Ablation of Go \( \alpha \) Overrides G\(_1\) Restriction Point Control through Ras/ERK/Cyclin D1-CDK Activities*

(Received for publication, February 4, 1997, and in revised form, April 30, 1997)

Jason D. Weber‡, Jie Cheng§, Daniel M. Raben§, Alice Gardner¶, and Joseph J. Baldassare‡

From the ‡Department of Cell and Molecular Biology and the §Department of Pharmacological and Physiological Sciences, St. Louis University School of Medicine, St. Louis, Missouri 63104 and the ¶Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

We have generated stable IIC9 cell lines, Goa1 and Goa2, that overexpress full-length antisense Go \( \alpha \) RNA. As shown previously, expression of antisense Go \( \alpha \) RNA ablated the \( \alpha \) subunit of the heterotrimeric G protein, Go, resulting in growth in the absence of mitogen. To better understand this change in IIC9 phenotype, we have characterized the signaling pathway and cell cycle events previously shown to be important in control of IIC9 G\(_1\)/S phase progression. In this paper we clearly demonstrate that ablation of Go \( \alpha \) results in growth, constitutively active Ras/ERK, elevated expression of cyclin D1, and constitutively active cyclin D1-CDK complexes, in all the absence of mitogen. Furthermore, these characteristics were abolished by the transient overexpression of the transducin heterotrimeric G protein \( \alpha \) subunit strongly suggesting the transformation of Go \( \alpha \)-ablated cells involves Go \( \beta\gamma \) subunits. This is the first study to implicate a heterotrimeric G protein in tumor suppression.

In IIC9 cells, a subclone of Chinese hamster embryo fibroblasts, platelet-derived growth factor (PDGF)\(^1\) is a potent mitogen (1). PDGF stimulates an increase in cyclin D1 expression concomitant with an increase in cyclin D1-CDK activity (1). Cyclin D1 is an important G\(_1\) protein in that its delayed early induction in response to mitogen is required for G\(_1\) progression (2). Microinjection of cyclin D1 antibodies or antisense cyclin D1 plasmids into normal fibroblasts arrests them in G\(_1\) but has no effect on cells already beyond the G\(_1\)/S boundary (3, 4). Thus, accumulation of cyclin D1 in G\(_1\) in response to mitogen is required for progression through the restriction point and entrance into S phase (2, 5, 6). Cyclin D1 preferentially binds to its catalytic partner, cyclin-dependent kinase 4 (CDK4), to form a holoenzyme (7–10). The activated cyclin D1-CDK4 complex preferentially binds to and phosphorylates the retinoblastoma gene product (pRb) (11–16). Hence, the mitogen-induced activation of cyclin D1-CDK4 complexes allows for progression through the restriction point in vivo presumably through the hyperphorylation and inactivation of pRb in concert with other G\(_1\) cyclin-CDK complexes.

We and others previously have shown that PDGF stimulates ERK1 activity in IIC9 and CCL39 cells and that inhibition of this activity is sufficient to cause the loss of PDGF-induced cyclin D1 expression, as well as a loss of cyclin D1-CDK activity (1). The loss of PDGF-induced cyclin D1-CDK activity was correlated with G\(_1\) growth arrest. Attention has focused on the mitogen-induced signals involved in cell growth and more recently those signals regulating mitogen-dependent induction cyclin D1 (1, 6). Expression of constitutively active mutant Ras has been shown to transform several cell types and elevate cyclin D1 expression (3, 17–19). The role of Ras proteins in the mitogenesis and transformation of cells is mediated, in part, by a downstream cascade of serine-threonine kinases that terminates with the activation of p42 and p44 MAPKs (ERK1, ERK2). Activated ERKs phosphorylate several nuclear factors that control gene expression. Evidence for the role of the Ras-mediated MAPK cascade is well documented with kinase-deficient mutants of Raf-1, MEK, and MAPKs inhibiting Ras transformation.

Recent evidence has shown that Go \( \alpha \) activates ERK (20). Go \( \alpha \) activation of ERK is mediated by a novel protein kinase C-dependent mitogenic signaling pathway which is independent of Ras activation. The studies reported here examine the effects of ablation of Go \( \alpha \) by overexpression of antisense Go \( \alpha \) RNA in IIC9 cells on the Ras/ERK pathway and cell cycle activities known to be important in mitogen-induced growth. Currently, there is evidence suggesting that \( \beta\gamma \) subunits from pertussis toxin-sensitive heterotrimeric G proteins are capable of ERK activation through a Ras-dependent signaling pathway (20–23). The \( \beta\gamma \)-mediated ERK activation is blocked by the expression of dominant negative Ras (22). Our data demonstrate that loss of Go \( \alpha \) expression in IIC9 cells results in unregulated growth by constitutively activating the Ras/ERK pathway, a pathway we and others have shown positively regulates cyclin D1-CDK activity through the increased expression of cyclin D1.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—IIC9 cells, a subclone of Chinese hamster embryo fibroblasts (46), were grown and maintained in DMEM (Life Technologies, Inc., Grand Island, NY) containing 10% fetal calf serum and 2 mm l-glutamine (all chemicals were obtained from Sigma, unless specified otherwise). Subconfluent cultures were growth-arrested by washing once with serum-free DMEM and cultured for 48–60 h in serum-free media. PDGF was obtained from Calbiochem (La Jolla, CA). Stable Go \( \alpha \) antisense transfectants were produced and maintained as described previously (48). Briefly, pcDNAI containing Go \( \alpha \) cDNA in an antisense orientation to the cytomegaviruses promoter was transfected into IIC9 cells using Lipofectamine™ protocol (Life Technologies, Inc.). Following an 18-h transfection period, cells were cultured for 48 h in DMEM containing 10% fetal calf serum to allow for the expression of neo-resistance gene products. Transfected IIC9 cells were grown for several weeks in selection media containing G418 (500 μg/ml). G418-resistant clones were isolated and subcultured in DMEM containing 10% fetal calf serum and 250 μg/ml G418. Several Go \( \alpha \)-ablated clones were isolated of which two, Goa1 and Goa2 (described in the

* This work was supported by National Institutes of Health Grants HL40901 (to J. J. B.) and GM51593 (to D. M. R.). 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.
Overriding the G₁ Restriction Point with G₀ Ablation

and washed once at room temperature with 5 mM MgCl₂, 0.1% Triton X-100, 20 mM sodium vanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin. The lysates were assayed for ERK activity as described above.

Cyclin D1/CDK Kinase Assay—Cyclin D1/CDK activity was assayed as described previously (8) with modifications. Briefly, growth-arrested IIC9 cells were stimulated with PDGF (10 ng/ml) and harvested at 0, 1, 2, and 4 h after stimulation. The cells were washed, resuspended in 1 ml of cold buffer (25 mM Hepes, 300 mM NaCl, 0.2 mM EDTA, 1.5 mM MgCl₂, 0.1% Triton X-100, 20 mM β-glycerophosphate, 0.1 mM sodium vanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.5 mM phenylmethylsulfonfluoride) and lysed with 0.5% Triton X-100 for 5 min on ice. The lysates were solubilized in solubilization buffer (0.5% Triton X-100, 20 mM β-glycerophosphate, 0.1 mM sodium vanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.5 mM phenylmethylsulfonfluoride) and washed twice with DMEM. Following primary incubation, cells were harvested and lysed by scraping in 500 μl of reaction buffer (50 mM Hepes, 10 mM EDTA, 2.5 mM EGTA, 10 mM β-glycerophosphate, 1 mM sodium fluoride, 0.1% Tween 20, 10% glycerol, 1 mM phenylmethylsulfonfluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin). Goa1 cells were grown in serum, serum-deprived for 48 h, and harvested by scraping in cold 1 × PBS. Lysates were sonicated briefly and insoluble material was pelleted by centrifugation at 10,000 × g for 10 min. Cyclin D1 monoclonal antibody (2 μg) was added to supernatants and incubated at 4 °C. After 1–2 h cyclin D1 complexes were precipitated for 2–3 h with protein G-Sepharose. Cyclin D1 immune complexes were washed 4 times with 1 ml of cold IP buffer and 2 times with 1 ml of cold wash buffer (50 mM Hepes, 10 mM MgCl₂, and 1% dithiothreitol). Cyclin D1 immune complexes were pelleted and SDS-polyacrylamide gels. Separated proteins were then transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were probed with a cyclin D1 polyclonal antibody (Santa Cruz Biotechnology). Goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate (Bio-Rad) was added as the secondary antibody and specific protein bands were visualized using ECL (Amersham) as recommended by the manufacturer.

Northen Blots—Total RNA was isolated from IIC9 and Goa1 cells (4–8 × 10⁶) cultured on 100-mm dishes with Trizol Reagent (Life Technologies, Inc.) using the manufacturer’s protocol. RNA was electrophoresed on 2% agarose/formamide gels. Formaldehyde was removed by washing gels in 0.5 M ammonium acetate. RNA was transferred onto Hybond N⁺ nylon membranes (Amerham) using the Turboblotter system (Schleicher & Schuell, Keene, NH). RNA was cross-linked onto membranes with an Ultraviolet Crosslinker (Amerham) using the manufacturers protocol. Transferred RNA was visualized using a methylene blue/sodium acetate stain. Randomly-labeled antisense cDNA (32P) probes (murine cDNA for cyclin D1 was a generous gift from Dr. Charles Sherr) were made using the Random Primed DNA Labeling Kit (Boehringer Mannheim, Germany). Blots were probed simultaneously with cyclin D1 and glyceraldehyde-3-phosphate dehydrogenase probes for 2 h at 65 °C using Rapid-hyb buffer (Amersham). The blots were washed once at room temperature with 5 × SSPE (20 mM EDTA, 1 × sodium phosphate buffer, 0.1% SDS), 0.2% SDS, and 0.1% SDS in autoradiography or direct quantitation with a PhosphorImager (Molecular Dynamics). More stringent washes were done at 65 °C with 0.5% SDS when necessary.

Thymidine Incorporation—Thymidine incorporation was performed as described previously (1) with minor modifications. Briefly, growth-arrested IIC9 cells were stimulated with PDGF (10 ng/ml) for approximately 20 h. Goa1 cells were serum-starved by washing twice with DMEM and incubated for 48 h in serum-free DMEM supplemented with 2 mM 1-glutamine. To grow-arrest Goa1 cells, aphidicolin (5 μg/ml) was added to serum-deprived cells as described above. Following a 24-h incubation, aphidicolin-arrested cells were released from arrest by washing twice with DMEM. Following primary incubation, cells were incubated for 3 h with 1 μCi of [3H]thymidine/ml (NEN Life Sciences Products). [3H]Labeled cells were washed twice with cold 1 × PBS and the DNA was precipitated by incubating the cells for 30 min with cold 5% trichloroacetic acid. The trichloroacetic acid-precipitated DNA was washed twice with cold 5% trichloroacetic acid and solubilized with 2% sodium bicarbonate, 0.1 N NaOH. The solution was neutralized by addition of one-fifth volume of 5% trichloroacetic acid and the trichloroacetic acid-precipitated [3H]DNA was quantitated by scintillation counting.

ERK Assay—Growth-arrested IIC9 cells were stimulated with mitogen at 37 °C. After 15 min the media was removed and the cells were washed with 1 × PBS. Goa1 cells were either grown in serum (10%), serum-starved for 48 h, arrested with aphidicolin (5 μg/ml), or released from aphidicolin arrest for 8 h as described above and washed with 1 × PBS. After washing, cells were lysed by scraping in 300 μl of solubilization buffer (20 mM Tris-HCl, pH 8, 1 mM sodium vanadate, 10% glycerol, 1 mM phenylmethylsulphonyl fluoride, 2 mM EDTA, 1% Triton X-100, 50 mM β-glycerophosphate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin). The lysates were assayed for ERK activity as described above.

RESULTS AND DISCUSSION

Goa1 Cells Do Not Exit the Cell Cycle upon Removal of Mitogen—We previously generated a panel of stable IIC9 clones (including Goa1 and Goa2 clones) overexpressing full-length G₀ RNA (48). We examined the phenotype of two of these clones, Goa1 and Goa2. In contrast to IIC9 cells, Goa1 and Goa2 cells do not express G₀ protein. Goa1 and Goa2 cells formed multiple foci in monolayer cultures and anchorage-independent colonies in soft agar (48).

To understand the mechanism for the transformed phenotype of these cells, we examined whether Goa1 cells growth arrested upon removal of mitogen. Goa1 cells did not grow arrest upon removal of serum (Fig. 1A) and flow cytometry showed Goa1 cells randomly distributed throughout the cell cycle with a slight majority of cells in S phase (data not shown). To arrest and synchronize populations of mitogen-independent Goa1 cells, aphidicolin, a novel DNA polymerase α inhibitor,
was utilized. Addition of aphidicolin in the absence of serum resulted in growth arrest within 12–16 h after treatment (Fig. 1B). Previous studies have shown that removal of aphidicolin by washing with serum-free media is sufficient to allow cells to enter S phase in the absence of mitogen within 4–8 h (5). However, aphidicolin-released cells arrest when they reach the subsequent G1/S restriction point of the next cycle. Goa1 cells resumed cell cycle progression after release from aphidicolin in serum-free media and continued through subsequent cycles in the absence of mitogen while IIC9 cells arrested at the proceeding G1/S restriction point (Fig. 1B) further demonstrating the mitogen-independent growth of Goa1 cells. Transient overexpression of Goα antisense RNA also resulted in mitogen-independent growth suggesting that the transformed phenotype of the stable Goa1 cell type is a result of Goα ablation and not a result of a deletion of another gene due to homologous recombination (data not shown).

Ablation of Goα Results in the Mitogen-independent Activation of Ras—IIC9 cells overexpressing constitutively activated Ras form multiple foci when grown in soft agar and do not growth arrest when serum-depleted (data not shown). To examine whether the Ras pathway was involved in the transformation of Goa1 cells we first examined Ras activation. In growth-arrested IIC9 cells, levels of activated Ras as determined by the ratio GTP/(GTP+GDP) associated with Ras were quite low (Fig. 2) and increased 6-fold within 5 min after the addition of PDGF (Fig. 2) or several other growth factors (data not shown). In contrast, Goa1 cells exhibited high levels of activated Ras in the absence of mitogen similar to levels found in IIC9 cells treated with PDGF (Fig. 2). Addition of PDGF did not increase the level of activated Ras above the levels seen in serum-depleted Goa1 cells. Similar results were observed with a second clonal Goα-ablated cell line (Goa2) (data not shown) indicating that ablation of Goα in IIC9 cells results in significant activation of Ras. These results are consistent with the observed inability of these cells to grow in the absence of mitogen and suggest that in certain cell types loss of Goα could result in neoplastic transformation.

Goa1 Cells Exhibit Constitutively Active ERK—Recent data from several laboratories has suggested the importance of ERK activation in Ras-dependent growth. We have previously demonstrated that suppression of PDGF-induced ERK activation...
Results reported are the mean ± S.D. (n = 4).

Goa1 cells exhibit constitutively active ERK. IIC9 and Goa1 cells were grown and serum-deprived for 48 h as described previously. IIC9 and Goa1 cells incubated in the absence (open bars) or presence (solid bars) of PDGF (10 ng/ml) for 15 min. Cells were assayed for ERK activity as described under "Experimental Procedures." Results reported are the mean ± S.D. (n = 4).

We next investigated the effect of aphidicolin arrest and release on cyclin D1 expression. Treatment of Goa1 cells with aphidicolin for 12 h resulted in sustained levels of cyclin D1 protein in the absence of mitogen (Fig. 4A). Matsushime et al. (5) previously showed that Bac1.2F5A macrophages released from aphidicolin arrest required the presence of growth factor (CSF-1) to sustain the expression of cyclin D1. Release of IIC9 cells from aphidicolin arrest in the absence of PDGF resulted in the rapid (within 5 h) decrease in the levels of cyclin D1 protein (Fig. 4A). However, cyclin D1 protein levels in Goa1 cells did not decrease significantly when released from aphidicolin in the absence of PDGF suggesting a significant difference in the requirement of sustained presence of growth factor for cyclin D1 expression. It is clear that the constitutive activation of the Ras/ERK pathway (Figs. 2 and 3) provides the sustained mitogenic signals required for the continued expression of cyclin D1. Cyclin D1 protein expression remained high through the next round of replication (approximately 24 h after aphidicolin release) (Fig. 4A). In the absence of mitogen, cyclin D1 mRNA levels in Goa1 cells were similar to the levels found in IIC9 cells treated with PDGF (Fig. 4B). Aphidicolin-treated and released...
Goa1 cells exhibited a 1.6-fold decrease in cyclin D1 mRNA expression although these levels were still 3-fold higher than serum-deprived IIC9 cells (Fig. 4B) providing further evidence for the positive role of the Ras/ERK pathway in sustaining cyclin D1 expression in Goa1 cells in the absence of mitogen.

Cyclin D1-CDK Complexes Are Constitutively Active—Phosphorylation of Rb by active cyclin D1-CDK complexes has been demonstrated to be required for progression through G1 in several cell types (12–16). Although several transformed tumor cells express abnormally high levels of cyclin D1, the role of cyclin D1 in tumor formation is unclear. However, cyclin D1-CDK activity is thought to play an important role in mitogen-induced progression of cells through G1. In the absence of mitogen, IIC9 cells contain low levels of cyclin D1-CDK activity (Fig. 4C). PDGF treatment induced a 6-fold increase in cyclin D1-CDK activity withi n 4 h (data not shown) and sustained this level of activity through 24 h (Fig. 4D). In contrast to IIC9 cells, Goa1 cells display significant cyclin D1-CDK activity in the absence of PDGF (10 ng/ml) for 12 h. Gray bars, in those cells treated with aphidicolin, aphidicolin was removed by washing with fresh media and the cells incubated in serum-free media for an additional 5 h. Cells were lysed and RNA extracted using Trizol as described previously. RNA was electrophoresed on 2% agarose/formaldehyde gels and transferred onto Hybond N* membranes. Membranes were probed simultaneously with [32P]CTP-labeled murine cyclin D1 and glyceraldehyde-3-phosphate dehydrogenase cDNA as described under “Experimental Procedures.” Results are reported as the mean ± S.D. (n = 3) of cyclin D1 normalized to glyceraldehyde-3-phosphate dehydrogenase as quantitated by a PhosphorImager. D, serum-deprived IIC9, Goa1, and Goa1/Gα cells were incubated in the absence (SS) or presence of PDGF (10 ng/ml) for 24 h and assayed for cyclin D1-CDK activity as described under “Experimental Procedures.” Gels were dried and scanned with a PhosphorImager and viewed using Lview Pro 1.1D© Software (MMedia Research Corp.).

Goa1 cells exhibited a 1.6-fold decrease in cyclin D1 mRNA expression although these levels were still 3-fold higher than serum-deprived IIC9 cells (Fig. 4B) providing further evidence for the positive role of the Ras/ERK pathway in sustaining cyclin D1 expression in Goa1 cells in the absence of mitogen.

Cyclin D1-CDK Complexes Are Constitutively Active—Phosphorylation of Rb by active cyclin D1-CDK complexes has been demonstrated to be required for progression through G1 in several cell types (12–16). Although several transformed tumor cells express abnormally high levels of cyclin D1, the role of cyclin D1 in tumor formation is unclear. However, cyclin D1-CDK activity is thought to play an important role in mitogen-induced progression of cells through G1. In the absence of mitogen, IIC9 cells contain low levels of cyclin D1-CDK activity (Fig. 4C). PDGF treatment induced a 6-fold increase in cyclin D1-CDK activity within 4 h (data not shown) and sustained this level of activity through 24 h (Fig. 4D). In contrast to IIC9 cells, Goa1 cells display significant cyclin D1-CDK activity in the absence of PDGF (10 ng/ml) for 24 h and assayed for cyclin D1-CDK activity as described under “Experimental Procedures.” Gels were dried and scanned with a PhosphorImager and viewed using Lview Pro 1.1D© Software (MMedia Research Corp.).
Overriding the G1 Restriction Point with Gα Ablation

Acknowledgments—We thank Dr. Charles Sherr for murine cyclin D1 cDNA and Dr. Mark Ewen for GST-Rb cDNA.

REFERENCES
1. Weber, J. D., Raben, D. M., Phillips, P. J., and Baldassare, J. J. (1997) Biochem J., in press.
2. Sherr, C. J. (1995) Trends Biochem Sci. 20, 187–190.
3. Quelle, D. E., Ashmun, R. A., Shurtleff, S. A., Kato, J. Y., Bar-Sagi, D., Roussel, M. F., and Sherr, C. J. (1993) Genes Dev. 7, 1559–1571.
4. Baldin, V., Lukas, J., Marcotte, M. J., Pagano, M., and Draetta, G. (1993) Genes Dev. 7, 812–821.
5. Matsuhashi, H., Roussel, M. F., Ashmun, R. A., and Sherr, C. J. (1991) Cell 65, 701–713.
6. Lavoie, J. N., L'Allemain, G., Brunet, A., Muller, R., and Pouyssegur, J. (1996) J. Biol. Chem. 271, 20608–20616.

2 J. D. Weber and J. J. Baldassare, unpublished observations.
3 A. Gardner, J. D. Weber, and J. J. Baldassare, unpublished observations.
Overriding the G₁ Restriction Point with G0α Ablation

7. Matsushime, H., Ewen, M. E., Strom, D. K., Kato, J.-Y., Hanks, S. K., Rousel, M. F., and Sherr, C. J. (1992) *Cell* 71, 323–334
8. Matsushime, H., Quelle, D. E., Shortleff, S. A., Shibuya, M., Sherr, C. J., and Kato, J.-Y. (1994) *Mol. Cell. Biol.* 14, 2066–2076
9. Sherr, C. J. (1994) *Cell* 79, 551–555
10. Bates, S., Bonetta, L., MacAllan, D., Parry, D., Holder, A., Dickson, C., and Peters, G. (1994) *Oncogene* 9, 71–79
11. Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsushime, H., Kato, J., and Livingston, D. M. (1995) *Cell* 73, 487–497
12. Dowdy, S. F., Hinds, P. W., Louie, K., Reed, S. I., Arnold, A., and Weinberg, R. A. (1993) *Cell* 73, 499–511
13. Kato, J.-Y., Matsushime, H., Hiebert, S. W., Sherr, C. J., and Ewen, M. E. (1993) *Genes Dev.* 7, 331–342
14. Resnitzky, D., Gossen, M., Bujard, H., and Reed, S. I. (1994) *Mol. Cell. Biol.* 14, 1669–1679
15. Ewen, M. E., Sluss, H. K., Sherr, C. J., and Kato, J.-Y. (1995) *Oncogene* 11, 211–219
16. Filmus, J., Robles, A. I., Shi, W., Wong, M. J., Colombo, L. L., and Conti, C. J. (1994) *Oncogene* 9, 3627–3633
17. Liu, J., Chao, J., Jiang, M., Yung, S., Yen, J. J., and Yang-Yen, H. (1995) *Mol. Oncogene* 14, 3654–3663
18. Inglese, J., Koch, W. J., Touhara, K., and Lefkowitz, R. J. (1995) *J. Biol. Chem.* 270, 211–219
19. Faure, M., Voyno-Yasenetskaya, T. A., and Bourne, H. R. (1994) *Nature* 369, 418–420
20. van Biesen, T., Hawes, B. E., Wang, Y.-Z., and Pledger, W. J. (1996) *Oncogene* 12, 127–134
21. Faure, M., Voyno-Yasenetskaya, T. A., and Bourne, H. R. (1994) *J. Biol. Chem.* 269, 7651–7654
22. Crespo, P., Xu, N., Simonds, W. F., and Gutkind, J. S. (1994) *Nature* 369, 418–420
23. Koch, W. J., Hawes, B. E., Allen, L. F., and Lefkowitz, R. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 12706–12710
24. Vouret-Craviari, V., Van Obberghen-Schilling, E., Seicmea, J. C., Van Obberghen, E., and Pouyssegur, J. (1993) *Biochem. J.* 290, 209–214
25. Albanese, C., Johnson, J., Watanabe, G., Eklund, N., Vu, D., Arnold, A., and Pestell, R. G. (1995) *J. Biol. Chem.* 270, 23589–23597
26. Li, Y. C., and Lieberman, M. W. (1989) *Oncogene* 4, 795–798
27. Hendy, P. F., Dowdy, S. F., Eaten, K. N., Arnold, A., and Weinberg, R. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 709–713
28. Lovec, H., Sewing, A., Lucibello, F. C., Muller, R., and Moroy, T. (1994) *Oncogene* 9, 323–326
29. Inglese, J., Koch, W. J., Touhara, K., and Lefkowitz, R. J. (1995) *Trends Biochem. Sci.* 20, 151–156
30. Murtagh, J. J., Eddy, R., Shows, T. B., Moss, J., and Vaughan, M. (1991) *Mol. Cell. Biol.* 11, 1146–1155
31. Callen, D. F., Doggett, N. A., Stallinge, R. L., Chen, L. Z., Whitmore, S. A., Lane, S. A., Nancarrow, J. K., Apostolou, S., Thompson, A. D., Lapeys, N. M., Eyre, H. J., Baker, E. G., Shen, Y., Holman, K., Phillips, H., Richards, R. I., and Sutherland, G. R. (1992) *Genomics* 13, 1178–1185
32. Wilkie, T. M., Gilbert, D. J., Olsen, A. S., Chen, X., Amatruda, T. T., Korenberg, J. R., Trasi, B. J., de Jong, P., Reed, R. E., Simon, M. I., Jenkins, N. A., and Copeland, N. G. (1992) *Nature Genet.* 1, 85–91
33. Doggett, N. A., Breuning, M. H., and Callen, D. F. (1996) *Cytogenet. Cell Genet.* 72, 271–283
34. Doggett, E. C., Rowe, S. T., Valentine, M., Parham, D., Meyer, W. H., and Thompson, E. I. (1996) *Cytogenet. Cell Genet.* 73, 87–90
35. Huff, V., Reeve, A. E., Leppert, M., Strong, L. C., Douglass, E. C., Geiser, C. F., Li, F. P., Meadows, A., Callen, D. F., Lenoir, G., and Saunders, G. F. (1992) *Cancer Res.* 52, 6117–6120
36. Bardi, G., Johansson, B., Pandis, N., Mandahl, N., Bak-Jensen, E., Lindstrom, C., Torqvist, A., Frederiksen, H., Andreu-Sandberg, A., Mitelman, F., and Heim, S. (1995) *Int. J. Cancer* 55, 422–429
37. Veronese, M. L., Bullrich, F., Negrini, M., and Croce, C. M. (1990) *Cancer Res.* 50, 728–732
38. Ananthawat-Jensson, K., Eyfjord, J. E., Ogmundsdottir, H. M., Petursdottir, I., and Steinarsdottir, M. (1996) *Cancer Genet. Cytogen.* 88, 1–7
39. Mandahl, N., Mertens, F., Willen, H., Rydholm, A., Broso, O., and Mitelman, F. (1984) *J. Cancer Res. Clin. Oncol.* 120, 707–711
40. Biggs, P. J., Wooster, R., Ford, D., Chapman, P., Mangion, J., Quirk, Y., Easton, D. F., Burn, J., and Stratton, M. R. (1995) *Nature* 371, 441–443
41. Newsham, I., Kindler-Rohrborn, A., Daub, D., and Cavenee, W. (1995) *Genes Chromosomes & Cancer* 12, 1–7
42. Tsuda, H., and Hirohashi, S. (1995) *Cancer Res.* 55, 3393–3398
43. Dorion-Bonet, F., Maualten, S., Hozstein, L., and Longy, M. (1995) *Genes Chromosomes & Cancer* 14, 171–181
44. Panagopoulos, I., Mandahl, N., Mitelman, F., and Aman, P. (1995) *Oncogene* 11, 1133–1137
45. McManus, A. P., Min, T., Swansbury, G. J., Gousterson, B. A., Pinkerton, C. R., and Shipley, J. M. (1996) *Cancer Genet. Cytogen.* 87, 179–181
46. Low, D. A., Scott, R. W., Baker, J. B., and Cunningham, D. D. (1982) *Nature* 298, 476–478
47. Downward, J., Graves, J. D., Warne, P. H., Rayter, S., and Cantrell, D. A. (1990) *Nature* 346, 719–723
48. Cheng, J., Weber, J. D., Baldassare, J. J., and Raben, D. M. (1997) *J. Biol. Chem.* 272, 17312–17319