Dimerization Effects in MAPK cascade

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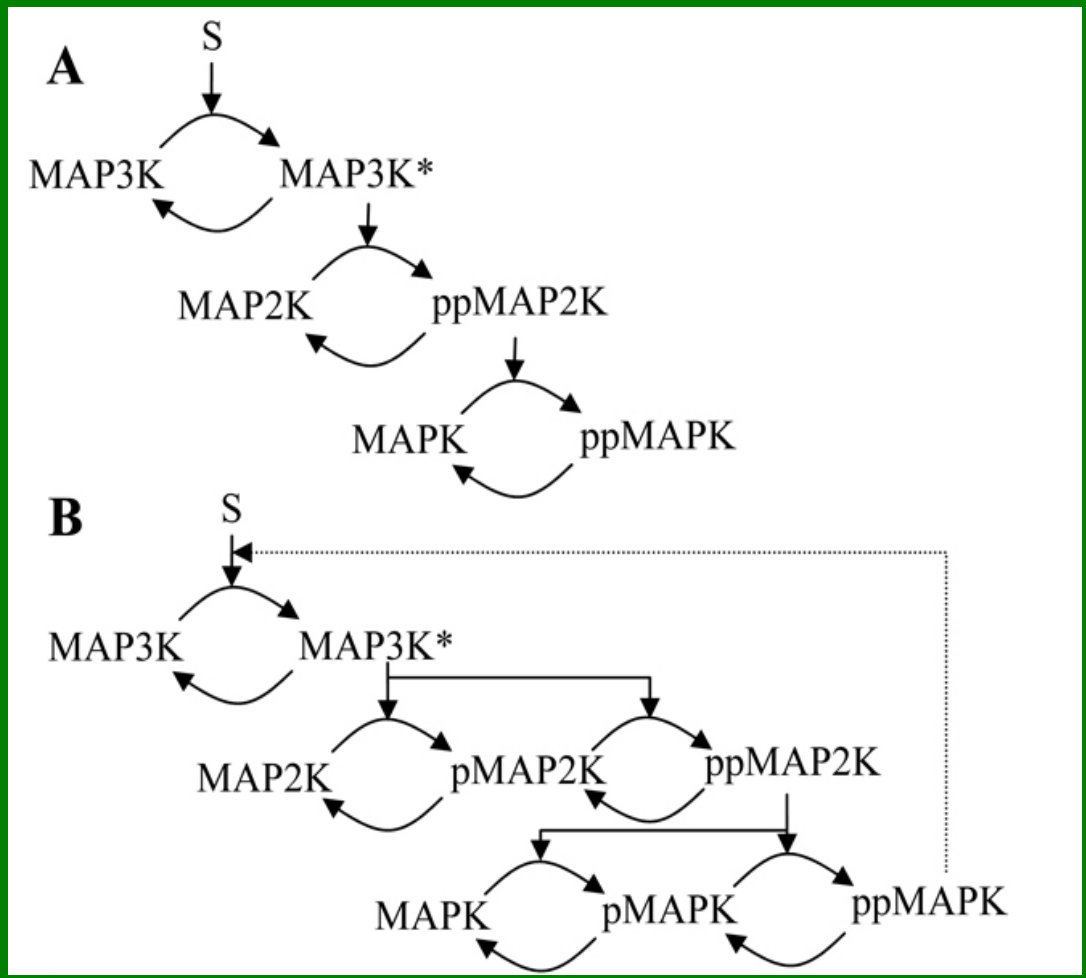
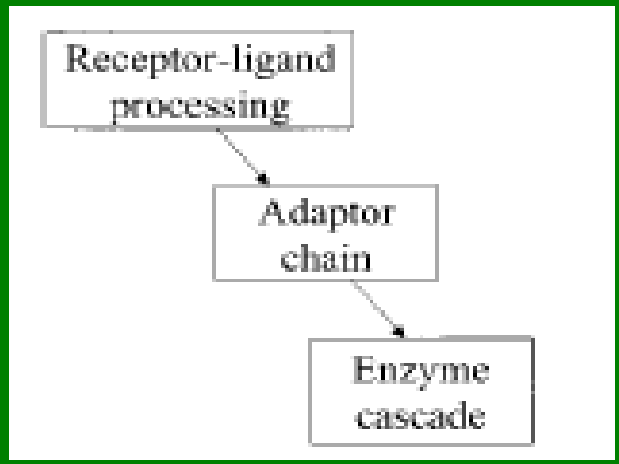
MAPK Core Architecture

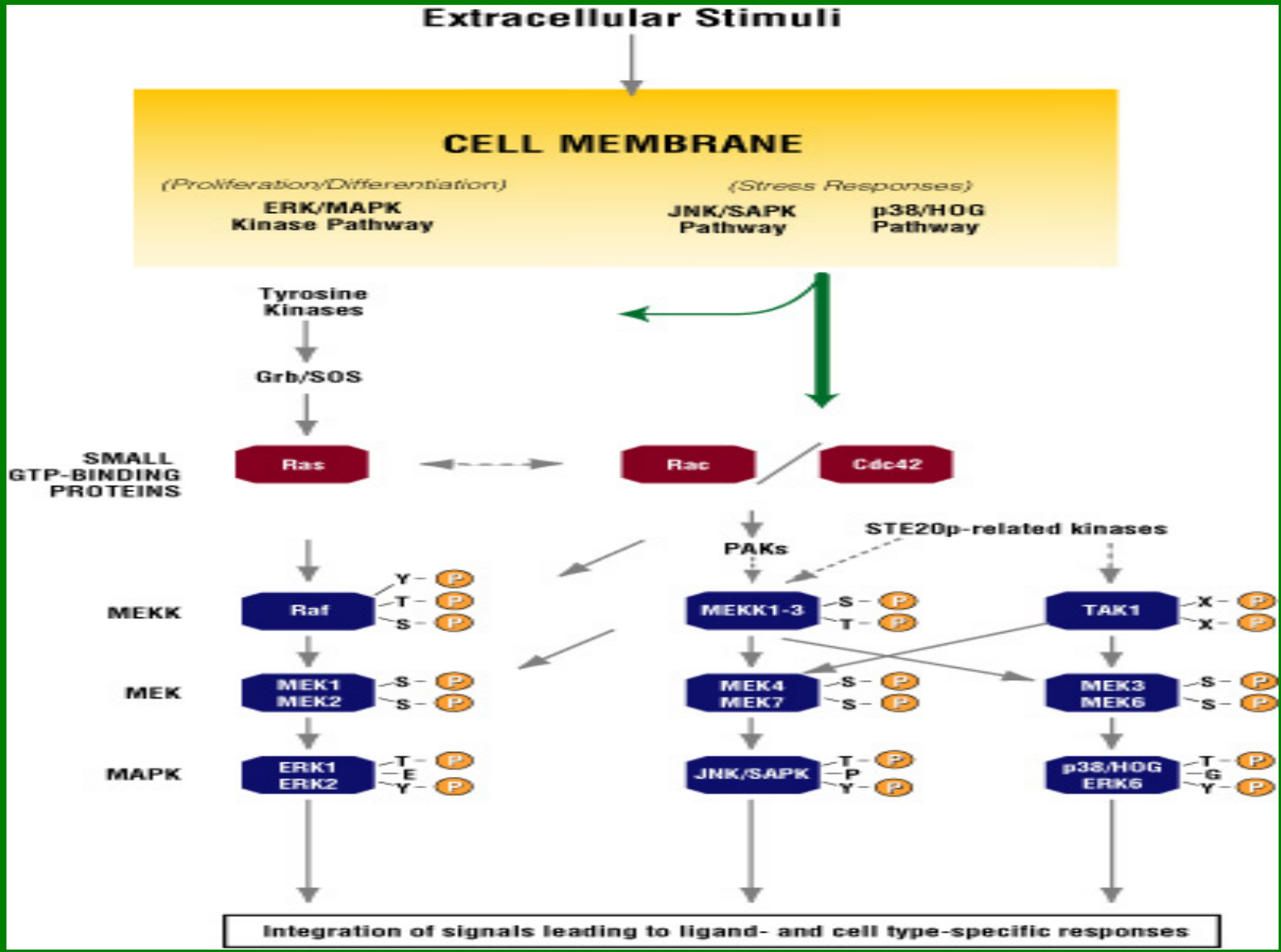




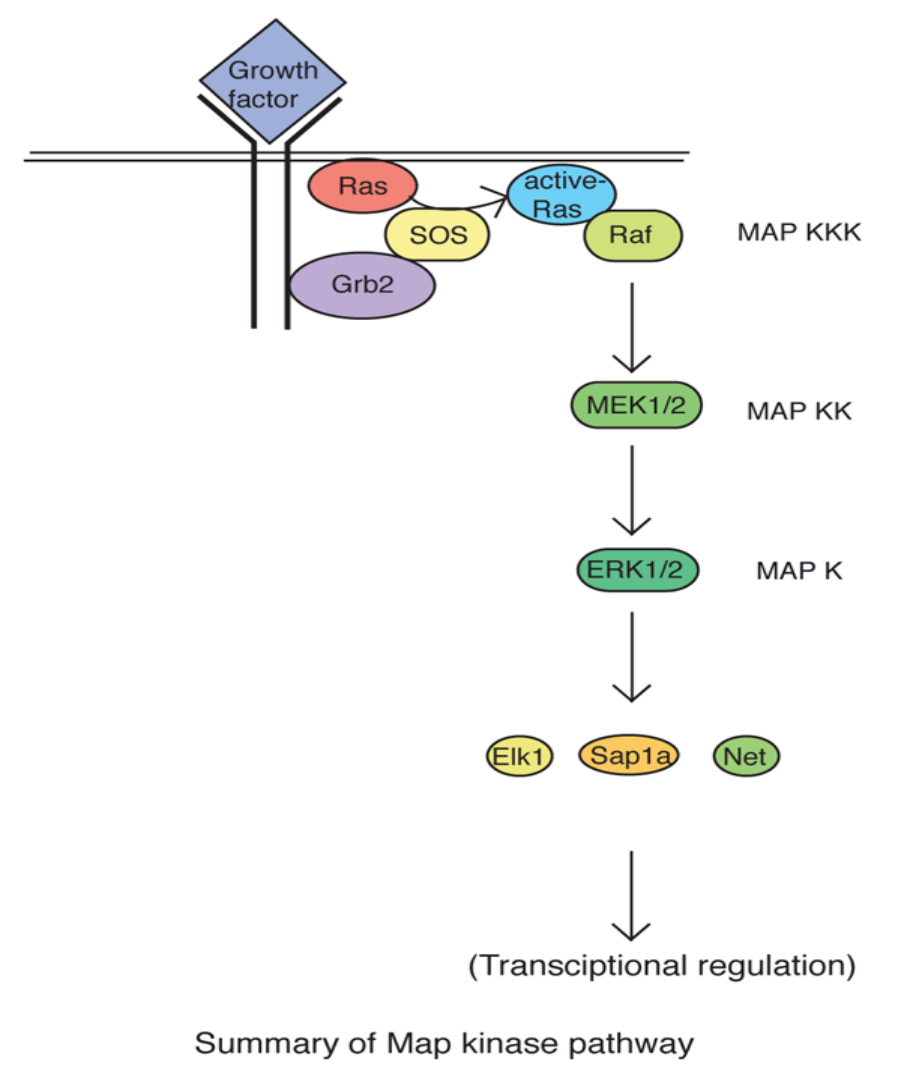
Function

- Responsible for transducing signals induced by
  - ERK1/2 – mitogens (growth factors)
  - JNK/p38 - heat shock, UV, osmotic stress
  - ERK 5 – responsible for cardiovascular development

MAPK Cascade



The Mechanism of Transduction



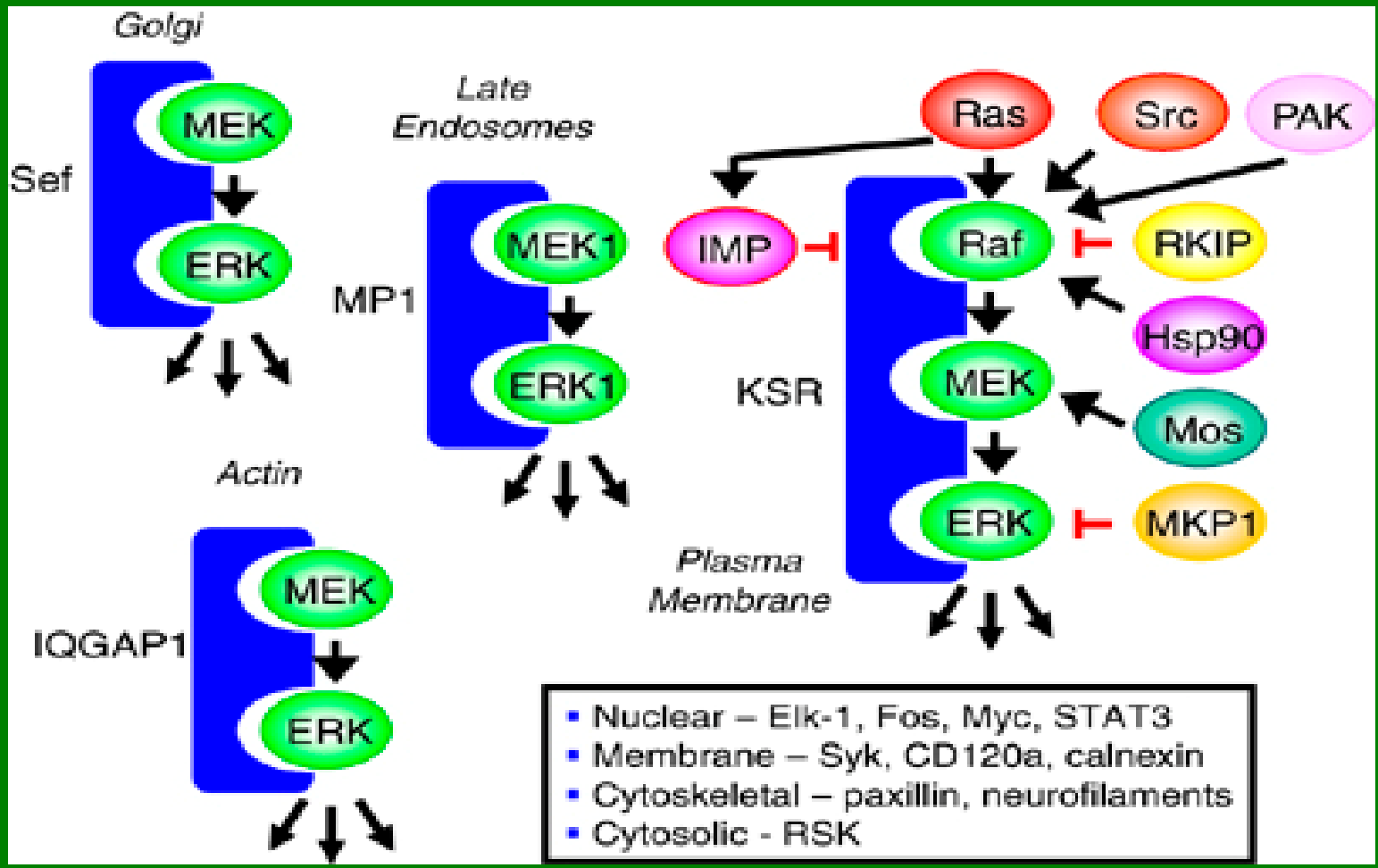
Summary of Map kinase pathway

MAPKKK:
A-Raf, B-Raf, C-Raf

MAPKK:
MEK1/2

MAPK:
ERK1/2

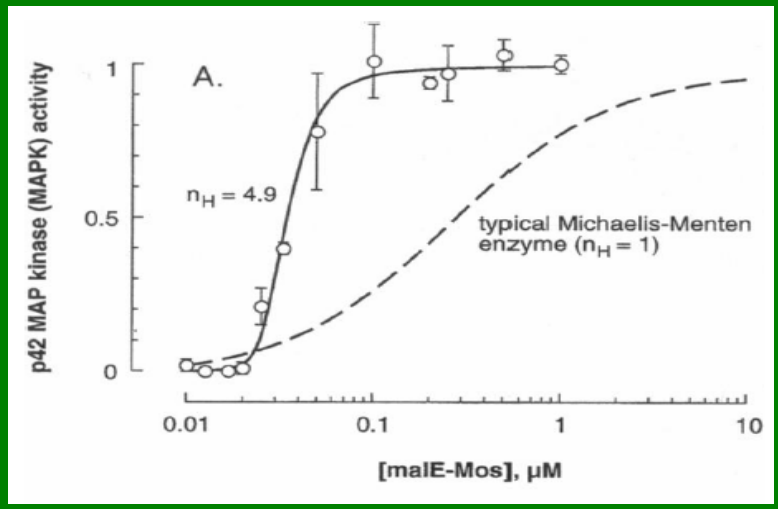
Scaffolds in MAPK Signalling



Key Dynamics I

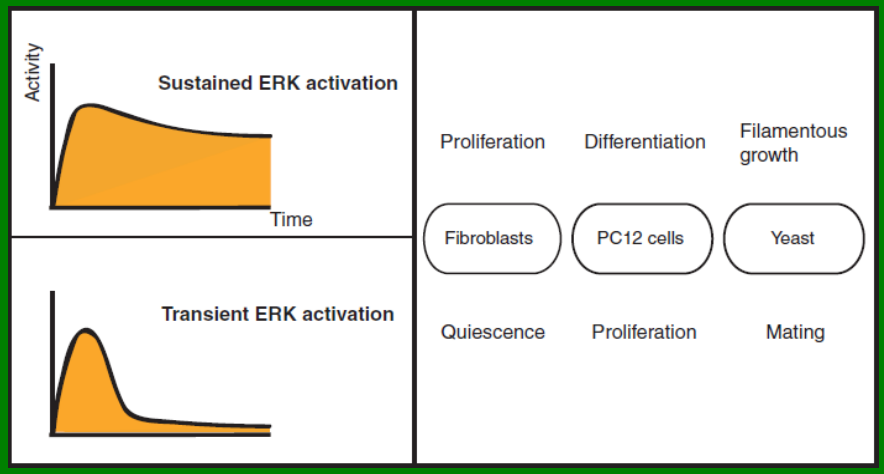
1) Ultrasensitivity ('non-linearity'):

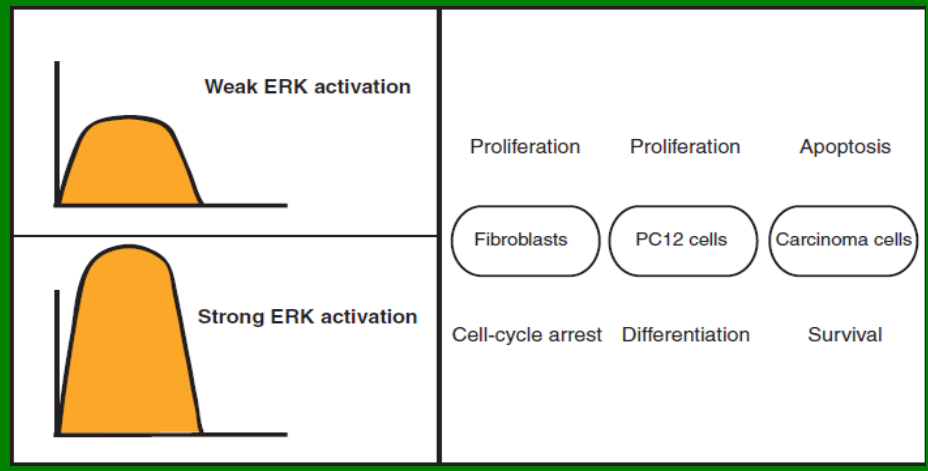
graded input -> "digital response"

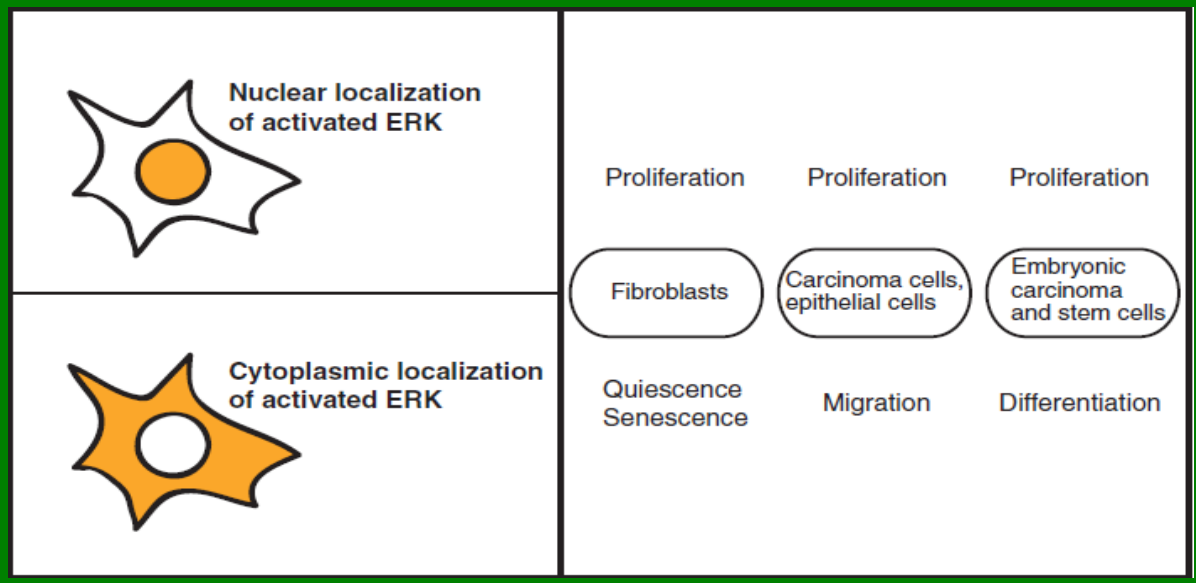


2) Transient vs. sustained response (negative feedback)

Output/Effect Relationship







ERK1/2 Cascade

- MAPKKK – A-Raf, B-Raf, C-Raf
- MAPKK – MEK1/2
- MAPK – ERK1/2
- Intensively investigated because of its involvement in cancer

Involvement in Human Disease

1) Involvement in Malignancies: RAS mutations in 15% of cancers, ERK upregulated in 30%

2) B-Raf Mutatations:

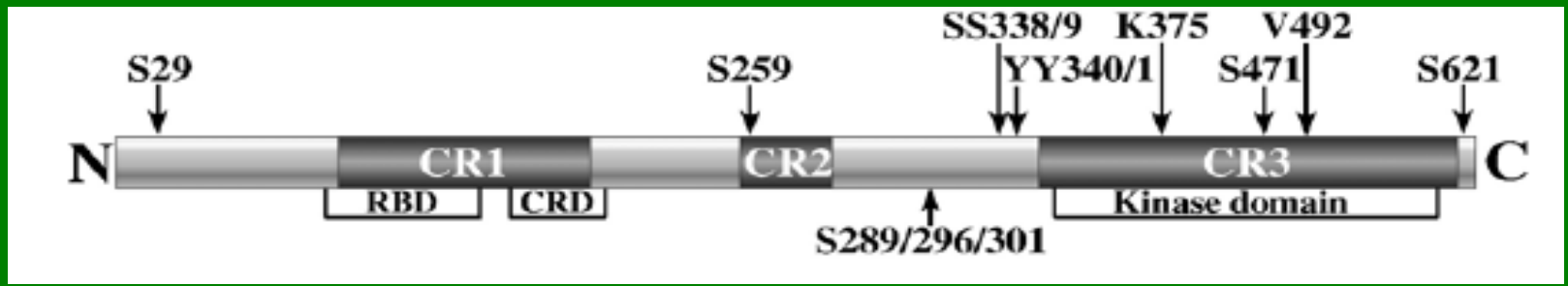
- melanoma (30–60%), thyroid cancer (30- 50%)
- colorectal cancer (5–20%) and
- ovarian cancer (~30%)
- others (1–3%)

Publication Statistics

1) RAS – 40154/5734
2) RAF – 8515/962
3) MEK – 7435/353
4) Erk – 20087/927
5) KRS – 153/7
6) IMP- 51/7

RAF Regulation

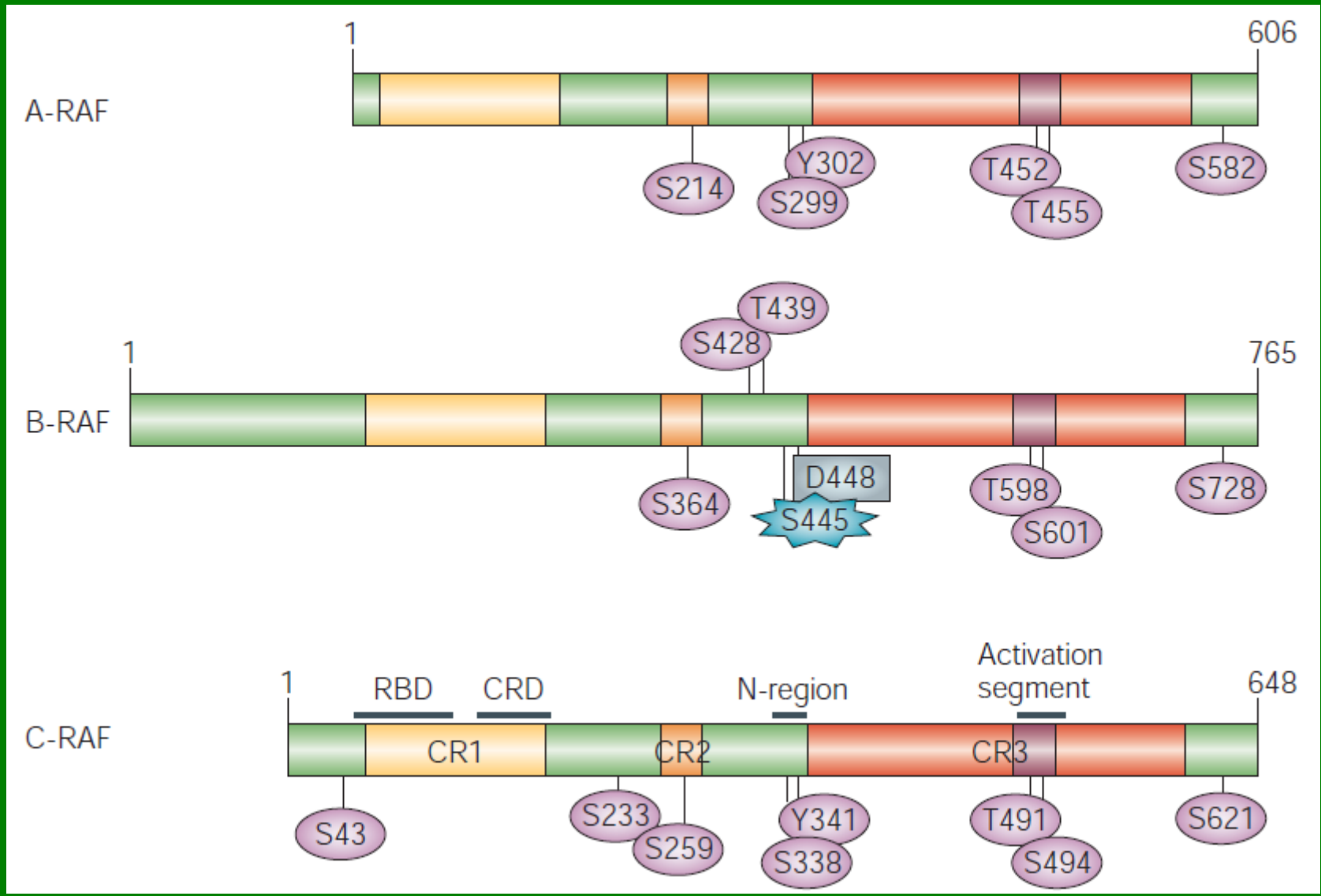
1. N-terminal autoinhibitory domain
2. C-terminal catalytic domain
3. All Raf proteins require dimerization, phosphorylation, and membrane recruitment for full activation



Differences between B-Raf and C-RAF

1. B-Raf Activation: requires only activation segment phosphorylation (T598, S601)
2. C-Raf Activation: activation segment (T491,S494), additionally S338 and T341
3. A-Raf follows a pattern similar to C-Raf

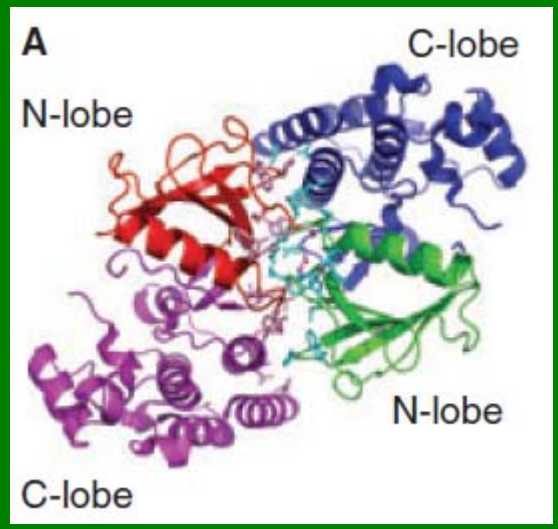
RAF Isoforms



Dimerization in MAPK

- RAS – dimerization at the membrane
- RAF – promoted by RAS and KSR
- MEK – one isoform represses the other
- Erk – distinct signalling roles

Raf Dimers

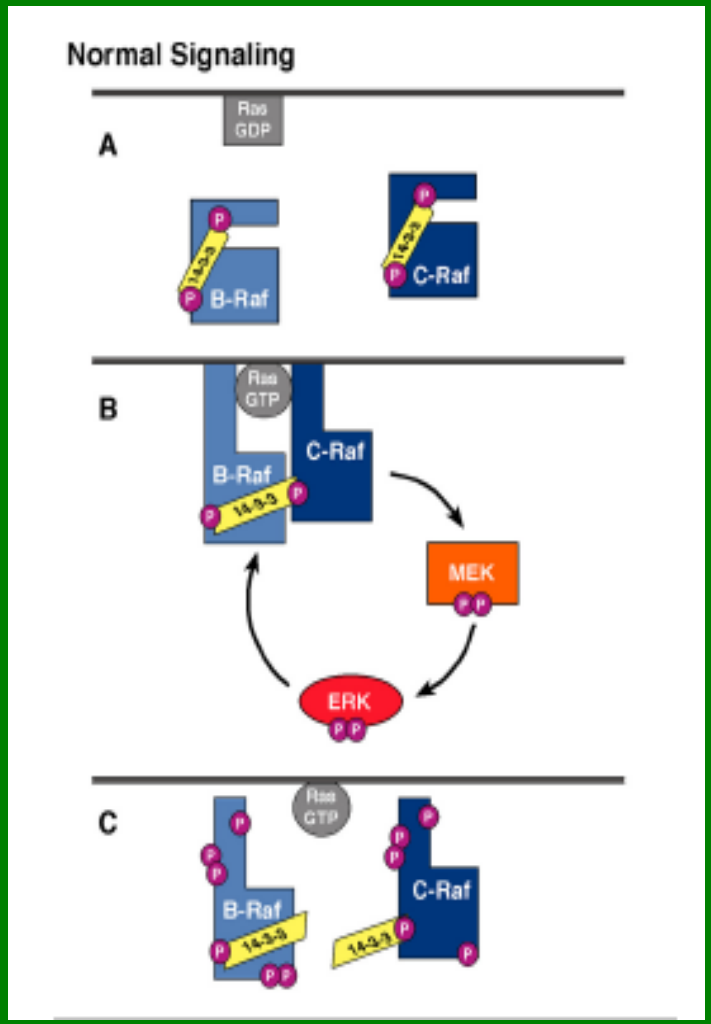


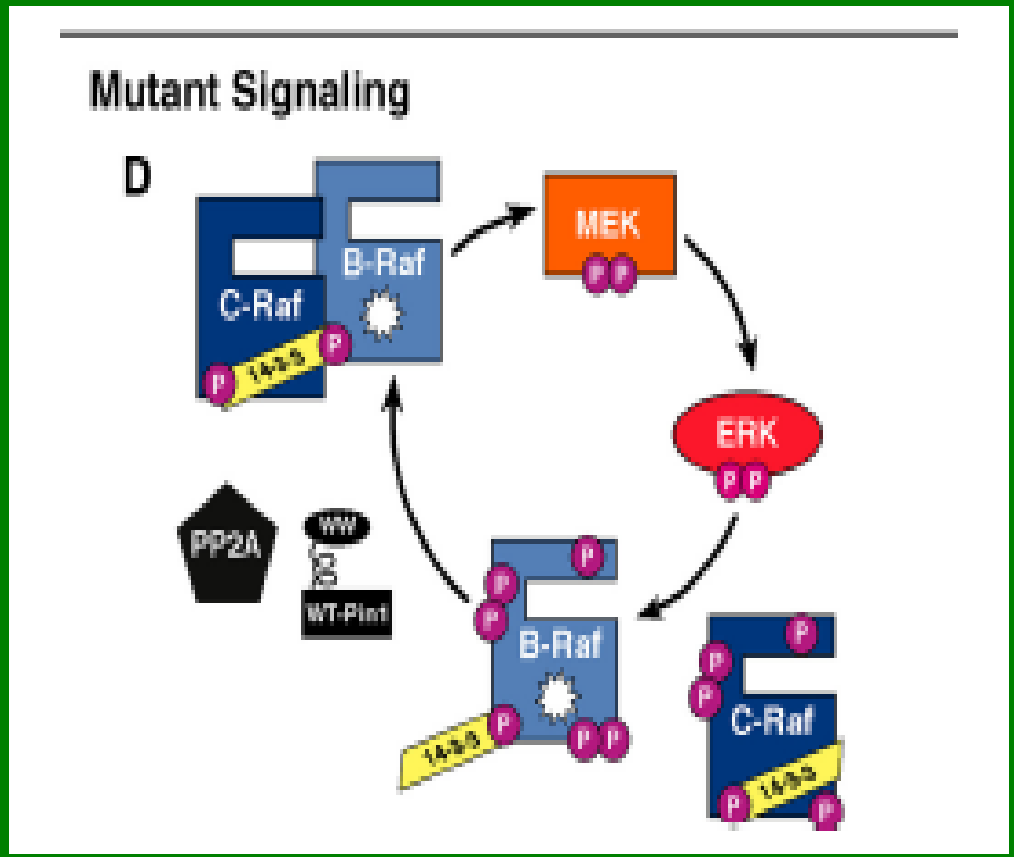
1. Homodimers
   - forced dimerization results in activation
   - unclear mechanism – side-to-side dimerization
2. Heterodimer – far more active than homodimers/monomers (50x-100x)
3. Play role in cancers / B-Raf Inhibitor Paradox

Raf Heterodimer

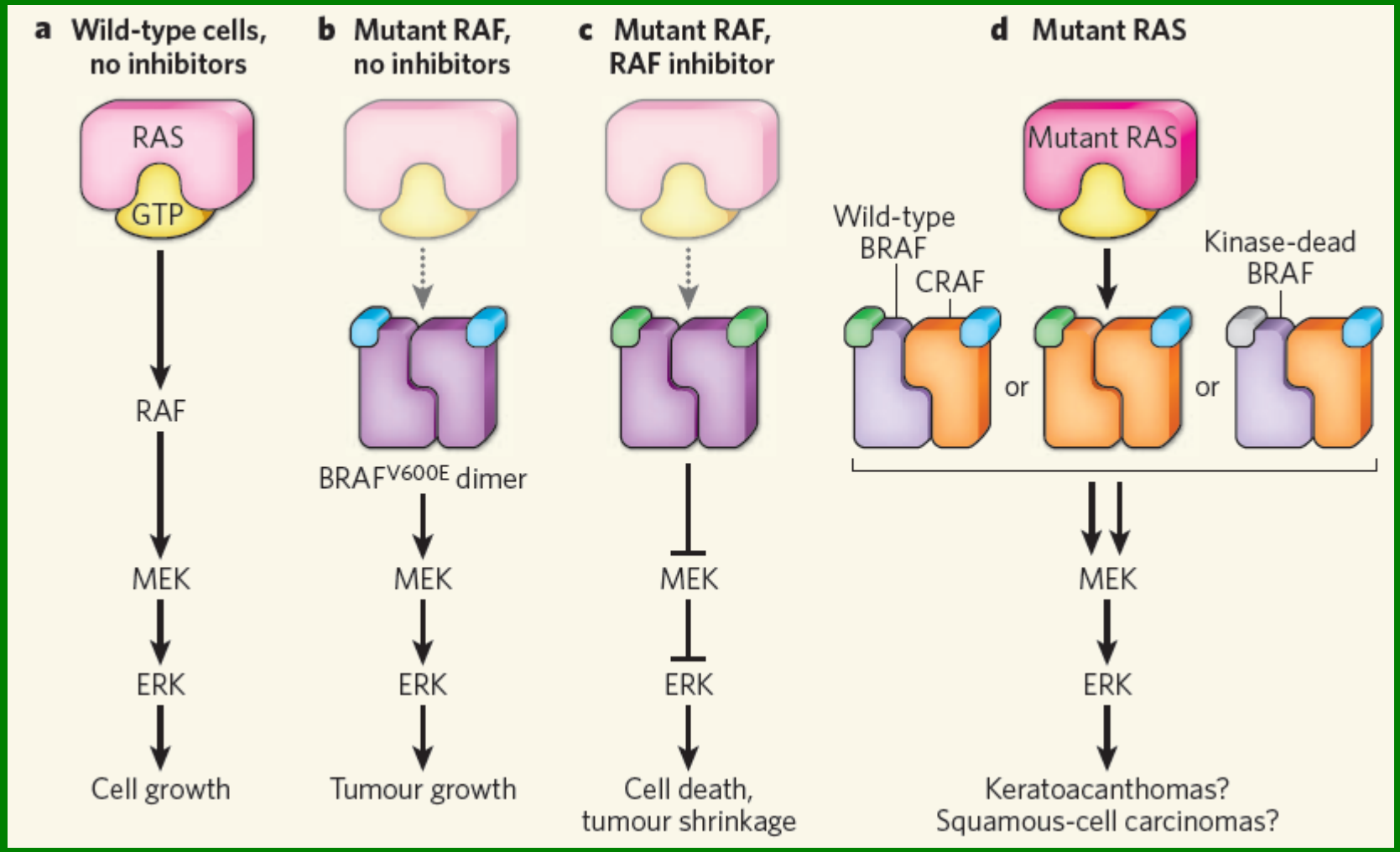
- Induced by RAS activation
- Negatively Regulated by Erk phosphorylation
- In certain cancers, mutant B-Raf constitutively binds and activates C-Raf
- Protomers in a dimer can transactivate each other – not certain if it is due to phosphorylation or conformation change

Raf Heterodimer Signalling



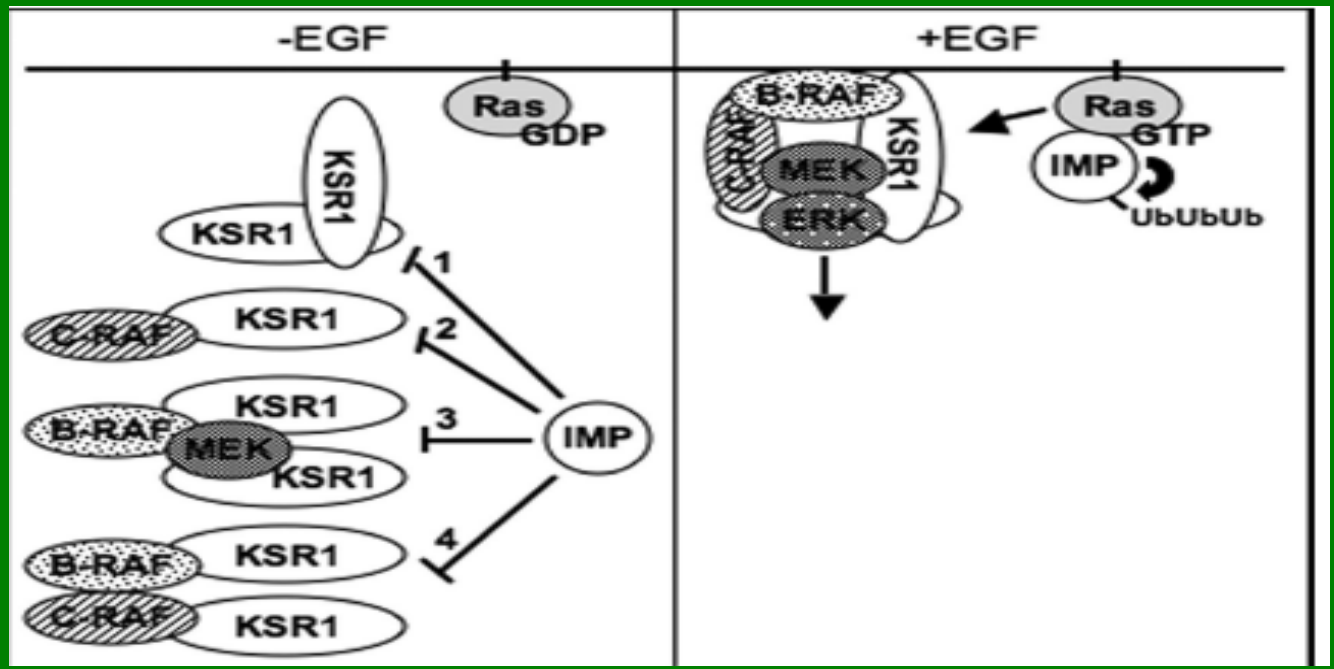


Raf Inhibitor Paradox

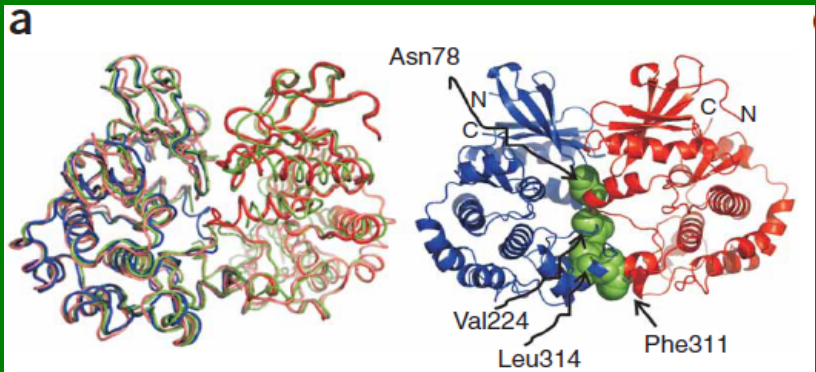


KSR Dimerization

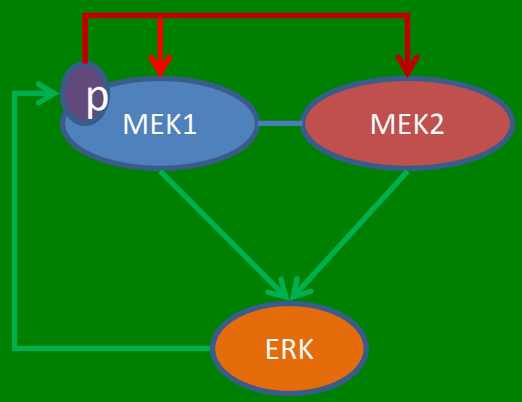
1. Inhibited by IMP
2. Upon Ras-Induced IMP1 Degradation dimerizes
3. KSR dimerization may promote Raf Dimerization



MEK1/2 Heterodimer



Complex	Predicted ΔG	Predicted K_d
Mek1–Mek1	−15.0	1.2×10^{-11}
Mek2–Mek2	−11.6	3.7×10^{-9}
Mek1–Mek2	−15.1	1.1×10^{-11}

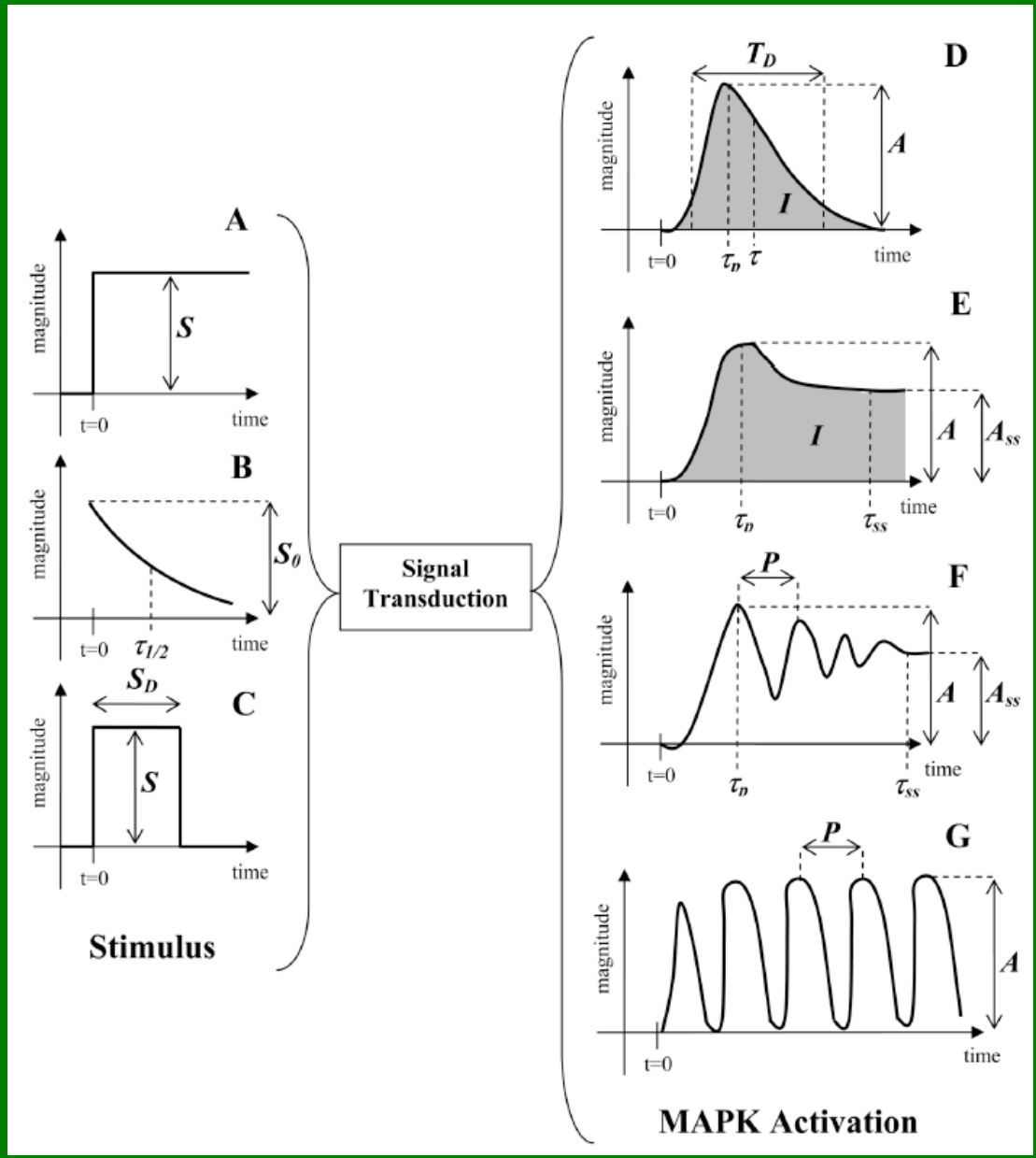


1. Mek1 decreases the activity of Mek2
2. Without Mek1, Mek2 activation is slighlty elevated but prolonged
3. Erk phosphorylation of Thr292 is required for both Mek1 and Mek2 attenuation
4. This mode of regulation is mediated via Mek1/2 heterodimerization

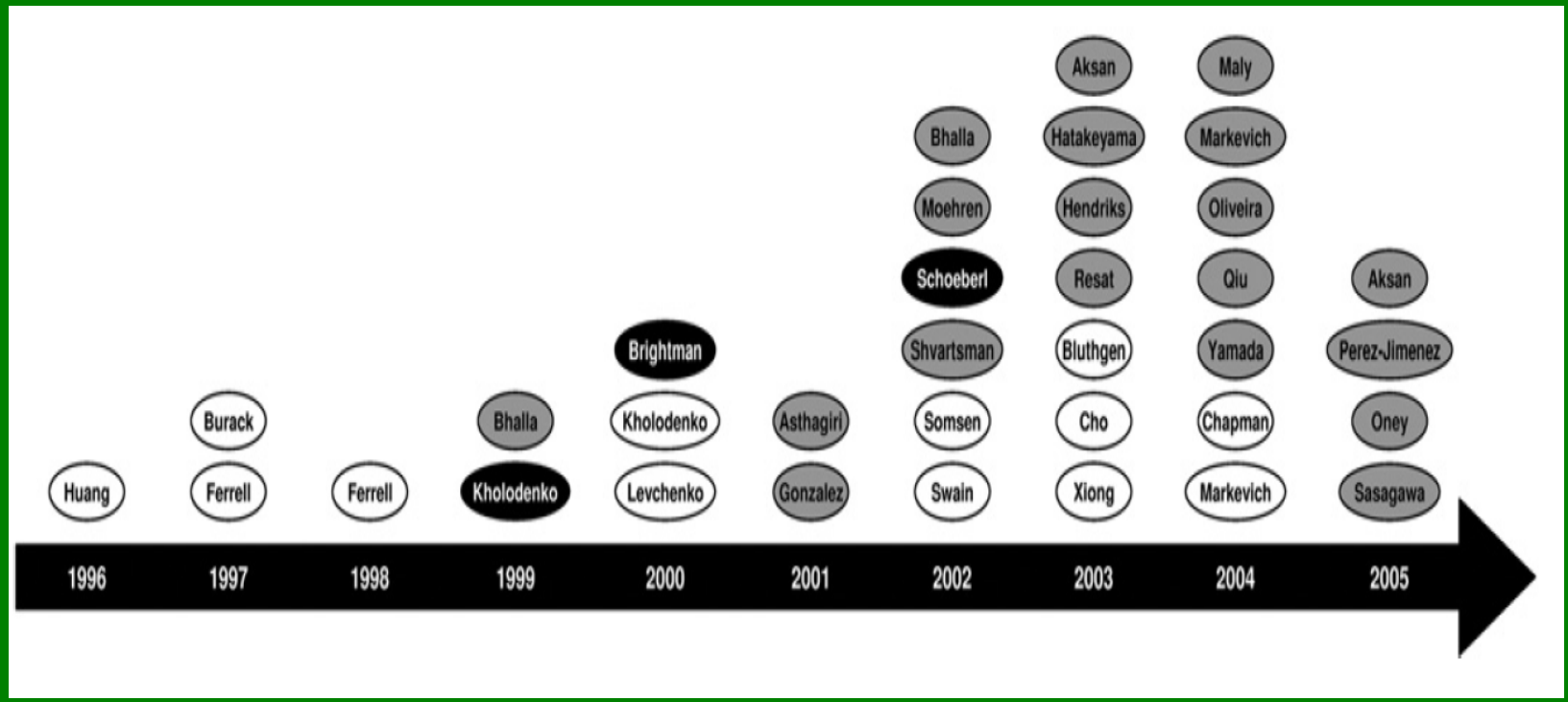
Erk Dimers

1. Upon activation Erk1 and Erk2 homodimerize (Erk1/2 heterodimer is unstable)
2. Dimers enter nucleus via active transport while monomers enter passively
2. Monomers activate nuclear substrates
3. Dimers phosphorylate cytoplasmic targets
4. Perhaps dimers anchor Erk in the cytoplasm

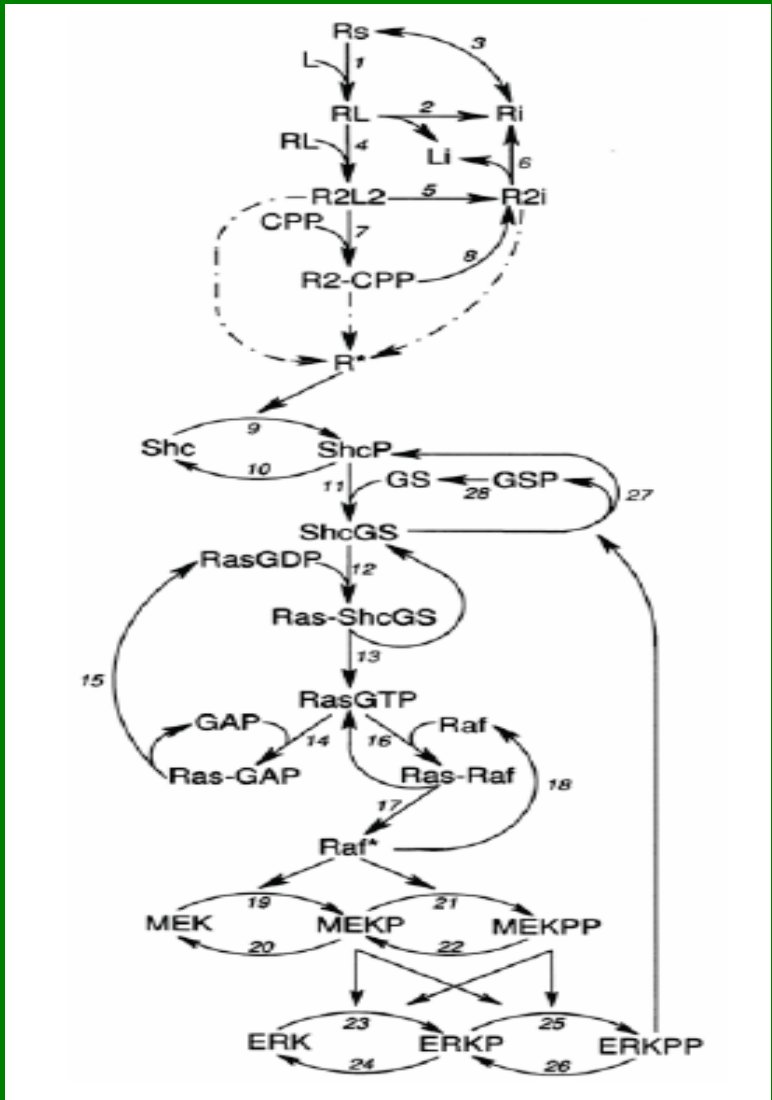
Key Dynamics II



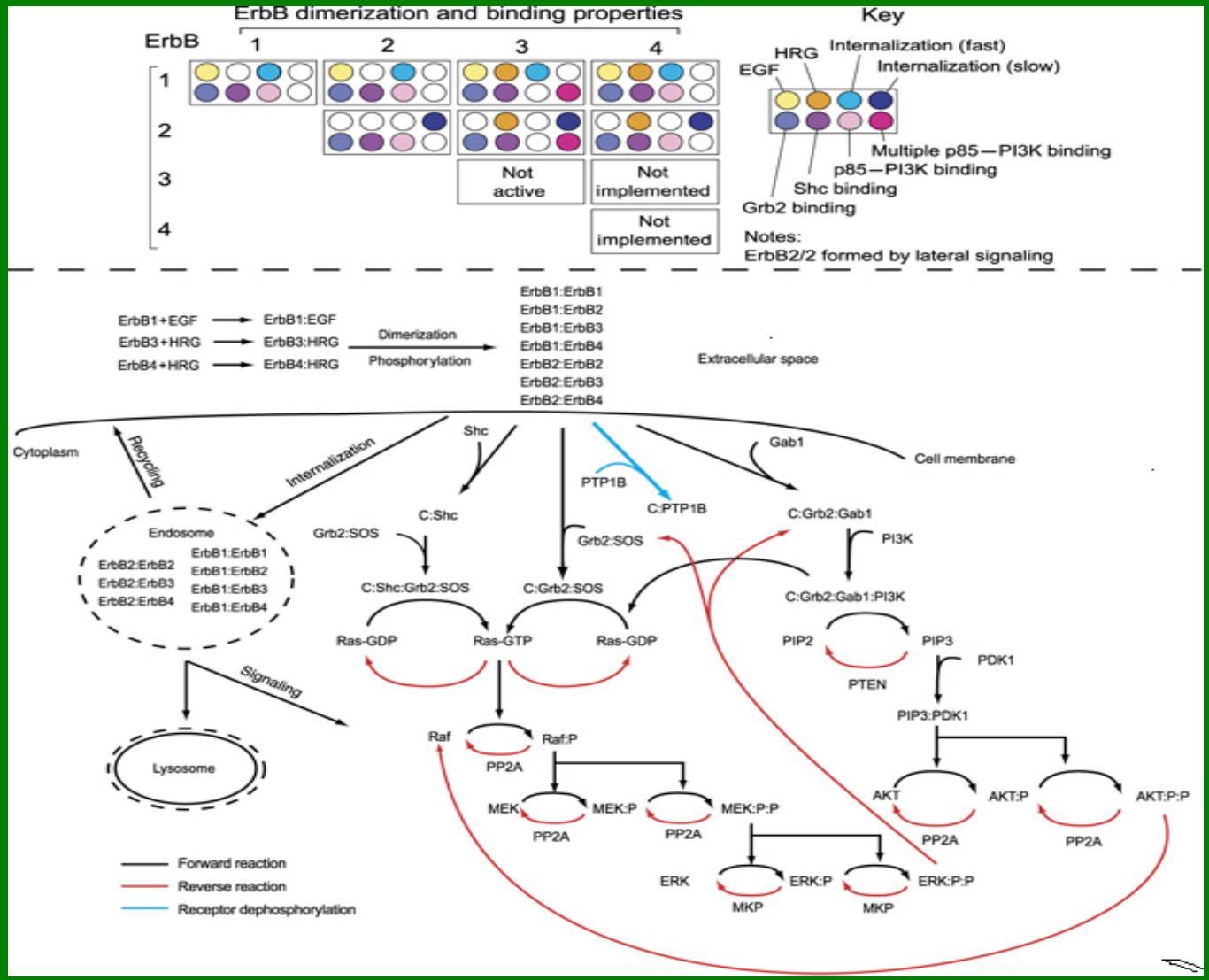
Modelling Efforts – Selected Models



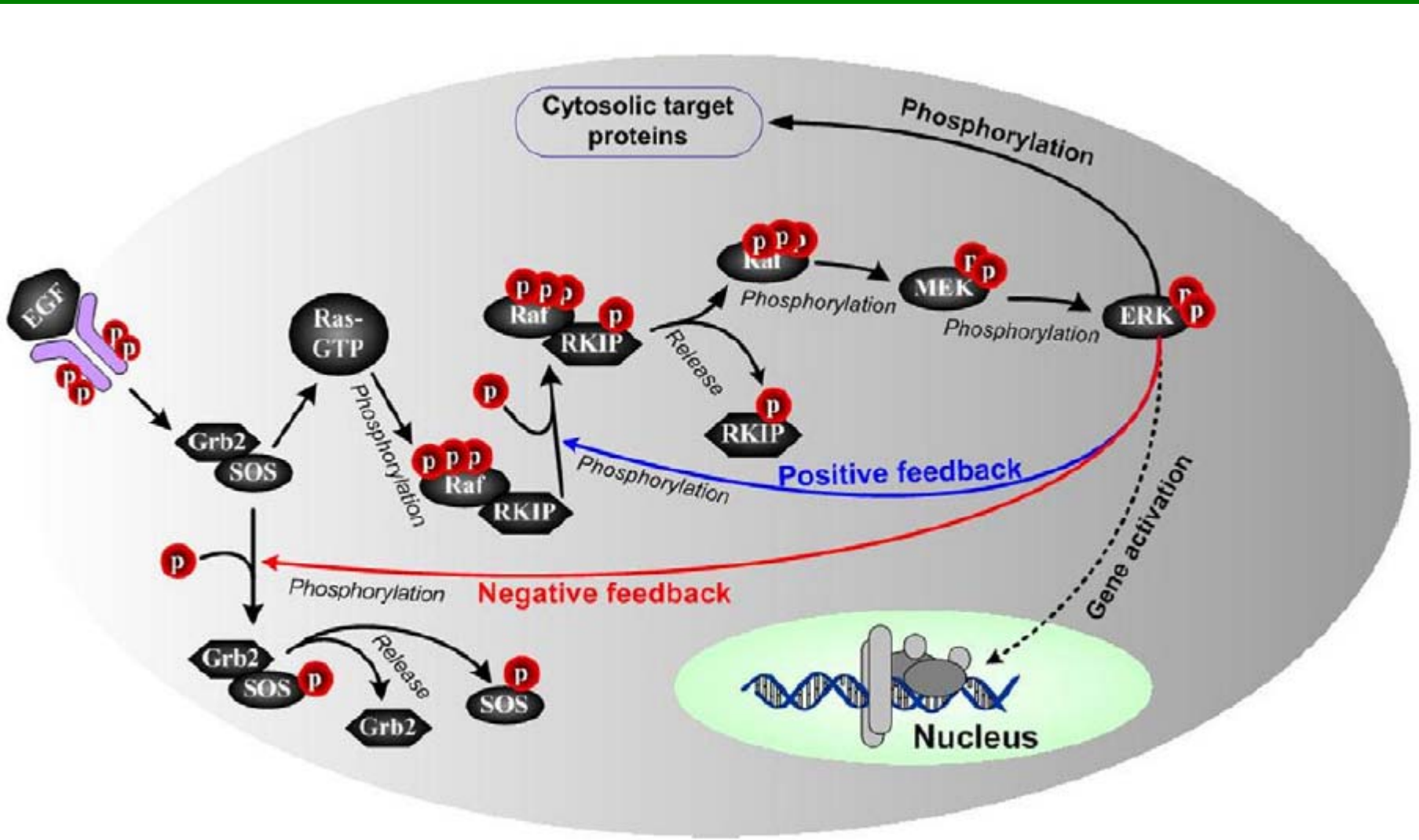
Brightman & Fell 2000



Schoeberl 2009



Shin 2009


Cytosolic target proteins
Phosphorylation
EGF
Grb2
SOS
Ras-GTP
Phosphorylation
Raf
RKIP
Release
Phosphorylation
MEK
Phosphorylation
ERK
RKIP
Phosphorylation
Positive feedback
Gene activation
Phosphorylation
Negative feedback
Grb2
SOS
Release
Grb2
SOS
Nucleus

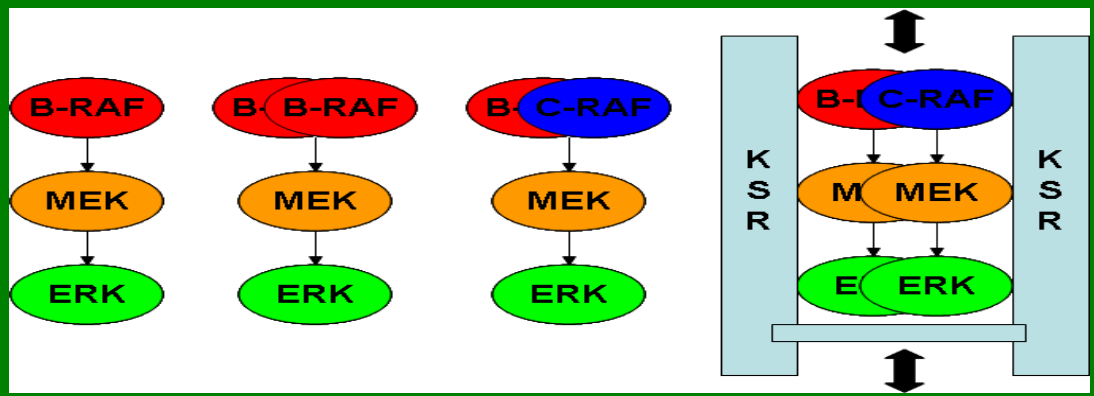
Typical Parameter Values

Table 2. Parameters and Their Typical Values[a]

parameter	description	value	units
n	cell density	3.3×10^4	cells/mL
L_0	initial ligand concentration	k_r/k_f	M
R_0	initial number of free receptors	10^5	no./cell
A_1^T, A_2^T	total number of each adaptor protein	10^4	no./cell
E_i^T, $i = 1-5$	total number of activating enzymes at stage i	10^4	no./cell
P_i^T, $i = 1-5$	total number of deactivating enzymes at each stage i	5×10^3	no./cell
k_f	receptor−ligand association rate constant	10^7	M^{-1} min^{-1}
k_r	receptor−ligand dissociation rate constant	0.3	min^{-1}
k_c	rate constant for dimerization of ligand-bound receptors	6×10^7	M^{-1} min^{-1}
k_u	rate constant for dissociation of dimers	60	min^{-1}
k_c^+	rate constant for activation of dimerized receptor−ligand complexes	50	min^{-1}
k_c^-	rate constant for deactivation of active receptor−ligand dimers	5	min^{-1}
k_f^1, k_f^2, k_f^{12}, k_c^{12}	association rate constants among adaptors	3×10^8	M^{-1} min^{-1}
k_r^1/k_f^1, k_r^2/k_f^2, k_r^{12}/k_f^{12}, k_d^{12}/k_u^{12}	equilibrium dissociation constant for adaptor interactions	10^{-7}	M
k_l^+, kp_i^+, k_x^+, k_z^+	enzyme−substrate association rate constant	6×10^8	M^{-1} min^{-1}
k_l^-, kp_i^-, k_x^-, k_z^-	enzyme−substrate dissociation rate constant	30	min^{-1}
$k_{cat,l}$, k_{cat,P_i}, $k_{cat,x}$, $k_{cat,z}$	rate constant for the formation of product from enzyme−substrate transition complex	6	min^{-1}

My Model(s)

1. Primary goal: account for dimerization, better understand the role of KSR
2. Primary premise: scaffolds (i.e. KSR or RAS) serve as a platform to induce/stabilize dimerization
3. Assumptions: a) scaffold itself is dimeric
   b) RAF dimers protect protomers from dephosphorylation
   c) RAF monomers are rapidly dephosphorylated
4. Variations of the model:
   a) RAS is the actual platform for RAF assembly
   b) KSR dimers may serve to sustain dimer population in the cytoplasm
   c) various modification of association rules



BioNetGen

1. Rules:

X(a,b) + Y(c,d) <-> X(a!1,b).Y(c!1,d) kf1,kr1

Y(d) + Z() -> Y(d~P) + Z() kf2

2. Emphasis on Domain Structure and Interactions
3. Combinatorial Complexity