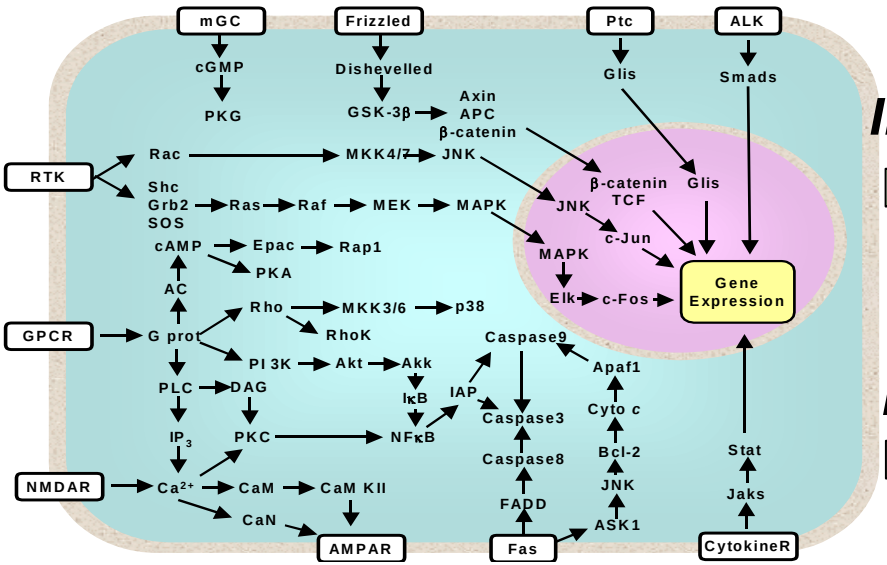
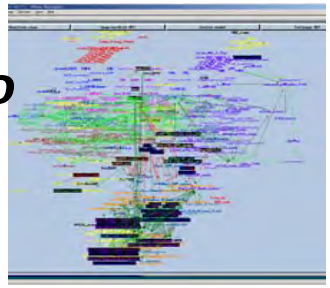
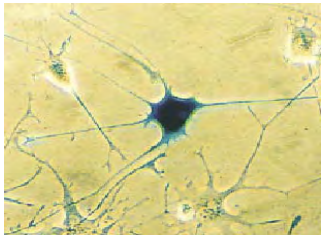
Temporal coding of ERK signaling networks



In silico



In vivo



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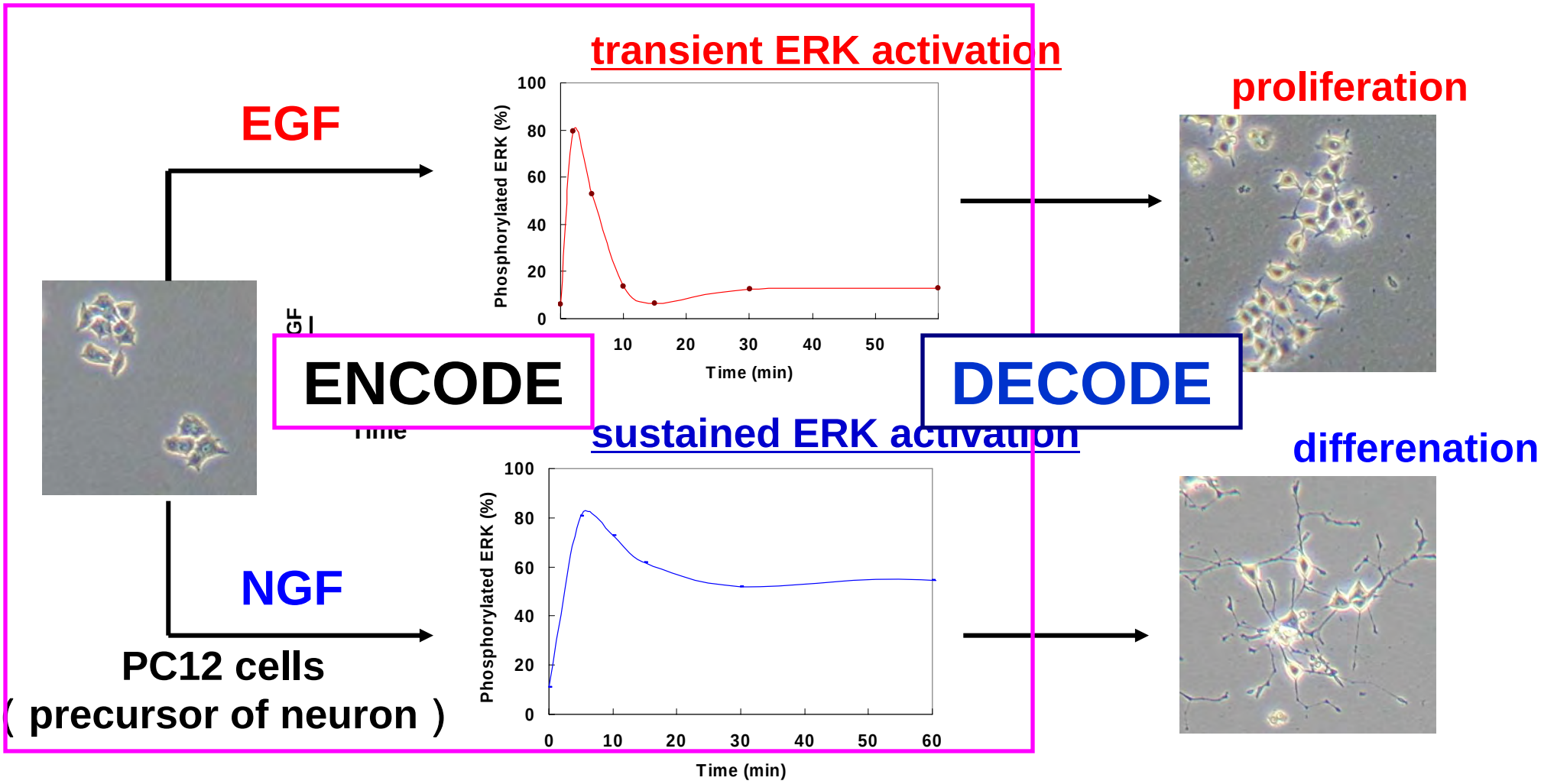
Cell Fate Determination

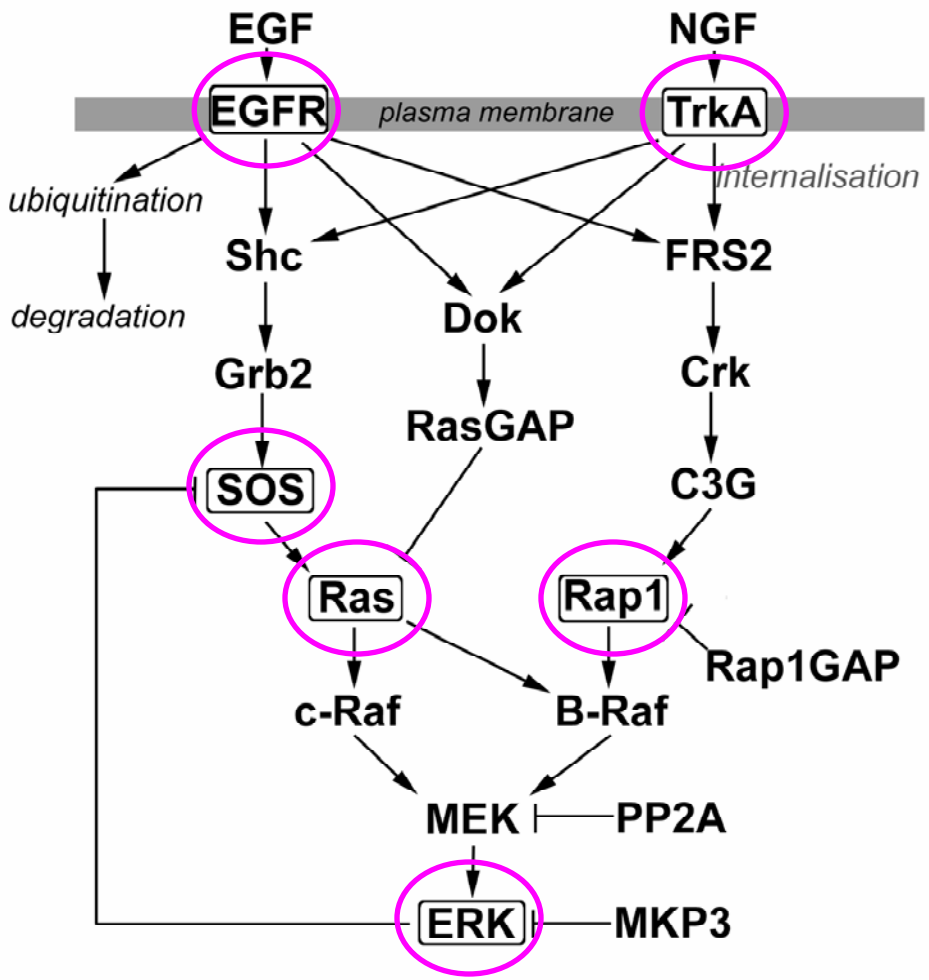
Temporal coding of ERK activation for growth and differentiation



Kinetic simulation model of ERK network

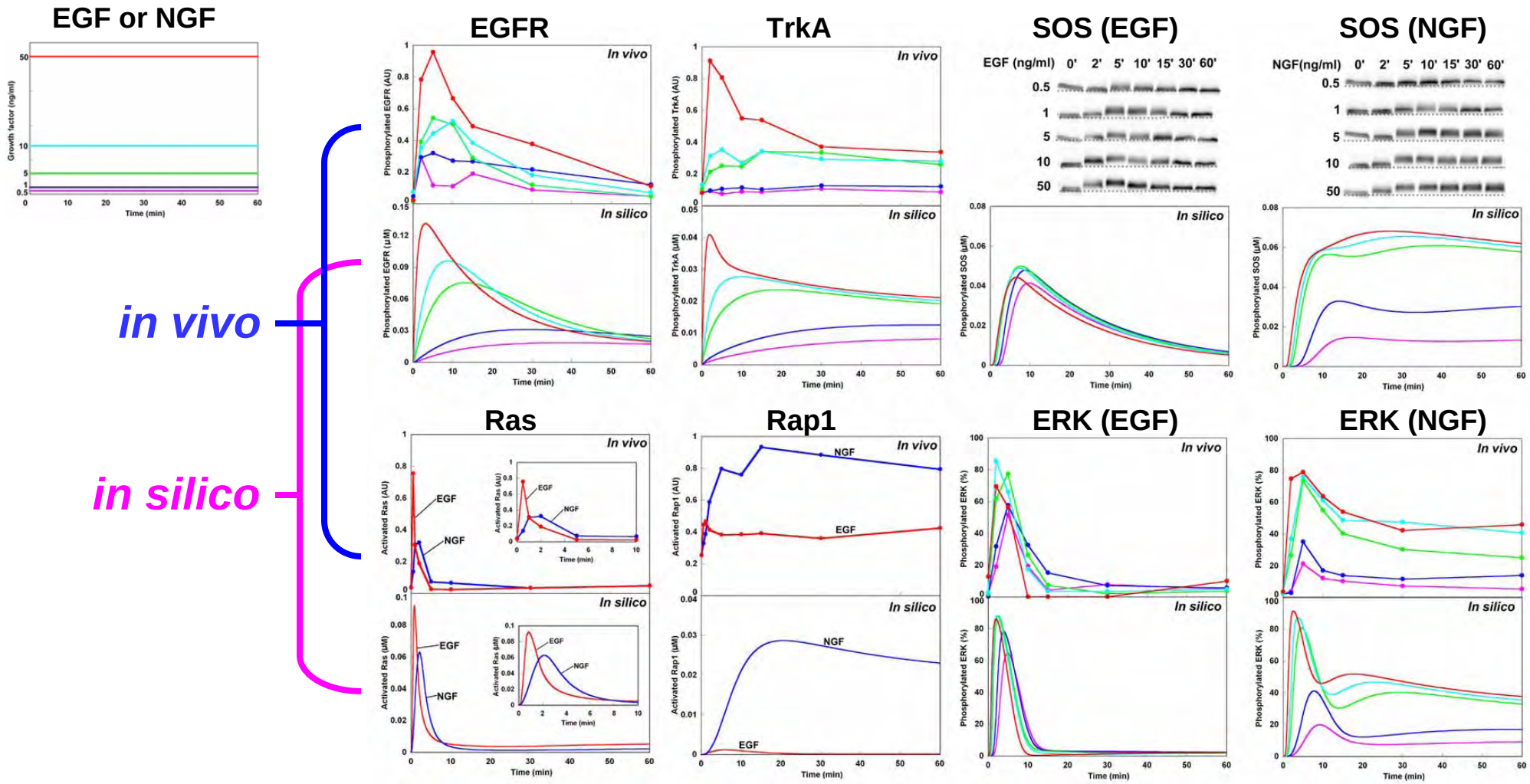
Screenshot of GENESIS/Kinetikit

$$\text{Ras} + \text{Raf} \underset{kb}{\overset{kf}{\rightleftharpoons}} \text{Ras} \cdot \text{Raf}$$



***In vivo* dynamics were measured.**

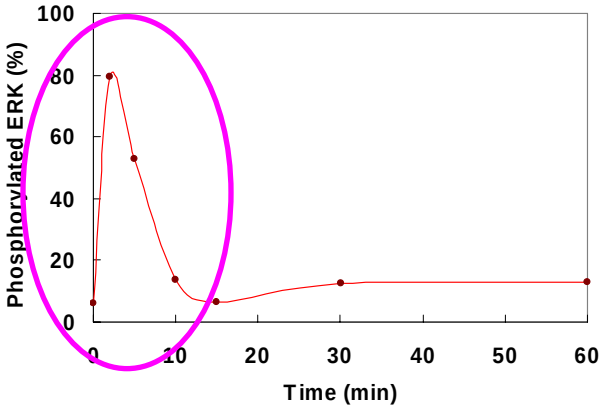
In vivo and in silico temporal dynamics of ERK signaling networks



Transient Ras and ERK activation

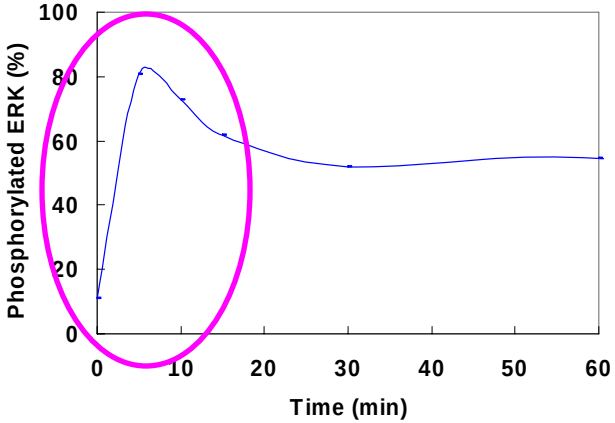
Transient ERK activation

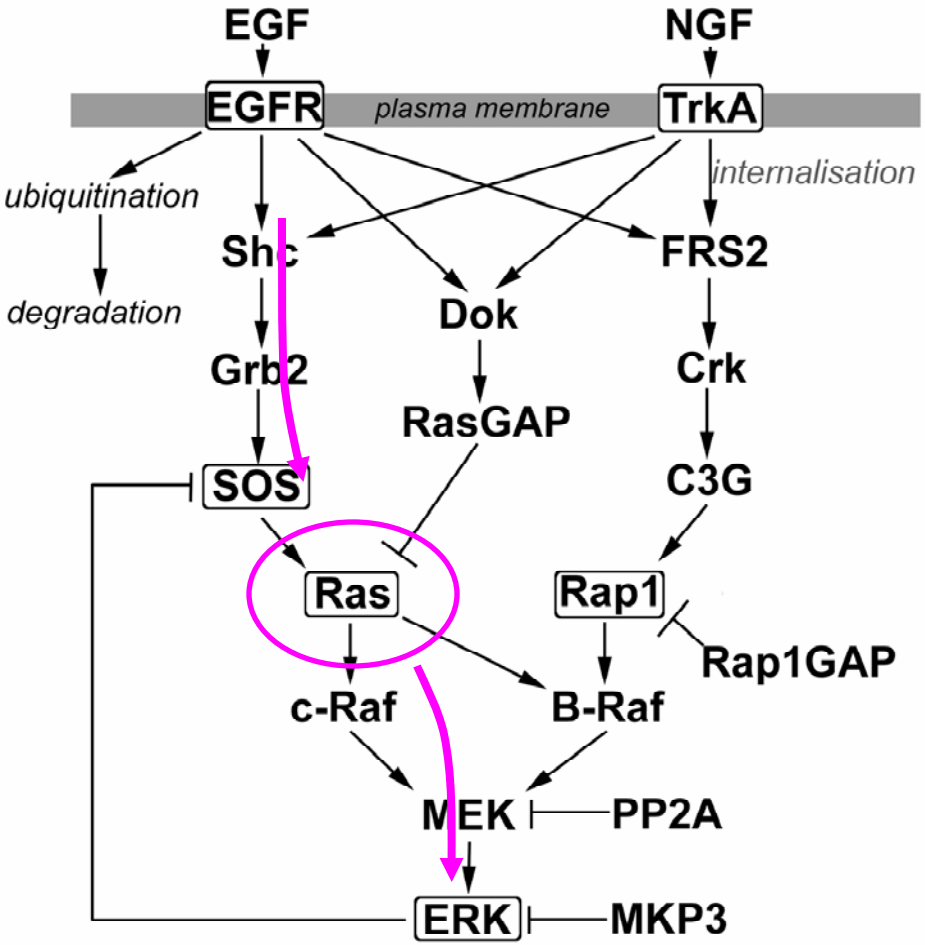
EGF



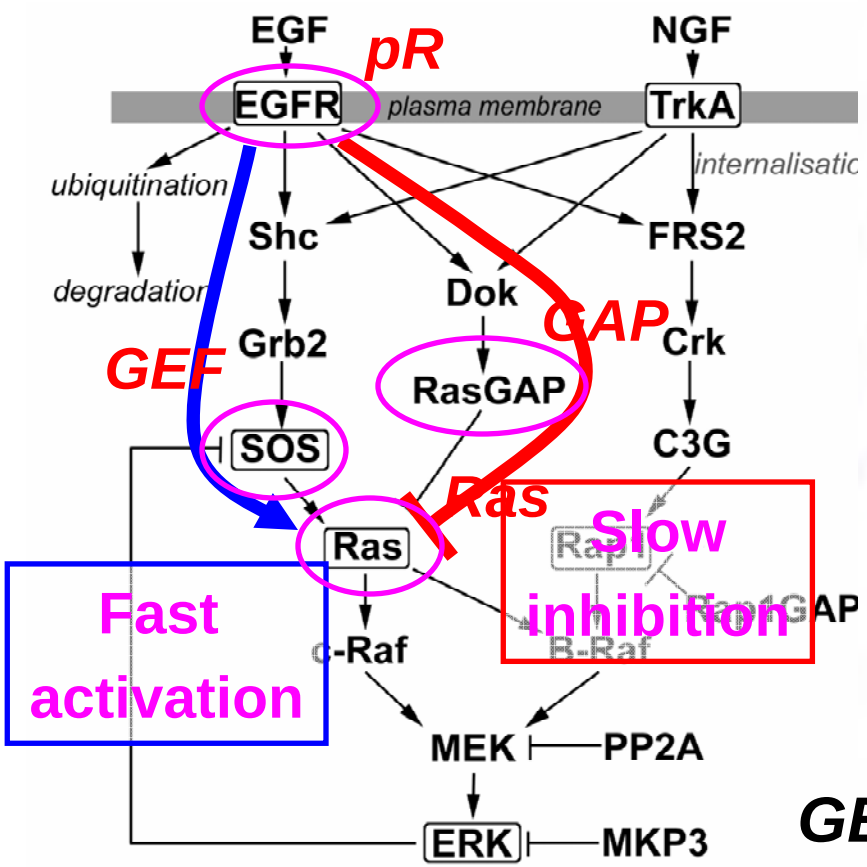
Sustained ERK activation

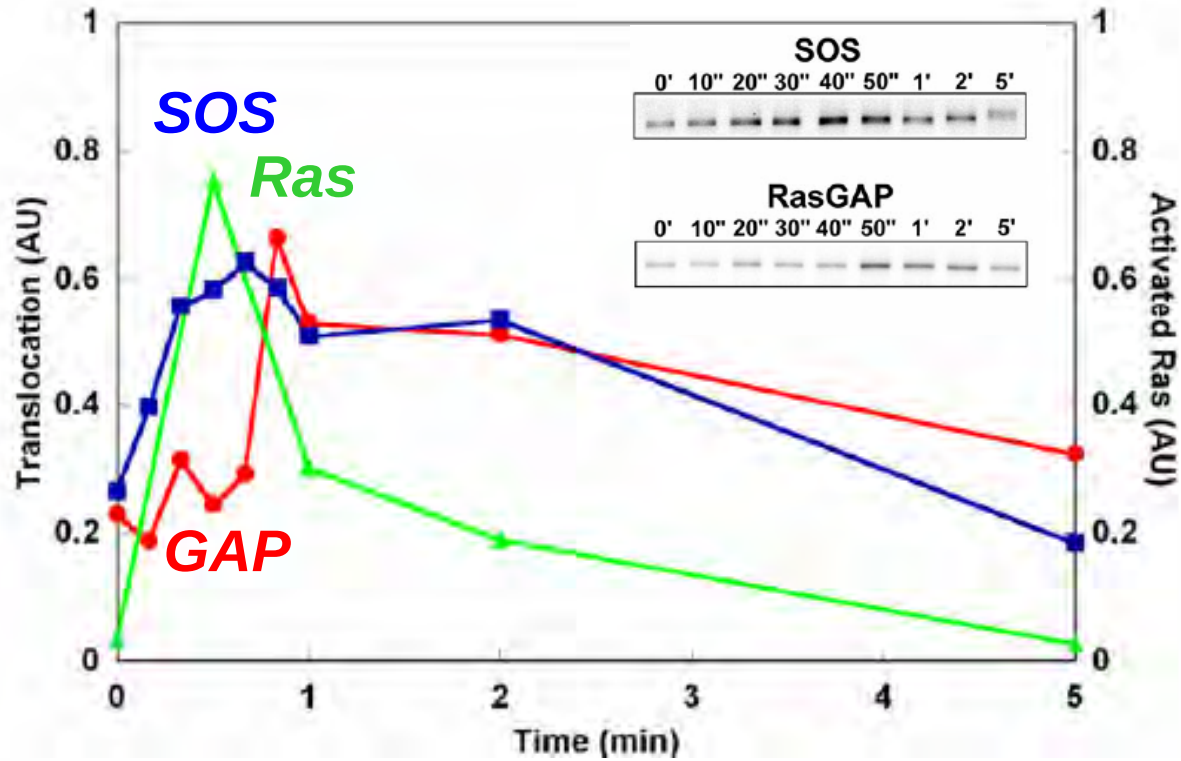
NGF





Transient Ras activation



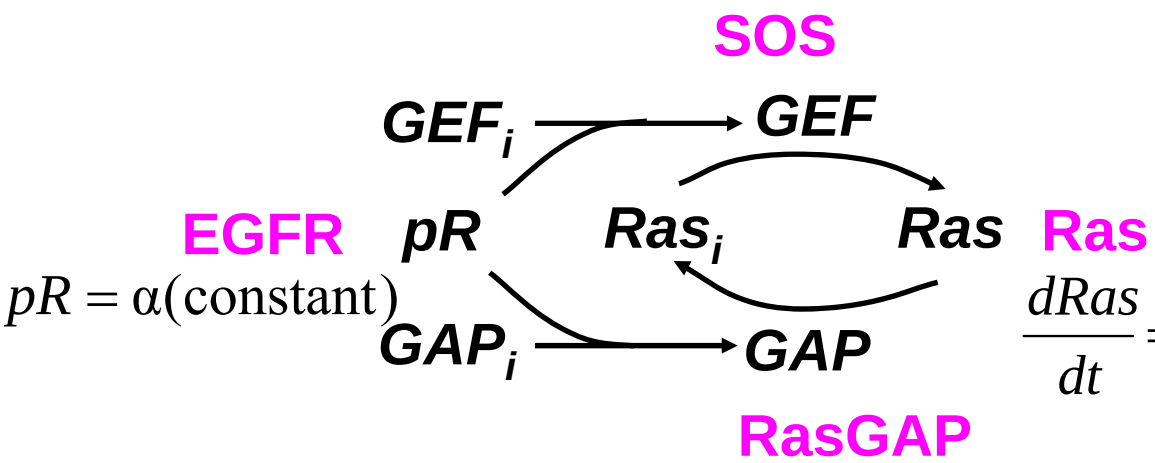


GEF: fast activation GAP: slow inhibition

Transient Ras activation

Simple Model of Ras

$$\frac{dGEF}{dt} = k_2\{pR - (1 + pR)GEF\}$$



$$pR = \alpha(\text{constant})$$

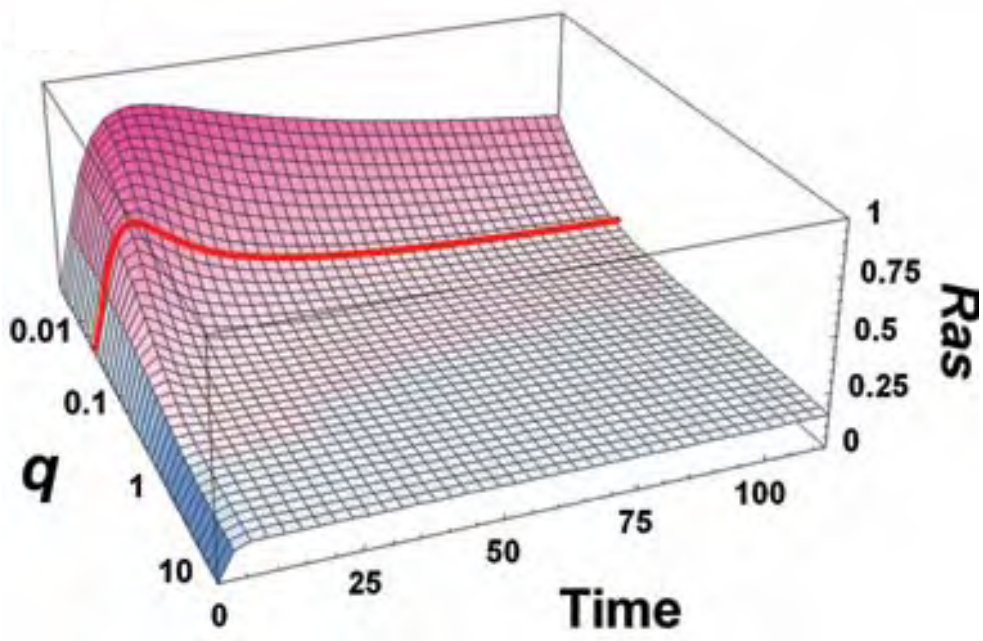
$$\frac{dRas}{dt} = k_6[\mathrm{GAP}_{\mathrm{total}}]\{\frac{GEF}{Ke} - (\frac{GEF}{Ke} + GAP)Ras\}$$

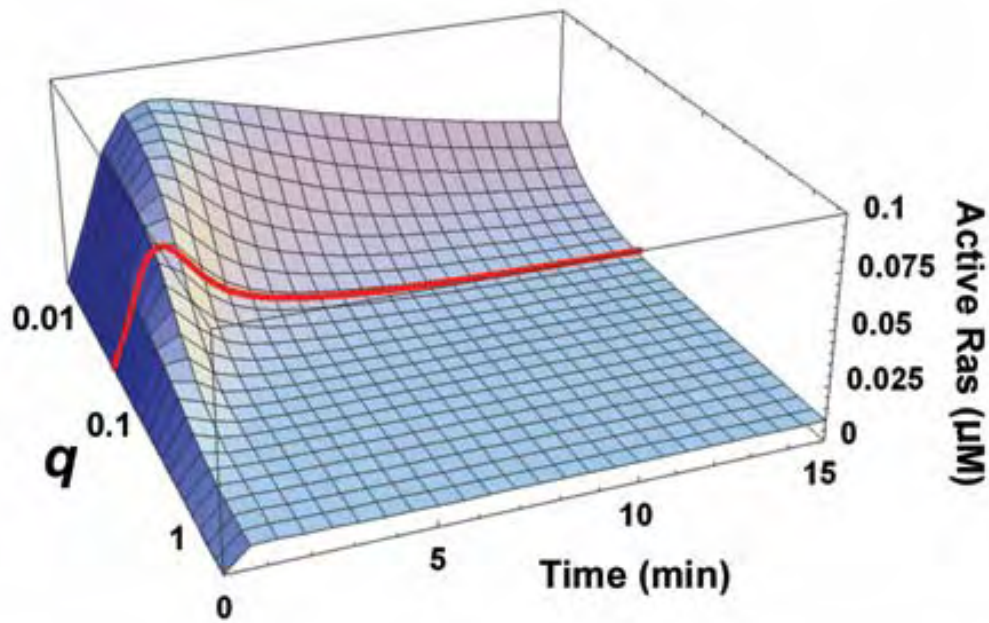
$$\frac{dGAP}{dt} = k_4\{p \cdot pR - (1 + p \cdot pR)GAP\}$$

The relative time constant of GAP to GEF $1/q = k_4/k_2$

$q < 1$, GEF is faster; $q > 1$, GAP is faster

Simple Model of Ras





Simple Ras model

q < 1: GEF is faster

Transient Ras activation ○

q > 1 : GAP is faster

Transient Ras activation ×

In silico model

q < 1: GEF is faster

Transient Ras activation ○

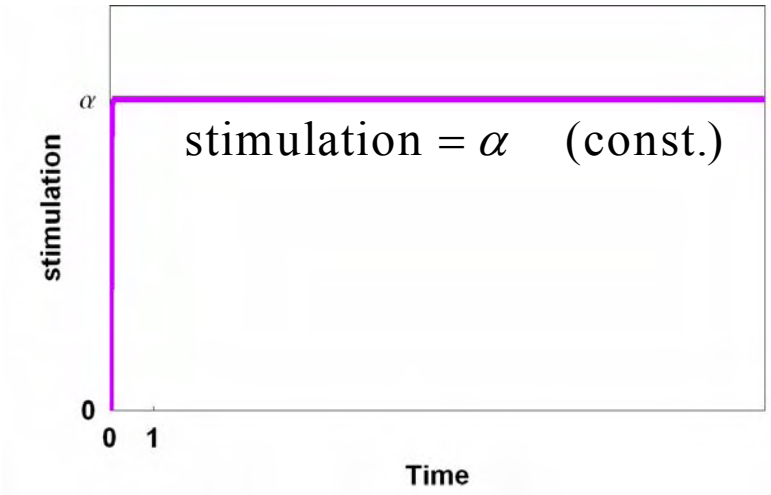
q > 1 : GAP is faster

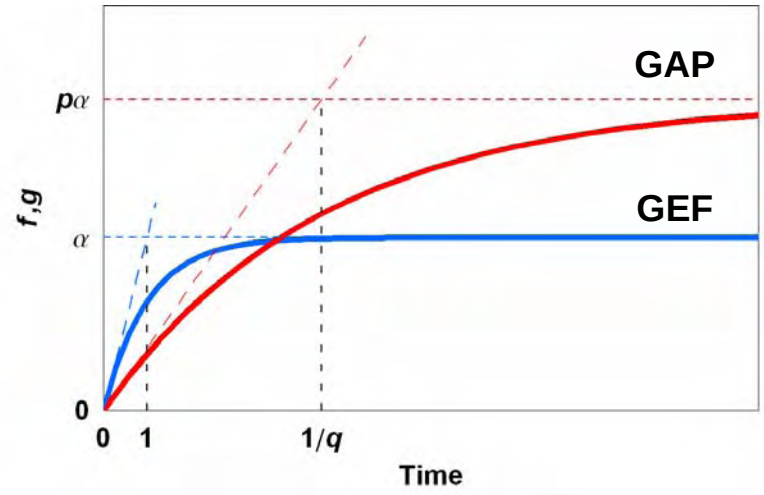
Transient Ras activation ×

Ramp stimulation

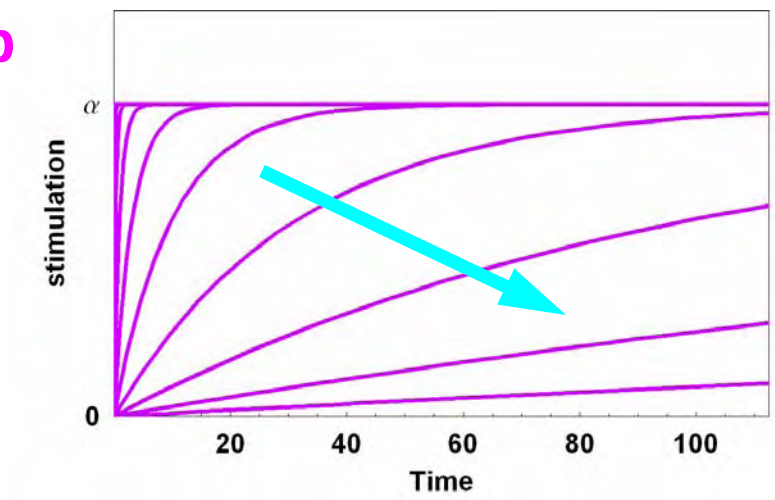
- How to change the relative time constant, q, in vivo-

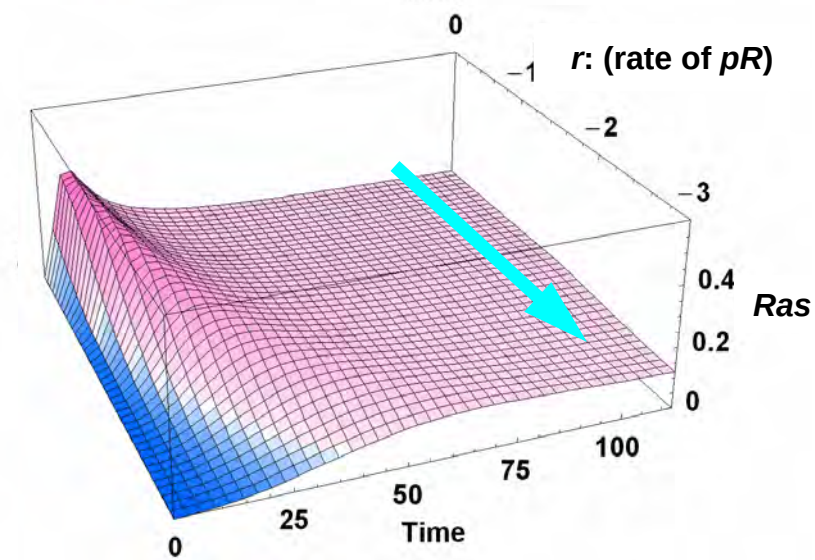
Step





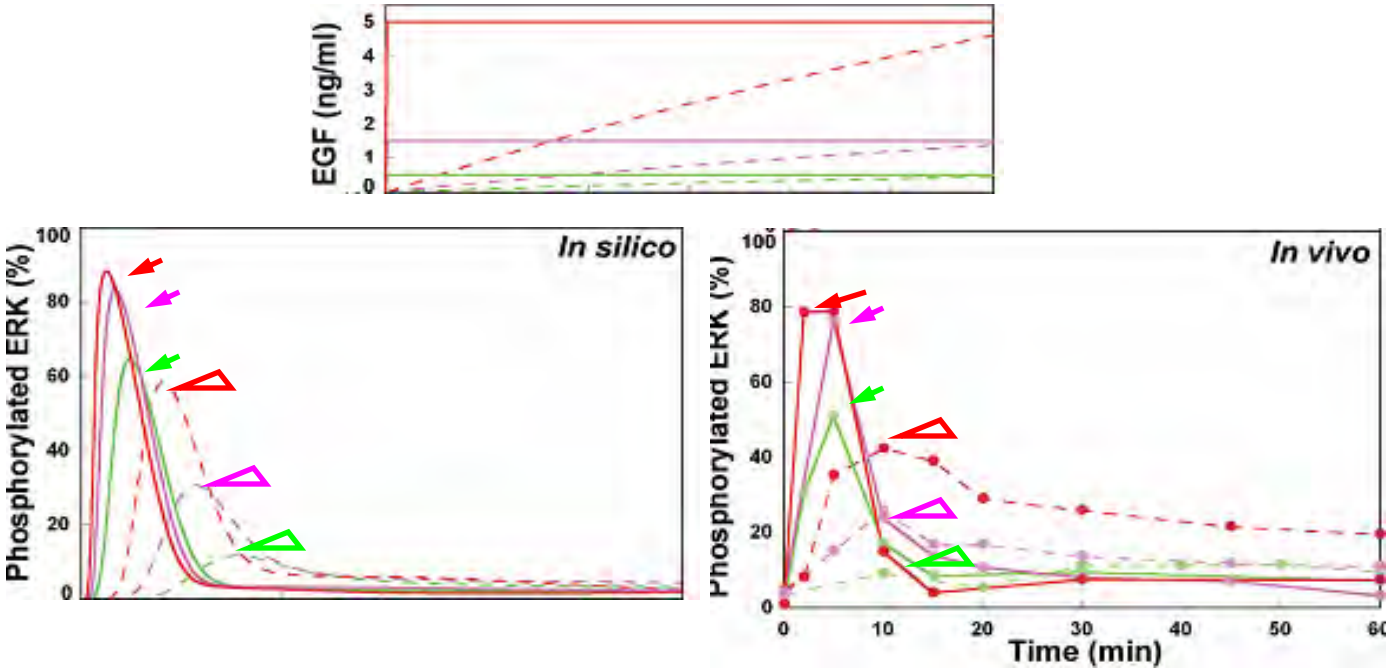
Ramp





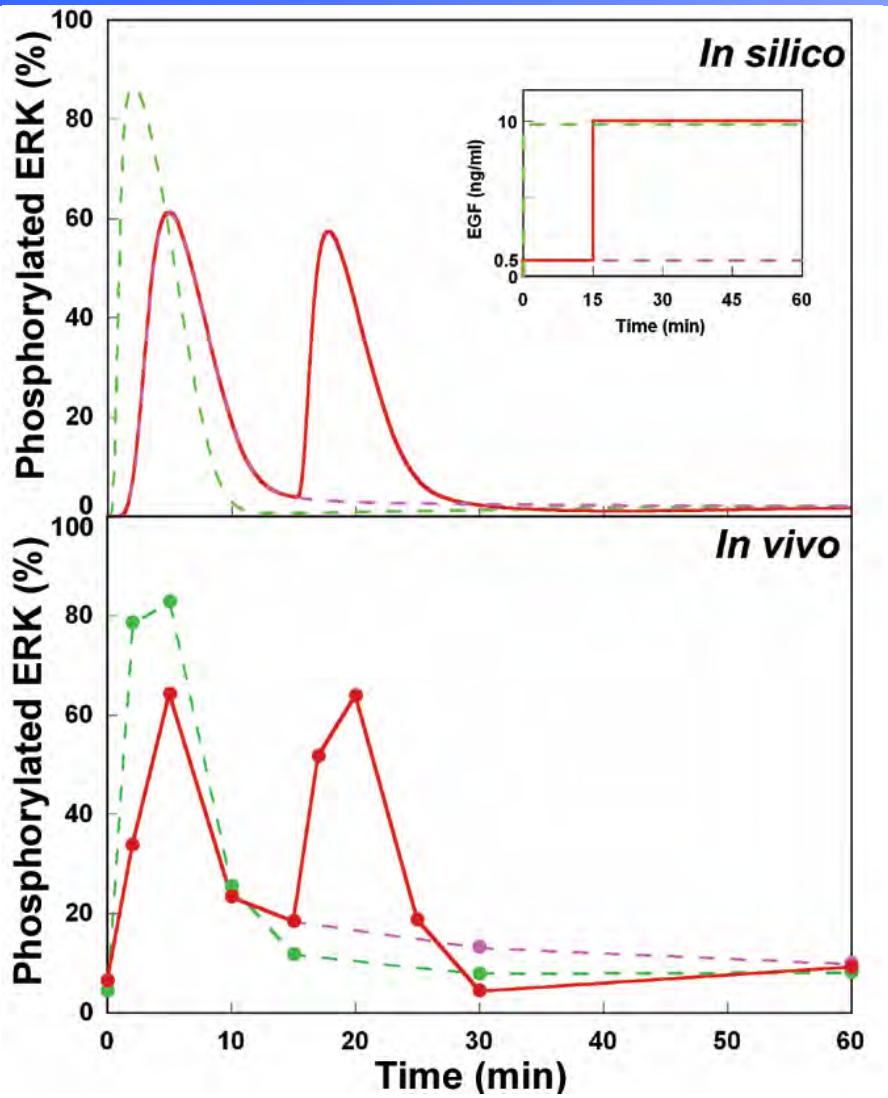
ERK dynamics respond to ramp stimuli

EGF stimulation



Ras system specifically captures temporal rate of EGF.

Stepwise increase of EGF in vivo and in silico



Rapid temporal rate

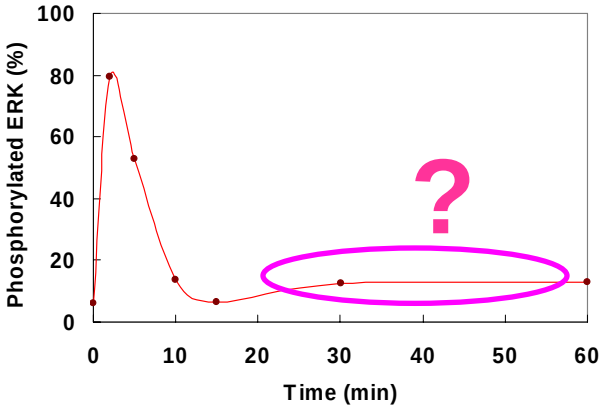
Difference in concentration

~~Threshold or absolute concentration~~

Dynamics of ERK networks

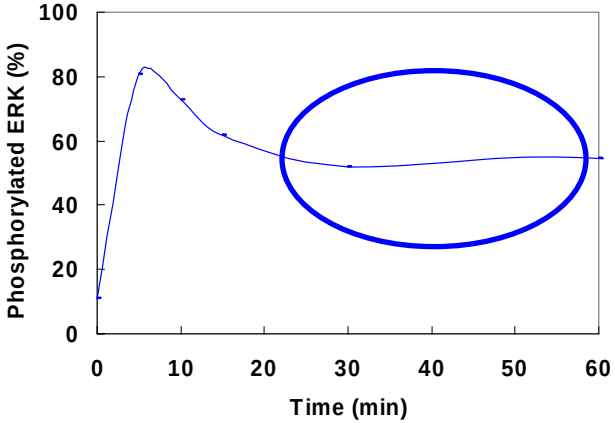
EGF

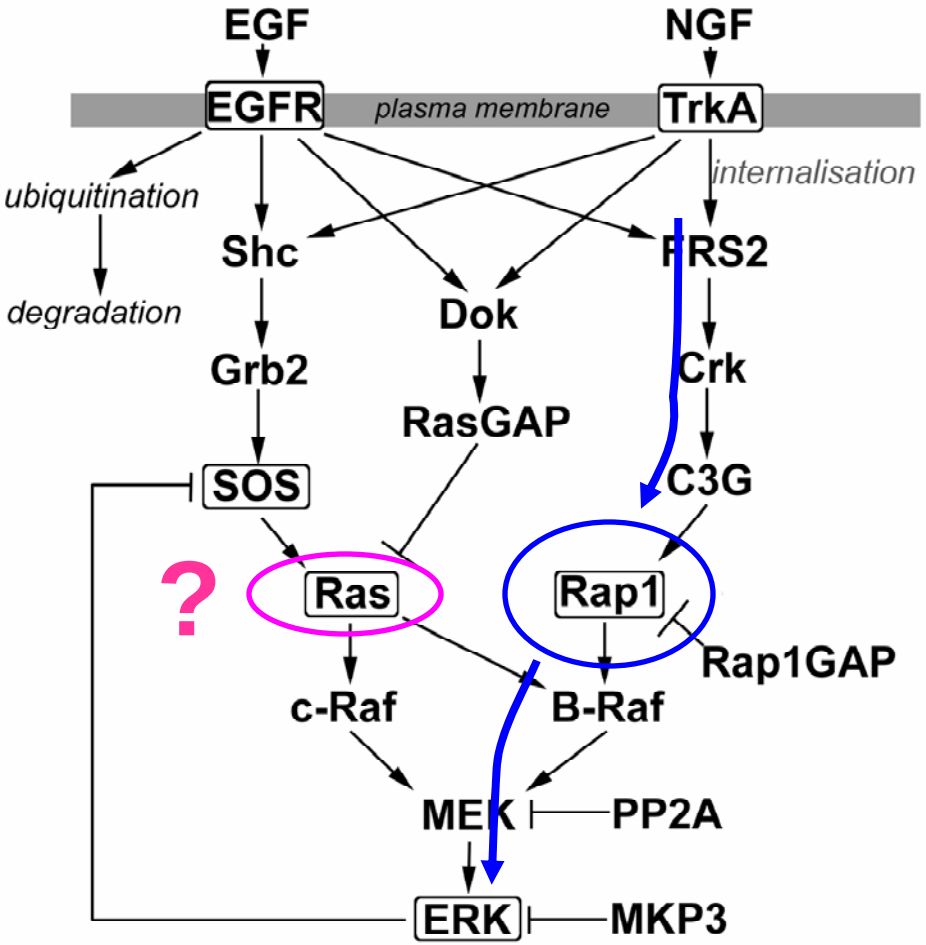
Transient ERK activation



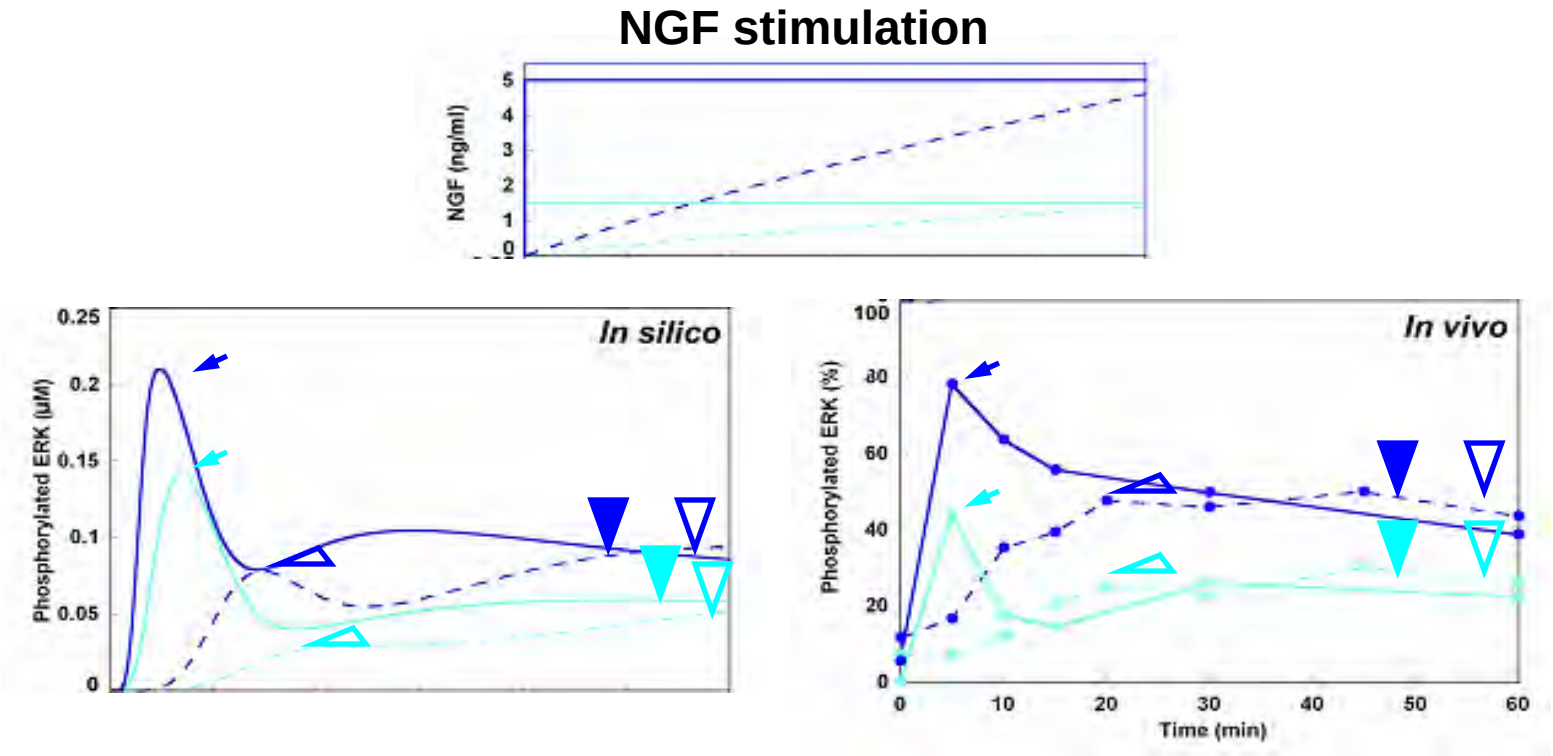
NGF

Sustained ERK activation



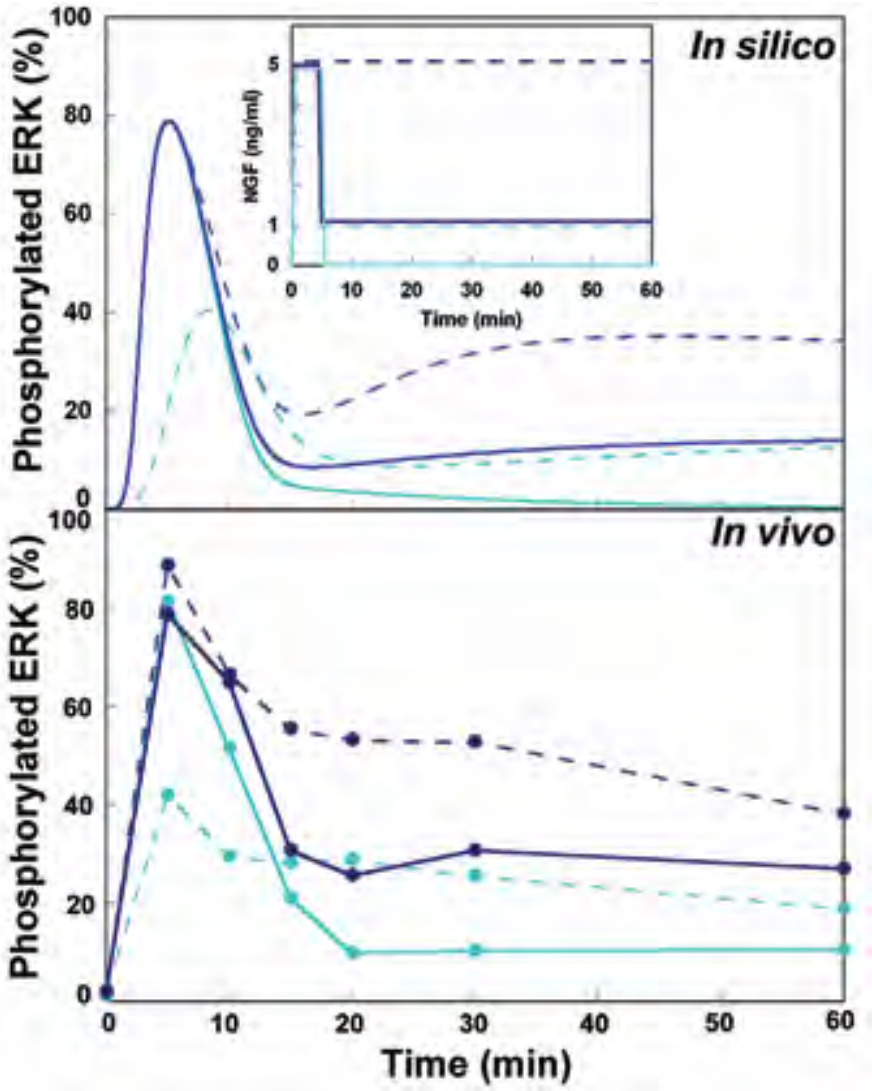


ERK dynamics respond to ramp stimuli



Sustained ERK activation → final concentration of NGF

Stepwise decrease of NGF in vivo and in silico



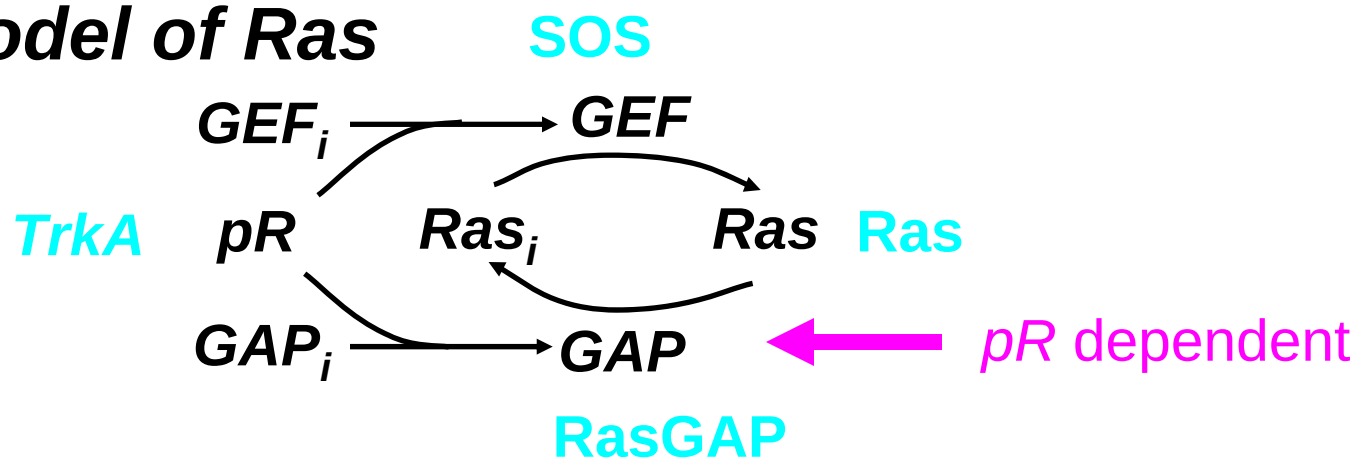
Final concentration

~~**Initial concentration**~~

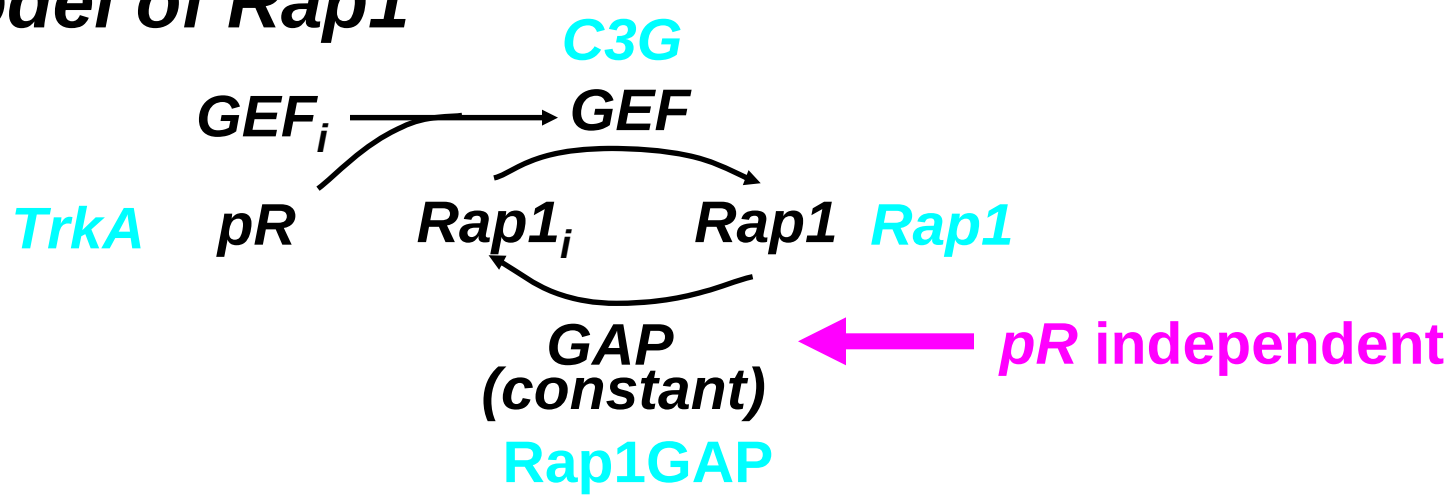
~~**Temporal rate**~~

Simple Model of Rap1 and Ras

Simple Model of Ras



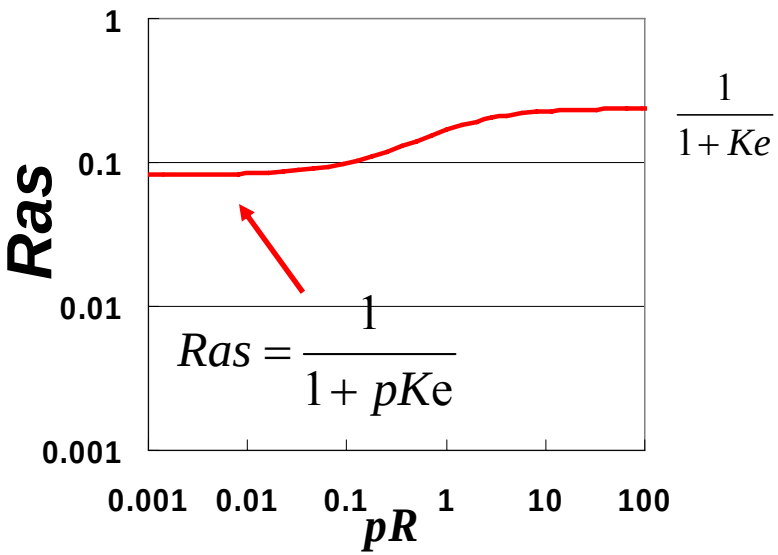
Simple Model of Rap1



Ras and Rap1 at steady state

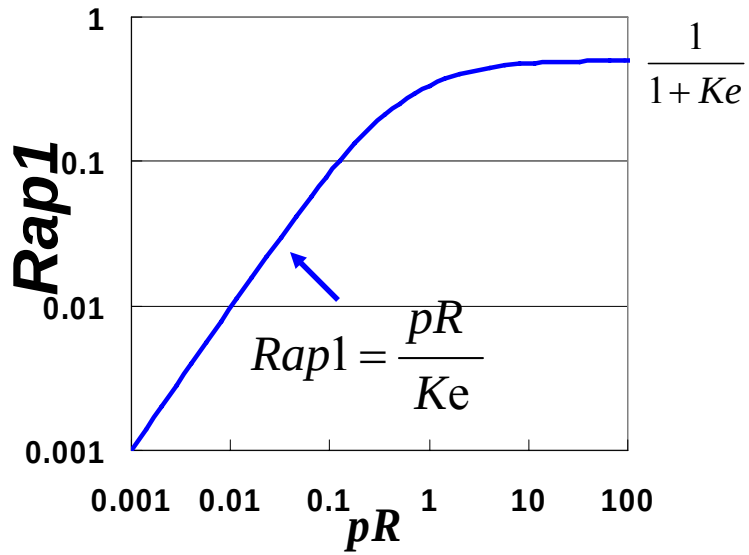
Ras

$$Ras = \frac{1}{1 + pKe\dfrac{1 + pR}{1 + p \cdot pR}}$$



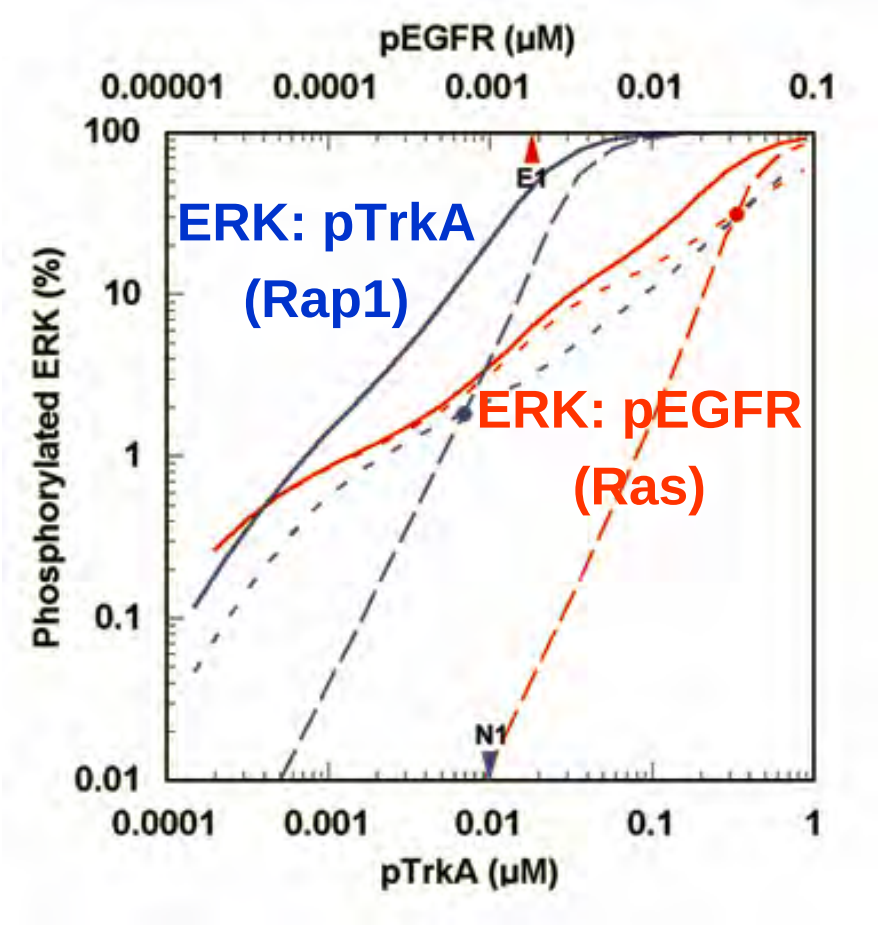
Rap1

$$Rap1 = \frac{pR}{(1 + Ke)\,pR + Ke}$$

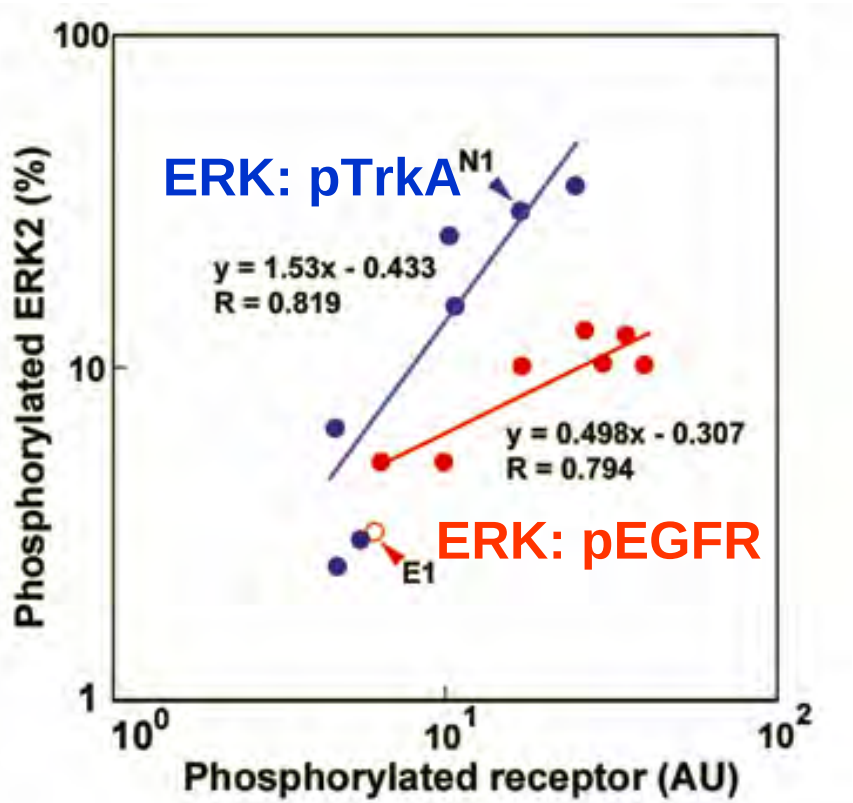


Sustained ERK activation against EGFR or TrkA in silico and in vivo

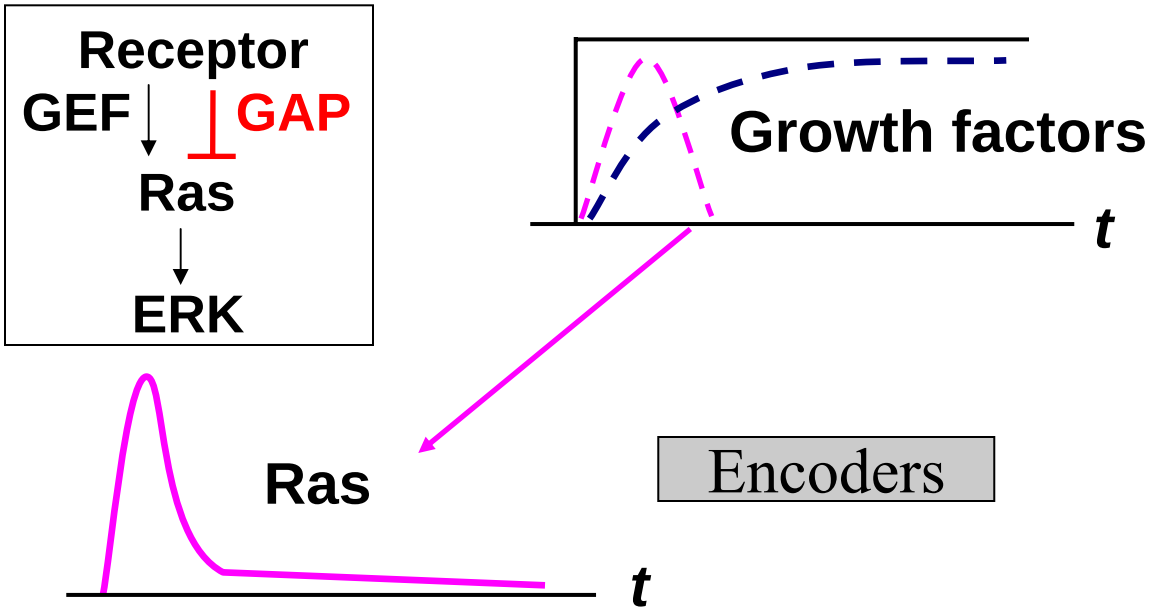
in silico



In vivo

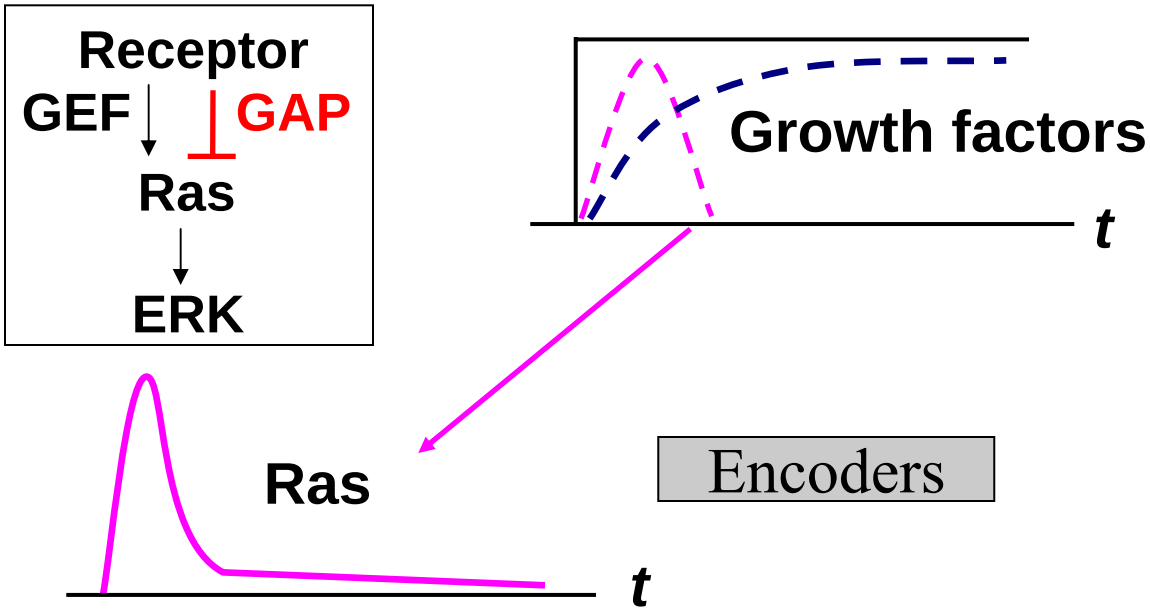


Distinct features of the Ras and Rap1 systems



Sasagawa, S. et al, Nat. Cell Biol. 2005, 7 (4), 365-373

Distinct features of the Ras and Rap1 systems

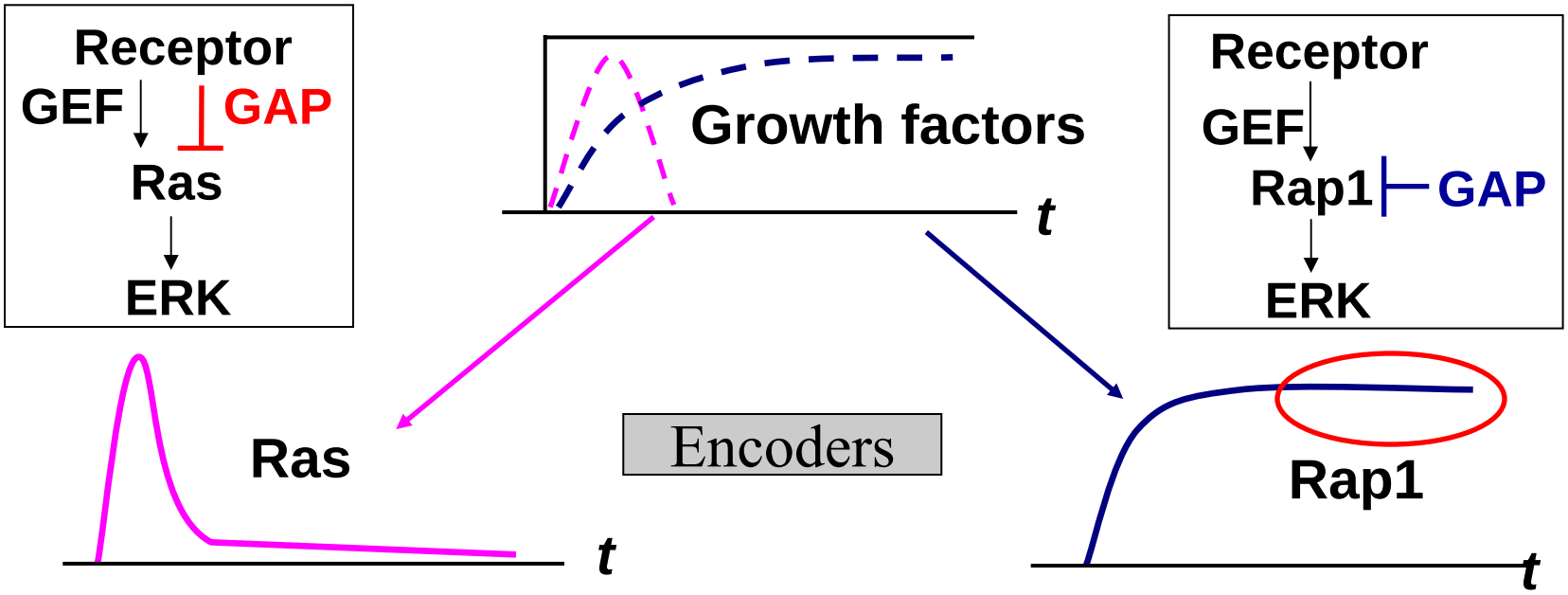


1. Transient peak: Rapid increase of GF

Rapid temporal patterns

Sasagawa, S. et al, Nat. Cell Biol. 2005, 7 (4), 365-373

Distinct features of the Ras and Rap1 systems



1. Transient peak: Rapid increase of GF

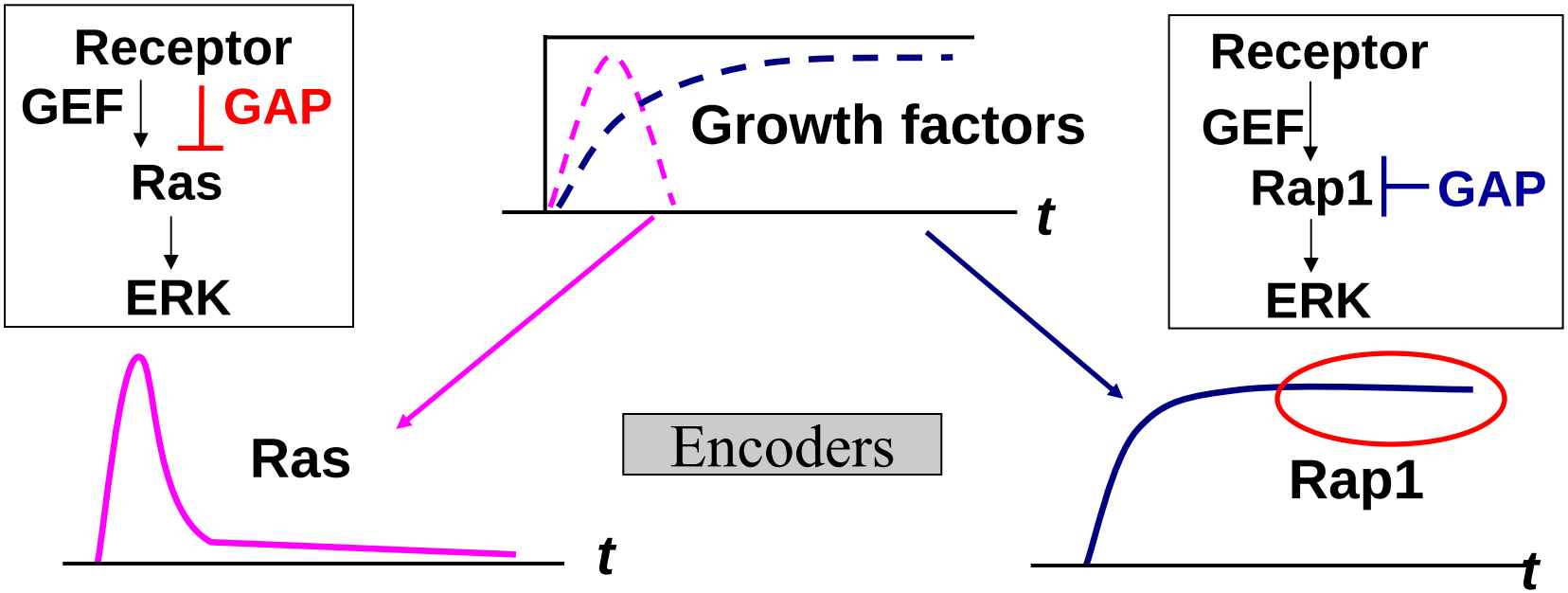
Rapid temporal patterns

2. Sustained activation

Independent from GF

Sasagawa, S. et al, Nat. Cell Biol. 2005, 7 (4), 365-373

Distinct features of the Ras and Rap1 systems

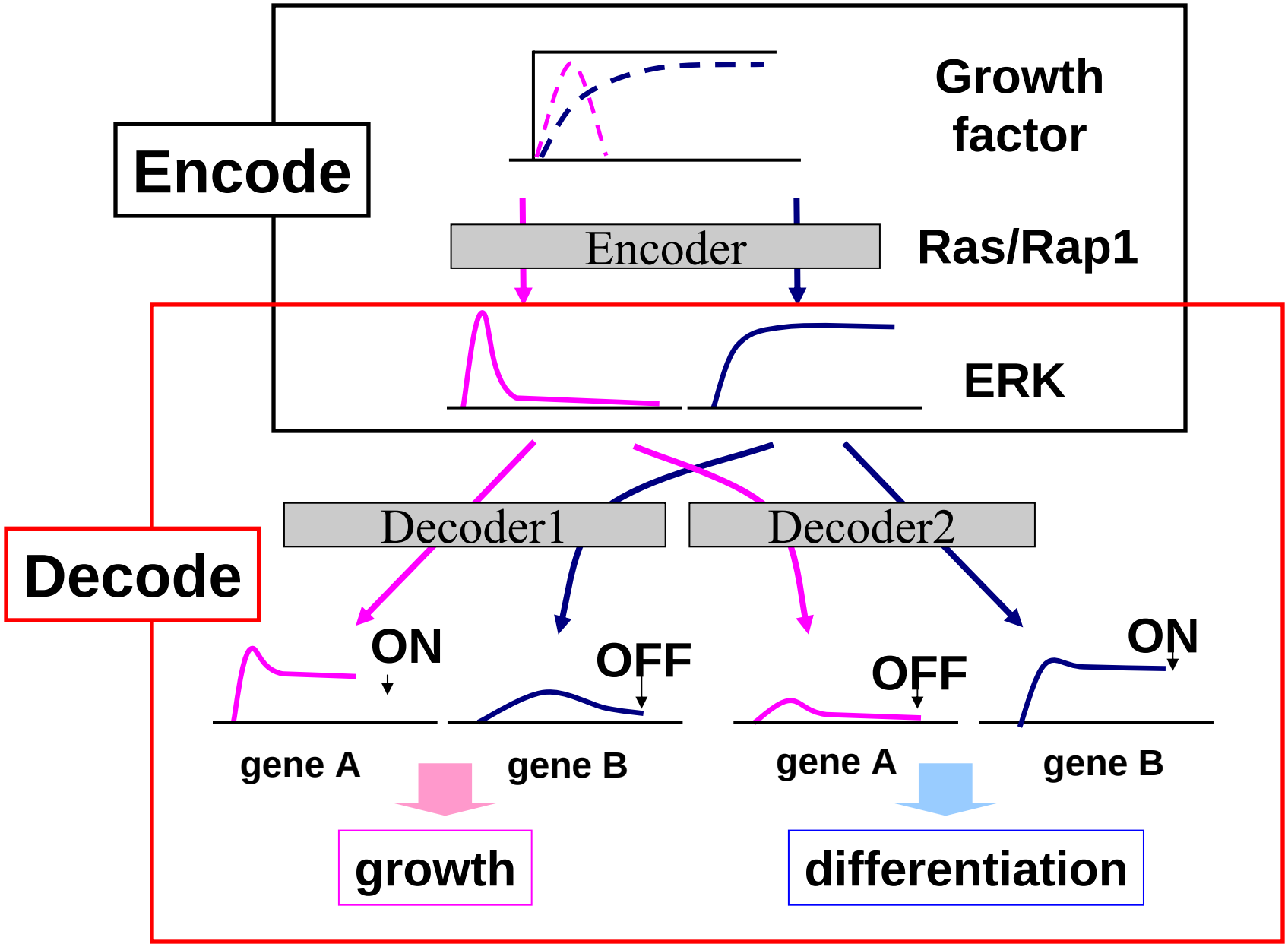


1. Transient peak: Rapid increase of GF
   Rapid temporal patterns
2. Sustained activation
   Independent from GF

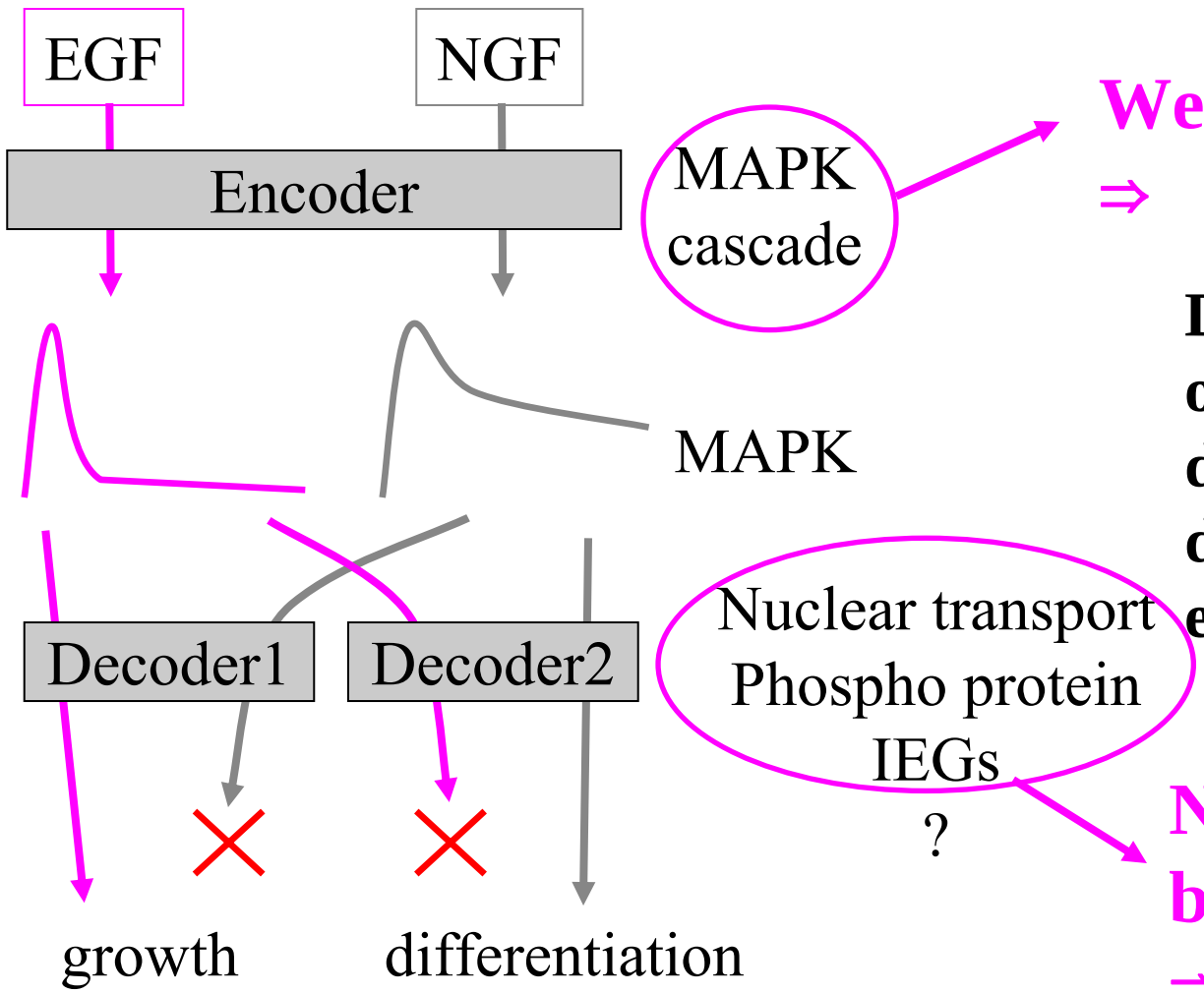
1. Sustained activation
   Final amplitude of GF
   Slow temporal patterns

Sasagawa, S. et al, Nat. Cell Biol. 2005, 7 (4), 365-373

Temporal coding of ERK signaling networks



Decoding of ERK signaling network



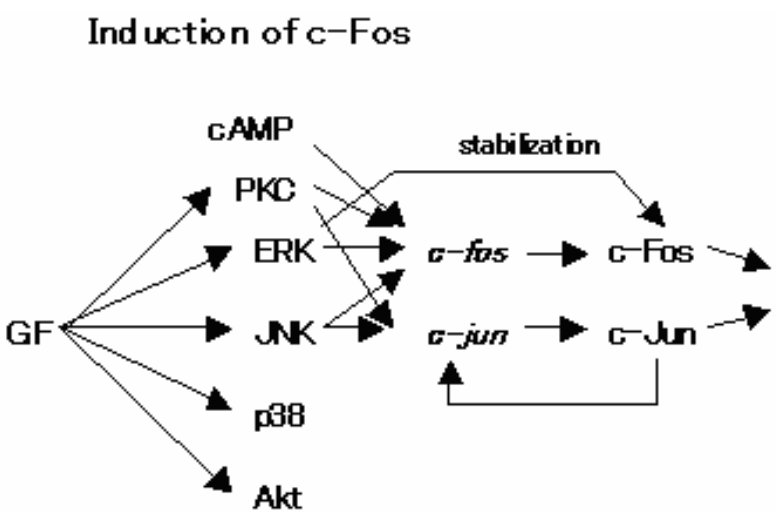
Well-defined network
⇒ Differential equation

Distinct temporal patterns of ERK should be differently decoded by downstream gene expression

Network is unknown, but they are related!
⇒ Statistical model

Statistical model of downstream of ERK

Temporal patterns tell the interactions of molecules

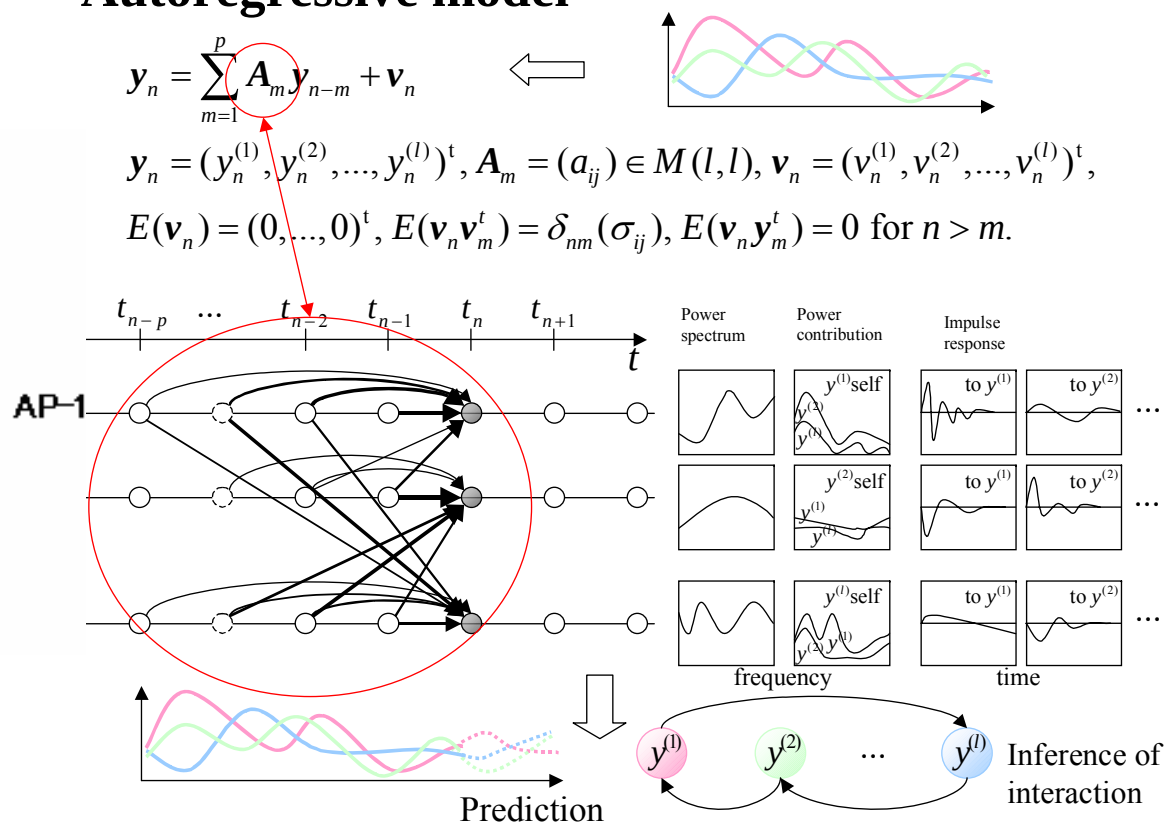


Autoregressive model

$$\boldsymbol{y}_n = \sum_{m=1}^{p} \boldsymbol{A}_m \boldsymbol{y}_{n-m} + \boldsymbol{v}_n$$

$$\boldsymbol{y}_n = (y_n^{(1)}, y_n^{(2)}, \ldots, y_n^{(l)})^{\mathrm{t}},\ \boldsymbol{A}_m = (a_{ij}) \in M(l,l),\ \boldsymbol{v}_n = (v_n^{(1)}, v_n^{(2)}, \ldots, v_n^{(l)})^{\mathrm{t}},$$

$$E(\boldsymbol{v}_n) = (0, \ldots, 0)^{\mathrm{t}},\ E(\boldsymbol{v}_n \boldsymbol{v}_m^t) = \delta_{nm}(\sigma_{ij}),\ E(\boldsymbol{v}_n \boldsymbol{y}_m^t) = 0 \text{ for } n > m.$$



Temporal patterns requires high throughput measurements

Minimal requirement of time points for AR model

0min ~ 120min 4 min interval	×	4 Signaling molecules	×	GF dose x3	×	n=3

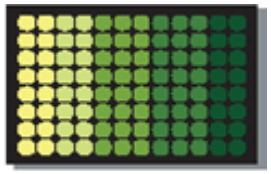
= 1116 points !

Automation by Robots + multiple immunostaining
= 96well X 6 plates

Automatic incubator

Cell culture
Stimulation
immunostaining

BiomekNX



HTP analysis

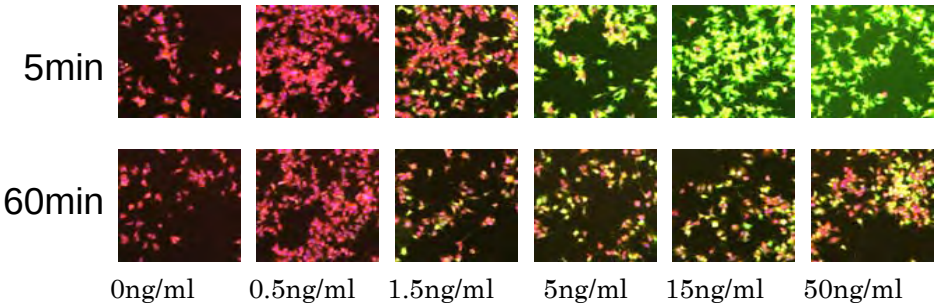
cellWoRx

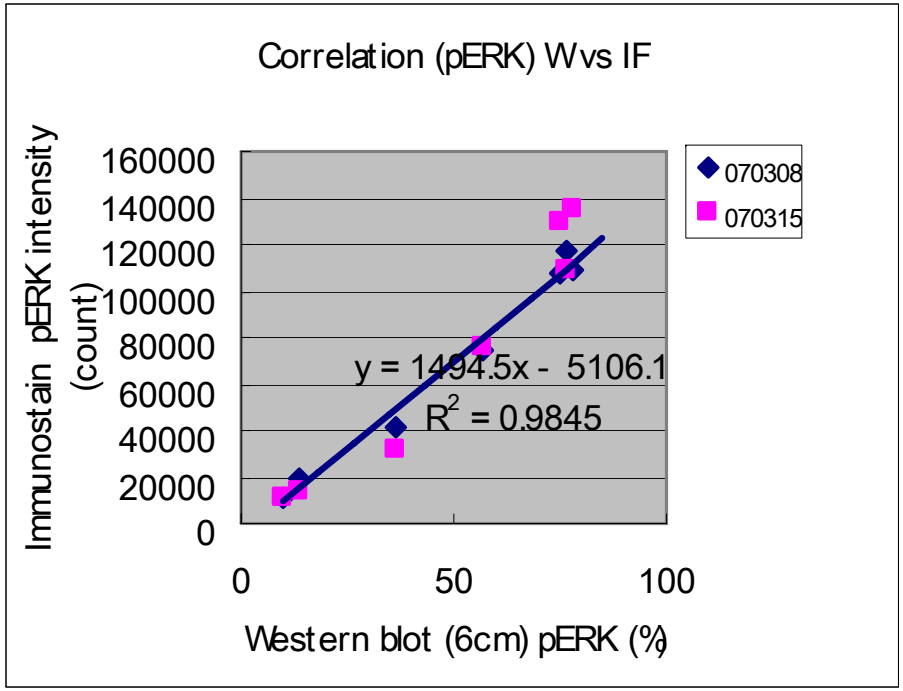
Quantitative measurement of pERK by immunostaining

NGF-induced ERK phosphorylation

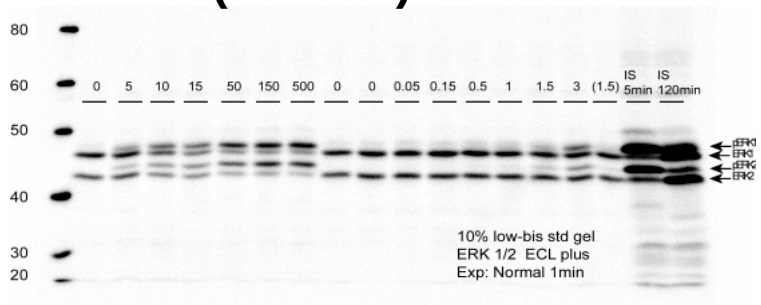
Immunostaining (Automatic)

Green, pERK Orange, c-Jun Red, cell





Western blot (manual)



High correlation between WB and IS
→ Quantitative measurement

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Yoko Shimao