A Spatial Model of ERK Nuclear Translocation

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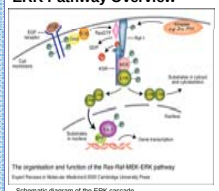
Abstract

The extracellular signal-regulated kinase (ERK, also known as MAPK, mitogen activated proetin kinase) is involved in many important cellular processes such as gene expression, cell spreading and tumor growth. In the final steps of the ERK cascade, phosphorylated ERK dissociates from active MEK and translocates into the nucleus. Various experiments have shown that MEK guides the localization of ERK. We constructed a spatial PDE-based model from a previous ODE model made by Fujioka et al. (2006) with the VirtualCell software. Our goal was explore two possible scenarios explaining the control of MEK (MAP kinase kinase) over ERK localization and to reproduce ERK nuclear translocation experiments done by Costa et al. (2006). Overexpression of ERK results in increased nuclear concentration of ERK with a decrease in its diffusion coefficient (Costa et al., 2006). Although this suggests that there is an agent which immobilizes ERK in the nucleus, previous ERK nuclear translocation models do not include this factor. In our simulations we tried to reproduce experiments of ERK nuclear translocation. We failed to observe sustained nuclear accumulation until the addition of a nuclear anchoring event, such as the binding of ERK to nuclear transcription factors. We also found that, through parameter variation, Raf concentration was key factor to reproduce the Costa experiments. Our simulations also highlight the spatial constrains that can affect ERK nuclear translocation, as the ratio of nuclear volume to cytoplasmic volume had to be increased to recapitulate the data of Costa et al., (2006) .

Background

ERK Pathway Overview

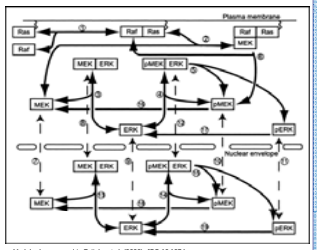


Schematic diagram of the ERK cascade

The ERK cascade can be activated by growth factors when they bind to receptor tyrosine kinases (RTK) located on the cell membrane. Two ligands for RTKs are fibroblast growth factor (FGF) and epidermal growth factor (EGF). Ligand binds and dimmerizes receptor. There is the recruitment of adapter proteins to form a complex that will activate the guanine nucleotide exchange factor (GEF), Son of Sevenless (SOS). The small G-protein Ras, which can be found GDP-bound in its inactive state or GTP-bound in its active state, is activated by SOS. Ras-GTP binds to and phosphorylates c-Raf which then activates MEK in the same manner. Upon activation, MEK is able to shuttle from the cytoplasm to the nucleus and back but some studies have shown that is mostly localized in the cytoplasm. Once activated, MEK phosphorylates the amino acids threonine and tyrosine on ERK, rendering ERK active. It is unclear whether the nuclear translocation of ERK is dependent on 1) the dissociation of cytoplasmic MEK or 2) on the ERK/MEK complex together enter the nucleus.

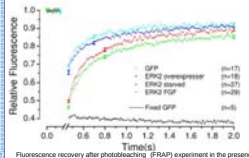
Computational Model of ERK nuclear translocation

Fujioka et al (2006) developed a ODE based model to recapitulate ERK nuclear translocation upon growth factor stimulation. They experimentally determined cellular concentrations, and kinetic constants. They also monitored nuclear translocation and dissociation constants using FRET techniques. The model reproduced ERK activation and nuclear translocation of the *in vivo* experiments. From their model, Fujioka et al., predicted pMEK and pERK sensitivity to c-Raf concentration, which was confirmed experimentally.



Model scheme used in Fujioka et al. (2006) JBC 10:1074

Regulation of ERK nuclear translocation

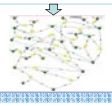


Fluorescence recovery after photobleaching (FRAP) experiment in the presence and absence of growth factor stimulation. Slower recovery implies lower mobility. Costa et al. (2006) Journal of Cell Science 119:4952

Since not much is known about the details of ERK nucleocytoplasmic shuttling, Costa et al., (2006) used ERK fluorescently tagged to visualize the dynamics of this process in live cells. Data shows that ERK nuclear accumulation requires a continuous upstream stimulus and that phosphatases rapidly counteract it. The Costa group shows that ERK diffusion is not free in the nucleus and it decreases after activation, suggesting the formation of low mobility complexes in the nucleus.

Methods

Input parameters, reactions and species for each experiment



Run simulation , analyze and compare results to experimental data from Costa et al., (2006) and adjust model

Results

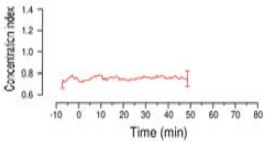
Figure 1. Timecourse of ERK Nuclear accumulation in untreated cells

Left: Time course of ERK2-GFP nuclear concentration in untreated control cells. The concentration index is defined as the average fluorescence of the nucleus (F_{Nuc}) minus the *BG* (average background) normalized to surrounding ring of thickness approximately equal to the nucleus radius (F_{Ring}). The CI was computed as:

$$CI = (F_{Nuc} - BG)/(F_{Ring} - BG)$$

Right: Simulations results of normalized nuclear to cytoplasmic ratio of total ERK concentration.
$CI=[ERK_{tot}]_{nuc}/([ERK_{tot}]_{cyto}*([ERK_{tot}]_{nuc}/[ERK_{tot}]_{cyto})_{max})$
In vivo experiment was simulated *in silico* by changing the $[RTK]_{active}$ to zero.

In Vivo



Costa et al., (2006) Journal of Cell Science 119 (23) 4952

In Silico

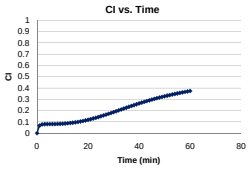
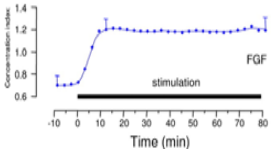


Figure 2: Timecourse of ERK nuclear translocation in the presence of constant stimulus

Left: Time course of ERK2-GFP nuclear concentration during constant stimulation with FGF.
Right: Normalized nuclear to cytoplasmic ratio of total ERK concentration.
In vivo experiment was simulated in silico by changing $[RTK]_{active}$ to 2 μM for the entire simulation.

Simulation was run using finite volume solver with a time-step 0.01 sec sampled every 60 sec



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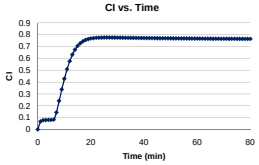
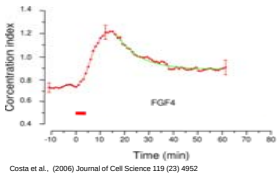


Figure 3: Timecourse of ERK nuclear translocation after transient 4 minute stimulus

Left: Time course of ERK2-GFP nuclear concentration after 4 minute stimulation with FGF.
Right: Normalized nuclear to cytoplasmic ratio of total ERK concentration.
In vivo experiment was simulated *in silico* by changing $[RTK]_{active}$ to 2 μM for 4 min. In order to reproduce the experimental data, we ranged the concentration of Raf.



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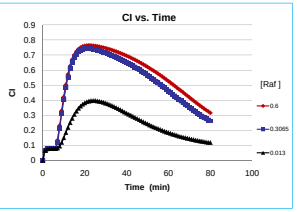
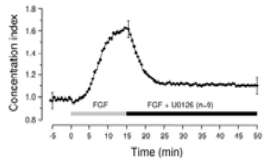


Figure 4: Timecourse of ERK nuclear translocation after treatment with FGF4 and inhibition of MEK with U0126 15 minutes post stimulation.

Left: Time course of ERK2-GFP nuclear concentration. Cells were constantly treated with FGF4 and with U0126 (MEK inhibitor) after 15 minutes of initial FGF stimulation.
Right: Normalized nuclear to cytoplasmic ratio of total ERK concentration. In vivo experiment was simulated *in silico* by adding a species to interact and inhibit MEK.



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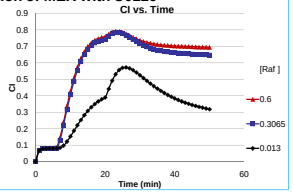
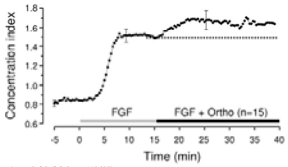
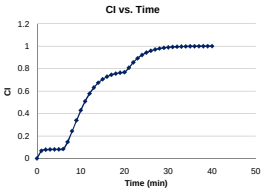


Figure 5: Timecourse of ERK nuclear translocation after treatment with FGF4 and inhibition of PTPs with orthovanadate 15 minutes post stimulation.

Left: Time course of ERK2-GFP nuclear concentration. Cells were constantly treated with FGF4 and with Orthovanadate (protein tyrosine phosphatase inhibitor) after 15 minutes of initial FGF stimulation
Right: Normalized nuclear to cytoplasmic ratio of total ERK concentration.
In vivo experiment was simulated *in silico* by decreasing PTP levels.

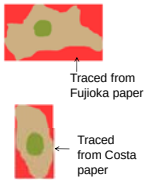


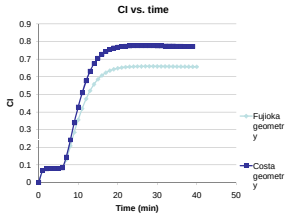
Journal of Cell Science 119 (23)



Results

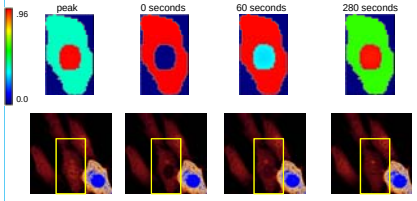
Figure 6. Effect of cytoplasmic volume to nuclear volume ratio on ERK nuclear translocation





Timecourse of ERK nuclear translocation upon constant stimulus in two different cellular geometries taken from Costa et al., and Fujioka et al.,

Figure 7. Comparison of ERK Nuclear Accumulation after FRAP



Top panel: Simulation results showing color-coded concentration of total ERK upon stimulation. Geometry was traced from image shown in bottom panel. Bottom panel: FRAP experiment of stimulated cells expressing ERK-GFP (Costa et al., unpublished data).

Conclusions

- The simulation results show that Raf concentration is a key parameter. The cell types used differed in the Fujioka and Costa experiments. In order to reproduce the Costa results we had to increase the Raf concentration from that used in the Fujioka simulation.
- In our simulations, an ERK anchor is needed in the nucleus so that we can have a sustained nuclear accumulation.
- Nucleus to cytoplasm volume ratio is an important factor. Increasing this ratio decreases the time it takes for the nucleus to reach a maximum ERK concentration and increases the maximum concentration level.

Acknowledgements

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References

1. Costa et al."Dynamic regulation of ERK2 nuclear translocation and mobility in living cells"*J. of Cell Science* 119, 4952-4963 (2006)
2. Fujioka et al. "Dynamics of the RAS/ERK map kinase cascade as monitored by fluorescence probes." *J. Biol. Chem*, 10.1074 (2006)