Activation of Gq protein-coupled receptors can either stimulate or inhibit cell growth. Previously, these opposite effects were explained by differences in the cell models. Here we show that activation of m3 muscarinic acetylcholine receptors ectopically expressed in NIH3T3 cells can cause stimulation and inhibition of growth in the same cell. A clonal cell line was selected from cells that formed foci agonist dependently (3T3/m3 cells). In quiescent 3T3/m3 cells, carbachol stimulated DNA synthesis. In contrast, when 3T3/m3 cells were growing, either due to the presence of serum or after transformation with oncogenic v-src, carbachol inhibited growth. This inhibition was not due to reduction of extracellular signal-regulated kinase activity because carbachol induced extracellular signal-regulated kinase phosphorylation in both quiescent and growing 3T3/m3 cells. Investigating the cell cycle mechanisms involved in growth inhibition, we found that carbachol treatment decreased cyclin D1 levels, increased p21cip1 expression, and led to hypophosphorylation of the retinoblastoma gene product (Rb). Proteasome inhibitors blocked the carbachol-induced degradation of cyclin D1. Effects on p21cip1 were blocked by a protein kinase C inhibitor. Thus, m3 muscarinic acetylcholine receptors couple to both growth-stimulatory and -inhibitory signaling pathways in NIH3T3 cells, and the observed effects of receptor activation depend on the context of cellular growth.

G protein-coupled receptors are found on virtually all cells and respond to a wide variety of regulatory molecules to influence differentiated cell functions including contraction, secretion, and ion transport. With the discovery that the mas oncoprotein coded for a G protein-coupled receptor (1), much interest has focused on the potential of these receptors to be involved in malignant transformation. More recently, activating mutations in thyroid-stimulating hormone receptors have been observed in ~30% of thyroid adenomas (2), and mutationally activated luteinizing hormone receptors have been identified in a form of precocious puberty that results from hyperplastic luteinizing hormone receptors (3). As expected, agonist treatment of quiescent m3 AchR-transfected cells led to hypophosphorylation of Rb, which ultimately leads to the initiation of the proliferative response (15). Thus, together, these observations provide a model for the induction of growth stimulatory and potentially transforming effects by m3 AchR activation.

Little is known concerning the growth-inhibitory mechanisms activated by these receptors. To investigate the growth-stimulatory and -inhibitory effects of Gq protein-linked receptors, we expressed m3 AchRs in NIH3T3 cells. Our studies confirmed that agonist treatment of m3 AchR-transfected cell populations leads to the formation of foci in a subset of receptor-bearing cells. As expected, agonist treatment of quiescent populations of these focus-competent cells stimulated DNA synthesis. However, when the same focus-competent cells were activated by stimulation with agonists, a biphasic response was observed, with an initial phase of cell growth followed by cell cycle arrest and cell death. This biphasic response is consistent with the involvement of both growth-stimulatory and -inhibitory pathways in the response to m3 AchR activation.
M3 Ach Receptor Activation Inhibits Growth in NIH3T3 Cells

FiguRe 1. Growth stimulation and inhibition in 3T3/m3 cells isolated from receptor-bearing foci. A, concentration dependence of carbachol-induced stimulation of DNA synthesis in quiescent 3T3/m3 cells. 3T3/m3 cells were made quiescent by serum starvation (24 h) and then stimulated with the indicated concentrations of carbachol for 24 h. [3H]Thymidine incorporation was assessed, and the results shown are expressed as the fold of incorporation in the control cultures and represent the mean ± S.E. for three separate experiments. Concentration (B) and time (C) dependence of carbachol-induced inhibition of DNA synthesis in growing 3T3/m3 cells is shown. Cells were cultured in the presence of 10% FBS and either the indicated concentrations of carbachol were included for 24 h (B) or 100 μM carbachol was added for the indicated times (C). Results shown are expressed as percentages of control.

EXPERIMENTAL PROCEDURES

Materials—The construction of the pTEJ-8 expression vector bearing human m3 AchR cDNA has been described previously (16). The plasmid bearing oncogenic v-src is a kind gift from Dr. Richard Jove (University of South Florida College of Medicine, Tampa, FL). Polyclonal antibodies to cyclin D1 and E, p21(2), cdk4, and ERK1/2 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and the Anti-AC-TVE(46) MAPK antibody was from Promega (Madison, WI). Monoclonal antibody to Rb was obtained from PharMingen (San Diego, CA). Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulins were purchased from Amersham Pharmacia Biotech. Carbachol and TPA were obtained from Sigma Chemical Co. The PKC inhibitor GF19023X (bisindolylmaleimide) was from LC Laboratories (Woburn, MA). The proteasome inhibitors PSI (N-benzoyloxycarbonyl-Ile-Glu(O-t-Bu)-Ala-leucinal) and LLnL (N-acetyl-Leu-Leu-norleucinal) were purchased from Peptide Institute Co. (Louisville, KY) and Sigma Chemical Co., respectively. Dulbecco’s modified Eagle’s medium, calf serum, fetal bovine serum (FBS), penicillin, streptomycin, amphotericin B, and Lipofectamine were obtained from Life Technologies, Inc.

Tissue Culture—The mouse NIH3T3 fibroblast cells (obtained from American Type Culture Collection, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum, penicillin, and streptomycin.

Foci Formation—NIH3T3 cells were transfected with 5 μg of pTEJ8-m3AchR using Lipofectamine and following the manufacturer’s recommended protocol. The day after transfection, the cells were replated into three new dishes. One dish was cultured in the presence of 100 μM carbachol, another dish was cultured in the presence of 0.8 μg/ml G418, and one dish was cultured in the absence of carbachol or G418. Foci were scored after 2–3 weeks. Individual foci were isolated with the aid of cloning cylinders and then selected in the presence of 0.8 μg/ml G418 to eliminate nontransfected cells. For replating assays, receptor-bearing cells were mixed with wild-type NIH3T3 cells at a ratio of 1:1000, and 106 cells were plated in a 35-mm dish. Cells were cultured in the presence or absence of 100 μM carbachol, and foci were scored after 2–3 weeks.

N-[3H]methylisocarbamoylamine Binding—The m3 Ach receptor binding assay was performed exactly as described previously (16).

Measurement of DNA Synthesis—DNA synthesis was estimated by measurement of [3H]thymidine incorporation into trichloroacetic acid-precipitable material. Cells growing in the absence or presence of 10% FBS were treated with carbachol (100 μM) for 24 h or as indicated, and [3H]thymidine (0.1 μCi/ml) was added during the last hour. Cells were precipitated twice with ice-cold 6% trichloroacetic acid. Cells were then removed with 0.1 N NaOH, and radioactivity was determined by liquid scintillation counting.

Immunoblotting—Cells were scraped in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 2.5 mM EGTA, 0.1% Tween 20, 1 mM dithiothreitol, 1 mM NaF, 0.1 mM sodium orthovanadate, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM β-glycerophosphate, 2 μg/ml aprotinin, and 5 μg/ml leupeptin), sonicated, and centrifuged to remove cellular debris. Bio-Rad protein assay reagent was used to determine the protein concentration. Samples of 20 μg of protein denatured with Laemmli buffer were subjected to electrophoresis on 7.5% (to detect Rb), 10% (to detect cyclin D1 and E, cdk4, phospho- and non-phospho-ERK1/2), and 12% (to detect p21cip1) SDS-polyacrylamide gels and transferred by electrophoresis to Hybond membranes (Amersham Pharmacia Biotech). The blots were then incubated with the respective primary antibody, and horseradish peroxidase-labeled secondary antibody was used for detection with ECL (Amersham Pharmacia Biotech).

RESULTS

Foci-competent 3T3/m3 Cells Show Both Inhibitory and Stimulatory Responses to Agonist Treatment—To study growth regulation induced by activation of a Gq protein-coupled receptor, we transfected NIH3T3 cells with the pTEJ8 expression vector bearing the human m3 AchR cDNA. A clonal cell line was isolated from foci formed in the presence of the receptor agonist carbachol (see “Experimental Procedures”), as reported previously (4). This cell line was designated 3T3/m3 and was further characterized. Binding assays indicated that these cells bound 623 ± 46 fmol of N-[3H]methylisocarbamoylamine per milligram of protein. This corresponds to a receptor density of approximately 112,000 receptors/cell. To investigate the effects of carbachol on DNA synthesis, 3T3/m3 cells were serum-starved for 24 h. Carbachol treatment of these quiescent 3T3/m3 cells led to a large (22-fold over the basal level) increase in thymidine incorporation within 24 h. This stimulation of DNA synthesis was concentration-dependent, with half-maximal effects observed at 4 μM, and maximal effects observed at 1 μM carbachol (Fig. 1A).

In contrast to the effects observed in quiescent 3T3/m3 cells, when these cells were treated with carbachol in the presence of serum, carbachol inhibited DNA synthesis. Inhibition of DNA synthesis was concentration-dependent, with half-maximal effects observed at 1 μM, and maximal effects observed at 100 μM carbachol (Fig. 1B). At maximal concentrations (100 μM), carbachol reduced DNA synthesis to 23 ± 7% of control. Inhibitory effects of m3 AchR activation on DNA synthesis were significant within 1 h and maximal after 12 h of carbachol treatment (Fig. 1C). At later times, DNA synthesis rates recovered (150 ±
M3 Ach Receptor Activation Inhibits Growth in NIH3T3 Cells

9.8% at 48 h). Thus, the inhibitory effect of carbachol in growing 3T3/m3 cells was transient.

To investigate the inhibitory effects of m3 AchR activation under different growth conditions, we transfected 3T3/m3 cells with oncogenic v-src (3T3/m3-src). Carbachol treatment of 3T3/m3-src cells led to a concentration-dependent inhibition of [3H]thymidine incorporation whether these cells were growing in the presence (data not shown) or absence of serum (Fig. 2A). Significant inhibitory effects were noted at 10 μM, half-maximal effects were observed at 0.8 μM, and maximal effects were seen with 1 mM carbachol. Treatment with 100 μM carbachol reduced DNA synthesis significantly within 1 h. Maximal inhibition (to 30 ± 4% of control) was observed at 12 h after treatment (Fig. 2B). Thus, the time course of onset of carbachol-induced inhibition of DNA synthesis was qualitatively similar in v-src-transformed cells and in 3T3/m3 cells growing in the presence of serum. Comparable results were also obtained in spontaneously transformed 3T3/m3 cells and when 3T3/m3 cells were growth-stimulated using fibroblast growth factor (data not shown).

3T3/m3 cells were either starved for 24 h or cultured in the presence of 10% FBS. Cells were then incubated with (+) or without (−) 100 μM carbachol (CCh) for the indicated times. The duration of ERK activation was longer in the serum-starved cells than in those growing in serum or transformed with v-src. Carbachol treatment had no effect on the ERK1/ERK2 protein levels in these cells (Fig. 3). Hence, the opposing effects of carbachol in quiescent and growing 3T3/m3 cells do not seem to be explainable by different effects of carbachol on the activation of ERKs.

m3 AchR Activation Increases Cyclin D1 Levels in Quiescent Cells but Decreases Cyclin D1 Levels in Growing Cells—We next investigated the effects of m3 AchR activation on cell cycle proteins. 3T3/m3 cells were either serum-starved or cultured in the presence of serum for 24 h before treatment with carbachol for various times. In quiescent 3T3/m3 cells, the basal levels of the G1-phase cyclins D1 and E were low. Treatment with carbachol led to an increase in the expression of these cyclins after 12–24 h. Carbachol also increased the level of the cdk inhibitor p21cip1 within 1 h (Fig. 4A). The expression of p21cip1 remained above control levels for at least 24 h. In quiescent cells, Rb existed primarily in the hypophosphorylated state. Carbachol treatment induced hyperphosphorylation of Rb at the same times that the cyclin D1 and E levels were increased. Levels of cdk4 were unaffected by carbachol treatment (Fig. 4A).

In contrast to what was observed in the quiescent cells, in 3T3/m3 cells growing in serum, carbachol significantly decreased cyclin D1 and E levels within 1 h (Fig. 4B). The observed decrease was transient. A maximal decrease was noted after 6 h, and after 12–24 h of carbachol treatment, cyclin D1 and E levels returned to control levels. Carbachol induced expression of p21cip1 in growing cells within 1 h for up to 24 h in a manner similar to that detected in the quiescent cells. Consistent with the observed time course of the carbachol-induced decrease of cyclin D1 and E levels, treatment with carbachol led to a transient hyperphosphorylation of Rb (Fig. 4B). Carbachol treatment had no effect on the expression of cdk4. These same effects on cyclins D1 and E, p21cip1, and Rb were also observed in 3T3/m3-src cells (data not shown). This suggests that the carbachol-induced inhibition of DNA synthesis is likely mediated by the effects on these cell cycle proteins.

The Carbachol-induced Increase of p21cip1 Is PKC-dependent—Several different pathways have been described leading to the induction of p21cip1 expression; some of these pathways involve PKC (17–20). PKC activity is increased by activation of m3 AchRs. Therefore, to investigate the role of PKC in the carbachol-induced up-regulation of p21cip1 observed in growing 3T3/m3 cells, we tested the effects of the PKC inhibitor GF109203X (Fig. 5). Growing 3T3/m3 cells were pretreated with the inhibitor for 30 min and then incubated for an additional 3 h with carbachol. Treatment with the inhibitor alone had no effect on p21cip1 protein levels. In contrast, GF109203X treatment completely blocked the carbachol-induced increase in p21cip1 expression. Furthermore, incubation of the cells for 3 h with TPA, which is known to activate PKC, mimicked the effect of carbachol on p21cip1 expression (Fig. 5). Thus, the

**FIG. 2.** Inhibition of DNA synthesis in serum-deprived 3T3/m3-src cells mediated by m3 AchRs. Concentration (A) and time (B) dependence of inhibition is shown. 3T3/m3-src cells were cultured in the absence of growth factors for 24 h, and the indicated concentrations of carbachol were included for an additional 24 h (A), or 100 μM carbachol was added for the indicated times (B). The data are presented as the percentage of incorporation into control cultures and represent the means ± S.E. of three experiments.

**FIG. 3.** Carbachol induces phosphorylation of ERKs in quiescent and growing 3T3/m3 cells. 3T3/m3 cells were either starved for 24 h or cultured in the presence of 10% FBS. Cells were then incubated with (+) or without (−) 100 μM carbachol (CCh) for the indicated times. Western blot analysis was performed using the antibody against anti-ACTIVE™ MAPK (phospho, top panel) or p42/p44 ERK (protein, bottom panel).
we examined the effects of m3 AchR activation on growing cells. Growth was stimulated either by serum or by transformation with v-src. In either condition, carbachol caused a large and rapid inhibition of DNA synthesis. This suggests that the growth-regulatory effects of m3 AchR activation depend on the growth context of the cells. An understanding of the mechanisms involved in these growth-inhibitory actions may provide new insight into cellular growth regulation.

In the present study, we investigated the mechanisms involved in the growth-inhibitory effects of m3 AchR activation. Because the ERKs are known to be important molecules in mediating mitogenic responses, we investigated the possibility that activation of the m3 AchR might inhibit this pathway in growing 3T3/m3 cells. However, carbachol led to an increase in ERK activation under all growth conditions. Differences were noted in the duration of ERK activation in serum-fed versus serum-starved cells. The duration of ERK activation seems to be an important issue for proliferative responses. Recently, Weber et al. (22) have shown that a sustained activation of the ERKs by platelet-derived growth factor was required for continued expression of cyclin D1 in IIC9 cells. On the other hand, Pumiglia and Decker (23) noted that sustained stimulation of the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase/ MAPK pathway by an inducible activated form of the Raf-1 proto-oncogene results in cell cycle arrest in NIH3T3 cells. In the current study, inhibition of DNA synthesis was observed within 1 h, a time point at which ERK phosphorylation was induced by carbachol under both growth conditions. Therefore, the observed differences in the duration of carbachol-induced ERK phosphorylation in quiescent and growing 3T3/m3 cells do not seem to be important for the observed differences in growth regulation. The most reasonable interpretation is that the effects of m3 AchR activation on the ERK pathway are not involved in the inhibitory actions.

Two responses that potentially explain the growth-inhibitory effects of m3 AchR activation are the decrease in the level of the G1 cyclin D1 and the induction of p21\textsuperscript{cip1} expression. These two mechanisms alone or in concert would be predicted to inhibit the phosphorylation of Rb and thereby block the entry into the S phase. The initial time course of p21\textsuperscript{cip1} induction and cyclin D1 reduction closely paralleled the decrease in cyclin E levels and the appearance of hypophosphorylated Rb in response to carbachol. This time course also correlated with the observed inhibition of DNA synthesis in 3T3/m3 cells. Thus, the effects of m3 AchR activation on these cell cycle proteins likely mediate the growth inhibition observed in 3T3/m3 cells.

p21\textsuperscript{cip1} is known as a potent cyclin-dependent kinase inhibitor and mediator of cell growth arrest (24, 25). The induction of p21\textsuperscript{cip1} expression by carbachol in 3T3/m3 cells was likely mediated by activation of protein kinase C because the specific PKC inhibitor GF109203X completely blocked the increase of p21\textsuperscript{cip1} levels. Additionally, the phorbol ester TPA mimicked
the carbachol effect and stimulated p21cip1 in a similar manner. Previously, Yamamoto et al. (26) found that TPA markedly inhibits DNA synthesis and proliferation of NIH3T3 cells. This could be due, at least in part, to the induction of p21cip1 expression demonstrated in this study. NIH3T3 cells have been shown to express PKC-α and PKC-ζ isozymes (6). The atypical PKC-ζ, which is not activated by either Ca2+ or diacylglycerol in vitro, has not been described to be involved in growth regulation. However, the classical α-isoform of PKC has been shown to raise p21cip1 levels and induce growth arrest in a human epithelial cell line (27). Stimulation of m3 AchRs increases PKC activity via accumulation of diacylglycerol generated by Goq-mediated activation of phospholipase C-β. Taken together, the data support an intracellular pathway leading to the observed p21cip1 induction by carbachol in 3T3/m3 cells that involves a Goq/PLC-β-induced activation of PKCa. The temporal pattern of p21cip1 induction did not correlate with the reversal of growth inhibition. Furthermore, p21cip1 induction was also observed when quiescent 3T3/m3 cells were treated with carbachol, which resulted in a stimulation of growth. Thus, the data do not support the hypothesis that induction of p21cip1 is the sole inhibitory mechanism activated by m3 AchR activation. p21cip1 is found in active cyclin/cdk complexes in proliferating cells such that the stoichiometry between p21cip1 and cyclin/cdk complexes determines the cell cycle-inhibitory behavior of p21cip1 (25, 28, 29). Hence, p21cip1 may be involved in the initial inhibitory effect, but subsequent changes in the stoichiometry of other cell cycle proteins may overcome this effect.

In contrast to the observed induction of cyclin D1 expression by carbachol in quiescent cells, treatment of growing 3T3/m3 cells with carbachol resulted in a profound decrease of cyclin D1 levels. The D-type cyclins are the first cyclins synthesized after mitogenic stimulation and are required and rate-limiting for G1 progression. They complex with their catalytic subunits cdk4 and cdk6, which function as Rb kinases, and hyperphosphorylation of Rb allows the progression through G1-S phase (30, 31). Withdrawal of growth factors leads to a rapid cyclin D1 destruction and G1-phase arrest (30). Decreased cyclin D1 levels have also been suggested to be involved in transforming growth factor-β-induced (32), rapamycin-induced (33), and cyclic AMP-induced (34) growth inhibition. We observed a rapid decrease in cyclin D1 after carbachol treatment of growing 3T3/m3 cells. Diehl et al. (21) suggested that a ubiquitin-proteasome pathway mediates cyclin D1 degradation. In the current study, proteasome inhibitors completely blocked carbachol-induced cyclin D1 degradation. The mechanisms involved in targeting cyclin D1 for ubiquitin-proteasome-mediated degradation are unknown. The carbachol-induced decrease in cyclin D1 was transient, and levels returned to control levels within 24 h. The time course of cyclin D1 degradation closely paralleled the observed inhibition of DNA synthesis. Thus, the effects of m3 AchR activation on cyclin D1 levels could explain both the initiation and the reversal of the observed effects on DNA synthesis. Further investigation will be necessary to understand the mechanisms involved in the effects of m3 AchR activation on cyclin D1.

The physiological relevance of the transforming or tumor-suppressing abilities of these receptors is unclear. The NIH3T3 cell model suffers from the fact that these cells are pre-neoplastic, are differentially sensitive to transformation by different oncogenes, and can “drift” and spontaneously assume a transformed phenotype. Expression of Gq protein-linked receptors was not able to induce transformation in Rat-1 cells, another common model of cellular transformation (35). Furthermore, conflicting results have previously been published with regard to the effect of expressing GTPase-deficient, constitutively active Goq subunits in NIH3T3 cells. Wu et al. (36) observed that mutant active Goq and Goq11 subunits caused cell death when transfected into NIH3T3 cells. In contrast, others have reported that constitutively active Goq could transform NIH3T3 cells (37, 38). Although it was reported that expression of the active Goq mutant caused more cell death than oncogenic transformation in NIH3T3 cells, this was interpreted to mean that low-level expression resulted in transformation and higher levels of expression caused cell death (38). Therefore, it is unclear how widespread or important the transforming effects might be. Another limitation to the ability of these receptors to mediate cellular transformation would likely be the requirement for sustained high concentrations of agonist. An alternative to high concentrations of agonist would be mutations that lead to persistent activation of the receptor or the G proteins. Mutant forms of Goq or other members of the Goq subunit family or receptors that couple to these G proteins have not been identified to date in any tumors or genetic disorder. If this pathway were highly transforming, then one would expect to find numerous examples, considering the ubiquitous nature of these receptors.

In summary, the current study shows that Gq protein-linked receptors couple to both inhibitory and stimulatory signaling pathways. Thus, activation of these receptors can have a variety of effects on cell growth, depending upon the cell model, the receptor level, the assay conditions, and the time of observation. These factors help to explain the apparent conflicts existing in the literature with regard to the growth effects of these receptors. An understanding of the growth inhibitory pathways activated by these receptors may lead to important new insights into cellular growth regulation in health and disease.

Acknowledgments—We thank Drs. Jessica Schwartz, Ormond MacDougald, and Paul Cook for critical review of the manuscript.

REFERENCES

1. Jackson, T. R., Blair, L. A., Marshall, J., Goedert, M., and Hanley, M. R. (1988) Nature 335, 437–440
2. Parma, J., Duprez, L., Van Sande, J., Cochaux, P., Gery, C., Mocell, J., Dumont, J., and Vassart, G. (1993) Nature 365, 649–651
3. Shenker, A., Laue, L., Kosugi, S., Merendino, J. J. J., Minegishi, T., and Cutler, G. R. Jr. (1993) Nature 365, 652–654
4. Gutkind, J. S., Novotny, E. A., Brann, M. R., and Robbins, K. C. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4703–4707
5. Mostowy, L. B., Sorisky, A., Brann, M. R., and Macara, I. G. (1994) Mol. Cell. Biol. 14, 7943–7952
6. Stephens, E. V., Kalinec, G., Brann, M. R., and Gutkind, J. S. (1993) Oncogene 19, 26
7. Felder, C. C., MacArthur, L., Ma, A. L., Gusovsky, F., and Kohn, E. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1706–1710
8. Detjen, K., Yang, J., and Logsdon, C. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10929–10933
9. Detjen, K., Fenrich, M., and Logsdon, C. D. (1997) Gastroenterology 112, 952–959
10. Gutkind, J. S., and Robbins, K. C. (1992) Biochem. Biophys. Res. Commun. 188, 155–161
11. Felder, C. C. (1995) FASEB J. 9, 619–625
12. Gutkind, J. S. (1996) J. Biol. Chem. 271, 1839–1842
13. Dhanasekaran, N., Taim, S. T., Derront, M. J., and Onesime, D. (1996) Oncogene 17, 1383–1394
14. Xu, N., McCormick, F., and Gutkind, J. S. (1994) Oncogene 9, 597–601
15. Peper, D. S., Upton, T. M., Ladha, M. H., Neuman, E., Zalvide, J., Bernards, R., DeCaprio, J. A., and Ewen, M. E. (1997) Nature 386, 177–181
16. Yang, J., Logsdon, C. D., Johansen, T. E., and Williams, J. A. (1993) Mol. Pharmacol. 44, 1158–1164
17. Detton, M. B., Yu, Y., and Wang, X. F. (1995) J. Biol. Chem. 270, 28623–28628
18. Li, R., Hannon, G. J., Beach, D., and Stillman, B. (1996) Curr. Biol. 6, 189–199
19. Sherr, C. J., and Roberts, J. M. (1995) Genes Dev. 9, 1149–1163
20. Livneh, E., and Fishman, D. D. (1997) Eur. J. Biochem. 248, 1–9
21. Diehl, J. A., Zindy, F., and Sherr, C. J. (1997) Genes Dev. 11, 957–972
22. Weber, J. D., Rabin, D. M., Phillips, P. J., and Baldassare, J. J. (1997) Biochem. J. 326, 61–68
23. Pumiglia, K. M., and Decker, S. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 448–452
24. Xiong, Y., Hannon, G. J., Zhang, H., Casse, D., Kobayashi, R., and Beach, D. (1993) Nature 366, 701–704
25. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) Cell 73, 805–816
26. Yamamoto, T., Tsuda, T., Hamamori, Y., Nishinoura, N., and Takai, Y. (1988)
M3 Ach Receptor Activation Inhibits Growth in NIH3T3 Cells

21706

J. Biochem. (Tokyo) 104, 53–56

27. Frey, M. R., Saxon, M. L., Zhao, X., Rollins, A., Evans, S. S., and Black, J. D. (1997) J. Biol. Chem. 272, 9424–9435

28. Zhang, H., Hannon, G. J., and Beach, D. (1994) Genes Dev. 8, 1750–1758

29. Hiyama, H., Iavarone, A., LaBaer, J., and Reeves, S. A. (1997) Oncogene 14, 2533–2542

30. Sherr, C. J. (1993) Cell 73, 1059–1065

31. Matsushima, H., Quelle, D. E., Shurtleff, S. A., Shibuya, M., Sherr, C. J., and Kato, J. Y. (1994) Mol. Cell. Biol. 14, 2066–2076

32. Ko, T. C., Yu, W., Sakai, T., Sheng, H., Shao, J., Beauchamp, R. D., and Thompson, E. A. (1998) Oncogene 16, 3445–3454

33. Hashemolhosseini, S., Nagamine, Y., Morley, S. J., Desrivieres, S., Mercep, L., and Ferrari, S. (1998) J. Biol. Chem. 273, 14424–14429

34. L’Allemain, G., Lavoie, J. N., Rivard, N., Baldin, V., and Pouyssegur, J. (1997) Oncogene 14, 1981–1990

35. Kahan, C., Julius, D., Pouyssegur, J., and Seuwen, K. (1992) Exp. Cell Res. 200, 523–527

36. Wu, D. Q., Lee, C. H., Rhee, S. G., and Simon, M. I. (1992) J. Biol. Chem. 267, 1811–1817

37. De Vivo, M., and Iyengar, R. (1994) J. Biol. Chem. 269, 19671–19674

38. Kaliniec, G., Nazarali, A. J., Hermouet, S., Xu, N., and Gutkind, J. S. (1992) Mol. Cell. Biol. 12, 4687–4693