The Sos1-Rac1 Signaling

**POSSIBLE INVOLVEMENT OF A VACUOLAR H\(^{+}\)-ATPase E SUBUNIT**

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Received for publication, March 16, 2001, and in revised form, July 23, 2001

The guanine nucleotide exchange factor mSos1 regulates cell growth, transformation, and differentiation (1, 2). mSos1 catalyzes the exchange of GDP for GTP on Ras through a carboxy-terminal domain homologous to a yeast CDC25. In addition to its catalytic activity, mSos1 is controlled by mSos1 in the cytoplasm, as determined by immunohistochemistry. mSos1 was found in the early endosome fraction together with V-ATPase E and Rac1, suggesting the functional involvement of mSos1/V-ATPase E complexes in the Rac1 activity at endosomes. Overexpression of V-ATPase E in COS cells enhanced the ability of mSos1 to promote the guanine nucleotide exchange activity for Rac1 and stimulated the kinase activity of Jun kinase, a downstream target of Rac1. Thus, the data indicate that V-ATPase E may participate in the regulation of the mSos1-dependent Rac1 signaling pathway involved in growth factor receptor-mediated cell growth control.

The guanine nucleotide exchange factor mSos1 regulates cell growth, transformation, and differentiation (1, 2). mSos1 catalyzes the exchange of GDP for GTP on Ras through a carboxy-terminal domain homologous to a yeast CDC25. In addition to this carboxy-terminal catalytic domain for Ras, the amino-terminal region of mSos1 contains a domain homologous to Dbl oncogene (DH) and a domain homologous to pleckstrin (PH). Dbl oncogene proteins were shown to have the guanine nucleotide exchange activity for Cdc42 and RhoA, which belong to the Rho GTPase family (3, 4). Similarly, the DH domain of Dbl-related molecules (5) appears to catalyze the GDP-GTP exchange reaction on Rho-like small GTP-binding proteins. Recently, the DH domain of mSos1 (Sos-DH) was reported to promote the GDP-GTP exchange reaction on Rac1, another member of the Rho GTPase family, and thereby induce the activation of Jun kinase (JNK) activity and membrane ruffling (6). Thus, mSos1 appears to have a dual role, activating Ras through the CDC25 domain and Rac1 through the DH domain. However, the function of the Sos-DH domain has not been fully established. In the conventional model, the binding of Grb2 to mSos1 at its COOH terminus and the subsequent translocation of mSos1 to the plasma membrane have been thought to occur in response to growth stimulation (7). Although Grb2 relieves an inhibitory effect of the mSos1 COOH terminus, several lines of evidence reveal that binding of mSos1 to Grb2 alone is not sufficient for activation of mSos1 (8–10). The overexpression of the NH2 terminus of mSos1 interfered with serum-, platelet-derived growth factor-, and EGF-dependent cellular DNA synthesis and suppressed the mitogen-activated protein kinase activation (11). This strongly supports the idea that the interaction between the mSos1-NH2-terminal sequence and a membrane target(s) could be required for the mSos1 activity and membrane targeting and that the NH2-terminal sequence is critical for physiological function of mSos1. However, to date, no protein has been identified that regulates the mSos1 activity through its interaction with the NH2 terminus of mSos1.

The vacuolar H\(^{+}\)-ATPase (V-ATPase) is a multimer enzyme complex composed of V1, a complex of at least seven different cytosolic components (A to H), which is responsible for the ATPase activity, and V0, a complex of at least four different subunits (a to d), which functions in proton translocation (12). The V-ATPase plays an important role in various cellular processes, including receptor-mediated endocytosis and maintenance of cytosolic pH (12). For example, the active V-ATPase pumps protons into the lumen of the vesicle and, thus, acidifies the vacuolar compartment. The low pH within endosomes generated by the V-ATPase is essential for ligand-receptor dissociation and receptor recycling. The V-ATPase also presents in the plasma membrane and exports protons out of the cells, leading to alkalization of the cytosol. The study on the biological role of the V-ATPase subunits has been performed by using lower organisms. Disruption of the B subunit resulted in a larval-lethal phenotype in *Drosophila* (13) and disruption of the VMA4, a yeast homologue of E subunit of the mammalian V-ATPase caused defects in bud morphology and actin distribution (14). The V-ATPase has been implicated in cell trans-
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Glutathione S-transferase (GST) fusion proteins were expressed in DH5α cells and induced by the addition of 0.5 mM isopropyl-thio-p-galactopyranoside. The harvested bacteria were homogenized in extraction buffer (PBS, 1% Triton X-100, 5 μg/ml leupeptin, 5 μM pepstatin), and extracts were mixed with 200 μl of glutathione-Sepharose 4B (Amersham Pharmacia Biotech) for 1 h. The Sepharose beads were washed with extraction buffer and used for GST pull-down assay or GST affinity chromatography.

**GST Pull Down Assay with 35S-Labeled Cells and Transfected Cells**—NIH3T3 cells (4 × 150-mm dishes) were serum-starved in methionine/cysteine-free medium including 0.5% fetal bovine serum for 1 h and metabolically labeled for 4 h with [35S]methionine. Lysates were separated into the cytosol (Cyto.) and membrane fraction (Memb.). Then each fraction was incubated with GST or GST-DH-coupled to glutathione-agarose beads. Proteins bound to the beads were resolved by SDS-PAGE and visualized by fluorography. GST pull-down assay, GST and GST-Sos-DH fusion proteins coupled to glutathione-agarose beads (20 μl) were incubated with the cytosol or membrane extracts for 2 h at 4 °C. After extensive washing in buffer A plus 20 mM NaCl, proteins retained to the beads were resolved by SDS-PAGE and visualized by fluorography. For analysis of mSos1-V-ATPase E association, COS cells were transfected with mSos1 and disrupted in lysis buffer (see “Immunoprecipitation and Kinase Assay”) 36 h after transfection. Lysates were incubated with GST-V-ATPase E-coupled resins for 2 h at 4 °C, and bound proteins were analyzed by immunoblotting.

**Yeast Two-hybrid Experiments**—The coding sequences for the Sos-DH domain and V-ATPase E were created by polymerase chain reaction and fused to a Lex A DNA binding domain of pEG202 and to a B42 transcriptional activation domain of pG42, respectively. Yeast two-hybrid screening was conducted according to the company’s protocol (CLONTECH, Palo Alto, CA). Briefly, pEG202-Sos-DH, pG42-5-V-ATPase E, and p8oplacZ were plated onto a glucose agar plate with the medium lacking His, Ura, Trp, and Leu (SD/Gal/Raf/HW/L) or glucose-containing agar plates with the medium lacking His, Ura, Trp, and Leu (SD/Gal/Raf/HW/L). Yeast cells were grown in 1 ml of culture media (SD/Leu/His/Trp/Raf/HW/L) or in SD/Gal/Raf/HW/L and visualized by silver staining after gel electrophoresis. For a large scale purification of p32, the membrane fraction was prepared from 30 rat brains.

**Amino Acid Sequencing of p32**—Purified p32 proteins were resolved by SDS-PAGE, transferred to a polyvinylidene difluoride membrane, and digested by endopeptidase Lys-C. The generated peptides were fractionated by C18 reversed phase column chromatography and subjected to amino acid sequencing (21).

**Materials and Methods**

**Plasmids**—The mouse V-ATPase E subunit cDNA was provided by G. Dean. V-ATPase E expression vectors were constructed by inserting the cDNA into BamHI and EcoRI sites of pcDNA3-myc plasmid (In Vitrogen, San Diego, CA) or by inserting the cDNA into KpnI and BamHI sites of pcG1-hemoglobin (provided by M. Tanaka). GST-V-ATPase E was created by inserting V-ATPase E cDNA into BamHI and EcoRI sites of pGEX4T (Amersham Pharmacia Biotech). pSRo3 carrying a full-length mSos1 and pGEX-Rac1 were obtained from A. Aronheim (18) and K. Kikuchi (19), respectively. The Sos-DH domain expression vector was constructed by inserting the cDNA fragment corresponding to residues 261–457 of mSos1 into BamHI and EcoRI sites of pcDNA3-myc plasmid or pGEX-T plasmid. A 7-residue cluster mutation (amino acid residues 351HIRDH357 for 351LHYFELL357 in mSos1) was introduced into the Sos-DH domain inserted into pGEX4T plasmid by using successive mutagenesis as described (11). Site-directed mutagenesis of V-ATPase E (where an asparagine 142 is changed to a glycine) was performed according to the method published (20).

**Transfection Experiments**—NIH3T3 or COS1 cells were transfected with various expression vector DNAs by using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s protocol. Transfected cells were grown in the culture medium for 36–42 h. Transfected cells were then subjected to immunoblotting assay of expressed proteins, GDP/GTP exchange activity assay for Rac1, in vitro kinase assay, in vitro reconstitution experiment, and immunofluorescence analysis as described below.

**Identification of GST Fusion Protein**—GST fusion proteins were expressed in DH5α cells and induced by the addition of 0.5 mM isopropyl-thio-p-galactopyranoside. The harvested bacteria were homogenized in extraction buffer (PBS, 1% Triton X-100, 5 μg/ml leupeptin, 5 μM pepstatin), and extracts were mixed with 200 μl of glutathione-Sepharose 4B (Amersham Pharmacia Biotech) for 1 h. The Sepharose beads were washed with extraction buffer and used for GST pull-down assay or GST affinity chromatography.
fluorescein isothiocyanate (Sigma) and anti-rabbit IgG antibodies labeled with fluorescein isothiocyanate or tetramethylrhodamine B isothiocyanate (Sigma). To detect F-actin, cells were stained with tetramethylrhodamine B isothiocyanate-labeled phalloidin (10 μg/ml) (Molecular Probes, Eugene, OR) for 1 h.

**Table 1**

Amino acid sequences of p32 peptides

Peptides isolated by digestion with endoproteinase Lys-C and sequenced by Edman degradation. Amino acid sequences were compared to those of mouse V-ATPase E subunit. Identical amino acids are shown as bold letters.

| Peptide | Amino acid sequence |
|---------|---------------------|
| 1       | XMEYIKE             |
| V-ATPase E | 14HMMAFIEQEANEK26 |
| 2       | AIPAFXEQEANEK       |
| 3       | HMTAFXEQEANEK       |
| V-ATPase E | 19GMEYIKE         |

**RESULTS**

**Identification of a Sos-DH Domain Binding Protein(s)**—To test whether the Sos-DH domain interacts with some cellular proteins other than Ral1 GTPase, we bacterially expressed the Sos-DH domain fused to GST and used it for affinity chromatography. When the GST-DH fusion protein bound to resins was incubated with cytosol or membrane fractions from NIH3T3 cells metabolically labeled with [35S]cysteine/methionine, 32- and 19-kDa proteins were detected to bind to GST-DH but not GST (Fig. 1). These proteins are localized in the membrane but not cytosol. Occasionally, the 40-kDa protein bound to the GST-DH. However, when bound proteins were dissociated with glutathione, 32- and 19-kDa proteins but not a 40-kDa protein were eluted reproducibly (data not shown). The 40-kDa protein appeared to interact with GST-DH resins nonspecifically. When rat brain crude extracts were tested for the binding study, the 32- and 19-kDa proteins were identified as GST-DH binding proteins (data not shown). Because the 32-kDa protein more prominently bound to GST-DH than the 19-kDa protein, we focused on the 32-kDa protein in this study.

To further characterize p32, we purified p32 proteins from rat brain membrane fractions by using GST-Sos-DH affinity...
chromatography. As shown in Fig. 2A, p32 proteins were eluted by buffer containing 200 mM NaCl from a GST-Sos-DH affinity column. Because a cluster of seven substitution mutations in the Sos-DH domain (351IIIIRDII357 for amino acid residues 351LHYFELL357) was reported to reduce the transforming activity of myristoylated Sos1 and the growth response of cells to EGF and platelet-derived growth factor (11), we tested whether Sos-DH interacts with V-ATPase E DNAs. V-ATPase E was detected by mouse antibodies and tetramethylrhodamine isothiocyanate-labeled anti-mouse IgG (a). mSos1 was detected by rabbit anti-mSos1 antibodies and fluorescein isothiocyanate-labeled anti-rabbit IgG (b). c shows a merged picture. The arrows indicate the colocalization of mSos1 and V-ATPase E.

3. Interaction of Sos and Sos-DH with V-ATPase E—To further confirm the result of in vitro interaction between Sos-DH and GST-V-ATPase E, we tested whether Sos-DH interacts with V-ATPase E in vivo using a Lex A-based two-hybrid system. V-ATPase E was fused to a Lex DNA binding domain and tested for interaction with the Sos-DH fused to a B42 transcriptional activation domain. Coexpression of a V-ATPase E-Lex A fusion with a Sos-DH-B42 fusion caused the growth of many cell transformants with increased β-galactosidase (Gal) activity. To confirm that p32 is V-ATPase E, the antibody against a COOH-terminal peptide derived from V-ATPase E was raised and used for immunoblotting analysis. The protein at the 32-kDa region obtained from GST-DH affinity chromatography reacted with an anti-V-ATPase E antibody, indicating that p32 is indeed the rat V-ATPase E (Fig. 2B). Interestingly, the sequences of peptide 2 and 3 showed similarity to the corresponding region of V-ATPase E, but their amino-terminal sequences were slightly different (Table 1), indicating that there might be isoforms of V-ATPase E. Consistent with our observation, microheterogeneity in V-ATPase E has previously been detected in the two-dimensional gel electrophoresis (26).
ity (Fig. 3A), indicating that V-ATPase E interacts with Sos DH. In the control experiments, the individual construct did not self-activate in this system. To determine whether mSos1 physiologically interacts with V-ATPase E, we immunoprecipitated endogenous mSos1 from COS cells and performed immunoblotting with antibodies against V-ATPase E. mSos1 coimmunoprecipitated with V-ATPase E (Fig. 3B). Reciprocally, V-ATPase E immunoprecipitated with its antibodies was complexed with mSos1 (Fig. 3B). In addition, GST-V-ATPase E fusion proteins-coupled resins were created and incubated with cell lysates from COS cells overexpressing wt mSos1. Immunoblot analysis demonstrated that mSos1 bound to the resins, indicating the interaction of mSos1 with V-ATPase E (Fig. 3C).

We next examined the subcellular localization of mSos1 and V-ATPase E. When NIH3T3 cells were cotransfected with mSos1 and the Myc-epitope-tagged V-ATPase E, mSos1 was partially colocalized with V-ATPase E in the cytoplasm (Fig. 3D), which is in agreement with the result of physical interaction between two proteins as described above. Preliminary studies using deletion mutants of V-ATPase E showed that mutants lacking amino acids 1–91 were defective in interaction with mSos1, implying that the Sos binding region may reside in the NH₂-terminal region of V-ATPase E.²

**Colocalization of Sos with V-ATPase E at Endosomes**—It is known that after growth stimulation of cells, ligand-growth factor receptor complexes are delivered to the early endosome where the V-ATPase-catalyzed acidification of this compartment leads to dissociation of the complexes (12). Because mSos1 appears to interact with V-ATPase E, anchoring on the peripheral domain of the V-ATPase at the endosome membrane, we examined whether mSos1 is detectable at the endosome upon growth stimulation. To this end, EGF receptor-mediated endocytosis was examined. 15 min after stimulation with EGF, endosome fractions were isolated and analyzed for the presence of mSos1 and the EGF receptor by immunoblotting. The data show that mSos1 and the EGF receptor were contained in endosome fractions (Fig. 4A). By using floatation on a sucrose-D₂O gradient, we next analyzed whether mSos1, V-ATPase E, and Rac1 are distributed in the early and late endosome fractions. Postnuclear cell lysates were fractionated, and the early and late endosomal fractions were subjected to gel electrophoresis and immunoblotting. Cells were incubated with horseradish peroxidase as a general marker for the endosome content (23), and the endosomal fractions were confirmed by the distribution of Rab5 and Rab7 as general markers for endosome content on the gradient. Fig. 4B shows that mSos1 was detected in the early endosomal fraction but not in the late endosomal fraction. By contrast, both V-ATPase E and Rac1 were detected in the early and late endosomal fractions. These data demonstrate that mSos1, V-ATPase E, and Rac1 were present in the early endosome, which is consistent with the biochemical observation that mSos1 binds to V-ATPase E (Fig. 3, B and C) and regulates the activity of Rac1 as described below.

**Effects of Overexpression of V-ATPase E on the Rac1 and JNK Activities**—Overexpression of the DH domain of Sos1 in COS cells has been reported to enhance the guanine nucleotide exchange activity of Rac1, resulting in the activation of JNK, a downstream target for Rac1 (6). Activation of JNK induces phosphorylation of transcription factors including C-Jun and ATF2, which regulate gene expression (27). To test whether overexpression of V-ATPase E also affects the Rac1 exchange activity and the JNK activity, we performed these assays by using a cell-free system. As in the case of mSos1 overexpression (Fig. 5A), cell lysates from COS cells overexpressing V-ATPase E increased the amount of GDP dissociated from Rac1, suggesting that V-ATPase E stimulates the activity of a guanine nucleotide exchange factor for Rac1 (Fig. 5B). To further confirm a mediating role of V-ATPase E in the regulation of mSos1 activity, a mouse V-ATPase E mutant with Asp-142 Gly was created. This aspartate is well conserved among various species from yeast to human. Substitution of Asp-145 with Gly in yeast V-ATPase E is known to disrupt its normal function, causing defects in bud morphology, actin distribution, and cytokinesis. As shown in Fig. 5C, the level of the nucleotide-releasing activity for Rac1 was higher in wild type V-ATPase E- and mSos1-cotransfected cells than that observed in mSos1-transfected cells. In contrast, a V-ATPase E Asp-142 significantly suppressed the mSos1-dependent nucleotide exchange activity. Furthermore, immunodepletion of endogenous mSos1 with anti-mSos1 antibodies completely abolished the ability of lysates from V-ATPase E-transfected cells to stimulate the guanine nucleotide exchange reaction on Rac1 (Fig. 5D). Thus, the data indicate that a nucleotide exchange factor involved in the V-ATPase E-induced activation of Rac1 is mSos1. Finally, we detected that cell lysates from the V-ATPase-transfected

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² J. Mitsushita and T. Kamata, unpublished data.
cells potentiated the ability of JNK to phosphorylate c-Jun proteins (Fig. 6). Similarly, ATF2 phosphorylation by JNK was enhanced by V-ATPase E overexpression (data not shown). Overexpression of Sos-DH as a positive control activated the JNK activity, as reported previously (6) (Fig. 6). Furthermore, actin stress fiber was dramatically disrupted, and cells exhibited a condensed morphology when V-ATPase E was overexpressed in NIH3T3 and COS cells (Fig. 7). This indicates that V-ATPase E contributes to actin cytoskeleton organization closely associated with the growth state of cells. Taken together, the results suggest that V-ATPase E regulates the mSos1-Rac1 signaling.

**DISCUSSION**

We have identified V-ATPase E as a protein that specifically interacts with the DH-domain of mSos1 by means of GST-Sos-DH affinity chromatography and a yeast two-hybrid assay. mSos1 communoprecipitated with V-ATPase E, associated with V-ATPase E in a cell-free system, and partially colocalized with V-ATPase E. Furthermore, mSos1 were present at endosomes after EGF stimulation of cells and cosedimented with V-ATPase E and Rac1 in the early endosome fraction. These data suggest that mSos1 can bind to V-ATPase E on the outer surface of endosomes during the process of growth factor receptor-mediated endocytosis. To our knowledge, this is the first report describing the interaction of mSos1 with a component of vacuolar type ATP-driven proton pump through its aminoterminal DH domain. The data are in agreement with the previous observation that activation of EGF receptor can lead to the localization of mSos1/Grb2 complexes at endosomes (23). Very recently, mSos1 has been shown to interact with the SH3A domain of intersectin, an endocytotic protein that is involved in clathrin-mediated endocytosis (28). Intersectin competes with Grb2 for binding to the carboxyl-terminal proline-rich domain of mSos1, and overexpression of intersectin results in the attenuation of EGF-induced Ras activation.

Originally, a subunit E of the V-ATPase has been characterized as an important regulatory protein in V-ATPase function (29). In *in vitro* reconstitution experiments, V-ATPase E promotes the ATPase activity of the cytosolic V	extsubscript{1} subcomplex of a vacuolar type ATPase. Furthermore, the temperature-sensitive yeast V-ATPase E mutant caused a decrease in the V-ATPase activity, which is associated with the defects in actin distribution, bud morphology, and cytokinesis (14). The present data suggest that in addition to the regulatory function for the V-ATPase, V-ATPase E may exert a role in the regulation of mSos1-Rac1-mediated growth signaling. This conclusion can be drawn from the following evidences. First, overexpression of V-ATPase E stimulated the mSos1-mediated release of GDP from Rac1, and a loss of function mutant of V-ATPase E was defective in this activity. Moreover, an increased level of V-
ATPase expression activated phosphorylation of a transcription factor c-Jun by JNK, a downstream effector of Rac1. Distribution of actin stress fiber was altered upon overexpression of V-ATPase E. Because small G-proteins are key elements in the reorganization of the actin cytoskeleton induced by growth factors, V-ATPase E might affect cytoskeletal organization through small G-proteins including Rac1.

We propose a model regarding the mechanism for mSos1-dependent Rac1 activation. After growth stimulation, ligand-induced growth factor receptor activation leads to its tyrosine phosphorylation and recruitment of Grb2-mSos1 complexes. Then ligand-mediated receptor internalization causes translocation of receptor-Grb2-mSos1 complexes to the endosome membrane (27, 28). Subsequently, the DH domain of Sos1 binds to V-ATPase E localized at the endosome, and this interaction may aid in activating the catalytic activity of mSos1, resulting in transmission of signals to Rac1. In this scheme, the DH domain of Sos1 is postulated to act as a binding site for V-ATPase E as well as a catalytic domain for the nucleotide exchange reaction on Rac1 (6). Presumably, the different amino acid residues within the peptide region are responsible for these two distinct functions. The noncatalytic, regulatory function of Sos-DH has also been implicated in forming a complex of the Sos1 NH2 terminus and a cellular machinery (11). The V-ATPase is associated with plasma membranes in some types of cells including macrophages and neutrophils (29), and granulocyte colony stimulating factor and phorbol esters up-regulate the V-ATPase at the plasma membrane of neutrophils, which exports protons out of the cells, resulting in alkalization of the cytosol (17). In our study, the plasma membrane of COS cells also contained V-ATPase E,3 implying the functional role of the plasma membrane-bound V-ATPase. Therefore, at present we do not rule out the possibility that the V-ATPase localized at the plasma membrane also participates in the regulation of the mSos1-Rac1 signaling.

Considering that V-ATPase E is involved in the endocytic function, it would be interesting to hypothesize that mSos1 may also play a role in endocytosis, perhaps through the mSos1-Rac1-signaling pathway. Various clathrin-mediated trafficking events are triggered after activation of small GTP-binding proteins by guanine nucleotide exchange factors. For example, Ral is required for the internalization of the EGF receptor (30). As for Rac1, Rac1 regulates transferrin receptor-mediated clathrin-coated vesicle formation (31), EGF-induced

3 K. Miura and T. Kamata, unpublished data.

FIG. 6. Activation of JNK by Sos-DH and V-ATPase E proteins. COS1 cells were transfected with the myc-Sos-DH or myc-V-ATPase E expression vector. After 36 h, cells were serum-starved for 12 h. Endogenous JNK1 was immunoprecipitated, and immune complexes were processed for the kinase assay using GST-c-Jun as a substrate. Aliquots of lysates were analyzed for expression of JNK1, Sos-DH, and V-ATPase E by immunoblotting using an anti-Myc antibody and polyclonal anti-JNK1 antibody. The common band (*) in all lanes indicates a protein nonspecifically bound to horseradish peroxidase-labeled anti-mouse IgG.

FIG. 7. Effects of overexpressed V-ATPase E on actin organization. NIH3T3 (A and B) or COS1 (C and D) cells were transfected with the c-myc-V-ATPase E. Cells were fixed at 36 h post-transfection and permeabilized. V-ATPase E was stained with mouse anti-c-Myc antibodies (A and C) and fluorescein isothiocyanate-labeled anti-mouse IgG, and actin was detected with tetramethylrhodamine B isothiocyanate-labeled phalloidin (B and D). The arrows indicate cells expressing V-ATPase E.
endocytosis via synaptojanin2 (32), and recycling of the Fc receptor (33). Whether the mSos1-V-ATPase E interaction is a necessary step for Rac1-mediated endocytosis remains to be determined.

Finally, our finding suggests that endocytosis may couple to a growth-signaling pathway mediated by small G proteins. Recently, it has been shown that membrane trafficking not only attenuates growth factor receptor signaling but also establishes and regulates the signaling pathways (34). For example, the importance of this intracellular process has been recognized by the findings that receptor tyrosine kinase-mediated signaling to mitogen-activated protein kinase involves endocytic trafficking (35). Thus, V-ATPase E appears to have a dual role in both endocytosis as well as signal transduction pathways.

Acknowledgments—We thank Drs. G. Dean, M. Tanaka, A. Aronheim, K. Kaibuchi, and T. Nikaido for the generous gifts of plasmid DNAs. We also thank Dr. I. Konishi for assistance with confocal microscope. Finally, we thank K. Yamada for preparation of the manuscript.

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J. Biol. Chem. 2001, 276:46276-46283.
doi: 10.1074/jbc.M102387200 originally published online September 17, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M102387200

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