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Cells That Overproduce Protein Kinase C Are More Susceptible to Transformation by an Activated H-*ras* Oncogene

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We recently developed rat fibroblast cell lines that stably overproduce high levels of the β_1 form of protein kinase C (PKC). These cells display several disorders in growth control and form small microscopic colonies in agar. In the present study we demonstrate that one of these cell lines, R6-PKC3, is extremely susceptible to transformation by an activated human bladder cancer c-H-*ras* oncogene (T24). Compared with control cell line R6-C1, T24-transfected R6-PKC3 cells yielded a 10-fold increase in the formation of large colonies in agar. Cell lines established from these colonies displayed a highly transformed morphology, expressed the T24-encoded p21 *ras* protein, continued to express high levels of PKC, and were highly tumorigenic in nude mice. These results provide genetic evidence that PKC mediates some of the effects of the c-H-*ras* oncogene on cell transformation. Data are also presented suggesting that optimum synergistic effects between c-H-*ras* and PKC require critical levels of their respective activities. These findings may be relevant to the process of multistage carcinogenesis in tissues containing cells with an activated c-H-*ras* oncogene.

The enzyme protein kinase C (PKC) plays a central role in signal transduction and mediates the tumor-promoting activity of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA), teleocidin, and related compounds (18, 25). The ability of TPA and teleocidin to enhance the transformation of rodent fibroblasts induced by a transfected c-H-*ras* oncogene suggests that PKC acts synergically with this oncogene in the process of cell transformation (5, 10, 11). Furthermore, several studies indicate that cellular levels of diacylglycerol (DAG) are elevated in *ras*-transformed fibroblasts (6, 20, 23, 26). In addition, microinjection of a *ras* p21 protein into frog oocytes causes an increase in cellular levels of DAG (15). Since DAG activates PKC (18), these findings suggest that *ras* oncogenes may produce some of their effects through the activation of PKC. Consistent with this idea are microinjection studies indicating that the mitogenic activity of H-*ras* p21 is dependent on PKC (16).

Gene-cloning studies indicate that the mammalian genome encodes at least six distinct forms of PKC (for a review, see reference 19). By transduction of the Rat 6 fibroblast cell line with a retrovirus-derived vector containing the coding sequence for the β_1 form of PKC, our laboratory recently developed cell lines that stably overexpress high levels of this specific form of PKC (9). One of these cell lines, R6-PKC3, produces about 50 times more PKC than does a control line, R6-C1, that carries the same vector but lacks the $PKC_{\beta 1}$ insert (9). In this report, we employed this cell system to address the question of whether cells that carry high levels of a specific form of PKC are altered in their susceptibility to transformation by an activated c-H-*ras* oncogene.

MATERIALS AND METHODS

Transformation assays. The R6-C1 cells are a control cell line that carries an integrated copy of the vector pMV7 but lacks a $PKC_{\beta 1}$ cDNA insert and has a normal level of PKC activity (9). The R6-PKC3 cell line carries an integrated copy of pMV7 with the $PKC_{\beta 1}$ cDNA insert and produces about 50 times the normal level of PKC (9). On day 0, the respective cell lines were seeded as monolayer cultures at a density of 5×10^5 cells per 90-mm plate. On day 1, these cultures were transfected with 1 μg of plasmid T24 DNA, containing the activated c-H-*ras* oncogene isolated from a human bladder cancer (8) or with 20 μg of genomic DNA from the normal Rat 6 fibroblast cell line, using previously described procedures (11). On day 4, the cultures were trypsinized, the cells were counted, and 2×10^5 cells were then seeded into 5 ml of a 0.3% Bacto-Agar suspension supplemented with Dulbecco modified Eagle medium plus 20% fetal bovine serum (Flow Laboratories, Inc.), in the absence or presence of TPA (100 ng/ml) (9). This suspension was overlaid above a layer of 10 ml of 0.5% agar in the same medium on a 90-mm plate. Triplicate plates were set up for each group. The cells were then refed every 4 days by pipetting 5 ml of Dulbecco modified Eagle medium containing 20% fetal bovine serum onto the top layer of agar, with or without TPA. At day 25, several large colonies were picked randomly, isolated from the agar plates with a micropipette, established as cell lines, and used as sources of DNA and RNA. The remaining plates were stained with the vital stain 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride hydrate (INT) (Sigma Chemical Co.) for 48 h at 37°C in a 5% CO_2 incubator (21), and the number of colonies greater than 0.4 mm in diameter observed with the naked eye were counted. The numbers of small (less than 0.3 mm in diameter) colonies present in the agar plates were also scored by microscopy (magnification, ×150).

Southern and Northern (RNA) blot analyses and p21-labeling studies. DNA and poly(A)$^+$ RNA were isolated from some of the T24-transformed cell lines described above that had been isolated from the agar plates, as well as from the parental R6-C1 and R6-PKC3 cells, and examined by Southern and Northern blot analysis using previously described methods (9, 11). Additional details are given in the legend to Fig. 3. To analyze the c-H-*ras* p21 proteins, cell cultures (5×10^5 cells per 60-mm plate) were metabolically labeled for 4 h with [^{35}S]methionine (250 μCi/ml) in methionine-free medium or with [^{32}P]phosphate (200 μCi/ml) in phosphate-free medium. Cell extracts were prepared and immunoprecipitated with the *ras* p21 monoclonal antibody Y13-259 (7).

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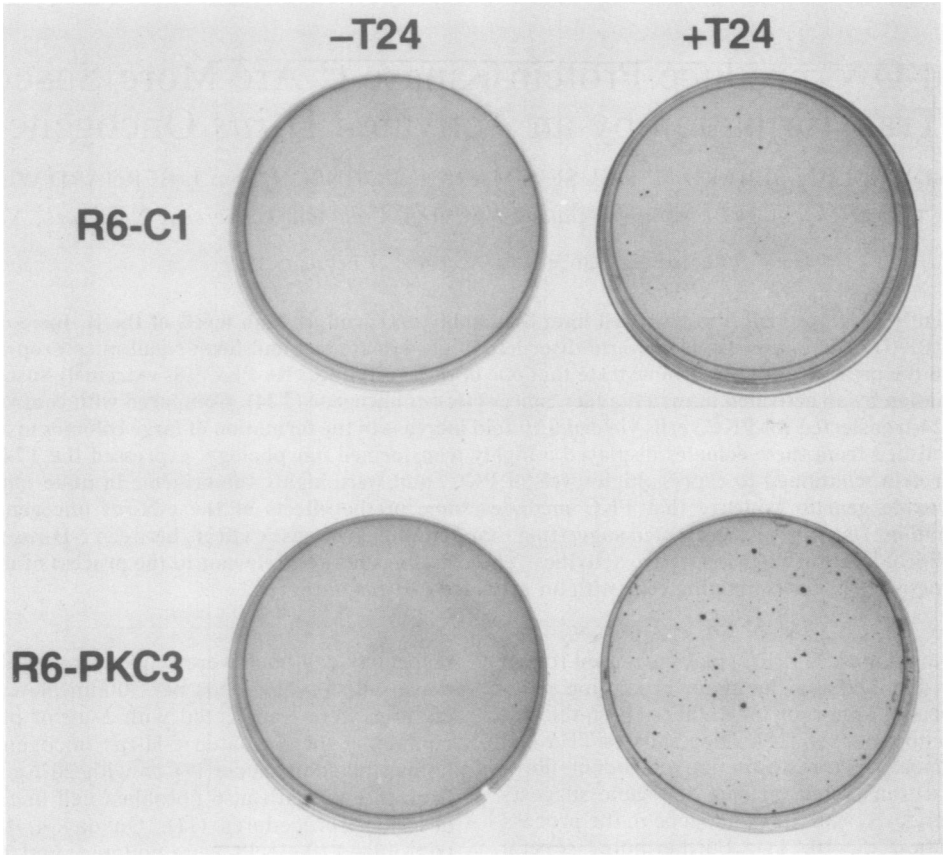

FIG. 1. Growth in agar of R6-C1 and R6-PKC3 cells following transfection with either normal Rat 6 genomic DNA (−T24) or T24 DNA containing an activated c-H-*ras* oncogene (+T24). For specific details, see Materials and Methods.

The immunoprecipitates were collected and analyzed by electrophoresis in sodium dodecyl sulfate-polyacrylamide (17.5%) and by autoradiography, as previously described (7).

RESULTS

Transformation of Rat 6 cells expressing a high level of PKC by the activated human c-H-*ras* oncogene. In our initial studies, we transfected R6-C1 or R6-PKC3 cells with plasmid T24 DNA containing the activated human c-H-*ras* oncogene isolated from a human bladder cancer cell line (8), grew the cells as monolayer cultures for 2 weeks, and then scored the plates for transformed foci (11). With the R6-C1 cells, this yielded a few small transformed foci per plate. These results are consistent with our previous evidence that normal Rat 6 cells are not very susceptible to T24-induced transformation in the absence of TPA or teleocidin (11). With R6-PKC3 cells, however, the transfected cultures developed highly transformed foci that were too numerous to count. It was also difficult to precisely score these foci, since at confluence this cell line forms dense clusters of cells (9). We decided, therefore, to quantitate the effects of T24 transfection by transferring cells into a 0.3% agar suspension on day 4 following the transfection procedure and then scoring for large anchorage-independent colonies 4 weeks later.

Figure 1 indicates that T24 transfection of the control R6-C1 cells yielded a few colonies that grew to a large size (>0.4 mm in diameter) in agar. With the high-PKC-expression cell line R6-PKC3, the yield of these large colonies following T24 transfection was about 10 times that obtained with the R6-C1 cells (Fig. 1 and Table 1). We have previously reported that R6-C1 cells do not form detectable colonies, and that R6-PKC3 cells form numerous small microscopically visible colonies (0.05 to 0.30 mm in diameter), when grown in 0.3% agar (9). This background did not interfere with scoring the large (0.4 to 1.2 mm in diameter) macroscopically visible colonies that appeared in agar plates containing cells previously transfected with the T24 oncogene (Fig. 2a). In the same study, a parallel set of cultures was grown in agar that contained TPA (100 ng/ml). With the T24-transfected R6-C1 cells, TPA resulted in about a 1.3-fold enhancement in the yield of large colonies. On the other

TABLE 1. Effect of overproduction of $PKC_{\beta 1}$ on the formation of colonies in soft agar induced by c-H-*ras* oncogene T24

Recipient cells	TPA treatment[a]	No. of large colonies[b]	
		−T24	+T24
R6-C1	−	0	681
	+	0	874
R6-PKC3	−	11	6,377
	+	0	392

[a] Agar plates contained 100 ng of TPA per ml (+) or no TPA (−).

[b] Total number of large (0.4- to 1.2-mm diameter) colonies that were macroscopically visible in agar plates containing cells that had been previously transfected with T24 DNA or control DNA (−T24), calculated per 5×10^5 transfected cells. Values on triplicate plates agreed with 10%. For additional details, see Materials and Methods.

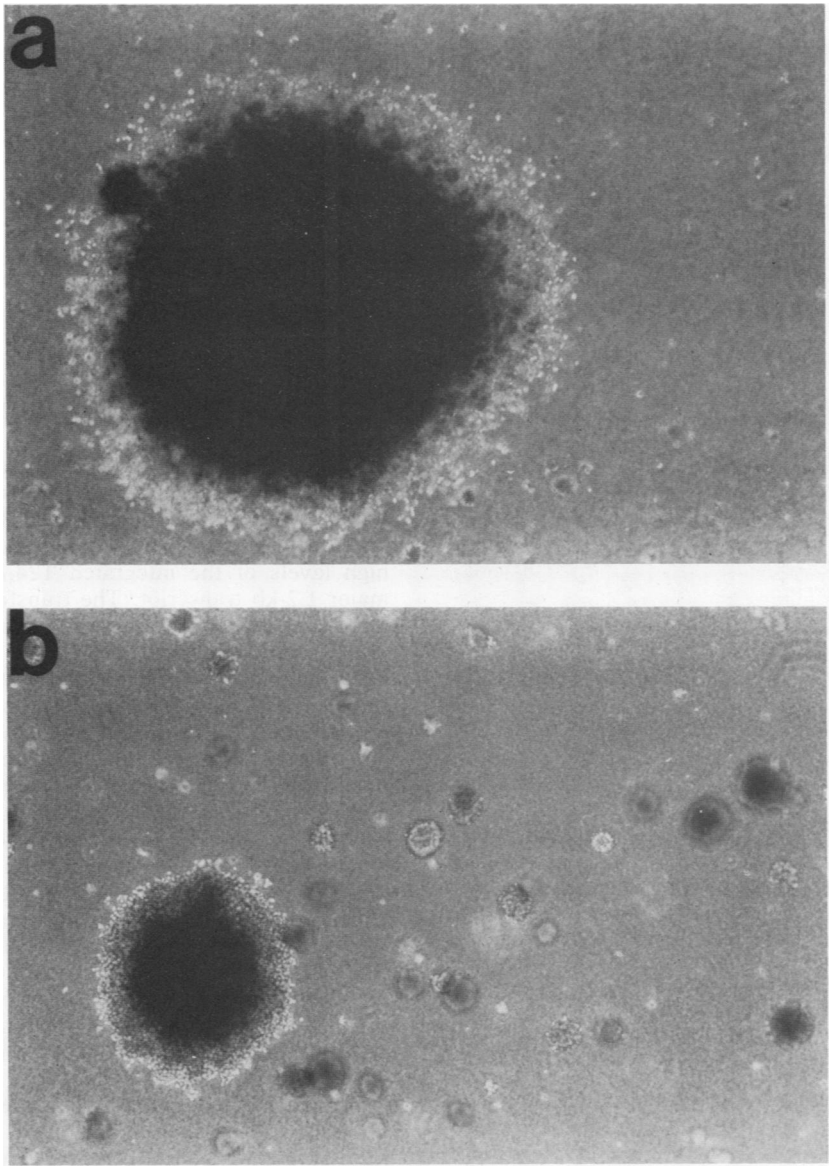

FIG. 2. Microscopic view (magnification, ×150) of an agar plate of T24-transfected R6-PKC3 cells in the absence or presence of 100 ng of TPA per ml on day 28 following the transfection procedure. In both panels, a large colony of T24-transformed cells is seen, against a background of numerous small colonies which are typical of the parental R6-PKC3 cell line. The latter colonies are larger and more numerous in panel b, reflecting the effects of TPA. For additional details, see Materials and Methods.

hand, with the T24-transfected R6-PKC3 cells TPA caused a marked inhibition (about 15-fold) in the yield of large colonies (Table 1). TPA is not toxic to R6-PKC3 cells, since in previous studies we found that the presence of TPA in the agar increased the number and size of the microscopically visible colonies (9). Microscopic examination of the R6-PKC3 agar plates in the present studies revealed a similar effect. Thus, with T24-transfected R6-PKC3 cells, TPA inhibited formation of the large colonies, whereas at the same time it stimulated the growth of the background of small colonies (Fig. 2b and Table 1). The inhibition of growth by TPA appears, therefore, to be specific to the R6-PKC3 cells that acquired the T24 sequence.

The studies described above indicate that cells that produce high levels of $PKC_{\beta 1}$ are extremely susceptible to transformation by an activated c-H-*ras* oncogene. This effect is not simply due to the fact that the R6-PKC3 cells are more susceptible than R6-C1 cells to transfection per se, since we found that both cell types gave approximately the same number of colonies resistant to the drug hygromycin after transfection with the bacterial drug resistance gene *hph* (data not shown here). Nor were the differences observed due to differences in cell density, since the same number of the two cell types were plated in the agar medium following the T24 transfection procedure. Furthermore, the results obtained are not peculiar to the agar selection procedure, since, as described above, R6-PKC3 cells were also much more susceptible to the induction of transformed foci by T24 transfection than were R6-C1 cells when both cell types were studied in monolayer cultures.

Patterns of integration and expression of the transfected c-H-*ras* gene in cells overproducing PKC. In further studies,

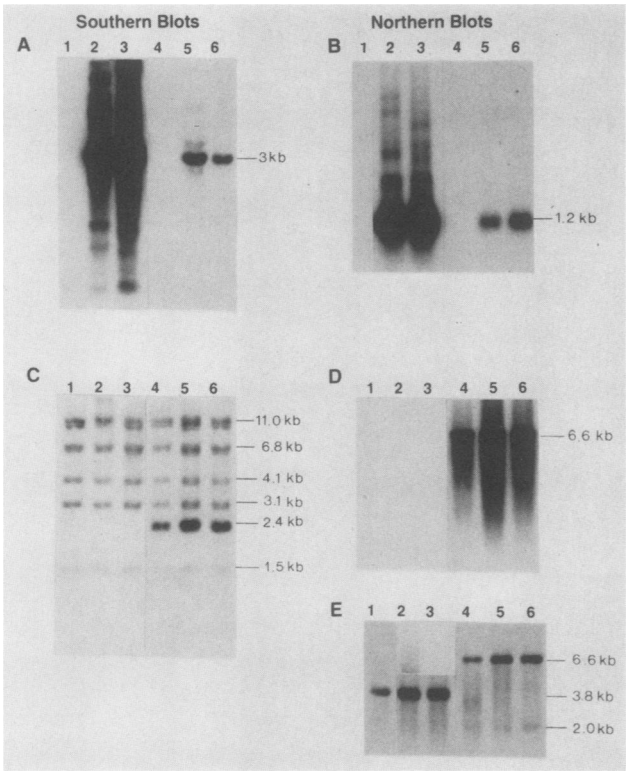

FIG. 3. Southern and Northern blot analyses. R6-C1 and R6-PKC3 cells were transfected with T24 DNA and grown in 0.3% agar, as described in Materials and Methods and the legend to Fig. 1. Several large colonies were picked form each group of agar plates and established as clonal T24-transformed cell lines. DNA and poly(A)$^+$ RNA were isolated from some of these cell lines, as well as from the parental R6-C1 and R6PKC3 cells, and examined by Southern and Northern blot analysis using previously described methods (4, 13). (A and C) Southern blot analyses of DNA samples digested with *Sac*I (A) or *Eco*RI (C) and hybridized to a ^{32}P-labeled probe for T24 or PKC$_{\beta 1}$, respectively. Each lane contained 20 μg of DNA. *Hin*dIII-digested λ DNA was used as a size marker. Assigned molecular sizes (in kilobases) are indicated. (B, D, and E) Northern blot analyses of poly(A)$^+$ RNAs hybridized to a ^{32}P-labeled probe for T24 (B), PKC$_{\beta 1}$ (D), or *neo* (E). Each lane contained 5 μg of poly(A)$^+$ RNA. The endogenous rRNAs (28 and 18S) revealed by ethidium bromide staining were used as size markers. Lanes: 1 and 4, DNA or RNA samples from parental cell lines R6-C1 and R6-PKC3, respectively; 2 and 3, samples from two T24-transformed R6-C1 clones; 5 and 6, samples from two T24-transformed R6-PKC3 cell lines. The ethidium bromide staining revealed equal amounts of RNA per lane in panels B, D, and E. The differences in band intensities were confirmed by densitometry.

several individual large colonies were randomly picked and isolated from the agar plates containing T24-transfected R6-C1 and R6-PKC3 cells and were established as clonal cell lines. All of these clones displayed a highly transformed morphology when grown in monolayer culture and also retained their anchorage-independent growth. Total cellular DNA was isolated from some of these clones and examined for integrated copies of PKC$_{\beta 1}$ and T24 DNA sequences by Southern blot analysis. The DNAs were digested with *Sac*I or *Eco*RI, electrophoresed, and then hybridized to ^{32}P-labeled T24 or PKC$_{\beta 1}$ probes, respectively (Fig. 3). All of the T24-transformed clones carried integrated T24 DNA sequences, with a major band of 3.0 kilobases (kb), indicating that they were not spontaneous transformants. Curiously, the abundance of T24 DNA sequences was much less in the clones derived from the R6-PKC3 cells than in the clones derived from the R6-C1 cells (Fig. 3A). This difference was observed in several additional clones that we examined (data not shown here). With the PKC$_{\beta 1}$ probe a distinct pattern of DNA bands (four major bands ranging in size from about 3 to 11 kb plus a fainter 1.5-kb band) was observed with the *Eco*RI-digested genomic DNAs obtained from both R6-C1 and T24-transformed R6-C1 cells (Fig. 3C). Therefore, these bands represent the endogenous genomic PKC$_{\beta 1}$ DNA sequence. The R6-PKC3 cells also displayed these bands, as well as an additional lower-molecular-size DNA band (about 2.4 kb) which represents the integrated PKC$_{\beta 1}$ cDNA sequence that we originally introduced into these cells (9). As shown in Fig. 3, the T24-transformed derivatives of R6-PKC3 cells retained this integrated cDNA sequence (Fig. 3C).

Poly(A)$^+$ RNAs were also isolated from the cell types described above and examined by Northern blot analyses for the expression of T24 and PKC$_{\beta 1}$ homologous sequences. The T24-transformed R6-C1 and R6-PKC3 cells expressed high levels of the integrated T24 sequence, displaying a major 1.2-kb transcript. The transformed R6-C1 cells contained much higher levels of this transcript than did the transformed R6-PKC3 cells (Fig. 3B), thus paralleling the differences seen in the number of integrated T24 DNA sequences (Fig. 3A). Neither the normal nor the T24-transformed R6-C1 cells contained detectable levels of PKC$_{\beta 1}$ homologous RNA. This is consistent with recent evidence that rodent fibroblasts normally express only the endogenous PKC$_{\alpha}$ gene (12). As previously described (9), the R6-PKC3 cells expressed high levels of a 6.6-kb PKC$_{\beta 1}$ homologous RNA, whose size is consistent with a full-length long terminal repeat (LTR)-to-LTR transcript of the integrated pMV7-PKC$_{\beta 1}$ construct. The T24-transformed R6-PKC3 cells continued to express high levels of this RNA (Fig. 3D) and also high levels of PKC enzyme activity (data not shown here).

It is of interest that the T24-transformed cells expressed somewhat higher levels of PKC$_{\beta 1}$ homologous RNA than did the nontransformed R6-PKC3 cells (Fig. 3D). This finding was even more apparent when the autoradiographs were developed for a shorter period of time. In view of these results, these same RNA samples were studied by Northern blot analysis, using a probe to the *neo* DNA sequence (Fig. 3E), since this sequence occurs in the LTR-to-LTR transcript made in cells containing the pMV7 vector (9). With both the R6-C1 cells and the R6-PKC3 cells, this transcript was also much more abundant in the T24-transformed cells than in their respective controls. Our finding that both the T24-transformed R6-C1 and R6-PKC3 cells expressed higher levels of transcripts from the integrated pMV7 constructs than their nontransformed counterparts (Fig. 3) suggests that expression of the *ras* oncogene enhanced the transcriptional activity of the 5′ Moloney murine sarcoma virus LTR sequence present in pMV7 constructs. Since this was seen with both the R6-C1 and R6-PKC3 T24-transformed cells, this effect is not dependent on the exogenous PKC$_{\beta 1}$ sequence. We have also found that the levels of these transcripts were increased about three- to fivefold when nontransformed R6-C1 or R6-PKC3 cells were treated with TPA (data not shown here). These results are consistent with other studies indicating that both an activated c-H-*ras* oncogene and TPA can enhance the expression of Moloney murine sarcoma virus LTR constructs in other cell systems (17, 24). H-*ras* and TPA can also activate the polyomavirus

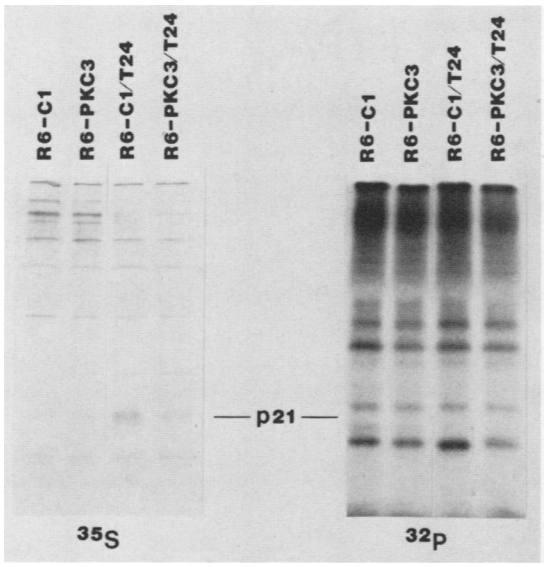

FIG. 4. Analyses of c-H-*ras* p21 proteins produced by the parental R6-C1 and R6-PKC3 cell lines and T24-transformed clones derived from these cells. Cell cultures were labeled with [^{35}S] methionine (^{35}S) or [^{32}P]phosphate (^{32}P), immunoprecipitated, and analyzed by gel electrophoresis, as described in Materials and Methods. The position of migration of the c-H-*ras* p21 protein is indicated.

enhancer in a myeloma cell line (24). The fact that this oncogene and TPA share these effects is consistent with the hypothesis that H-*ras* exerts its cellular effects, at least in part, through PKC.

In view of the marked differences in the abundance of T24 transcripts seen between T24-transformed R6-C1 and R6-PKC3 cells, it was of interest to examine the levels of the c-H-*ras*-encoded p21 protein, using immunoprecipitation with a monoclonal antibody to the p21 protein and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (7). Figure 4 (left) indicates that when prelabeled with [^{35}S]methionine the non-T24-transformed R6-C1 and R6-PKC3 cells displayed only a faint radioactive p21 band. This band was more intense in the T24-transformed derivatives of these cells (Fig. 4, left), reflecting expression of the transfected T24 sequence. It was particularly intense in the R6-C1 cells, which is consistent with the high level of T24 RNA seen in these cells (Fig. 3). In parallel studies, the same cells were labeled with $^{32}P_i$. When immunoprecipates were prepared using an H-*ras*-specific antiserum and the proteins were resolved by gel electrophoresis, as described in Fig. 4, no radioactivity was detected in the p21 protein band obtained from normal or T24-transformed R6-C1 or R6-PKC3 cells (Fig. 4, right). Thus, overproduction of $PKC_{\beta 1}$ does not appear to enhance T24 transformation simply by increasing phosphorylation of the p21 *ras* protein, at least within the limits of sensitivity that could be detected in the present study. Other investigators have reported that *ras* p21 proteins are substrates for in vitro phosphorylation by PKC (13), but this does not appear to be the case in Rat 6 cells. Our findings are consistent with previous studies indicating that although TPA treatment resulted in phosphorylation of the c-K-*ras* p21 protein in a mouse adrenocortical cell line that expressed high levels of p21, this was not the case with the c-H-*ras* or v-H-*ras* proteins (1). Studies are in progress to identify other phosphoproteins that may play a role in the synergistic interaction between PKC and H-*ras* in the process of cell transformation.

The fact that the T24-transformed R6-PKC3 clones contained fewer integrated copies of the T24 DNA sequence, and had lower levels of T24 RNA and its encoded p21 protein, than did the corresponding T24-transformed R6-C1 cells (Fig. 3 and studies on additional clones not shown here) might be explained by two alternative mechanisms. The first is that, since the R6-PKC3 cells are more sensitive to T24 transformation than the R6-C1 cells, the former cells become transformed with fewer copies of T24 DNA. The second is that when cells express high activity of PKC, high expression of T24 is actually cytotoxic. This would explain why we did not obtain any clones that expressed high levels of both PKC and the H-*ras* oncogene. The latter explanation is consistent with the results shown in Table 1 indicating that TPA treatment, which activates PKC (18), inhibited the yield of T24-transformed cells obtained with R6-PKC3, but this was not the case with the R6-C1 cells. In additional studies, the growth in agar of two T24-transformed R6-C1 clones and two T24-transformed R6-PKC3 clones was studied in the presence and absence of TPA (100 ng/ml) (Fig. 5). We found that TPA markedly inhibited (by about 70%) the colony-forming ability of the latter clones but had little or no effect on the former clones. Although further studies are required, these results favor the second explanation mentioned above. We suggest that optimum synergistic interactions between PKC and an activated c-H-*ras* oncogene require a proper balance between their respective functions. If this is true, then during multistage carcinogenesis, cellular levels of PKC and its endogenous activator(s) may be important determinants of their response to a mutated c-H-*ras* oncogene.

Tumorigenicity studies were also done in nude mice by injecting 10^6 cells subcutaneously, in quadruplicate sites, for each cell type. With R6-C1 cells, no tumors were detected up to 150 days postinjection. With R6-PKC3 cells tumors did occur, but only after about 38 days. With both T24-transformed R6-C1 and T24-transformed R6-PK3 cells, tumors (about 1 cm in diameter) were apparent within 9 days. When the T24-transformed cells were injected at 10^5 cells per site, it was apparent that the T24-transformed R6-PKC3 cells grew more rapidly than the T24-transformed R6-C1 cells.

DISCUSSION

Although previous studies (6, 15, 16, 20, 23, 26) suggested a synergistic interaction between *ras* oncogenes and PKC, the present studies provide the first genetic evidence for this synergy, in a stable cell transformation system. We are quite confident that the increased susceptibility of the R6-PKC3 cells to transformation by an activated c-H-*ras* oncogene is due to their high level of expression of $PKC_{\beta 1}$, rather than to fortuitous clonal variations, for the following reasons. (i) In parallel studies, this cell line displayed a 10-fold-greater frequency in c-H-*ras*-induced transformation than either the parental Rat 6 cells or a randomly picked vector control line R6-C1 (Table 1 and additional studies not shown here). (ii) The R6-PCK5 cell line, which expresses a much lower level of $PKC_{\beta 1}$ (9), did not show this increased susceptibility (data not shown here). It may also be significant that R6-PKC3 cells have lost the normal refractory response to TPA treatment but R6-PKC5 cells retain this response (9). (iii) The c-H-*ras*-transformed derivative of R6-PKC3 cells continued to express high levels of PKC, suggesting a synergistic interaction between the *ras* oncogene and PKC in maintenance of the transformed state. Furthermore, this

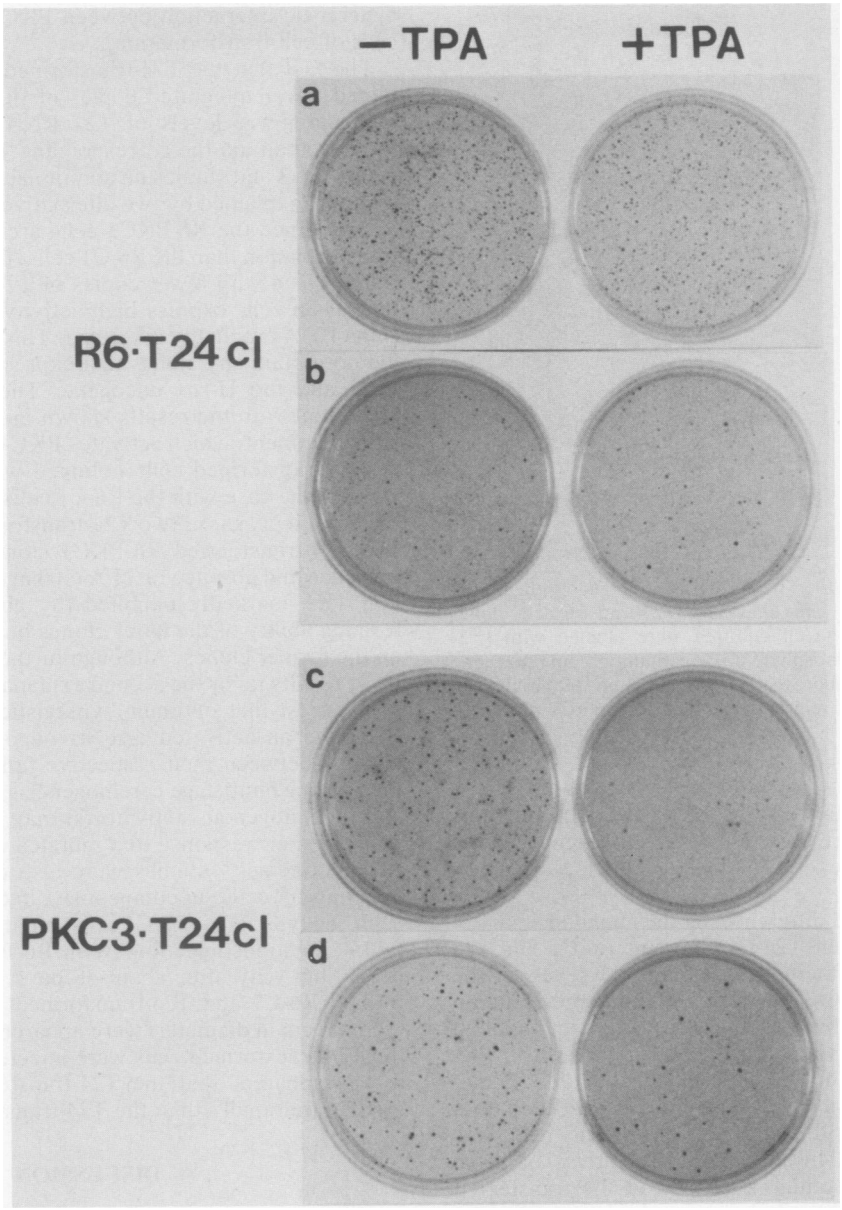

FIG. 5. Soft-agar cultures of isolated T24-transformed clones grown in the absence (−) or presence (+) of TPA (100 ng/ml). Two T24-transformed R6-C1 clones (a and b) and two T24-transformed R6-PKC3 clones (c and d) were seeded separately at 2×10^4 cells per 60-mm plate in a 2-ml suspension of 0.3% Bacto-Agar in growth medium. Cells were fed twice a week by overlaying the agar with 2 ml of the growth medium with or without the addition of TPA. The plates were stained and photographed on day 14. For additional experimental details, see Materials and Methods.

interaction is not confined to the rat fibroblast system, since in recent studies we have found that two clonal derivatives of rat liver epithelial cells that express high levels of $PKC_{\beta 1}$ are also much more susceptible to transformation by the activated c-H-*ras* oncogene than are the parental rat liver epithelial cells (L. L. Hsieh, S. Hoshina, and I. B. Weinstein, unpublished studies).

It is difficult to formulate the exact mechanism by which a high level of PKC enhances *ras*-induced cell transformation, since the biochemical function in mammalian cells of the p21 proteins encoded by *ras* oncogenes is not known. These proteins display significant sequence homology with the α subunits of known regulatory G proteins and also share certain functional similarities with these proteins, since they bind GTP and GDP and have GTPase activity (for a review, see reference 4). The GTPase activity is enhanced by a recently identified *ras* p21-binding protein designated GAP (3, 22). Several studies indicate that *ras*-transformed cells have increased cellular levels of DAG (6, 20, 23, 26). Since phospholipase C, the enzyme that generates DAG, appears to be regulated by a G protein, it has been suggested that *ras* p21 proteins may function by regulating the activity of phospholipase C (2). There is, however, no direct evidence for this hypothesis. The effect of *ras* oncogenes on cellular levels of DAG could be secondary to the process of cell transformation, since cells transformed by other oncogenes can also display increased levels of DAG (26). Nevertheless, even if the c-H-*ras* oncogene increases cellular levels of

DAG via an indirect effect, this could explain the findings in the present study, since increased levels of DAG would lead to activation of the excess $PKC_{\beta 1}$ molecules present in R6-PKC3 cells, thus resulting in a synergistic effect. This formulation is consistent with the fact that normal Rat 6 cells have low levels of PKC (9) and display a low frequency of transformation when transfected with T24 DNA (11). It is also consistent with the finding that flat revertants of *ras*-transformed NIH 3T3 cells (which continue to express the mutant p21 *ras* protein) have subnormal levels of PKC (14). Presumably, in the latter cells there is insufficient PKC activity to optimally support the action of *ras*. We should emphasize, however, that the present data do not exclude other explanations for the synergy between PKC and H-*ras*. For example, a study by Yu et al. (27) suggests that *ras* oncogenes may act downstream from PKC. It is possible, therefore, that PKC alters the expression of other cellular genes that synergize with the H-*ras* p21 protein to produce cell transformation.

Hopefully, the present system will facilitate further studies on the mechanism of action of the *ras* oncogenes and the role of PKC in mediating cell transformation. Since PKC is a multigene family (19), it will be of interest to determine whether overexpression of other specific forms of PKC also synergizes with an activated H-*ras* oncogene and whether or not cells that overexpress various forms of PKC are more susceptible to transformation by other types of oncogenes.

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