The integrin β subunit cytoplasmic domains are important for activation-dependent cell adhesion and adhesion-dependent signaling events. We report an interaction between integrin β subunit cytoplasmic domain and Rack1, a Trp-Asp (WD) repeat protein that has been shown to bind activated protein kinase C. The Rack1-binding site on integrin β2 subunit resides within a conserved, membrane-proximal region. In the yeast two-hybrid assay, WD repeats five to seven of Rack1 (Rack1-WD5/7) interact with integrin β1, β2, and β2 cytoplasmic domain. In eukaryotic cells, Rack1 co-immunoprecipitates with at least two different β integrins, β1 integrins in 293T cells and β2 integrins in JY lymphoblastoid cells. Whereas Rack1-WD5/7 binds integrins constitutively, the association of full-length Rack1 to integrins in vivo requires a treatment with phorbol esters, which promotes cell spreading and adhesion. These findings suggest that Rack1 may link protein kinase C directly to integrins and participate in the regulation of integrin functions.

Integrins are αβ heterodimeric adhesion receptors that mediate attachment of cells to the extracellular matrix and specific cell counter-receptors (1). Various extracellular stimuli have been shown to affect the adhesiveness of integrins and regulate attachment of cells to the extracellular matrix (2). This process known as an activation-dependent cell adhesion is best illustrated in leukocytes where the attachment of integrin LFA1 (αMβ2) to intercellular cell adhesion molecule-1 (ICAM-1) substrates can be promoted by cross-linking T cell receptors or stimulating cells with phorbol 12-myristate 13-acetate (PMA) (3). Upon binding to the extracellular matrix, integrins induce signals required for the reorganization of actin cytoskeleton and the formation of focal adhesion complexes (4–6). The adhesion-dependent clustering of integrins leads to the activation of nonreceptor tyrosine kinase focal adhesion kinase and Ras/mitogen-activated protein kinase pathway, the stimulation of inositol lipid metabolism, an increase in intracellular Ca²⁺ and pH, and the activation of PKC (4, 7, 8).

Each subunit of integrins consists of a large extracellular ligand-binding domain, a transmembrane domain, and a short cytoplasmic domain that lacks any enzymatic activity. Although the cytoplasmic domains of α subunits are variable in size and sequence, the cytoplasmic domains of β subunits are more conserved in size and sequence. In particular, three conserved regions, termed cyto-1, cyto-2, and cyto-3, found in β integrin cytoplasmic domains have been implicated in the recruitment of integrins to the focal adhesion plaques and the regulation of adhesive functions of integrins (9–11). Although both integrin subunits are required for the ligand binding, the interaction between intracellular proteins and integrin cytoplasmic domain can occur in the absence of subunit association. Studies have shown that chimeric molecules composed of the β integrin cytoplasmic domains and the extracellular domain of the interleukin-2 receptor can be directed to the focal adhesion plaques and activate focal adhesion kinase in the absence of ligand binding (12). Based on these findings, it has been hypothesized that the integrin β subunit cytoplasmic domain provides binding sites for proteins involved in the regulation of integrin functions. Proteins that have been shown to directly interact with β subunit cytoplasmic domains include known cytoskeletal proteins α-actinin (13), paxillin (14), talin (11, 15), and filamin (16); protein kinases focal adhesion kinase (14) and integrin-linked kinase-1 (ILK-1) (17); and potential regulatory proteins such as β3-endonexin (18), cytobasin-1 (19), and integrin cytoplasmic domain-associated protein-1 (lcap-1) (20).

In this study, we report a direct association of receptor for activated protein kinase C (Rack1) to the integrin β subunit cytoplasmic domain. Rack1 consists of seven repeating units of WD motifs presumed to be involved in protein-protein interaction (2, 21). Deletion studies indicate that the C-terminal three WD motifs (WD-5, WD-6, and WD-7) of Rack1 interact with the conserved membrane-proximal region of β subunit cytoplasmic domain. Interestingly, interaction of Rack1 with integrins in vivo requires a treatment with PMA, which promotes cell spreading and integrin-dependent cell adhesion (3, 22). Our finding suggests a direct linkage between integrins and PKC through Rack1 and further implicates PKC in integrin-mediated cell signaling.

**MATERIALS AND METHODS**

**Antibodies and Cell Lines**—The anti-β integrin antibody producing hybridoma cell line, TS2/16, was generously provided by Dr. M. Hemler (Dana Farber Cancer Institute, Boston, MA). The mouse hybridoma cell line TS1/18 producing anti-β integrin antibody was obtained from the ATCC (Rockville, MD). The mAb TS2/16 and TS1/18 recognize the extracellular domains of β1 and β2 integrins, respectively. Anti-Rack1 antibody was purchased from Transduction Labs. The anti-αL antibody, directed against the integrin αL cytoplasmic domain, was described previously (20).

293T cells, a derivative of human kidney embryonal fibroblast containing SV40 T antigen, were obtained from Dr. K. Shuai (University of California at Los Angeles) and cultured in Dulbecco’s modified Eagle’s...
medium + 5% fetal calf serum (BioWhittaker). JY cells, an Epstein-Barr virus-transformed human lymphoid cell line, were obtained from Dr. T. Springer (Harvard Medical School, Boston, MA) and cultured in RPMI + 10% fetal calf serum.

**Yeast Genetic Screening**—The yeast genetic screening for the isolation of proteins interacting with the cytoplasmic domain of β2 integrin was carried out essentially as described previously (20, 23). The C-terminal 46 amino acids (724–769) of β2 integrin were cloned in frame into LexA coding sequence to generate a “bait” plasmid, pNlex-2cyto. pNLex-β2cyto, pNLex-β2cyto and a JY cDNA library in the pJG45 yeast expression vector (23) were introduced into a yeast strain containing a chromosomal copy of the Leu2 gene (EGY48 (Matα trp1 ura3-52 leu2::pLeu2-lexAop6(UAS leu2)) and an episomal β-galactosidase gene (as a JK103 plasmid) under the control of a synthetic promoter with LexA-binding sites (23). Yeast transformants were selected for the formation of blue colonies on 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside indicator plates with leucine. The interacting cDNA clones were rescued from the selected yeast transformants as described previously (23).

Rack1-ΔBH1 was constructed by cloning the C-terminal BamHI fragment of Rack1 (amino acids 204–317) into the pGJ45 vector (23). The β2 and β1 integrin cytoplasmic domain and the β2 integrin cytoplasmic domain mutants were generated by polymerase chain reaction and cloned into the pNLex vector. All constructs used in this study were verified by direct DNA sequence analysis.

**In Vitro Interaction Assay**—For in vitro GST interaction assays, the β2 integrin cytoplasmic domain (amino acids 724–769), a truncation mutant β2(E18) (amino acids 750–769), and the αc subunit cytoplasmic domain cytoplasmic domain were individually expressed as GST fusion proteins using the bacterial expression vector pGEX4T1 (Pharmacia Biotech Inc.) (24). Rack1-WD5/7 was synthesized in vitro using a co-transcription/translation system (Promega) and added to approximately 2 μg of GST fusion proteins and incubated overnight at 4 °C in NET (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 2 mM MgCl2) containing 0.5% Triton X-100 in NET. The bound proteins were eluted by boiling in SDS sample buffer, subjected to SDS/polyacrylamide gel electrophoresis, and then transferred to a nitrocellulose membrane (Bio-Rad). The filter membrane was probed with an anti-Rack1 antibody (0.06 μg/ml) and then developed using an enhanced chemiluminescence method (Amersham Corp.).

**Eukaryotic Expression and in Vivo Interaction Assay**—A full-length Rack1 cDNA was obtained from a JY cDNA library using a polymerase chain reaction and cloned into the eukaryotic expression vectors pcDNA3 (Invitrogen) and pEBG (20). 5–10 μg of plasmid DNA was transfected into 293T cells by using a calcium phosphate precipitation method (24). 48 h post-transfection, cells were lysed in TBSM (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, and 2 mM MgCl2 containing 0.5% Nonidet P-40, leupeptin, aprotnin, and phenylmethysulfonyl fluoride. When indicated, the cells were treated with PMA (100 ng/ml, stock solution 1 mg/ml in Me2SO) for 30 min at 37 °C prior to lysis. Detergent insoluble materials were removed by centrifugation at 12,000 × g for 15 min. 500 μg of the cleared lysates were mixed with an equal volume of TBSM and incubated with glutathione-Sepharose beads for 3 h at 4 °C. Beads were washed with TBSM + 0.25% Nonidet P-40 once and TBSM alone three times. Co-precipitation of β2 integrins with the bound GST-Rack1 was analyzed by immunoblotting with the mAb TS2/16 (anti-β2 integrin). Immuno precipitation of β2 integrins was carried out by incubating the lysates with 4 μg/ml of the mAb TS2/16 for 3 h at 4 °C, in the presence of protein G-agarose beads (Life Technologies, Inc.). JY cell lysates were prepared as above, and LFA1 was immunoprecipitated using the mAb TS 1/18 (anti-β2 integrin). Co-immunoprecipitation of Rack1 with the integrins was analyzed by immunoblotting using an anti-Rack1 antibody.

**RESULTS**

**Rack1 Interacts with the Integrin β Subunit Cytoplasmic Domain**—The yeast two-hybrid assays were used to identify cellular proteins that interact with the cytoplasmic domain of β2 integrin. A bait construct, pNLex-β2cyto, which contains the entire cytoplasmic domain (amino acids 724–769) of β2 subunit was introduced into EGY48, a yeast strain harboring Leu2 and β-galactosidase genes under the control of a synthetic promoter with LexA-binding sites (23). The resulting strain was used as a host for transformation with a human B cell (JY cells) cDNA library in the pJG45 yeast expression vector. From ~8 × 105 primary transformants, four overlapping cDNAs were found to interact with the cytoplasmic domain of β2 integrin but not with the control baits containing unrelated sequences (data not shown). Sequence analysis revealed that all four clones encode overlapping regions of the C-terminal portion of the heterotrimeric Gβ, superfamily protein, Rack1.

**WD Domain 5–7 of Rack1 Interacts with the Membrane-proximal Region of Integrin β Subunit Cytoplasmic Domain**—Rack1 is composed of 7 repeating units of WD motif (21). Like Gβ proteins, Rack1 is expected to form a β-propeller structure, where each WD repeat composed of four anti-parallel β strands make up a blade of the propeller (25–27). The four overlapping Rack1 cDNA clones isolated in yeast two-hybrid screening all contained WD-5 through WD-7, suggesting these three C-terminal WD repeats are sufficient for binding to the β2 integrin cytoplasmic domain (Fig. 1). A deletion of 22 additional amino acids corresponding to the N-terminal portion of WD-5 (Rack1-ΔBH1) completely abolished the interaction. Therefore, we conclude that WD-5 is necessary for the integrin binding. The 22 amino acids deleted in Rack1-ΔBH1 includes the fourth β strand and the loop that are located on the solvent exposed outer surface of the β-propeller (21, 26, 27). Interestingly none of the isolated Rack1 clones contained the WD motifs N-terminal to WD-5. The full-length Rack1 failed to interact with the baits containing the β integrin cytoplasmic domains in yeast, raising a possibility that the interaction between the full-length Rack1 and the β integrins may be regulated.
Inducible Binding of Rack1 and β Integrins

| Bait       | Sequences                                      | Interaction |
|------------|------------------------------------------------|-------------|
| β1         | LLTVNLALHLSDLKELGKLQKQDENFLFSATTTVVPSKFAES    | +           |
| β2A        | KALDLHSLAYRELFEKELQKQDENFLFSATTTVVPSKFAES     | +           |
| β2B        | HLSGRELAEKELQKQDENFLFSATTTVVPSKFAES           | -           |
| β2ALI/EEE  | Keesfllskayrekepelqkqdenflefsatttvvpskfaes    | -           |
| β2Δ744     | KALDLHSLAYRELFEKELQKQDENFLFSATTTVVPSKFAES     | +           |
| β2(E16)    | DNLPSFALSTTVVPSKFAES                           | -           |
| β3         | KLNNRIRDEFRKGLQPQEEGRRSARYSANNLYPKPSSTRTAVYFTESYNKNTVD + |

Fig. 2. Interaction between Rack1 and mutant integrin β subunit constructs. The ability of Rack1-WD5/7 to bind various integrin β subunit constructs in the yeast two-hybrid assay is summarized. A truncated β2 cytoplasmic domain corresponding to amino acid positions 724–743 (underlined) interacted with Rack-WD5/7 as well as the intact β2 cytoplasmic domain.

In a converse set of experiments we delineated the Rack1-binding site on the β2 integrin cytoplasmic domain by testing the interaction between Rack1-WD5/7 (amino acids 182–317) and various β2 integrin cytoplasmic domain mutants (Fig. 2). A C-terminal deletion of the β2 integrin cytoplasmic domain up to amino acid 744 (β2Δ744), which removes the major phosphorylation sites at Ser\(_{743}\) and three adjacent threonines at 758–760, did not affect the binding of Rack1-WD5/7. However, a small deletion of 4 amino acids in the membrane-proximal portion of β2 integrin cytoplasmic domain (β2B) or a replacement of the corresponding KALI to KEEE (β2ALI/EEE) completely abolished the interaction, suggesting the Rack1-binding site resides within a 20-amino acid region (amino acids 724–743) of the β2 integrin cytoplasmic domain. The membrane-proximal region of the β integrin cytoplasmic domains is highly conserved, and not surprisingly, Rack1-WD5/7 was able to interact with two other integrins, β1 and β3.

Rack1-WD5/7 Binds Integrin β2 Cytoplasmic Domain in Vitro—To confirm the interaction between integrin β subunit and Rack1 observed in yeast, Rack1-WD5/7 was synthesized in vitro and incubated with GST fusion proteins containing the β2 integrin cytoplasmic domains (Fig. 3). Rack1-WD5/7 associated with a GST fusion protein encoding the complete cytoplasmic domain of integrin β2 (GST-β2A, amino acids 724–749, Fig. 3, lane 2) but not with GST fusion proteins encoding truncated forms of integrin β2 cytoplasmic domain (GST-β2B, amino acids 728–769, and GST-β2(E16), amino acids 750–769, Fig. 3, lanes 1 and 3) or a GST fusion protein encoding the cytoplasmic domain of integrin α\(_{c}\) (Fig. 3, lane 4). The results of in vitro binding assays were identical to the findings in the yeast two-hybrid interaction studies.

Integrins Form an Inducible Complex with Full-length Rack1 in Vivo—To demonstrate the interaction of Rack1 and integrins in vivo, Rack1-WD5/7 or a full-length Rack1 were expressed as GST fusion proteins in 293T cells. The transfected cells were lysed in a buffer containing nonionic detergent and incubated with glutathione-Sepharose beads to purify GST-Rack fusion proteins. The binding of β2 integrins to the GST-Rack fusion proteins were analyzed by immunoblotting with the mAb TS2/16 (anti-β2). The β2 integrins were co-precipitated with GST-Rack1-WD5/7 (Fig. 4A, lane 2) but not with GST alone used as the controls (Fig. 4A, lane 1). Expression of equivalent amounts of the GST fusion proteins in 293T cells was confirmed by immunoblotting with anti-GST antibody (Fig. 4A, bottom panel).

In two-hybrid screening, only partial Rack1 clones, none of which contained the WD motifs N-terminal to the WD-4, were isolated. We subsequently confirmed that the β2 integrin cytoplasmic domain does not interact with the full-length Rack1 in yeast (see Fig. 1). These results indicate that the presence of Rack1 WD-1 to WD-3 may interfere with the integrin binding. In view of the previous report that Rack1 binds PKC only in the presence of Ca\(^{2+}\) and lipid to activate PKC (21), we reasoned that the binding of full-length Rack1 to integrins in vivo is conditional and may require activation of PKC. As in yeast, we failed to detect association of integrins with the transfected GST-Rack1 in 293T cells (Fig. 4A, lane 4). However, upon stimulation with PMA, a complex formation between the β2 integrins and GST-Rack1 became readily detectable (Fig. 4A, lane 5).
Inducible Binding of Rack1 and β Integrins

In a converse set of experiments, 293T cells were transfected with pcDNA3/Rack1, and the complex formation between Rack1 and β1 integrins was tested by immunoprecipitation with the mAb TS2/16 (anti-β1) followed by a Western analysis using anti-Rack1 antibody (Fig. 4B). As suggested in GST interaction assays, Rack1 and β1 integrins formed a complex following a PMA treatment (Fig. 4B, lane 5). In the unstimulated state, there was no detectable association between the two (Fig. 4B, lane 6). The amount of background Rack1 precipitated by control antibodies did not change significantly upon PMA stimulation (Fig. 4B, lanes 3 and 4). Furthermore, focal adhesion kinase or Paxillin, two proteins known to co-localize with integrins to the focal adhesion plaques, were not detected in the immune complex formed by anti-β1 antibody (data not shown).

To demonstrate that Rack1 can associate with different β subunits, as suggested in the yeast two-hybrid assay, we conducted the immune complex analysis in JY cells that expresses LFA1 (αLβ2). The association of Rack1 with LFA1 can be clearly seen only after PMA treatment (Fig. 5, lane 1 and 2). Immunoprecipitation of equivalent amounts of LFA1 was confirmed by re-probing the blot with anti-αL antibody (Fig. 5, bottom panel). These results clearly demonstrate a specific association of Rack1 with different β integrins in response to a stimulus that is known to enhance integrin-mediated cell adhesion.

**DISCUSSION**

Integrins have been proposed as crucial transmembrane proteins linking cytoskeleton to the extracellular matrix. Numerous studies have demonstrated that integrin-dependent cell adhesion is regulated in response to extracellular stimuli. In platelets, agonist stimulation induces conformational changes in the extracellular ligand-binding domain of the platelet integrin αIIbβ3, leading to an affinity state (7). A similar affinity modulation has not been clearly demonstrated for other integrins. Nonetheless, integrin-dependent cell adhesion of many cell types is enhanced when cell spreading is augmented by a PMA treatment (3, 22, 30). Both of these responses require integrin cytoplasmic domains, suggesting that the interaction between integrins and cellular proteins plays an important role in the regulation of cell adhesion. To gain insights into cell adhesion regulation, we sought to identify cellular proteins that directly associate with integrin β subunit cytoplasmic domains. In the present study, we report isolation of Rack1 as an integrin-binding protein. Our evidence for the interaction of Rack1 with integrins is based on (i) the interaction of the two proteins in a yeast two-hybrid assay, (ii) the binding of *in vitro* translated C-terminal portion of Rack1 to bacterially expressed GST fusion protein containing the β2 integrin cytoplasmic domain, and (iii) the detection of a complex between Rack1 and integrins *in vivo*.

Rack1 is a member of the G protein-coupled receptor (GPCR) superfamily, composed exclusively of seven WD repeats forming an overall β propeller structure (27). WD repeats represent structural motifs formed by four anti-parallel β strands and have been implicated in protein-protein interaction (25). Although the precise function of Rack1 is not known, Rack1 binds to PKC in the presence of Ca²⁺ and lipid *in vitro* and has been proposed as an intracellular receptor for activated protein kinase C responsible for subcellular localization of PKC (21). Overlapping cDNA isolates from initial yeast screening indicated that the WD repeats 5–7 are sufficient for its interaction with β integrins. Rack1-ΔBH1, lacking a part of WD repeat 5, failed to bind β2 integrin. The region deleted in Rack1-ΔBH1 corresponds to the outer exposed surface of the β propeller that would be available to make contacts with other proteins.

The region of the integrin β2 cytoplasmic domain responsible for the Rack1 binding lies within the membrane-proximal 20-amino acid region (residues 724–743). This region is conserved among different β subunits, and not surprisingly, Rack1 interacts with at least two other β subunits, β1 and β3. The presence of three apolar residues immediately following a Lys residue at the membrane-cytosol interface appears to be critical for the Rack1 binding. Either a 4-amino acid deletion in this region or a substitution of KALI to KEEE prevented the Rack1 interaction. Several functions, including localization of β1 integrins to the focal adhesion plaques (9) and affinity regulation of β2 integrins (28), have been assigned to the membrane-proximal region. It will be of interest to determine whether these known integrin functions require Rack1.

The most intriguing finding of our study is that the association between Rack1 and integrins *in vivo* is inducible and requires a PMA treatment. Integrin β subunits are known to undergo phosphorylation on serines and threonines upon PMA treatment (29). We, however, do not favor the possibility that the phosphorylation of β cytoplasmic domain is responsible for the recruitment of Rack1 because Rack1-ΔBH5/7 binds integrins constitutively both *in vitro* and *in vivo*, and the deletion of major phosphorylation sites on β cytoplasmic domain does not affect this interaction. Based on a previously reported function of Rack1 as a cytoplasmic receptor of activated PKC (21), it is conceivable that upon a PMA treatment, activated PKC binds and phosphorylates Rack1, allowing subsequent recruitment of Rack1 to the integrin cytoplasmic domain.

Cell stimulation with PMA or receptor activation frequently increases integrin avidity and promotes cell spreading (22, 30). The functional consequences of integrin-Rack1 association remains to be seen. In addition to Rack1, two other proteins, α-actinin (31) and calreticulin (32), have been shown to bind integrin cytoplasmic domain in an inducible manner. α-Actinin through linking integrins to the actin cytoskeleton may function to modulate integrin avidity. Calreticulin, unlike Rack1 or α-actinin, which bind to β subunit cytoplasmic domains, interacts with α subunits through a high conserved short KXG-FKFR motif (33). The precise function of calreticulin remains to be seen. However, functional knock-out of calreticulin by introducing anti-calreticulin antibody into cells or gene disruption interferes with integrin-dependent cell adhesion (32, 34). The presence of multiple putative protein-protein interaction WD domains may allow Rack1 to function as a scaffold protein to recruit PKC and/or other proteins to the membrane at the site of membrane-cytoskeletal junction by its ability to bind inte-
grin cytoplasmic domain. Rack1 may therefore be an integral player in this membrane-cytoskeletal association and participate in regulation of cell adhesion.

Acknowledgments—We are grateful to R. Brent (Massachusetts General Hospital) for providing the yeast strains and plasmids for the yeast genetic screening. We are also grateful to M. Hemler (Dana-Farber Cancer Institute), T. Springer (Harvard), and K. Shuai (University of California at Los Angeles) for providing reagents and C. Denny for comments on the manuscript.

REFERENCES

1. Hynes, R. O. (1992) Cell 69, 11–25
2. Springer, T. A. (1994) Cell 76, 301–314
3. Dustin, M. L., and Springer, T. A. (1989) Nature 341, 619–624
4. Clark, E. A., and Brugge, J. S. (1995) Science 268, 233–239
5. Yamada, K. M., and Miyamoto, S. (1995) Curr. Opin. Cell Biol. 7, 681–689
6. Burridge, K., and Chrzanowska-Wodnicka, M. (1996) Annu. Rev. Cell Dev. Biol. 12, 463–519
7. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–599
8. Laffranchi, R. M., and Yamada, K. M. (1996) J. Cell. Biochem. 61, 543–553
9. Rezza, A. A., Hayashi, Y., and Herwitz, A. F. (1992) J. Cell Biol. 117, 1321–1330
10. O'Toole, T. E., Ylanne, J., and Culley, B. M. (1995) J. Biol. Chem. 270, 8553–8558
11. Dedhar, S., and Hannigan, G. E. (1996) Curr. Opin. Cell Biol. 8, 657–669
12. LaFlamme, S. E., Thomas, L. A., Yamada, S. S., and Yamada, K. M. (1994) J. Cell Biol. 126, 1287–1298
13. Otey, C. A., Vasquez, G. B., Burridge, K., and Erickson, B. W. (1993) J. Biol. Chem. 268, 21193–21197
14. Schaller, M. D., Otey, C. A., Hildebrand, J. D., and Parsons, J. T. (1995) J. Cell Biol. 130, 1181–1187
15. Herwitz, A., Duggan, K., Buck, C., Beckerle, M. C., and Burridge, K. (1986) Nature 320, 531–533
16. Sharma, C. P., Ezell, R. M., and Arnaout, M. A. (1995) J. Immunol. 154, 3461–3470
17. Hanning, G. E., Leung-Hagesteijn, C., Fritz-Gibbon, L., Coppolino, M. G., Radeva, G., Filmus, J., Bell, J. C., and Dedhar, S. (1996) Nature 379, 91–96
18. Shattil, S. J., O'Toole, T., Eigenthaler, M., Thon, V., Williams, M., Babior, B. M., and Ginsberg, M. H. (1995) J. Cell Biol. 131, 807–816
19. Kalamzade, N., Nagel, W., Schaller, B., Zeitmann, L., Goslars, S., Stockinger, H., and Seed, B. (1996) Cell 86, 233–242
20. Chang, D. D., Wang, C., Smith, H., and Liu, J. (1997) J. Cell Biol. 138, 1149–1157
21. Ron, D., Shen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 839–843
22. Stewart, M. P., Cabanas, C., and Hogg, N. (1996) J. Immunol. 156, 1810–1817
23. Guyon, J., Golemiis, E., Chertkov, H., and Brent, R. (1993) Cell 75, 791–803
24. Ausubel, F. M., Baxis, K., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) Current Protocols in Molecular Biology, pp. 911–919, John Wiley & Sons, Inc., New York
25. Neer, J. E., Schmidt, C. J., Nambudripad, R., and Smith, T. F. (1994) Nature 371, 297–300
26. Lambricht, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) Nature 379, 311–319
27. Wall, M. A., Coleman, D. E., Lee, E., Iniguez-Lluhi, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1995) Cell 83, 1047–1058
28. Hughes, P. E., O'Toole, T. E., Ylanne, J., Shattil, S. J., and Ginsberg, M. H. (1995) J. Biol. Chem. 270, 12411–12417
29. Hibbs, M. L., Lakes, S., Stacker, S. A., Wallace, R. W., and Springer, T. A. (1991) J. Exp. Med. 174, 1227–1238
30. Paul, S. E., Kovach, N. L., Harlan, J. M., and Ginsberg, M. H. (1994) J. Exp. Med. 179, 1307–1316
31. Pavan, P. M., and Ladbrose, M. (1993) J. Immunol. 151, 3795–3807
32. Coppolino, M., Leung-Hagesteijn, C., Dedhar, S., and Wilkins, J. (1995) J. Biol. Chem. 270, 23132–23138
33. Rojiani, M., Finlay, B. B., Gray, V., and Dedhar, S. (1991) Biochemistry 30, 3859–3866
34. Coppolino, G. G., Woodside, M. J., Demeurix, N., Grinstein, S., St-Arnaud, R., and Dedhar, S. (1997) Nature 386, 843–846