Signaling

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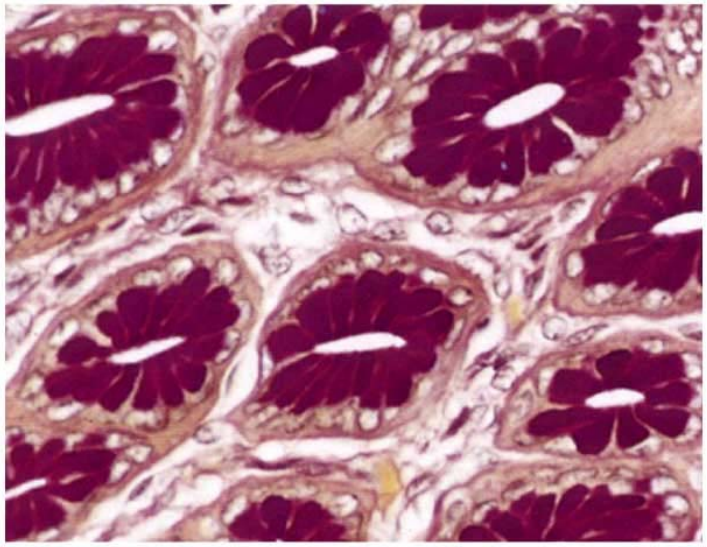
Growth Factor Receptors and Other Signaling Pathways

What we will cover today:

- -How signaling proteins recognize upstream and downstream partners
- -How this process has been altered in cancer cells
- -Mechanisms of initiating signals through cellular GF receptors

Cell-Cell Communication

- Maintenance of tissue architecture (cell type, stage of differentiation, cell number)
- Response to stimuli or changes in the status quo
  - Control of cell growth (#)
  - Control of motility



Stomach epithelium: mucus-secreting cells

Figs 2-6C The Biology of Cancer (Garland Science 2007)

Turning on Proliferation in Normal Tissues

- Normal cells (and tissues) have capacity to proliferate (and to stop proliferating) when appropriate
- How is the decision to proliferate communicated?

Signal Transduction

Relay of signal from extracellular environment to interior of cell

- Relay very fast
- Usually through series of protein-protein interactions
- Frequently involves phosphorylation of signaling proteins
- Different messages use different pathways

Signaling Pathways

- Growth factor receptors (GFRs): proliferation
- Wnt/β–catenin: differentiation, proliferation
- Integrins: cell-cell contact, adhesion
- NFKB: proliferation, blocks apoptosis
- Notch: cell fate determination

Generic Signaling Pathway

Ligand (extracellular)

↓

Receptor (cell surface)

↓

Series of signal transducers (cytoplasmic)

↓

Transcription factors (nuclear)

↓

Gene targets (nuclear)

Growth Factor Receptor Signaling Pathway(s)

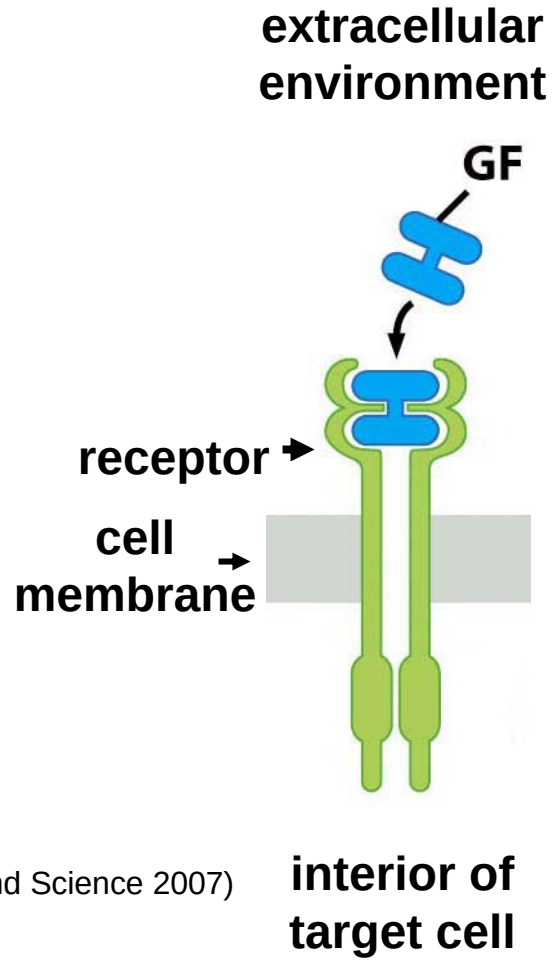
Autocrine, Paracrine and Endocrine Signals

Refer to signaling paths of hormones or factors released by one cell and acting upon:

- the same cell - ?
- a nearby cell in the same tissue - ?
- a cell in a distant tissue - ?

Initiation of the Proliferation Signal in Normal Tissues

- ***Growth factors* (GFs)**: proteins released by one cell that signal to another cell (target cell) that it is time to divide
- Receptor on target cell = sensor (detects growth factors in the extracellular environment)
- Receptor transfers information from growth factor to interior of cell



Figs 5-12 The Biology of Cancer (Garland Science 2007)

Activation of Growth by PDGF Signaling Pathway in Normal Cells

- Platelet derived growth factor (PDGF): potent attractant and *mitogen** for fibroblasts
- Wounding assay (normal cells): monolayer of fibroblasts, before (L) and after (R) PDGF
- Bottom: same, except cells are PDGF receptor-negative

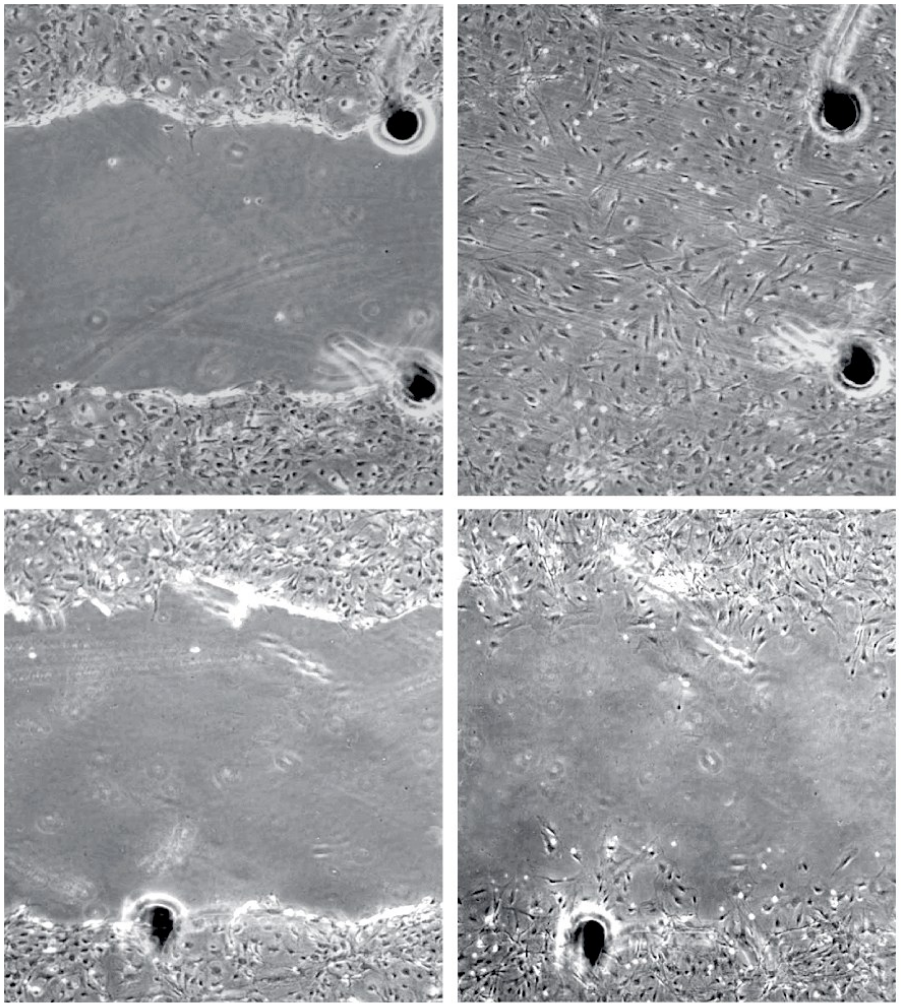
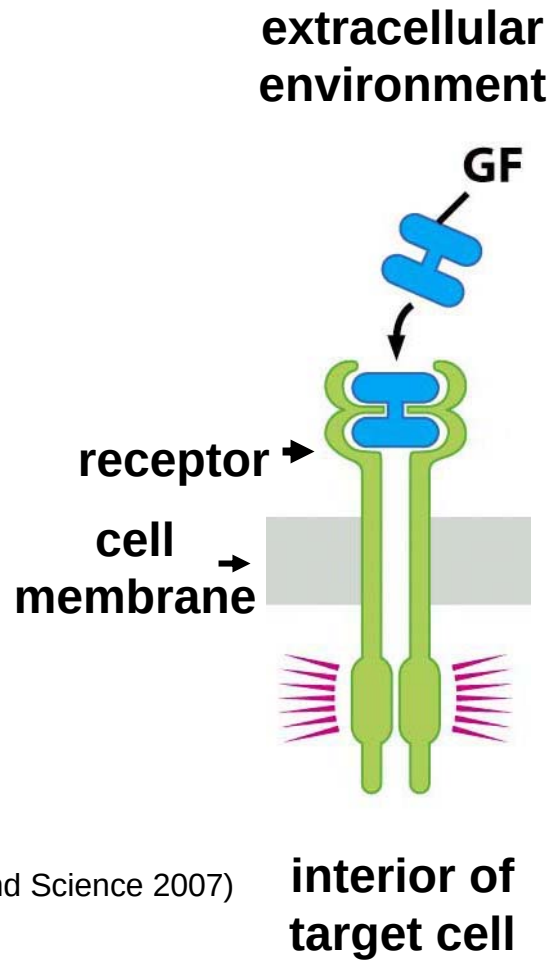


Figure 5-4a The Biology of Cancer (© Garland Science 2007)

**Mitogen*: an agent that induces cell proliferation

Initiation of the Proliferation Signal in Normal Tissues (cont'd)

- *Growth factors* (GFs): proteins released by one cell that signal to another cell (target cell) that it is time to divide
- Receptor on target cell = sensor (detects growth factors in the extracellular environment)
- Receptor transfers information from growth factor to interior of cell



Figs 5-12 The Biology of Cancer (Garland Science 2007)

Growth Signaling in Normal Cells: Phosphorylation of Receptors

- Certain amino acids within proteins can be modified by covalent attachment of phosphate group (PO_4): *phosphorylation* alters activity
- *Kinases*: enzymes that catalyze phosphorylation
- Most protein phosphorylation at serine and threonine
- Phosphorylated tyrosine (p-tyr) rare: first identified in studies with Src

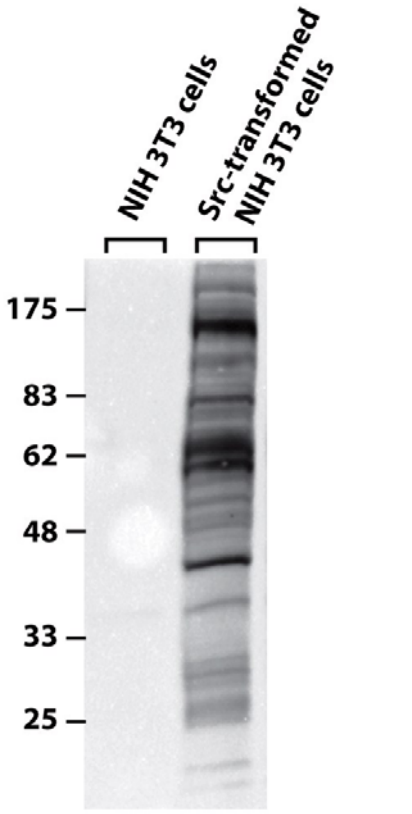
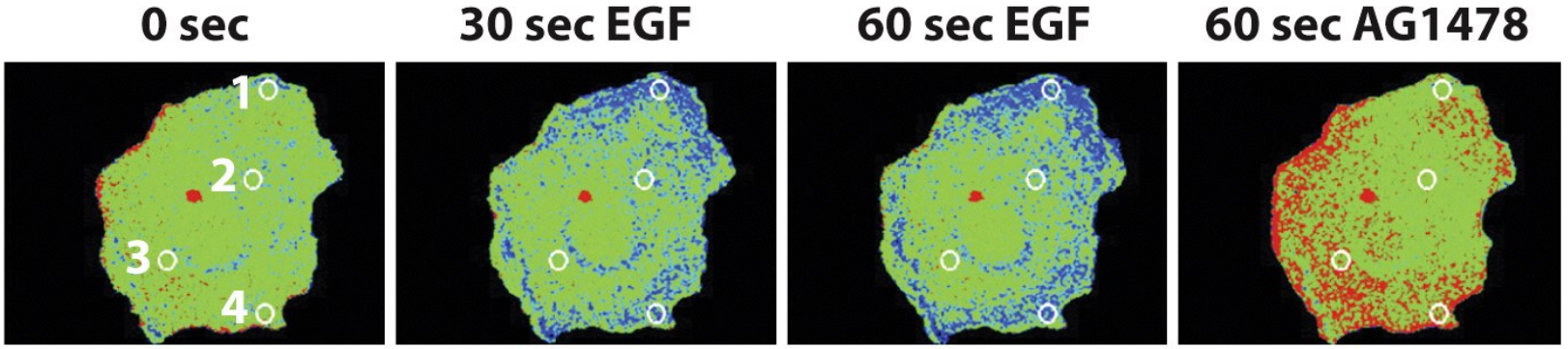


Figure 5-7a The Biology of Cancer (© Garland Science 2007)

P-tyr-containing proteins in extracts of normal or Src-transformed cells

Growth Signaling in Normal Cells: Tyrosine Phosphorylation

- P-Tyr: mitogenic signal
- GFRs frequently have tyrosine kinase activity
- Tyrosine phosphorylation occurs extremely quickly after exposure to growth factors
- Receptor itself frequently phosphorylated on tyrosine



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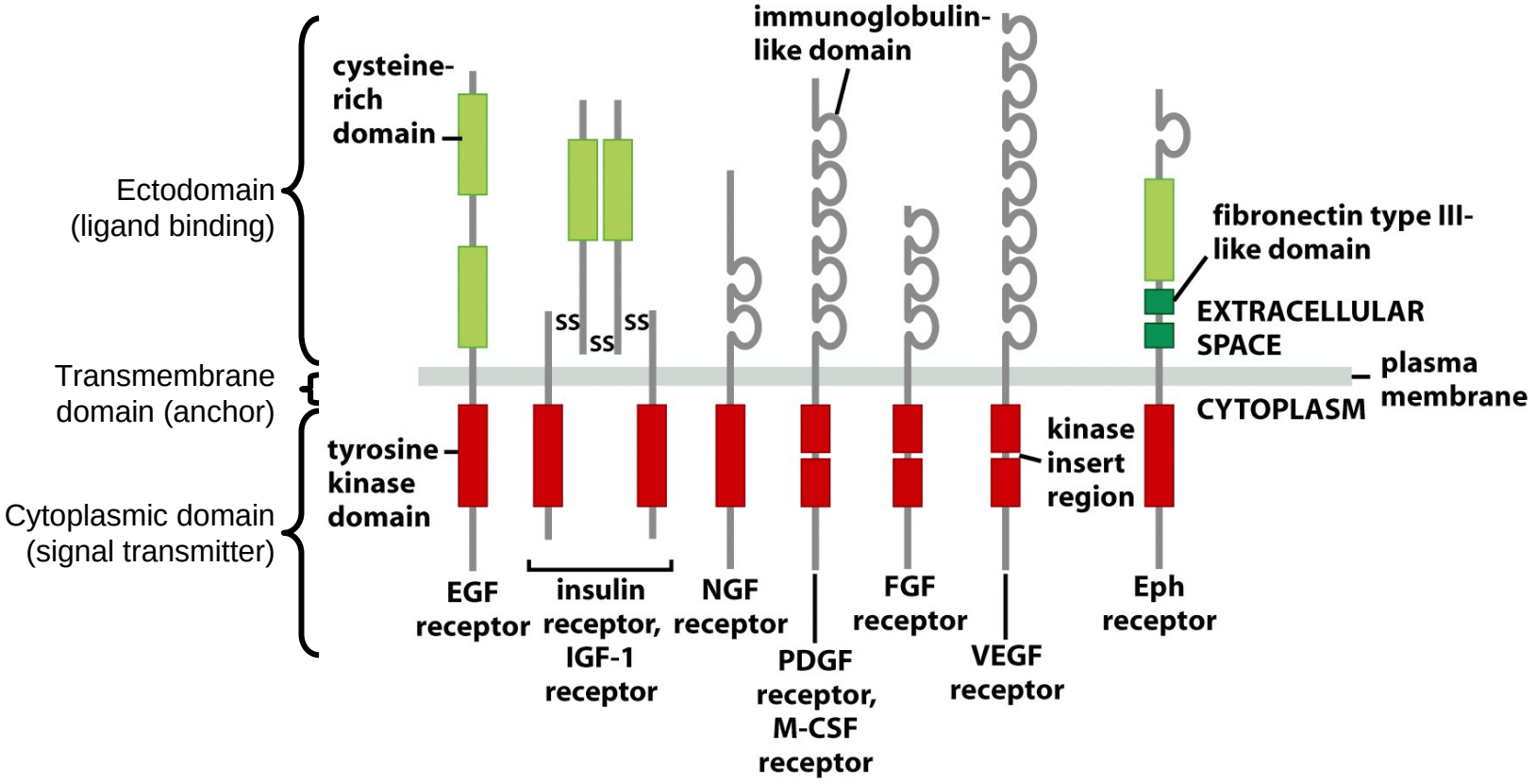
Blue: overexpression of pTyr-EGFR; red: underexpression

Receptor Tyrosine Kinase Families

Structure of RTK families



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How Are Receptors Activated?

Simple Dimerization Model for Receptor Activation

- GF dimer binds to receptor monomer
- Complex moves through membrane until it encounters another receptor monomer
- GF dimer acts as bridge to dimerize the receptor
- Dimerization induces phosphorylation of Tyr on receptor
- Catalytic site in TK domain exposed, allowing access to substrates (the next contact in the signal relay) and/or other sites on receptor partner

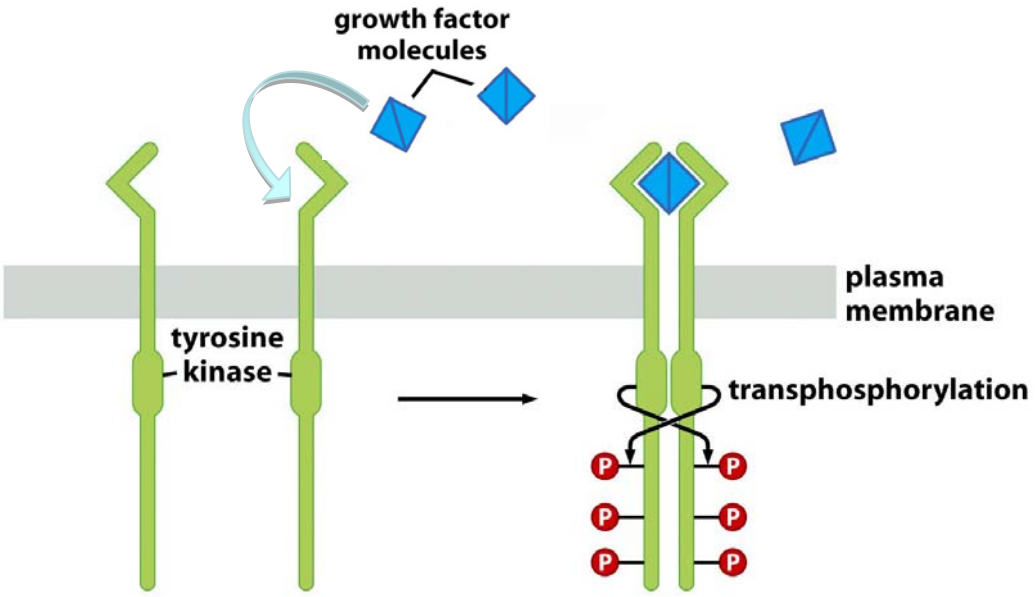


Figure 5-15 The Biology of Cancer (© Garland Science 2007)

Mechanisms of Disrupting Controlled Growth Factor Signaling in Cancer…..
At the Level of the Receptor GF Interaction

Autocrine Signaling

- Cancer cells release growth factors into immediate extracellular environment
- GF binds to receptors on same or neighboring cells

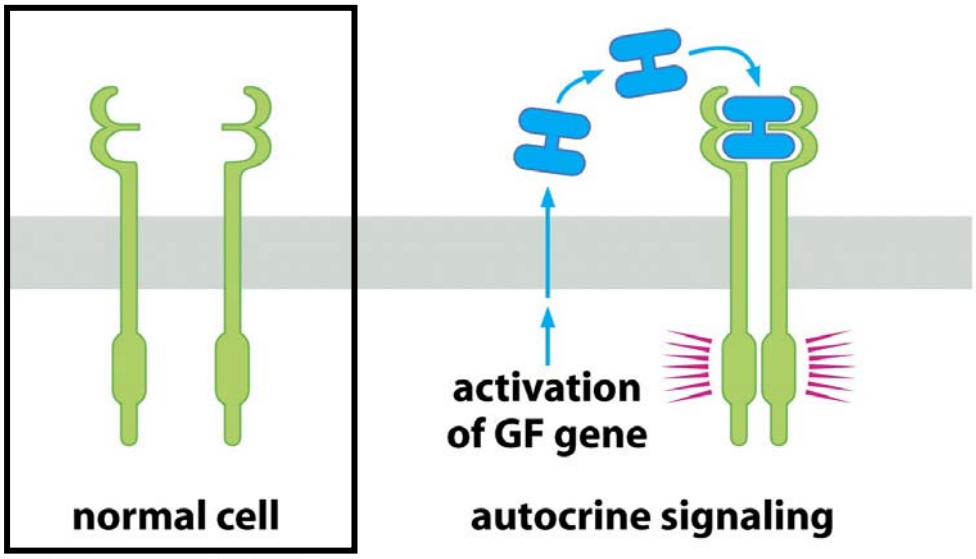


Figure 5-12b The Biology of Cancer (© Garland Science 2007)

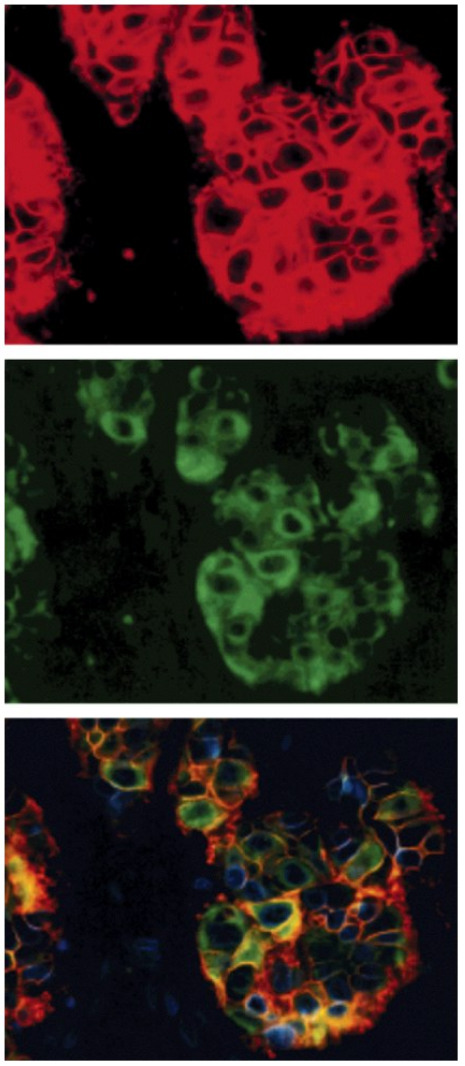
Autocrine Growth Loops in Tumor Cells

- Dangerous for tissue organization: cell controls its own proliferation independent of normal growth signals
- How?
  - Cell type naturally expresses the GF receptor: during transformation, growth factor gene activated
  - Or converse: receptor expression activated in cells already producing the growth factor

Autocrine Signaling in Breast Cancer

Successive sections of invasive human breast cancer stained for
- Red: epidermal growth factor receptor (EGFR)
- Green: ligand of EGFR (TGF-α)

- Cells that express both receptor and ligand appear yellow when images superimposed



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Autocrine GFs Produced by Many Types of Human Tumors

- PDGF produced by some sarcomas (PDGF-R normally on fibroblasts)
- >1 autocrine growth loop possible: extreme case: Kaposi's sarcoma tumors
  - 7 different cellular GFs and their receptors
  - GFs for two additional autocrine loops contributed by HHV-8 (the virus associated with Kaposi's sarcoma)

Growth Factor-Independent Signaling

- Mutations that enhance dimerization lead to enhanced signaling, such as…

1. Higher concentration of GFRs on surface of cancer cells than on normal cells
   - Elevated transcription
   - Gene amplification
   - Decreased cycling away from surface
2. Structural alterations in GFR

Deregulation of Receptor Firing

- Mutations (fusions, deletions, point mutations) in receptor protein

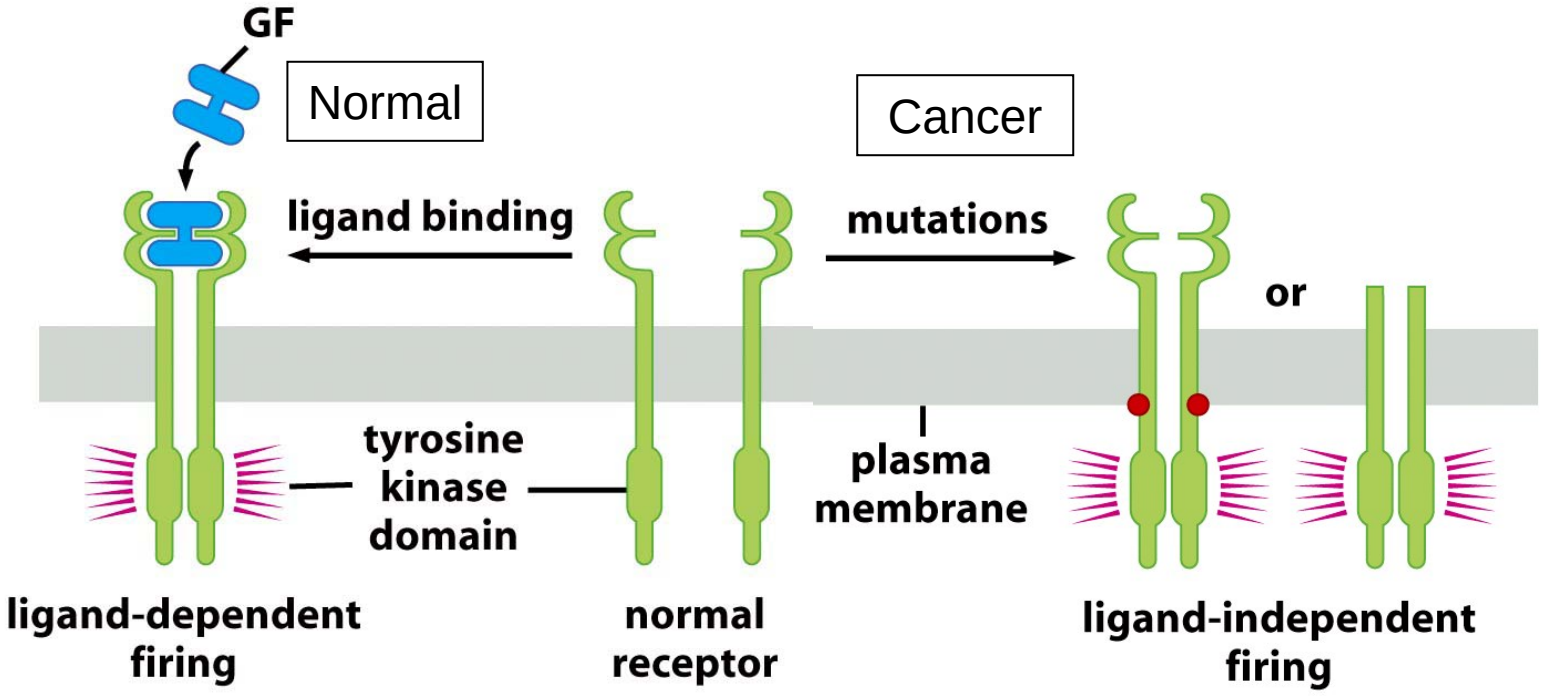
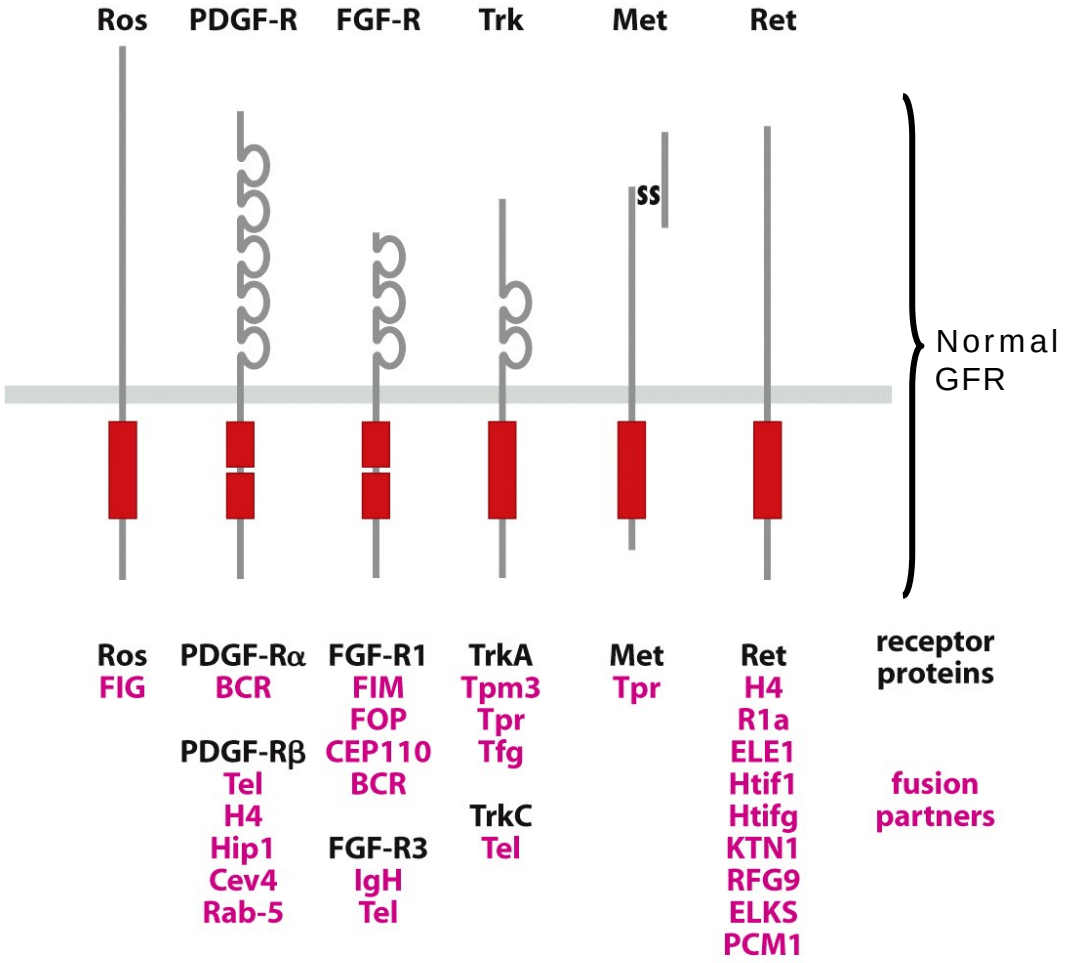


Figure 5-12a The Biology of Cancer (© Garland Science 2007)

Gene Fusion Causing Constitutively Dimerized Receptors in Tumor Cells

- Fusion of TK domain of receptor genes (black type) to unrelated genes encoding proteins that normally form dimers or oligomers (pink type)
- Hybrid protein dimerizes with itself, relays signal



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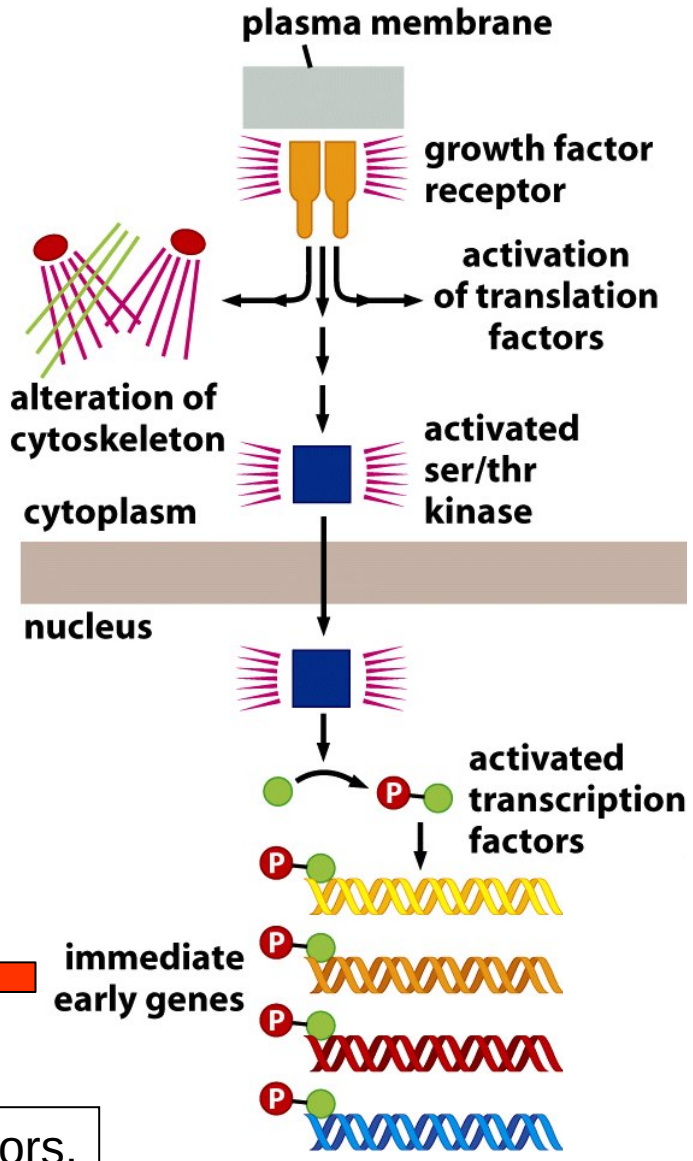
Signal Transducers in Normal Cells

Mitogenic Signaling Cascade



Amplification of signal

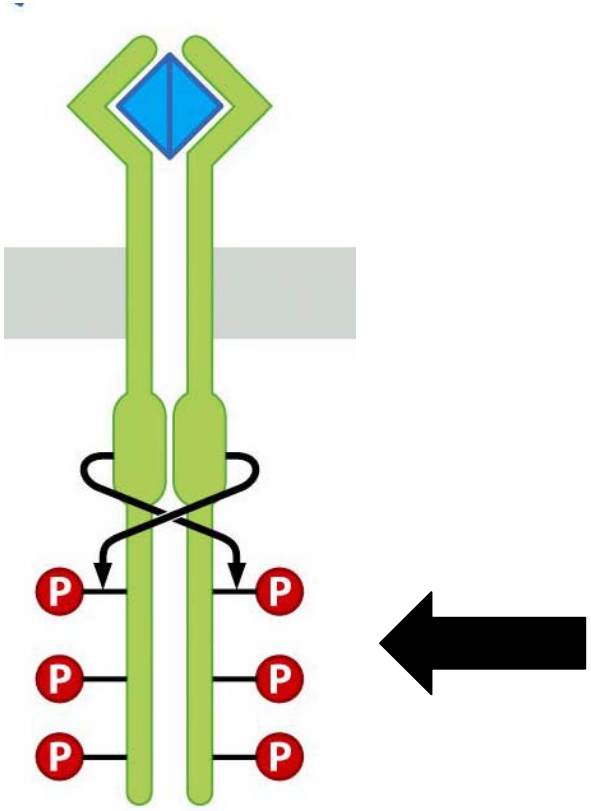
50-100 genes expressed within 30 min

Encode transcription factors, cytoskeletal proteins

Growth, motility, survival

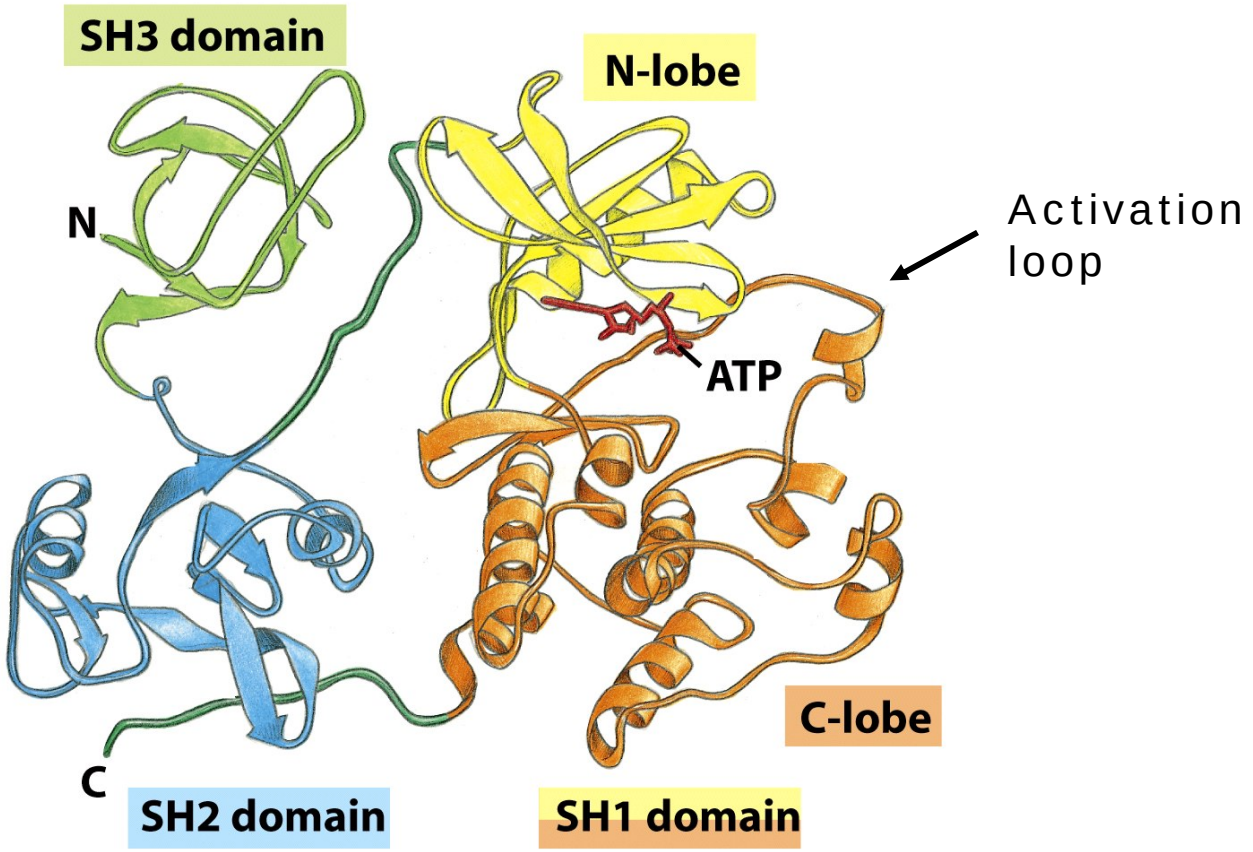
How are Signals Relayed from receptor-GF to Intracellular Destination?

Signal Relay



- Intracellular protein docks on activated receptor
- Intracellular protein relays the signal to next partner

Src Kinase as Model for Signal Transducing Molecule



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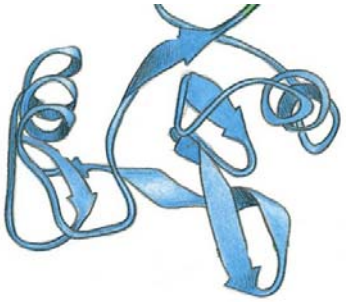
Structure of Src protein

SH2 Interacts with p-Tyr Proteins

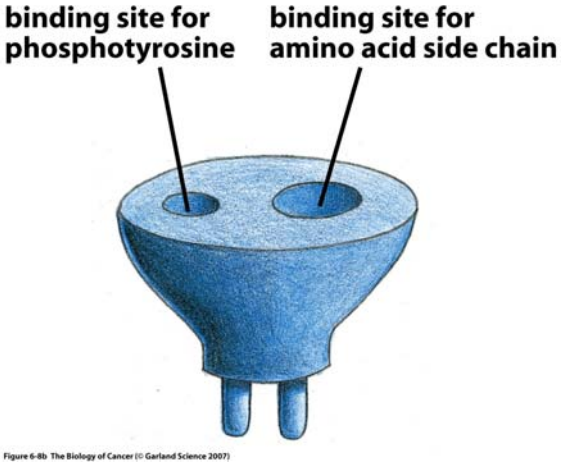


SH2 domain

- >100 distinct SH2 domains, each part of different protein
- All SH2 domains bind p-Tyr followed by 3-6 amino acids embedded within partner protein at its C-terminal site
- Each SH2 domain specific for different set of 3-6 amino acids

- SH2 domains found on variety of signaling proteins always in combination with other domains

➢ SH2 is independent module



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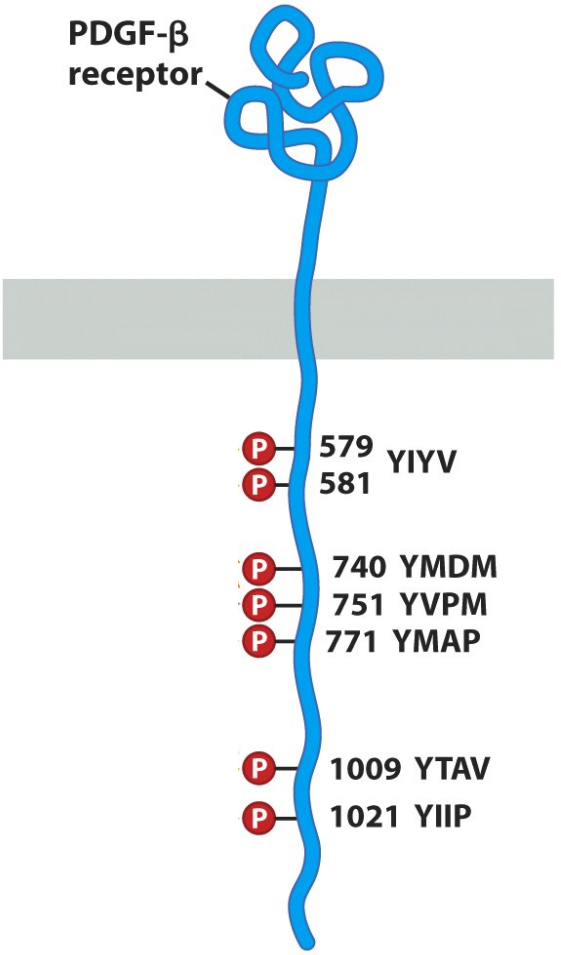
Docking of Signal Transducing Proteins on pTyr-Receptors

- SH2-containing proteins home to activated receptor at specific sites
- SH2-containing proteins tethered to receptor might be substrates for the RTK, and/or
- Localization may be more important: tethered SH2 proteins interact with other membrane-associated proteins or phospholipids at higher rate than if they weren't in close proximity



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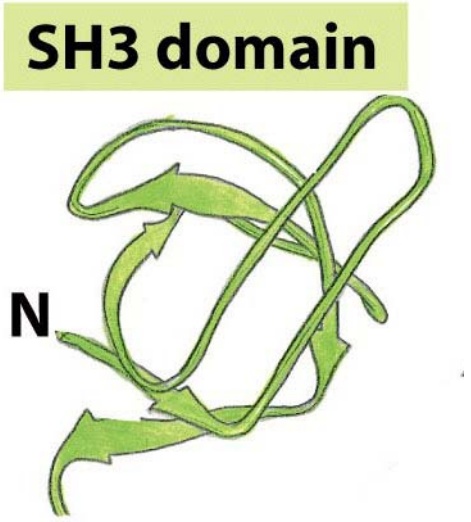
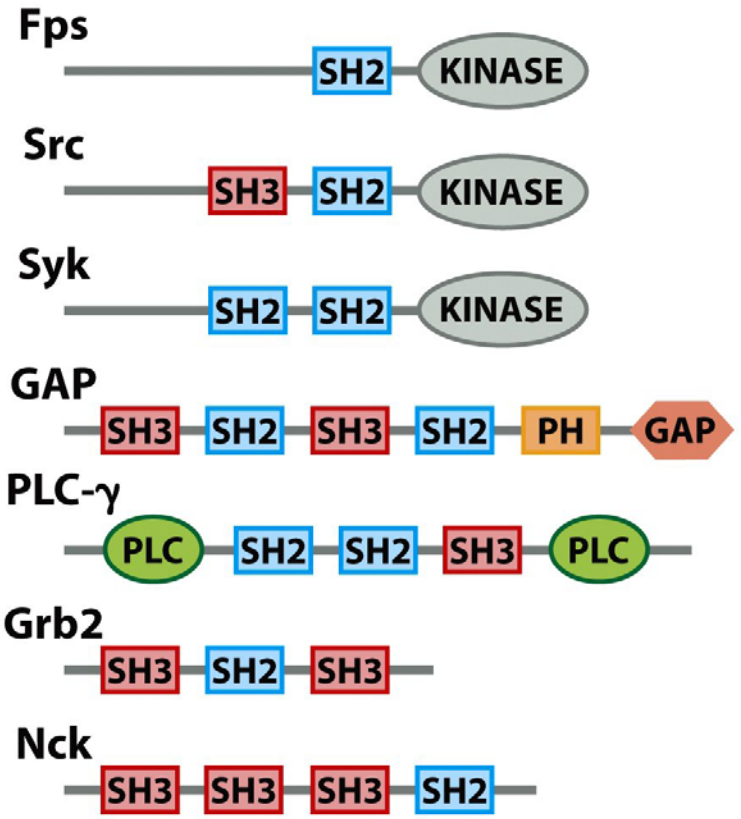
SH3 Domain Interactions

- > 250 genes encode proteins with SH3 domains
- SH3 domains bind to proline-rich sequences in partner proteins
- ➢ SH3 acts as independent module



Protein Interaction Domains as Modules

- Most signaling molecules interacting with RTKs have ≥1 SH2 domain (some also have ≥1 SH3 domain)
- Some signaling molecules have enzyme activities
- Some have no catalytic function and act as bridges (*adaptor* proteins)



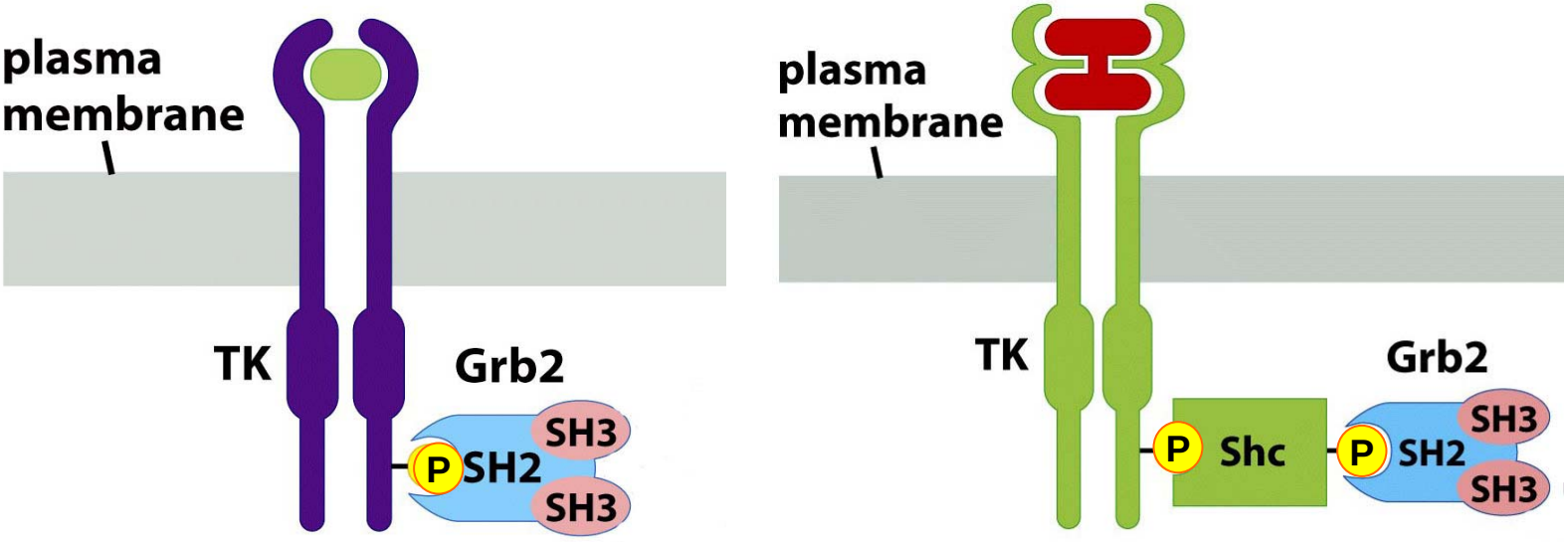
Rectangles: protein-protein binding modules; Oblongs: catalytic domains

Binding Domains Associated with Mitogenic Signals

Name of domain	"Ligand"
SH2	pTyr plus 3-6 amino acids at C-terminal side
PTB	pTyr plus 3 amino acids at the N-terminal side
SH3	proline-rich
14-3-3	phosphoserine
Bromo	acetylated lysine
PH (pleckstrin homology)	membrane phospho-inositides (eg, PIP3)

Early Steps of Mitogen Signaling Pathway

- Grb2 and Shc are adaptors linking receptors to the next signal in relay



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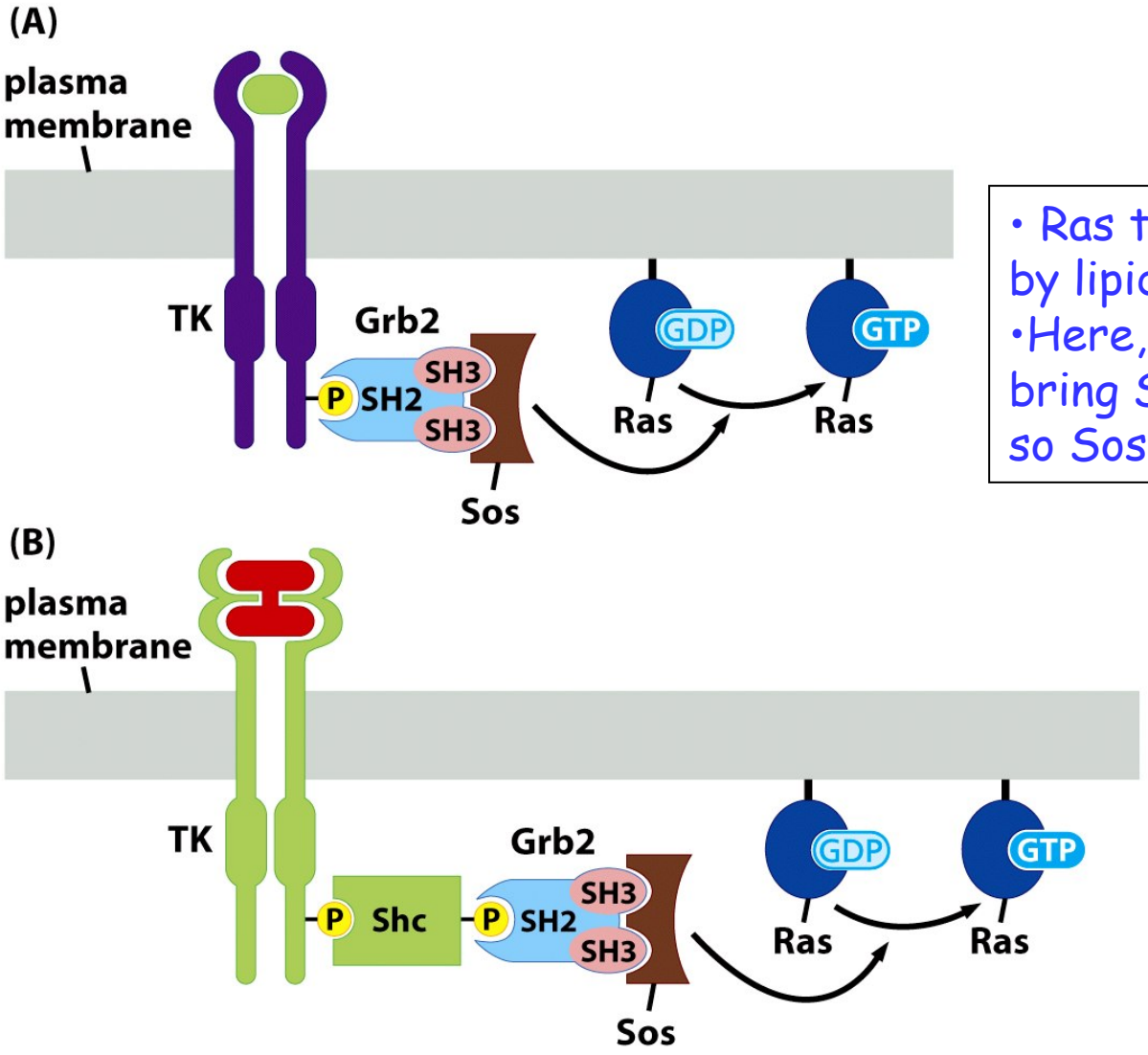
Growth Factor Receptor Signal Relayed To Ras



- Ras tethered to membrane by lipid tail
- Here, role of adaptor is to bring Sos close to membrane so Sos can activate Ras

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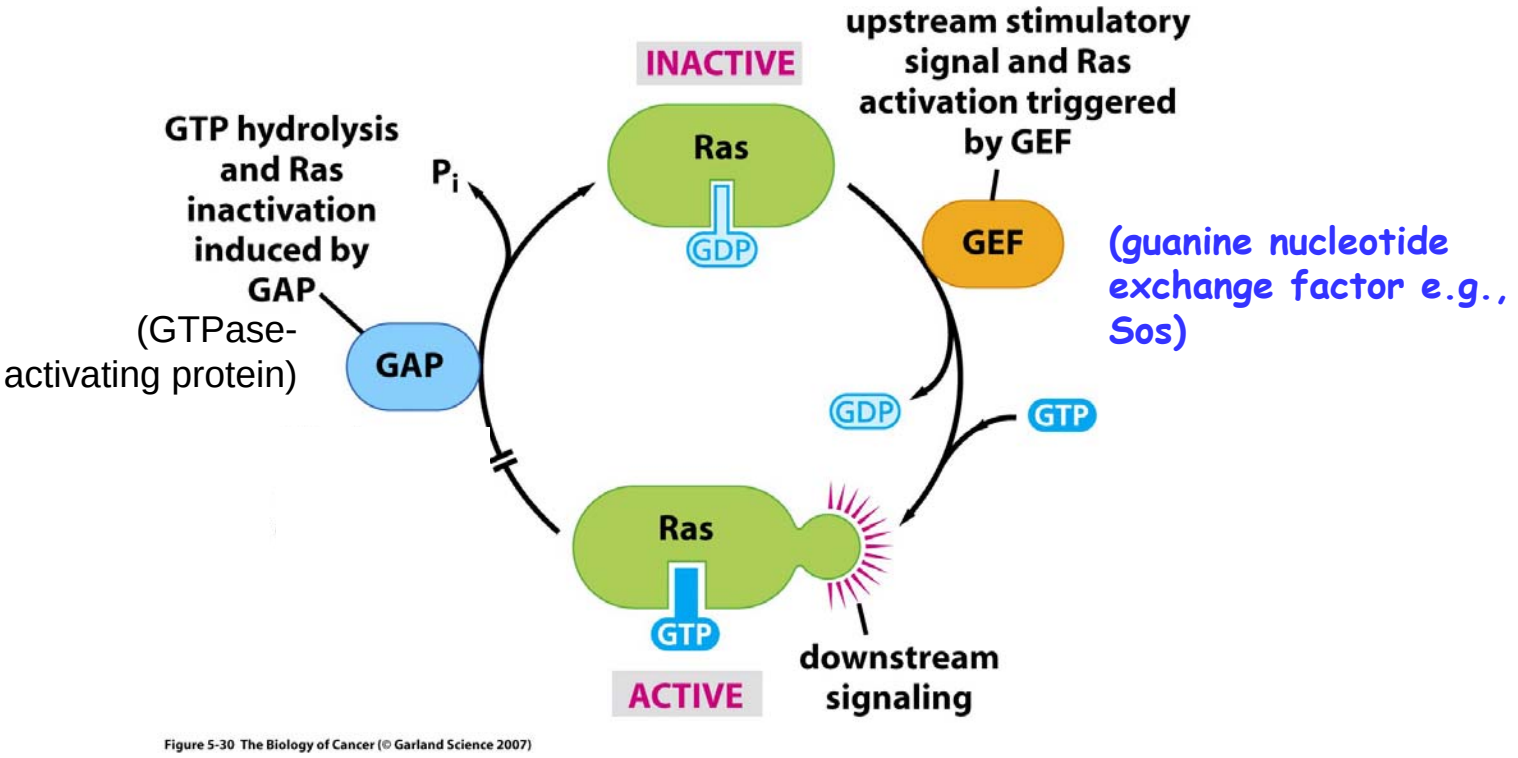
Ras Activation is Controlled by a Binary Switch



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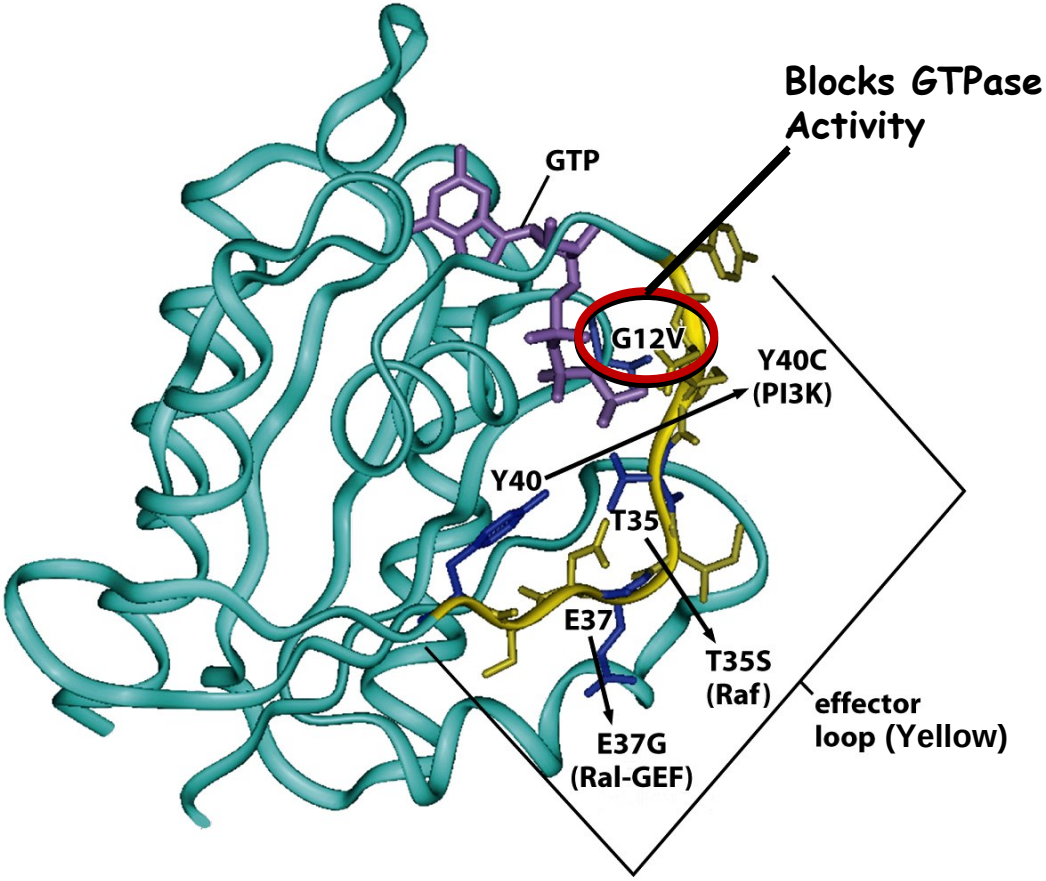
GDP = inactive
GTP = active

GTP-bound Ras Transmits Signal to downstream partners.

Activated Ras Structure

- GTP-bound Ras exposes effector loop for binding to downstream partners
- Binding to Ras tethers partners to membrane and/or induces conformational changes in partners that activates them



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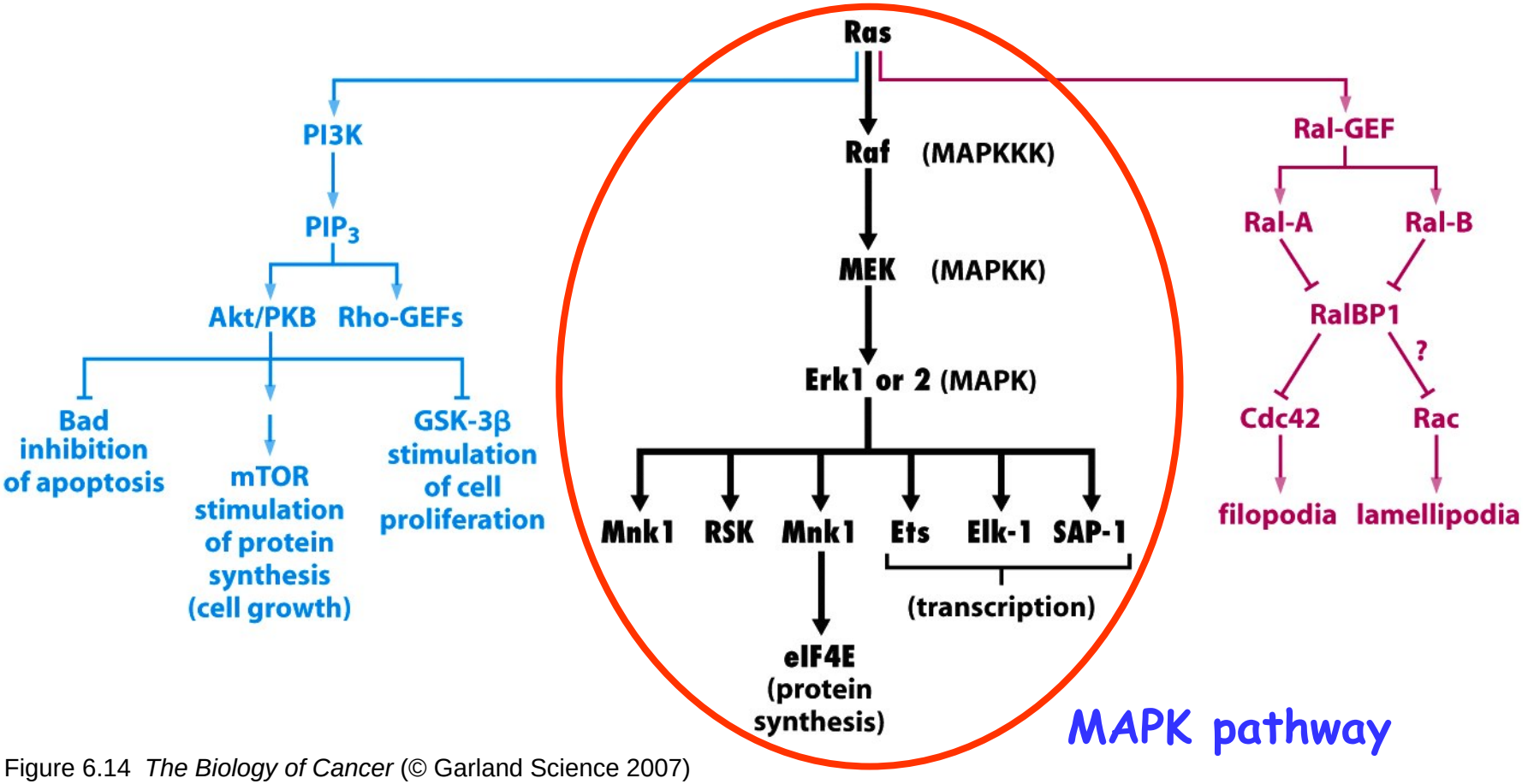
Ras Signaling can be Relayed down Three Different Pathways



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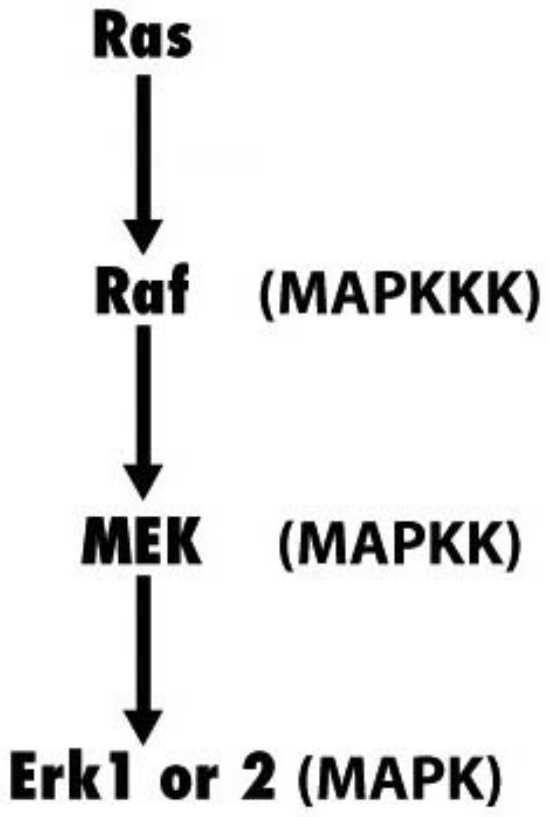
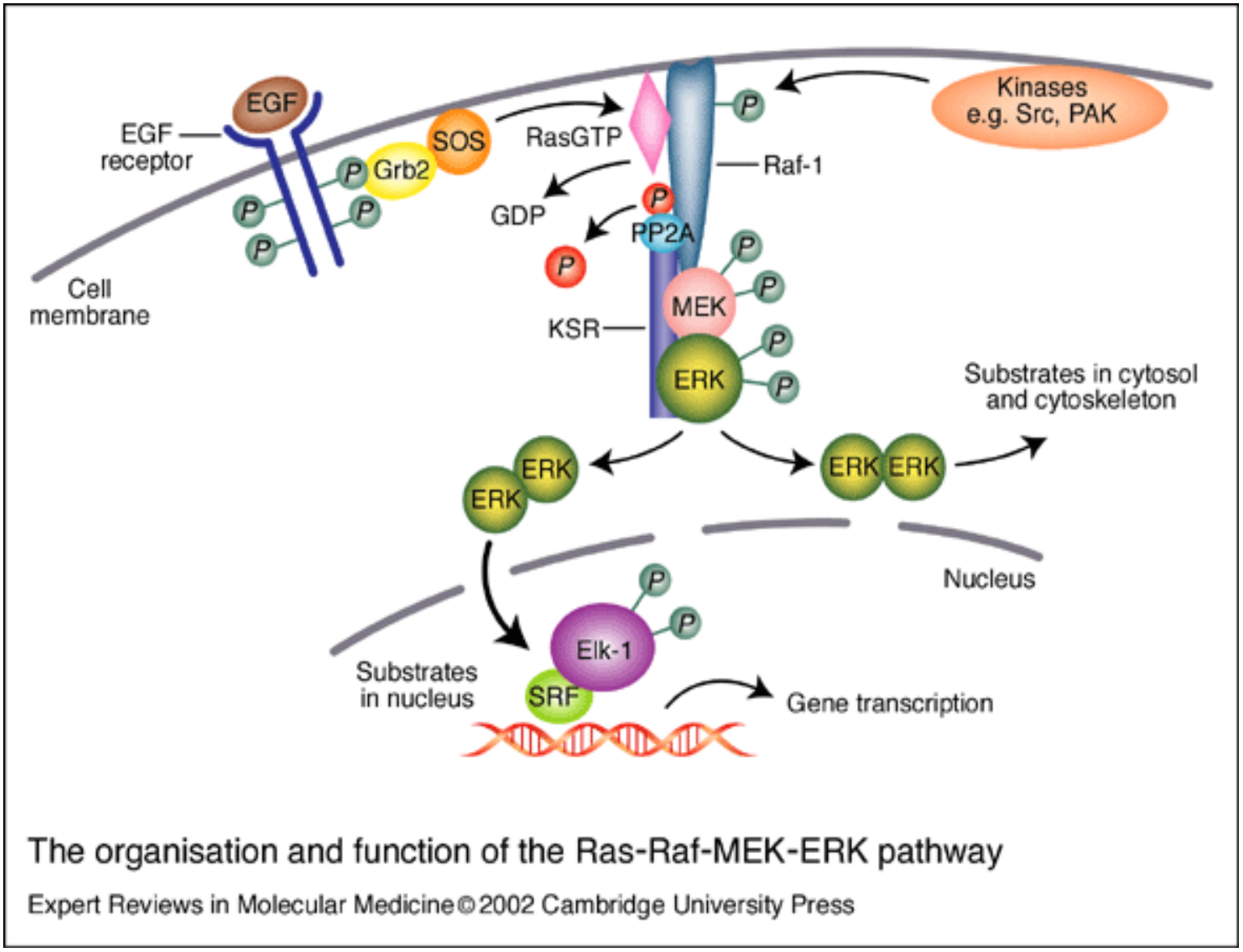
Mitogen Activated Protein Kinase (MAPK) Signaling Pathway



- MAPK pathways highly conserved (not just proliferation signal)
- 3-tiered signaling unit
- Raf is a ser/thr kinase
- MEK is a dual-specific kinase (both ser/thr and tyr are substrates)

The MAP Kinase Pathway

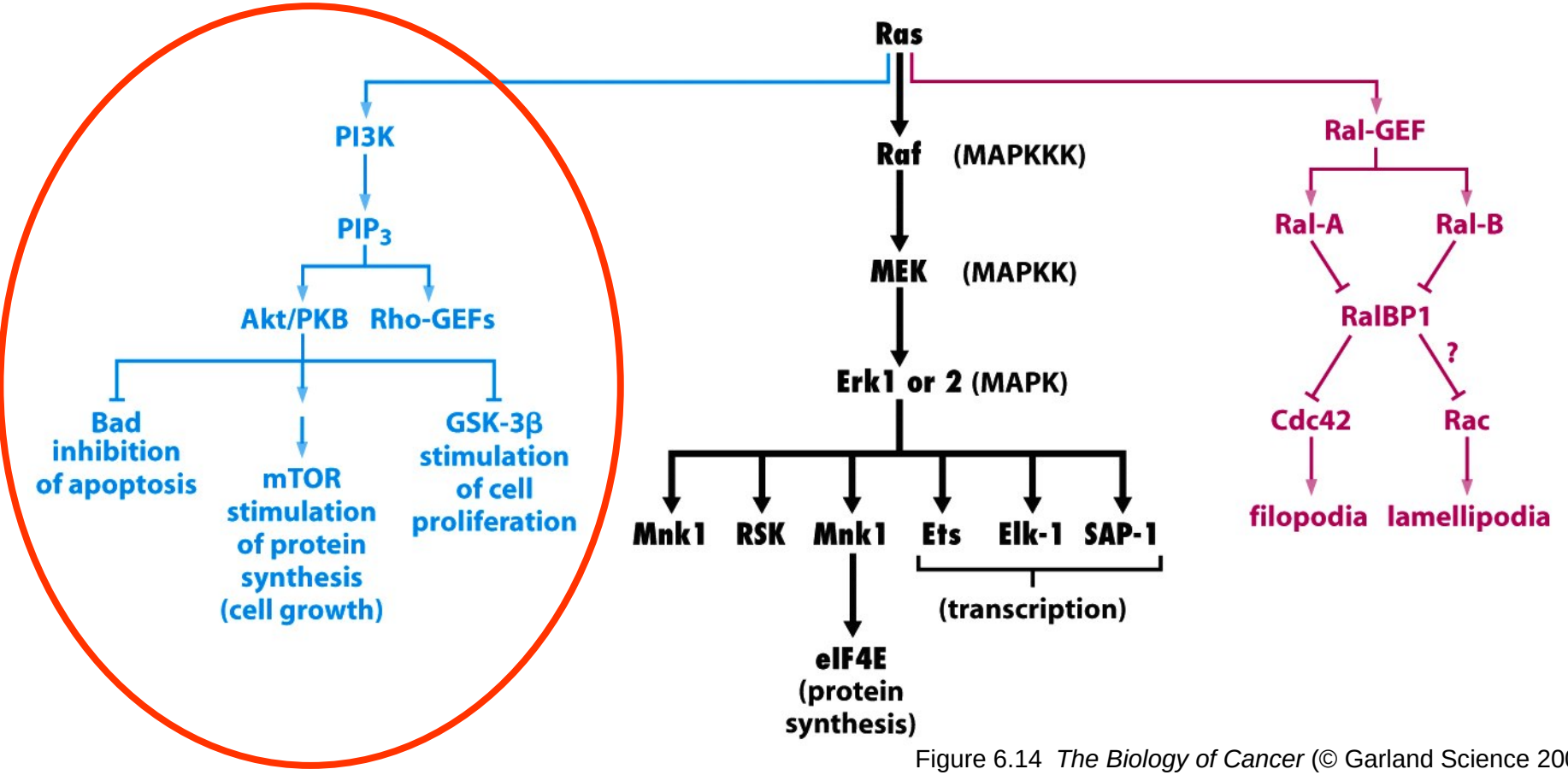


The organisation and function of the Ras-Raf-MEK-ERK pathway

Activities of Erk

- Phosphorylates cytoplasmic substrates
- Translocates to the nucleus and phosphorylates transcription factors (Ets, Elk-1, SAP-1)
- Phosphorylates a kinase that in turn activates protein synthesis machinery
- Phosphorylates other downstream kinases that activate transcription factors
  - TFs activate growth promoting genes and contribute to anchorage independence

Ras Pathway Controlling Inositol Lipids and Akt/PKB Kinase



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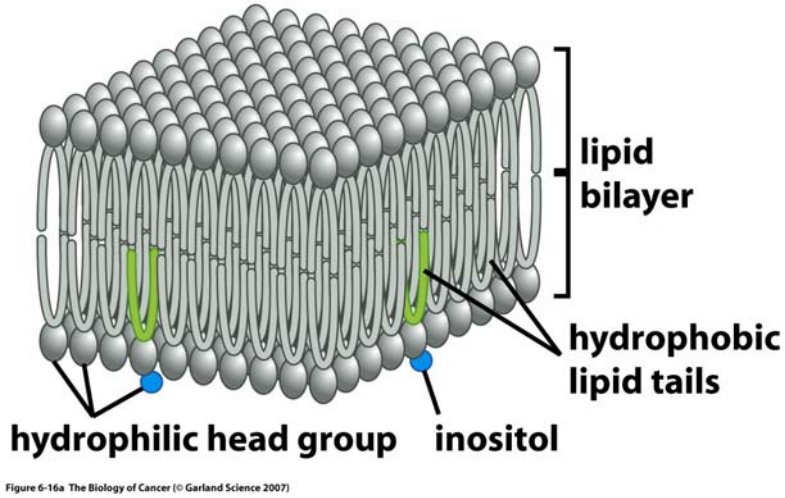
Inositol Lipids and Mitogenic Signaling

- Phospholipids can have more than just structural function in membrane: cytoplasmic "head" of phospholipid can bind inositol



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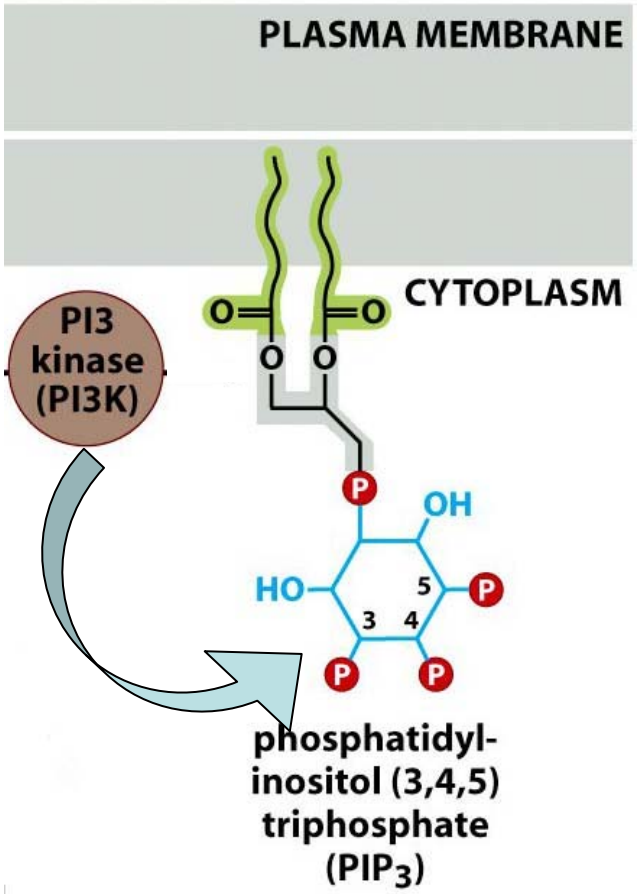
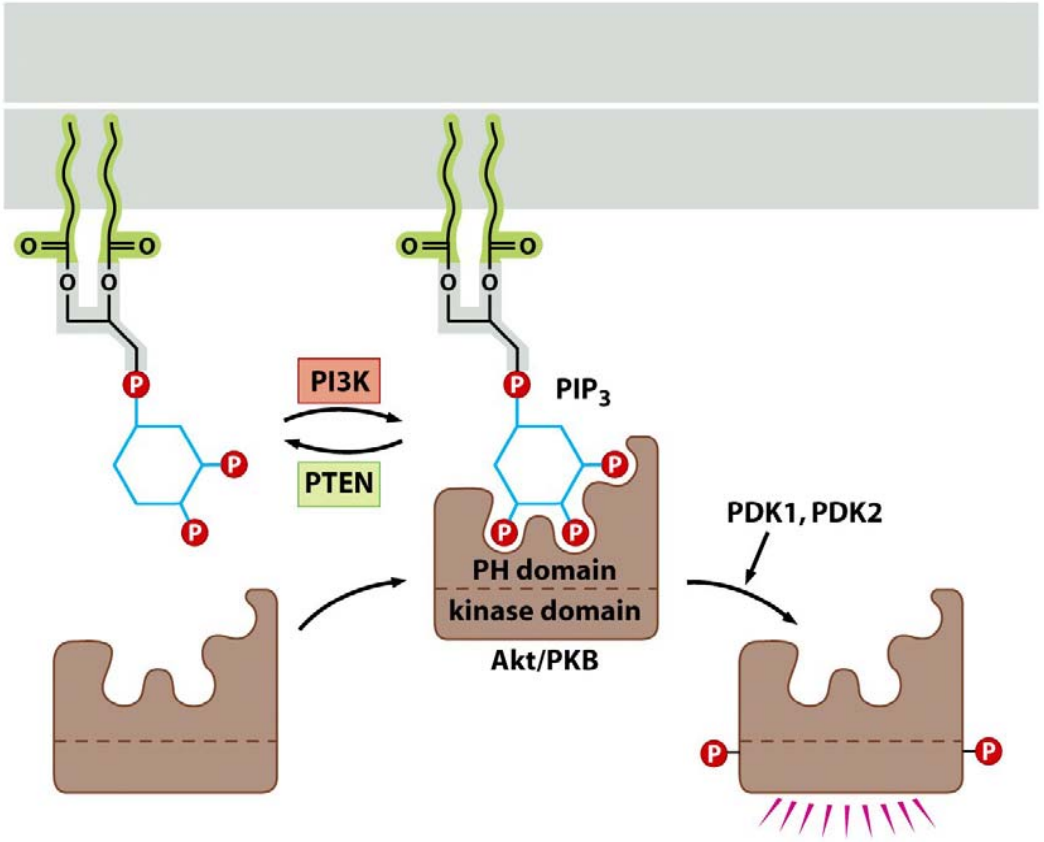
- Release of phosphorylated inositol (IP_3) from the membrane: intracellular signaling ("second messenger") but also…

Inositol Lipids and Mitogenic Signaling (cont'd)

- Phosphorylation by PI3 kinase generates PIP_3 which remains in membrane
- PI3 kinase activity enhanced by Ras binding
- PIP_3 acts as anchor for certain proteins to bind to plasma membrane



Docking of PH Domain of Akt/PKB to PIP_3



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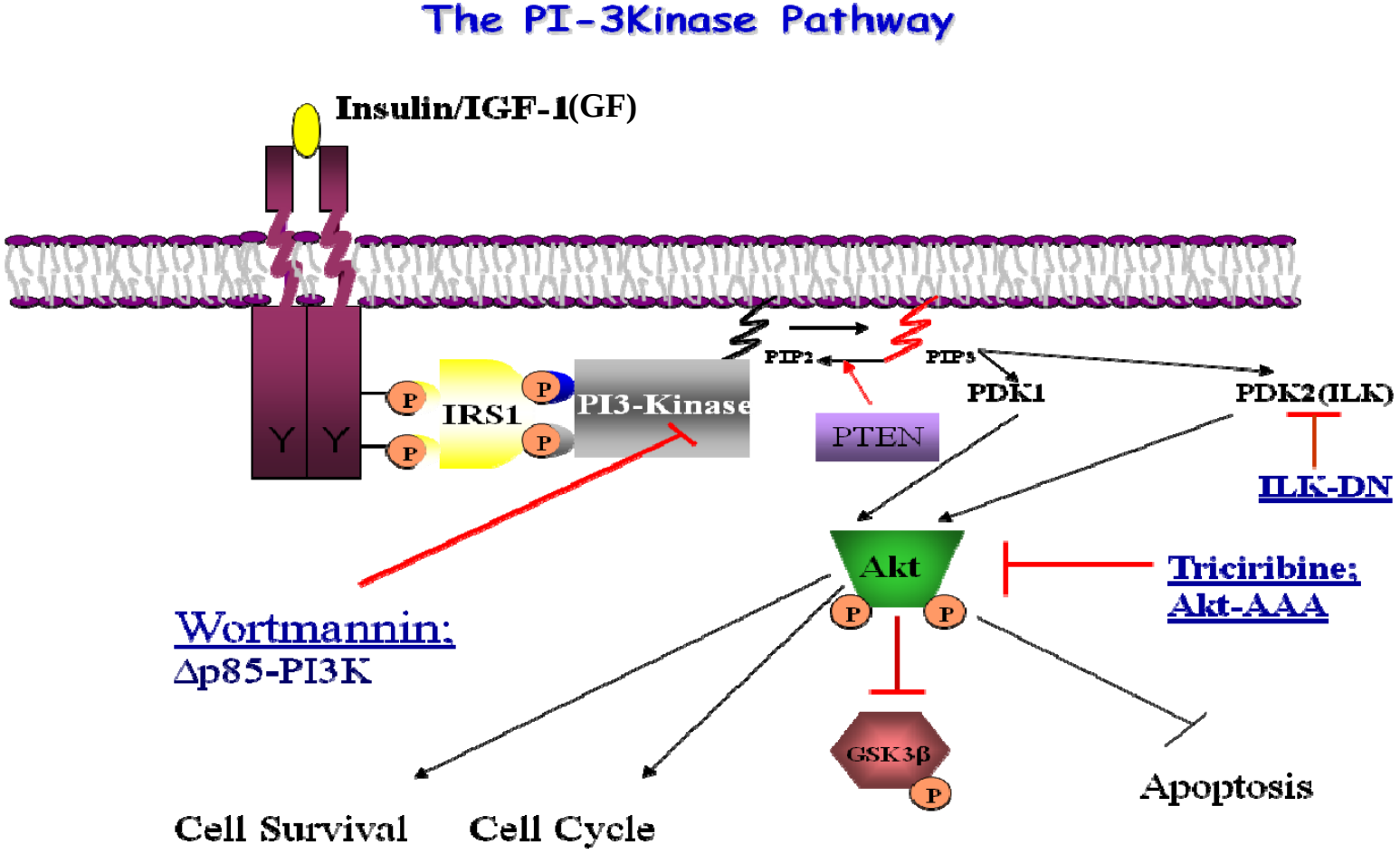
PI3 Kinase Pathway

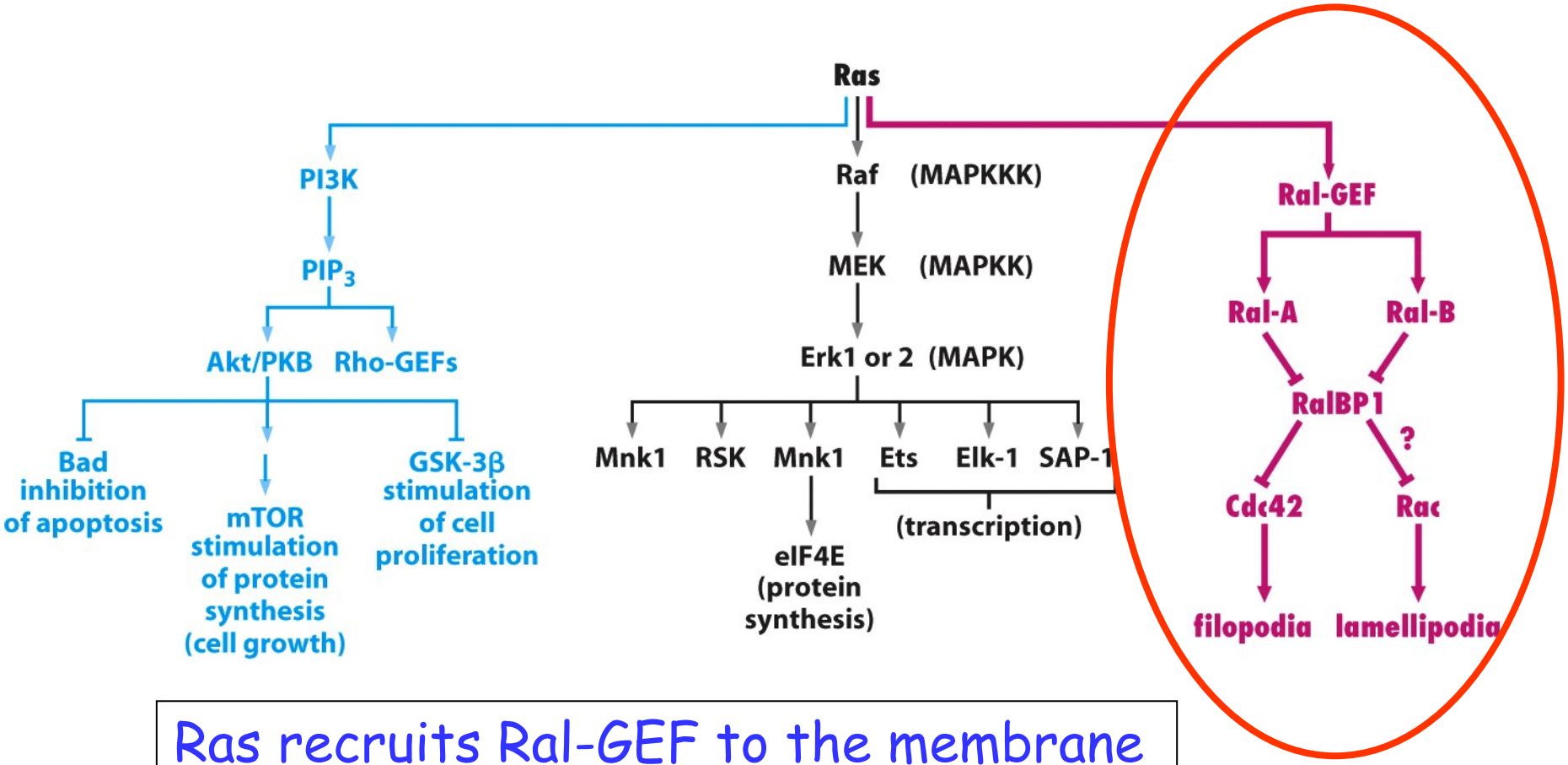


- PTEN mutation is linked to numerous cancers

What does Akt/PKB do?

- Inhibits pro-apoptotic proteins by phosphorylating them
  → prevents apoptosis
- Inhibits anti-proliferative proteins by phosphorylating them
  →causes cells to divide
  → stimulates protein synthesis

The Ras-regulated Ral Pathway



Ras recruits Ral-GEF to the membrane
→→ motility

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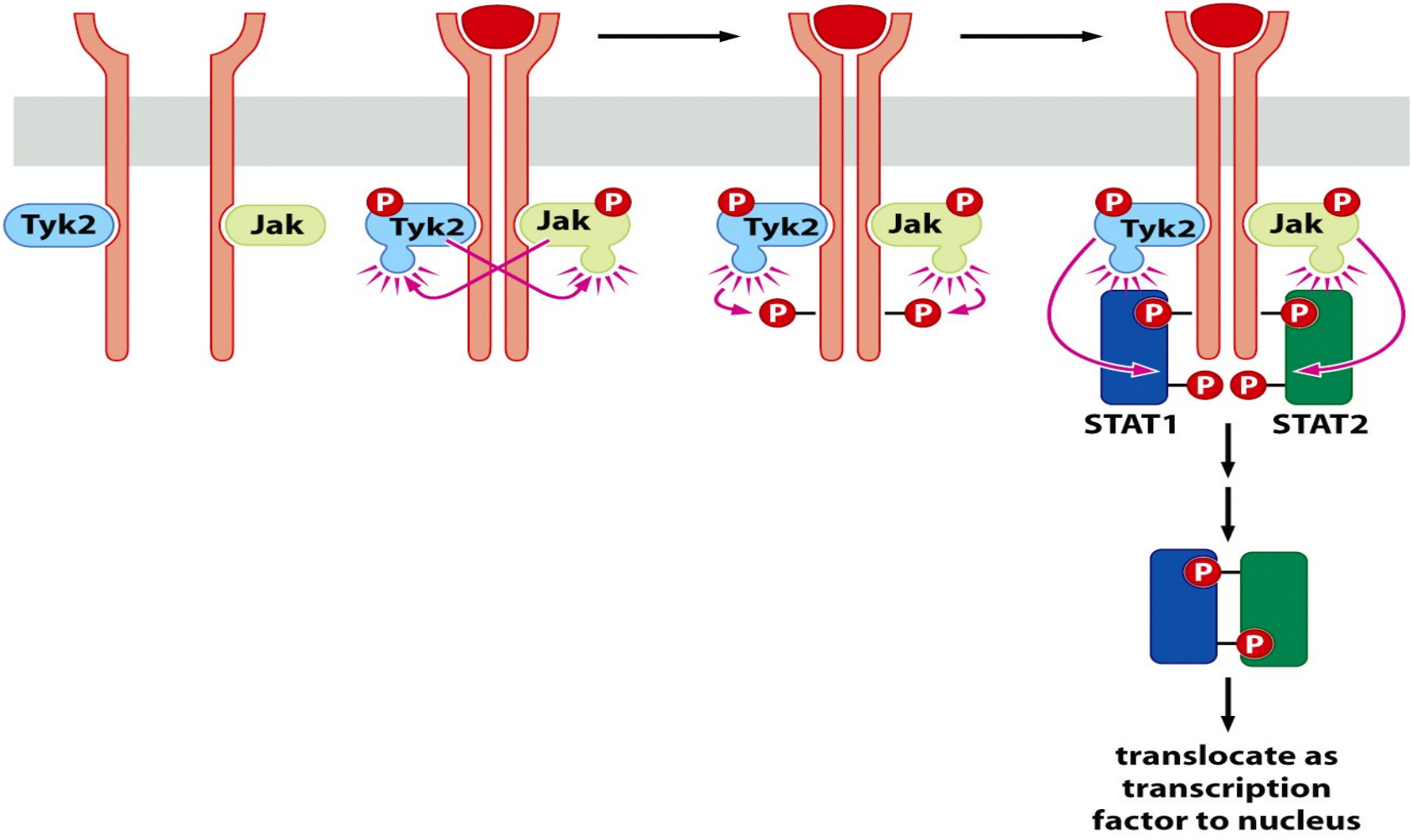
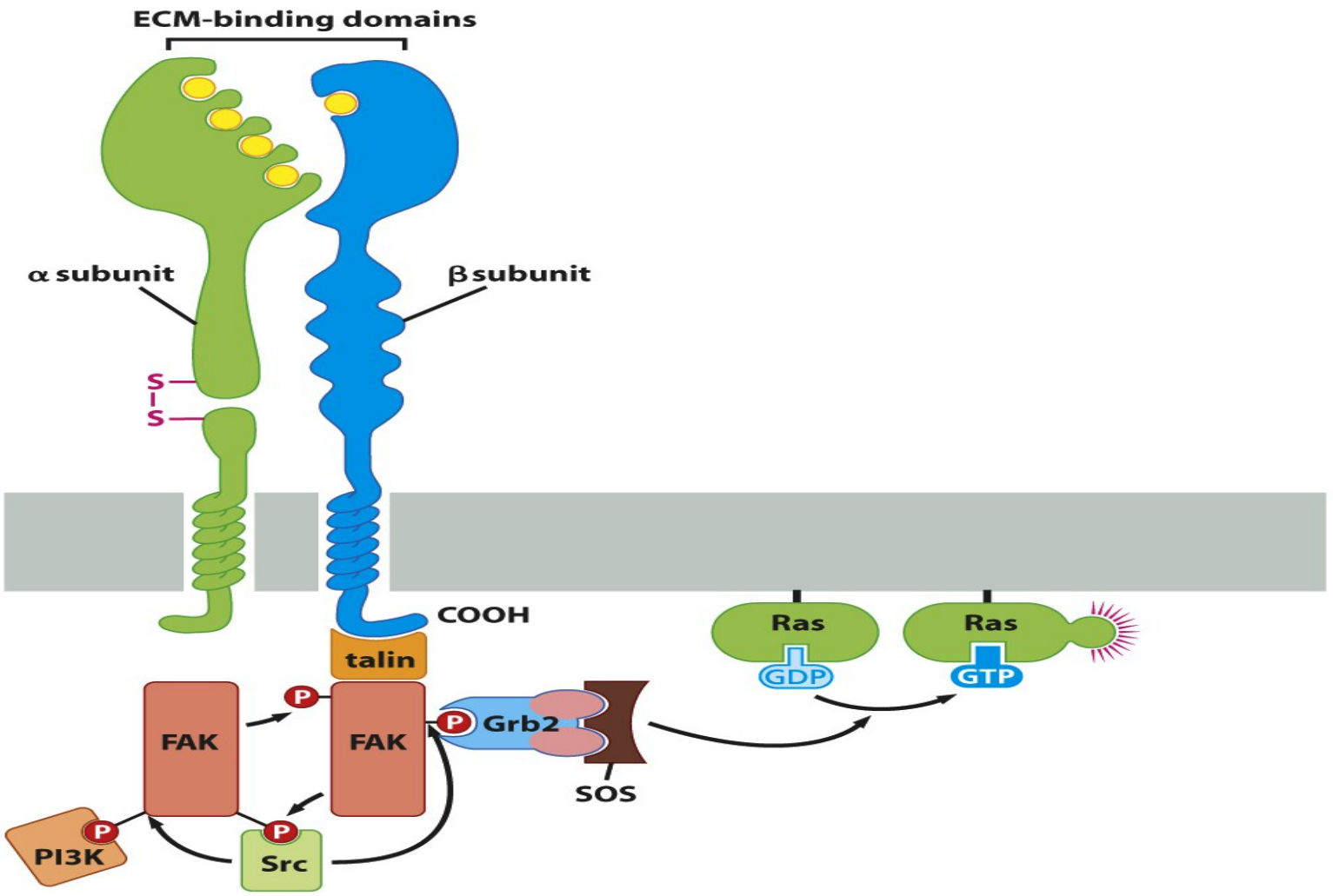
Signaling Directly from Plasma Membrane to Nucleus

The Jak-STAT Pathway

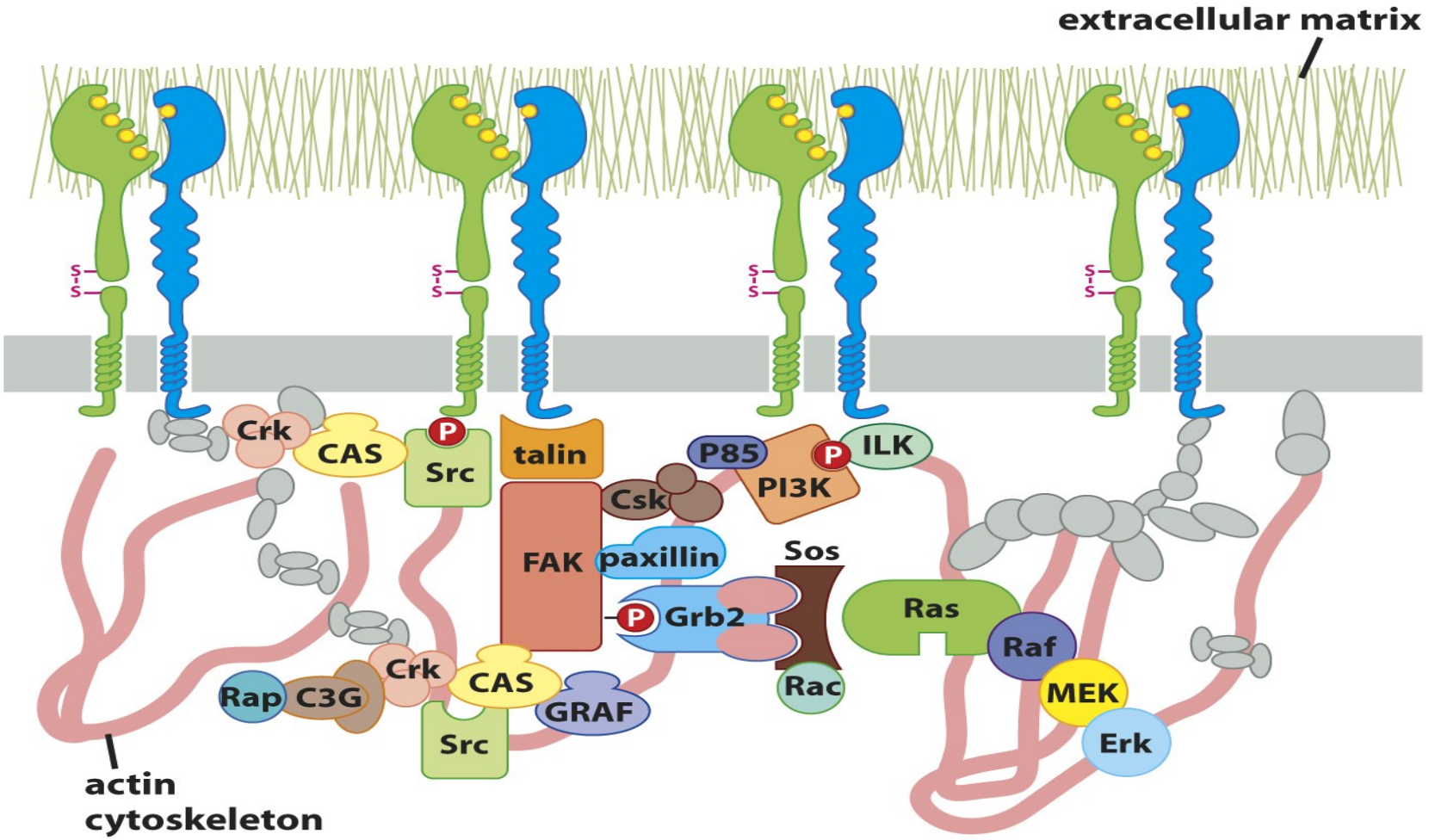


Signaling From Cell Adhesion Receptors

Integrin Signaling



Cell Adhesion Receptor and GFR Signals Converge



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How can cells keep their signals straight?

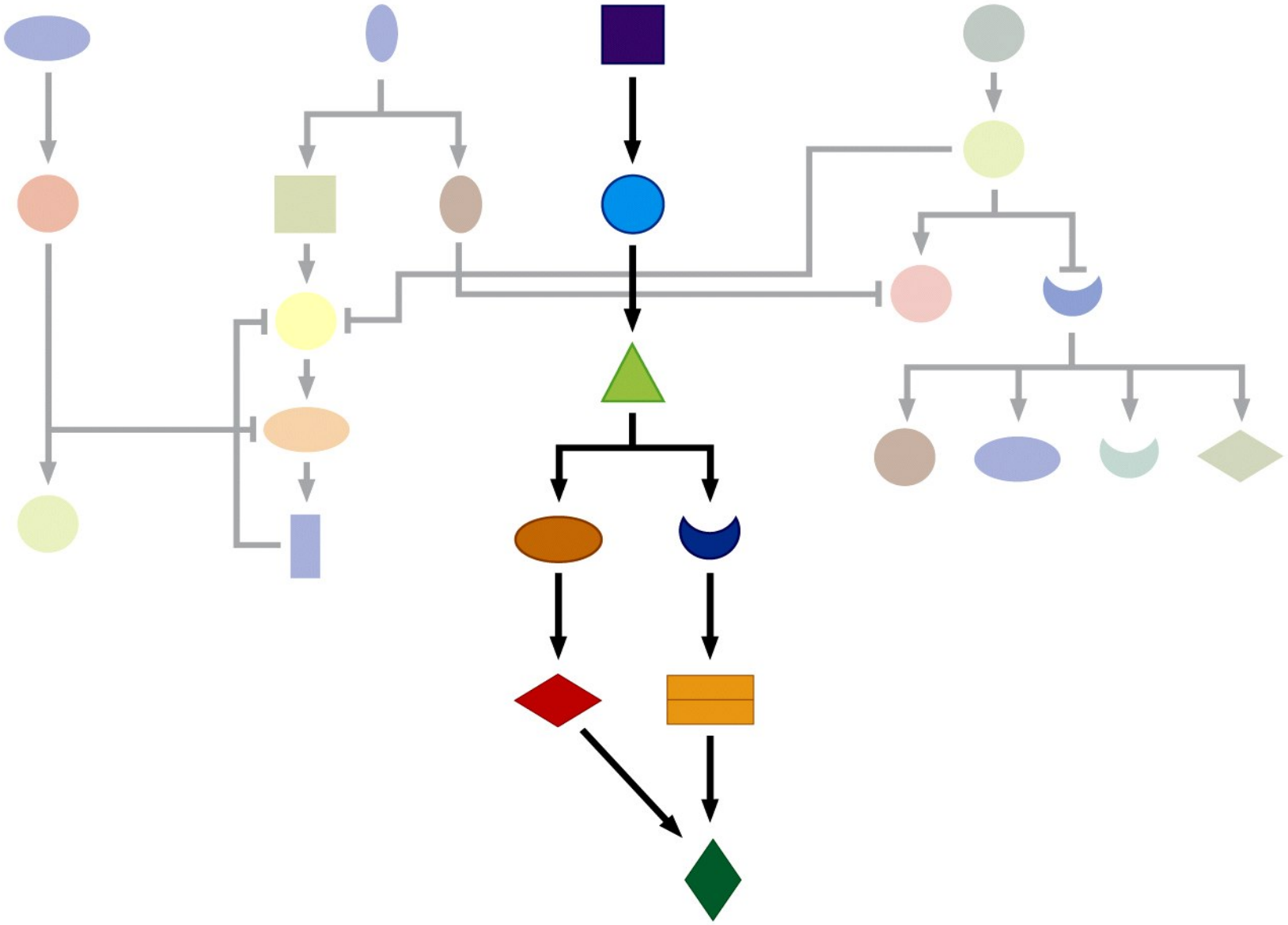
Why do different cells behave so differently if they use the same signaling pathways??

- Different spectrum of receptors on each cell
- Similar but non-identical forms of many signaling proteins (e.g.,multiple ras genes)
- Multiple concurrent signaling pathways (cell type specific?)
- Positive and negative feedback loops modulate flux of signal (over 100 phosphatases (remove phosphates from pTyr proteins) that we know little about)
- Intracellular diffusion rates & locations of signaling molecules important but not understood
- The whole process is ***dynamic***

Modulating the Signal

- Localization
- Concentration
- Activity (by dimerization, GTP binding, phosphorylation, proteolytic cleavage) of signaling molecules can be used to regulate and fine tune the signal

• Essentially all 3 of these are dysregulated in cancer



Summary

- Growth factors bind receptors, induce signal to divide
- Proliferation signal frequently controlled by receptor tyrosine kinases (RTKs)
- Most RTKs dimerize to transmit signal
- Hyperactive growth signaling common in cancer: over-expression of GFs or GFRs, mutation in GFR proteins
- Targets of mitogenic signaling are genes involved in growth and differentiation, and proteins important for motility, metabolism,and protein synthesis
- SH2 domains in signal transducers bind specific pTyr sites in signaling partners; SH3 domains bind proline-rich regions
- Kinases important in many signaling pathways
- Each cell uses multiple signaling pathways to control proliferation
- Activation of signaling pathways associated with many kinds of cancer