in cell membrane

Receptor Tyrosine Kinases

These receptors undergo autophosphorylation (catalyze their own phosphorylation) and can transphosphorylate other proteins in response to a ligand.

new - not many marketed drugs, but investigational ones

Classes of Receptor Tyrosine kinases (RTKs)

1- Epidermal growth factor (EGF-R) receptor family includes:

No ligand yet

dimer ✓

- EGFR1 (ErbB1)
- EGFR2 (ErbB2, HER2/neu)
- EGFR3,4 (ErbB3,4 or HER3,4)

"Er" means human

2- Insulin Receptor

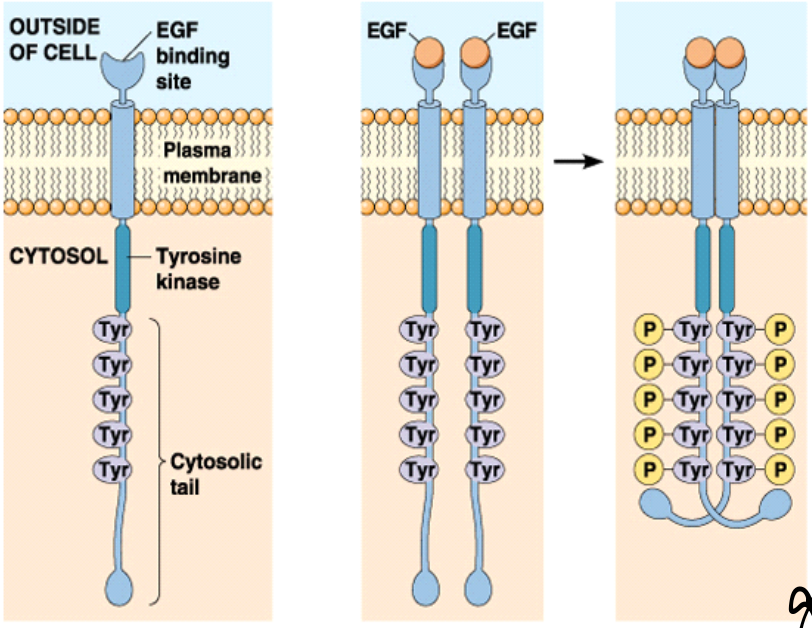
3- Platelet-derived growth factor (PDGF)

4- Fibroblast growth factor (FGF)

5- Vascular endothelial growth factor (VEGF)

Receptor tyrosine kinases

The interaction of the external domain of a receptor tyrosine kinase with the ligand, often a growth factor, up regulates the enzymatic activity of the intracellular catalytic domain, which causes tyrosine phosph orylation of cytoplasmic signaling molecules

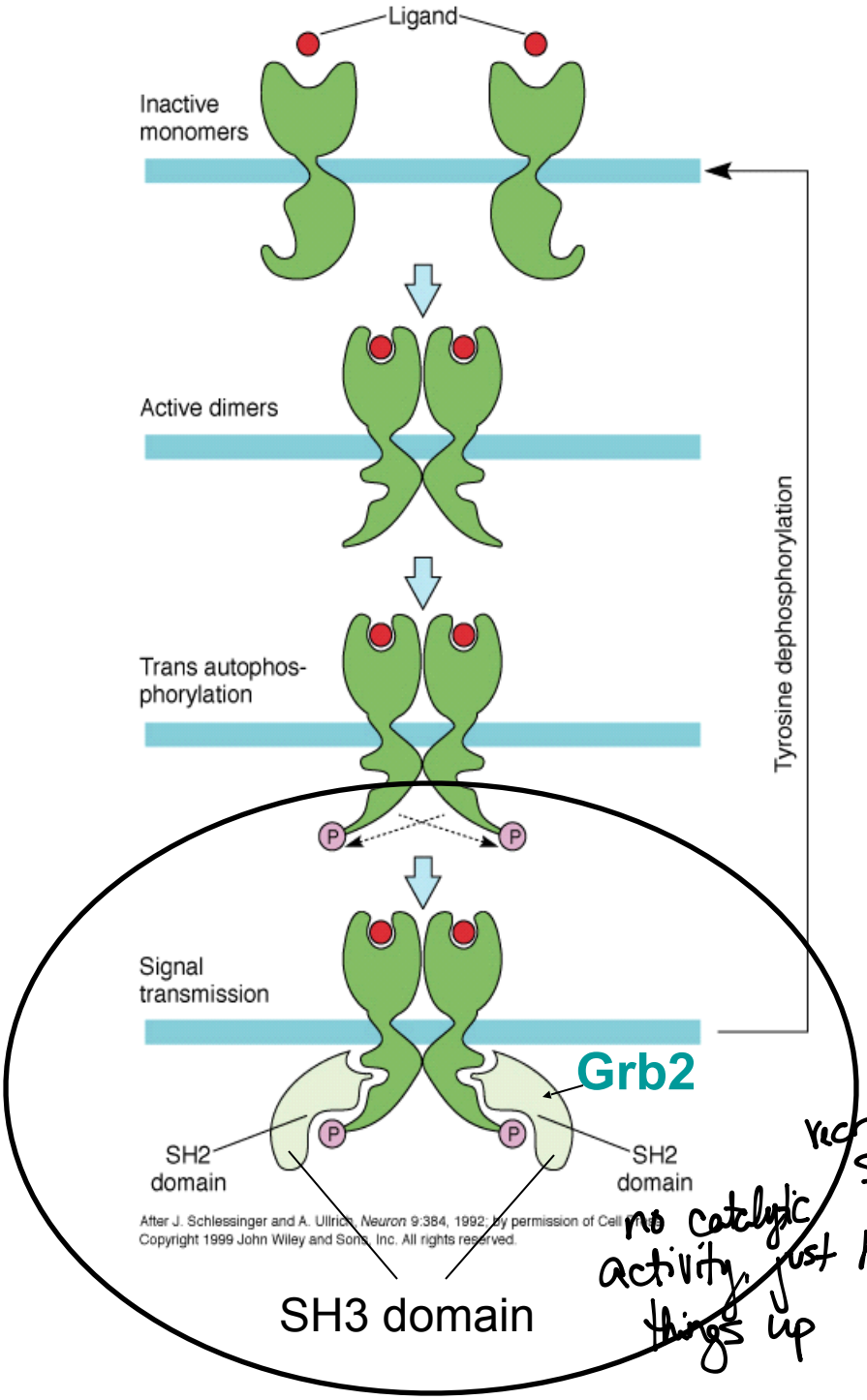


(a) Structure of the epidermal growth factor (EGF) receptor

(b) Activation of the EGF receptor

good for setting up signal cascade

Fig. 15.23



Grb2 is an adaptor protein containing both SH2 and SH3 domains

-SH2 Domains

-Small protein module (150 amino acids) including some highly conserved basic aa (can tell if has SH2 in this motif)

-mediates protein – protein interaction

-these domains found in a wide variety of proteins including those with “catalytic domains” & structural proteins

SH2 domains recognize phosphotyrosine binding sites.

Q: How do you achieve specificity of protein protein interactions?

A: Different SH2 domains have different affinities for a given phosphotyrosine (surrounding sequences can confer specificity)

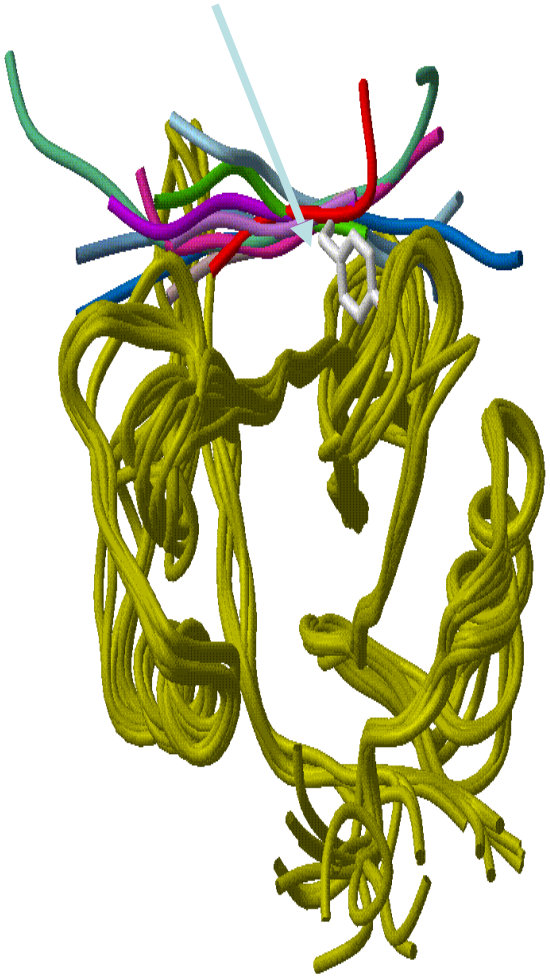
Determining specificity substrates & adaptor proteins.

The sequences C-terminal to the consensus phosphotyrosine binding domain determine if a protein will bind to an RTK and (if it is a substrate) be phosphorylated.

The Src homology 2 (SH2) domain has been found in a number of signal transduction pathways.

eg. on a tyrosine kinase receptor

Phospho-tyrosine



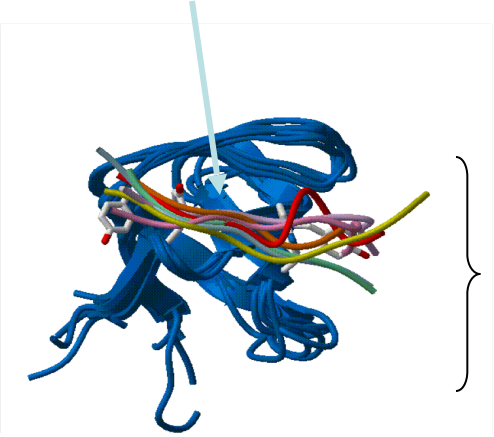
SH2 Domain

Its primary function is to bind phosphotyrosines and in doing so localizing different proteins necessary to transmit the proper function Pawson, 1997.

homology is gene called SARC

eg. on Grb2

Polyproline helix



SH3

SH3 domain

β-barrel of 5-6 anti-parallel β-strands.

Binds a polyproline helix

(binds other ppis of proteins)

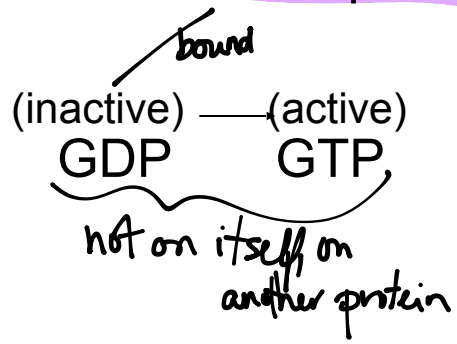
SH3 Domains

Mediate protein: protein interaction

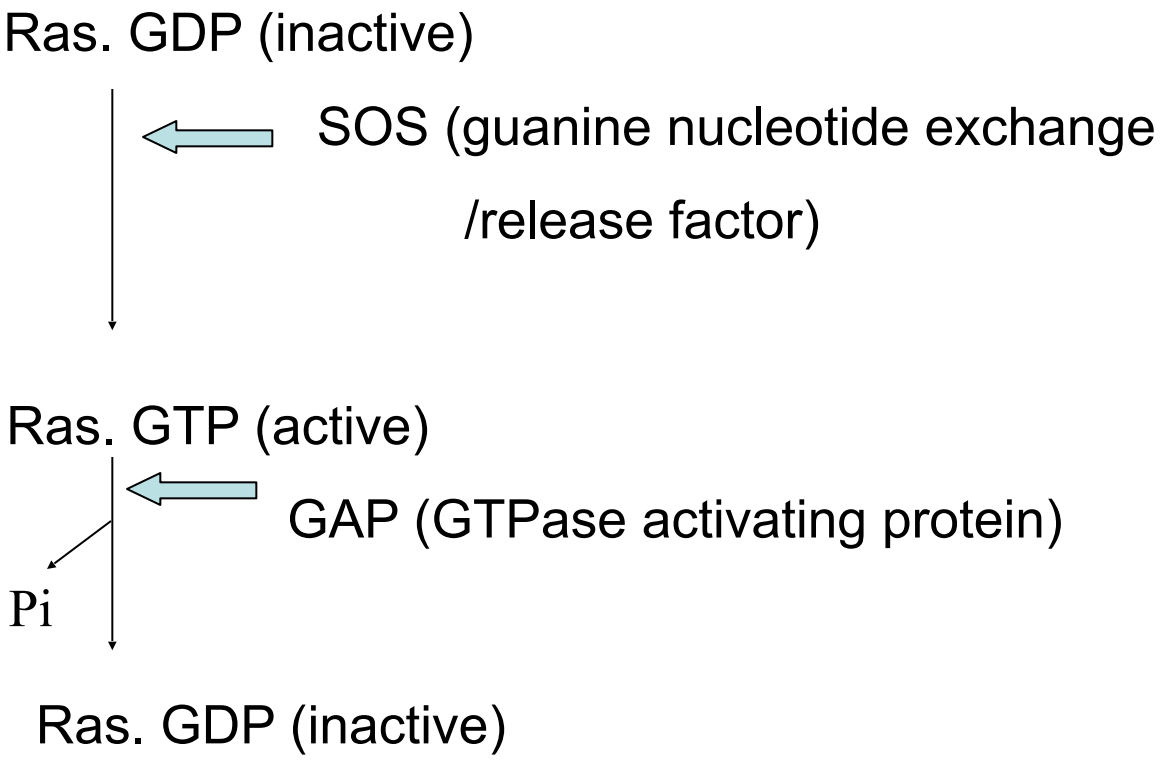
Bind to proline-rich regions in other proteins

After the phophorylated RTK binds to Grb2
Grb2 interacts with SOS through the Grb2 SH3 domain

SOS is a guanine nucleotide exchange protein that facilitates the activation of the Ras protein



The Ras GTPase Cycle



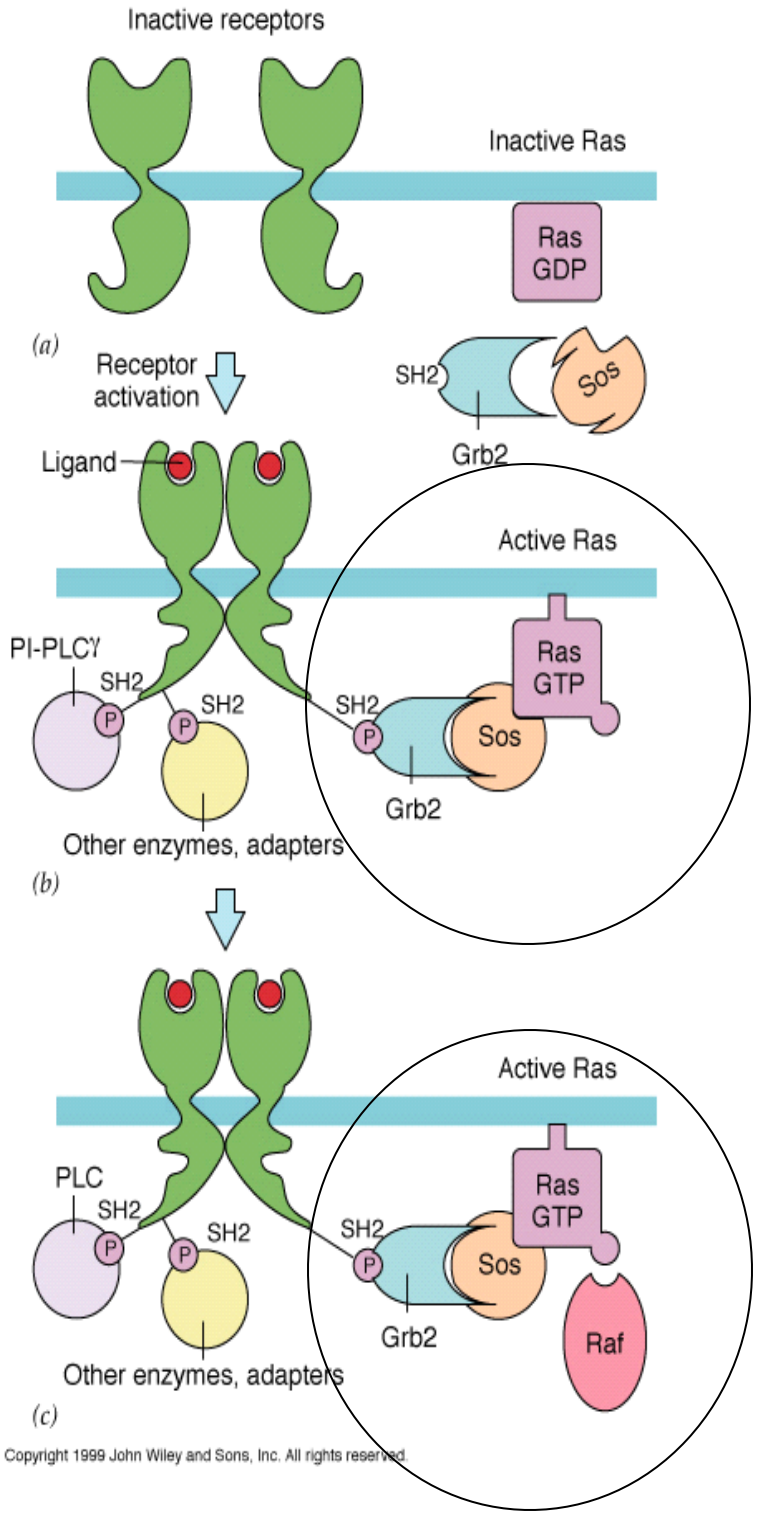
Ras is an important intermediate in RTK signal transduction

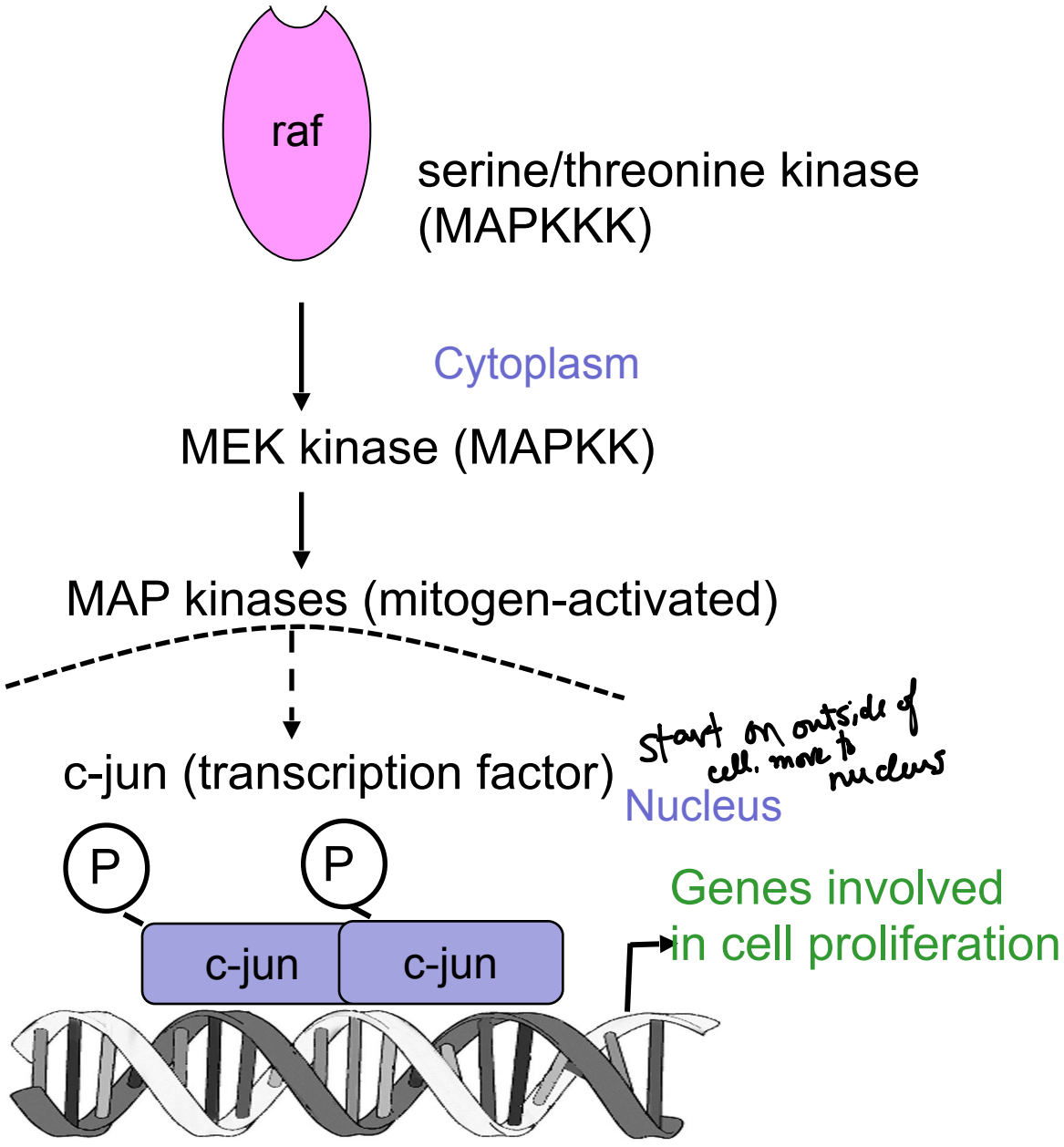
- Ras is a small G-protein (monomeric 21-kD) responsible for activating the Raf-1 kinase

- Mutant Ras proteins are unable to dissociate GTP, so they are stuck in the ON or proliferative state: *ras* (*gene*) mutations found in 30% of human cancers.

- mutations in Ras-GAPs can lead to disease

Intracellular signalling by receptor tyrosine kinases




raf
serine/threonine kinase
(MAPKKK)
Cytoplasm
MEK kinase (MAPKK)
MAP kinases (mitogen-activated)
c-jun (transcription factor)
start on outside of cell, move to nucleus
Nucleus
P
P
c-jun
c-jun
Genes involved
in cell proliferation

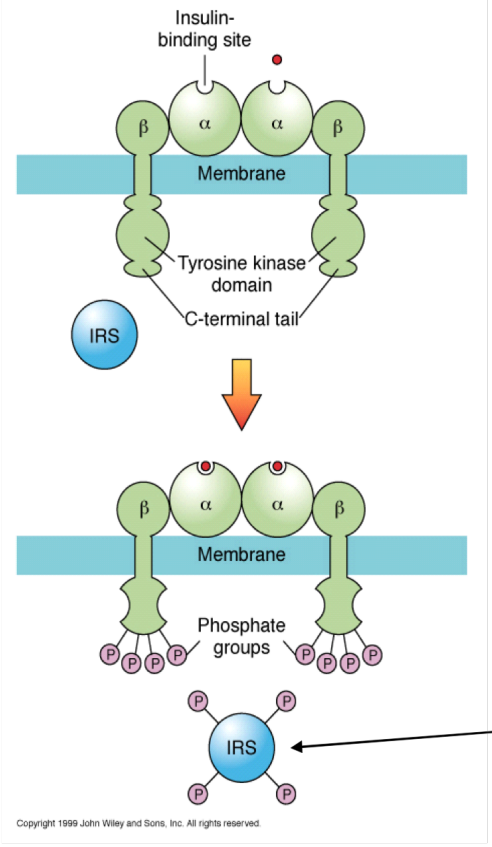
Signaling by RTKs: Summary

*Raf-1 is key intermediate to all ErF

- RTK is activated by ligand
- Receptor phosphorylation and binding to Grb2
- Grb2 binds SOS to activate Ras by exchanging GDP for GTP
- Activated Ras then causes a change in conformation of the Raf-1 kinase resulting in its activation
- Raf-1 then activates the MAP kinase family which eventually results in the activation of transcription factors in the nucleus
- These transcription factors stimulate the transcription of genes involved in cell proliferation

The insulin RTK is a special case:

Response of the insulin receptor kinase (IRK) to ligand binding Fig. 15.20



- Heterotetramer (2α, 2β)

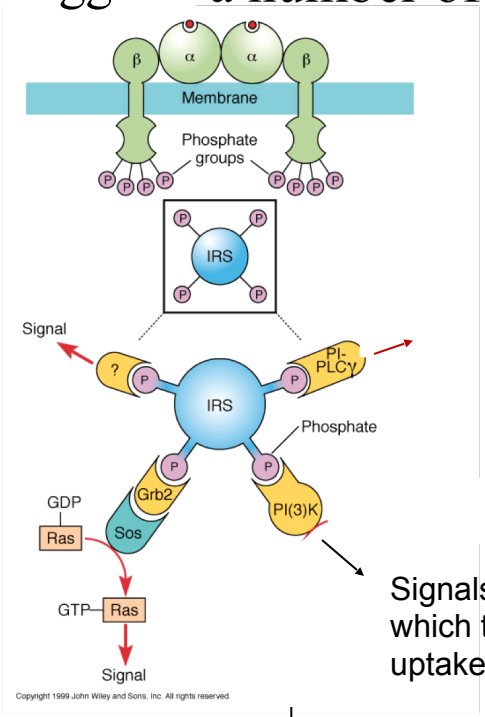
bound by disulfide bridges

- Insulin binding leads to change in structure (different from other RTKs)
- Conformation change activates β-subunit TK activity

? β subunit phosphorylates Tyr residues on cytoplasmic domains as well as downstream substrates (IRS)

insulin receptor substrate
transduct^{n} molecule

Once Tyr-Phosphorylated, the IRK activity triggers a number of signaling pathways.

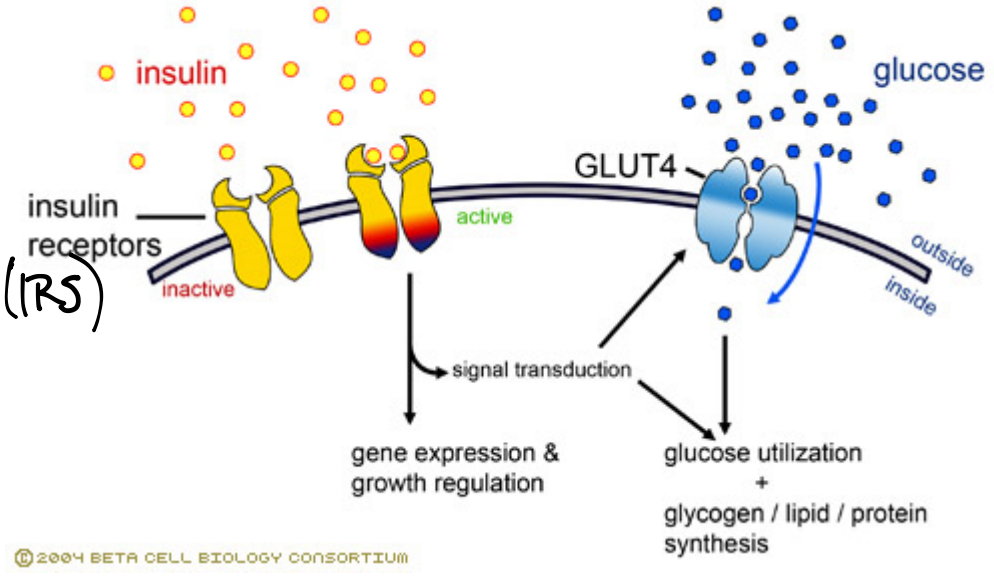


Signals to other kinases which then control glucose uptake

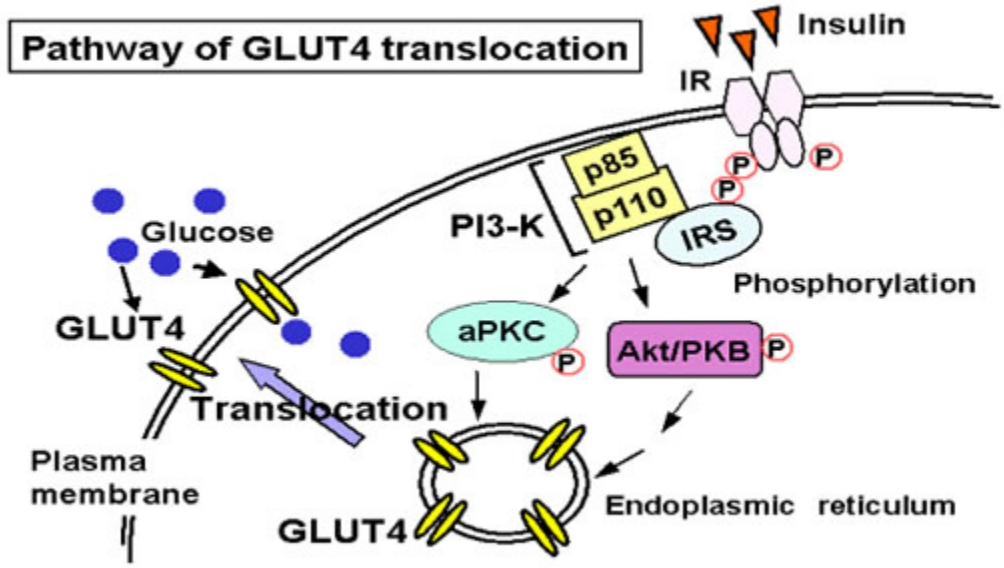
Growth/proliferation
Survival (antiapoptosis)
Cell motility
Glucose metabolism- stimulates movement of Glucose transporter proteins to cell membrane

regulate food metabolism

In skeletal muscle and adipose (fat) tissue insulin stimulates both gene expression & glucose uptake



Molecular mechanism of insulin-stimulated GLUT4 membrane fusion



GLUT4 storage vesicles

(allow glucose uptake into cell)

Insulin Therapeutics

Diabetes:

Type I- (juvenile)- destruction of beta cells of the pancreas that make insulin

Type II- Adult onset- often associated with obesity and insulin resistance and can involve autoimmunity to the insulin or receptor defects

Insulin actions:

Liver- The liver provides glucose during fasting therefore insulin stimulates the liver to store glucose in the form of glycogen

Skeletal muscle: depends on insulin for glucose uptake to provide energy

Adipose: insulin stimulates the conversion of glucose to fatty acids and storage as triglycerides (that's how sugar can cause obesity)

Diabetes results in many pathologies….

Acute:

•hyperglycemia resulting in glycosuria (sugar urine), osmotic diuresis and dehydration

•electrolyte depletion

•increased lipolysis stimulated by glucagon leading to ketone production in the liver → ketoacidosis

•abnormal respiration, coma, death

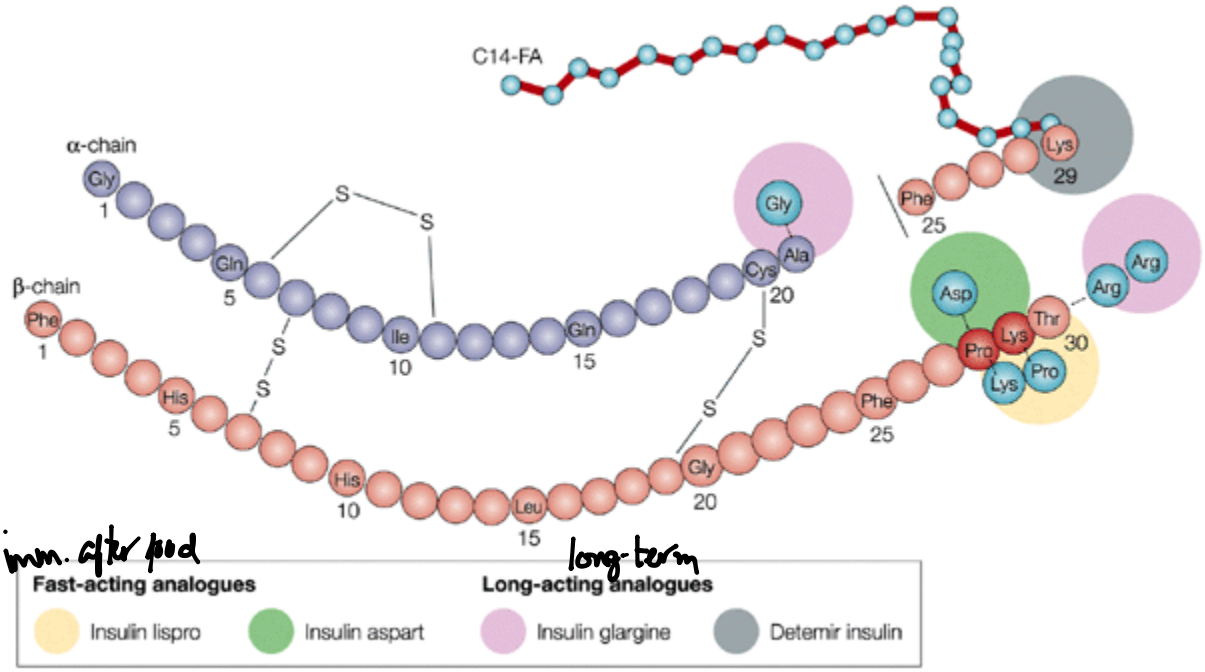
Chronic:

•Many vascular issues due to high blood glucose levels that result in glycosylated proteins

•Coronary artery disease

insulin that will handle spikes and long term glucose levels

Human Insulin and Insulin analogues



imm. after food

long-term

Pro-Lys switch

	B-28 Pro-Lys B29 Lys-Pro	B28 Pro-Asp	A21 Ala-Gly B-30 C-terminal Arg additions	B-30 removed A-29 FA addition
				Fatty acid
	Rapid	Rapid	Long-acting	Long-acting
Onset:	min	min	1hr	1-2hr

Post-prandial hyperglycemia

Maintenance
(slow release due to aggregation of molecules therefore dissociation is required)

Activation of RTKs can ultimately lead to cell division or differentiation.

Since RTKs can function as growth factor receptors they are potential oncogenes (cancer promoting).

Tyrosine Kinase Receptors & Cancer

- Many cancer cells have either increased numbers of Tyrosine Kinase receptors (amplification) or they have acquired a mutation that makes it constantly active (constitutive activity)

Examples:

EGF-Receptor (EGFR1) (mutated or overexpressed in many epithelial cancers)

Erb-B2 (EGFR-2/Her2/neu) – Receptor (amplified/mutant in many breast cancers)

also some brain cancers

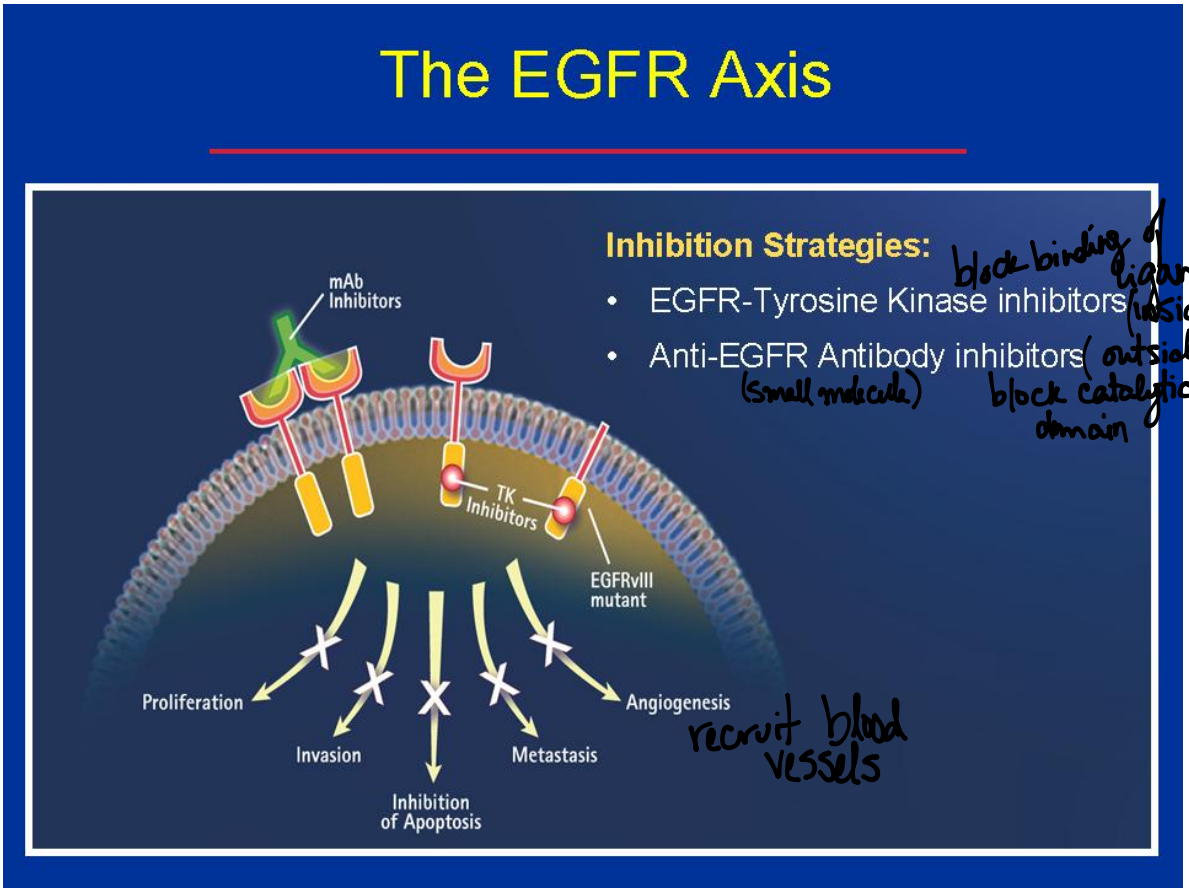
-B3 } -both of these receptors are

-B4 } heterodimer partners with -B2

About 15-20% of breast cancers overexpress HER2 (*neu*)

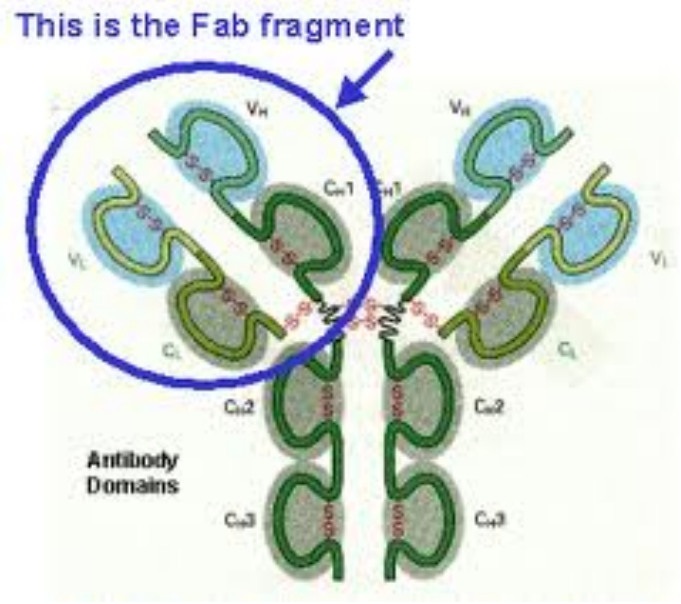
And other cancers overexpress other receptor tyrosine kinase types.

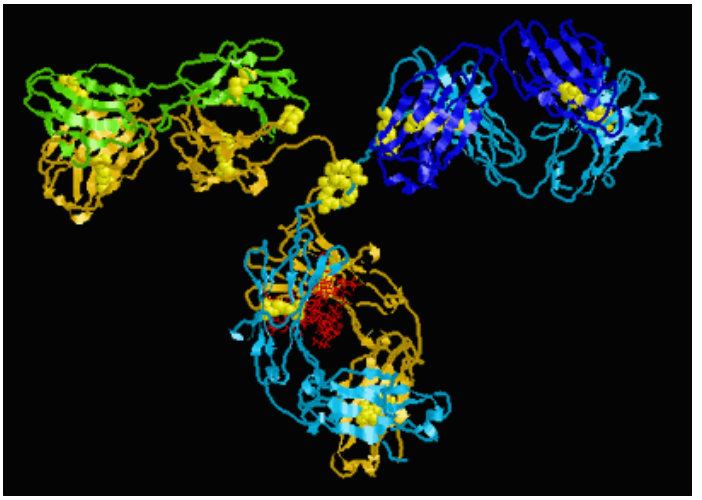
Therapeutic strategies to target the RTK family



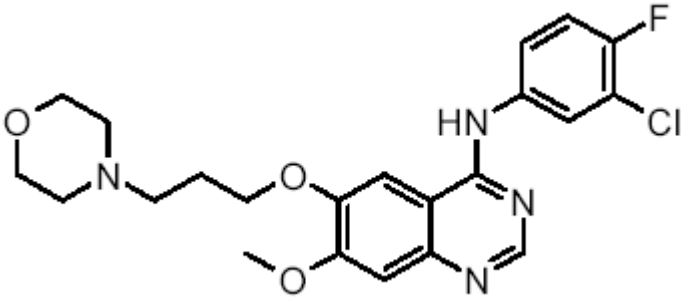
every event above needed for cancer cell to grow

~ 145,000 Da





RTK Monoclonal Antibody-based therapies



447 Da

Small Molecule Inhibitors: Gefitinib (Iressa™)

Inhibitors of RTKs can be monoclonal antibodies or small molecule drugs….

v. hard to get mol. for 1 catalytic domain

EGFR inhibitors in cancer therapeutics

Monoclonal antibodies	RTK Target	Cancer site
Trastuzumab (*Herceptin*, ~~Genentech~~)	ERBB2 (Her2-neu)	breast
Bevicizumab (*Avastin*, Genetech)	VEGFR	colorectal some lung cancers
Small molecules		
Imatinib (*Gleevec*, Novartis)	PDGFR & others	some leukemias, GI tumors
Erlotinib (*Tarceva*, Genentech)	EGFR	some lung cancers
Gefitinib (*Iressa,* Astra-Zeneca)	EGFR	some lung cancers

most successful

monoclonal antibody

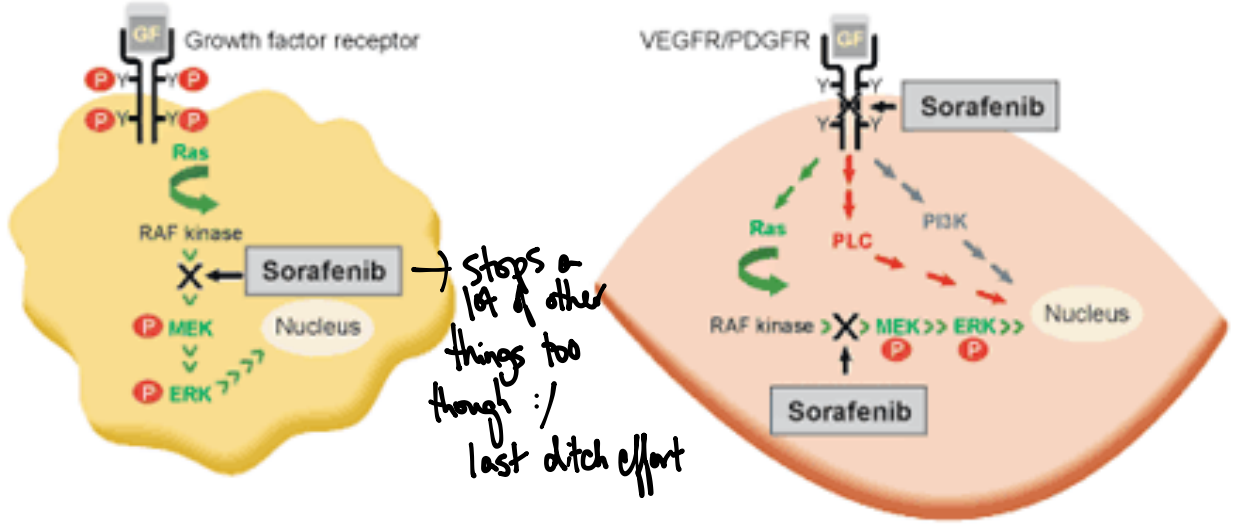
not to remember

Sm. mol. inhibitor

successful = ↑ survival time, not curing disease, used in chemo + ↓ chemo time

Sorenfenib blocks the Raf kinase (and VEGFR and PDGFR)

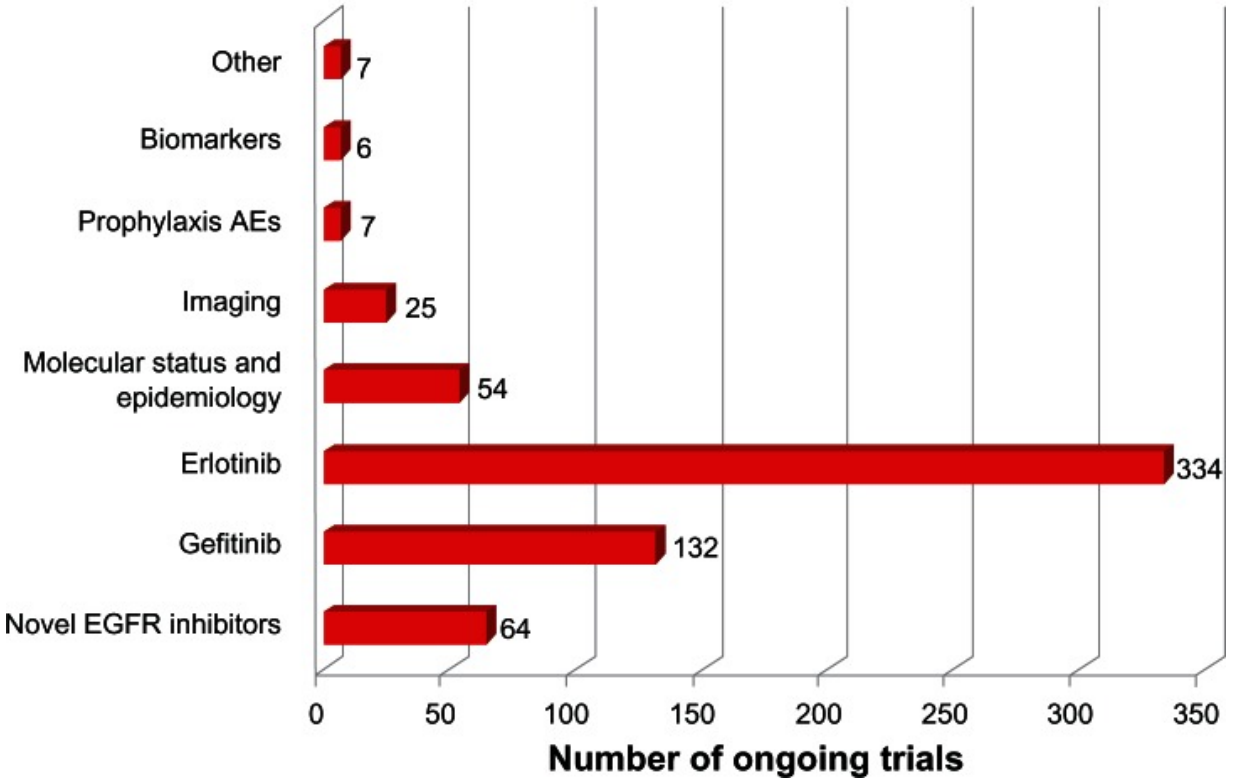
bottleneck for Tyr-Kin receptor



stops a lot of other things too though :/ last ditch effort

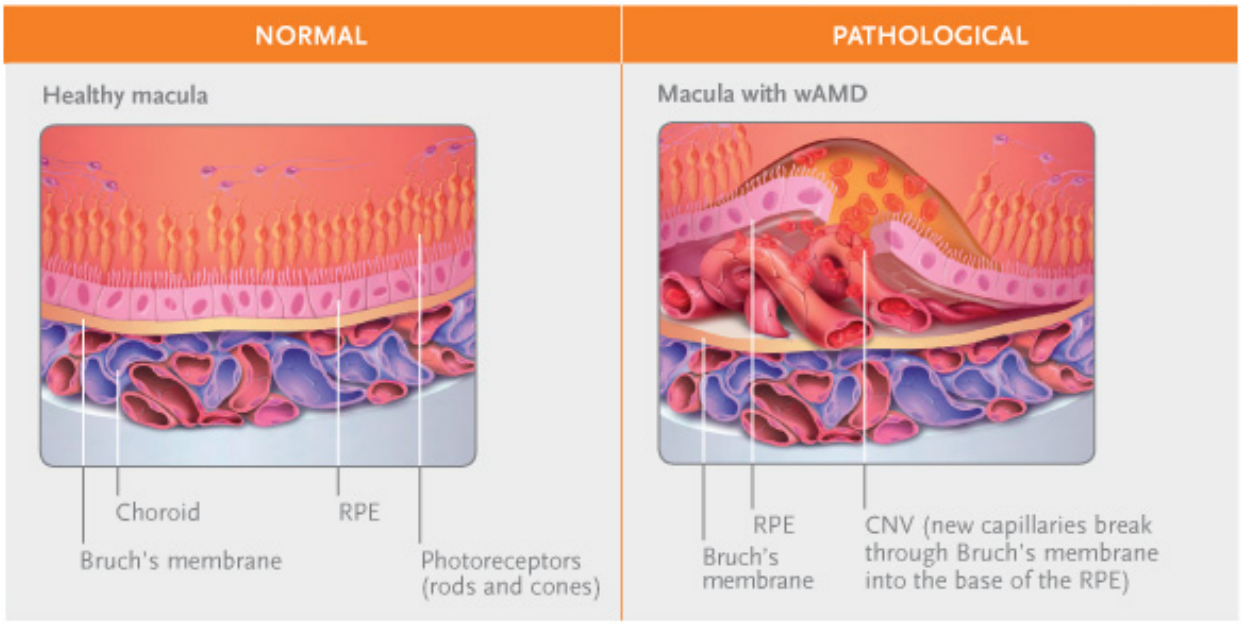
BAY 43-9006 inhibits tumor cell proliferation by targeting the RAF/MEK/ERK pathway at the level of RAF kinase.

BAY 43-9006 exerts an antiangiogenic effect by targeting the receptor tyrosine kinases VEGFR-2 and PDGFR and their associated signaling cascades.



Onco Targets Ther. 2013; 6: 563–576.

Vascular endothelial growth factor (VEGF) can cause “Wet Macular Degeneration” and loss of central vision



CNV, choroidal neovascularization; RPE, retinal pigment epithelium; VEGF, vascular endothelial growth factor; wAMD, neovascular (wet) age-related macular degeneration

The macula is in the center of the retina (the layer of tissue on the inside back wall of your eyeball). Wet macular degeneration is a chronic eye disease that causes vision loss in the center of the field of vision caused by abnormal blood vessels that leak fluid or blood into the region of the macula.

Which RTK inhibitor would you prescribe???

Avastin

Receptor Downregulation

-bound ligand results in endocytosis of tyrosine kinase receptors → eventual degradation

↓

if degradation excedes synthesis

↓

net receptor down regulation

attenuation of cellular response