Immunofluorescent Localization of a 39,000-dalton Substrate of Tyrosine Protein Kinases to the Cytoplasmic Surface of the Plasma Membrane

ERICH A. NIGG,* JONATHAN A. COOPER,‡ and TONY HUNTER‡

*Department of Biology, University of California, San Diego, La Jolla, California 92093; and ‡Tumor Virology Laboratory, The Salk Institute, San Diego, California 92138. Dr. Nigg's present address is Institute for Cell Biology, ETH Hönggerberg, Zurich, Switzerland.

ABSTRACT The intracellular distribution of p39, a 39,000-dalton substrate for a number of tyrosine protein kinases, has been determined by indirect immunofluorescence microscopy. No binding of anti-p39 antibodies to intact cells was observed, indicating that this protein is not accessible to antibody on the cell surface. Following detergent permeabilization of formaldehyde-fixed cells, a reasonably uniform cytoplasmic labeling was observed. This fluorescence was most pronounced in membrane ruffles, especially in the leading lamellae of migrating cells, and in areas of cell-cell contact. Brief permeabilization of cells with detergent prior to formaldehyde fixation resulted in the appearance of a reticular lattice. An identical staining pattern was observed when fluorescently-labeled lectins were used as plasma membrane markers, but not when antibodies to a variety of cytoskeletal proteins were used. Taken together, these results indicate that p39 is, at least in part, located at the cytoplasmic surface of the plasma membrane. Immunolabeling of Rous sarcoma virus-transformed cells with anti-p39 antibodies resulted in fluorescent staining patterns indistinguishable from those observed in untransformed cells. It is conceivable that p39 plays some structural role within a protein network underlying the plasma membrane.

Various RNA tumor viruses express transforming gene products with protein kinase activities specific for tyrosine residues on cellular target proteins (1). Similarly, two growth factors, epidermal growth factor (EGF) (2) and platelet-derived growth factor (PDGF) (3), have recently been shown to stimulate cellular tyrosine protein kinase activities. In the case of EGF, this activity may reside within the membrane receptor for the growth factor (4, 5). It is likely that modification of tyrosines in specific cellular proteins plays a major role in certain cases of viral transformation and cell growth control. To understand the molecular events leading to cell transformation and growth stimulation, it will therefore be necessary to identify and characterize intracellular target proteins for tyrosine protein kinases.

One protein, variously estimated between 34,000 and 39,000 daltons (6–9), hereinafter named p39, has been described as a major substrate for the tyrosine protein kinases induced by both sarcoma viruses (6–13) and EGF (14–16). In Rous sarcoma virus (RSV)-transformed fibroblasts, ~7–10% of the population of p39 is found to be phosphorylated on a single tyrosine residue and a similar fraction is phosphorylated at serine (7, 8, 12, 17). Moreover, it has been shown that purified p39 can act as a substrate for the isolated Rous transforming protein, pp60c-src in vitro (7). The same tyrosine residue in p39 becomes phosphorylated under either in vitro or in vivo conditions (7). p39 is not phosphorylated, however, in untransformed cells or in cells transformed by agents that do not induce tyrosine protein kinase activities (6, 11).

p39 is a relatively abundant cellular protein, representing ~0.3% of the total methionine in cellular polypeptides (7, 12, 17), yet its function(s) in the cell is completely unknown. Initial efforts to characterize p39 have mainly been aimed at determining its intracellular location. Cell fractionation studies have indicated that a p39 population may be associated with membranes (18) and/or detergent-insoluble cytoskeletal structures (18, 19). There is no evidence for posttranslational alterations in the size of p39, suggesting that it is neither extensively glycosylated nor is it synthesized with a cleavable signal peptide.
Here, using antibodies raised against partially purified p39 (17), we have investigated the intracellular distribution of p39 by indirect immunofluorescence microscopy. Our results indicate that p39 is associated with the cytoplasmic surface of the plasma membrane in cultured fibroblasts and epithelial cells.

**Materials and Methods**

**Cell Culture:** Normal rat kidney cells (NRK cells) and RSV B77-transformed NRK cells were grown as described previously (20). Dog kidney cells (MDCK) were grown in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal calf serum and antibiotics. Chick embryo fibroblasts were prepared and cultured as described previously (21) and 1881 lymphoma cells were grown in DME supplemented with 10% calf serum and 5 x 10^{-3} M 2-mercaptoethanol.

**Immunocchemical Reagents and Fluorescein-conjugated Lectins:** The purification of p39 and the production of rabbit anti-p39 antisera were described previously (17). Antiserum was also raised to p39 purified through an SDS gel. For this purpose, carboxymethyl-cellulose-purified material from ~2 x 10^{13} chick embryo fibroblasts was lyophilized, dissolved in SDS gel sample buffer, and electrophoresed through a 15% polyacrylamide gel. The p39 band was excised, homogenized in 8 ml saline and 1-ml samples, emulsified with 2 ml of complete Freund's adjuvant, and injected into a New Zealand white rabbit at 4-wk intervals. The positive serum was obtained 1 wk after the second injection.

IgG fractions were prepared by passing sera over DEAE-Affigel Blue (Bio-Rad Laboratories, Richmond, CA), according to the manufacturer's instructions, and concentrated by vacuum dialysis to a concentration of ~15 mg/ml. For preabsorption on 1881 lymphoma cells, 20 μl serum was incubated for 30 min at 0°C with 10^6 cells that had been fixed for 5 min with 4% formaldehyde in Tris-buffered saline at 25°C and permeabilized for 5 min with 0.5% Nonidet P-40 (Shell Chemical Company, New York, NY) in buffered sucrose (18). Guinea pig antibodies to chicken vimentin were raised against vimentin extracted from 14-d chick embryos (22) and purified by preparative gel electrophoresis. The specificity of the antibodies for vimentin was confirmed by immunoprecipitation and immunoblotting (E. A. Nigg and S. F. Singer, unpublished data). Actin was fluorescently labeled using 7-nitrobenz-2-oxa-1,3-diazole phalohidin (NBD-phalloidin) (Molecular Probes, Inc., Junction City, OR) at twice the concentration recommended by the manufacturer. Rhodamine- and fluorescein-conjugated goat antibodies against rabbit IgG and guinea pig IgG were prepared as described by Brandtzaeg (23). Fluorescein-conjugated concanavalin A (Con A) and wheat germ agglutinin (WGA) were prepared as described (24), and were a kind gift of Dr. Pamela Maher, (University of California, San Diego).

**Indirect Immunofluorescence Experiments:** Cells were cultured on 18- or 22-mm square coverslips for at least 40 hr prior to immunofluorescence. Cells were treated in either of two ways: (a) Pretreatment: cells were fixed for 5 min at room temperature with 3% formaldehyde, 2% sucrose in phosphate-buffered saline (PBS), pH 7.6, washed three times in PBS, and extracted for 5 min with ice-cold 0.5% Triton X-100 in 20 mM HEPES, 300 mM sucrose, 3 mM MgCl₂, 50 mM NaCl, pH 7.4; (b) pre-extraction: cells were extracted for exactly 30 s with the ice-cold Triton X-100 buffer described above, then washed quickly in the 3% formaldehyde fixative described above, and fixed in this fixative for 5 min. The fluorescence intensity of p39 antibodies was not greatly affected by the permeabilization procedure. However, since the local fluorescent signal reflects not only p39 concentration but also accessibility to antibodies, the fluorescence intensity is not a good index of p39 retained through extraction. When chick embryo cells, grown on coverslips and labeled with [35S] methionine, were extracted under these conditions, 48% of immunoprecipitable p39 remained. However, since removing the soluble fraction from the residual material took an additional 30 s after the original 30-s extraction, the fraction of p39 in the cells prepared for immunofluorescence may be underestimated.

Fig. 1 illustrates the effect of the lymphoma cell preabsorption step on the immunofluorescent staining patterns produced by anti-p39 antibodies in cultured NRK cells. The nonabsorbed sera gave rise to some fluorescent staining of 1881 lymphoma cells, which is presumably caused either by nonspecific staining of IgG or by the presence of antibodies reacting with proteins contaminating the p39 used for immunization (Fig. 1A). As would be anticipated, this immunofluorescent staining was almost completely abolished by extensive preabsorption on 1881 lymphoma cells (Fig. 1B). In contrast, when immunofluorescence labeling was carried out in NRK cells that were either fixed with formaldehyde prior to permeabilization with Triton X-100 (prefixed cells) (Fig. 1, C and D) or briefly exposed to Triton X-100 (30 s at 4°C) before fixation with formaldehyde (preextracted cells) (Fig. 1, E and F), the only obvious effect of preabsorption was a reduction of diffuse staining in the region of the nucleus.

**Results**

**Specificity of Anti-p39 Antibodies**

The partial purification of the 39,000-dalton protein, the production of antisera, and a characterization of their specificity have been published (17). These antibodies immunoprecipitate p39 together with small amounts of proteins of molecular weights ~50,000 and 35,000 but react specifically with p39 when denatured cell proteins are immobilized on nitrocellulose (17, 25). For the present immunofluorescence studies, antibodies raised to partially purified p39 were enriched for p39-specific molecules by preabsorption on fixed and permeabilized 1881 lymphoma cells that lack p39 but contain the contaminating proteins (25). More striking was the dependence of the observed immunofluorescence patterns upon the experimental conditions chosen to prepare the cells for incubation with anti-p39 antibodies. In cells that had been fixed prior to permeabilization by detergent (Fig. 1, C and D), a relatively diffuse fluorescence was apparent over most of the cell. Labeling was most pronounced around the nucleus and in membrane ruffles, particularly in the leading lamellae of migrating cells (arrows in Fig. 1, C and D). Similar results were obtained when cells were fixed and permeabilized simultaneously by a sequential treatment with methanol for 5 min at −20°C and acetone for 20 s at −20°C (not shown). By contrast, in cells that had been exposed to detergent for 30 s and then promptly fixed (Fig. 1, E and F), a reticular network extending over the whole cell became apparent. Because the reticular pattern was very characteristic, we chose the corresponding experimental conditions to extend our tests of the specificity of the anti-p39 antibodies.

Similar serpiginous immunofluorescence staining patterns were obtained with prepermeabilized cells from several different species using antibodies from two rabbits, even though the animals had been immunized with widely different preparations of p39: One of the rabbits had been injected with a preparation of p39, partially purified by ion-exchange chromatography (17), whereas the other animal had been immunized with p39, purified further by preparative SDS polyacrylamide gel electrophoresis and elution from a gel slice (see Materials and Methods). Antibodies from the former rabbit recognized p39 both in immunoprecipitation and in immunoblotting assays and were used for all the immunofluorescence experiments shown here. Antibodies from the latter rabbit did not immunoprecipitate native p39 but did react with this protein in immunoblots and gave very similar immunofluorescence.
cent staining patterns. Serum from a nonresponsive rabbit failed to recognize p39, either in immunofluorescence or in immunochemical assays. Antibodies from a rabbit immunized with a 46,000-dalton protein unrelated to p39, but also identified as a substrate of certain viral tyrosine protein kinases (17), produced immunofluorescent staining patterns entirely distinct from those observed with the anti-p39 reagents. Moreover, the staining by anti-46,000-dalton protein antibodies was not dependent on the order of cell permeabilization and fixation (not shown).

Fig. 2 shows additional specificity controls. A reticular network, similar to that seen in NRK cells, was also observed in
pre-extracted chick embryo fibroblasts (Fig. 2A). When preimmune serum was used, an extremely low level of very diffuse background staining was apparent in the cytoplasm, and some faint labeling of nuclei could be seen (Fig. 2B). Most importantly, immunofluorescent labeling by anti-p39 antibodies was completely abolished after preincubation of the antibodies with an excess of highly purified p39 (Fig. 2C). The extent of purity of the p39 used in this competition experiment is illustrated by the gel electropherogram in Fig. 2E. On the basis of these experiments we conclude that the antibodies used in the present study are indeed highly specific for p39 and that the reticular lattice stained in pre-extracted cells must contain p39.

### Association of p39 with Cytoplasmic Surface of the Plasma Membrane

While studying the immunofluorescent labeling of p39 in NRK cells and in chick embryo fibroblasts, we occasionally observed marked labeling of cell-cell contacts. To examine this location of p39 more closely, we turned to truly epithelial cells, characterized by polygonal morphology and extensive, readily visualized cell-cell contact areas. The anti-p39 immunofluorescent labeling patterns of MDCK cells that had been either prefixed (Fig. 3A) or pre-extracted for 2 min at 4°C with 0.5% Triton X-100 (Fig. 3B) both showed a pronounced labeling of cell-cell contact areas. No fluorescence was detectable after immunolabeling of intact MDCK cells by anti-p39 antibodies (Fig. 3, C and D). Similarly, negative results were obtained when labeling intact chick embryo fibroblasts or RSV-transformed NRK cells (not shown). Fluorescence was never observed unless the cells were extracted with detergent, indicating that p39 is absent (or at least inaccessible to antibody) at the cell surface.

To investigate the origin of the serpiginous structures stained by anti-p39 antibodies in pre-extracted cells, we first carried out a series of double indirect immunofluorescence experiments using reagents directed against known cytoskeletal proteins together with antibodies to p39 (Fig. 4). Little detailed correspondence was observed between the distributions of p39 and vimentin (Fig. 4, A and B), vinculin (not shown), or actin (Fig. 4, C and D). Moreover, pre-extraction of cells with detergent for prolonged times (>10 min) resulted in almost complete loss of fluorescence arising from anti-p39 antibodies, whereas actin filaments could still be readily seen in the same cells (not

**Figure 2** Specificity of immunofluorescent staining pre-extracted chick embryo fibroblasts by anti-p39 antibodies. Chick embryo fibroblasts fixed after pre-extraction with detergent were stained as described in Materials and Methods. Anti-p39 serum was preabsorbed on 1881 lymphoma cells and used for immunolabeling at 1:75 dilution (A and C). Preimmune IgG was used at 150 μg/ml (B). Secondary antibodies were rhodamine-conjugated goat antibodies against rabbit IgG. (A) Cells stained with preabsorbed anti-p39 serum; (B) cells stained with preimmune IgG; (C) cells stained with preabsorbed anti-p39 serum that had been incubated for 10 min at 4°C with 1 μg of highly purified p39 antigen prior to use. (D) The same field as shown in C viewed by Nomarksi optics. The scale and magnification are the same as in Fig. 1. (E) SDS gel electrophorogram stained by Coomassie Brilliant Blue, illustrating the extent of purity of the p39 antigen used for the blocking experiment shown in C and D. 0.4 μg protein was applied to the gel. The small arrowhead denotes the top of the gel.
shown). Occasionally, however, there seemed to be some over-
all alignment between the reticular arrays and microfilament
bundles (Fig. 4, C and D, arrows; Fig. 2A).

While characterizing the nature of the spongiform network
stained by anti-p39 antibodies, we learned about the immu-
nofluorescence results obtained independently by other groups
([25a], K. Shriver, personal communication) that, together with
our own cell fractionation data (18), suggested that p39 might
be associated with the plasma membrane. To examine the
possibility that the reticular lattice seen after immunolabeling
of pre-extracted cells with anti-p39 antibodies might represent
remnants of the plasma membrane, we carried out fluorescent
labeling studies with reagents directed to the plasma mem-
brane. When fluorescently labeled lectins were used as markers
for plasma membrane glycoproteins, very similar patterns to
those produced by anti-p39 antibodies were observed (Fig. 5).
Double-labeling of NRK cells with anti-p39 antibodies (Fig.
5A) and fluorescent WGA (Fig. 5B) produced virtually super-
imposable images (note patterns indicated by arrows). Most
important, the same dependence of the immunofluorescent
labeling patterns on the experimental conditions (i.e., prefLxa-
tion vs. pre-extraction) was observed for WGA, Con A, or anti-
p39 antibodies as exemplified by the labeling of NRK cells by
fluorescent Con A after pre-extraction (Fig. 5C) and prefixa-
tion (Fig. 5D). Neither WGA nor Con A can be considered
absolutely specific markers for the plasma membrane under
the conditions used here, because they also label glycoproteins
in the extracellular matrix and in endomembranous systems.
Nevertheless, the plasma membrane is clearly a major site of
reaction for Con A and almost the exclusive site of reaction for
WGA (26). Moreover, when intact cells were briefly incubated
with fluorescein-conjugated WGA or Con A and washed prior
to detergent extraction and formaldehyde fixation, the typical
spongiform lattice was also observed, whereas virtually no
labeling of glycoproteins occurred in the cell interior (not
shown).

In addition, we found that antibodies specific for components
of the rough endoplasmic reticulum and the Golgi apparatus
(27) produced patterns of immunofluorescent labeling different
from one another and the reticular structures seen with anti-
p39 antibodies in pre-extracted cells. On the basis of these
results, we conclude that it is the plasma membrane that is
stained in similar ways by both anti-p39 antibodies and flu-
orescently labeled lectins. Clearly, however, p39 resides on the
cytoplasmic surface of the membrane, whereas the carbohy-
drake moieties recognized by lectins are exposed at the external
surface of the plasma membrane.

In other experiments (not shown), we studied the partitioning

![Figure 3](image-url)
of p39 in a detergent-water phase-separation assay (28) designed to provide an operational criterion for distinguishing integral from peripheral membrane proteins. In this assay, an originally homogeneous aqueous solution containing the protein(s) of interest and the nonionic detergent Triton X-114 is warmed above 20°C. Above this temperature, the solution separates into two distinct phases, an aqueous phase and a detergent phase, which can be physically separated and analyzed for their protein content. It has been demonstrated that a series of bona fide integral membrane proteins, when present during the formation of the two phases, partition into the detergent phase, whereas peripheral membrane proteins or other water-soluble proteins partition into the aqueous phase (28). In similar experiments, p39 partitioned almost exclusively into the aqueous phase, which suggests that it is not an integral membrane protein but rather is peripherally associated with the membrane and/or with cytoskeletal proteins underlying the membrane.

**Distribution of p39 in RSV-transformed Cells**

A p39 population becomes phosphorylated on tyrosine residues when cells are transformed by certain sarcoma viruses or after some cultured cells are treated with EGF or PDGF. It therefore was of interest to examine whether or not changes in the intracellular distribution of p39 might be detected in appropriate cells. Immunofluorescent labeling of p39 in prefixed NRK cells transformed by the B77 strain of RSV showed uniform labeling over the entire cell body (Fig. 6A), whereas the typical reticular network appeared after fluorescent staining of pre-extracted RSV-transformed cells (Fig. 6B). Cell-cell contact staining was also occasionally apparent under either experimental condition, when crowded cultures were examined (not shown). Analysis of several pairs of normal and transformed cells indicates that, at the resolution level of immunofluorescence microscopy, no major differences in the intracellular distribution of p39 can be detected between transformed and untransformed cells.

**DISCUSSION**

The determination of the cellular location of p39 may give some clues as to the function(s) of this protein and to the effect of tyrosine phosphorylation on these functions. Here, we have

![Figure 4](https://example.com/figure4)
investigated the intracellular distribution of p39 by using indirect immunofluorescence microscopy. No p39 could be detected by labeling intact cultured cells, indicating that p39 is not accessible to antibodies and is most probably not exposed at the cell surface. This conclusion agrees with our previous finding that p39 is not degraded during trypsin treatment of intact cells (18). However, two lines of evidence from this study suggest that a significant population of p39 is associated with the plasma membrane. First, with cells prepared conventionally for immunofluorescence, a rather uniform labeling of the entire cell was observed. Staining was most pronounced in regions of high plasma membrane density: in membrane ruffles, especially at the leading lamellae of moving cells, and in cells of epithelial origin, at cell-cell contacts. Similar immunofluorescence observations have also been made by other workers ([25a, 28a] K. Shriver, personal communication). Second, the fluorescent patterns produced by anti-p39 antibodies and by reagents to other membrane proteins were altered concordantly if permeabilization preceded fixation.

Brief extraction of cells, under conditions designed to maximize structural preservation (29), prior to fixing and labeling with anti-p39 antibodies, revealed a striking reticular pattern that was distinct from the patterns of known cytoskeletal systems in the same cells. Fluorescent staining of cell-surface lectin-receptors revealed that the pre-extraction procedure resulted in the formation of the same reticular structures. This suggests that membrane-associated proteins are reorganized when membrane lipids are extracted: possibly they collapse onto one another or onto submembraneous structures. This might explain the occasional alignment of actin-containing microfilament bundles and parts of the p39 lattice. When we consider that the glycoproteins recognized by the lectins are exposed at the cell surface, whereas p39 reacts exclusively with antibodies at the cytoplasmic surface of the plasma membrane, their congruent arrangement in pre-extracted cells is remarkable and certainly supports the conclusion that at least a fraction of p39 is associated with the plasma membrane.

A factor contributing to the appearance of the reticular network during pre-extraction may be the removal of a subpopulation of membrane-associated p39 with a diffuse localization. Our extraction conditions solubilize ~50% of p39 from chick embryo fibroblasts, as demonstrated by immunoprecipitation of the soluble and insoluble fractions. Potentially, solubilized proteins could associate artefactually with submembraneous structures during the pre-extraction procedure, although we are not aware of any examples of this. Many other

![Figure 5](image-url)
proteins with isoelectric points similar to that of p39 are extracted quantitatively under our conditions (18). Moreover, the concentration of p39 in regions of plasma membrane activity in prefixed cells suggests that at least some of the p39 present in the reticular structures was membrane-associated before permeabilization.

The present immunofluorescence results agree well with recent data on the distribution of p39 during cell fractionation. Following hypotonic lysis of cells, a major fraction of p39 is found in the particulate pelletable material, whereas detergent extraction results in the solubilization of variable fractions of p39, depending on the exact conditions used for extraction (18, 19). Our studies and another report have indicated that p39 is associated with a cytoskeletal fraction, defined operationally as material adhering to the substratum after extraction of attached cells with nonionic detergents (18, 19). In contrast, when cells are removed from the substratum before extraction, much of the p39 population is soluble. The cell fractionation results can now be reconciled, since cytoskeletal residues remaining after detergent extraction of cells clearly contain remnants of the plasma membrane, as well as intracellular organelles. The detergent soluble fraction of p39 from suspended cells could also be membrane associated, since cytoskeletal/plasma membrane contacts are organized differently in rounded and flattened cells (30). The small fraction of p39 that is not particulate following hypotonic lysis may constitute a soluble pool. A soluble population of p39 might contribute to the diffuse cytoplasmic immunofluorescence observed in prefixed cells, and possibly coexist with the membrane-associated protein in a dynamic equilibrium.

Besides p39, another substrate for tyrosine protein kinases, vinculin (31), is concentrated in membrane proximal structures (32-35). The transforming protein of RSV, pp60 

Transformation by RSV results in tyrosine phosphorylation of ~10–15% of the population of p39 (12, 17). Phosphorylated and unphosphorylated p39 co-distribute in cell fractionation experiments (18). We were unable to detect any differences in the immunofluorescent labeling patterns produced by anti-p39 antibodies in untransformed and RSV-transformed cells. The effect of phosphorylation on p39 function remains to be determined.

p39 may appear concentrated at sites of cell-cell contacts in polygonal cells and in membrane ruffles partly because of the accumulation of fluorescence that results from the opportunity to view these particular membrane areas tangentially. Actin, in addition to being arranged in microfilaments and distributed diffusely within the cytoplasm, is also concentrated in membrane ruffles, the leading lamellae of migrating cells, and in areas of cell-cell contact (40–42), much like p39. It is possible therefore that p39 is not directly associated with the plasma membrane but is bound to cytoskeletal structures underlying the membrane. Immunoelectron microscopy would be necessary to distinguish these possibilities.

In view of the relative abundance of p39 in fibroblasts, it would appear that a structural role for it within a protein matrix underlying the plasma membrane is plausible. From the amount of p39 in the cell, one can calculate that this single protein could account for as much as 3% of a monomolecular layer covering the entire inner surface of the plasma membrane. However, p39 is absent from certain lymphoma cell lines (25), and preliminary evidence has been obtained that p39 is also scarce in some tissues including brain, liver, and muscle, but is abundant in gut, lung, and thymus (K. L. Gould and J. A. Cooper, unpublished results). This argues against the suggestion that p39 is identical to malic dehydrogenase (43), an enzyme vital to basic cellular metabolism and unlikely to be absent from any cell type. Whatever function one wants to envisage for p39, this function has to be compatible with both its subcellular location and its tissue distribution.

We thank Mike Bishop and Kathy Shriver for communication of results prior to publication. We are grateful to Drs. D. Louvard and A. Knippe for their gift of antibodies to Golgi apparatus and rough endoplasmic reticulum, and to Dr. P. Mahet for fluorescein-conjugated lectins. E. A. Nigg is indebted to S. J. Singer for the opportunity to work in his laboratory and for his helpful suggestions.
REFERENCES

1. Hunter, T., and B. M. Sefton. 1982. Protein kinases and viral transformation. In The Molecular Genetics of Tumors, and Viruses. P. Cohen and S. Van Hengsingen, editors. Elsevier Biomedical Press, Amsterdam. 333-366.

2. Carpenter, G. L., King, Jr., and S. Cohen. 1979. Rapid enhancement of protein phosphorylation in A-431 cell membrane preparations by epidermal growth factor. J. Biol. Chem. 254:4884-4891.

3. Etkin, B., and W. Steward, A. Wasteson, and C.-H. Heldin. 1982. Stimulation of tyrosine phosphorylation of a cellular substrate for transformation-specific protein phosphorylation from shed plasma vesicles. J. Biol. Chem. 257:1101-1102.

4. Bahrow, S. A., S. Cohen, and J. V. Stavros. 1982. Affinity labeling of the protein kinase presenting nuclear localization signal. J. Biol. Chem. 257:2851-2854.

5. Kobayashi, N, and A. Kaji. 1980. Phosphoprotein associated with activation of the epidermal growth factor receptor in fibroblasts. J. Biol. Chem. 255:419-420.

6. Radke, K., and G. S. Martin. 1979. Transformation by Rous sarcoma virus: effects of p38 gene expression on the synthesis and phosphorylation of cellular glycoproteins. Proc. Natl. Acad. Sci. USA. 76:5121-5126.

7. Erikson, E., and R. L. Erikson. 1980. Identification of a cellular protein substrate phosphorylated by the avian sarcoma virus transforming gene product. Cell 21:429-436.

8. Cooper, J. A., and T. Hunter. 1981. Changes in protein phosphorylation in Rous sarcoma virus transformed chicken embryo cells. Mol. Cell. Biol. 1:165-178.

9. Kobayashi, N., and A. Kaji. 1980. Phosphoprotein associated with activation of the src gene product in myogenic cells. Biochem. Biophys. Res. Commun. 93:278-284.

10. Erikson, E., R. Cook, G. J. Miller, and R. L. Erikson. 1981. The same normal cell protein is phosphorylated after transformation by avian sarcoma viruses with unrelated transforming genes. Mol. Cell. Biol. 1:43-50.

11. Cooper, J. A., and T. Hunter. 1981. Four different classes of retroviruses induce phosphorylation of tyrosine present in similar cellular proteins. Mol. Cell. Biol. 1:394-407.

12. Radke, K., T. Gilmore, and G. S. Martin. 1980. Transformation by Rous sarcoma virus: a cellular substrate for transformation-specific protein phosphorylation contains phosphotyrosine. Cell 21:821-828.

13. Watson, T. J., Guyden, T.-H. Kang, K. Radke, T. Gilmore, and G. S. Martin. 1980. A strain of Fujinami sarcoma virus which is temperature-sensitive in protein phosphorylation. Cell 22:767-774.

14. Hunter, T., and J. A. Cooper. 1981. Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. Cell 24:741-752.

15. Cooper, J. A., and T. Hunter. 1981. Similaties and differences between the effects of epidermal growth factor and Rous sarcoma virus. J. Cell Biol. 91:878-883.

16. Eriksson, E., E. J. Shaysky, and R. L. Erikson. 1981. Evidence that viral transforming gene products and epidermal growth factor stimulate phosphorylation of the same cellular protein with similar specificity. J. Biol. Chem. 256:13381-13384.

17. Cooper, J. A., and T. Hunter. 1983. Identification and characterization of cellular targets for tyrosine protein kinases. J. Biol. Chem. 258:1108-1115.

18. Cooper, J. A., and T. Hunter. 1982. Discrete primary locations of a tyrosine protein kinase and of three proteins that contain phosphotyrosine in virally transformed chick fibroblasts. J. Cell Biol. 94:287-296.

19. Cheng, Y.-S. E., and R. P. Chen. 1981. Detection of phosphotyrosine-containing 36,000 dalton protein in the framework of cells transformed with Rous sarcoma virus. Proc. Natl. Acad. Sci. USA. 78:2388-2392.

20. Nigg, E. A., B. M. Sefton, T. Hunter, G. Walter, and S. J. Singer. 1982. Immunoassay localization of the transforming protein of Rous sarcoma virus using antibodies directed at a synthetic src peptide. Mol. Cell. Biol. 2:1209-1216.

21. Sefton, B. M., K. Keenan, and T. Hunter. 1978. Comparison of the expression of the src gene of Rous sarcoma virus in vivo and in vitro. J. Virol. 28:957-971.

22. Runyon-Walter Winchell Cancer Fund fellowship.

23. Acknowledgments. The authors are grateful to Alphonse murine leukemia virus for providing a major 36,000 dalton tyrosine protein kinase gene product, E. Erikson and R. L. Erikson for antibody to cellular substrate for transformation-specific protein phosphorylation, T. Hunter and G. Walter for the antibody to a synthetic src peptide, and C. F. Tse for anti-vinculin antibody to Rous sarcoma virus-transformed rat cells and D. Warm u for advice on microinjection.

24. Stavros, J. V., G. Rainthaler, and H. Hinssen. 1981. Organization of actin meshworks in cultured kidney epithelial cells. J. Cell Biol. 92:92-107.

25. Pinto da Silva, P., R. R. Terrisi, and B. Kachar. 1981. Freeze-fracture cytometry of the localization of wheat germ agglutinin and concanavalin A binding sites on freeze-fractured pancreatic cells. J. Cell Biol. 91:361-372.

26. Lowry, D., H. Reggio, and G. Warren. 1981. Antibodies to the Golgi complex and the rough endoplasmic reticulum. J. Cell Biol. 92:95-217.

27. Border, C. 1981. Phase separation involves a major membrane protein in Triton X-114 solution. J. Biol. Chem. 256:1604-1607.

28. Greenberg, M. E., and G. M. Edelman. 1983. The 43-KD pp60src substrate is located at the plasma membrane in normal and RSV-transformed cells. In Proc. Natl. Acad. Sci. USA. 80:3560-3564.

29. Leach, R. K., L. Ransone, Y. Kaufmann, and S. Perman. 1977. A cytoskeletal structure associated with polyribosomes obtained from Hela cells. Cell 10:87-78.

30. Pernazza, A., A. Buson, J. Edelstein, and I. Vale. 1982. The cytoplasmic and nuclear architecture in cells and tissues: form, function, and mode of assembly. Curr. Top. Dev. Biol. 18:1-33.

31. Sefton, B. M., J. A. Cooper, E. H. Ball, and S. J. Singer. 1981. Vinculin; a cytoskeletal target of the transforming protein of Rous sarcoma virus. Cell 24:165-174.

32. David-Pindyck, T., and S. J. Singer. 1980. Altered distribution of the cytoskeletal proteins vinculin and -actin in cultured fibroblasts transformed by Rous sarcoma virus. Proc. Natl. Acad. Sci. USA. 77:6867-6869.

33. Skriver, K., and K. Rosenthal. 1981. Organization of pp60src and selected cytoskeletal proteins within adhesion plaques and junctions of Rous sarcoma virus-transformed rat cells. Cell Biol. 90:525-535.

34. Sefton, B. M. 1979. A 130K protein from chicken gizzard: its location at the terminus of microfilament bundles in cultured chicken cells. Cell 18:193-205.

35. Budnitz, K. M., and J. Feramisco. 1980. Microinjection and localization of a 130K protein in living fibroblasts: a relationship to actin and fibronectin. Cell 19:587-595.

36. Willingham, M. C., G. Jay, and L. Fast. 1981. Organization of pp60src and selected cytoskeletal proteins within adhesion plaques and junctions of Rous sarcoma virus-transformed rat cells. Cell Biol. 90:525-535.

37. Courmier, S., R. Ralston, K. Alitalo, and J. M. Bishop. 1983. The subcellular localization of an abundant substrate (p38) for tyrosine-specific protein kinases. Mol. Cell. Biol. 3:540-549.

38. Brandt, P., and R. S. Molday. 1977. Binding of concanavalin A to Rous sarcoma virus and its implication in cell-surface labeling studies. FEBS. (Fed. Eur. Biochem. Soc.) Lett. 4:391-394.

39. Sefton, B. M., J. A. Cooper, and T. Hunter. 1983. Some lymphoid cell lines transformed by Abelson murine leukemia virus lack a major 36,000 dalton tyrosine protein kinase gene product. In Living Cells and Viruses. P. Cohen and S. Van Hengsingen, editors. Elsevier Biomedical Press. Amsterdam. 333-366.

40. Barchi, R. L., and S. J. Singer. 1982. Phosphorylation of tyrosine protein kinase gene product, E. Erikson and R. L. Erikson for antibody to cellular substrate for transformation-specific protein phosphorylation, T. Hunter and G. Walter for the antibody to a synthetic src peptide, and C. F. Tse for anti-vinculin antibody to Rous sarcoma virus-transformed rat cells and D. Warm u for advice on microinjection.

41. Crossen, J. D., and G. M. Edelman. 1983. The 43-KD pp60src substrate is located at the plasma membrane in normal and RSV-transformed cells. In Proc. Natl. Acad. Sci. USA. 80:3560-3564.