Important Signaling Pathways That Have a Role in Cancers

- TGF-β/SMAD signaling
- PI3K/AKT pathway
- MAPK pathways
- Wnt / β-Catenin signaling
- Cytokine signaling

TGF-B Signaling

TGF-β Signaling

- TGF- β family
  - TGF –β
  - Bone Morphogenetic protein (BMP)
  - Decapentapelagic (dpp)
  - Mullerian Inhibitory Substance
  - Inhibin /Activin
- Functions
  - controls the growth and differentiation of epithelial cells
  - Often growth inhibitory – mainly through the induction of the transcription of CDK inhibitor $p15^{INK4B}$ and repression of C-Myc

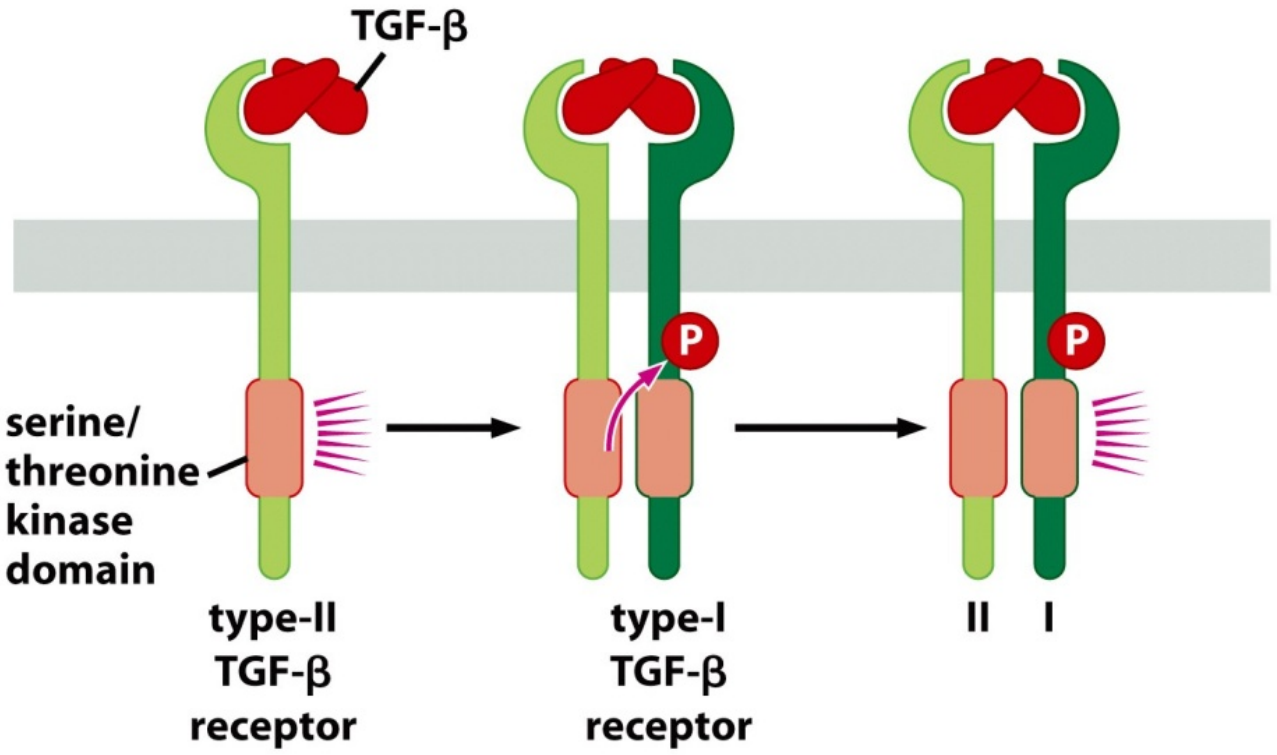
TGF- β Receptor

TYPE I Receptor	TYPE II Receptor
• 53 KDa • Serine/Threonine Kinase • Activated by phosphorylation by Type II receptor	• 75 KDa • Constitutive Serine Kinase • Related in sequence to Type I receptor • TGF- β binds directly

TGF- β Binding to its Receptor

Dimeric ligand binds to type II receptor, leading to heterooligomerization - > receptor tetramer with **2 copies** each of TGF- β RI and TGF- β RII

Leads to transphosphorylation of serine and threonine residues in the cytoplasmic region of TGF- β RI



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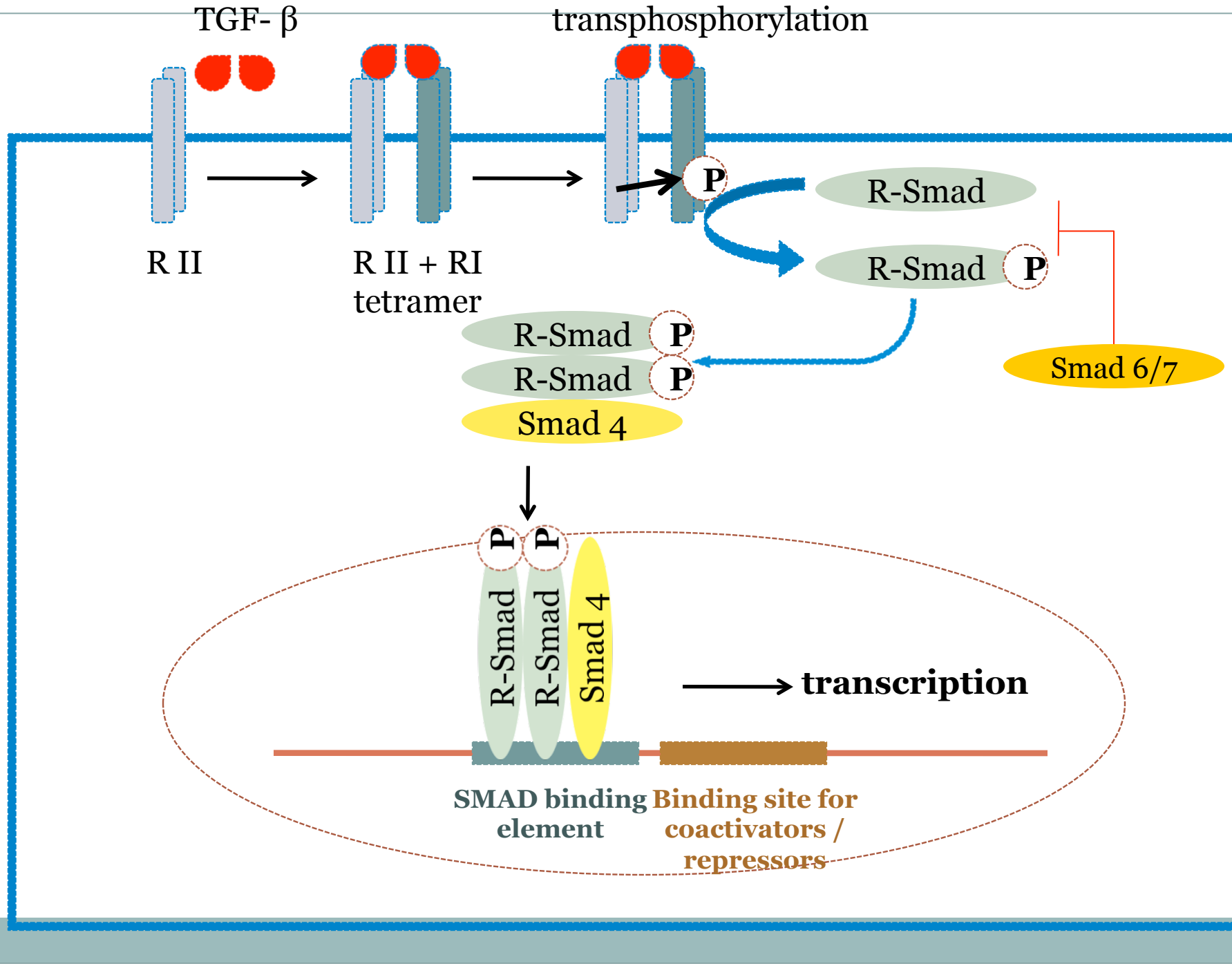
SMAD Proteins

- **Regulatory SMADs** (R-SMADs)
  - SMAD 1, 2, 3, 5 8
- **Comediator SMAD** (Co-SMAD)
  - SMAD4
- **Inhibitory SMADs** (I-SMADs)
  - SMAD 6 and 7

- SMADs are transcription factors that are activated by oligomerization with other SMADs
- MH1 domain – DNA binding
- MH2 domain– SMAD oligomerization and receptor interaction


TGF- β
transphosphorylation
R II
R II + RI
tetramer
P
R-Smad
R-Smad
P
Smad 6/7
R-Smad
P
R-Smad
P
Smad 4
P
P
R-Smad
R-Smad
Smad 4
transcription
SMAD binding element
Binding site for coactivators / repressors

Aberrations in Cancers

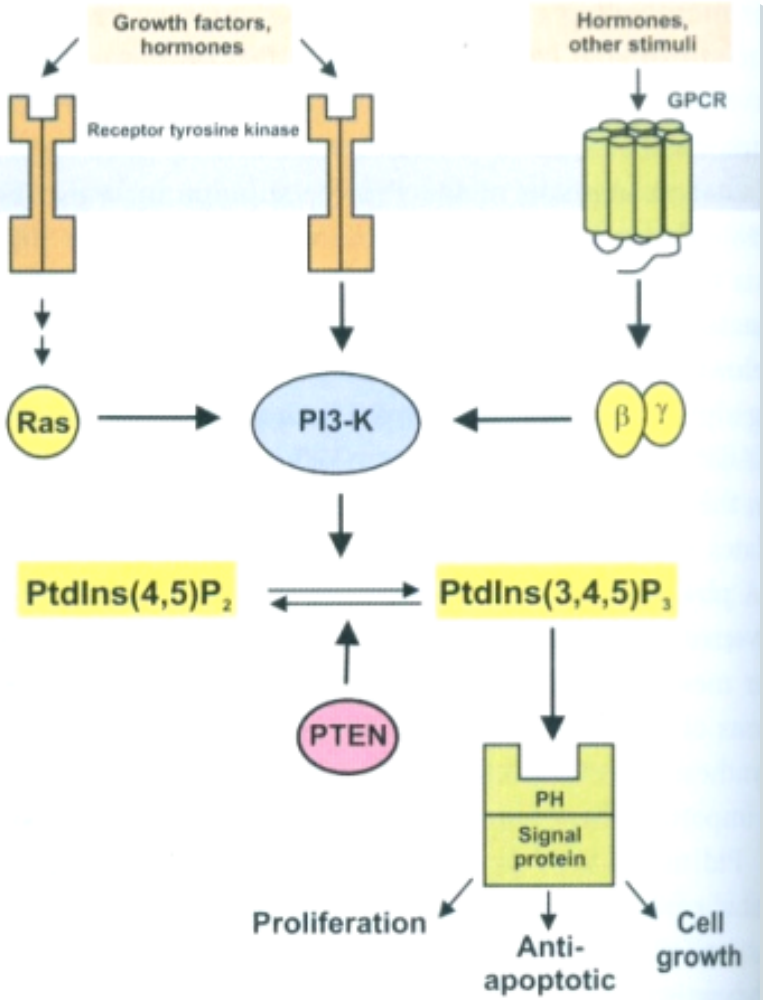
- In **colon cancer**, one third of tumors have mutated TGF- β receptors, and the remainder of the tumors have mutations in the signaling pathway activated by TGF- β
- Cancer cells can become insensitive to TGF- β due to loss of expression and mutations in SMAD proteins

PI3K / AKT Pathway

Phosphatidyl Inositol-3-Kinase (PI3K)

- Catalyzes the phosphorylation of the inositol part of phosphatidylinositol derivatives at the 3’ position
- PI3K catalyzes PtdIns(4,5)P2 → PtdIns(3,4,5)P3
- PIP3 – a membrane-localized second messenger
  - Cell growth control
  - Chemotaxis
  - Glycogen synthesis

Activation of PI3K



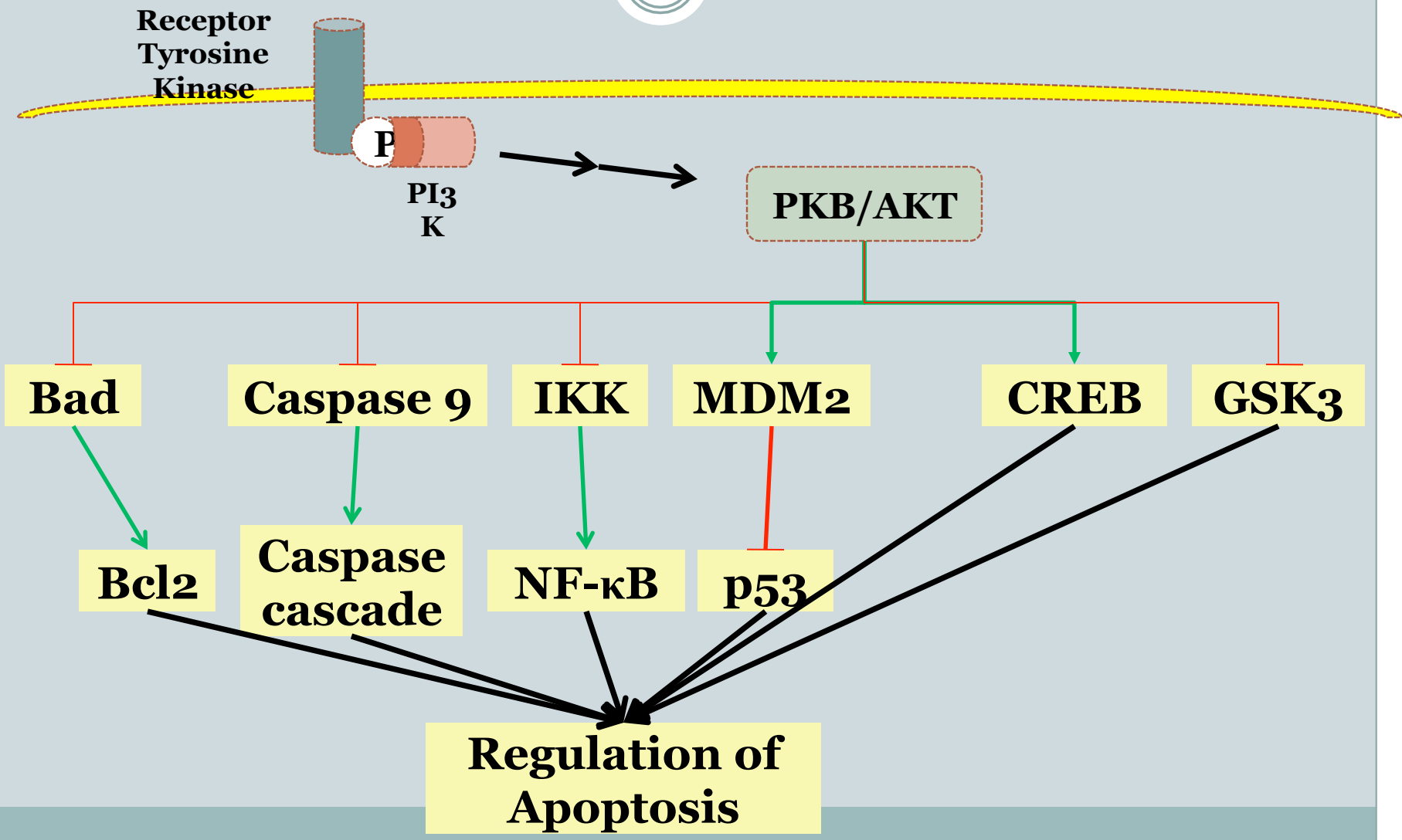
- PI3K is activated mainly via 3 pathways
  - Interaction with activated Receptor Tyrosine Kinases
  - Activation in the Ras Pathway
  - Activation by the Gβγ dimer (GPCR signaling)
- PTEN is a tumor suppressor that negates the effect of PI3K by promoting conversion of PtdIns(3,4,5)P3→ PtdIns(4,5)P2

Biochemistry of Signal Transduction and Regulation; Gerhard Krauss

Akt Kinase / Protein Kinase B

- Activated by binding of PIP3 to the PH domain
- Directly or indirectly involved in cell proliferation and prevention of apoptosis
- Also mediates the metabolic effects of insulin
  - Glucose transport
  - Lipid metabolism
  - Glycogen synthesis
  - Protein synthesis

AKT and Apoptosis



Aberrations in Cancers

- PTEN (loss of function) mutations are common in colorectal cancers
- Activating mutations in PI3K and / or AKT lead to constitutive activation of this pathway in many cancers
- Numerous components of the PI3K/AKT pathway are targeted by amplification, mutation and translocation more frequently than any other pathway, with the exception of RB and p53

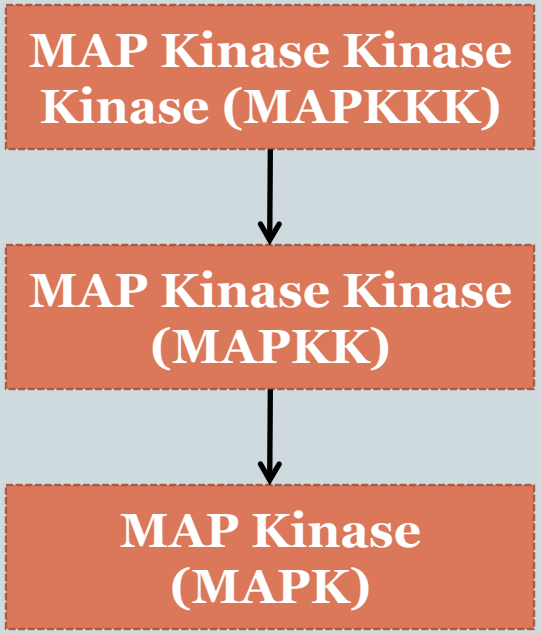
MAP Kinase Pathways

Mitogen Activated Protein Kinases

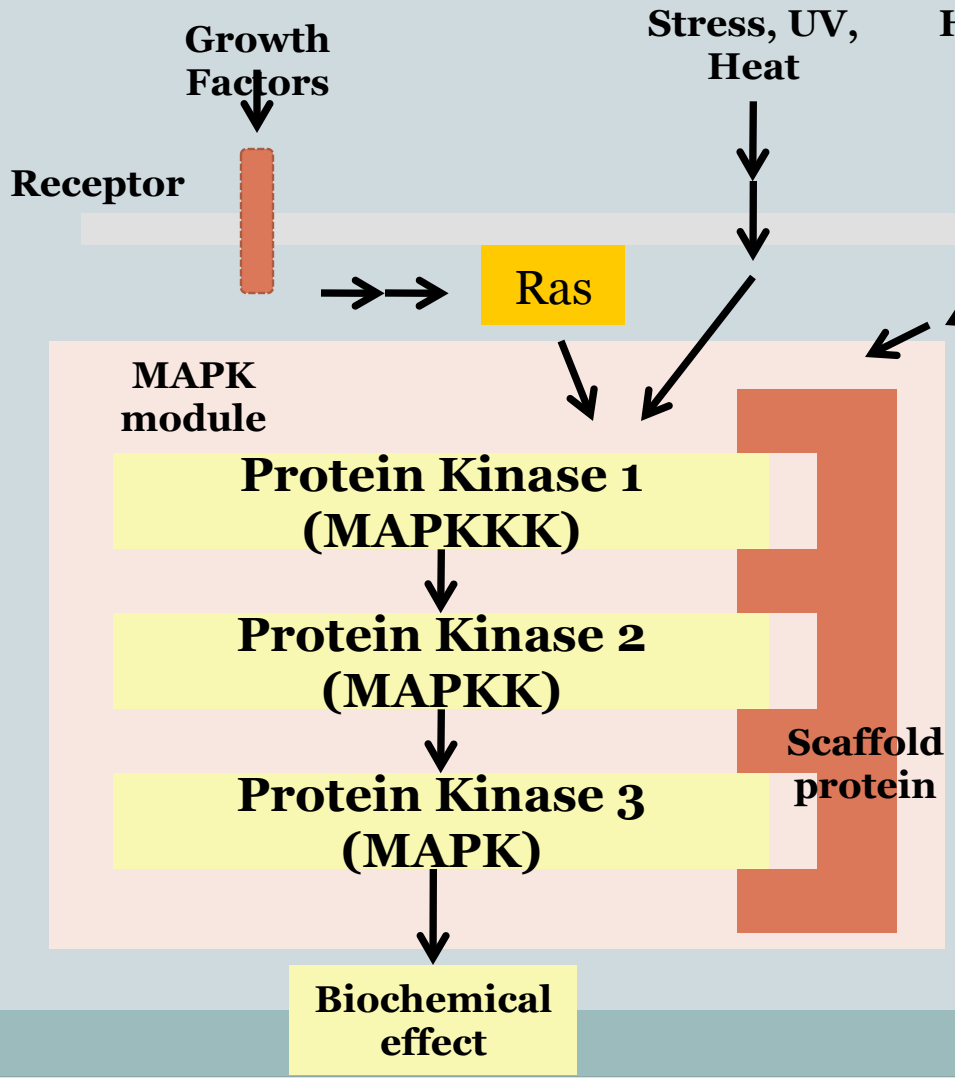
- In response to growth inducing signals (eg: Insulin, growth factors)
- Five main MAPK pathways
  - ERK 1/2
  - C-Jun N-terminal kinases (JNK 1,2,3)
  - p38 kinases
  - ERK 3, 4
  - ERK 5

The Kinase Cascade

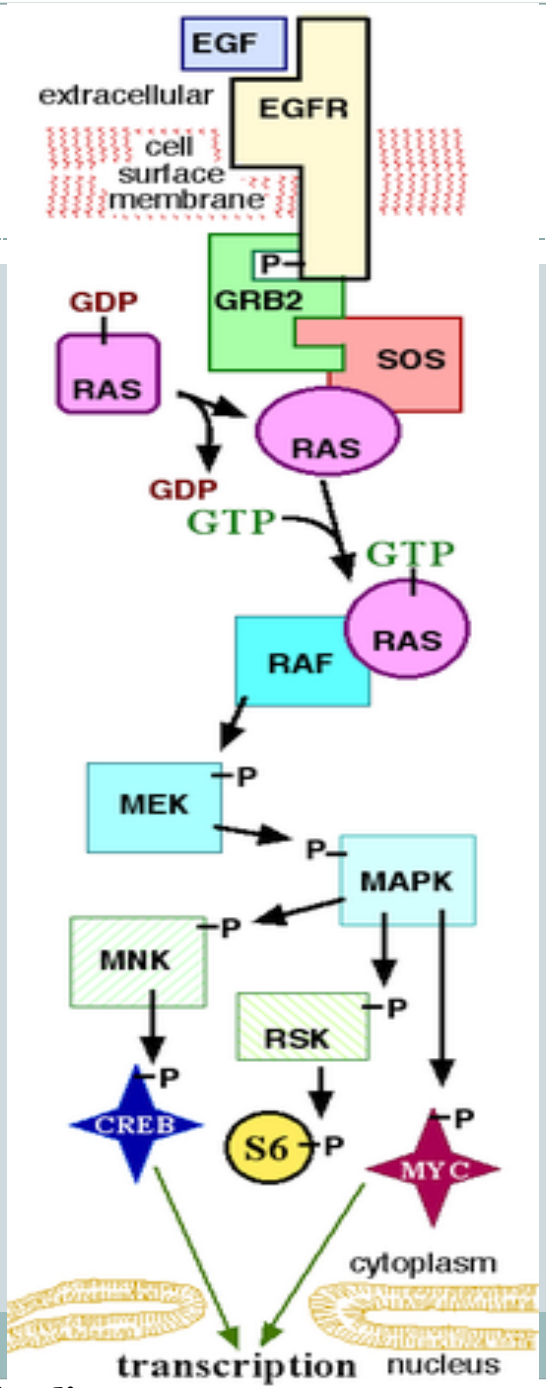
- Typically consists of three protein kinases; their phosphorylation occurs in a cascade
- Recall what was said about signaling cascades



The Protein Kinases of MAPK Pathway are Often Organized into Modules With the Help of Scaffold Proteins



How does the scaffold protein help?



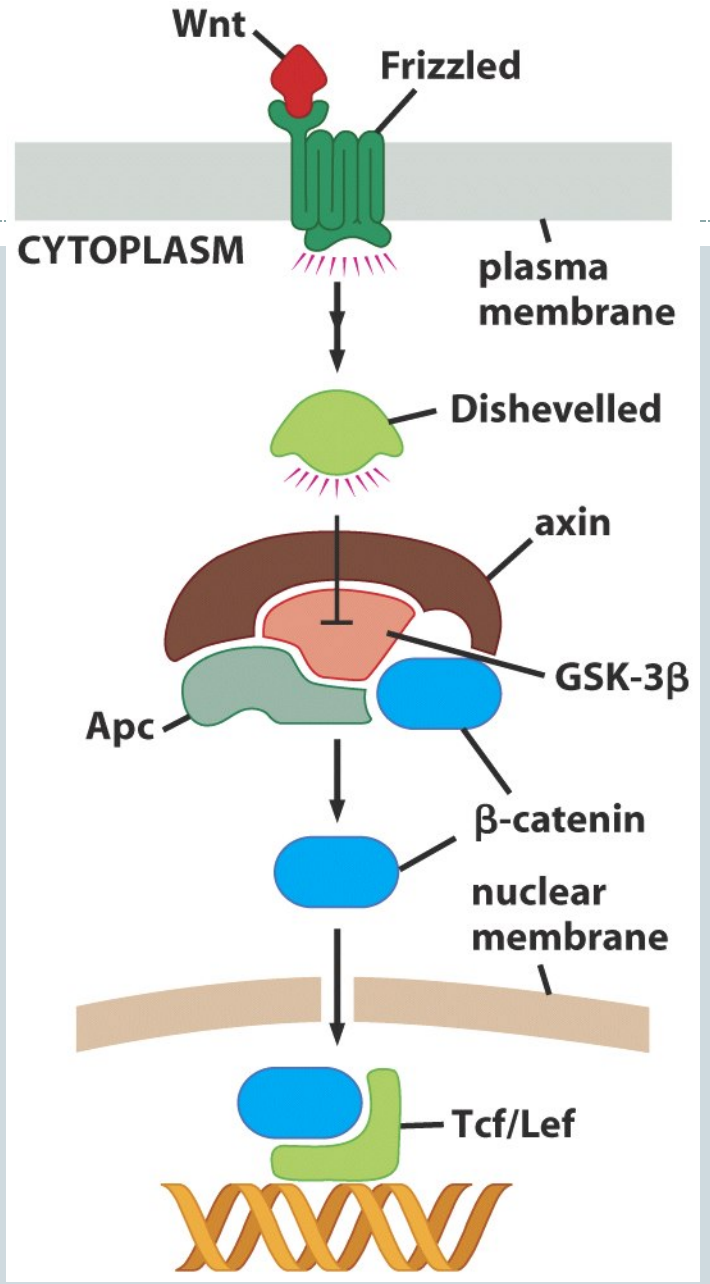
- RAF = MAPKKK
- MEK = MAPKK

Example of MAPK Pathway – The EGFR Pathway

En.Wikipedia.org

Wnt / β-Catenin Signaling

- **Wnt** genes encode secreted lipoproteins → act as ligands for the Frizzled (Fz) receptor
- Important in **development** (morphogenic signaling)
- Some components are inactivated / mutated in cancers
  - Wnts
  - APC (Adenomatous Polyposis Coli gene)
  - Axin
  - β –Catenin



- β-Catenin –effector molecule that translocates to nucleus, helps in transcription
- **Axin/GSK-3/APC complex – degrades β-Catenin**

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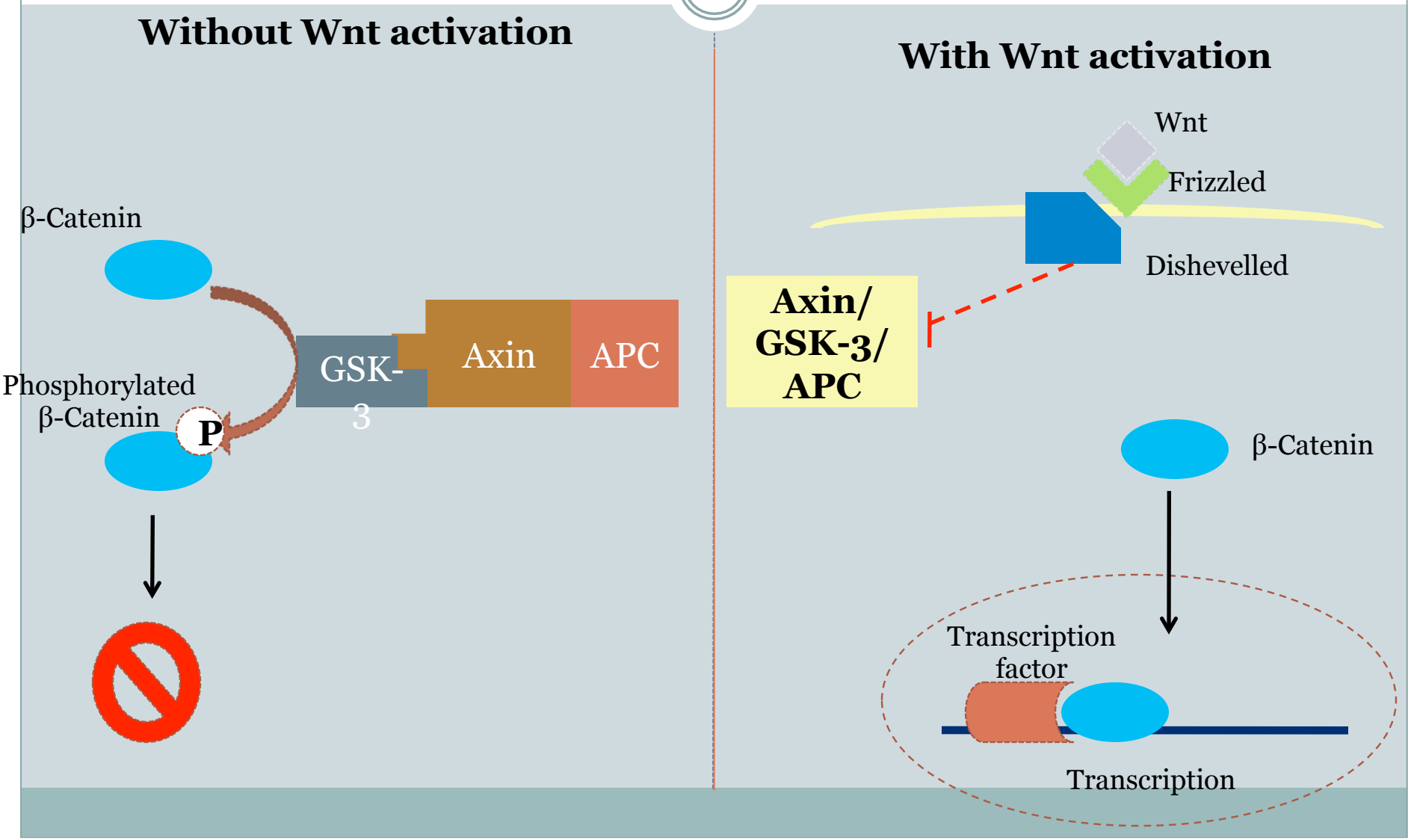
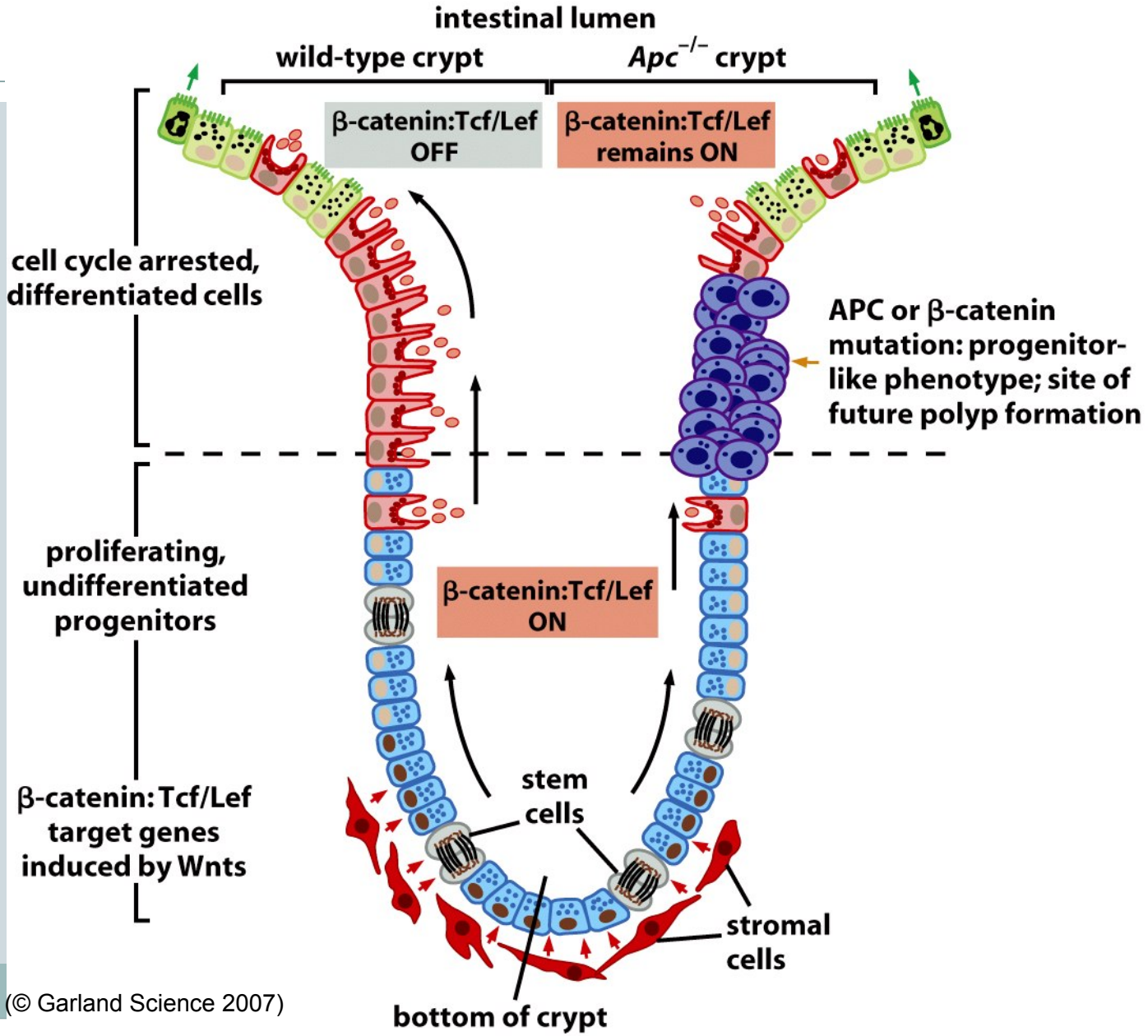
Wnt Signaling Prevents Degradation of β-Catenin



APC and B-Catenin in Polyp Formation



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Aberrations in Cancers

- Overexpression and activation of growth factor receptors, eg. EGFR, result in activation of the MAPK pathway
- Mutations of RAF protein (a MAPKKK) can result in constitutive MAPK signaling

Aberrations in Cancers

- Expression of Wnts increased 4 to 10-fold in many breast cancers
- Increased nuclear translocation of B-Catenin is seen in >20% of prostate cancers
- Some prostate cancers have a mutant B-Catenin that is resistant to phosphorylation by GSK-3, increasing its half-life
- **Defective APC protein leads to defective B-catenin degradation in virtually all colon carcinomas**
- Loss of one allele of APC gene occurs in most familial polyposis syndromes of colon → The *polyp:colon cancer sequence* is another example of **Knudson' s Two-hit hypothesis**

Cytokines and the JAK/STAT Pathway

Cytokines

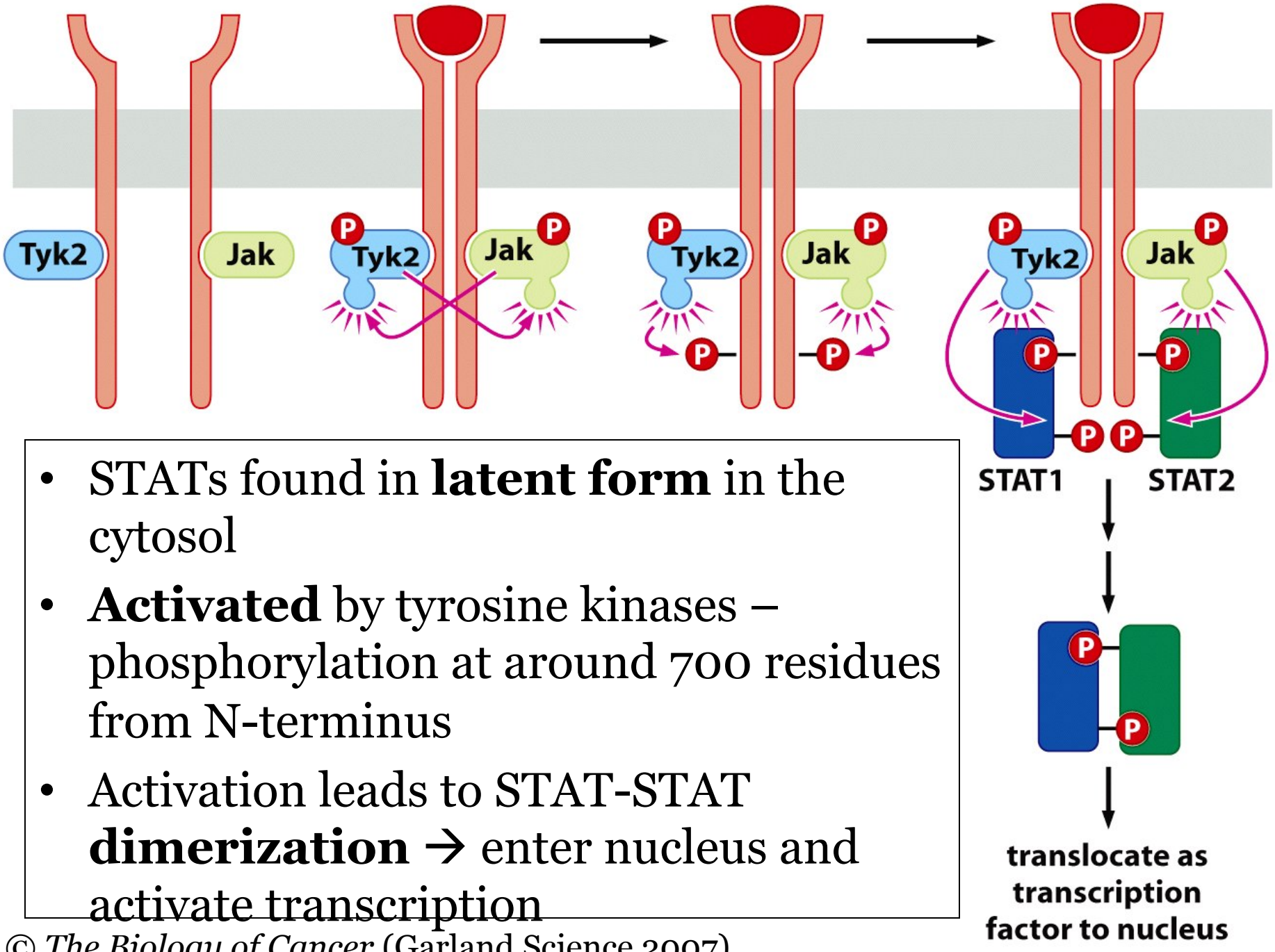
- Important role in immune functions, neuronal, hematopoietic and embryonal development
- Examples
  - Interleukins (ILs)
  - Erythropoietin (EPO)
  - Interferons (IFNs)
  - Tumor Necrosis Factor (TNF)
- Often act locally, on cells of the same or similar type as the cytokine-producing cell

Cytokine Receptors are Associated With Tyrosine Kinases

- Cytokine receptors have no intrinsic kinase activity
- Instead, **ligand binding leads to conformational change** in the cytoplasmic domain of the receptor → activation of the tyrosine kinase that is associated with the receptor
- **Janus Kinase** (JAK) family proteins are such tyrosine kinases that are associated with cytokine receptors
- JAKs can phosphorylate STATs in addition to substrates in other mitogenic pathways

The JAK-STAT Pathway

- STAT proteins are transcription factors found in the cytosol; can be **activated by tyrosine kinases**
  - JAKs
  - RTKs (eg: EGFR, PDGFR)
  - Non-RTKs (Src kinase)
  - GPCRs (indirectly)
- STATs activate transcription of
  - Myc
  - Cyclin D2, D3
  - BCL-XL
- The JAK-STAT pathway different from other pathways – very small number of components involved → What are the advantages and disadvantages of such a short pathway?



- STATs found in **latent form** in the cytosol
- **Activated** by tyrosine kinases – phosphorylation at around 700 residues from N-terminus
- Activation leads to STAT-STAT **dimerization** → enter nucleus and activate transcription

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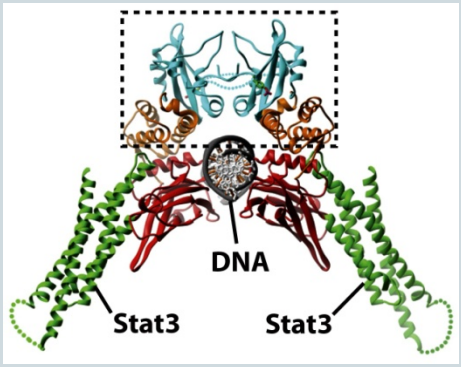
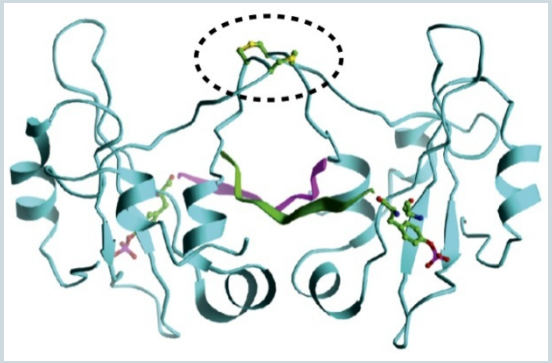
Aberrations in Cancers

- STAT3 – constitutively activated in many cancers
  - Melanomas (constitutive activation of Src)
  - Majority of breast cancers
  - Most head and neck cancers
- Using site-directed mutagenesis, two STAT subunits can be covalently attached → constitutive activation due to formation of a "dimer"





STAT-STAT dimer

Covalently linked STAT-STAT dimer

Further Reading

- The Biology of Cancer; Robert A. Weinberg
- **Loss of expression, and mutations of Smad 2 and Smad 4 in human cervical cancer;** Tessy T Maliekal, Marie-Lue Antony, Asha Nair, Ramasamy Paulmurugan and Devarajan Karunagaran; *Oncogene*
- KEGG pathways - http://www.genome.jp/kegg/pathway.html