1 was subjected to immunoprecipitation, and in vitro kinase reactions were performed in 10 mM Tris, pH 7.5, 10 mM Mg<sub>Cl</sub><sub>2</sub>, 100 μM ATP including 5 μCi of [γ-<sup>32</sup>P]ATP at 30°C for 30 min. Reactions were stopped by addition of SDS sample buffer and incubation at 98°C for 5 min. In vitro <sup>32</sup>P labeling was performed using 1 μCi/ml in phosphate-free DMEM for 6 h as detailed previously (19). Radiolabeled FLAG-ASAP1 was isolated by immunoprecipitation and processed identically to in vitro labeled species. Proteins were separated by 8% SDS-PAGE, transferred to nitrocellulose, and analyzed using a PhosphorImager (Molecular Image FX Pro, Bio-Rad). Two-dimensional phosphopeptide mapping was performed as described previously (19). Briefly, 3<sup>2</sup>–<sup>3</sup>–<sup>3</sup>–labeled ASAP1 bands were identified and in situ subjected to tryptic digestion. Phosphoamino acid acid composition was determined, and positions of phosphorylated residues were determined by Edman sequencing. By using this information, phosphorylation sites were predicted and confirmed by site-directed mutagenesis and two-dimensional phosphopeptide mapping of mutants.

**Affinity-purified FLAG-ASAP1**—Frat1 was expressed in *E. coli* BL21 (DE3) together with N-myristoyltransferase and purified in the myristoylated form in three steps as follows: (i) precipitation with 35% ammonium sulfate saturation; (ii) DEAE-Sepharose chromatography; and (iii) phosphopeptide mapping. FLAG-ASAP1 was then subjected to affinity-purified anti-FLAG M2 affinity matrix following the manufacturer’s instructions (Sigma) (20). Phosphorylation states were determined, and positions of phosphorylated residues were determined by Edman sequencing. By using this information, phosphorylation sites were predicted and confirmed by site-directed mutagenesis and two-dimensional phosphopeptide mapping of mutants.
Identification of ASAP1 SH3 domain as an interaction partner of PRNK in a yeast two-hybrid screening. A, schematic representation of Pyk2 and ASAP1 proteins. Pyk2 is composed of an N-terminal FERM domain, a central kinase domain, two proline-rich regions, and a C-terminal FAT domain. ASAP1 consists of a PH domain followed by an Arf GAP domain, three ankyrin repeats, three proline-rich sequences, and an SH3 domain. The C-terminal fragment of Pyk2 (PRNK) that was used as bait in yeast two-hybrid screening and the ASAP1 SH3 domain identified as a PRNK-interacting sequence are framed with broken lines. B, yeasts were co-transformed with different combinations of bait vectors containing LexA, LexA-PRNK, or LexA-LEF and prey vectors containing VP16, VP16-ASAP1 SH3, or VP16-β-catenin (LEF-interacting protein). Liquid cultures of co-transformants were assayed for β-galactosidase activity (in arbitrary units; AU) with o-nitrophenyl β-D-galactopyranoside as a substrate. The absorbance measured at 410 nm indicates β-galactosidase activity and correlates with the strength of the protein-protein interaction. Yeast transformed with LexA-LEF and VP16-β-catenin served as positive control, whereas transformants containing non-interacting LexA-PRNK and VP16-β-catenin fusion proteins were used as a negative control.

RESULTS

Identification of ASAP1 as an Interaction Partner of Pyk2—In order to improve understanding of how Pyk2 mediates its diverse cellular functions, we performed a yeast two-hybrid screening using the C-terminal part of Pyk2 (PRNK) that contains several potential protein-protein interaction sites as bait (Fig. 1A). We screened 3 × 10⁶ clones of an embryonic mouse cDNA library using the LexA system (17) and identified 30 clones that were positive in growth and filter as well as liquid β-galactosidase assays. Three independent clones encoded cDNAs covering the SH3 domain of ASAP1, a phospholipid-dependent GTPase-activating protein for small Arf GTPases. ASAP1 consist of a PH domain followed by an Arf GAP domain, three ankyrin repeats, three proline-rich sequences, eight E/DLPKKP repeats, and an SH3 domain (Fig. 1A). It is a 130-kDa protein that is expressed in many tissues but is most abundant in testis, brain, lung, and spleen (12). Activation of ASAP1 involves PI(4,5)P₂ binding to the PH domain and may be implicated in regulating the actin cytoskeleton (24, 25).

The Pyk2-ASAP1 interaction and its specificity were confirmed by re-transformation of the empty library vector, ASAP1 SH3 domain, and a β-catenin in LexA, LexA-PRNK, as well as in LexA-LEF yeast, subsequent growth (Fig. 1B), and β-galactosidase assay (Fig. 1C). The β-catenin-LEF interaction (26) and a C-terminal FAK fragment (FRNK, FAK-related non-kinase) that has been shown previously (13) to interact with ASAP1 SH3 domain served as positive controls. Only the PRNK-ASAP1 SH3 domain and positive controls resulted in growth of yeast and expression of β-galactosidase (Fig. 1, B and C). Altogether, our yeast two-hybrid data suggest a specific and tight interaction between the C-terminal Pyk2 fragment PRNK and the ASAP1 SH3 domain.

In Vitro binding of PRNK and Pyk2 to the SH3 Domain of ASAP1—To confirm the Pyk2-ASAP1 interaction found in the yeast two-hybrid screen and to more precisely map domains...
that mediate binding, we expressed Pyk2, PRNK, a mutant form PRNK-P859A in which the proline-rich region is disturbed, and the SH3 domain of ASAP1 as GST fusion proteins in *E. coli*, purified them on glutathione-Sepharose, and used these baits to pull down mammalian proteins expressed in HEK293T cells. In support of our yeast two-hybrid data, the GST-PRNK fusion protein bound to full-length ASAP1 expressed in HEK293T cells, whereas interaction was abrogated when proline 859 in GST-PRNK was mutated to alanine or GST alone was used as bait (Fig. 2A). These results were confirmed in a reverse experiment using a GST fusion protein of the ASAP1 SH3 domain that bound to full-length Pyk2 and PRNK but not to PRNK-P859A expressed in HEK293T cells (Fig. 2B). Comparable levels of the different GST baits were used for pull downs as demonstrated by Ponceau S staining of nitrocellulose membranes before proceeding with Western blots (Fig. 2A and B, lower panel). Furthermore, expression of corresponding binding proteins in HEK293T cells was confirmed by Western blotting using either anti-FLAG or anti-Pyk2/PRNK antibodies (Fig. 2A, upper panel, and B, middle panel). In summary, these data from *in vitro* interaction assays suggest specific binding of ASAP1 to proline-rich sequences in Pyk2 surrounding proline 859.

SH3 domain-mediated interactions are frequently of moderate affinity and specificity (27–29). We therefore sought to test the specificity of the ASAP1-Pyk2 interaction by using a panel of different SH3 domains fused to GST in pull-down assays with lysates from PRNK and Pyk2 expressing HEK293T cells. GST alone served as a negative control, and GST-Grb2 that can bind to Pyk2 via multiple interactions was used as a positive control (4). Comparable levels of the different GST baits were applied for the pull down as demonstrated by Ponceau S staining of nitrocellulose membranes before proceeding with Western blots (Fig. 3, lower panels) and by probing membranes with an anti-GST antibody (not shown). In this setting, PRNK and Pyk2 did bind only to Grb2 and ASAP1 baits but not to the SH3 domains of Ras-GAP, Abl, Src, phospholipase-Cγ, p85, and Crk suggesting a high degree of specificity of the SH3 domain-mediated association of ASAP1 and Pyk2 (Fig. 3, upper panel).

**Co-immunoprecipitation of Pyk2 and ASAP1**—To confirm the interaction of full-length proteins in living cells and to map further the contact sites we used, HEK293T cells were co-transfected with different FLAG-ASAP1 and Pyk2 constructs. Isolation of ASAP1 by immunoprecipitation and Western blotting with anti-Pyk2 antibodies revealed binding of full-length FLAG-ASAP1 to Pyk2 in HEK293T cells (Fig. 4A, left upper panel). No corresponding band was seen when Pyk2 cDNA was omitted from the transfections and when an ASAP1 construct lacking the SH3 domain (ASAP1-ΔSH3) was co-expressed with Pyk2. In a reverse experiment, FLAG-ASAP1 but not FLAG-ASAP1-ΔSH3 could be detected in Pyk2 immunoprecipitates from co-transfected cells (Fig. 4A, right upper panel). Immunoprecipitation of equal amounts of Pyk2 and ASAP1 (Fig. 4A, lower panels) and expression levels of Pyk2, ASAP1, and ASAP1-ΔSH3 in HEK293T cells (Fig. 4B) were verified by Western blotting using anti-Pyk2 or anti-FLAG antibodies.

For a more precise mapping of the region in Pyk2 that interacts with the ASAP1 SH3 domain, we disrupted both proline-rich regions of Pyk2 individually or in combination by site-directed mutagenesis. Co-immunoprecipitation experiments either with anti-FLAG or with anti-Pyk2 antibodies revealed a moderate reduction of ASAP1 binding to Pyk2-P859A where the binding motif of the second proline-rich region was mutated (Fig. 4C, upper panels). A more dramatic effect was observed upon disruption of the first proline-rich domain of Pyk2 (Pyk2-P717A), and binding of ASAP1 was
completely abolished in the corresponding double mutant. Quality of the immunoprecipitation was controlled by re-probing blots with antibodies against bait proteins (Fig. 4C, lower panels), and comparable quantities of FLAG-ASAP1 and Pyk2 proteins in cell lysates were demonstrated by Western blotting using corresponding antibodies (Fig. 4D). These experiments demonstrate binding of ASAP1 via its SH3 domain to the second and more effectively to the first proline-rich domain of Pyk2. To validate our co-immunoprecipitation strategy, we also reproduced the previously described interactions between ASAP1 and the tyrosine kinases Src and FAK (Refs. 12 and 13 and data not shown).

To verify that Pyk2 and ASAP1 can also interact when expressed at endogenous levels, we performed co-immunoprecipitations with lysates from PC12 cells and mouse embryonic fibroblasts (MEF). Immunoprecipitation of Pyk2 and subsequent Western blotting with anti-ASAP1 antibodies revealed an association of Pyk2 and ASAP1 in both cell types (Fig. 5A and B, upper panels). Pyk2-ASAP1 complex formation seemed to be constitutive because it did not change upon activation of Pyk2 by stimulation of cells with either bradykinin or the phorbol ester PMA. As a control, we used an antibody against an unrelated cytosolic kinase (extracellular signal-regulated kinase) that did not co-precipitate ASAP1 (Fig. 5, control IP).

Equal levels of Pyk2 immunoprecipitation and expression of Pyk2 and ASAP1 in PC12 and MEF cells were shown by Western blotting (Fig. 5, A and B). Unfortunately, commercially available anti-ASAP1 antibodies...
performed poorly in immunoprecipitations preventing the reciprocal experiment.

In summary, our co-immunoprecipitation studies confirmed a specific complex formation between full-length Pyk2 and ASAP1 in mammalian cells and mapped interaction sites to the SH3 domain of ASAP1 and primarily to the first and secondarily to the second proline-rich region of Pyk2.

**Tyrosine Phosphorylation of ASAP1 by Pyk2**—As Pyk2 is a tyrosine kinase, we investigated whether ASAP1 could be a substrate for Pyk2-mediated tyrosine phosphorylation. Immunoprecipitation of ASAP1 from HEK293T cells co-transfected with Pyk2 and subsequent blotting with anti-phosphotyrosine antibodies revealed a robust increase in tyrosine phosphorylation of ASAP1, whereas there was no tyrosine phosphorylation of ASAP1 evident in the absence of Pyk2 (Fig. 6A, upper panel). In agreement with our interaction studies, ASAP1 tyrosine phosphorylation was almost undetectable when isolated from cells co-expressing Pyk2 variants with single mutations in any of the proline-rich domains and completely blocked in the presence of the corresponding double mutant (Fig. 6A, upper panel; note that some ASAP1 tyrosine phosphorylation was seen with Pyk2-P859A upon longer exposure times). Furthermore, a kinase-inactive mutant of Pyk2 (PKM), although still able to bind ASAP1 (not shown), did not induce its tyrosine phosphorylation. In agreement with former studies FAK, although able to bind ASAP1 (Ref. 13 and data not shown), did not induce any tyrosine phosphorylation (Fig. 6A). In addition to Pyk2, we also detected a tyrosine-phosphorylated 100-kDa protein (pp100) of yet unknown identity in ASAP1 immunoprecipitates. Immunoprecipitation of equal amounts of FLAG-ASAP1 as well as expression levels of Pyk2 and hemagglutinin-FAK were controlled by Western blotting using respective antibodies (Fig. 6A, middle and lower panel).

Src has been shown to bind to and phosphorylate ASAP1, and many Pyk2 actions are tightly linked to Src (1, 3, 4, 12). We therefore wondered whether Pyk2 directly phosphorylated ASAP1 or whether it recruited Src to ASAP1 that subsequently executes the phosphorylation reaction. The finding that ASAP1 tyrosine phosphorylation is almost undetectable upon co-expression with Pyk2-Y402F that is unable to bind and activate Src may support the latter hypothesis (Fig. 6B, upper panel). Furthermore, co-expression of Pyk2 and Src with ASAP1 amplified the ASAP1 tyrosine phosphorylation, and a kinase-inactive dominant-interfering mutant of Src (SrcK−) slightly reduced the effect of Pyk2. In agreement with Brown et al. (12) Src may also phosphorylate ASAP1 independently of Pyk2 as shown by co-expression of ASAP1 with Src alone or in combination with dominant-interfering Pyk2 mutants Pyk2-Y402F and PKM. Comparable immunoprecipitation of FLAG-ASAP1 and expression of FLAG-ASAP1, Pyk, and Src variants was shown by Western blotting with respective antibodies (Fig. 6B, lower panels).

To challenge the hypothesis that Src and not Pyk2 is the kinase that phosphorylates ASAP1, we performed in vitro kinase reactions with FLAG-ASAP1 complexes isolated from HEK293T cells by immunoprecipitation (cf. Fig. 6B). Addition of [γ-32P]ATP to these complexes led to a strong increase in phosphorylation of ASAP1 by Pyk2 (Fig. 7A). Interestingly, we were unable to detect significant amounts of Src protein or Src kinase activity in these complexes (not shown), suggesting that Pyk2 can directly phosphorylate ASAP1. In support of this notion, Pyk2-Y402F, which is unable to recruit and activate Src, binds ASAP1 in HEK293T cells and increased ASAP1 phosphorylation in vitro. Furthermore, a dominant-interfering kinase-inactive Src mutant did not affect in vitro phosphorylation of ASAP1 by Pyk2, and doses of up to 10 μM of the fairly specific Src inhibitor PP1 in the kinase reaction had no effect on the Pyk2-mediated ASAP1 phosphorylation (Fig. 7A and not shown). No ASAP1 phosphorylation was detected when the reaction was performed with ASAP1-PKM complexes containing kinase-inactive Pyk2 or ASAP1 and FAK (Fig. 7A and not shown).

In summary, our data suggest that Pyk2 increases tyrosine phosphorylation of ASAP1. In vitro Src is not necessary for this process, and Pyk2 can directly phosphorylate ASAP1. In vivo Src contributes to the tyrosine phosphorylation of ASAP1 either by directly phosphorylating ASAP1 or by enhancing Pyk2 activity toward ASAP1, e.g., by an activating phosphorylation of Pyk2 as has been suggested previously (1, 30, 31).

**Mapping of ASAP1 Tyrosine Phosphorylation Sites**—To identify amino acids in ASAP1 that are phosphorylated by Pyk2, we applied a two-dimensional phosphopeptide mapping technique (19, 32). ASAP1-Pyk2 complexes were isolated by immunoprecipitation and subjected to in vitro kinase reactions using...
expression of employed constructs was confirmed by Western blotting (WB) of cell lysates using corresponding antibodies. To control levels of immunoprecipitates, membranes were re-probed with an anti-FLAG antibody. The SDS-PAGE and transfer onto nitrocellulose membranes, tyrosine-phosphorylated proteins present in immune complexes were detected with an anti-phosphotyrosine antibody (PY99). We wondered whether the same residues are also phosphorylated in vivo and therefore performed a similar two-dimensional phosphopeptide mapping experiment with ASAP1 phosphorylated in vivo. We performed a similar analysis of the phosphorylation pattern of ASAP1 phosphorylated in vitro. However, we were unable to see the equivalent phosphotyrosine 782-containing peptide 1 in our two-dimensional phosphopeptide maps performed with ASAP1 phosphorylated in intact cells. Therefore, we first sought to confirm that the additional phosphopeptide we detected indeed contained phosphorylated Tyr-308. The corresponding phosphopeptide was extracted and subjected to phosphoamino acid analysis and Edman sequencing. Similar to peptide 2 from the in vitro experiment (cf. Fig. 7C), 32P was exclusively incorporated into phosphotyrosine and found in sequencing cycle 6 (not shown). To confirm further the identity of this peptide and to prove that tyrosine 308 is an in vivo Pyk2 phosphorylation site in ASAP1, we performed a similar analysis of the phosphorylation pattern of the ASAP1-Y308F mutant expressed alone or together with Pyk2 (Fig. 7F, right panels). In agreement with our hypothesis, the amount of total tyrosine phosphorylation was decreased (Fig. 7E, lower panels), and the phosphotyrosine-containing peptide was absent in this ASAP1 mutant lacking tyrosine 308 (Fig. 7F, right panels, dashed circles). We therefore confirmed that at least tyrosine 308 that we identified as a major ASAP1 phosphorylation site is also phosphorylated by Pyk2 in intact cells.

ASAP1 Activity Is Enhanced by Phosphoinositol 4,5-Bisphosphate, and Phospholipid Binding of ASAP1 Is Modulated by Tyrosine Phosphorylation—Previous data suggested a role of phospholipids, particularly of PI(4,5)P₂, in regulating ASAP1 Arf-GAP activity (24). To confirm this hypothesis, we purified myristoylated Arf1 expressed in E. coli and FLAG-ASAP1 from transiently transfected HEK293T cells close to homogeneity,
Identification of ASAP1 tyrosine phosphorylation sites by two-dimensional phosphopeptide mapping. A, HEK293T cells co-transfected with indicated constructs (2 μg of Pyk2, 6 μg of Src, and 2 μg of ASAP1/10-cm dish) were lysed and subjected to an anti-FLAG immunoprecipitation (IP) to isolate FLAG-ASAP1 and associated proteins. In vitro kinase reactions (IVKR) were performed by incubating complexes with 5 μCi of [γ-32P]ATP in kinase buffer for 30 min at 30 °C. Reactions were stopped, and samples were subjected to 8% SDS-PAGE, transferred onto nitrocellulose membrane, and analyzed using a PhosphorImager (Molecular Imager® FX, Bio-Rad). 32P-Labeled FLAG-ASAP1 was digested in situ with trypsin. B, one fraction of resulting peptides was hydrolyzed, subjected to phosphoamino acid analysis, and visualized using a PhosphorImager. Phosphorylated amino acids were identified by co-migrations with ninhydrin-stained standards that are indicated by dashed circles. C, the second phosphopeptide fraction was separated on TLC plates by high voltage electrophoresis and ascending chromatography. A circled cross illustrates where samples were applied; + and − indicate the polarity during electrophoresis. Phosphopeptides were localized by PhosphorImager analysis, and the position of phosphorylation sites was predicted as described under “Experimental Procedures.” The same procedure was used to demonstrate absence of major Pyk2 phosphorylation sites in ASAP1-Y308F and ASAP1-Y308F/Y782F mutants. D, HEK293T cells co-transfected with the indicated constructs (2 μg of pcDNA3, 2 μg of ASAP1, or 2 μg of ASAP1-Y308F and 2 μg of Pyk2/10-cm dish) were labeled with 1 mCi/ml [32P]orthophosphate, lysed, and subjected to an anti-FLAG immunoprecipitation. Following 8% SDS-PAGE and transfer to nitrocellulose membrane, [32P]-labeled FLAG-ASAP1 or FLAG-ASAP1-Y308F and associated proteins were detected using a PhosphorImager. Phosphoamino acid (E) and phosphopeptide (F) analysis of in situ digested [32P]-labeled FLAG-ASAP1 or FLAG-ASAP1-Y308F were done as described under B and C, respectively.
and we performed a fluorimetric GTPase assay with GTP-loaded Arf1. In agreement with Kam et al. (24) we found that ASAP1 displayed only poor Arf1-GAP activity in the absence of phosphoinositides (Fig. 8A). However, GAP activity of ASAP1 was strongly augmented by addition of PI(4,5)P2 to the liposomes indicated by a reduction of intrinsic Arf1 fluorescence upon GTP hydrolysis. As a control we used GTPγS, a non-hydrolyzable GTP analogue that did not lead to any significant change of Arf1 fluorescence upon addition of ASAP1. Thus, ASAP1 activity seems to be largely dependent on the presence of PI(4,5)P2.

The PH domain of ASAP1 has been shown to be critical of phosphoinositide binding and allosteric activation of ASAP1 (24). Interestingly, tyrosine 308 that we identified as a major in vitro and in vivo Pyk2 phosphorylation site in ASAP1 is in proximity to the PH domain. We therefore wondered whether introduction of a negative charge by phosphorylation of this tyrosine could affect binding properties of the PH domain. To test this hypothesis we isolated FLAG-ASAP1 as well as FLAG-ASAP1-Pyk2 and FLAG-ASAP1-Y308F/Y782F-Pyk2 complexes from transfected HEK293T cells by affinity chromatography. To increase tyrosine phosphorylation, we subjected them to in vitro kinase reactions by addition of ATP. As shown in the upper panel of Fig. 8B, tyrosine phosphorylation of ASAP1 in complex with Pyk2 significantly increased as detected by Western blotting (WB) with an anti-phosphotyrosine antibody (pY99). ASAP1 levels were controlled using an anti-ASAP1 antibody. C, protein-lipid overlay assays were performed with immobilized phospholipids (100 pmol/spot) incubated with 0.5 μg/ml ASAP1, phosphorylated ASAP1, or phosphorylated ASAP1-Y308F/Y782F. Binding of FLAG-tagged ASAP1 or ASAP1-Y308F/Y782F to immobilized lipids was detected with an anti-FLAG antibody. LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; LPC, lysophosphocholine; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

**FIG. 8.** Tyrosine phosphorylation regulates phospholipid binding of ASAP1. A, purified Arf1 was loaded with GTP or GTPγS in reaction buffer containing liposomes with or without PI(4,5)P2. Affinity-purified ASAP1 (2 μg) was injected, and GTP hydrolysis was followed as decrease in Arf1 intrinsic tryptophan fluorescence at 340 nm (in arbitrary units; AU) upon excitation at 297.5 nm. B, affinity-purified ASAP1, ASAP1-Pyk2, or ASAP1-Y308F/Y782F-Pyk2 complexes were subjected to kinase reactions with or without 100 μM ATP for 40 min at 30 °C. Thereafter, samples were subjected to 8% SDS-PAGE and analyzed by Western blotting (WB) with an anti-phosphotyrosine antibody (pY99). ASAP1 levels were controlled using an anti-ASAP1 antibody. C, protein-lipid overlay assays were performed with immobilized phospholipids (100 pmol/spot) incubated with 0.5 μg/ml ASAP1, phosphorylated ASAP1, or phosphorylated ASAP1-Y308F/Y782F. Binding of FLAG-tagged ASAP1 or ASAP1-Y308F/Y782F to immobilized lipids was detected with an anti-FLAG antibody. LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; LPC, lysophosphocholine; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine.
phosphoinositides PI(3)P, PI(4)P, PI(5)P, PI(3,5)P₂, and PI(4,5)P₂, whereas weaker binding to PI(3,4)P₂, PI(3,4,5)P₃, and phosphatidylinositol (PS) was observed (Fig. 8C). In contrast, tyrosine-phosphorylated ASAP1 did not bind to PI(3,4)P₂, PI(3,4,5)P₃, and PS and revealed significantly reduced interaction with PI(3)P and PI(4,5)P₂, whereas binding to PI(4)P, PI(5)P, and PI(3,5)P₂ seemed to be only marginally affected. Interestingly, the ASAP1-Y308F/Y782F mutant, although still partially phosphorylated by Pyk2, displayed a phospholipid binding profile very similar to non-phosphorylated wild-type ASAP1. These results demonstrate that Pyk2-mediated tyrosine phosphorylation of tyrosine 308 and 782 leads to a modulation of ASAP1 Arf-GAP activity by Pyk2-mediated tyrosine phosphorylation. We have mapped two major Pyk2 phosphorylation sites, tyrosine 308 and 782 in ASAP1, and could demonstrate that mutation of the corresponding residues did not only reduce Pyk2-mediated phosphorylation but also abolished the effect of Pyk2 on ASAP1 phospholipid binding profile and Arf1-GAP activity. We found that in vitro Src can phosphorylate similar residues in ASAP1 (not shown), and we hypothesized that Src-mediated phosphorylation may have a similar functional consequence on ASAP1 GAP activity. However, we did show that, at least in vitro, Src is not necessary for the Pyk2-induced ASAP1 phosphorylation because Pyk2 lacking the Src-binding site Tyr-402 is still able to phosphorylate ASAP1. Furthermore, intervention with Src function using either pharmacological inhibitors or dominant-negative constructs did not block the Pyk2-mediated ASAP1 tyrosine phosphorylation in vitro nor in HEK293T cells (not shown). The lack of ASAP1 tyrosine phosphorylation in cells expressing Pyk2-Y402F could be due to Src-dependent effects on Pyk2 activity, e.g. by phosphorylation of tyrosine residues in the Pyk2 kinase domain that may be crucial for full Pyk2 activity (1, 30, 31). Indeed, we found that, at least in vitro, Src can phosphorylate tyrosine residues in Pyk2 that are different from Pyk2 auto-phosphorylation sites, tyrosine 308 and 782 of ASAP1, are significantly phosphorylated by Pyk2, only reduce Pyk2-mediated phosphorylation but also abolished the effect of Pyk2 on ASAP1 GAP activity (Fig. 9, right panel). These results strongly suggest a modulation of ASAP1 Arf1-GAP activity by Pyk2-mediated phosphorylation of tyrosine 308 and 782 and provide a first molecular mechanism for a cross-talk between tyrosine kinases and small GTPases of the Arf family.

**DISCUSSION**

In a yeast two-hybrid screen, we identified ASAP1 as a new binding partner of Pyk2. Interaction sites were mapped to the SH3 domain of ASAP1 and the proline-rich regions of Pyk2. ASAP1 can be directly phosphorylated by Pyk2 resulting in a reduction of its Arf-GTPase activity. We therefore suggest a role of Pyk2 in regulating Arf function and potentially vesicular trafficking, and we provide a molecular mechanism how tyrosine kinases can modulate signaling of small Arf GTPases.

Pyk2 has been shown previously to engage several proteins through its C-terminal domain that contains proline-rich sequences able to bind SH3 domain partners and a FAT domain that overlaps with contact sites for paxillin (1). One of the SH3 domain partners of Pyk2 is the adapter protein p130Cas (Crk-associated substrate) that binds to both proline-rich regions in Pyk2, whereas the GTPase-activating proteins Graf (GTPase regulator associated with FAK) and PGSAP (PH and SH3 domain containing Rho-GAP) seem to prefer the second proline-rich motif (33, 34). Our results show that ASAP1 can bind to both proline-rich regions but has a higher affinity to the first motif. Thus ASAP1 may be one of the few Pyk2 interaction partners that favor the first proline-rich region sequence allowing the assembly of multiprotein complexes that are believed to regulate most cellular functions (35). In addition, our co-immunoprecipitation studies in PC12 and MEF cells with endogenously expressed Pyk2 and ASAP1 suggest that the observed interaction is not a yeast two-hybrid or overexpression artifact. However, low ASAP1 expression and moderate quality of commercial ASAP1 antibodies so far prevented further detailed functional studies in these cells. To overcome this limitation, we are planning to develop own high affinity ASAP1 antibodies for future studies.

ASAP1 was originally purified from bovine brain as a phosphatidylinositol 4,5-bisphosphate-dependent Arf-GAP, and it was independently identified in a yeast two-hybrid screening as a binding partner of the tyrosine kinase Src (12). Although functional consequences of the ASAP1-Src interaction have remained elusive, we here provide evidence for a negative control of ASAP1 Arf-GAP activity by Pyk2-mediated tyrosine phosphorylation. We have mapped two major Pyk2 phosphorylation sites, tyrosine 308 and 782 in ASAP1, and could demonstrate that mutation of the corresponding residues did not only reduce Pyk2-mediated phosphorylation but also abolished the effect of Pyk2 on ASAP1 phospholipid binding profile and Arf1-GAP activity. We found that in vitro Src can phosphorylate similar residues in ASAP1 (not shown), and we hypothesized that Src-mediated phosphorylation may have a similar functional consequence on ASAP1 GAP activity. However, we did show that, at least in vitro, Src is not necessary for the Pyk2-induced ASAP1 phosphorylation because Pyk2 lacking the Src-binding site Tyr-402 is still able to phosphorylate ASAP1. Furthermore, intervention with Src function using either pharmacological inhibitors or dominant-negative constructs did not block the Pyk2-mediated ASAP1 tyrosine phosphorylation in vitro nor in HEK293T cells (not shown). The lack of ASAP1 tyrosine phosphorylation in cells expressing Pyk2-Y402F could be due to Src-dependent effects on Pyk2 activity, e.g. by phosphorylation of tyrosine residues in the Pyk2 kinase domain that may be crucial for full Pyk2 activity (1, 30, 31). Indeed, we found that, at least in vitro, Src can phosphorylate tyrosine residues in Pyk2 that are different from Pyk2 auto-phosphorylation sites and may be involved in regulation of Pyk2 kinase activity. Alternatively, another protein partner may be recruited via phosphotyrosine 402 that is necessary for cellular Pyk2 activity. We are currently trying to challenge this hypothesis by searching for specific binding partners to phosphorylated tyrosine 402 using genetic screens and classical biochemical methods. However, it also clear that Src can phosphorylate ASAP1 independent of Pyk2, and it is possible that in vivo Src cooperates with Pyk2 in phosphorylating ASAP1, as it has been shown for several other proteins that are tyrosine-phosphorylated upon activation of Pyk2 (1).

Whereas in vitro both residues, tyrosine 308 and 782 of ASAP1, are significantly phosphorylated by Pyk2, only phosphotyrosine 308 could be confirmed as a phosphorylation site in intact cells. This might either be due to a lower stoichiometry of the Tyr-782 phosphorylation as already suggested by the two-dimensional maps with in vitro phosphorylated ASAP1 or the phosphotyrosine 782 peptide could be masked by phosphoserine-containing peptides with similar migration behavior. Alternatively, Tyr-782 may not be phosphorylated to a significant extent in intact cells. Currently, we are trying to discriminate between these possibilities by analyzing phosphopeptides from in vivo phosphorylated ASAP1 enriched by anti-phosphotyrosine affinity chromatography. Because our cellular ³²P-labeling experiments could not exclude a phosphorylation of Tyr-782 that we observed in vitro, we decided to use the double mutant ASAP1-Y308F/Y782F for subsequent biochemical assays.

*2 A. Kruljac-Letunic, A. Kallin, and A. Blaukat, manuscript in preparation.
Tyrosine 308 that we identified as a major ASAP1 phosphorylation site in vitro and in intact cells is in proximity to the PH domain, which is, together with PI(4,5)P₂, essential for ASAP1 activity (12, 24). We hypothesized that introduction of a negative charge by Pyk2-mediated tyrosine phosphorylation could affect PI(4,5)P₂ binding and Arf-GAP activity. Indeed, phosphorylation of tyrosine 308 and 782 diminished ASAP1 binding to PI(4,5)P₂ and other phospholipids, such as PI(3)P, PI(3,4,5)P₃, and PS. Decreased PI(4,5)P₂ binding was paralleled by a significant reduction in Arf1-GAP activity of ASAP1. In agreement with these findings, we observed an increase of cellular Arf1-GTP levels upon overexpression of Pyk2 that may result from an inhibition of the ASAP1 Arf-GAP activity (not shown). A similar observation has been described for the Pyk2-mediated inhibition of PSGAP Cdc42-GAP activity that led to an increase in Cdc42-GTP levels. However, in contrast to our studies on ASAP1, molecular mechanisms of this phenomenon have remained elusive (34). Recently, the cytoskeletal protein gelsolin has been described as another example for modulation of phospholipid binding by Pyk2 phosphorylation. Interestingly, in this case Pyk2 phosphorylation seem to increase affinity of gelsolin to PI(4,5)P₂ (36).

Arf GTPases are involved in the regulation of membrane trafficking, actin cytoskeleton re-organization, as well as phospholipase D and phosphatidylinositol-4-phosphate 5-kinase activation, leading to the generation of phosphaticid acid and PI(4,5)P₂. In this respect it is intriguing that tyrosine kinases have been implicated in the activation of phospholipase D (37, 38) and that an increase in cellular PI(4,5)P₂ levels has been observed upon overexpression of Pyk2 in HEK293 cells (39). The interesting question whether Pyk2-mediated modulation of ASAP1 and thus Arf activity is indeed involved in these important Arf functions as well as the physiological relevance of the described molecular mechanisms remains to be addressed by further studies.

Acknowledgments—We are grateful to Ivan Dikic, Giulio Superti-Furga, Paul Randazzo, and Jürgen Behrens for providing reagents. We thank Christer Wernstedt, Karin Meyer, Jakub Świercz, and Daniel Gommel for technical help. We also thank Stefan Offermanns for critically reading the manuscript and supporting the work.

References

1. Avraham, H., Park, S. Y., Schinkmann, K., and Avraham, S. (2000) Cell Signal. 12, 123–133
2. Matsuya, M., Sasaki, H., Aoto, H., Mitaka, T., Nagura, K., Ohba, T., Ishino, M., Takahashi, S., Suzuki, R., and Sasaki, T. (1998) J. Biol. Chem. 273, 1003–1014
3. Dikic, I., Patzer, A. G., Blaike, P., Obermeier, A., Ulrich, A., Schlessinger, J., and Margolis, B. (1995) J. Biol. Chem. 270, 15125–15129
4. Blaukat, A., Ivanovic-Dikic, I., Gronroos, E., Dolfi, F., Tokiwa, G., Vuori, K., and Dikic, I. (1999) J. Biol. Chem. 274, 14493–14501
5. Kuhn, R. A., and Gilman, A. G. (1986) J. Biol. Chem. 261, 7906–7911
6. Donaldson, J. G., and Kluasner, R. D. (1994) Curr. Opin. Cell Biol. 6, 527–532
7. Rothman, J. E. (1994) Nature 372, 55–63
8. Sohn, K., Orci, L., Ravazzola, M., Amherdt, M., Bremser, M., Letteipeil, F., Stierler, K., Helms, J. B., and Wieland, F. T. (1996) J. Cell Biol. 135, 1239–1248
9. Norman, J. C., Jones, D., Barry, S. T., Holt, M. R., Cockcroft, S., and Critchley, D. I. (1998) J. Cell Biol. 141, 1109–1126
10. Cukierman, E., Huber, I., Rotman, M., and Casset, D. (1995) Science 270, 1999–2002
11. Moss, J., and Vaughan, M. (1995) J. Biol. Chem. 270, 12327–12330
12. Brown, M. T., Andrade, J., Radhakrishna, H., Donaldson, J. G., Cooper, J. A., and Randazzo, P. A. (1998) Mol. Cell. Biol. 18, 7038–7051
13. Liu, Y., Lajinos, J. C., Martin, K. H., Karginov, A. V., and Parsons, J. T. (2002) Biochim. Biophys. Acta 1556, 75–85
14. Andreev, J., Simon, J. P., Sabatini, D. D., Kam, J., Plowan, G., Randazzo, P. A., and Schlessinger, J. (1999) Mol. Cell. Biol. 19, 2338–2350
15. Ivanovic-Dikic, I., Gronroos, E., Blaukat, A., Barth, B. U., and Dikic, I. (2000) Nat. Cell Biol. 2, 574–581
16. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2002) Cell Struct. Function. 27, 47–61
17. Vojtek, A. B., Cooper, J. A., and Hollenberg, S. M. (1997) in The Yeast Two-hybrid System (Bartel, P. L., and Fields, S., eds) pp. 29–42, Oxford University Press, Oxford, UK
18. Gietz, R. D., and Schiebel, R. H. (1991) Yeast 7, 253–263
19. Blaukat, A., Pizard, A., Breit, A., Wernstedt, C., Alhenc-Gelas, F., Muller-Esterl, W., and Dikic, I. (2001) J. Biol. Chem. 276, 40431–40440
20. Franco, M., Chardin, P., Chabre, M., and París, S. (1995) J. Biol. Chem. 270, 1337–1341
21. Dowler, S., Kular, G., and Alesi, D. R. (2002) Sci. STKE 129, PE6
22. Avraham, H., Beraud-Dufour, S., Chardin, P., and Chabre, M. (1997) Biochemistry 36, 4675–4684
23. Paris, S., Beraud-Dufour, S., Robinneau, S., Bigay, J., Avraham, B., Chabre, M., and Chardin, P. (1997) J. Biol. Chem. 272, 22221–22226
24. Kam, J. L., Miura, K., Jackson, T. R., Gruschus, J., Roller, P., Stauffer, S., Clark, J., Aneja, R., and Randazzo, P. A. (2000) J. Biol. Chem. 275, 9653–9663
25. Blaukat, A. P., Andrade, J., Miura, K., Brown, M. T., Long, Y. Q., Stauffer, S., Roller, P., and Cooper, J. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4011–4016
26. Behrens, J., van Kries, J. P., Kohl, M., Bruhn, L., Wedlich, D., Grosschedl, R., and Birchmeier, W. (1996) Nature 382, 638–642
27. Ricks, R. J., Botfield, M. C., Weng, Z., Taylor, J. A., Green, O. M., Brugge, J. S., and Zoller, M. J. (1994) EMBO J. 13, 5598–5604
28. Yu, H., Chen, J. K., Feng, S., Dalgarno, D. C., Brauer, A. W., and Schreiber, S. L. (1994) Cell 76, 933–945
29. Pawson, T. (1995) Nature 373, 573–580
30. Girault, J. A., Costa, A., Derkinderen, P., Studler, J. M., and Toutant, M. (1999) Trends Neurosci. 22, 257–263
31. Nakamura, T., Yamashita, H., Nagano, Y., Takahashi, T., Avraham, S., Avraham, H., Matsumoto, M., and Nakamura, S. (2002) FEBS Lett. 521, 180–184
32. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) Methods Enzymol. 201, 119–149
33. Ohba, T., Ishino, M., Aoto, H., and Sasaki, T. (1998) Biochem. J. 330, 1249–1254
34. Ben, R. X., Du, Q. S., Huang, Y. Z., Ao, S. Z., Mei, L., and Xiong, W. C. (2001) J. Cell Biol. 153, 971–984
35. Gavin, A. C., and Superti-Furga, G. (2003) Curr. Opin. Cell Biol. 15, 21–27
36. Wang, Q., Xie, Y., Du, Q. S., Wu, X. J., Feng, X., Mei, L., McDonald, J. M., and Xiong, W. C. (2003) J. Cell Biol. 160, 565–575
37. Houle, M. G., and Bourgouin, S. (1999) Biochem. Biophys. Acta 1439, 135–149
38. Exton, J. H. (2002) FEBS Lett. 531, 58–61
39. Oude Wepenink, P. A., Hinz, M., Reitemeyer, K., Schmidt, M., and Jakobs, K. H. (2000) Naxys-Schmieloeber's Arch. Pharmacol. 363, R62

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J. Biol. Chem. 2003, 278:29560-29570.
doi: 10.1074/jbc.M302278200 originally published online May 27, 2003

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