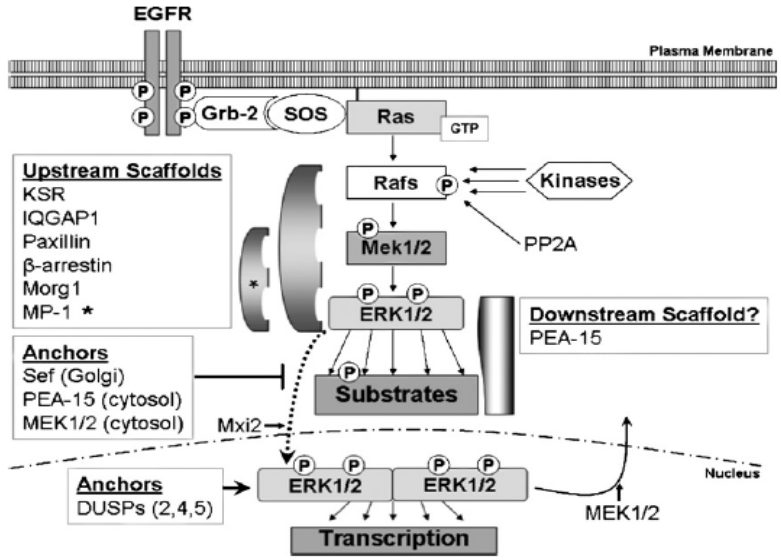
# Shuttling
# citoplasma <---> nucleus

1

ERK regulation by scaffolds and anchors

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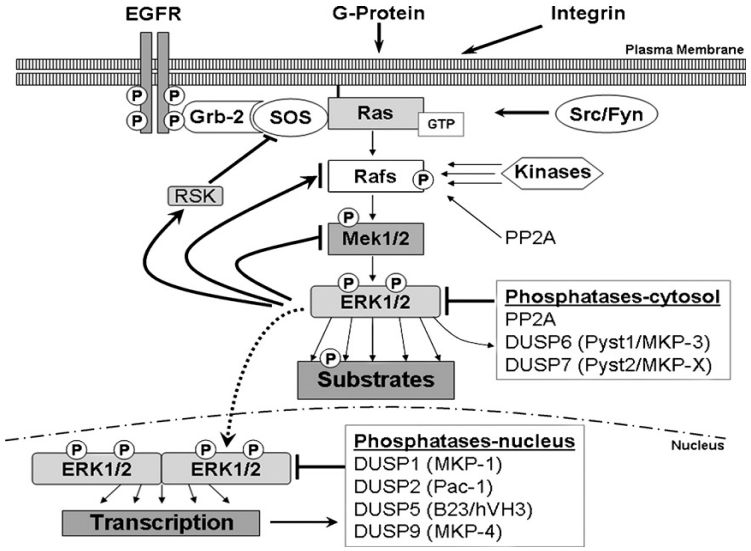
J.W. Ramos / The International Journal of Biochemistry & Cell Biology 40 (2008) 2707–2719

ERK Pathway regulation by feedback loops and phosphatases

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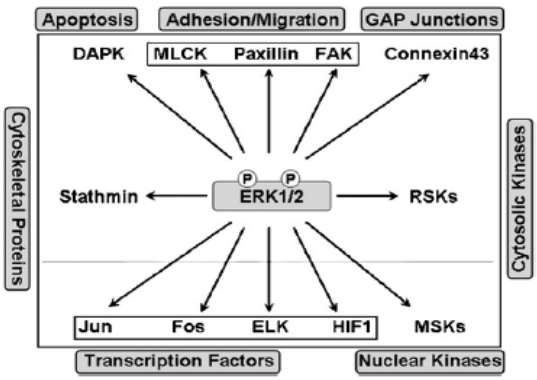
J.W. Ramos / The International Journal of Biochemistry & Cell Biology 40 (2008) 2707–2719

ERK has more than 100 known substrates. The diversity of these substrates is indicated by showing examples of substrates with divergent functions. ERK targets both transcription factors and kinases in the nucleus. It can also phosphorylate various kinases and structural proteins in the cytosol, while at the plasma membrane it targets proteins that regulate cell adhesion, cell–cell communication, and cell survival. The outcome of activation of the ERK pathway in a given cell will therefore be determined in part by where the active ERK is targeted in the cell and which substrates it has access to at those locations.




4

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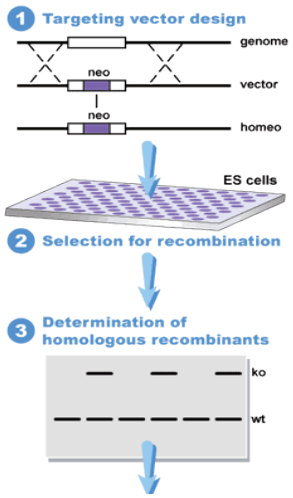
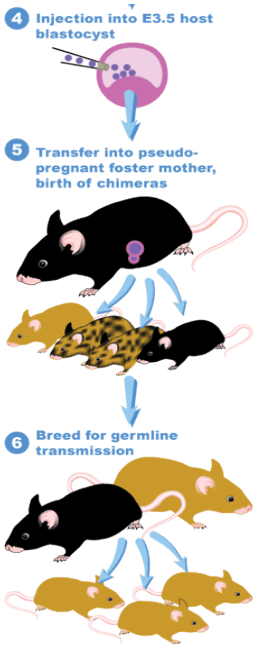
ERK1-deficient mice are viable, fertile, with normal size, but manifest a deficit in thymocyte maturation. Moreover, these mice exhibit an elevated synaptic plasticity in the striatum, which could be a result of a stimulus-dependent elevation in ERK2 phosphorylation, which was observed in neurons as well as fibroblasts of these mice.

ERK2-deficient mice die early in development, showing that ERK1 can't compensate for ERK2 in the embryo

General Outline for the Production of knockout mice by homologous recombination?



ERK1 and ERK2 are also very similar proteins, with about 70% similarity between them, which is higher in the kinase domain and lower in the flanking N- and
C-terminal regions. Numerous studies using anti-ERK and anti-phosphoERK antibodies revealed that the two isoforms are expressed in essentially all cells and tissues in variable relative amounts, whereby ERK2 is the predominant isoform in most cells. Usually, both proteins share similar activation kinetics, cellular localization, and a set of substrates, indicating that under most circumstances the two isoforms function in a similar fashion. However, as described for MEKs,
some differences between the two isoforms do exist under certain restricted conditions, and those differences are best exemplified by the use of ERK1 and ERK2 knockout mice.

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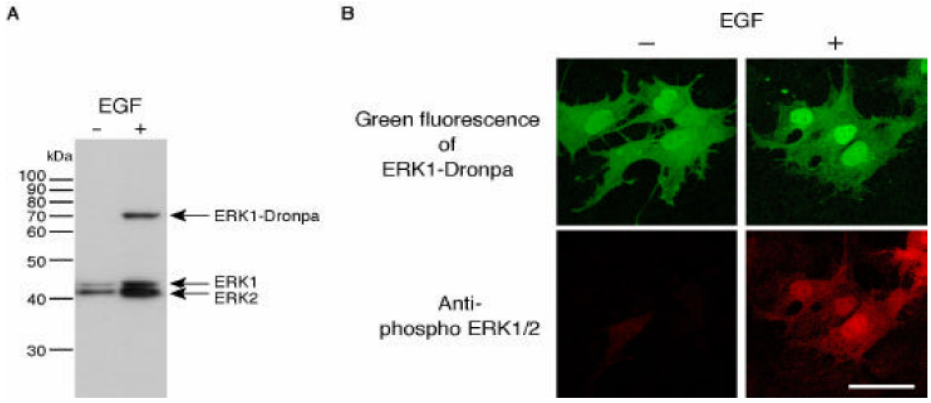
# Regulated Fast Nucleocytoplasmic Shuttling Observed by Reversible Protein Highlighting

**Ryoko Ando, Hideaki Mizuno, Atsushi Miyawaki***

*Science* **306**, 1370 (2004)

8

EGF-dependent phosphorylation and nuclear accumulation of ERK1-Dronpa in COS7 cells.
Dronpa is a GFP variant

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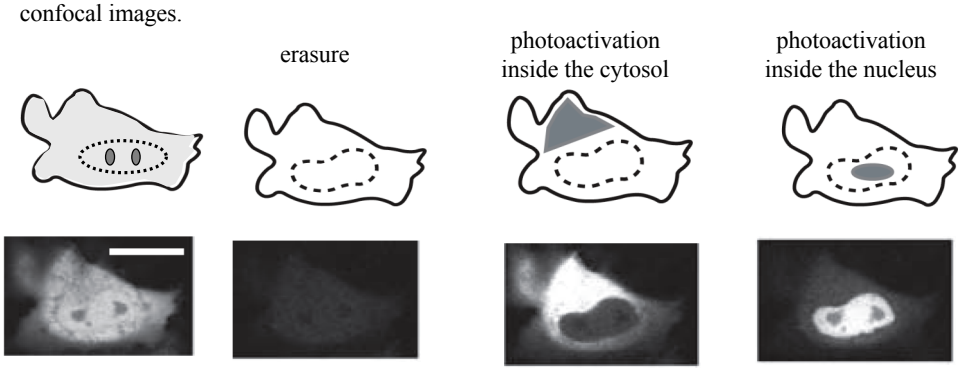
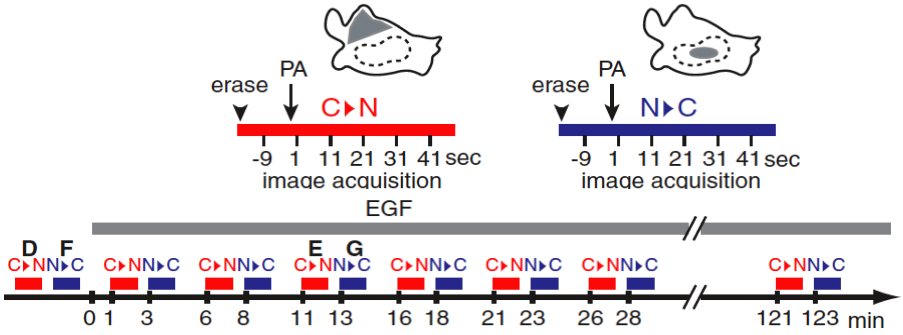
Each experimental period consisted of erasure, photoactivation, and acquisition of a series of confocal images.



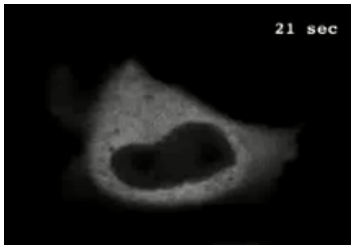

Monitoring nuclear import (C ----> N)

Monitoring nuclear export (N ----> C)

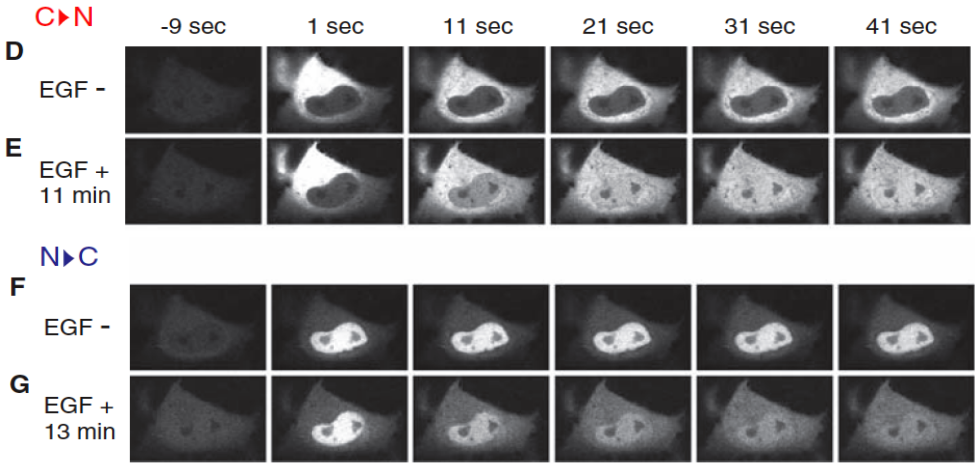
10




This movie shows the nuclear import and export of ERK1-Dronpa in COS7 cells stimulated with 100 ng/ml EGF. Images of two representative monitoring experiments (t = 11 and 13 min)




11

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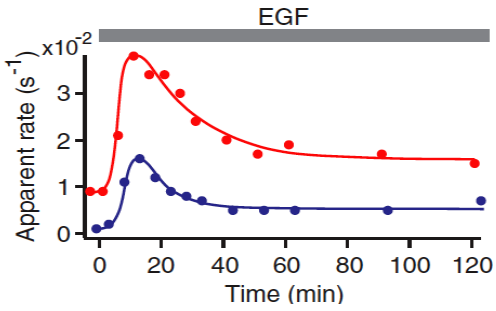
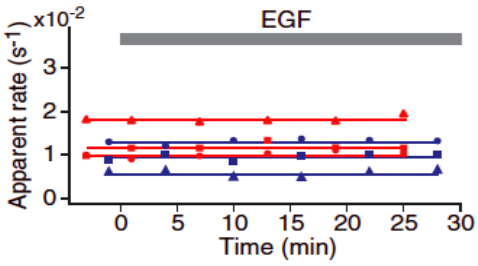
Time courses of the nuclear influx (red) and efflux (blue) rates of ERK1-Dronpa and importin-dronpa during during stimulation with 100 ng/ml EGF

ERK-1




importin-dronpa

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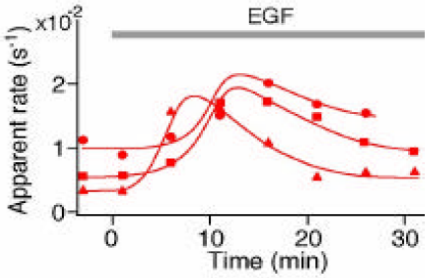
Notably, variation in the initial shuttling rate between different cells was observed. Thus, any changes in movement must be assessed using data from a single cell, because measurements are affected by the geometry of the cells and marked regions.




Time courses of the nuclear influx (red) rates of ERK1-Dronpa during stimulation with 10 ng/ml EGF obtained from different three COS7 cells (circles, squares, and triangles).

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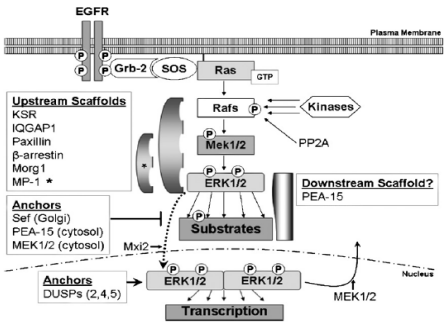
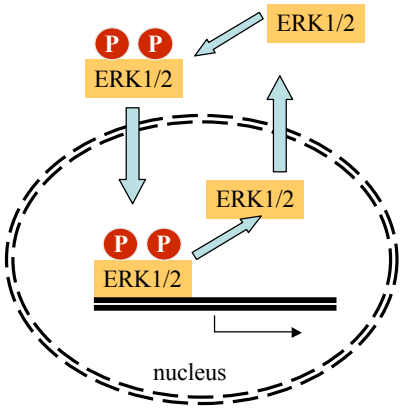
Similar regulation was observed for ERK2-Dronpa in COS7 cells and HeLa cells that were stimulated with EGF.




Assuming that ERK principally undergoes inactivation within the nucleus, fast circulation across the nuclear envelope is predicted to more effectively increase gene expression than does simple nuclear retention.

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