Transforming and c-fos Promoter/Enhancer-stimulating Activities of a Stimulatory GDP/GTP Exchange Protein for Small GTP-binding Proteins*

Hiroyuki Fujioka, Kozo Kaibuchi, Kiyohiko Kishi, Takeshi Yamamoto, Motohiro Kawamura, Tsuyoshi Sakoda, Takakazu Mizuno, and Yoshimi Takaï

*From the Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan

smg GDP dissociation stimulator (GDS) is a stimulatory GDP/GTP exchange protein for a group of ras p21-like small GTP-binding proteins (G proteins) including c-Ki-ras p21, smg p21A, smg p21B, and rhoA p21. smg GDS converts the GDP-bound inactive form to the GTP-bound active form of each small G protein by stimulating their GDP/GTP exchange reaction in a cell-free system. The point-mutated c-Ki-ras p21 (c-Ki-rasV1112)p21 is known to strongly transform NIH/3T3 cells and to markedly stimulate the c-fos promoter/enhancer in this cell line, whereas the normal c-Ki-ras p21 is weak in these activities. In the present study, we examined the effect of smg GDS on these activities to explore its physiological function. Overexpression of both smg GDS and c-Ki-ras p21 strongly transformed NIH/3T3 cells, whereas overexpression of either smg GDS or c-Ki-ras p21 alone weakly transformed the cells. Furthermore, overexpression of both smg GDS and c-Ki-ras p21 markedly stimulated the c-fos promoter/enhancer in NIH/3T3 cells, whereas overexpression of either smg GDS or c-Ki-ras p21 alone weakly stimulated it. These results indicate that smg GDS transforms NIH/3T3 cells and stimulates the c-fos promoter/enhancer in this cell line in cooperation with c-Ki-ras p21.

There is a superfamily of ras p21-like small G proteins1 (1–3). Small G proteins have GDP-bound inactive and GTP-bound active forms that are interconvertible by GDP/GTP exchange and GTPase reactions (2, 3). The GDP/GTP exchange and GTPase reactions are regulated by GDP/GTP exchange protein and GAP, respectively (2, 3). There are stimulatory and inhibitory GDP/GTP exchange proteins, named GDS and GDP dissociation inhibitor, respectively (2, 3). smg GDS is originally found as a stimulatory GDP/GTP exchange protein for smg p21A (identical to the rap1A and Krev-1 proteins) and smg p21B (identical to the rap1B protein) (4, 5), but our recent studies have revealed that smg GDS is also active on c-Ki-ras p21 and rhoA p21 (6). Several lines of evidence suggest that the GDP-bound active form of ras p21, which is produced by use of a point mutation or a non-hydrolyzable GTP analogue such as GTPγS, transforms NIH/3T3 cells and stimulates the c-fos promoter/enhancer in NIH/3T3 cells and PC-12 cells, whereas the GDP-bound inactive form of ras p21 is weak in these activities (1, 7–9). To explore the physiological function of smg GDS, we attempted to express smg GDS in NIH/3T3 cells and to examine its activities to transform the cells and to stimulate the c-fos promoter/enhancer. Here we report that smg GDS transforms NIH/3T3 cells and stimulates the c-fos promoter/enhancer in this cell line in cooperation with c-Ki-ras p21.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—pSRaneo and pCEV4 expression plasmids were donated from A. Miyajima (DNAX Research Institute, Palo Alto, CA) and T. Yokota (University of Tokyo, Tokyo, Japan), respectively. c-fos-luciferase and pTKCAT were donated from M. Muramatsu and K. Aris (University of Tokyo, Tokyo, Japan). c-Ki-ras (4B) cDNA was a gift from R. A. Weinberg (Whitehead Institute for Biochemical Research, Cambridge, MA). c-Ha-ras cDNA was a gift from F. Tannoy (University of Chicago, Chicago, IL). The anti-ras p21 monoclonal antibody (RASK-4) was provided by H. Shiku (Nagasaki University, Nagasaki, Japan). The anti-smg GDS monoclonal antibody was made by immunizing rabbits with smg GDS. Other materials and chemicals were obtained from commercial sources.

Construction of Plasmids—Expression plasmids, pSRa-GDS, pSRa-Ki-ras, and pSRa-Ki-rasV1112 were constructed by the following procedures. The 1.7-kilobase fragment containing the complete smg GDS coding region and the 0.6-kilobase fragment containing the complete c-Ki-ras or c-Ki-rasV1112 coding region with the BamHI sites upstream of the initiator methionine codon and downstream of the termination codon were synthesized by polymerase chain reaction as described (5). These fragments were digested by BamHI and ligated into the BamHI site of a pSRaneo plasmid which was constructed by insertion of the SRa promoter and the polyadenylation site into pSV2neo (10). The SRa promoter is composed of the SV40 early promoter and the R-U5 segment of the human T cell leukemia virus-I long terminal repeat (10). The SRa promoter is very strong in the cells expressing SV40-large T antigen such as COS7 cells, but not strong in other types of cells. Expression plasmids pCEV4-GDS, pCEV4-Ki-ras, pCEV4-Ki-rasV1112, pCEV4-Ha-ras, and pCEV4-Ha-rasV1112 were constructed by the same procedures as described above except that a pCEV4, a derivative of pSRa296, was used instead of a pSRaneo.

Cells and Transfection—NIH/3T3 cells were obtained from M. Wigler (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). NIH/3T3 cells were grown in DMEM supplemented with 10% calf serum in a 37 °C, 5% CO2 humidified environment. Transfections of

926
plasmids into NIH/3T3 cells were performed as described (11). To obtain the cell lines expressing either neo alone, smg GDS alone, c-Ki-ras p21 alone, c-Ki-ras"ras112 p21 alone, smg GDS plus c-Ki-ras"ras112 p21, or smg GDS plus c-Ki-ras"ras112 p21, the cells were transfected with either pSRneo, pSRa-GDS, pSRa-Ki-ras, pSRa-Ki-ras"ras112, pSRa-GDS plus pSRa-Ki-ras, or pSRa-GDS plus pSRa-Ki-ras"ras112 respectively. After transfection, cells were fed with a growth medium containing an effective concentration of 450 mg/ml G418 antibiotic. Colonies of the cells resistant to G418 antibiotic were picked at 14–17 days for expansion. Subsequently, the cells expressing the desired gene products among the G418-resistant cells were further selected by immunoblot analysis.

Focus-forming Assay: To assess the transforming activity of smg GDS, c-Ki-ras p21, and c-Ki-ras"ras112 p21, pSRa-GDS, pSRa-Ki-ras, or pSRa-Ki-ras"ras112 (0.1, 1, or 10 μg) with pSRneo (20 μg) was transfected into NIH/3T3 cells, seeded 24 h earlier at a density of 1.2 × 10^5 cells in 100-mm diameter dishes in DMEM supplemented with 10% calf serum (11). Cultures were maintained with twice weekly changes of DMEM supplemented with 5% calf serum. Monolayers were screened 14–21 days after transfection for focus formation.

Immunoblot Analysis: For immunoblot analysis, 5 × 10^6 cells of each cell line were plated in 100-mm diameter dishes and cultured in DMEM supplemented with 10% calf serum for 4 days. Immunoblot analysis of the cell lysate of each cell line was carried out by use of the anti-smg GDS monospecific polyclonal antibody or the anti-ras p21 monoclonal antibody (RASK-4) as described (12).

Low Serum Growth Assay: To assess the growth rate of various NIH/3T3 cell lines in low serum, 3.5 × 10^5 cells of each cell line were plated in 100-mm diameter dishes and cultured in DMEM supplemented with 10% calf serum. Twelve hours later, cells were trypsinized and counted using a hemacytometer (Burker-Turk) to ensure accurate plating. At this time, the growth medium of the other dishes was changed to DMEM supplemented with 0.5% calf serum. Thereafter, at selected intervals, cells were trypsinized and counted. Cells were fed every other day during the growth assay. To observe the morphological changes of NIH/3T3 cell lines in low serum, 1 × 10^6 cells of each cell line were plated in 100-mm diameter dishes and cultured as described above. After 10 days, they were photographed at a magnification of ×40.

Anchorage-independent Growth Assay: To assess the anchorage-independent growth state of NIH/3T3 cell lines in soft agar, 1 × 10^4 cells of each cell line were suspended in 2 ml of 0.3% agarose in DMEM supplemented with 10% calf serum and overlaid above a layer of 4 ml of 0.5% agarose in the same medium in 60-mm diameter dishes as described (13). After 14 days, they were photographed at a magnification of ×40.

Nude Mouse Tumorigenicity Assay: To assess the tumorigenicity of NIH/3T3 cell lines, the growing cells were resuspended at a concentration of 2.5 × 10^6 cells/ml. Approximately 5 × 10^6 cells were injected into the left and right flank of 4–8-week-old CD-1 athymic nude mice (five mice were used for each cell line). Mice were examined once or twice a week for tumor formation and growth. Tumors greater than 3 mm in the largest diameter by 30 days postinjection were counted. Mice were sacrificed, and tumors were excised aseptically for analysis.

Luciferase and CAT Assays—c-fos-luciferase and pTKCAT were transfected into NIH/3T3 cells with various constructs. Luciferase and CAT activities derived from the transfected cells were assayed as described (14, 15).

RESULTS

The smg GDS, c-Ki-ras, and c-Ki-ras"ras112 cDNAs were introduced into the pSRneo expression plasmid to construct pSRa-GDS, pSRa-Ki-ras, and pSRa-Ki-ras"ras112, respectively. Attempts to induce foci in NIH/3T3 cells by transfection with pSRa-GDS and/or pSRa-Ki-ras were unsuccessful in many experiments, although parallel transfection with pSRa-Ki-ras"ras112 consistently yielded many foci (50–100 per 0.1 μg of the plasmid) of transfected cells. Therefore, cell lines constitutively expressing various combinations of smg GDS, c-Ki-ras p21, and c-Ki-ras"ras112 p21 were generated so that their effect could be directly studied. These constructs were transfected into NIH/3T3 cells, and the transfected cells were selected for resistance to G418 antibiotic. To determine the levels of smg GDS, c-Ki-ras p21, and c-Ki-ras"ras112 p21, each of G418-resistant cell lines was subjected to immunoblot analysis using anti-smg GDS and anti-ras p21 antibodies. The stable NIH/3T3 cell lines expressing neo alone, smg GDS alone, c-Ki-ras p21 alone, smg GDS plus c-Ki-ras p21, c-Ki-ras"ras112 p21 alone, and smg GDS plus c-Ki-ras"ras112 p21 were obtained and named here 3T3NEO/1–3, 3T3GDS/1–3, 3T3ras/1–3, 3T3rasGDS/1–5, 3T3ras"ras112/1–3, and 3T3ras"rasGDS/1–3 cell lines, respectively (Fig. 1, A and B, Table I). Each cell line expressed each protein over 2–15-fold as compared with the control cells. To characterize each cell line, we measured the three biological properties with cellular transformation: foci formation, growth in low serum, and anchorage-independent growth in soft agar. All of these cell lines grew in DMEM containing 10% calf serum in an anchorage-dependent manner. In the medium containing 0.5% calf serum, neither control, 3T3NEO/1–3, nor 3T3ras/3 cells grew (Fig. 2, Fig. 3A, Table I). Under the same conditions, both 3T3GDS/1–3 and 3T3ras/1–2 cells grew slowly, underwent slightly morphological change, and weakly developed foci. In contrast, 3T3rasGDS/1–5 cells as well as 3T3ras"ras112/1–3 and 3T3ras"rasGDS/1–3 cells grew well, underwent morphological change, and developed dense foci. The morphology of 3T3rasGDS/1–5 cells was apparently similar to that observed for 3T3ras/1–3 and 3T3rasGDS/1–3 cells. In soft agar, 3T3rasGDS/1–5, 3T3ras"ras112/1–3, and 3T3ras"rasGDS/1–3 cells formed medium or large colonies quite efficiently, whereas control or 3T3NEO/1–3 cells failed to grow and persisted as a single cell (Fig. 3B, Table I). 3T3GDS/1–3 and 3T3ras/1–3 cells formed low and varying numbers of very small colonies. The growth efficiency in soft agar of 3T3ras"ras112/1–3 and 3T3ras"rasGDS/1–3 cells was generally better than that of 3T3rasGDS/1–5 cells. The colony sizes of 3T3rasGDS/1 cells were greater than those of 3T3rasGDS/2–5 cells, presumably reflecting the higher levels of smg GDS and c-Ki-ras p21 in the transfected cells. When 3T3rasGDS/1, 3T3ras"ras112/1, and 3T3ras"rasGDS/1 cells were inoculated into nude mice, all the mice produced tumors. The time of tumor appearance varied slightly with the different cell lines but centered around 2–3 weeks. The majority of the tumors attained diameters of at least 1 cm.
### TABLE I

| Cell line          | Tranfected construct | Relative smg GDS level | Relative ras p21 level | Growth in low serum | Colony-forming efficiency |
|--------------------|----------------------|------------------------|------------------------|---------------------|---------------------------|
| Control NIH/3T3    | None                 | -                      | -                      | -                   | <0.1                      |
| 3T3NEO/1           | pSRa-neo             | -                      | -                      | -                   | <0.1                      |
| 3T3GDS/1           | pSRa-GDS             | ++                     | +                      | +                   | <0.5                      |
| 3T3ras/1           | pSRa-Ki-ras          | -                      | +++                   | +                   | <0.5                      |
| 3T3rasGDS/1        | pSRa-GDS plus        | ++                     | ++                    | ++                  | 32                        |
| 3T3ras"12          | pSRa-Ki-ras"12      | -                      | +                     | ++                  | 18                        |
| 3T3rasGDS/1        | pSRa-GDS plus        | ++                     | ++                    | ++                  | 16                        |
| 3T3ras"12 GDS/1    | pSRa-GDS plus"12    | +                      | ++                    | ++                  | 13                        |
| 3T3rasGDS/1        | pSRa-GDS plus"12    | +                      | ++                    | ++                  | 10                        |

*The levels of smg GDS and ras p21 were estimated by qualitative analysis of immunoblots shown in Fig. 1.

**+,** cells did not grow; **+,** cells grew slowly and weakly developed foci; ++, cells grew well and developed dense foci.

† Ancephalization-independent growth state was expressed as colony-forming efficiency. Colonies comprising >50 cells were scored at 14 days.

---

The growth rate of various NIH/3T3 cell lines in low serum is shown in Fig. 2. Initially, cell lines grew slowly and weakly developed foci, but with time, they grew well and developed dense foci. The levels of smg GDS and ras p21 were estimated by qualitative analysis of immunoblots shown in Fig. 1.

Histopathologically, all the tumors were high-grade fibrosarcoma. In contrast, control, 3T3NEO/1, 3T3GDS/1, or 3T3ras/1 cells showed a very low incidence of tumor formation under these conditions (0/5 for control cells; 1/5 for 3T3NEO/1 cells; 1/5 for 3T3GDS/1 cells; 1/5 for 3T3ras/1 cells).

We and others have shown that the point-mutated ras p21 stimulates the c-fos promoter/enhancer (7–9). In the next set of experiments, we examined whether smg GDS showed the similar effect. A fusion gene (c-fos-luciferase) containing 445 base pairs of the c-fos 5′-flanking sequence ligated to the coding sequence of the luciferase gene was used as a reporter of transcriptional activity of the c-fos promoter/enhancer (15). Consistent with our earlier observation, transfection of pCEV4-Ki-ras"12 or pCEV4-Ha-ras"12 into NIH/3T3 cells increased luciferase activity derived from c-fos-luciferase (Fig. 4). Under the same conditions, transfection of either pCEV4-GDS, pCEV4-Ki-ras, or pCEV4-Ha-ras alone conferred a small increment of luciferase activity. Cotransfection of pCEV4-GDS and pCEV4-Ki-ras synergistically increased lu-
FIG. 4. Activation of c-fos-luciferase in NIH/3T3 cells. c-fos-luciferase (4 μg) and pTKCAT (4 μg) were transfected into NIH/3T3 cells with either pCEV4-GDS (8 μg), pCEV4-Ki-ras (2 μg), pCEV4-Ha-ras (2 μg), pCEV4-GDS (8 μg) plus pCEV4-Ki-ras (2 μg), pCEV4-GDS (8 μg) plus pCEV4-Ha-ras (2 μg), pCEV4-Ki-ras<sup>Δ112</sup> (2 μg), pCEV4-Ha-ras<sup>Δ112</sup> (2 μg), pCEV4-GDS (8 μg) plus pCEV4-Ki-ras<sup>Δ112</sup> (2 μg), or pCEV4-GDS (8 μg) plus pCEV4-Ha-ras<sup>Δ112</sup> (2 μg). After transfection, the luciferase and CAT activities derived from the transfected cells were assayed. The CAT activity was not shown here. Column 1, pCEV4; column 2, pCEV4-GDS; column 3, pCEV4-Ki-ras; column 4, pCEV4-Ha-ras; column 5, pCEV4-GDS plus pCEV4-Ki-ras; column 6, pCEV4-GDS plus pCEV4-Ha-ras; column 7, pCEV4-Ki-ras<sup>Δ112</sup>; column 8, pCEV4-Ha-ras<sup>Δ112</sup>; column 9, pCEV4-GDS plus pCEV4-Ki-ras<sup>Δ112</sup>; column 10, pCEV4-GDS plus pCEV4-Ha-ras<sup>Δ112</sup>. The results shown are representative of three independent experiments.

ciferase activity to the extent similar to that observed when the cells were transfected with pCEV4-Ki-ras<sup>Δ112</sup>. On the other hand, cotransfection of pCEV4-GDS and pCEV4-Ha-ras did not synergistically increase luciferase activity. pCEV4 harboring the smg GDS cDNA in the reversed orientation did not show this action. Luciferase activity derived from the luciferase construct lacking the c-fos 5′-flanking sequence was negligible in the cells transfected with the smg GDS and c-Ki-ras constructs. Another fusion gene (pTKCAT) containing the thymidine kinase promoter/enhancer ligated to the CAT gene was used as an internal control to standardize the transfection efficiency (15). CAT activity derived from pTKCAT was almost the same among these transfected cells.

**DISCUSSION**

We have shown here that smg GDS, which converts the GDP-bound form to the GTP-bound form of c-Ki-ras p21 in a cell-free system, transforms NIH/3T3 cells and stimulates the c-fos promoter/enhancer in NIH/3T3 cells in cooperation with c-Ki-ras p21. These observations indicate that smg GDS is active on c-Ki-ras p21 in an intact cell system as well as in a cell-free system. The GTP-bound form of ras p21 has been shown to be the active form on the basis of several lines of evidence. 1) The point-mutated ras p21 with a reduced GTPase activity is active in transforming and differentiating cells, and this type of point-mutated ras p21 is found in human cancers (16-18); 2) ras p21 in the form complexed with a non-hydrolyzable GTP analogue is active in differentiating cells (19); 3) ras p21 GAP, which stimulates the GTPase activity of normal ras p21, does not stimulate the GTPase activity of the point-mutated ras p21 (20, 21); and 4) the neurofibromatosis gene (NF1) product has GAP activity (22, 23). Our present results are consistent with these earlier observations. It has recently been shown that transfection of the expression plasmid carrying the C-terminal part of Saccharomyces cerevisiae SCD2S, whose gene product is known to stimulate the GDP/GTP exchange reaction of c-Ha-ras p21, enhances expression of human immunodeficiency virus long terminal repeat-CAT in Chinese hamster ovary cells (24). Our results are also in good agreement with this observation.

The cells expressing smg GDS or c-Ki-ras p21 alone grow slowly, undergo slightly morphological change, and weakly develop foci in low serum, but they hardly grow or form colonies in soft agar. Moreover, transfection of the smg GDS or c-Ki-ras p21 cDNA does not strongly stimulate the c-fos promoter/enhancer. The exact reasons why overexpression of smg GDS alone or c-Ki-ras p21 alone neither fully transforms NIH/3T3 cells nor strongly stimulates the c-fos promoter/enhancer are not known, but it is possible that in the smg GDS-overexpressing cells the amount of endogenous c-Ki-ras p21 is not sufficient to exhibit these activities and that in the c-Ki-ras p21-overexpressing cells the amount of endogenous smg GDS to act on the overexpressed c-Ki-ras p21 is not sufficient to exhibit these activities. It is also possible that, since smg GDS is active on not only c-Ki-ras p21 but also other small G proteins such as smg p21A, smg p21B, and rhoA p21, these small G proteins interfere with the interaction of smg GDS with c-Ki-ras p21 in the cells overexpressing smg GDS (6).

Several investigators have already shown that overexpression of c-Ki-ras p21 is sufficient to promote transformation in the focus-forming assay (1). However, transfection of the c-Ki-ras p21 cDNA into NIH/3T3 cells hardly induces foci in the focus-forming assay in the present study. Although the exact reason for this discrepancy is not known, weakness of the SRα promoter in the expression plasmids may partly account for it. Cotransfection of the smg GDS and c-Ki-ras p21 cDNAs into NIH/3T3 cells hardly induces foci in the focus-forming assay either, whereas the cells overexpressing smg GDS and c-Ki-ras p21 undergo morphological alterations and develop dense foci. The exact reason for this discrepancy is not known, but it is likely that the expression levels of smg GDS and c-Ki-ras p21 may not be high enough to transform the cells in the focus-forming assay due to weakness of the SRα promoter and that the cells overexpressing smg GDS and c-Ki-ras p21 in amounts enough to show transforming properties can be obtained by G418 selection. Alternatively, it is possible that competition from the untransfected NIH/3T3 cells prevents the focus formation of the cells transfected with the smg GDS and c-Ki-ras p21 cDNAs in the focus-forming assay and that G418 selection eliminates the untransfected cells and allows the cells expressing smg GDS and c-Ki-ras p21 to proliferate more effectively.

It has recently been shown that c-Ha-ras p21 and c-Ki-ras p21 are converted from the GDP-bound form to the GTP-bound form upon stimulation of Swiss 3T3 cells by platelet-derived growth factor or epidermal growth factor and of Jurkat cells by a phorbol ester (25-27). Therefore, it is likely that the conversion from the GDP-bound inactive form to the GTP-bound active form of c-Ki-ras p21 is continuously stimulated by c-Ha-ras p21. However, it is practically difficult to compare the ratio of the GDP-bound form to the GTP-bound form of ras.
p21 in the various NIH/3T3 cell lines because the expression levels of c-Ki-ras p21 are different from one another and each cell line has the endogenous Ha-ras p21 and N-ras p21. In a preliminary experiment, we have found that cotransfection of the smg GDS and c-Ki-ras p21 cDNAs into COS7 cells increases the ratio of the GTP-bound form to the GDP-bound form of ras p21.

We have previously shown that smg p21B is phosphorylated by protein kinase A at the serine residue in the C-terminal region and that this phosphorylation initiates the smg GDS-induced transformation of smg p21B (28-31). It is not known how the smg GDS-induced activation of c-Ki-ras p21 is initiated by growth factors contained in calf serum. It has been shown that c-Ki-ras p21 is phosphorylated by protein kinases C and A at the serine residue in the C-terminal region (32). Therefore, it is possible by analogy with smg p21B that the phosphorylation of c-Ki-ras p21 also initiates the smg GDS-induced activation of c-Ki-ras p21. If this is the case, it could be speculated that intracellular signal transduction systems directly modify the smg GDS substrates including smg p21A, smg p21B, c-Ki-ras p21, and rhoA p21 rather than smg GDS itself, eventually leading to the smg GDS-induced activation of these small G proteins.

The point-mutated ras p21 is detected in 50% of colon adenocarcinomas, 90% of pancreatic adenocarcinomas, 30% of lung adenocarcinomas, 50% of thyroid tumors, and 30% of myeloid leukemia (18). It is possible that smg GDS could be changed to be always active on c-Ki-ras p21 even without a growth factor signal. It is tempting to speculate that some abnormality could be found in smg GDS in these cancers whose ras p21 is not mutated.

Acknowledgments—We thank Drs. A. Miyajima (DNAX Research Institute, Palo Alto, CA) and T. Yokota (University of Tokyo, Tokyo, Japan) for their kind gifts of pSRaneo and pCEV4 expression plasmids. We are indebted to Drs. H. Hara and J. Oyha (Mitsubishi Kasei Corp., Yokohama, Japan) for performing the nude mouse tumorigenicity assay and to Dr. S. Maeda (Kobe University, Kobe, Japan) for performing histopathological analysis of the tumors. We are grateful to J. Yamaguchi for secretarial assistance.

REFERENCES
1. Barbacid, M. (1987) Annu. Rev. Biochem. 56, 779-827.
2. Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) Nature 348, 125-132.
3. Takai, Y., Kajibuchi, K., Kikuchi, A., and Kawata, M. (1991) Int. Rev. Cytol., in press.
4. Yasui, T., Kajibuchi, K., Mizuno, T., Hiroyoshi, M., Shirakati, H., and Takai, Y. (1989) J. Biol. Chem. 264, 16626-16634.
5. Kajibuchi, K., Mizuno, T., Fujisaka, H., Yamamoto, T., Kishi, K., Fukumoto, Y., Horii, Y., and Takai, Y. (1991) Mol. Cell. Biol. 11, 2875-2889.
6. Mizuno, T., Kajibuchi, K., Yamamoto, T., Kawamura, M., Sakoda, T., Fujisaka, H., Matsuzawa, Y., and Takai, Y. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 6447-6451.
7. Schönhals, A., Herrlich, P., Rahmsdorf, H. J., and Ponta, H. (1988) Cell 54, 325-334.
8. Fukumoto, Y., Kajibuchi, K., Oku, N., Horii, Y., and Takai, Y. (1990) J. Biol. Chem. 265, 774-780.
9. Oka, N., Kajibuchi, K., Fukumoto, Y., Horii, Y., Fujisaka, H., and Takai, Y. (1989) FEBS Lett. 269, 148-152.
10. Takebe, Y., Seiki, M., Fujisawa, J., Hori, Y., Koyata, K., Arai, K., Yoshida, M., and Arui, N. (1988) Mol. Cell. Biol. 8, 466-472.
11. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745-2752.
12. Yoshida, K., Hamatani, K., Koide, H., Ikekita, H., Nakamura, N., Akiyumi, M., Tsuchiyama, H., Nakayama, S., and Shiku, H. (1988) Cancer Res. 48, 5523-5529.
13. Williamsen, B. M., Papageorge, A. C., Kung, H., Bekesi, E., Robins, T. J., Johnson, M., Vass, W. C., and Lowy, D. R. (1986) Mol. Cell. Biol. 6, 2646-2654.
14. de Wet, J. R., Wood, K. V., Delacou, M., Helinski, D. R., and Subramani, S. (1987) Mol. Cell. Biol. 7, 725-737.
15. Kajibuchi, K., Fukumoto, Y., Oka, N., Horii, Y., Yamamoto, T., Toyoshima, K., and Takai, Y. (1989) J. Biol. Chem. 264, 20665-20670.
16. Gibbs, J. B., Sigal, I. S., Poe, M., and Scolnick, E. M. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 5704-5708.
17. Lacal, J. C., Srivastava, S. K., Anderson, P. S., and Aaronson, S. A. (1986) Cell 44, 609-617.
18. Bae, J. J. (1988) Cancer Res. 49, 4682-4689.
19. Satoh, T., Endo, M., Nakamura, S., and Kaziro, Y. (1988) FEBS Lett. 236, 185-189.
20. Albie, L., Lowy, D. R., Williamsen, B. M., Der, C. J., and McCormick, F. (1988) Science 240, 518-521.
21. Schaber, M. D., Garkey, V. M., Boylan, D., Hill, W. S., Scolnick, E. M., Ullrich, A., Dull, T. M., Sigal, I. S., and Gibbs, J. B. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 306-315.
22. Xu, G., Lin, B., Takaha, R., Dunn, D., Wood, D., Gesteland, R., White, R., Weiss, R., and Tamanoi, F. (1990) Cell 63, 555-564.
23. Martin, G. A., Vlodkovich, D., Bollag, G., McCabe, P. C., Crosier, W. J., Haubruck, H., Conroy, L., Clark, R., O'Connell, P., Cawthon, R. M., Inris, M. A., and McCormick, F. (1990) Cell 83, 843-854.
24. Rey, I., Schweiger, F., Bartal, I., Camonis, J., Boy-Marcotte, E., Guibaud, R., Jacquet, M., and Tocque, B. (1991) Oncogene 6, 347-349.
25. Satoh, T., Endo, M., Nakamura, M., Nakamura, S., and Kaziro, Y. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5965-5967.
26. Satoh, T., Endo, M., Nakamura, M., Akiyama, T., Yamamoto, T., and Kaziro, Y. (1990) Cancer Res. 50, 4682-4689.
27. Downward, J., Graves, J. D., Warne, P. H., Rayter, S., and Cantrell, D. A. (1990) Nature 346, 719-723.
28. Hoshijima, M., Kikuchi, A., Kawata, M., Ohmori, T., Hashimoto, E., Yamamura, H., and Takai, Y. (1988) Biochem. Biophys. Res. Commun. 157, 851-860.
29. Kawata, M., Kikuchi, A., Hoshijima, M., Yamamoto, K., Hashimoto, E., Yamamura, H., and Takai, Y. (1989) J. Biol. Chem. 264, 16588-16595.
30. Hata, Y., Kajibuchi, K., Kawamura, S., Hiroyoshi, M., Shirakati, H., and Takai, Y. (1991) J. Biol. Chem. 266, 2767-2777.
31. Isoh, T., Kajibuchi, K., Sasaki, T., and Takai, Y. (1991) Biochem. Biophys. Res. Commun. 177, 1329-1334.
32. Balbinger, R., Forth, M. E., and Rosen, O. M. (1987) J. Biol. Chem. 262, 2688-2695.