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Mitogen-activated protein kinase activation resulting from selective oncogene expression in NIH 3T3 and Rat 1a cells

(transformation/protein kinases/oncoproteins)

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ABSTRACT **Mitogen-activated protein kinases (MAPKs) are serine/threonine kinases that are rapidly activated in response to a variety of growth factors in many cell types. MAPKs are activated by phosphorylation of both tyrosine and threonine residues. They are proposed to be key integrators of growth factor receptor transduction systems involving conversion of tyrosine kinase signals to serine/threonine kinase activation. We have studied the influence of specific oncogenes on the regulation of MAPK activity in NIH 3T3 and Rat 1a fibroblasts. In NIH 3T3 cells, *ras* or *raf* oncogene expression, but not *gip2* oncogene expression, induces a significant constitutive MAPK activation. In contrast, in Rat 1a cells, *gip2*, but not *ras* or *raf* oncogene expression, induces a strong constitutive MAPK activation. The findings indicate that, in a cell type-selective manner, different oncoproteins are capable of causing the constitutive activation of MAPK. However, the magnitude of oncogene-induced MAPK activation is not directly correlated with cellular transformation in either cell type. It appears that expression of only a subset of transforming oncogenes in a specific cell type is able to alter the regulation of the MAPK activation pathway. Thus, the network of cytoplasmic serine/threonine kinases will be differentially regulated when the same oncogene is expressed in different cell types.**

Growth factor receptor signals initiated at the plasma membrane must traverse the cytoplasm and converge in the nucleus. The convergence of these signals alters the expression of specific genes and commits a cell to mitogenesis. One mechanism that growth factor receptors use in their signal transduction is to recruit and activate specific cytoplasmic serine/threonine protein kinases. Representative examples of serine/threonine protein kinases that are activated in response to many different growth factors include protein kinase C, Ca^{2+}-calmodulin protein kinase, Raf, and mitogen-activated protein kinases (MAPKs) (1–3).

Attention has been focused on the MAPKs because of their activation by many different mitogens and the correlation of MAPK regulation with cell cycle progression (4–6). Both tyrosine kinase-encoded growth factor receptors [i.e., epidermal growth factor (EGF) and platelet-derived growth factor receptors] and pertussis toxin-sensitive guanine nucleotide inhibitor factor (G_i)-coupled receptors (i.e., thrombin receptor) are capable of inducing the rapid activation of MAPKs (7). The mechanisms involved in activation of MAPKs by different receptors remain poorly defined but involve, at least in part, phosphorylation of MAPK polypeptides at both tyrosine and threonine residues (8–10). Evidence exists that these phosphorylations may involve both heterologous protein kinases and autophosphorylation (11–13). The number of protein kinases and other regulatory events that regulate MAPK activation is not well defined. The function of MAPKs in signal transduction pathways controlling mitogenesis is also poorly defined, but evidence exists that MAPKs might regulate the activity of proteins such as Rsk 90 (14), c-Myc (15), and the EGF receptor (16), a diverse set of gene products all of which are components in mitogenic signal transduction pathways.

Many transforming oncogenes encode mutant proteins whose cognate wild-type protooncogene products are components of signal transduction networks that are activated in response to growth factor receptor stimulation (1, 17, 18). It is possible, therefore, that specific oncoproteins might influence the regulation of MAPKs. Recently, we demonstrated that expression of the *gip2* oncoprotein in Rat 1a cells resulted in constitutive activation of MAPK (19). In this report, we demonstrate that oncogenes capable of transforming NIH 3T3 and Rat 1a cells differentially activate MAPKs in the two cell types. The results show that the influence of specific oncogene expression on cytoplasmic signal transducing serine/threonine protein kinases can be quite variable in different cell types.

METHODS

MAPK Assay. Appropriate Rat 1a or NIH 3T3 cell clones ($2–4 \times 10^6$ cells) were seeded in 10-cm dishes in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum and 5% calf serum. Twenty-four hours later, the cells were placed in DMEM with 0.1% bovine serum albumin. The cells were incubated for 16 hr to achieve quiescence and then challenged in the presence or absence of the appropriate growth factor. After 5 min of growth factor stimulation, the cells were rinsed three times and harvested by scraping in ice-cold phosphate-buffered saline (PBS). Cell lysis was accomplished with 0.5% Triton X-100 in 50 mM β-glycerophosphate, pH 7.2/100 μM sodium vanadate/2 mM $MgCl_2$/1 mM EGTA/leupeptin (10 μg/ml)/aprotinin (0.02 unit/ml)/1 mM dithiothreitol. The cell lysates were centrifuged for 5 min in a microcentrifuge and the supernatants were normalized for protein content. Aliquots of protein normalized lysates (0.5 ml) were loaded onto a Mono Q FPLC column equilibrated in 50 mM β-glycerophosphate, pH 7.2/100 μM sodium vanadate/1 mM EGTA/1 mM dithiothreitol. Proteins were eluted with a linear gradient of NaCl (0–0.35 M) and 1-ml fractions were collected for assay. Twenty-microliter aliquots of the fractions were assayed with the MAPK EGF receptor synthetic peptide $EGFR^{662-681}$ (15 min; 30°C) (20, 21).

Phosphotyrosine Immunoblotting. Fractions (900 μl) eluted from the Mono Q column (see above) were assayed for phosphotyrosine-containing proteins as described (19).

Chloramphenicol Acetyltransferase (CAT) Assay. Rat 1a and NIH 3T3 cells were seeded in 100-mm dishes (10^6 cells

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Abbreviations: MAPK, mitogen-activated protein kinase; EGF, epidermal growth factor; CAT, chloramphenicol acetyltransferase.

per dish). Twenty-four hours later, the cells were rinsed once and fresh DMEM containing 10% fetal calf serum was added. Transfections were performed by the calcium phosphate coprecipitation procedure (22). The cells were washed 12 hr posttransfection with PBS and placed in medium containing 10% fetal calf serum. The cells were harvested 48 hr posttransfection and lysed by five cycles of freeze–thaw in dry ice/methanol followed by 5 min at 37°C. Lysates were centrifuged at 10,000 × *g* for 10 min at 4°C and supernatants were collected for assay of CAT activity as described by Gorman *et al.* (23).

Measurement of DNA Synthesis. Appropriate Rat 1a and NIH 3T3 clones were seeded (2×10^4 cells per well) in 24-well plates and cultured as described above to obtain quiescence. Cells were then stimulated with 10% fetal calf serum, and, 16 hr later, 0.5 μCi of [^{3}H]thymidine was added to each well (1 Ci = 37 GBq). After 4 hr, cells were washed four times in Hanks' balanced salt solution and solubilized in 0.1% SDS. DNA was collected on glass fiber filters, and trichloroacetic acid-precipitable counts were quantified by scintillation counting.

Soft Agar Colony Growth. The indicated Rat 1a and NIH 3T3 cells were cloned in soft agar (5000 cells per 60-mm dish) in DMEM containing 10% fetal calf serum. Twelve to 15 days later, colonies were counted in triplicate dishes for each clone.

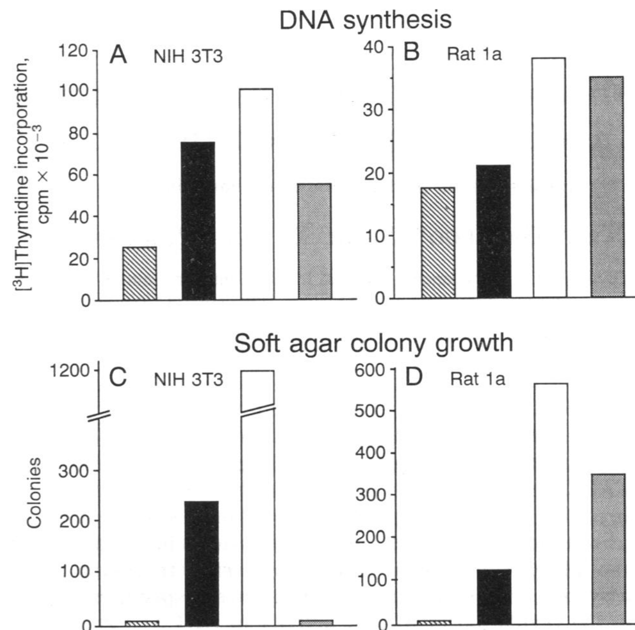

FIG. 1. Measurement of DNA synthesis and soft agar colony growth of NIH 3T3 and Rat 1a cells. NIH 3T3 and Rat 1a cells were infected with retrovirus encoding the v-*raf* (solid bars), v-*ras* (open bars), or α_{i2}Q205L (stippled bars) oncogenes or virus with no cDNA insert (hatched bars) (24). After selection in G418, appropriate NIH 3T3 and Rat 1a cell clones were characterized for oncoprotein expression. Clones expressing the indicated oncoproteins were then used for measurement of DNA synthesis and growth in soft agar. For measurement of DNA synthesis, the numbers represent the mean of duplicate determinations, which varied by <5%, and are representative of two or three independent clones for each cell type and oncoprotein. For soft agar colony growth, the numbers represent the average between the number of colonies in triplicate dishes, which varied by <10%. The wild-type NIH 3T3 and Rat 1a cells formed a small number of minute colonies that were 5–10 times smaller in size than the colonies observed with the oncoprotein-expressing clones.

RESULTS

Growth Regulation in Oncogene Transfected Cells. Expression of the *gip2*, *raf*, and *ras* oncogenes in NIH 3T3 and Rat 1a cells causes a loss of normal growth control in both cell types. However, the phenotypic consequence of expression of the three oncogenes in the two cell types is distinguishable.

Fig. 1 (*A* and *B*) shows that expression of *gip2*, *ras*, or *raf* results in enhanced rates of DNA synthesis in both Rat 1a and NIH 3T3 cells relative to control cells not expressing the oncogenes. The influence of the *raf* oncogene product on enhancing DNA synthesis was modest in several Rat 1a clones. With Rat 1a cells, all three oncogenes are also capable of inducing growth in soft agar, consistent with each of the oncogenes inducing anchorage-independent growth characteristic of the transformed phenotype (Fig. 1*D*). In contrast, only *ras* and *raf* induce anchorage-independent growth in NIH 3T3 cells (Fig. 1*C*). Expression of the *gip2* oncogene is unable to induce anchorage-independent growth of NIH 3T3 cells, even though *gip2* is capable of enhancing the rate of DNA synthesis in NIH 3T3 cells (Fig. 1*A*). Thus, the ability of *gip2* to alter the growth control of NIH 3T3 cells is more restricted than either *ras* or *raf*. This is consistent with a restricted cell-type transformation potential of the *gip2* oncogene (24–26). To date, *gip2* has only been found in neuroendocrine tumors (25), while *ras* oncogenes are found in a broad range of tumors of different tissue origin (27).

Oncogene Influences on c-*jun* Transactivation Functions. A more direct biochemical measurement of the action of transforming oncogenes is the augmentation of c-Jun transactivation functions (28). Jun is a component of the AP-1 complex (29, 30), whose phosphorylation is markedly increased in Ha-*ras* transformed cells (31). The increased Jun phosphorylation correlates with increased AP-1 activity (31). It is proposed that the increased phosphorylation of Jun results from a Ha-Ras-induced protein kinase cascade and that this may be a general mechanism for collaboration among transforming and immortalizing oncogenes (31).

In stably transfected Rat 1a clones, *gip2*, *ras*, and *raf* expression resulted in the activation of c-Jun (Fig. 2). This is readily demonstrated by using a collagenase promoter–CAT reporter construct (29–31) that measures the c-Jun-dependent transactivation function of the AP-1 complex. The collagenase promoter-driven CAT activity was similar in clones stably expressing the three transforming oncogenes tested.

In contrast to the results in Rat 1a cells, only *ras* and *raf* expression in NIH 3T3 cells resulted in enhanced AP-1 activity. Expression of *gip2* in NIH 3T3 cells did not result in activation of c-Jun and enhanced AP-1 transactivation function. This observation supports the cell type-specific transforming function of oncogenes. The *gip2* oncogene is capable of causing a transformed phenotype in Rat 1a cells but not in NIH 3T3 cells. Correspondingly, *gip2* expression leads to c-Jun activation in Rat 1a cells but not in NIH 3T3 cells. This finding suggests that the protein kinases that mediate the series of events leading to c-Jun transactivation are regulated differently in Rat 1a and NIH 3T3 cells. Thus, differences in transformation properties, including DNA synthesis, soft agar colony growth, and c-Jun activation, were observed between the two cell types. These differences were observed despite similar levels of oncoprotein expression as determined by immunoblotting (ref. 26; data not shown). The inference is that the gene products involved in regulating signals initiated by *gip2* that control these protein kinase pathways are different in the two cell types.

Oncogene and Cell Type-Specific MAPK Regulation. The identity and number of serine/threonine protein kinases whose activity might be regulated by the *gip2* oncogene product is unknown. However, in anti-phosphotyrosine immunoblots of *gip2* transformed cells, a 42-kDa polypeptide exhibited a constitutively elevated phosphotyrosine content (data not shown). The 42-kDa polypeptide whose phosphotyrosine content was increased in *gip2* transformed cells corresponds to p42 MAPK (19). Fig. 3*A* shows that MAPK is constitutively activated 3- to 5-fold in *gip2* transformed

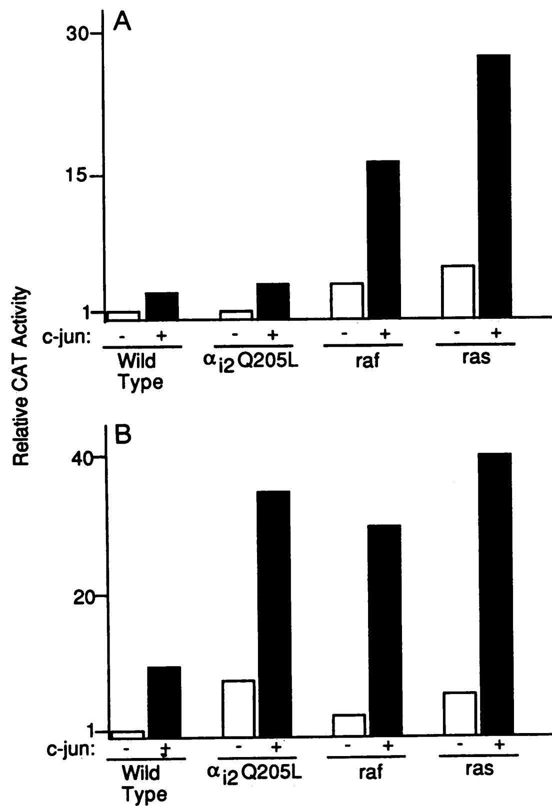

FIG. 2. Oncoprotein stimulation of c-Jun activity. NIH 3T3 (*A*) or Rat 1a (*B*) cells expressing the indicated oncoproteins were transfected with the AP-1-dependent collagenase–CAT reporter (0.5 μg per plate) in the presence or absence of Rous sarcoma virus c-*jun* (5 μg per plate). CAT activity was measured as described and autoradiograms were quantified by densitometry. Relative CAT activity for each oncoprotein and cell type is representative of two independent experiments.

cells relative to the activity measured in wild-type Rat 1a cells. Fractionation of MAPK from Rat 1a cells with Mono Q FPLC reveals a major peak of activity that corresponds to the 42-kDa MAPK and a minor peak of activity that corresponds to the p44 MAPK polypeptide (19, 32). In *gip2* transformed cells, the increased MAPK enzyme activity correlates with a similar 3- to 5-fold increase in phosphotyrosine content of the MAPK polypeptide (Fig. 3*B*). The constitutively activated MAPK activity correlates well with the increased tyrosine phosphorylation of the MAPK polypeptide, which is part of the established mechanism for MAPK activation (8, 9). No change in total immunoreactive p42 MAPK polypeptide levels was detected in the transformed cells relative to wild-type cells (data not shown).

Interestingly, neither *ras* nor *raf* transformed Rat 1a cells showed a significantly elevated MAPK activity (Fig. 3*A*). This contrasts with the MAPK activity in *gip2* transformed cells, which was strongly constitutively activated relative to the MAPK activity measured in wild-type Rat 1a cells (Fig. 3*A*). Relative to EGF or thrombin-stimulated MAPK activity in Rat 1a cells (Fig. 3*C*), expression of the *gip2* oncogene product is capable of constitutively activating MAPK to 50–60% of maximal growth factor-stimulated levels. Expression of the *ras* and *raf* oncogene products resulted in very modest changes in MAPK activity in Rat 1a cells, even though they induce a transformed phenotype. The results indicate that the *gip2* oncogene product is selectively able to alter the regulation of MAPK in Rat 1a cells, whereas two other transforming oncogenes *ras* and *raf* do not significantly alter MAPK regulation. This finding demonstrates that the activity of mitogen-regulated cytoplasmic protein kinases is selectively and stably modified as a consequence of specific oncogene expression.

In striking contrast to the findings in Rat 1a cells, *ras* and *raf* expression in NIH 3T3 cells resulted in a significant constitutive activation of MAPK (Fig. 4*A*). Expression of *gip2* in NIH 3T3 cells did not result in MAPK activation (Fig. 4*A*). The activation state of MAPK in *raf* transformed NIH 3T3 cells is dramatic and is greater than that observed with EGF stimulation (Fig. 4 *A* and *C*). MAPK activation in *ras* transformed NIH 3T3 cells was more modest but significant relative to the MAPK activity in lysates from *raf*-expressing NIH 3T3 cells. In both *raf* and *ras* transformed NIH 3T3 cells, the increased MAPK enzyme activity correlates with a constitutive increase in phosphotyrosine content of the p42 MAPK polypeptide (Fig. 4*B*). The phosphotyrosine content of p42 MAPK from *gip2*-expressing NIH 3T3 cells was undetectable, similar to control wild-type cells. The total immunoreactive p42 MAPK polypeptide levels were similar for all of the NIH 3T3 cell clones (data not shown). The results indicate the presence of unique effectors responding

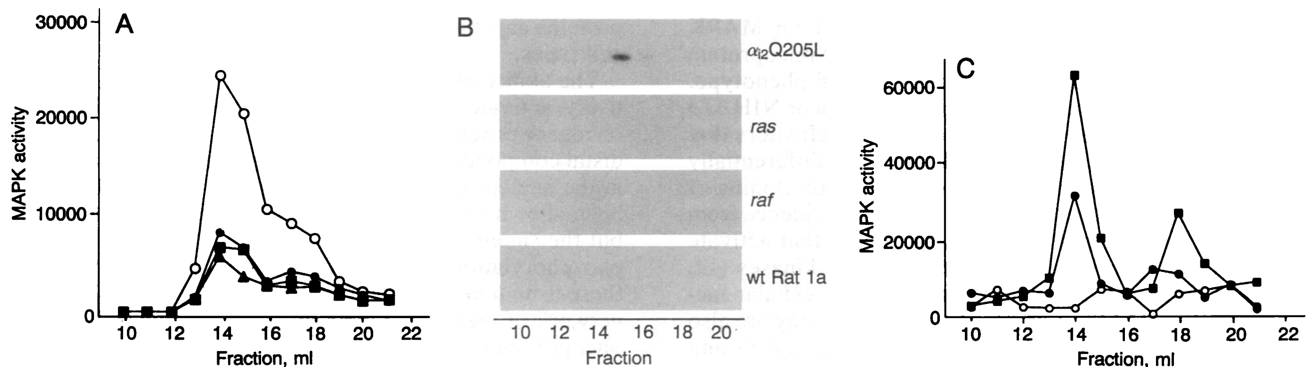

FIG. 3. MAPK activity (cpm per 15 min per 20-μl aliquot) in transformed Rat 1a cells. (*A*) Basal MAPK activity was measured after Mono Q FPLC fractionation of lysates from wild-type Rat 1a cells (▲) and clones were transformed with α_{i2}Q205L (○), v-*ras* (●), or v-*raf* (■) after 24 hr of serum starvation of the cells. (*B*) Autoradiographs show increased phosphotyrosine content in the p42 MAPK polypeptide from α_{i2}Q205L transformed Rat 1a cells compared with control wild-type (wt), v-*ras*, and v-*raf* transformed cells. Immunoblotting with anti-p42 MAPK antibody indicated a similar level of the polypeptide in all four clones (data not shown). (*C*) Serum-starved wild-type Rat 1a cells were challenged for 5 min with buffer alone, EGF (20 ng/ml), or thrombin (0.1 unit/ml). Cell lysates were then prepared, resolved by Mono Q FPLC, and assayed for MAPK activity. The constitutive MAPK activity (cpm per 15 min per 20-μl aliquot) (○) observed with α_{i2}Q205L-expressing Rat 1a cells was 50–60% of the activity observed with EGF (■) or thrombin (●) stimulation (compare *A* and *C*). Peak fractions for MAPK activity and phosphotyrosine content of the p42 polypeptide corresponded to fraction 14 for each condition. Additional chromatographic purification and immunoreactivity demonstrated that the activity measured was directly related to p42 MAPK and not a contaminating kinase recognizing the peptide substrate (ref. 21; data not shown).

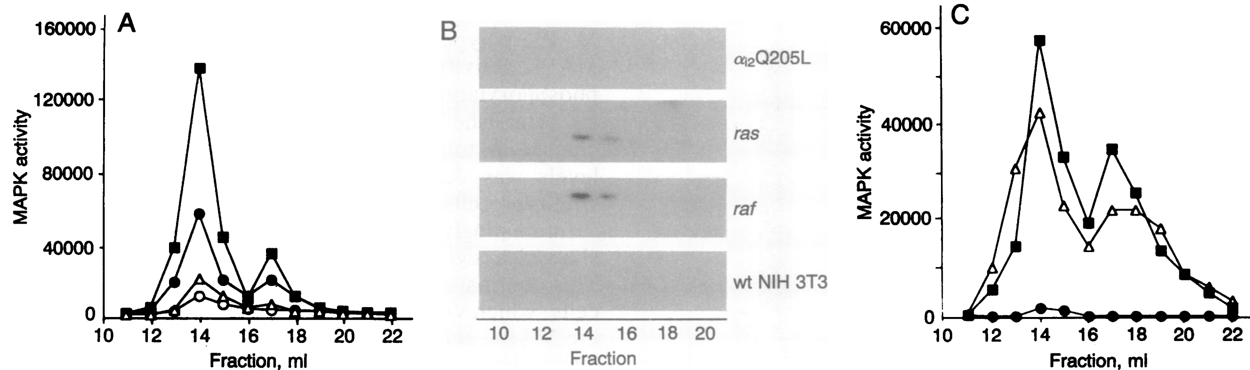

FIG. 4. MAPK activity (cpm per 15 min per 20-μl aliquot) in transformed NIH 3T3 cells. (*A*) Basal MAPK activity was measured after Mono Q FPLC fractionation of lysates from wild-type NIH 3T3 cells (○) and clones expressing v-*raf* (■), v-*ras* (●), or α_{i2}Q205L (△) as was done for Rat 1a cells in Fig. 3. MAPK is constitutively activated in v-Raf- and v-Ras-expressing cells but not in α_{i2}Q205L-expressing cells relative to wild-type NIH 3T3 cells. (*B*) Autoradiographs show increased phosphotyrosine content in the p42 MAPK polypeptide from v-*raf* and v-*ras* transformed cells but not in α_{i2}Q205L-expressing NIH 3T3 cells. wt, Wild type. (*C*) Serum-starved wild-type NIH 3T3 cells were challenged for 5 min with buffer alone (●), EGF (20 ng/ml) (■), phorbol 12-myristate 13-acetate (200 nm) (△). Cell lysates were prepared and resolved by Mono Q FPLC and assayed for MAPK activity as described. MAPK activity (cpm per 15 min per 20-μl aliquot) in v-*ras* transformed NIH 3T3 cells was similar to that observed with EGF and phorbol ester. MAPK activity from v-*raf* transformed cells was reproducibly greater than that observed with acute EGF or phorbol ester stimulation of NIH 3T3 cells. Peak fractions for MAPK activity and phosphotyrosine content of the p42 polypeptide corresponded to fraction 14 for each condition.

to the *ras* and *raf* oncogene products in NIH 3T3 cells that are significantly different in Rat 1a cells. Differential regulation of MAPK in Rat 1a and NIH 3T3 cells expressing *gip2, ras*, or *raf* indicates there will be multiple cell-specific mechanisms for the regulation of MAPK.

DISCUSSION

Previously, we demonstrated that expression of the *gip2* oncogene constitutively activated p42 MAPK in Rat 1a cells (19). Here, we have demonstrated that three different oncogenes in a cell type-specific fashion are able to constitutively activate p42 MAPK. MAPK activation by *gip2* in Rat 1a but not NIH 3T3 cells, and *ras* and *raf* activation of MAPK in NIH 3T3 cells with modest effects in Rat 1a cells, indicates there must be multiple cell-specific mechanisms for regulation of MAPKs. This finding is intriguing because EGF activates MAPK in both cell types, presumably by a common pathway, whereas the constitutive MAPK activation resulting from selected oncogene expression is cell type specific. The findings in Rat 1a cells, where *gip2, ras*, and *raf* all induced transformation, demonstrate that the constitutive activation of MAPK by *gip2* with little effect on MAPK activity by *ras* or *raf* is a function of the specific oncoprotein and not secondary to assuming the transformed phenotype.

The different oncoproteins expressed in Rat 1a or NIH 3T3 cells apparently alter the regulation of specific effectors that control MAPK activity and are predicted to be differentially expressed in the two cell types. The isolation or cloning of these effectors has not yet been accomplished; evidence from other laboratories suggests the cellular factors that activate MAPK may be tyrosine and serine/threonine kinases (12, 13). Our preliminary studies characterizing the cellular factors that mediate MAPK activation indicate that they are also constitutively activated in *gip2* transformed Rat 1a cells and *raf* transformed NIH 3T3 cells.

We also found that phorbol ester-induced down regulation of protein kinase C did not blunt the constitutive MAPK activity in either *gip2* transformed Rat 1a or *raf* transformed NIH 3T3 cells (data not shown). The findings are similar to reports describing phorbol ester-induced activation of MAPK but show that protein kinase C down regulation does not inhibit growth factor-stimulated MAPK activity (33). The oncogene-induced change in MAPK regulation, similar to EGF stimulation, appears to be independent of the phorbol ester-regulated pathway that is down regulated in response to long-term phorbol 12-myristate 13-acetate treatment. The oncoprotein effects on the MAPK regulatory pathway are therefore most likely similar to that for growth factors such as EGF and thrombin.

The fact that expression of the *ras* oncogene resulted in MAPK activation in NIH 3T3 cells was not totally unexpected. Acute phorbol ester activation of MAPK in NIH 3T3 cells was inhibited by the *ras* GTPase-activating protein GAP (34). In PC12 cells a dominant negative *ras* (Asn-17 Ha-ras; ref. 35) inhibits nerve growth factor activation of MAPK (36, 37). Cumulatively, these two results indicate that a functional Ras signal system is required for both the phorbol ester- and growth factor-regulated pathways leading to activation of MAPKs. Thus, expression of an activated Ras in the appropriate cell type is predicted to alter the regulation of MAPK activity, a result we have observed with NIH 3T3 cells. A similar finding with Ras was recently characterized in Swiss 3T3 cells scrape-loaded with the Ras oncoprotein (38). Why expression of v-*ras* in Rat 1a cells results in such a modest MAPK activation relative to that observed in NIH 3T3 cells is unclear, but it is most likely related to differences in the proteins capable of influencing MAPK regulation in the two cell types.

The ability of *raf* expression in NIH 3T3 cells to constitutively activate MAPK was an unexpected result. The *raf* oncogene product has been generally considered to be a more distal component in the series of signaling events converging in the nucleus to regulate gene expression (39). Raf has also been shown to be phosphorylated *in vitro* by MAPKs (40), but the significance in terms of Raf activity regulated by its phosphorylation by MAPKs remains unclear. At present, there is no *a priori* reason to place Raf distal to MAPKs in a network of signaling events initiated by growth factors and oncoproteins. This is particularly true with the growing awareness that there are one or more regulatory enzymes between Ras and MAPK (12, 13). Wood *et al.* (37) demonstrated that MAPK, Raf-1, and Rsk activation by growth factors required a functional Ras signal transduction pathway. This suggests some level of commonality in the initial regulatory signal events controlling the activation of MAPK and Raf-1. MAPK and Raf may be controlled by parallel but integrated signal transduction pathways involving protein kinases (37). MAPKs have also been shown to have cytoplasmic and nuclear localizations (41) and to phosphorylate a host of both cytoplasmic and nuclear substrates (14–16, 19,

41, 42). It is clear that expression of the *raf* oncogene results in activation of MAPK in NIH 3T3 cells, indicating that either MAPK or components of the MAPK regulatory pathway are regulated by Raf. The involvement of protein serine/threonine kinases in the regulation of MAPKs is consistent with a role for Raf-like kinases in this pathway (12, 13). Despite our current lack of understanding of the number of proteins involved in this signaling network, it is clear that the *raf* oncogene product is capable of recruiting and activating additional growth factor-regulated serine/threonine kinases like the MAPKs. Just as with tyrosine kinases, this result indicates that serine/threonine kinase oncoproteins themselves activate additional kinases involved in mitogenic signal transduction.

Finally, our observations that *gip2*, *ras*, and *raf* constitutively activate MAPK in a cell-specific manner may provide insight into cell type-specific oncogenesis. Activated *ras* oncogenes contribute to tumorigenesis in a broad range of cell types but not in every tissue (27). Similarly, changes in c-*myc* expression, a transcription factor found in almost all cell types, is found in many but not all neoplasms (27). The *gip2* oncogene appears very limited in the tissues in which it can contribute to oncogenesis and has been identified only in tumors of neuroendocrine origin (25). It is likely that specific oncogenes are identified only in tumors in which the protooncogene product functions prominently in stimulating mitogenic signal pathways (3). The differential ability of oncogenes to stimulate cell proliferation in different cell types must, in turn, be related to the background of gene products expressed in that cell type that are required for mitogenic signaling. If only a subset of the gene products required for a mitogenic response are expressed or selectively recruited for activation in response to a specific oncoprotein, then a significant increase in cell proliferation will not be observed. Established fibroblast cell lines like Rat 1a and NIH 3T3 are differentially primed to convert to a transformed-like phenotype when the regulation of a limited set of cytoplasmic response pathways is altered. In these established cell lines, the differential regulation of MAPKs by three different oncogenes is clearly demonstrated. It must be noted that there is not a correlation with constitutive MAPK activation and transformation. In Rat 1a cells *raf* and *ras* expression induces transformation but only modest MAPK activation, indicating that altered regulation of alternative pathways regulated by these oncoproteins is probably sufficient for transformation. This finding, however, does not exclude the possibility that MAPKs may contribute to transformation of specific cell types by different oncogenes. In NIH 3T3 cells, for example, both *raf* and *ras* induced a constitutive MAPK activation. Genetic approaches using dominant negative MAPKs or gene inactivation studies will be required to determine the necessity of MAPKs in the transformation process induced by expression of specific oncogenes.

The number of identified growth factor-regulated serine/threonine kinases (i.e., MAPKs, Raf, Rsk-90, etc.) continues to grow (36, 37). Our findings demonstrate that the growth factor-regulated kinases are targets for oncoproteins resulting in perturbation of normal cellular regulation of mitogenic signaling. It is likely that different growth factor-regulated cytoplasmic and nuclear serine/threonine kinases will be activated in tumors of different tissue origin as a direct consequence of specific oncogene expression. Screening of tissues in different stages of tumor progression for the activation of growth factor-regulated serine/threonine protein kinases might provide a biochemical measure of oncogene contributions to the transformation process.

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