ADP-ribosylation of the G Protein Rho Inhibits Integrin Regulation of Tumor Cell Growth*

(Received for publication, August 24, 1995, and in revised form, March 1, 1996)

Taturo Udagawa and Bradley W. McIntyre‡

From the Department of Immunology, The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030

The integrins are a family of adhesion receptors that play a role in the interaction of cells with the extracellular matrix (1, 2). Cellular interactions with the extracellular matrix play a role in diverse processes such as differentiation (3, 4), lymphocyte activation (5), and tumor cell dissemination and metastasis (6). Collagen is a major component of the extracellular matrix that serves as a scaffold for cell binding but also plays a role in cell differentiation and growth (7).

In vitro, interaction of cells with a collagen matrix can induce arachidonic acid production in HeLa cells (8) and glandular differentiation in a human colon carcinoma line (9). Clustering of the α2β1 integrin, a receptor for collagen, induces stimulation of tyrosine phosphorylation and accumulation of GTP bound Ras in a human lymphoblastic cell line (10). The interaction of cells with the extracellular matrix can also regulate growth by creating a permissive effect for the action of mitogens (11, 12). Therefore, specific interaction between integrins and matrix components provide another level of growth regulation. A potential mediator of integrin signaling is the small GTP binding protein Rho. It is a member of the Ras family of GTPases that regulates the formation of actin stress fibers in response to growth factors (13). Integrins can also regulate the formation of actin stress fibers (14), which suggests a convergence of integrin and Rho signaling pathways.

A role in mitogen signaling has been demonstrated for Rho using Val-14 mutations, analogous to Val-12 oncogenic mutations in Ras, which induced transformation of fibroblasts (15). Amplification of Rho by transfection resulted in cells that exhibited increased tumorigenicity, higher saturation density, and reduced serum dependence (16). Mutations in Db1, a guanine exchange factor for Rho, also induced cellular transformation (17, 18). Recent reports have demonstrated the induction of phospholipid levels in untransformed fibroblasts upon adhesion to fibronectin by a mechanism involving Rho. This pathway may provide a mechanism for integrating adhesion with soluble growth factors that generate phospholipid derived second messenger signals (19, 20). These findings suggest a role for Rho in the regulation of both cell growth and cell architecture. A means for elucidating the function of Rho has employed the use of the ADP-ribosylation exoenzyme C3 from Clostridium botulinum. C3 could efficiently modify Rho A, B, and C byribosylation to alter their function, presumably by inhibiting interaction with downstream effectors (21, 22). The other Rho family proteins such as Rac-1 and CDC42 are poor substrates in vitro (23, 24), and therefore C3 provides a sensitive means for elucidating Rho function.

In these studies, we investigated a role for integrins and Rho in growth regulation of a tumor line. Cytin A and cyclin D are regulators of S phase progression (25, 26). They have been implicated in tumorigenesis (25, 27), and cyclin A and D has also been shown to serve as a link between adhesion and cell cycle progression (28). Using a gastric tumor line ST2 whose proliferation was previously shown to be regulated by adhesion (29), we have demonstrated specific β1 integrin-dependent regulation of cyclin A expression, cell cycle progression, and phospholipid synthesis on collagen matrix using monoclonal antibodies that block these events. We also demonstrated the involvement of Rho in this integrin-mediated process by inactivating Rho in tumor cells using C3 exoenzyme. These studies address novel mechanisms relating an integrin and Rho in mediating tumor growth in response to matrix.

MATERIALS AND METHODS

Cell Lines and Reagents—ST2 and ST7 are tumor lines established from separate patients diagnosed with gastric adenocarcinoma. A375 is derived from melanoma, MG-63 from an osteosarcoma, and MCF-7 from a mammary epithelial adenocarcinoma (ATCC, Rockville, MD). HMEC-1 is a human microvascular endothelial cell line immortalized with SV40 large T antigen (30). The astrocytoma 131-IN1 and glioblastoma Fogerty were gifts of Drs. I. J. Fidler and Janet Price, respectively (University of Texas, M. D. Anderson Cancer Center, Houston, TX). All cell lines were maintained in complete RPMI medium supplemented with 10% FBS, 2 mEq l-glutamine, 1 mEq sodium pyruvate, and 100

* This work was supported by National Institutes of Health Grant 62596. 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.

‡ To whom correspondence should be addressed: Dept. of Immunology, Box 180, The University of Texas, M. D. Anderson Cancer Center, Houston, TX 77030. Tel.: 713-792-8739; Fax: 713-745-0846.

1 The abbreviations used are: FBS, fetal bovine serum; PBS, phosphate-buffered saline; polyHEMA, poly(2-hydroxyethyl methacrylate); PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate.
Integrin-mediated Growth Regulation

Propidium Iodide Staining—To analyze cellular DNA content, cells were fixed in ice-cold 70% ethanol for 30 min and then resuspended in PBS containing 50 μg/ml propidium iodide (Sigma) and 25 μg/ml RNase. After 30 min at room temperature, the cells were analyzed on an EPICS Profile (Coulter, Hialeah, FL). The Multicycle program (Phoenix Flow Systems, San Diego, CA) was used to analyze the histograms.

Northern Blot Analysis—Total RNA was extracted by the method of Huang and High (35). Briefly, after transfer of cells to microcentrifuge tubes, 2 × 10⁶ cells were lysed gently in 0.5 ml of solution I containing 2% SDS, 200 mM Tris-HCl, pH 7.5, and 1 mM EDTA at room temperature. 150 μl of ice-cold solution II containing 42.9% (w/v) potassium phosphate and 11.2% (w/v) acetic acid was added, mixed, and placed on ice for 5 min to precipitate DNA and proteins. The insoluble material was pelleted at 20,000 g for 3 min. The supernatant was extracted with phenol/chloroform (1:1) according to standard procedures (36) and precipitated with isopropanol. Approximately 10 μg of total RNA was electrophoresed on formaldehyde gels, transferred to nylon membranes (DuPont NEN) by vacuum transfer (LKB, Bromma, Sweden), and hybridized according to standard procedures (36) with 50 ng of cDNA labeled with [α-³²P]ATP using a random prime labeling kit (Boehringer Mannheim).

Phospholipid Analysis—Cells (2 × 10⁶) were washed three times in phosphate free RPMI (ICN) containing 5% bovine serum albumin supplemented with 2 mM l-glutamine, 1 mM sodium pyruvate and then incubated in the same medium containing 100 μg/ml of [³²P]orthophosphate with PBS added (DuPont NEN) for 1 h at 37°C in a 5% CO₂ humidified incubator. The samples were cooled by placing the tissue culture plates on ice, and adherent cells were treated briefly for 2 min with 0.5% (w/v) trypsin and 0.1% EDTA diluted in PBS. After pelleting in microcentrifuge tubes, the cells were lysed in 180 μl of methanol/1 N HCl (1:1). Chloroform (150 μl) was added and vortexed vigorously for 10 s. The samples were centrifuged at 20,000 g for 5 min at room temperature to separate phases, and the bottom organic layer was removed. 10 μl of this sample was spotted onto a flexible 1B-F silica gel plates (J. T. Baker, Phillipsburg, NJ) pretreated with 1% oxalic acid and developed in buffer composed of chloroform/methanol/29.5% ammonium hydroxide/water (9:7:0.3:1.7). Radioactive spots were visualized by autoradiography, and phosphatidylinositol phosphate and phosphatidylglycerol bisphosphate were identified by comparison with phospholipid standards.

RESULTS

C3 Exonuclease Inhibits Cell Proliferation—Given a role for Rho in the regulation of cell morphology and growth, we initially investigated the effect of inhibiting Rho by culturing the cells in the presence of C3 exonuclease from C. botulinum, which was previously demonstrated to specifically inhibit Rho by ADP-ribosylation (21, 22). In Fig. 1, increasing amounts of C3 exonuclease were added to cultures of cells plated on substrate. After 5 days in culture, cell number was determined, and relative cell number was expressed as a ratio of the number of cells cultured with C3 versus the number of cells cultured without C3. Cell proliferation was inhibited dose-dependently, with maximal inhibition at approximately 30–50 μg/ml (Fig. 1A). This demonstrated that C3 was taken up by the cells to ribosylate and inactivate Rho in situ. Using 50 μg/ml C3 not only inhibited ST2 but also exhibited cytostatic activity for a number of other cell lines as well (Fig. 1B).

C3 Catalyzed Ribosylation Induces Alterations in Cell Morphology—Cells cultured in the presence of 50 μg/ml of C3 exonuclease for 24 h exhibited pronounced alteration in morphology, showing slimmer processes and numerous micro-spikes relative to nontreated cells (Fig. 2). Using the same isolation procedure as for C3, a control preparation from untransformed bacteria had no effect on either morphology or proliferation, demonstrating that the effects were not due to contaminating bacterial components (not shown). These results further suggested that Rho was ribosylated by the C3 taken up by the cells from the culture medium. To demonstrate this, whole cell lysates prepared from cells cultured in the absence or the presence of 50 μg/ml C3 were subjected to in vitro C3 catalyzed ribosylation using [³²P]NAD as a ribose donor followed by SDS electrophoresis. In Fig. 3, the resulting autoradiogram revealed...
that C3 specifically labeled a species that migrated with an apparent molecular mass of approximately 25 kDa on a 11.25% polyacrylamide gel; this is consistent with previously published reports of the specificity for C3 catalyzed ribosylation of Rho (23, 24, 37). Lysates from cells cultured in the presence of C3 exoenzyme (50 μg/ml) after 5 days in culture, the number of cells in each well was determined as described under “Materials and Methods,” and the percentage of inhibition was determined as 100% × (1 - cell number cultured with C3/cell number cultured without C3). The values are expressed as means of triplicate ± S.E.

We next investigated the effect of adhesion and C3 on cell cycle progression at the transcriptional level by analyzing the expression of cyclin A and cyclin D, two regulators of cell cycle progression that have been implicated in tumorigenesis (25, 38). We first investigated the effect of integrin-mediated adhesion to collagen on the expression of cyclin A message. ST2 cells were initially deprived of adhesion for 30 h by transferring the cells to culture dishes coated with polyHEMA, a substance used to inhibit cell attachment (11) (Fig. 5A). By culturing the cells in the absence of adhesion (11, 39), the effect of adhesion to specific substrates can then be analyzed. Upon transfer from polyHEMA to collagen-coated dishes, the cells expressed low levels of cyclin A (Fig. 5A, lane 1). However, by 18 h on collagen-coated dishes (lane 2), the expected 2.7- and 1.8-kilobase alternatively polyadenylated forms of cyclin A mRNA (25) were induced significantly, and their levels continued to rise 24 h (lane 3) and 36 h (lane 4) after plating. To demonstrate that integrin-mediated attachment to collagen was required for induction, in Fig. 5B, the cells were initially deprived of adhesion for 30 h to induce low levels of cyclin A message (lane 1). The cells were then plated on collagen (lane 2 and 3) or maintained on polyHEMA (lane 4 and 5) either in the absence (lane 2 and 4) or the presence of an anti-β1 integrin monoclonal antibody (lane 3 and 5). After 24 h, cyclin A expression was induced when the cells were plated on collagen (lane 2) but was blocked in the

**Fig. 1. Inhibition of proliferation by C3.** A, dose-dependent inhibition by C3 exoenzyme. Cells (4000 cells/well) were plated in triplicate in 96-well plates coated with collagen (see “Materials and Methods”) with the indicated concentrations of C3. After 5 days in culture, cell number was determined as described under “Materials and Methods,” and relative cell number was calculated by dividing the number of cells treated with C3 by the number of cells that were left untreated. B, C3 inhibition of proliferation. Cells (4000 cells/well) were plated in 96-well tissue culture plates in the absence or the presence of C3 exoenzyme (50 μg/ml). After 5 days in culture, the number of cells in each well was determined as described under “Materials and Methods,” and the percentage of inhibition was determined as 100% × (1 - cell number cultured with C3/cell number cultured without C3). The values are expressed as means of triplicate ± S.E.

**Fig. 2. C3 induced morphological changes.** The indicated cell lines were plated on collagen-coated wells either in the presence or the absence of 50 μg/ml C3 and incubated for 24 h at 37°C in a 5% CO2 humidified incubator.

**Fig. 3. Culturing cells in the presence of C3 induces ADP-ribosylation of Rho.** ST7 (lanes 1 and 2), ST2 (lanes 3 and 4), A375 (lanes 5 and 6), HMEC-1 (lanes 7 and 8), 131-IN1 (lanes 9 and 10), and FOGERTY (lanes 11 and 12) were left either untreated (lanes 1, 3, 5, 7, 9, and 11) or treated with 50 μg/ml C3 (lanes 2, 4, 6, 8, 10, and 12) for 3 days, harvested by brief trypsination, and transferred to microcentrifuge tubes. The cells were then ADP-ribosylated in vitro and separated by SDS-polyacrylamide gel electrophoresis as described under “Materials and Methods.”
The expression of cyclins A and D1 (26, 38). In Fig. 6, the presence of anti-

**Fig. 4.** Cell cycle analysis of C3-treated cells. ST2 cells were plated on collagen either in the presence (A) or the absence (B) of C3, incubated for 48 h, and then analyzed by propidium iodide staining and flow cytometry as described under "Materials and Methods."
sion of cyclin D, by relieving requirements for G<sub>0</sub> to G<sub>1</sub> cell cycle transition, has been proposed as a mechanism contributing to tumorigenesis (38, 42). These results indicate that tumor proliferation may also require integrin signaling and Rho to achieve progression into S phase.

**β1 Integrin-dependent Phospholipid Induction Is Inhibited by C3**—The contribution of adhesion to endogenous phospholipid synthesis was determined for ST2 by measuring ³²P incorporation into phospholipids. In Fig. 7, ST2 cells were incubated on polyHEMA for 24 h either in the presence or the absence of C3 exoenzyme and then maintained on polyHEMA or replated on collagen-coated dishes in the presence or the absence of anti-β1 integrin monoclonal antibodies. Phospholipid levels were then determined by ³²P incorporation, and the labeled products analyzed by thin layer chromatography. Significant induction of phospholipids including PIP and PIP<sub>2</sub> were seen when cells were transferred from polyHEMA (lane 1) to collagen-coated dishes and incubated (lane 2) for 3 h. Anti-β1 integrin monoclonal antibodies could block the induction of phospholipids when plated on collagen (lane 4). The induction of phospholipids could also be inhibited if the cells were preincubated in the presence of 50 μg/ml C3 exoenzyme for 24 h on polyHEMA prior to plating cells on collagen (lane 6). C3 treatment alone also slightly but consistently inhibited phospholipid levels (compare lanes 1 and 5). This suggests that a separate pathway mediated by integrin as well as Rho can lead to phospholipid induction. Both integrin-mediated adhesion as well as a functional Rho, however, were required for efficient induction of phospholipids.

**Decreased Phospholipid Levels Correlate with Reduced Proliferation of Tumor Cells in the Absence of Adhesion**—In Fig. 8, a panel of tumor cells were either maintained on substrate or transferred to polyHEMA-coated dishes for 24 h, and their phospholipid synthesis levels were measured using ³²P incorporation. The level of phospholipid synthesis including PIP and PIP<sub>2</sub> was reduced markedly in the absence of adhesion for a panel of tumor lines (Fig. 8A). We also measured the proliferation of these cells after 5 days and found that the cells plated on polyHEMA, which showed reduced phospholipid including PIP and PIP<sub>2</sub> levels on polyHEMA, correlated with reduced proliferation relative to cells maintained on substrate (Fig. 8B).

**DISCUSSIONS**

Numerous examples have demonstrated integrin signaling, including clustering of the fibronectin receptor leading to protein tyrosine phosphorylation of focal adhesion tyrosine kinase (43) and cytoplasmic alkalinization (44). In peripheral blood T cells, antibodies to integrins or matrix components have been shown to possess growth stimulatory activities (5, 45).

In these studies we have shown that cyclin A and phospholipids were regulated by adhesion to collagen, and the induction was inhibited by an anti-β1 integrin monoclonal antibody. ADP-ribosylation of Rho by C3 exoenzyme inhibited proliferation, induced G<sub>0</sub>/G<sub>1</sub> accumulation, and also inhibited integrin-mediated induction of phospholipid synthesis as well as cell cycle progression as measured by the expression of cyclin A. The ability of ADP-ribosylation of Rho to inhibit integrin-mediated cell cycle progression demonstrates a convergence of integrin and Rho signaling pathways leading to positive regu-
liation of tumor cell growth as a consequence of cell attachment to substratum. Cyclin A expression was also inhibited by culturing the cells under low serum (0.5%) conditions (Fig. 6A), and this correlated with G2/G0 cell cycle arrest (not shown). These results demonstrate an additional level of cooperativity between integrins, Rho, and soluble serum factors leading to cell cycle progression. The generation of second messengers leading to proliferation induced by many growth factors relies on a pool of phospholipids (46) and therefore, the synthesis of phospholipids induced by integrins in tumor cells may play a pivotal role in the integration of growth factors and adhesion leading to growth stimulation.

The profound morphological alterations accompanying ADP-ribosylation of Rho is consistent with alterations in the level of phospholipids. Phospholipid can alter the function of cytoskeletal components including α-actinin (47), gelsolin (48), profilactin (49), and talin (50), and therefore, changes in morphology induced by C3 ribosylation of Rho may in part be explained by alterations in phospholipid levels induced by C3 treatment. Cells maintained in suspension overnight show reduced phospholipid synthesis and correspondingly showed similar alterations in morphology, which was evident upon reattachment to collagen coated plastic. This alteration was transient, and the cells resumed normal morphology after several hours on collagen (not shown).

These results are in agreement with those of other investigators who demonstrated that enhanced inositol lipid synthesis and platelet-derived growth factor-induced inositol lipid synthesis breakdown in response to fibronectin involved the regulation of phosphatidylinositol 4-phosphate 5-kinase by Rho (19, 20). Our studies using intact cells demonstrated that integrin-mediated adhesion could regulate not only PI(2)P but also PI(3)P as well. Treatment of cells with C3 also inhibited the synthesis of PI(2)P and PI(3)P. The observed differences may be due to more complex molecular interactions in intact cells in which, for example integrin-mediated induction of phospholipids may influence downstream activation of protein kinase C, which in turn, may indirectly alter phospholipid metabolism.

Various studies have implicated a role for integrins in cancer. The mechanism by which adhesion molecules promote metastasis is not clear but may involve adhesion to platelets to escape immunologic detection (51). Integrins can mediate extravasation of cells from circulation into tissue to sequester them from immunologic detection or physical damage caused by shear forces under conditions of flow (6). An additional role for integrins in promoting metastasis may involve generation of growth promoting signals. Anchorage-independent growth is a function of the suppression of the apoptotic pathway (52) and or the activation of growth promoting pathways. The growth-promoting effect on tumors through integrins may therefore occur as a separate event in the context of mutations leading to suppression of apoptosis.

A number of tumors have been shown to overexpress growth factor receptors. Karyotype analysis of ST2 has revealed the amplification of chromosome 7p, which carries the gene for the epidermal growth factor receptor (29). Therefore, the epithelial growth factor receptor may play a relevant role in contributing to tumor proliferation of ST2, and its overexpression may facilitate rapid tumor growth under conditions of limiting soluble factors (53, 54). The binding of growth factors can lead to phospholipid turnover and the generation of second messengers, which ultimately act on components that regulate cell cycle progression and cell division. Several components that respond to growth factor stimulation and that have also been implicated to play a role in tumorigenesis include cyclin A and cyclin D. Results using ST2 demonstrated that cyclin D expression is disregulated and may contribute to its tumorigenicity. Cyclin D is required for exit from G0 to G1, and therefore, constitutive expression of cyclin D may result in the inability of ST2 to remain in G0, such that the cells will, in the absence of physiologic stimulation, progress to G1. Full progression into S phase not only requires cyclin D but also the expression of cyclin A. In contrast to cyclin D1, however, cyclin A expression in ST2 cells required not only soluble factors (serum) but also integrin-mediated adhesion. The ability of cyclin A to mediate adhesive signals has been shown in untransformed fibroblast lines (28), and therefore, integrin-mediated adhesion contributing to tumor proliferation may be a feature that may be retained upon cell transformation in some cases.

REFERENCES

1. Albelda, S. M., and Buck, C. A. (1990) FASEB J. 4, 2888–2880
2. Fuchs, R. O. (1992) Cell 69, 11–26
3. Jones, P. L., Schmidhauser, C., and Bissell, M. J. (1993) Crit. Rev. Eukaryotic Gene Expression 3, 137–154
4. Adams, J., and Watts, F. (1993) Development 117, 1183–1198
5. Matsuyama, T., Yamada, A., Kay, J., Yamada, K. M., Akiyama, S. K., Schlossman, S. F., and Morimoto, C. (1989) J. Exp. Med. 170, 1133–1148
6. Ruosdahl, E., and Giancotti, F. G. (1989) Cancer Cells 1, 119–125
7. Kleinman, H. K., Kebe, R. J., and Martin, G. R. (1981) J. Cell Biol. 88, 473–485
8. Chun, J.-S., and jacobson, B. S. (1993) Mol. Biol. Cell 4, 271–281
9. Pignatelli, M., and Bodmer, W. F. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5561–5565
10. Kapron-Bras, C., Fitz-Gibbon, L., Jeevaratnam, P., Wilkins, J., and Dedhar, S. (1992) J. Biol. Chem. 267, 7070–7074
11. Folkman, J., and Moscona, A. (1978) Nature 273, 345–349
12. Otsuka, H., and Moscovitz, M. (1975) J. Cell. Physiol. 87, 213–220
13. Ridley, A. J., and Hall, A. (1993) Cell 70, 389–399
14. Bauer, J. S., Varner, J., Schreiner, C., Kornberg, L., Nicholas, R., and Juliano, R. L. (1993) J. Cell Biol. 122, 209–221
15. Perona, R., Esteve, P., Jiamen, B., Ballestro, R. P., Ramon y Cajal, S., and Solano, J. C. (1993) Oncogene 8, 1295–1292
16. Avraham, H., and Weinberg, R. A. (1989) Mol. Cell. Biol. 9, 2058–2066
17. Ruggiero, M., Srivastava, S. K., Fleming, T. P., Ron, D., and Eva, A. (1989) Oncogene 4, 767–771
18. Hart, M. J., Eva, A., Zangrilli, D., Aaronson, S. A., Evans, T., Cerione, R. A., and Zheng, Y. (1994) J. Biol. Chem. 269, 62–65
19. Chong, L. D., Traynor-Kaplan, A., Bokoch, G. M., and Schwartz, M. A. (1994) Cell 79, 507–513
20. McNamme, H., Inger, D. E., and Schwartz, M. A. (1993) J. Cell Biol. 121, 673–678
21. Martin, C., Koch, G. J., and Aktories, K. (1992) FEBS Lett. 297, 95–99
22. Leonard, D., Hart, M. J., Platko, J. V., Eva, A., Henzel, W. D., Evans, T., and Cerione, R. A. (1992) J. Cell Biol. 117, 561–574
23. Martin, R., Tomhave, E., Davis, P. J., Uhring, R. J., Snyderman, R., and Diddersby, J. R. (1992) J. Biochem. 207, 536–547
24. Just, I., Mohr, C., Schallhein, G., Menard, L., Diddersby, J. R., Vandekerckhove, J., van Damme, J., and Aktories, K. (1992) J. Cell Biol. 167, 10274–10280
25. Wang, J., Chenreves, X., Henglein, B., and Bröckel, C. (1996) Nature 343, 555–559
26. Xiong, Y., Connolly, T., Futterer, B., and Beach, D. (1993) Cell 65, 691–699
27. Hartwell, L. (1992) Cell 71, 533–546
28. Guadagno, T. M., Ohtsubo, M., Roberts, J. M., and Assoian, R. K. (1990) Science 242, 1572–1575
29. Udagawa, T., Hopwood, V. L., Pathak, S., and McIntyre, B. W. (1995) Clin. Exp. Metastasis 13, 427–438
30. Ades, E. W., Candal, F. J., Swerlick, R. A., George, V. G., Summers, S., Bosse, D. C., and Lawley, T. J. (1993) Exp. Dermatol. 2, 20701–20704
31. Bednarczyk, J. L., Wygant, J. N., Szabo, M. C., Molinari-Storey, L., Renz, M., D. C., and Lawley, T. J. (1992) Exp. Dermatol. 2, 20701–20704
32. Popoff, M., Boquet, P., Gill, D. M., and Eklund, M. W. (1990) J. Cell Biol. 115, 1419–1425
33. Sherr, C. J. (1994) Cell 79, 551–555
34. Paterson, H. F., Self, A. J., Marinos, G., Pignatelli, M., and Bodmer, W. F. (1988) Oncogene 7, 93–99
35. beach, D. (1993) J. Biol. Chem. 268, 345–349
36. Schwartz, M. A., Lechene, C., and Ingber, D. E. (1993) Proc. Natl. Acad. Sci. U.S.A. 88, 7489–7493
37. Davis, L. S., Oppenheimer-Marks, N., Bednarczyk, J. L., McIntyre, B. W., and Lipsky, P. E. (1990) J. Immunol. 145, 785–793
38. Berdridge, M. J. (1987) Biochim. Biophys. Acta 907, 33–45
47. Fukami, K., Furuhashi, K., Inagaki, M., Endo, T., Hatano, S., and Takenawa, T. (1992) Nature 359, 150–152
48. Janmey, P. A., and Stossel, T. P. (1987) Nature 325, 362–364
49. Lassing, I., and Lindberg, U. (1985) Nature 314, 472–474
50. Goldmann, W. H., Niggli, V., Kaufmann, S., and Isenberg, G. (1992) Biochem-istry 31, 7665–7671
51. Karpatkin, S., Ambrogio, C., and Pearlstein, E. (1988) Prog. Clin. Biol. Res. 283, 585–606
52. Guinebault, C., Payrastre, B., Racaud-Sultan, C., Mazarguil, H., Breton, M., Mauco, G., Plantavid, M., and Chap, H. (1995) J. Cell Biol. 129, 831–842
53. Helseth, E., Brogger, A., Dalen, A., Fure, H., Johansen, S. G., Lier, M. E., Skandsen, T., Unsgaard, G., and Vik, R. (1990) Acta Pathol. Microbiol. Immunol. Scand. 98, 996–1004
54. Sauter, G., Haley, J., Chew, K., Kerschmann, R., Moore, D., Carroll, P., Moch, H., Gudat, F., Mihatsch, M. J., and Waldman, F. (1994) Int. J. Cancer 57, 508–514