The Nuclear Localization of ERK2 Occurs by Mechanisms Both Independent of and Dependent on Energy

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The mitogen-activated protein (MAP) kinases ERK1 and ERK2 often accumulate in the nuclei of stimulated cells to mediate changes in transcription. The mechanisms underlying stimulus-dependent redistribution of these kinases remain unclear. We have used a permeabilized cell reconstitution assay in HeLa cells and human foreskin fibroblasts to explore the processes by which ERK2 enters and exits the nucleus. We previously reported that entry of unphosphorylated ERK2 into the nucleus occurs by facilitated diffusion not requiring cytosolic transport factors. We find that export, like import, can occur by an energy- and carrier-independent mechanism. An energy-dependent mechanism of ERK2 export can also be distinguished, mediated at least in part through the exportin CRM1. We have also examined import and export of thio-phosphorylated, active ERK2. Import of active ERK2 is significantly enhanced by the addition of exogenous transport factors and an energy regeneration system. These studies support a model in which multiple constitutive and regulated processes control the subcellular distribution of ERK2.

Many signaling events are controlled by acute changes in the activities of proteins due to covalent modifications. The appropriate localization of regulatory molecules and the enzymes that modify them is crucial for coordinated cellular responses. The subcellular localization of ERK1/2 is tightly regulated. ERK1/2 affect regulatory processes in cell compartments including the cytoskeleton, the plasma membrane, and the nucleus. The stimulus-induced nuclear uptake of ERK1/2 is essential for some of the phenotypic programs to which they contribute including differentiation, transformation, and other events requiring altered transcription or other nuclear functions. Improper localization of ERK1/2 has been found in certain cancers, highlighting the importance of elucidating the mechanisms regulating their localization.

Chen et al. (1) first observed that inactive ERK2 is present in the cytoplasm of resting cells and that upon stimulation, a fraction of total ERK2 accumulates in the nucleus; similar observations were subsequently reported by others with ERK1 and ERK2 (2–4). Microinjection of thiophosphorylated ERK2 (thio-pERK2) into the cytoplasm of REF52 cells resulted in its rapid translocation to the nucleus (5), suggesting that activation of ERK2 is sufficient for its nuclear localization. In support of this finding, inhibition of ERK2 phosphorylation by the MEK inhibitor PD98059 reduced ERK2 nuclear localization (6).

Activation of ERK1/2 does not ensure their nuclear localization. Endogenous inactive ERK1/2 are also found in the nucleus, and active ERK1/2 are observed in the cytoplasm (7–9). Microinjection of unphosphorylated ERK2 into the cytoplasm of fibroblasts led to its nuclear localization within 2 min and redistribution to the cytoplasm by 5–10 min, suggesting that ERK2 can rapidly enter and exit the nucleus in unstimulated cells (5). Overexpressed inactive ERK2 accumulates in the nucleus and has been used to assay for factors that promote cytoplasmic retention (10, 11). These later findings suggest that nuclear localization of ERK1/2 can be uncoupled from their activation.

Import reconstitution assays with unphosphorylated ERK2 showed that ERK2 enters the nucleus by an energy- and carrier-independent facilitated mechanism (12, 13). Direct interactions with nuclear pore proteins were inferred from pull-down assays. More recently, fluorescence recovery after photobleaching was used to confirm the energy-independent movement of ERK2 into the nucleus (14). Fluorescence resonance energy transfer demonstrated that ERK2 interacts with CRM1 in the cytoplasm. This finding supports earlier suggestions that MEK1 is involved in cytoplasmic anchoring of ERK2 (15, 16). Phosphorylation of ERK2 releases it from a MEK1 complex, presumably increasing the free pool of ERK2 available to enter the nucleus by the facilitated, energy-independent mechanism. Together these studies suggest an important role for anchoring proteins including MEK1 in the subcellular redistribution of ERK2.

In addition to cytoplasmic anchoring, MEK1 has been proposed to cause nuclear export of ERK2. MEK1 has a nuclear export sequence (NES) that enables its export via the export receptor CRM1 (16, 17). A MEK1 mutant in which the NES was disrupted caused nuclear retention of microinjected ERK2, whereas wild-type MEK1 enhanced cytoplasmic localization of ERK2 (16, 17). Thus, it was proposed that inactive ERK2 can be exported from the nucleus by association with MEK1. Leptomycin B (LMB), an inhibitor of CRM1-mediated export, reduced loss of ERK2 from the nucleus, suggesting that ERK2 export is CRM1- and energy-dependent. Several studies have shown that the localizations of ERK2 and MEK1 are sensitive to LMB (9, 17–19). Because the dually phosphorylated form of ERK2 does not bind MEK1, it was suggested that the active form of ERK2 is not exported from the nucleus (17).

It is not clear whether the behavior of MEK1 is consistent with a role in ERK1/2 export. MEK1 has been found primarily in the cytoplasm of cells. Disabling its NES causes its nuclear accumulation, suggesting that it shuttles between the two compartments (17, 20, 21). However, no significant translocation of MEK1 into the nucleus has been detected upon stimulation (14, 20). Thus, the question remains as to whether quantities of MEK1 that enter the nucleus or the kinetics of its entry or exit are sufficient to drive ERK2 export.
In aggregate, these studies suggest that there is continuous nuclear-cytoplasmic shuttling of the free pool of ERK2 by facilitated diffusion. The active form binds to proteins in the nucleus and, as a consequence, is less readily exported. Anchoring ERK2 in different compartments of the cell determines the size of the pool available for relocalization.

We analyzed import and export in separate reconstitution assays to gain further insight into the mechanisms of ERK2 nuclear transport. Our results identify additional regulated events that must be added to the current model.

**MATERIALS AND METHODS**

**Constructs and Recombinant Proteins**—The preparation of GFP-ERK2, thio-pERK2, MEK1, all with His6 tags, CRM1, Ran, p10/NTF2, karyopherin-β1, karyopherin-α2, and rhodamine-labeled bovine serum albumin (BSA) containing a synthetic nuclear localization sequence (NLS) was as described before (5, 13, 22).

**Cell Culture**—HeLa cells and BJ fibroblasts (human foreskin fibroblasts immortalized with h-TERT (23)) were grown on coverslips for 24 h in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 100 units/ml penicillin/streptomycin at 37 °C under 10% CO2. As indicated, cells were treated with 10–20 nM (5.5–11 ng/ml) LMB (LC Laboratories) for 4–6 h prior to use in import assays. Serum was removed for 2 h prior to use in import assays with thio-pERK2.

**RNAi**—CRM1 expression was reduced with RNAi as described (24). Small interfering RNA oligonucleotides generated toward three CRM1 sequences (CRM1-1, 5'-ATACGTTTGGCCCTATT-3'; CRM1-2, 5'-ATATGTTTGGGTATCTGA-3'; CRM1-3, 5'-TTACTCATCTGGATTATGT-3') and toward WNK4 were synthesized by the UT Southwestern core facility. HeLa cells were grown on coverslips to 30–50% confluence as above but without antibiotics. 100 nM CRM1-1, CRM1-2, or CRM1-3 double-stranded-RNA oligonucleotides were added, and cells were grown for 24 h. In some cases, a second transfection with 100 nM oligonucleotide was performed. Cells were harvested after an additional 12–24 h. Aliquots of cell lysates were immunoblotted with the CRM1 antibody (BD Transduction Laboratories) or ERK1/2 antibody (25) to verify that CRM1 expression was reduced without affecting ERK1/2 expression.

**Import and Export Assays**—Import assays were performed as described before (13), and export assays were according to Ref. 26. In each experiment, duplicate coverslips were examined under all conditions. Unless otherwise indicated, experiments were performed at least three times. Cells

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**FIGURE 1.** Export of GFP-ERK2 in BJ fibroblast and HeLa cells. A, import of GFP-ERK2 into nuclei of BJ fibroblasts was for 15 min in transport buffer with no added factors. For export, cells were incubated in transport buffer alone (−C+E) or in transport buffer containing 2.5 mg/ml cytosol and energy (+C+E) for the indicated times. B, import and export were performed using HeLa cells as above. C, fluorescence intensity of GFP-ERK2 quantified from two independent experiments in BJ cells is shown. Import was for 15 min in transport buffer. Export was for the indicated times in the presence or absence of cytosol and energy. D, fluorescence intensity of NLS-BSA quantified in BJ cell nuclei is presented. Import was for 15 min in transport buffer containing cytosol and energy. Export was for 30 min in the presence or absence of cytosol and energy. Duplicate coverslips were examined for each condition.
were washed in transport buffer (TB) (20 mM Hepes-KOH, pH 7.3, 110 mM potassium acetate, 2 mM magnesium acetate, 1 mM EGTA, and 2 mM diethiothreitol), once unless otherwise specified, and permeabilized with 70 μg/ml digitonin in TB for 5 min. The import reaction was performed in a 40-μl reaction mix that contained one or more of the following components as specified in the figure legends: TB with 10 mg/ml BSA; 0.8 μM recombinant substrate (GFP-ERK2 or thio-pERK2); 0.14 μM TRITC-NLS-BSA; HeLa cell cytosol dialyzed against TB (2.5 mg/ml or as specified); energy- (ATP/GTP regenerating system consisting of 1 mM ATP, 1 mM GTP, 5 mM phosphocreatine, and 20 units/ml creatine phosphokinase); 4 units of apyrase; 0.5 mg/ml wheat germ agglutinin (WGA); and recombinant transport factors kapβ2 (0.5 μM), kapβ1 (0.25 μM), Ran (2 μM), and p10/NTF2 (0.4 μM). Import was for 15 min unless otherwise specified and was terminated with 0.25 ml of TB. Cells were fixed or used for export assays. For export assays, cells that had been preincubated with import substrate were transferred to a 40-μl reaction mix that contained TB with 10 mg/ml BSA and one or more of the following as specified in the figure legends: cytosol, energy, WGA, or apyrase at the same concentrations as above. Export was for 30 min unless otherwise specified. The reaction was terminated with 0.25 ml of TB. Cells were fixed in 3% paraformaldehyde for 10 min, and coverslips were mounted using Aqua PolyMount (Polysciences, Inc.).

For indirect immunofluorescence, fixed cells were repermeabilized in 0.5% Triton X-100 for 10 min. Blocking was performed in PBTA (1× phosphate-buffered saline, 0.1% Tween 20, 10 mg/ml BSA) for 1 h at room temperature. The PERK1/2 antibody (Sigma) was used at 1:300 for 24 h at 4 °C, and the Alexa Fluor 546 anti-mouse secondary antibody (Molecular Probes) was used at 1:3000 or the MEK1 or MEK2 antibodies, A2227 or A2228 (22) were used at 1:300 for 24 h at 4 °C, and the Alexa Fluor 546 anti-rabbit secondary antibody (Molecular Probes) was used at 1:3000 for 1 h at room temperature. Washes between the incubations were performed with 1× phosphate-buffered saline or 1× PBTA for 15 min at room temperature.

**Fluorescence Microscopy**—GFP, TRITC, and Alexa Fluor fluorophores were visualized by fluorescence microscopy using a Zeiss Axioskop 2 Plus microscope, and images were acquired using a Hamamatsu digital CCD camera (C4742-95). Exposures for all conditions within an experiment were constant, and fluorescence intensity within the nucleus was quantified using Slidebook 4.1 software (Intelligent Imaging Innovations, Inc.).

**RESULTS**

*Characterization of ERK2 Export*—The mechanism of ERK2 export from the nucleus has not been thoroughly examined. ERK2 has no identifiable NES, but a CRM1-dependent nuclear export process has been reported based on sensitivity to LMB (17). However, one study indicated that ERK2 was exported in the absence of transport factors and energy, suggesting that export of ERK2 may not be mediated via CRM1 (12). To explore mechanisms of ERK2 export, an export reconstitution assay was performed following import of a GFP-tagged fusion protein of ERK2 in permeabilized BJ fibroblasts and HeLa cells. BJ fibroblasts behave like primary human foreskin fibroblasts in that the nuclear accumulation of ERK1/2 occurs only in response to certain stimuli (9). Because import of unphosphorylated GFP-ERK2 into nuclei of permeabilized cells occurs in an energy- and carrier-independent manner, GFP-ERK2 was allowed to accumulate in the nucleus for 15 min without the addition of cytosol or an ATP/GTP regenerating system (energy). The cells were then incubated for different times in transport buffer with an ATP/GTP-regenerating system (+E) either without (−C) or with (+C) cytosol as a source of transport factors. The amount of GFP-ERK2 remaining in the nucleus was detected by fluorescence microscopy. Reduced nuclear fluorescence from GFP-ERK2 in BJ fibroblasts (Fig. 1A) or HeLa cells (Fig. 1B) was detected as early as 5 min under both conditions. Quantification of fluorescence intensity in three independent experiments with BJ cells indicated ~85% export by 30 min in the presence of cytosol and energy (Fig. 1C). Longer incubations resulted in little additional export. GFP-ERK2 was intact at the end of the export time course as assessed by incubation with permeabilized cells for up to 45 min (data not shown). In the absence of cytosol and energy, less export is detected (Fig. 1C). Experiments with thio-pERK2 indicate that it is also exported in the absence of cytosol and energy (data not shown). Rhodamine-labeled NLS-BSA was used as the model import substrate. It is import via the conventional import mechanism in an energy- and cytosol-dependent manner and, as expected, was not exported from nuclei of BJ cells under the conditions used even after 2 h (Fig. 1D and data not shown).

To determine whether loss of GFP-ERK2, a 68-kDa protein, from the nucleus is through the nuclear pore complex, we performed an export experiment with GFP-ERK2 in which WGA was added after the initial import period. WGA blocks nuclear transport through the nuclear pore complex by binding to carbohydrate moieties on nucleoporins (27). The addition of WGA blocked loss of GFP-ERK2 from the nucleus (Fig. 2).
This confirms that GFP-ERK2 is being exported through the nuclear pore complex and that our conditions of permeabilization do not grossly damage the nuclear membrane.

Export of GFP-ERK2 Occurs by Two Distinguishable Processes—We observed significant export of GFP-ERK2 in the absence of cytosol and energy, suggesting that export can occur by a passive process much like the import process characterized previously. We also observed increased export in the presence of energy and cytosol, suggesting that there may be more than one export mechanism (Fig. 1, A–C).

To characterize the energy and carrier dependence of export further, we performed the export assay using GFP-ERK2 in BJ fibroblasts in the presence or absence of cytosolic factors or energy. After 30 min, little fluorescence in the nucleus was detected when both cytosol and energy were present in the export mix (Fig. 3). The absence of energy, i.e. no exogenously added ATP/GTP-regenerating system or the inclusion of apyrase to scavenge nucleotides, reduced export. The absence of cytosolic factors also reduced export, although to a lesser extent. We obtained similar results in HeLa cells (data not shown), indicating that this is not a cell type-specific event. We tested the effects of varying cytosol concentration and found a minimal effect. At 0.5 mg/ml cytosol, export was inhibited ~20%; this effect was reversed as cytosol concentration was further increased (data not shown).

Export of GFP-ERK2 by the Energy-dependent Process Is Mediated by CRM1—CRM1 mediates energy-dependent transport of many cargo proteins from the nucleus (28). Because there is an energy/cytosol dependence of export of GFP-ERK2, we examined the involvement of CRM1 in this process. We knocked down the expression of CRM1 using RNA interference in HeLa cells. Expression of CRM1 was reduced from 75 to 90% in four experiments (Fig. 4, A). We observed inhibition of export at 15 and 30 min in cells transfected with double-stranded RNA targeting CRM1 (Fig. 4, B and C, labeled CRM1 RNAi (+) and − (control) respectively). Quantification of the fluorescence intensity in the nucleus showed ~30% inhibition at 15 min and a maximum of 45% at 30 min. RNAi with oligonucleotides that suppress expression of the protein kinase WNK4 had no effect on export of ERK2 (data not shown). CRM1 RNAi neither significantly altered the import of GFP-ERK2 (Fig. 4, B and C) nor did it affect the amount of NLS-BSA in the nucleus under import or export conditions (Fig. 4, D and E). CRM1-mediated export should be dependent on a source of Ran-GTP, provided by the cytosol, as well as an energy regeneration system to provide GTP. Thus, export
was also performed in the absence of cytosol and energy to determine whether these components were required for the export we detected (Fig. 4F). Consistent with a role for CRM1 in the energy-dependent export of GFP-ERK2, little or no inhibition of energy-independent export was evident under these conditions.

Finally, we performed export assays with GFP-ERK2 in HeLa cells treated with 10 or 20 nM LMB for 4–6 h. We observed a maximum of 50% inhibition of export (with cytosol and energy) at 30 min after treatment with LMB (+LMB), as compared with export lacking LMB (−LMB) (Fig. 5, A and B). Neither import nor export carried out in the absence of cytosol and energy were affected by LMB (Fig. 5, A and B), consistent with the results of CRM1 RNAi. NLS-BSA was used as the nuclear localized control, and no effect of LMB on its localization was observed during the import or export process (Fig. 5, C and D).

**Export of ERK2 Occurs Even with Impaired MEK1 Binding**—Several experiments were performed to characterize the actions of MEK1 on this system. The addition of an equimolar amount of recombinant MEK1 to the import mixture reduced the import of GFP-ERK2 (Fig. 6A). Because this occurred rapidly and in the absence of added transport factors, the reduced nuclear staining is probably due to inhibition of import rather than enhanced export. In the absence of added exogenous MEK1, immunofluorescent staining of the washed permeabilized HeLa cells after import of GFP-ERK2 revealed a significant amount of endogenous MEK1 and MEK2 (Fig. 6B) in the nuclei. MEK1 and MEK2 were largely retained in HeLa nuclei following export of GFP-ERK2 in the presence of cytosol and energy (Fig. 6B). A comparable experiment in BJ fibroblasts also showed detectable nuclear MEK1 and MEK2 but suggested that MEK1 was more readily lost from B) cell nuclei during the time necessary for export (data not shown).

We also examined export of an ERK2 mutant Y261N that is impaired in binding to MEK1 and has been shown to accumulate in nuclei of transfected cells (11). This mutant was imported, albeit more slowly than wild-type ERK2. Its export was not significantly different from that of wild-type ERK2.

**Phosphorylated ERK2 Can Enter the Nucleus in the Absence of Transport Factors and Energy via the Nuclear Pore Complex**—We previously showed that thio-pERK2 competes for import with GFP-ERK2 and NLS-BSA, in the absence of cytosolic factors and energy (13). These experiments suggest a similarity in the mechanism of import of unphosphorylated and phosphorylated ERK2. Studies in intact cells suggested that phosphorylation promoted ERK2 import (5); this

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finding was interpreted to suggest that ERK2 enters the nucleus via an active import mechanism. Thus, we also investigated the mechanism of entry of phosphorylated ERK2 into the nucleus using import reconstitution assays.

To examine import, B) cells were permeabilized and incubated in the import mixture for 20 min in the presence or absence of recombinant transport factors (TF) and energy. To reduce any endogenous phospho-myelinated ERK2, cells were starved for 2 h prior to permeabilization and/or washed in transport buffer prior to the addition of thio-pERK2. Thio-phosphorylated proteins are more resistant to dephosphorylation than phospho-proteins (5). Thus, thio-pERK2 was used as the import substrate. Import of thio-pERK2 was detected with an anti-pERK1/2 antibodies (Fig. 8). From the difference in nuclear uptake observed (Fig. 7C, far right), it seems likely that this effect depends on residual transport factors that remain following permeabilization. In support of this interpretation, the addition of transport factors did not further enhance uptake (not shown). To confirm that the increase in fluorescence intensity within the nucleus was due to phosphorylation of endogenous ERK2 in the presence of energy, permeabilized cells were incubated in the import mixture with an energy regeneration system without thio-pERK2. No fluorescence above background was observed (Fig. 7C, far right). We also performed import assays in the presence of WGA. WGA blocked most of the fluorescence seen in the nuclei in the presence of energy (Fig. 7C).

This suggests that, in addition to an energy- and carrier-independent import process, thio-pERK2 also utilizes an energy-dependent mechanism for nuclear translocation not detected for unphosphorylated ERK2 using this same assay system (13). From the difference in nuclear uptake observed, the energy-dependent process appears to be the predominant mechanism.

Phosphorylated ERK2 Can Exit the Nucleus in the Absence of Transport Factors and Energy—We examined the export of thio-pERK2 in the absence of energy and cytosol. Thio-pERK2 was exported from the nucleus in the absence of transport factors or energy-regenerating capacity (Fig. 8, A and B), indicating that phosphorylation alone is sufficient to retain ERK2 in the nucleus. Incubation of thio-pERK2 with cytosol for 30 min did not significantly reduce its immunoreactivity with pERK1/2 antibodies (Fig. 8C). Thus, dephosphorylation of thio-pERK2 by cytosolic phosphoprotein phosphatases was not the cause of loss of thio-pERK2 in import assays.

DISCUSSION

We have used import and export assays in permeabilized cells to evaluate mechanisms of ERK2 entry into and exit from the nucleus. Much of the previous work directed at understanding these processes employed overexpressed proteins that may behave differently from
endogenous proteins or small molecule inhibitors that may block the functions of unrelated components in the cell.

The following model best captures the current findings with ERK2 and is likely to apply also to ERK1. ERK2 in the unphosphorylated state enters the nucleus by an energy-independent mechanism facilitated by direct interaction with nucleoporins (12, 13). An effect of transport factors and energy has not been detected, suggesting that this is the primary entry mechanism for the unphosphorylated protein. Entry is very rapid in reconstitution assays and as reported using photobleaching in intact cells (14). Proteins that bind ERK2 in the cytosol inhibit its entry by tethering it at sites distant from the nuclear membrane or, like PEA-15, by blocking its ability to interact with nucleoporins (18). ERK2 can also exit the nucleus by an energy-independent process as suggested previously (12), presumably by the same nucleoporin-mediated mechanism. Thus, there is a constant exchange of ERK2 between the cytoplasm and the nucleus. In the case of export, a second process dependent on energy and transport factors including CRM1 also exists. Like unphosphorylated ERK2, phosphorylated ERK2 can enter and exit the nucleus by an energy- and carrier-independent mechanism. However, a significant component of entry of phosphorylated ERK2 requires energy. Energy appears to increase the rate and extent of nuclear uptake. This process presumably promotes the nuclear uptake of active ERK2 selectively over the unphosphorylated form. Interactions specific for the phosphorylated form of ERK2 would then be expected to enhance retention of the active form in the nucleus.

The factors required to mediate the steps we have identified are present in unstimulated cells following permeabilization. Residual amounts of transport proteins are present, but factors that are dependent on stimulation, due to either covalent modification or induction, are not required for these processes. Additional regulation is expected to be introduced under stimulated conditions. Active ERK2 binds kinetochores, topoisomerase II, and many transcription factors, as well as an unidentified newly synthesized protein (6, 29–31), all of which may serve to sequester active ERK2 from the readily exchanging pool.

Energy-dependent import mediated via importins requires direct binding of cargo proteins through their NLS to import factors. ERK2 has no obvious NLS. Thus, as for export, the energy-dependent process of import of unphosphorylated ERK2 may be mediated by binding to other NLS-containing proteins; phosphorylation of ERK2 would be expected to enhance interaction with such proteins. It is also possible that phosphorylation un masks an otherwise silent NLS in ERK2.

The energy-dependent component of ERK2 export is mediated, at least in part, by CRM1. Thus far, we have been unable to show any direct interaction between ERK2 and CRM1 (data not shown). It is possible that binding of ERK2 to other karyopherin-β family members bypasses the need for an NES. There is precedent for direct interactions of MAPKs with transport factors. The Drosophila ERK (D-ERK) binds to the homolog of importin7 (DIM-7) (32), and the yeast p38 MAPK HOG1 binds to a karyopherin-β family member, NMD5 (33). It seems more likely that ERK2, which lacks a recognizable NES, requires binding to other NES-containing proteins to be exported via the classical export machinery. One such protein is MEK1. Our results support a role for MEK1 as a cytoplasmic anchor, as originally proposed (15), but do not yet provide a clear picture of its significance in ERK2 export. MEK1 is present in the nuclei of permeabilized cells and might participate in the active export process as it has a leucine-rich NES and is exported via CRM1. Our results suggest that MEK1 does not enter the nucleus readily when complexed to ERK2, nor is it apparently exported as readily as is ERK2 in the presence of energy and cytosolic factors. Reduced MEK1 binding does not detectably decrease ERK2 export. Nevertheless, these findings are insufficient to rule out a role for MEK1 in the CRM1-dependent component of ERK2 export.

In summary, we have shown that active ERK2 enters the nucleus by two processes; one is energy-dependent, and the other is energy-independent. The energy-dependent process is enhanced by transport factors. The energy-independent process is facilitated by interaction of ERK2 with nucleoporins. Two processes, one energy-dependent and one energy-independent, also appear to mediate export of ERK2 from the nucleus. It seems likely that differential regulation of these processes would be sufficient to achieve a range of stimulus-induced localizations such as have been reported in different cell types in response to different ligands.

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