Focal adhesion kinase (FAK) is a protein tyrosine kinase enriched in focal adhesions, which plays a critical role in integrin-dependent cell motility and survival. The crucial step in its activation is autophosphorylation on Tyr-397, which promotes the recruitment of several enzymes including Src family kinases and the activation of multiple signaling pathways. We found in a yeast two-hybrid screen that the N-terminal domain of FAK interacted with protein inhibitor of activated STAT1 (PIAS1). This interaction was confirmed and shown to be direct using in vitro assays. PIAS1 was co-immunoprecipitated with FAK from transfected cells and brain extracts. PIAS1 has recently been recognized as a small ubiquitin-like modifier (SUMO) ligase. In the presence of PIAS1 and SUMO-1, FAK was sumoylated in intact cells, whereas PYK2, a closely related enzyme, was not. Sumoylation occurred on Lys-152, a residue conserved in FAK during evolution. Sumoylated FAK, like PIAS1, was recovered predominantly from the nuclear fraction. Sumoylation did not require the catalytic activity or autophosphorylation of FAK. In contrast, sumoylation increased dramatically the ability of FAK to autophosphorylate in intact cells and in immune precipitate kinase assays. Endogenous FAK was sumoylated in the presence of PIAS1 and SUMO-1 independently of cell adhesion, and autophosphorylation of sumoylated FAK was persistently increased in suspended cells. These observations show that sumoylation controls the activity of a protein kinase and suggest that FAK may play a novel role in signaling between the plasma membrane and the nucleus.

To elucidate the role and partners of the N-terminal domain of FAK, we undertook a yeast two-hybrid screen and identified a novel interaction partner, PIAS1, a protein initially cloned as a specific inhibitor of activated STAT1 (24). PIAS1 proteins have recently been shown to be ligases for small ubiquitin-like modifier proteins (SUMO) (25, 26). We report that PIAS1 induces the conjugation of SUMO-1 to FAK on Lys-152. SUMO-1 conjugation to FAK enhances the autophosphorylation capacity of the protein in vitro and in cells, independently of adhesion, suggesting a novel mode of regulation of this enzyme.
against a recombinant fusion protein, GST-ΔPIAS1, encompassing residues 402–651 of human PIAS1. Serum SLA1 for specific immunoprecipitation of transfected FAK and serum against PYK2 were as described (27, 28). Anti-stathmin polyclonal antibody was a gift from A. Maucler (INSERM 440, Paris).

Yeast Two-hybrid Screen—The N-terminal domains of rat FAK (amino acids 1–361) and PYK2 (amino acids 2–364) were cloned in pB7M16 and used as baits in L40 yeast strain co-transformed with a human brain cDNA library (Clontech). Transformants were plated on agar selection medium lacking tryptophan, leucine, and histidine. Colonies were isolated and retested for growth in minimal medium and for β-galactosidase activity. Clones positive for both tests were used for retransformation of yeast strains expressing heterologous baits, including GST-PYK2, to determine the specificity of the interactions.

Constructs and Site-directed Mutagenesis—GST-PIAS1 and GST-ΔPIAS1 were obtained by subcloning full-length PIAS1 from pSG5-PIAS1 (a kind gift from J. Tan, University of North Carolina) and ΔPIAS1 from pACT2 prey plasmid, respectively, into pGEX-4T-1 and pGEX-4T-2 (Amersham Biosciences). GST-N-FAK was made by ligating a BamHI-BglII fragment comprising FAK sequence (amino acids 1–361) from pLexA-N-FAK into BamHI-digested pGEX-4T-1 plasmid. For cell transfection, an HA-tagged PIAS1 plasmid was constructed by insertion of full-length PIAS1 from pSG5-PIAS1 digested with EcoRI and XhoI into the pcDNA3-HA. Substitution of Lys-152 of FAK with an arginine was carried out with QuickChange (Stratagene).

Cell Culture, Transfection, and Fractionation—COS-7 and NIH-3T3 cells were cultured and transfected with 8 μg of DNA per 100-mm diameter culture dish as described (23). For triple transfections, the amounts of transfected plasmids were: 2 μg of pBK-FAK (23) or pBK-CMV3-PYK2, 2 μg of pSG5-SUMO-1 (a generous gift from J. Seeler, Pasteur Institute), and the indicated amounts of pcDNA3-HA-PIAS1. Total DNA quantity was maintained constant with empty pDNA3-HA. Cells were lysed 48 h after transfection. Comparisons of attached and suspended cells (23) and cell partitioning (29) were as described.

Biochemical Procedures—Extracts from freshly dissected tissues (~50 mg) or cell lysates were prepared in modified radioimmune precipitation assay buffer as described (23), except for the presence, when indicated, of 10 mM N-ethylmaleimide (NEM, Sigma). After clarification with 100 μl of Sephacryl beads, the supernatants were used for immunoprecipitation or GST pull-down in radioimmune precipitation assay buffer (2 μg of GST fusion protein on glutathione-Sepharose 4B (Amersham Biosciences)500 μl of cell lysate). Recombinant GST and GST fusion proteins were produced in BL21(DE3) Escherichia coli strain (Stratagene) and purified on glutathione-Sepharose 4B. GST-PIAS1 was in vitro translated using T7 Tnt-Quick (Promega) in the presence of [35S]methionine. Binding reactions were carried out at 4 °C for 16 h in a buffer containing 50 mM Tris-HCl pH 7.8, 150 mM NaCl, 10% glycerol, 0.5 mM EDTA, 5 mM MgCl2, and protease inhibitors (Complete, Roche Molecular Biochemicals). For "far Western" assays, purified recombinant proteins were resolved by SDS-PAGE without previous boiling, transferred onto a nitrocellulose membrane, and stained with Ponceau red. Proteins were partly renatured by incubation in buffer (Hepes, pH 7.4, MgCl2 10 mM, NaCl 50 mM, EDTA 1 mM, dithiothreitol 1 mM, glycerol 10%) for 24 h. After saturation of nonspecific sites with 5% nonfat dry milk, the membrane was incubated overnight with purified recombinant GST-N-FAK (75 ng/cm2 of membrane), washed extensively, and processed for immunoblotting using anti-FAK antibody A-17. The other procedures were as described (23, 30).

RESULTS AND DISCUSSION

Identification of a Direct Interaction between PIAS1 and FAK N-terminal Domain by Yeast Two-hybrid and in Vitro Assays—A yeast two-hybrid screen of a human brain cDNA library using the N-terminal domain of FAK as a bait yielded 407 positive clones of 2 × 10^6 transformants, among which 125 were randomly selected for further analysis. They were tested for specificity using three different baits: DNA-binding domain of LexA alone or fused in-frame with the N-terminal domain of PYK2 (a kinase closely related to FAK) or with lamin (used as a control for "sticky" preys). Only 36 clones satisfied the specificity criteria yielding His+, LacZ+ colonies exclusively with FAK N-terminal domain as bait. Several of these clones coded for the same proteins and following restriction analysis and sequencing we identified five different proteins as potential partners of FAK N-terminal domain. Two independent clones were found to encode human PIAS1 C-terminal region (amino acids 403–651, here referred to as ΔPIAS1). PIAS1 is the member of a small protein family with a central RING-finger-like motif and a less conserved C-terminal region (corresponding to ΔPIAS1) (31). PIAS1 regulates the activity of several transcription factors including STAT1 (24), p53 (25), c-Jun (25), LEPF1 (32), Sp3 (26), and androgen receptors, (33–35) but its interaction with signaling enzymes has not been reported.

To confirm and further characterize the interaction between FAK and PIAS1, we tested the ability of GST fusion proteins comprising full-length PIAS1 or ΔPIAS1 to bind FAK. In GST pull-down experiments, transfected FAK protein was retained by GST-PIAS1 or GST-ΔPIAS1 but not by GST (Fig. 1A, lanes 6–7).
Fig. 2. Direct association and coimmunoprecipitation of FAK and PIAS1. A, direct association between recombinant FAK and PIAS1 was tested by far Western blot. GST fusion proteins (~2 µg each) and molecular weight markers were resolved by SDS-PAGE, transferred to nitrocellulose, and partially renatured, and the membrane was incubated with soluble GST-N-FAK. Proteins were stained with Ponceau red (left panel), and GST-N-FAK binding to protein bands was detected by FAK immunoblotting (right panel). GST-PIAS1 (arrow) and GST-ΔPIAS1 (arrowhead) were specifically labeled, whereas GST-PEA15 and GST-Kiaa316, two negative controls and molecular weight markers (M, × 10^3 indicated on the right) were not labeled (dots). (B) COS-7 cells were transfected with vector alone or HA-tagged PIAS1 with or without FAK. Immunoprecipitation (IP) was carried out with anti-PIAS1 serum, and FAK was detected with a monoclonal anti-FAK antibody (upper panel). FAK was found in PIAS1 immune precipitate from cotransfected cells (arrowhead). The amounts of HA-PIAS1 in the immune precipitate (middle panel) and of FAK in cell lysates (lower panel) were checked by immunoblotting. C, rat brain hippocampus was homogenized in modified radioimmunoprecipitation assay buffer and subjected to immunoprecipitation with anti-PIAS1 or pre-immune (Pre-Im) serum, followed by FAK immunoblotting.

3, 5, and 7). In cells transfected with the vector alone, endogenous FAK was also retained by GST-PIAS1 or GST-ΔPIAS1 (Fig. 1A, lanes 2 and 6). These results confirmed that ΔPIAS1 was sufficient for binding of FAK. Conversely, GST-N-FAK was able to bind full-length in vitro translated [35S]PIAS1 (Fig. 1B, lane 2), whereas GST control beads did not (Fig. 1B, lane 3). Thus, full-length PIAS1 and FAK were able to bind to each other, showing that the corresponding interacting surfaces are fully accessible in the entire proteins. GST pull-down assays demonstrated that PIAS1 interacted similarly with endogenous FAK from various rat tissues, which express different alternatively spliced isoforms of FAK (36) (Fig. 1C).

Although the above results suggested that the FAK-PIAS1 interaction was direct, the existence of an unidentified bridging partner could not be excluded completely. To provide unequivocal evidence that the interaction was direct, we used soluble GST-N-FAK as a probe to carry out a far Western blot of purified recombinant proteins: GST-PIAS1, GST-ΔPIAS1, GST-N-kiaa316 (N-terminal region of a hypothetical protein similar to N-FAK; Ref. 18), or GST-PEA15 (unrelated protein). GST-N-FAK labeled GST-PIAS1 and GST-ΔPIAS1 protein bands (Fig. 2A, right panel) but not the other irrelevant proteins present on the same membrane, including the molecular weight markers (see Fig. 2A, left panel). This experiment demonstrated that the interaction between FAK and PIAS1 did not require an additional protein.

FAK and PIAS1 Are Associated in Transfected Cells and Brain Extracts—We analyzed the association between FAK and PIAS1 transfected in COS-7 cells, using immunoprecipitation with anti-PIAS1 serum followed by immunoblotting with an anti-FAK monoclonal antibody. FAK was detected in the PIAS1 immune precipitate when the two proteins were cotransfected (Fig. 2B). A specific interaction between FAK and PIAS1 was also detected in rat hippocampal extracts subjected to immunoprecipitation with anti-PIAS1 serum followed by FAK immunoblotting (Fig. 2C). These results demonstrated that FAK and PIAS1 proteins interact in transfected cells and that the endogenous proteins also interact in brain. In both cases the amounts of co-immunoprecipitated protein were low,
suggesting that only a minor proportion of FAK is associated with PIAS1.

**FAK Is Modified by SUMO-1 in the Presence of PIAS1—**
PIAS1 has been shown to interact with SUMO-1 and its E2 conjugase, Ub9, leading to the SUMO modification of several proteins including p53 (25, 37, 38). SUMO is a small protein that can be linked covalently to the ϵ-amino group of a lysine residue on a substrate protein via an enzymatic pathway resembling that of ubiquitination, although sumoylation appears to play multiple roles that are distinct from those of ubiquitination (39). We explored the possibility that PIAS1 can function as an E3 SUMO ligase toward FAK, using COS-7 cells co-transfected with FAK and increasing amounts of PIAS1. Co-transfection of SUMO-1 was needed in these experiments, because COS-7 cells contain very small amounts of unconjugated endogenous SUMO-1 (39). N-Ethylmaleimide, a cysteine alkylating agent that inhibits SUMO-deconjugating enzymes (40, 41), was added to the lysis buffer to stabilize sumoylated proteins. Sumoylation of FAK, which migrates normally as a 125-kDa band, was investigated by examining the appearance of an immunoreactive band with an increased molecular mass corresponding to the FAK-SUMO conjugate (Fig. 3). A FAK-immunoreactive band migrating at about 145 kDa appeared when the amount of co-transfected PIAS1 was increased in a dose-dependent manner (Fig. 3A). The 145-kDa band was also immunoreactive with an anti-SUMO monoclonal antibody (Fig. 3B, lanes 3–5), indicating that it corresponded to sumoylated FAK. To unequivocally establish that the high molecular mass band corresponded to sumoylated FAK, transfected cell extracts were immunoprecipitated with an anti-FAK serum and immunoblotted with anti-FAK (Fig. 3C) and anti-SUMO-1 monoclonal antibodies (Fig. 3D). The 145-kDa protein was immunoprecipitated as efficiently as FAK protein and detected by both anti-FAK (Fig. 3C, lanes 3–5) and anti-SUMO-1 antibodies (Fig. 3D, lanes 3–5), confirming that it corresponded to SUMO-1-modified FAK. The migration of the SUMO-modified FAK was consistent with the addition of a single SUMO-1 molecule. These data demonstrate that in transfected cells FAK is sumoylated in the presence of PIAS1, which most probably functions as an E3 SUMO ligase.

**PIAS1 Induces Sumoylation of FAK at Lys-152—**
Sumoylation of proteins occurs at specific lysine residues, in most cases embedded in a consensus sequence, ΨKXE (42), in which Ψ is any amino acid. In some cases Glu (E) is replaced by Asp (D) (43, 44). We found in the FAK sequence a single matching motif surrounding Lys-152 (Fig. 4A). It is remarkable that this motif was conserved, not only among FAK proteins of different vertebrate species in which the sequence identity is very high, but also in *Drosophila* and Anopheles, in which there is much less overall conservation in the N terminus (Fig. 4A). To examine whether Lys-152 was the acceptor site for conjugation of SUMO, we mutated this residue to arginine. The mutant FAK protein, FAK K152R, was not modified by SUMO-1 (Fig. 4B, lane 4). Even longer exposure times failed to reveal any traces of SUMO-1-modified FAK, indicating that Lys-152 was the only sumoylation site. Mutation of Lys-152 prevented the attachment of SUMO-1 to FAK but not its interaction with PIAS1, because FAK K152R...
and wild type FAK were similarly able to interact with GST-PIAS1 (Fig. 4C, lanes 2 and 3). PYK2, a tyrosine kinase closely related to FAK (45% sequence identity), but which lacks a lysine corresponding to Lys-152 (Fig. 4A) did not interact with PIAS1 in yeast two hybrid (see above), was not sumoylated in COS-7 cells (data not shown). Altogether, these results demonstrated that PIAS1 induces the sumoylation of FAK on Lys-152, a site conserved in FAK molecules but absent from PYK2.

Sumoylated FAK Is Enriched in the Nuclear Fraction and Sumoylation Is Independent of Its Autophosphorylation and Kinase Activity—Sumoylation occurs mainly in the nucleus (39). In transfected COS-7 cells, PIAS1, a predominantly nuclear protein (45), was almost exclusively found in the nuclear soluble fraction (Fig. 4D, lane 5). The bulk of sumoylated-FAK was also in this nuclear fraction (Fig. 4D, lane 5), suggesting that sumoylation of FAK occurred in the nucleus, as reported for most sumoylated proteins (39).

Because FAK is a tyrosine kinase regulated by autophosphorylation, we examined whether phosphorylation and/or kinase activity were important for its sumoylation. Neither autophosphorylation of FAK nor its kinase activity was required for its sumoylation, as shown by the study of FAK molecules with mutations Y397F, K454R (kinase dead mutant), or both (Fig. 4E, lanes 4, 6, and 8). In addition, splice variants of FAK with an increased capacity to undergo autophosphorylation (36) were sumoylated to the same extent as the standard isoform (data not shown). Taken together, these results show that conjugation of SUMO-1 to FAK is a process independent of its autophosphorylation and catalytic activity.

Sumoylation of FAK Increases Its Autophosphorylation—Sumoylation has been reported to exert various effects on substrate proteins. It can modulate protein-protein interactions (46), compete ubiquitination if both occur on the same residue (47, 48), control subcellular localization (49), or alter the biological activity of transcription factors such as p53, c-Jun, or Sp3 (25, 26, 50–52) or of enzymes including HDAC1 (53). Because the autophosphorylation of FAK is an essential step in its activation and function, we examined whether it was altered by SUMO-1 modification. We compared the level of phosphorylation of sumoylated and nonsumoylated FAK in COS-7 cells co-transfected with HA-PIAS1 and SUMO-1 using an antibody that specifically recognizes the phosphorylated form of Tyr-397 (Fig. 5A). For various proportions of sumoylated FAK (Fig. 5A, left panel), a markedly higher Tyr-397 phosphorylation signal was detected in FAK-SUMO as compared with FAK (Fig. 5A, right panel). Binding of PIAS1 by itself did not alter autophosphorylation, because FAK K152R, which binds PIAS1 but is not sumoylated (see above), gave a similar pattern of autophosphorylation (Fig. 5B, left panel).
ylation as wild type FAK (Fig. 5B). Because sumoylation did not depend on phosphorylation (see above), these results show that sumoylation of FAK increased the phosphorylation level of Tyr-397 in intact cells. FAK autoprophosphorylation on Tyr-397 is known to create a high affinity binding site for the Src homology 2 domain of Src and Fyn (11). These kinases phosphorylate other tyrosine residues in FAK, including Tyr-576 and Tyr-577 in the activation loop of the kinase domain, thereby enhancing intermolecular phosphorylation of Tyr-397 (54). However, the effects of FAK sumoylation did not appear to involve Src family kinases, because they were unchanged in the presence of an inhibitor of these enzymes, and phosphorylation of Tyr-577 in sumoylated FAK was low (data not shown). Increased phosphorylation levels of Tyr-397 in cells could result from stimulation of its phosphorylation or inhibition of its dephosphorylation. To gain further insight into the mechanistic enhancement of phosphorylation of FAK-SUMO, we performed an in vitro kinase assay. FAK-SUMO and FAK were immunoprecipitated, and the immune complexes were dephosphorylated by the action of GST-PTP-β tyrosine phosphatase and incubated in the presence of [γ-32P]ATP (30). After SDS-PAGE, proteins were transferred onto nitrocellulose and analyzed by immunoblotting for FAK and by autoradiography (Fig. 5C, left and middle panels). The amount of 32P incorporated in FAK-SUMO was higher than in nonsumoylated FAK (438 ± 58%, mean ± S.E., n = 14, p < 0.002. Student t test, cpm corrected for the amounts of the corresponding protein). Immunoblotting of the membrane with P-Tyr-397 antibody confirmed that the increased incorporation of 32P in sumoylated FAK correlated with high levels of immunoactivity for P-Tyr-397 (Fig. 5C, right panel). Thus, the increased level of phosphorylation of FAK-SUMO on Tyr-397 compared with FAK can be accounted for by an increase in its intrinsic ability to autophosphorylate. In contrast, sumoylation only moderately increased the activity of FAK toward an exogenous substrate, poly-Glu-Tyr (4:1) in vitro (FAK 613 ± 37 cpm, sumoylated FAK 1010 ± 28 cpm, n = 3, p < 0.001). Although the mechanisms of activation of FAK autophosphorylation by the conjugation of SUMO are not known, they may involve a modulation of the regulatory role of the N-terminal FERM domain and/or a facilitation of the intermolecular interactions (22, 23).

**Sumoylation Promotes Autophosphorylation of Endogenous FAK Independently of Cell Adhesion**—When we examined cells transiently expressing PIAS1 and SUMO-1, we found that a significant proportion of endogenous FAK was sumoylated (Fig. 5C). As a consequence of transfecting FAK, endogenous sumoylated FAK was enriched in the nuclear fraction (data not shown). Because FAK activation is promoted by integrin engagement and is cell adhesion-dependent (5), we examined the consequences of adhesion on sumoylation. The amount of endogenous FAK in the sumoylated form was similar in attached or suspended cells in the presence of PIAS1 and SUMO-1 (Fig. 5E, left panel). Moreover, whereas Tyr-397 of unsumoylated FAK was markedly dephosphorylated following cell suspension, autophosphorylation of SUMO-FAK was unchanged (Fig. 5E, right panel). These results indicate that sumoylation of FAK and the resulting increased autophosphorylation occur and/or persist independently of cell adhesion.

**Possible Functional Implications of FAK Interaction with PIAS1 and Sumoylation**—Our data revealing that PIAS1 interacts with FAK and triggers its sumoylation, presumably in the nucleus, are surprising since FAK is a cytoplasmic protein that has a well-characterized function at the plasma membrane. However, the presence of truncated forms of FAK and of the full-length protein in the nucleus has already been reported, suggesting that FAK may have a role in the nucleus under some circumstances (55, 56). An attractive hypothesis would thus be that FAK undergoes a nucleocytoplasmic cycling that allows its nuclear sumoylation in the presence of PIAS1. Interestingly, the related protein PYK2 has recently been shown to undergo a regulated accumulation in the nucleus (57, 58), although its mechanisms remain to be established. Similarly, it will be important to determine whether the fraction of FAK in the nucleus can be regulated by post-translational modifications and/or interaction with other proteins, including PIAS1. Unfortunately little is known about the regulation of PIAS1 expression or activity. A number of proteins associated with plasma membrane adhesion receptors are known to undergo a regulated translocation to the nucleus and to alter nuclear function (59, 60). It is tempting to speculate that FAK may have a similar function and could represent the first example of a tyrosine kinase capable to signal at focal adhesions as well as in the nucleus. Because autophosphorylation is the critical step in FAK activation and because sumoylation stimulates dramatically FAK autophosphorylation, PIAS1-catalyzed sumoylation of FAK might represent an additional regulatory step controlling the function of FAK in the nucleus. To establish this function it will be critical to identify possible targets of FAK in the nucleus or possibly in its vicinity (61). In summary, our results show that FAK can be sumoylated in the presence of PIAS1, presumably following nuclear translocation, suggesting that FAK may play a novel role in signaling between the plasma membrane and the nucleus.

**Acknowledgments**—We thank Jacques Camonis for the gift of yeast two-hybrid plasmids, advice, and stimulating discussions, Jiann-An Tan for the pSG5-PIAS1 plasmid, Jacob Seeler for pSG5-SUMO-1, Alexandre Maucuer and André Sobel for stathmin antibodies, and Sylvie Clain for assistance in figure preparation.

REFERENCES

1. Schaller, M. D., Borgman, C. A., Cobb, B. S., Vines, R. H., Reynolds, A. B., and Parsons, J. T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5192–5196
2. Hans, S. K., Calalb, M. B., Harper, M. C., and Patel, S. K. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8487–8491
3. Hans, S. K., and Polte, T. R. (1997) Bioessays 19, 137–145
4. Parsons, J. T., Martin, K. H., Slack, J. K., Taylor, J. M., and Weed, S. A. (2000) Oncogene 19, 5606–5613
5. Schaller, M. D. (2001) Biochem. Biophys. Acta 1540, 1–21
6. Girault, J. A., Costa, A., Derkindenh, P., Studler, J. M., and Toutant, M. (1999) Trends Neurosci. 22, 257–263
7. Ito, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobe, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., and Yamamoto, T. (1995) Nature 377, 539–544
8. Frisch, S. M., Vucur, K., Ruoslahti, E., and Chan-Hui, P. Y. (1996) J. Cell Biol. 134, 783–799
9. Sieg, D. J., Hauck, C. R., and Schlaepfer, D. D. (1999) J. Cell Sci. 112, 2577–2580
10. Isha, D. A., Mitra, S. K., Hauck, C. R., Strehlow, D. N., Nelson, J. A., Ilic, D., Huang, S., Li, E., Nemirov, G. R., Leng, J., Spencer, K. S., Chersah, D. A., and Schlaepfer, D. D. (2003) J. Cell Biol. 160, 753–767
11. Cobb, B. S., Schaller, M. D., Leu, T. H., and Parsons, J. T. (1994) Mol. Cell. Biol. 14, 147–155
12. Han, D. C., and Guan, J. L. (1999) J. Biol. Chem. 274, 24425–24430
13. Chen, H. C., and Guan, J. L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10148–10152
14. Akagi, T., Muruta, K., Shishido, T., and Hanausaa, H. (2002) Mol. Cell. Biol. 22, 7015–7023
15. Calalb, M. B., Polte, T. R., and Hans, S. K. (1995) Mol. Cell. Biol. 15, 954–963
16. Schlaepfer, D. D., Jones, K. C., and Hunter, T. (1998) Mol. Cell. Biol. 18, 2571–2585
17. Hildebrand, J. D., Schaller, M. D., and Parsons, J. T. (1993) J. Cell Biol. 123, 993–1005
18. Girault, J. A., Labesse, G., Morrow, J. P., and Callebaut, I. (1999) Trends Biochem. Sci. 24, 54–57
19. Poulet, P., Gautreau, A., Gadara, G., Girault, J. A., Louvard, D., and Arpin, M. (2001) J. Biol. Chem. 276, 57696–57701
20. Schaller, M. D., Otey, C. A., Hildebrand, J. D., and Parsons, J. T. (1995) J. Cell Biol. 120, 1181–1187
21. Eide, B. L., Turck, C. W., and Escobedo, J. A. (1995) Mol. Cell. Biol. 15, 2827–2837
22. Chan, P. Y., Kanner, S. B., Whitney, G., and Aruffo, A. (1994) J. Biol. Chem. 269, 20567–20574
23. Toutant, M., Costa, A., Studler, J. M., Kadare, G., Carmona, M., and Girault, J. A. (2002) Mol. Cell. Biol. 22, 7731–7743
24. Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D., and Shuai, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10626–10631
25. Schmidt, D., and Muller, S. (2002) Proc. Natl. Acad. Sci. U. S. A. **99**, 2872–2877
26. Sapetschnig, A., Rischitor, G., Braun, H., Doll, A., Schergaut, M., Melchior, F., and Suske, G. (2002) *EMBO J.* **21**, 5206–5215
27. Derkinderen, P., Toutant, M., Burgaya, F., Le Bert, M., Siciliano, J. C., de Francis, V., Gelman, M., and Girault, J. A. (1996) *Science* **273**, 1719–1722
28. Siciliano, J. C., Toutant, M., Derkinderen, P., Sasaki, T., and Girault, J. A. (1996) *J. Biol. Chem.* **271**, 28720–28725
29. Kagey, M. H., Melhuish, T. A., and Wotton, D. (2003) *Cell* **113**, 127–137
30. Toutant, M., Studler, J. M., Burgaya, F., Costa, A., Ezan, P., Gelman, M., and Girault, J. A. (2000) *Biochem. J.* **348**, 119–128
31. Shuai, K. (2000) *Oncogene* **19**, 2638–2644
32. Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F., and Grosschedl, R. (2001) *Genes Dev.* **15**, 3088–3103
33. Melchior, F. (2000) *Annu. Rev. Cell Dev. Biol.* **16**, 591–626
34. Li, S. J., and Hochstrasser, M. (1999) *Nature* **398**, 246–251
35. Gong, L., Kamitani, T., Millas, S., and Yeh, E. T. (2000) *J. Biol. Chem.* **275**, 14212–14216
36. Hochstrasser, M. (2001) *Cell* **107**, 5–8
37. Johnson, E. S., andBlobel, G. (1999) *J. Cell Biol.* **147**, 981–994
38. Buschmann, T., Fuchs, S. Y., Lee, C. G., Pan, Z. Q., and Ronai, Z. (2000) *Cell* **101**, 755–762
39. Shuai, K., Ma, H., and Firtel, R. A. (2002) *Dev. Cell* **2**, 745–756
40. Muller, S., Berger, M., Lehembre, F., Seeler, J. S., Haupt, Y., and Dejean, A. (2000) *J. Biol. Chem.* **275**, 13321–13329
41. Rodriguez, M. S., Destro, J. M., Lain, S., Midgley, C. A., Lane, D. P., and Hay, R. T. (1999) *EMBO J.* **18**, 6455–6461
42. Buschmann, T., Fuchs, S. Y., Lee, C. G., Pan, Z. Q., and Ronai, Z. (2000) *Cell* **101**, 755–762
43. Lambert, A., Basile, G., and Matunis, M. J. (2002) *J. Biol. Chem.* **277**, 42981–42986
44. Kotaja, N., Karvonen, U., Janne, O. A., and Palvimo, J. J. (2002) *Mol. Cell.* **Biol.** **22**, 5222–5234
45. Desterro, J. M., Rodriguez, M. S., and Hay, R. T. (1998) *EMBO J.* **17**, 5206–5215
46. Matsui, M. J., Wu, J., and Blobel, G. (1998) *J. Cell Biol.* **140**, 499–509
47. Destro, J. M., Rodriguez, M. S., and Hay, R. T. (1998) *Mol. Cell* **2**, 233–239
48. Matunis, M. J., Wu, J., and Blobel, G. (1998) *J. Cell Biol.* **140**, 499–509
49. Johnson, E. S., and Blobel, G. (1999) *J. Cell Biol.* **147**, 981–994
50. Rui, H. L., Fan, E., Zhou, H. M., Xu, Z., Zhang, Y., and Lin, S. C. (2002) *J. Biol. Chem.* **277**, 42981–42986
51. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2000) *J. Biol. Chem.* **275**, 13321–13329
52. Rodriguez, M. S., Desterro, J. M., and Hay, R. T. (1999) *EMBO J.* **18**, 6455–6461
53. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2000) *J. Biol. Chem.* **275**, 13321–13329
54. Melchior, F. (2000) *Annu. Rev. Cell Dev. Biol.* **16**, 591–626
55. Melchior, F. (2000) *Annu. Rev. Cell Dev. Biol.* **16**, 591–626
56. Hofmann, K., and Stoffel, M. (1999) *Science* **283**, 1669–1672
57. Matunis, M. J., Wu, J., and Blobel, G. (1998) *J. Cell Biol.* **140**, 499–509
58. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2000) *J. Biol. Chem.* **275**, 13321–13329
59. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2000) *J. Biol. Chem.* **275**, 13321–13329
60. Aoto, H., Sasaki, H., Ishino, M., and Sasaki, T. (2000) *J. Biol. Chem.* **275**, 13321–13329
61. Xie, Z., Sanada, K., Samuels, B. A., Shih, H., and Tsai, L. H. (2003) *Cell* **114**, 469–482
62. Sobel, A., Baturin, M. C., Beretta, L., Cnheyweiss, H., Doye, V., and Peyro-Saint-Paul, H. (1989) *J. Biol. Chem.* **264**, 3765–3772