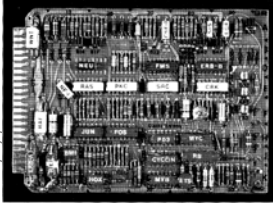


Cell Signaling II: A circuitous pursuit

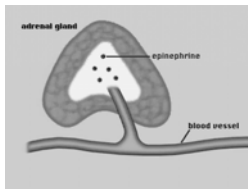


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I will post my lecture notes on
my web site:
<http://www.crch.org/Faculty/jramos>

A fanciful model of the circuitry involved in cell signaling, with the extracellular factors on top and the transcription factors at the bottom.
From *Genes and the Biology of Cancer*, Varmus and Weinberg, 1993

Epinephrine binds β adrenergic receptors on liver cells to stimulate increase in glucose levels in response to stress

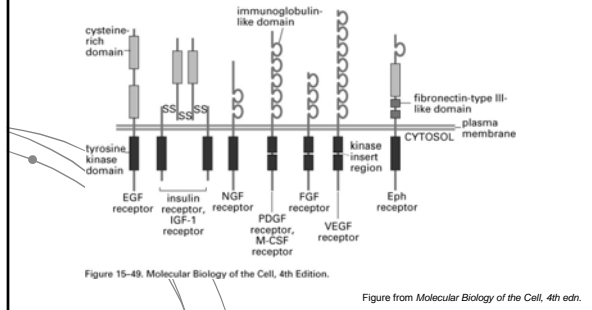


PKA=A Kinase=cAPK

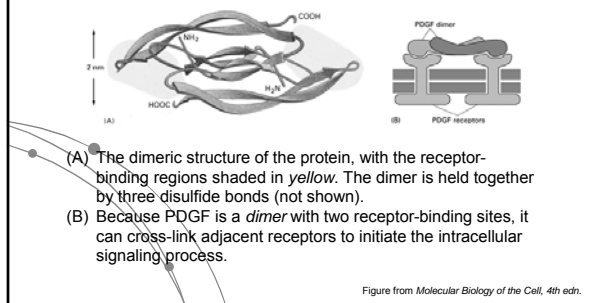
Receptor tyrosine kinases and Ras

- RTK pathways are involved in regulation of :
 - cell proliferation (EGF) and differentiation (FGF)
 - promotion of cell survival (NGF)
 - modulation of cellular metabolism (Insulin)
- RTKs transmit a hormone signal to **Ras**, a GTPase switch protein that passes on the signal on to downstream components

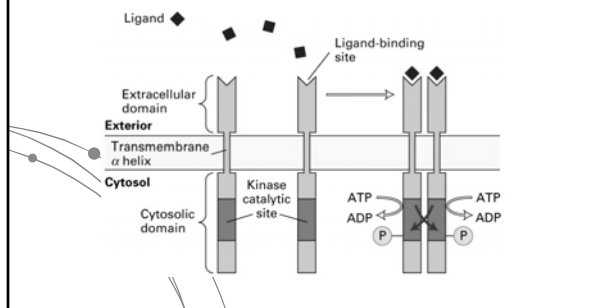
Six subfamilies of tyrosine kinase receptors



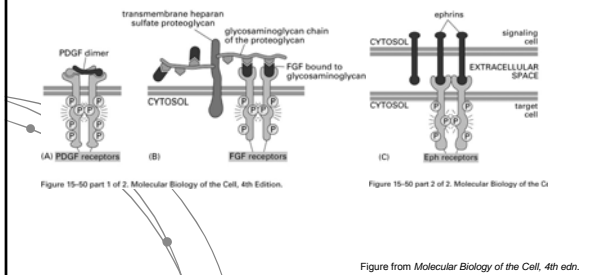
Structure of Platelet derived growth factor



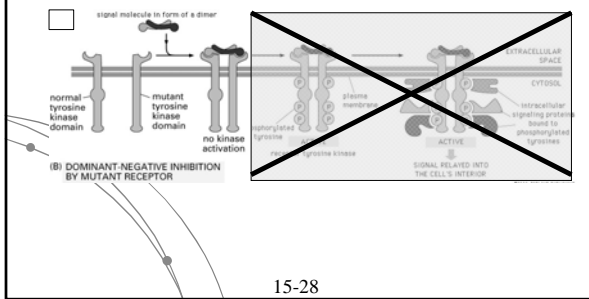
Ligand binding leads to autophosphorylation of RTKs



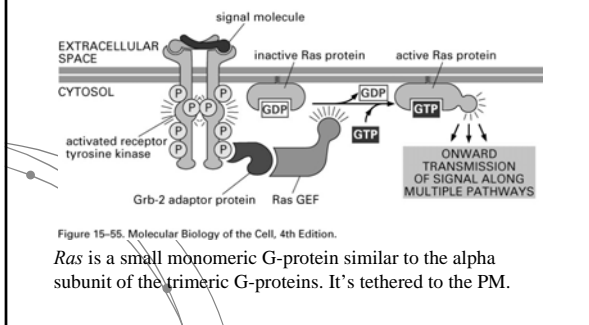
Different Ligands induce RTK dimerization



Activation of receptor tyrosine kinase: forms complex



Activation of Ras by an activated receptor kinase





H-Ras

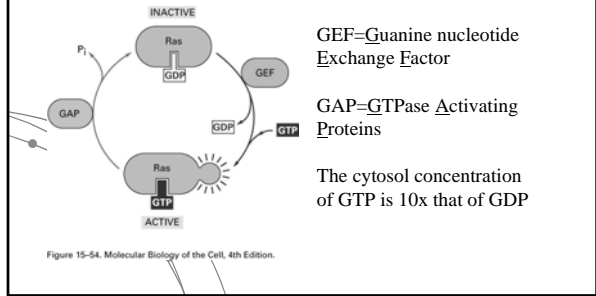
- H-Ras is a small GTPase that controls both proliferation and differentiation pathways.
- A single amino acid mutation of Ras is found in more than 30% of all cancers.
- As many as 90% of certain human tumors like pancreatic carcinomas have mutant Ras!
- Activates Raf/MAP kinase, PI 3 Kinase, RaIGDS

Ras Superfamily of small GTPases

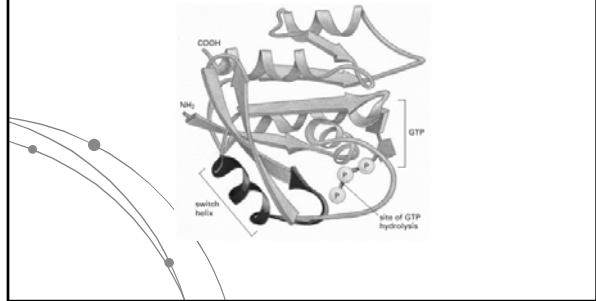
Ras	Rab	Rho	Arf	Rad	Ran	Rheb	Rit	κB-Ras
H-Ras	Rab1A	RhoA	Arf1	Rad	Ran	Rheb	RIT	κB-Ras1
K-Ras	Rab1B	RhoB	Arf2	Gem			Rin	κB-Ras2
N-Ras	Rab2	RhoC	Arf3	Kir			Ric	
R-Ras	Rab3A	RhoD	Arf4	Rem1				
TC21	Rab3B	RhoE	Arf5	Rem2				
M-Ras	Rab4	RhoG	Arf6					
Rap1A	Rab5A	Rnd1	Arf7					
Rap1B	Rab5B	Rnd2						
Rap2A	Rab6	Rac1						
Rap2B	Rab7	Rac2						
RalA	Rab8	Rac3						
RalB	Rab9	Cdc42						
	Rab10	TC10						
	Etc.	TCL						
		TTF						
		Chp						

All are highly similar and are regulated by GTP binding- they have different effectors and locations in the cell

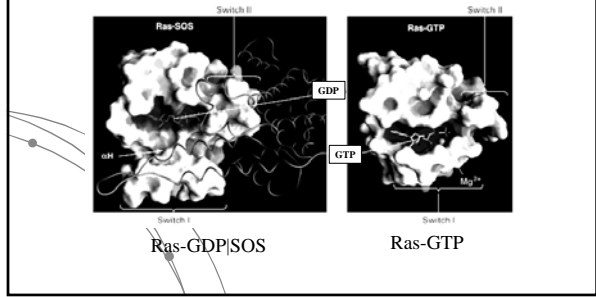
Ras cycles between active and inactive forms



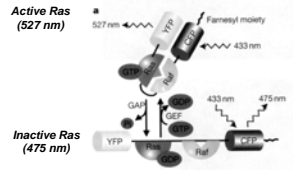
Structure of Ras in GTP-bound form



Structures of Ras-GDP-Sos complex and Ras-GTP



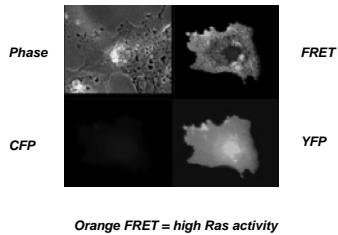
Flourescence Resonance Energy Transfer (FRET)



Single protein contains Ras, the Ras binding domain of Raf, and two fluorophores: Yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP). Excite CFP—the energy emitted by CFP is partly captured by YFP which emits energy that peaks at 527 nm. Similar setup for Rap1, another small GTPase.

N. Mochizuki, et al., Nature 411:1065-1068 (2001)

EGF induced Ras activation... The Movie



Orange FRET = high Ras activity

N. Mochizuki, et al., Nature 411:1065-1068 (2001)

Ras and Rap1 activation in living cells: different location of active GTPases

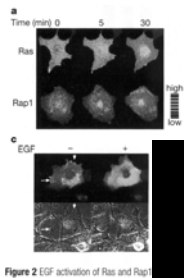
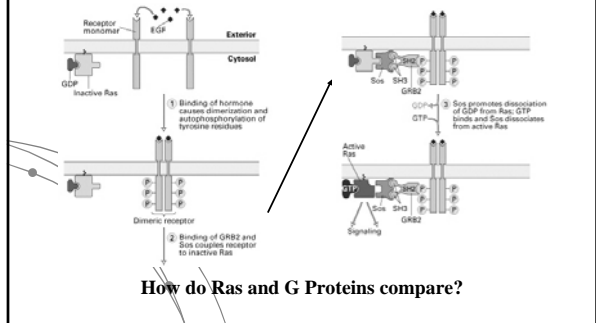


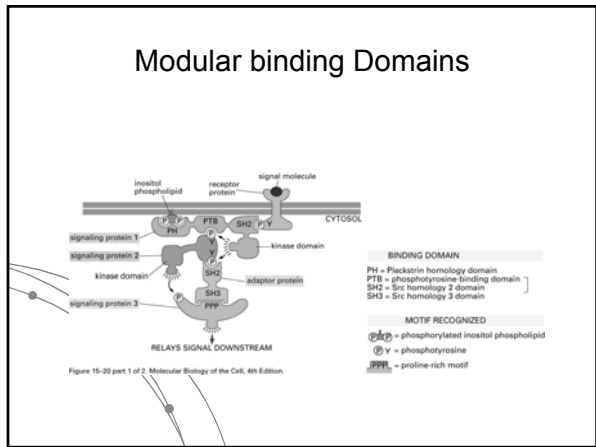
Figure 2 EGF activation of Ras and Rap1

N. Mochizuki, et al., Nature 411:1065-1068 (2001)

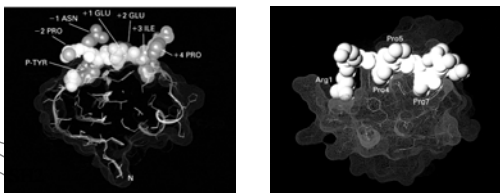
An adapter protein and GEF link most activated RTKs to Ras



Modular binding Domains



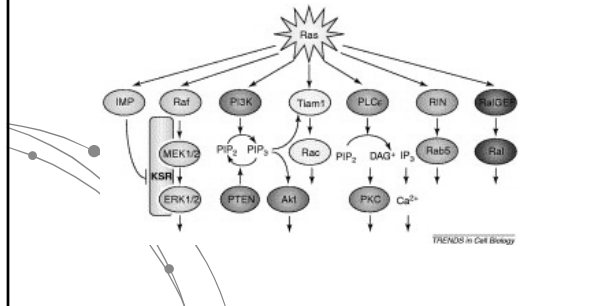
Models of SH2 and SH3 domains bound to short target peptides



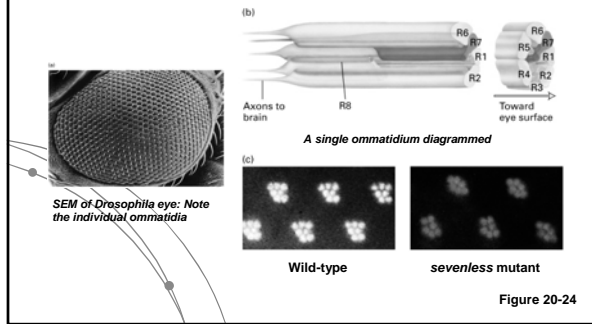
SH2 domain in GRB2 adapter protein binds to a specific phosphotyrosine peptide (Src here) in an activated tyrosine kinase. RTKs

Proline rich sequence in Sos, a GEF, binds to SH3 domains in GRB2.

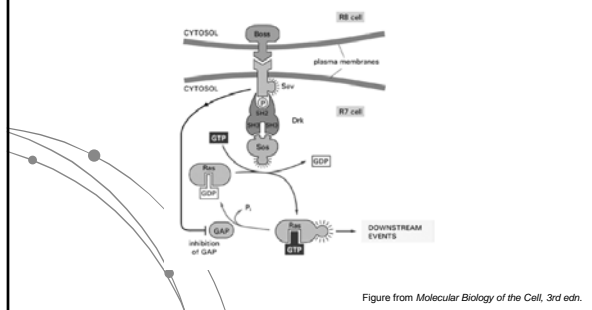
Ras effectors involved in cancer



Analysis of eye development in *Drosophila* has provided insight into RTK signaling pathways



Early cell-signaling events in R7 development



MAP kinase pathways

- Activated Ras induces a kinase signal cascade that culminates in activation of MAP kinase
- MAP kinase is a serine/threonine kinase that can translocate into the nucleus and phosphorylate many different proteins, including transcription factors that regulate gene expression

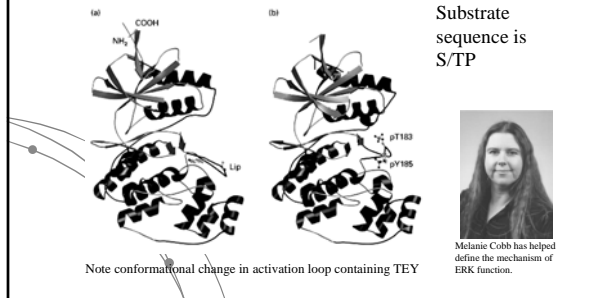
A Ras activated phosphorylation cascade

Ras also activates PI3 kinase and RaIGDS pathways.

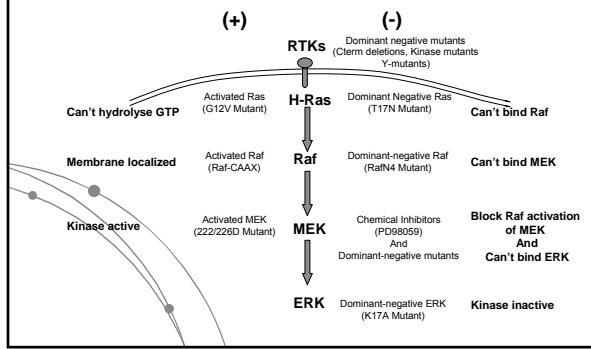
Figure 15-56. Molecular Biology of the Cell, 4th Edition.

Signals pass from activated Ras to a cascade of protein kinases

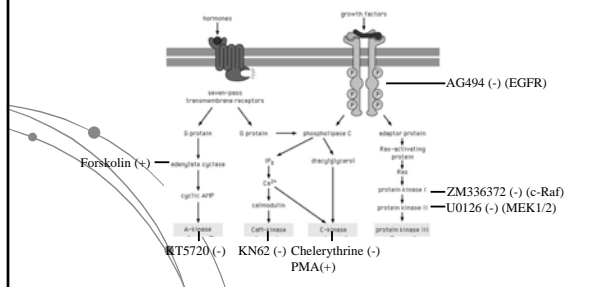
Phosphorylation of a tyrosine and a threonine activates MAP kinase

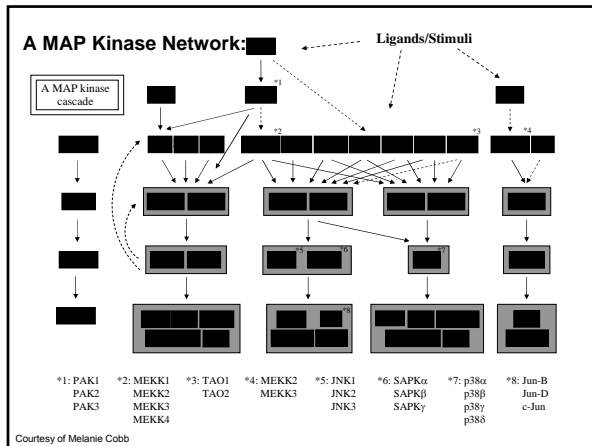


Selected signaling tools



Drugs can be used to block or activate specific kinases

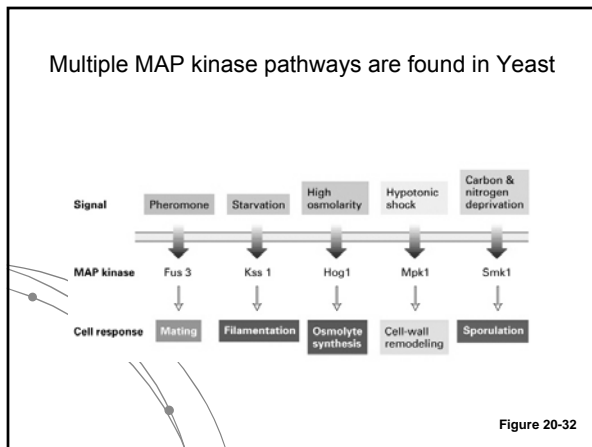




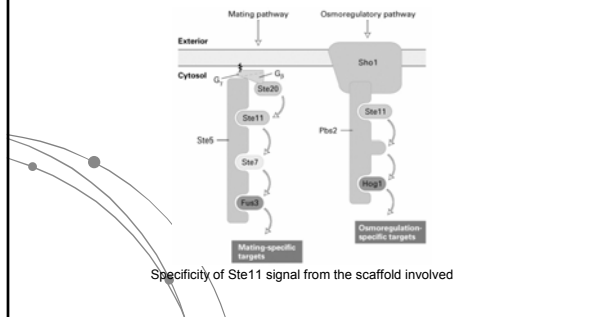
Much work on MAP kinases has been done in yeast

Mating pathway in *S. cerevisiae*
Two secreted peptide pheromones called α and α factors control mating between haploid yeast cells. The receptors for these factors are GPCR that activate MAP kinase pathways.

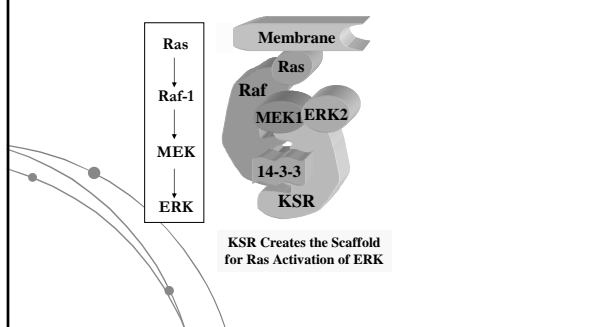
Figure from *Molecular Biology of the Cell*, 3rd edn.



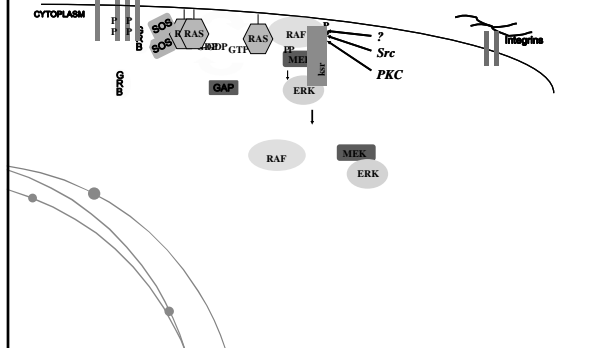
Scaffolds organize MAP kinase pathways:Yeast

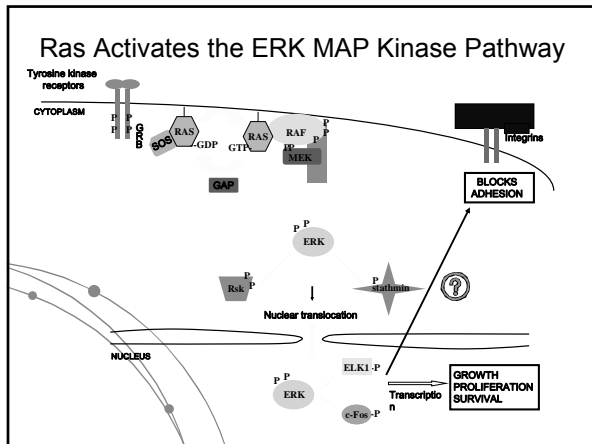


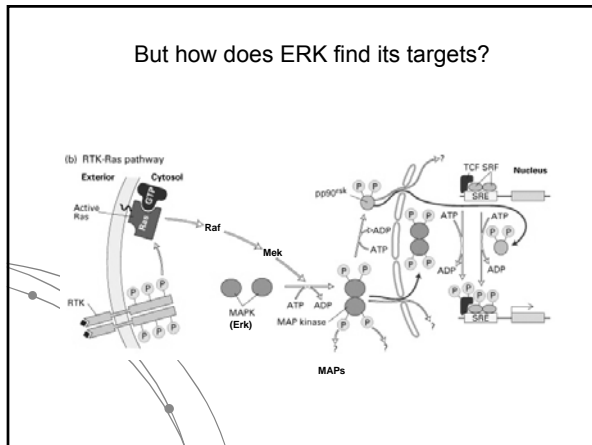
Scaffolds organize MAP kinase pathways:Vertebrates

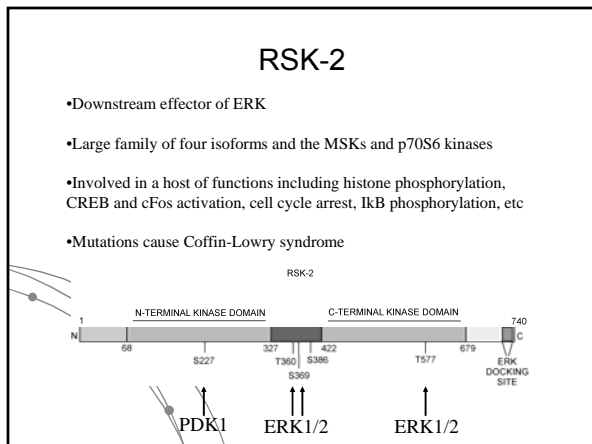


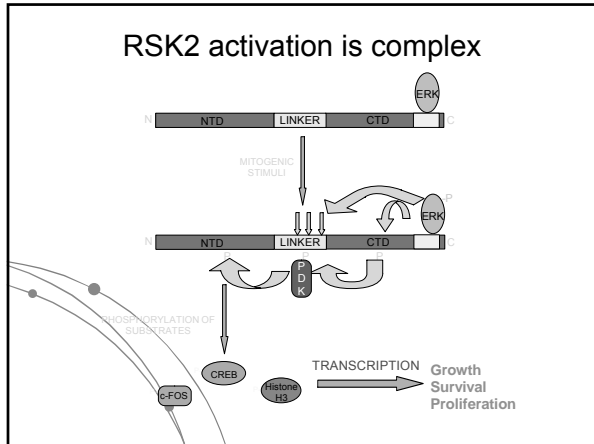
Ras Activates the ERK MAP Kinase Pathway

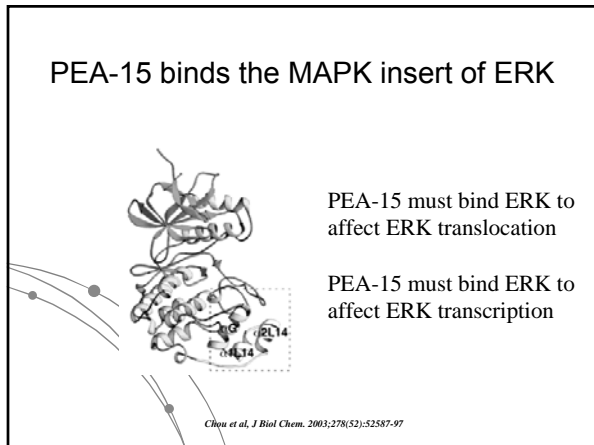


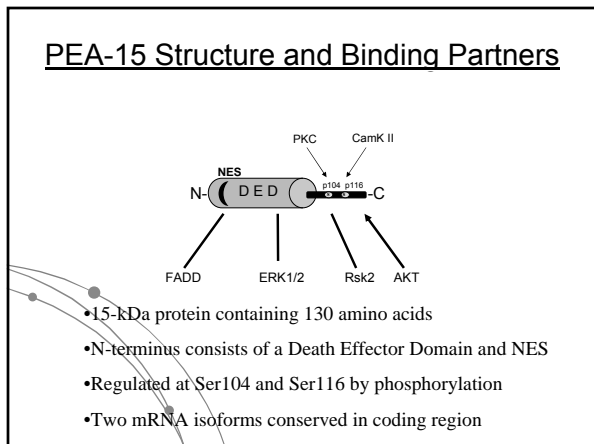




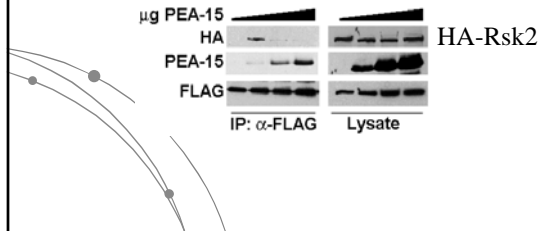




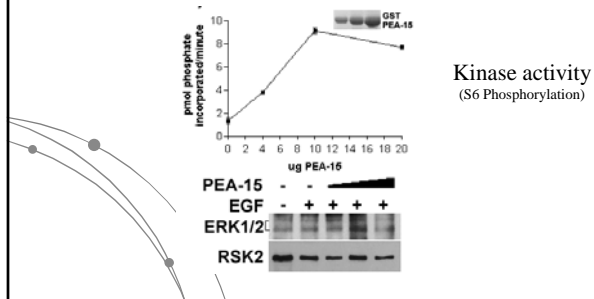




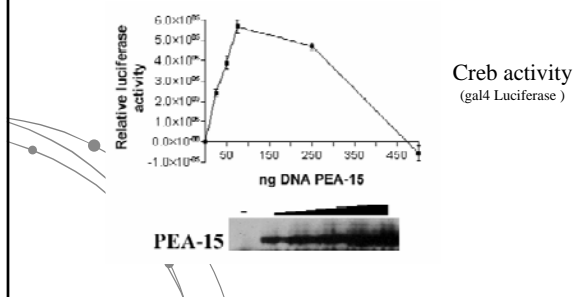
PEA-15 enhances ERK binding to Rsk2



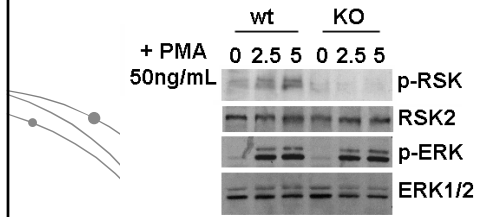
PEA-15 increases Rsk2 kinase activity in a concentration dependent manner



PEA-15 increases Rsk2 signaling in a concentration dependent manner

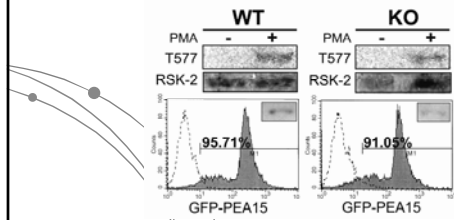


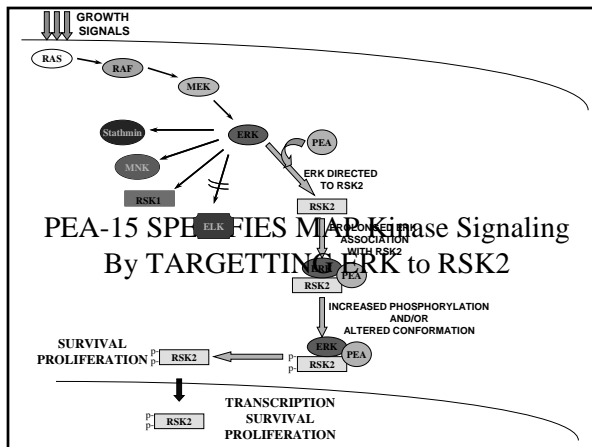
PEA-15 null mice have reduced activation of Rsk2 in thymocytes



Re-expression of PEA-15 rescues RSK2 activation

Primary lymphocyte cultures
Transient Transfection





Interaction and regulation of signaling pathways

- The effects of activation of GPCRs and RTKs is more complicated than a simple step-by-step cascade
- Interaction of different signaling pathways permits fine-tuning of cellular activities

Four parallel intracellular pathways and their connections

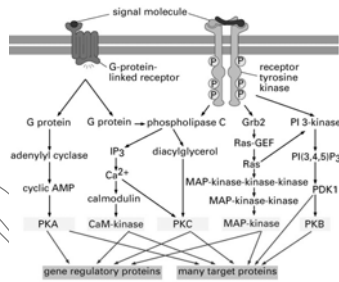


Figure 15-61. Molecular Biology of the Cell, 4th Edition.

Two simple mechanisms of signal integration

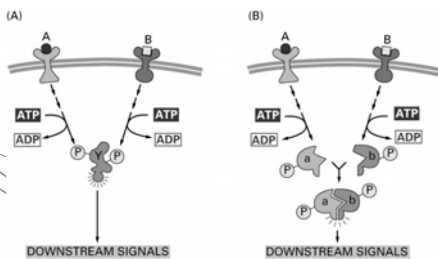


Figure 15-18. Molecular Biology of the Cell, 4th Edition.
