Serum-induced Translocation of Mitogen-activated Protein Kinase to the Cell Surface Ruffling Membrane and the Nucleus

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Abstract. The mitogen-activated protein (MAP) kinase signal transduction pathway represents an important mechanism by which growth factors regulate cell function. Targets of the MAP kinase pathway are located within several cellular compartments. Signal transduction therefore requires the localization of MAP kinase in each sub-cellular compartment that contains physiologically relevant substrates. Here, we show that serum treatment causes the translocation of two human MAP kinase isoforms, p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK}, from the cytosol into the nucleus. In addition, we report that p41\textsuperscript{MAPK} (but not p40\textsuperscript{MAPK}) is localized at the cell surface ruffling membrane in serum-treated cells.

To investigate whether the protein kinase activity of MAP kinase is required for serum-induced redistribution within the cell, we constructed mutated kinase-negative forms of p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK}. The kinase-negative MAP kinases were not observed to localize to the cell surface ruffling membrane. In contrast, the kinase-negative MAP kinases were observed to be translocated to the nucleus. Intrinsic MAP kinase activity is therefore required only for localization at the cell surface and is not required for transport into the nucleus.

Together, these data demonstrate that the pattern of serum-induced redistribution of p40\textsuperscript{MAPK} is different from p41\textsuperscript{MAPK}. Thus, in addition to common targets of signal transduction, it is possible that these MAP kinase isoforms may differentially regulate targets located in distinct sub-cellular compartments.

Cellular proliferation and differentiation is regulated by the action of growth factors that bind to specific receptors expressed at the surface of responsive cells. Growth factor binding to these receptors initiates multiple signal transduction pathways that control cell physiology. One mechanism of signal transduction by growth factor receptors is represented by the mitogen-activated protein (MAP)\textsuperscript{K} kinase pathway (2, 15, 19, 46). In the case of signal transduction by the EGF receptor it has been demonstrated that this pathway is complex and consists of multiple steps including the adaptor protein (GRB2/SEM-5 (38, 45), a guanine nucleotide exchange protein (41), Ras (36, 55, 58), and a protein kinase cascade involving a MAP kinase kinase kinase (c-Raf-1 [21, 32, 34], c-Mos [47], Ste11 [35], or other protein kinase [26, 40]), MAP kinase kinase (5, 18, 33, 50, 56, 59), and MAP kinase (2, 15, 19, 46).

Growth factor-activated MAP kinase phosphorylates substrate proteins in vivo. These substrates represent targets of the MAP kinase signal transduction pathway (19). In vitro analysis has demonstrated that MAP kinase exhibits a restricted substrate specificity (3, 14, 19, 27). However, several MAP kinase substrates have been identified (19). These substrates include: (a) cell surface proteins such as the EGF receptor (44, 53) and phospholipase A2 (37, 43); (b) the cytoskeletal proteins tau (22) and caldesmon (12); and (c) the nuclear proteins c-Jun (3, 13, 48), c-Myc (3, 51, 52), NF-IL6 (42), ATF-2 (1), TA/J (11), and p62/Jr/Elk-1 (25, 39). The targets of the MAP kinase signal transduction pathway are therefore located in the nucleus, the cytoplasm and at the cell surface. Analysis of sub-cellular distribution has demonstrated the presence of MAP kinase within the cytoplasm and the nucleus, but not at the cell surface (3, 10, 49, 52). A fundamental question about the process of signal transduction is therefore the identity of the mechanism that allows MAP kinase to couple to targets localized at the cell surface and within different cellular compartments.

The purpose of this study was to investigate the sub-cellular localization of the MAP kinase isoforms p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK}. We report that these MAP kinases are located in the cytosol of serum-starved cells. However, serum treatment causes translocation of these MAP kinases from the...
cytosol into the nucleus. Significantly, p41\textsuperscript{\textit{\alpha}k} (but not p40\textsuperscript{\textit{\alpha}k}) was also observed to be translocated to the cell surface ruffling membrane. The difference between the serum-induced reduction of p40\textsuperscript{\textit{\alpha}k} and p41\textsuperscript{\textit{\alpha}k} suggests that these MAP kinase isofoms may be coupled to distinct targets of signal transduction in addition to common signaling pathways.

**Materials and Methods**

**Plasmids**

The plasmids pCHI10 and pUC13 were from Pharmacacia-LKB Biotechnol-
yogy Inc. (Piscataway, NJ). The plasmid pGEM-Luc was from Promega (Madison, WI). The plasmids pGALA/Myc and pG5EILuc have been de-
scribed previously (3, 52). The plasmid pMyc contains the human c-myc gene under the control of an SV-40 promoter (30). The plasmid pG5EILuc has been described previously (52).

The plasmid pCMV-p41\textsuperscript{\textit{\alpha}k} was constructed using a 1,992-bp Espl re-
striction fragment of the human p41\textsuperscript{\textit{\alpha}k} cDNA (28) (available from Gen-
Bank under accession number Z1695) cloned as a blunt-ended fragment in the Smal site of the polylinker of the expression vector pCMV5 (4). The protein sequence of human p41\textsuperscript{\textit{\alpha}k} has a calculated molecular mass of 41 kD and is 96.8% identical to the rat ERK2 protein kinase (7, 28). Mutation of p41\textsuperscript{\textit{\alpha}k} at the ATP binding site to inhibit protein ki-

The protein kinases p40\textsuperscript{\textit{\alpha}k} and IM1\textsuperscript{\textit{\alpha}k} were isolated from transfected COS-7 cells by sequential chromatography on Mono-Q and phenyl-

**Cell Culture and Transfection Assays**

COS-7 cells were subcultured in DME supplemented with 5% FBS (GIBCO BRL, Gaithersburg, MD). Transfections were performed using the DEAE-Dextran method as previously described (3). The cells were tryp-
istinized 24 h after transfection, seeded into 100-mm dishes or 4-well tissue culture chambers mounted on a glass slide (Lab-tek, Naperville, IL), and incubated in DME containing 5% FBS for 24 h. Serum-starved cells were transferred to medium without serum during the last 18 h of incubation. Se-

**Measurement of Myc-directed Transactivation of Reporter Gene Expression**

Transactivation assays were performed using 0.02 µg of pGALAMyc activa-
tor plasmid and 2 µg of a luciferase reporter plasmid (pG5EILuc) as previ-

**ISOLATION OF PROTEIN KINASES**

The protein kinases p40\textsuperscript{\textit{\alpha}k} and p41\textsuperscript{\textit{\alpha}k} were isolated from transfected COS-7 cells by sequential chromatography on Mono-Q and phenyl-

**MAP Kinase Assays**

Protein kinase activity was measured using a synthetic peptide substrate based on the sequence of the EGF receptor surrounding the Thr-

The plasmid pCMV-p40\textsuperscript{\textit{\alpha}k} was constructed using a 1,940-bp SacII-EspI restriction fragment of the human p40\textsuperscript{\textit{\alpha}k} cDNA (28) (available from Gen-

**Stoichiometry of EGF Receptor Phosphorylation**

COS cells seeded in 35-mm dishes were labeled with [\textsuperscript{32}P]Phosphate by in-

The total DNA in all transfections was maintained at 10 µg using pUC13 as carrier DNA. The cells were harvested 48 h after transfe-

**Western Blot Analysis**

Cells were solubilized in lysis buffer (25 mM Hepes, pH 7.5, 5 mM EDTA, 1 mM PMSF and 10 µg/ml leupeptin) and the extracts were centrifuged at 100,000 g for 20 min at 4°C. The supernatant containing that MAP kinase activity was collected. Phosphorylation assays were performed using 25 mM Hepes, pH 7, 10 mM MgCl\textsubscript{2}, 50 µM ATP\textsuperscript{[\textit{\gamma}P]} (10 Ci/mmole), 1 mg/ml synthetic peptide, and 10 µl of kinase preparation in a final volume of 25 µl. Reactions were terminated after 20 min at 22°C by the addition of 10 µ1 of 95% formic acid containing 75 mM ATP. The phosphorylated synthetic peptide was iso-

The optical density at 420 nm was measured using a spectrophotometer (Pharmacacia-LKB Biotechnology Inc.).

The Journal of Cell Biology, Volume 122, 1993
Immunocytochemistry

Transfected cells were plated into 4-well tissue culture chambers mounted on a glass slide (Lab-tek) and incubated for 10 h in DME supplemented with 5% FBS. To examine the effect of serum on the cellular distribution of MAP kinases, the cells were washed and incubated in serum-free DME for 18 h, and then they were permeabilized with 0.2% Triton X-100 in KRH (5 min, 22°C), washed three times and incubated for 1 h in KRH containing the primary antibody and 20% horse serum at 22°C. The primary antibodies and the dilutions used were: (a) a mouse monoclonal anti-c-Myc antibody (Ab-2; Oncogene Science, Inc., Manhassett, NY) diluted 1:20; (b) a mouse monoclonal anti-MAP/ERK kinase antibody (2Yned Laboratories, Inc.) diluted 1:500; and (c) a sheep polyclonal anti-EGF receptor antibody diluted 1:200. The anti-EGF receptor antibody was purified from serum that was obtained after immunization of a sheep with a bacterially expressed carboxy-terminal fragment of the human EGF receptor as an antigen.

After the incubation with primary antibodies, the cells were washed three times and incubated for 1 h at 22°C in KRH containing secondary antibodies and 20% horse serum. The secondary antibodies and the dilutions used were: (a) a rhodamine-conjugated rabbit anti-mouse Ig antibody (Boehringer Mannheim Corp., Indianapolis, IN) diluted 1:500; (b) an FITC-conjugated goat anti-mouse Ig antibody (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) diluted 1:300; and (c) an FITC-conjugated rabbit anti-EGF antibody ICN Immunochromics, Costa Mesa, CA) diluted 1:500. Actin filaments were stained with 5 U/ml of rhodamine-labeled phalloidin (Molecular Probes, Inc., Eugene, OR) during the incubation with the secondary antibody. The cells were then washed with KRH and mounted onto slides with a solution of 87.5% glycerol, 10% PBS, and 2.5% DABCO. Beads (200 nm) with a broad fluorescent emission (Molecular Probes, Inc.) were added to the mounting medium as fiduciary markers.

Control experiments were performed to assess the specificity of each antibody used for indirect immunofluorescence experiments. No fluorescence was detected using the secondary antibodies in the absence of primary antibody. To confirm the presence of ruffling membranes after serum stimulation we stained the actin filaments in the cells with rhodamine-labeled phalloidin. FITC-conjugated WGA (Molecular Probes, Inc.) was also used to visualize the cell surface ruffling membrane. It was found that the expression of both the wild-type and kinase-negative forms of the MAP kinases p40

Biochemical Characterization of p40

Two isoforms of the human ERK2 protein kinase that are expressed by HeLa cells have been identified by molecular cloning (28). These protein kinases share a high degree of sequence identity with the exception that p41

Figure 1. Comparison of the NH2-terminal region of p41

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expression of 1041 and 41 in cultured cells. Extracts were prepared from COS-7 cells transfected with pCMV5 (Control), pCMV-p41mapk, pCMV-41(Ala5Ala5)mapk, pCMV-p40mapk, or pCMV-p40(Ala4Ala5)mapk. MAP kinase expression was examined by Western blot analysis (30 μg of the cell extract) using a monoclonal anti-MAP kinase antibody and the enhanced chemiluminescence detection procedure. Quantitation of the Western blot was performed using 125I-labeled protein A to detect the immune complexes and a Phosphorimager with ImageQuant software (Molecular Dynamics Inc.). The level of over-expression of the transfected MAP kinase was approximately 20-fold over the level of the endogenous MAP kinase. Similar data were obtained in three separate experiments.

The partially purified protein kinases were then applied to a hydrophobic interaction column. This column was washed with high salt and the bound proteins were eluted using a decreasing salt gradient in the presence of ethylene glycol. A single peak of protein kinase activity was eluted from the column in each case. However, the elution of p40mapk from the hydrophobic interaction column was found to be modestly retarded in comparison to that of p41mapk (Fig. 5). Together, these data demonstrate that the chromatographic properties of p40mapk and p41mapk are very similar.

Figure 3. Expression of p41mapk and p40mapk causes an increase in MAP kinase activity. Extracts were prepared from COS-7 cells that were mock-transfected (Control) or transfected with plasmid vectors to express wild-type and kinase-negative forms of p40mapk and p41mapk. MAP kinase activity was measured using a synthetic peptide substrate based on the primary sequence of the EGF receptor surrounding the phosphorylation site Thr62. The results are presented as the mean of data obtained in three independent experiments.

In further studies to compare the properties of p40mapk and p41mapk, we examined the effect of the expression of these kinases on a physiologically relevant target of the MAP kinase signal transduction pathway. In previous studies we have demonstrated that c-Myc is a substrate for phosphorylation by MAP kinase at Ser-62 and that the phosphorylation at this site positively regulates the c-Myc transactivation domain (51, 52). We therefore examined the effect of the expression of p40mapk and p41mapk on the c-Myc transactivation
Figure 5. Phenyl-superose chromatography of 1>41~ and p400~. Peak fractions of MAP kinase activity obtained after anion exchange chromatography (Fig. 4) were applied to a phenyl-Superose column. (A) Protein kinase activity of 1>41 (O) and 1>41 mapk (D) was measured using the synthetic peptide KRELVEPLTC~PSG-EAPNQALLR as a substrate. The protein kinase activity in the flow through (FT) and in the eluated fractions is presented. The concentration of NaCl in the eluate is indicated (---). (B) Fractions corresponding to the peak of protein kinase activity were analyzed by SDS-PAGE, transferred to Immobilon-P, and probed with MAP kinase antibody using enhanced chemiluminescence detection.

Figure 6. Effect of 1>41 and p44~ on c-Myc-directed transactivation of reporter gene expression. The effect of transient expression of wild-type and mutated (kinase-negative) forms of 1>41 and p44~ was investigated. COS-7 cells were co-transfected with 0.02 #g of the activator plasmid pGAL4/Myc, 2 #g of the reporter plasmid pGL3BLuc and 1 #g of a β-galactosidase expression vector (pCMV-β-gal) together with either 2 #g of pCMV5 (Control), pCMV-p14/1, pCMV-p41(Ala42Ala43)~ or pCMV-p40(Ala42Ala43)~. The cells were grown in medium containing 5% FBS and extracts were prepared 48 h posttransfection. Differences in transfection efficiency were estimated by measurement of the β-galactosidase activity (OD value) in 5 μl cell extract. Luciferase activities (light units) in 5 μl of cell extract were measured and are presented as (Light Units/OD). Each data point represents the mean ± S.E. of determinations obtained from three independent transfections. Similar data were obtained in three separate experiments.

Figure 7. Cellular Localization of MAP kinase Substrates

Several substrates for MAP kinase have been identified that are located in distinct subcellular compartments. The c-Myc protein is an example of a substrate that is found within the nucleus (3). Immunofluorescence, digital imaging microscopy, and image restoration procedures confirmed that the c-Myc protein was localized within the nucleus (Fig. 7). In contrast to the nuclear location of c-Myc, the EGF receptor is an example of a MAP kinase substrate that is present at the cell surface (16, 44, 53). Immunofluorescence analysis demonstrated that the EGF receptor was expressed at the cell surface with a relatively uniform distribution (Fig. 7).

In further experiments, the effect of serum treatment on the localization of these MAP kinase substrates was examined. It was found that serum caused no significant alteration in the nuclear location of c-Myc (Fig. 7). However, serum caused a redistribution of the EGF receptor to the cell surface ruffling membranes which were visualized by staining cellular actin with rhodamine-labeled phalloidin (Fig. 7). Together, these data demonstrate that MAP kinase substrates are located within several cellular compartments in serum-treated cells, including the nucleus and the cell surface.
Figure 7. Localization of MAP kinase substrate proteins. Transfected COS-7 cells were serum-starved for 18 h and then treated without (A, C, and E) or with (B, D, and F) 10% FBS for 30 min. The cells were then fixed and analyzed by indirect immunofluorescence using a digital imaging microscope and image restoration techniques. Images were obtained for c-Myc (A and B) and the EGF receptor (C and D). Actin filaments were visualized using rhodamine-conjugated phalloidin (E and F). The figure presents representative images of the fluorescence detected from single cells.

Figure 8. Analysis of the distribution of endogenous MAP kinase. COS cells were treated with 10% FBS for 30 min. The cells were then fixed and analyzed by indirect immunofluorescence using a mouse monoclonal anti-MAP kinase antibody and a rhodamine-conjugated goat anti-mouse Ig antibody. The figure presents a representative image of the fluorescence detected from a single cell by digital imaging microscopy and processed for image restoration.
crease in the level of p40-~ in the nucleus (Fig. 10). This are located within each of these cellular compartments.

Sub-cellular Localization of p40-~

To investigate the localization of p40-~, we performed immunofluorescence analysis using a monoclonal anti-MAP kinase antibody. The protein kinase p40-~ was visualized in the cytosol and the nuclear region of serum-starved cells (Fig. 10). Treatment of the cells with serum was associated with a decreased level of p40-~ in the cytosol and an increase in the level of p40-~ in the nucleus (Fig. 10). This observation implies that serum treatment induces the translocation of p40-~ from the cytosol into the nucleus.

To achieve a quantitative understanding of the sub-cellular distribution of p40-~, we analyzed the fluorescence images obtained by digital imaging microscopy. The method we used allowed the compilation of data from several individual cells and the calculation of the average fluorescence in discrete sub-cellular regions (Fig. 12). This quantitative analysis of the immunofluorescence demonstrated that the treatment of cells with serum caused a significant redistribution of cytosolic p40-~ to the nucleus (Figs. 12 and 13). Significantly, no apparent localization of p40-~ to the surface ruffling membrane of serum-treated cells was detected (Fig. 10).

Sub-cellular Localization of p41~p k

The protein kinase p41~p k was detected in the cytosol of serum-starved cells (Fig. 11). A low level of p41~p k was also detected in the nuclear region (Fig. 11). Treatment of the cells with serum caused a marked increase in the nuclear localization of p41~p k and a corresponding decrease in the level of p41~p k in the cytosol (Fig. 11). In addition, it was observed that p41~p k was localized at the border of serum-treated cells. This location at the cell border coincided with the cell surface ruffling membranes which were visualized by staining with rhodamine-labeled phalloidin and FITC-conjugated WGA (data not shown). The cell surface and nuclear localization of p41~p k observed using the digital imaging microscope and image restoration procedures (Fig. 11) was also observed by analysis of the immunofluorescence by laser scanning confocal microscopy (Fig. 14). Quantitative analysis of the immunofluorescence demonstrated that serum caused a significant redistribution of p41~p k to the nuclear region and to the cell surface ruffling membrane (Figs. 12 and 13).

Sub-cellular Localization of p40~p k

MAP Kinase Activity Is Required for Redistribution to the Cell Surface, but not for Translocation to the Nucleus

The MAP kinase isoforms p40~p k and p41~p k were observed to redistribute from the cytosol to the nucleus after treatment of cells with serum (Figs. 10-13). The kinase-negative mutant forms of p40~p k and p41~p k also exhibited serum-stimulated translocation into the nucleus (Figs. 10-13). Thus, kinase activity is not required for the serum-induced nuclear translocation of p40~p k and p41~p k. In contrast, kinase activity was required for the serum-stimulated redistribution of MAP kinase to the cell surface ruffling membrane. Furthermore, the cell surface redistribution was found only for p41~p k and was not observed for p40~p k (Figs. 10 and 11). Together, these observations demonstrate that there are distinct structural requirements for the serum-stimulated redistribution of MAP kinases to different cellular compartments.

Discussion

There has been much interest in the role of MAP kinases in signal transduction because these enzymes appear to integrate signaling pathways that are initiated by many types of cell surface receptors (2, 15, 17, 19, 46). Molecular cloning
Figure 10. Localization of the ERK2 isoform p40\textsuperscript{ERK}. Cells expressing wild-type (A and B) and kinase-negative mutant (C and D) forms of p40\textsuperscript{ERK} were serum starved for 18 h and then treated without (A and C) or with (B and D) 10% FBS for 30 min. The cells were fixed and analyzed by indirect immunofluorescence using a mouse monoclonal anti-MAP kinase antibody and an FITC-conjugated goat anti-mouse Ig antibody. All images were obtained by digital imaging microscopy and processed for image restoration. The figure presents representative images of the fluorescence detected from single cells.

Figure 11. Localization of the ERK2 isoform p41\textsuperscript{ERK}. Cells expressing wild-type (A and B) and kinase-negative (C and D) forms of p41\textsuperscript{ERK}. The cells were serum-starved for 18 h and then treated without (A and C) or with (B and D) 10% FBS for 30 min. The cells were then fixed and analyzed by indirect immunofluorescence microscopy using a mouse monoclonal anti-MAP kinase antibody and an FITC-conjugated goat anti-mouse Ig antibody. All images were obtained by digital imaging microscopy. The figure presents representative images of the fluorescence detected from single cells.
of MAP kinases has revealed the presence of isoforms that exhibit heterogeneous expression in human tissues (6, 7, 28). These isoforms have similar in vitro substrate specificity (3, 14, 19, 27) and may have similar functions in vivo. However, as MAP kinase substrates are located within several cellular compartments, it is possible that these MAP kinase isoforms may have distinct roles during signal transduction because of differences in sub-cellular localization.

We have previously described two isoforms of the human ERK2 protein kinase that are expressed in HeLa cells. These isoforms were designated p40\textsuperscript{ERK2} and p41\textsuperscript{ERK2} according to their predicted molecular mass (28). Analysis of the sequence of these protein kinases indicates that they share a high degree of sequence identity except that p41\textsuperscript{ERK2} has a short NH\textsubscript{2}-terminal Ala-rich extension that is absent in p40\textsuperscript{ERK2} (Fig. 1). To examine the significance of this difference in structure, we compared the properties of p40\textsuperscript{ERK2} and p41\textsuperscript{ERK2}. It was found that these protein kinases exhibited similar chromatographic properties (Figs. 4 and 5) and that both isoforms were able to transduce a signal to the nucleus (Fig. 6). Thus, the biochemical properties of p40\textsuperscript{ERK2} and p41\textsuperscript{ERK2} are very similar. However, in further studies using immunofluorescence and digital imaging microscopy, it was found that these MAP kinase isoforms differ in their sub-cellular localization. This observation suggests that these MAP kinase isoforms may couple to distinct signal transduction targets in addition to common pathways.

**Serum-induced Redistribution of MAP Kinase to the Nucleus**

The nucleus has been demonstrated to be a target of the MAP kinase signal transduction pathway (52). Physiological functions of MAP kinases in the nucleus are likely to include the regulation of gene expression (19). For example, c-Myc, NF-IL6, c-Jun, TAL1 and p62\textsuperscript{TCF}/Elk-1 have been demonstrated to be phosphorylated by MAP kinases (1, 3, 11, 13, 25, 30, 39, 42, 48, 51, 52). In serum-starved cells only a very low level of MAP kinase is detected in the nucleus. However, treatment of cells with serum causes the redistribution of MAP kinase from the cytosol to the nucleus (3, 10, 49, 52). Previous investigations of the cellular distribution of MAP kinases have been performed without reference to the analysis of individual isoforms (3, 10, 49). Here we show that serum treatment caused the redistribution of two different MAP kinase isoforms (p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK}) from the cytosol to the nucleus (Figs. 10 and 11). This serum-stimulated nuclear translocation was also observed for the kinase-negative forms of p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK} (Figs. 10 and 11). Together, these data demonstrate that serum causes the nuclear redistribution of both p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK} and that intrinsic MAP kinase activity is not required for the nuclear accumulation.

The mechanism of serum-induced translocation of MAP kinases remains unknown because a nuclear localization sequence within the primary structure of p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK} has not been identified (28). However, three general mechanisms can be proposed: (a) MAP kinase may enter the nucleus because of a general increase in nuclear transport in serum-treated cells. We are, however, unaware of any precedent for this form of regulation of nuclear localization. (b) It is possible that MAP kinases may have a cryptic nuclear localization sequence that is exposed by the treatment of cells with serum. Kinase-negative mutant forms of p40\textsuperscript{MAPK} and p41\textsuperscript{MAPK} are translocated into the nucleus (Figs. 10 and 11). Thus, intrinsic kinase activation is not required for translocation. However, it is possible that the mechanisms of MAP kinase activation (Thr and Tyr phosphorylation) may act as a signal for translocation. (c) If MAP kinase lacks a functional nuclear localization sequence, it is possible that this signal may be provided by association with another protein. Proteins that could account for this activity have not been identified, but one candidate is represented by MAP kinase (5, 18, 33, 50, 56, 59).

**Serum-induced Redistribution of MAP Kinase to the Cell Surface Ruffling Membrane**

The formation of cell surface membrane ruffles is a common event that is observed after the treatment of cells with growth factors. Recent studies have established an important role for small GTP-binding proteins (rho and rac) in the formation of the membrane ruffles and the reorganization of the cytoskeleton (10, 49). It is possible that the localization of MAP kinase in the membrane ruffles may be related to the regulation of the cytoskeleton in this region of the cell (12, 22, 29). Alternatively, the serum-stimulated redistribution of MAP kinase to the membrane ruffles may function to couple the MAP kinase signal transduction pathway to targets located at the cell surface. Examples of potential targets of MAP kinase at the cell surface (19) include the EGF receptor (16, 44, 53) and phospholipase A\textsubscript{2} (37, 43).

Analysis of the cellular distribution of p41\textsuperscript{MAPK} demonstrated that this protein kinase is localized at the cell surface ruffling membrane after serum-stimulation (Fig. 11). However, the cell surface translocation of p40\textsuperscript{MAPK} was not observed (Fig. 10). This observation suggests that the NH\textsubscript{2}-terminal region of p41\textsuperscript{MAPK} is required for localization at the cell surface (Figs. 10 and 11). Thus, it is possible that the 12-amino acid NH\textsubscript{2}-terminal extension could act as a "signal sequence" for cell surface membrane localization. However, strong evidence against this hypothesis was obtained from the analysis of the kinase-negative MAP kinases which did not localize to the cell surface ruffling membranes after serum treatment. This observation demonstrates that the 12-amino acid NH\textsubscript{2}-terminal extension found in the primary sequence of p41\textsuperscript{MAPK} is not sufficient to confer serum-dependent localization at the cell surface. Together, these data indicate that the cell surface localization of p41\textsuperscript{MAPK} in serum-stimulated cells requires both protein kinase activation and the NH\textsubscript{2}-terminal region of p41\textsuperscript{MAPK}. Further studies are required to identify structures within the cell surface ruffling membrane that are required for the cell surface localization of p41\textsuperscript{MAPK}.

We thank Margaret Shepard for secretarial assistance.

This work was supported by grants from the National Institutes of Health (CA58396 and GM37845) and by a grant from the National Science Foundation (BIR-920002). R. J. Davis is an Investigator of the Howard Hughes Medical Institute. F. A. Gonzalez was the recipient of a postdoctoral fellowship from the National Science Foundation.

Received for publication 7 April 1993 and in revised form 22 June 1993.
A Cell Surface

**Figure 13.** Comparison of MAP kinase distribution in control and serum-treated cells. The relative molecular density of MAP kinase in the nucleus (A) and at the cell surface ruffling membrane (B) is presented (mean ± SD, n = 8). The density of MAP kinase was normalized by dividing the average pixel intensity (Fig. 10) of region 15 (nucleus) and region 1 (cell surface ruffling membrane) by the average pixel intensity of region 12 (a cytosolic serum-independent region).

B Nucleus

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