Cell Signaling
PHYA-Sem-II-CC3

JAK-STAT
MAP-Kinase
SMAD
……Cell Signalling Pathways

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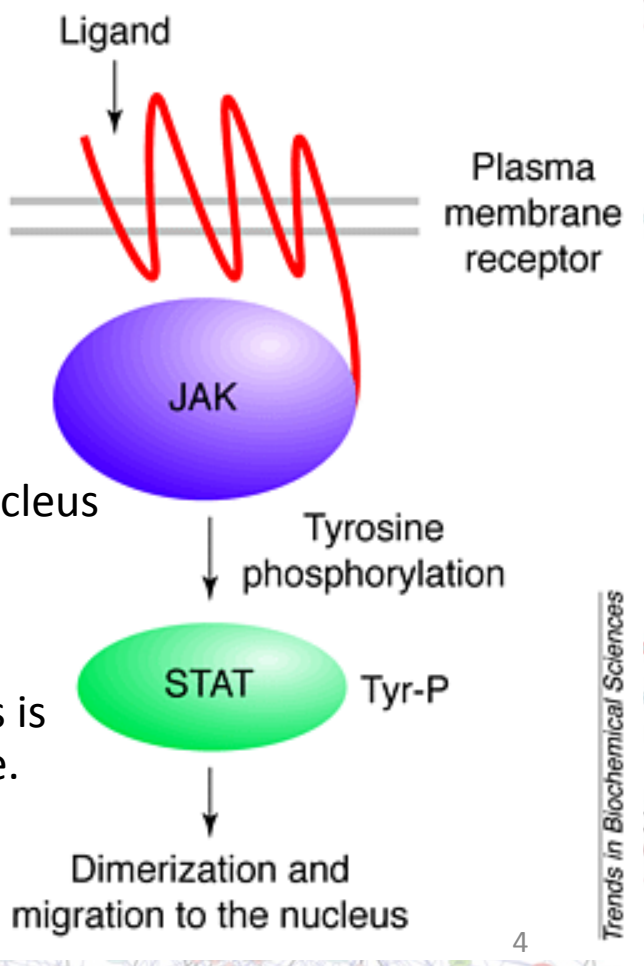
- **Janus Activated Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway**

- **JAK-STAT signalling pathway** is a chain of interactions between proteins in a cell, and is involved in processes
- immunity
- cell division
- cell death
- tumour formation

- **Disrupted JAK-STAT signalling may lead to a variety of diseases**
- skin conditions
- cancers
- disorders affecting the immune system

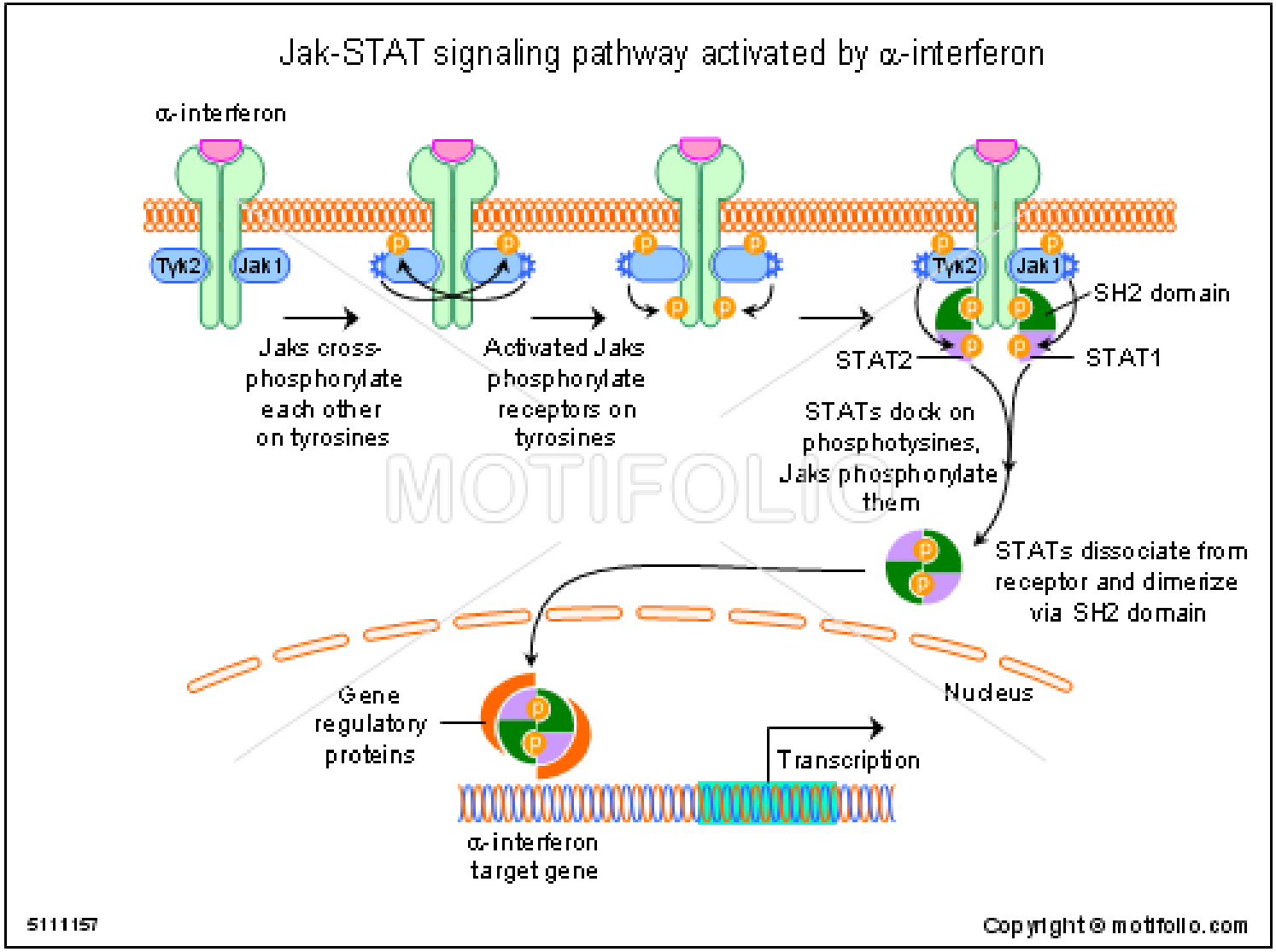
STATs - a signal responsive TF family

- STATs: **S**ignal **T**ransducers and **A**ctivators of **T**ranscription
  - two functions given in the name
- 1. Transducers for signals from many cytokines
  - Broad spectrum of biological effects
- 2. Transcriptional activators
  - characteristic activation mechanism
  - activation at the cell membrane, response in the nucleus
- Rapid signal response
  - The activation/deactivation cycle of STAT molecules is quite short, about 15 min for an individual molecule.



JAK–STAT pathway

- Sequential tyrosine phosphorylations
  - Receptor dimerization allows transphosphorylation and activation of Janus kinases (JAKs).
  - This is followed by phosphorylation of receptor tails and the recruitment of the STAT proteins through their SH-2 domains. STAT tyrosine phosphorylation then occurs.
  - Dimerization of activated (tyrosine phosphorylated) STAT is followed by nuclear entry.


Jak-STAT signaling pathway activated by α-interferon
α-interferon
Tyk2
Jak1
Jaks cross-phosphorylate each other on tyrosines
Activated Jaks phosphorylate receptors on tyrosines
SH2 domain
STAT2
STAT1
STATs dock on phosphotysines, Jaks phosphorylate them
STATs dissociate from receptor and dimerize via SH2 domain
Nucleus
Gene regulatory proteins
Transcription
α-interferon target gene
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Mechanism of Action:

- The binding of various ligands, usually cytokines, such as interferons and interleukins, to cell-surface receptors, causes the receptors to dimerize, which brings the receptor-associated JAKs into close proximity.

- The JAKs then phosphorylate each other on tyrosine residues located in regions called activation loops, through a process called transphosphorylation, which increases the activity of their kinase domains.

- The activated JAKs then phosphorylate tyrosine residues on the receptor, creating binding sites for proteins possessing SH2 domains.

- STATs then bind to the phosphorylated tyrosines on the receptor using their SH2 domains, and then they are tyrosine-phosphorylated by JAKs, causing the STATs to dissociate from the receptor.

- These activated STATs form hetero- or homodimers, where the SH2 domain of each STAT binds the phosphorylated tyrosine of the opposite STAT, and the dimer then translocates to the cell nucleus to induce transcription of target genes.

Role of JAK-STAT in Cytokine receptor signalling

- cytokines are substances produced by immune cells that can alter the activity of neighbouring cells. For example, JAK3 activation in response to IL-2 is vital for lymphocyte development and function.

- The JAK-STAT pathway in cytokine receptor signalling can activate STATs, which can bind to DNA and allow the transcription of genes involved in immune cell division, survival, activation and recruitment. For example, STAT1 can enable the transcription of genes which inhibit cell division and stimulate inflammation.

- Also, STAT4 is able to activate NK cells (natural killer cells), and STAT5 can drive the formation of white blood cells.

- In response to cytokines, such as IL-4, JAK-STAT signalling is also able to stimulate STAT6, which can promote B-cell proliferation, immune cell survival, and the production of an antibody called IgE.

Role of JAK-STAT in Development

- JAK-STAT signalling plays an important role in animal development. The pathway can promote blood cell division, as well as differentiation (the process of a cell becoming more specialised).

- In some flies with faulty JAK genes, too much blood cell division can occur, potentially resulting in leukaemia.

- JAK-STAT signalling has also been associated with excessive white blood cell division in humans and mice.

- **Map Kinase / Ras-Raf-MEK-ERK pathway**
  (Map Kinase = mitogen-activated protein kinases)

- It is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell.

- It is an emzyme that translocates the signals to the nucleus and activates many transcriptional factors by phosphorylating many different proteins that regulate expression of important cell cycle and differentiation specific proteins.

- MAPK (mitogen-activated protein kinases, originally called ERK, extracellular signal-regulated kinases), which communicate by adding phosphate groups to a neighboring protein (phosphorylating it), which acts as an "on" or "off" switch.

Mechanism :

Ras activation

- Receptor-linked tyrosine kinases such as the epidermal growth factor receptor (EGFR) are activated by extracellular ligands, such as epidermal growth factor (EGF). Binding of EGF to the EGFR activates the tyrosine kinase activity of the cytoplasmic domain of the receptor.
- The EGFR becomes phosphorylated on tyrosine residues. Docking proteins such as GRB2 contain an SH2 domain that binds to the phosphotyrosine residues of the activated receptor.
- GRB2 binds to the guanine nucleotide exchange factor SOS by way of the two SH3 domains of GRB2. When the GRB2-SOS complex docks to phosphorylated EGFR, SOS becomes activated.
- Activated SOS then promotes the removal of GDP from a member of the Ras subfamily (most notably H-Ras or K-Ras). Ras can then bind GTP and become active.
- Apart from EGFR, other cell surface receptors that can activate this pathway via GRB2 include Trk A/B, Fibroblast growth factor receptor (FGFR) and PDGFR.

Contd.

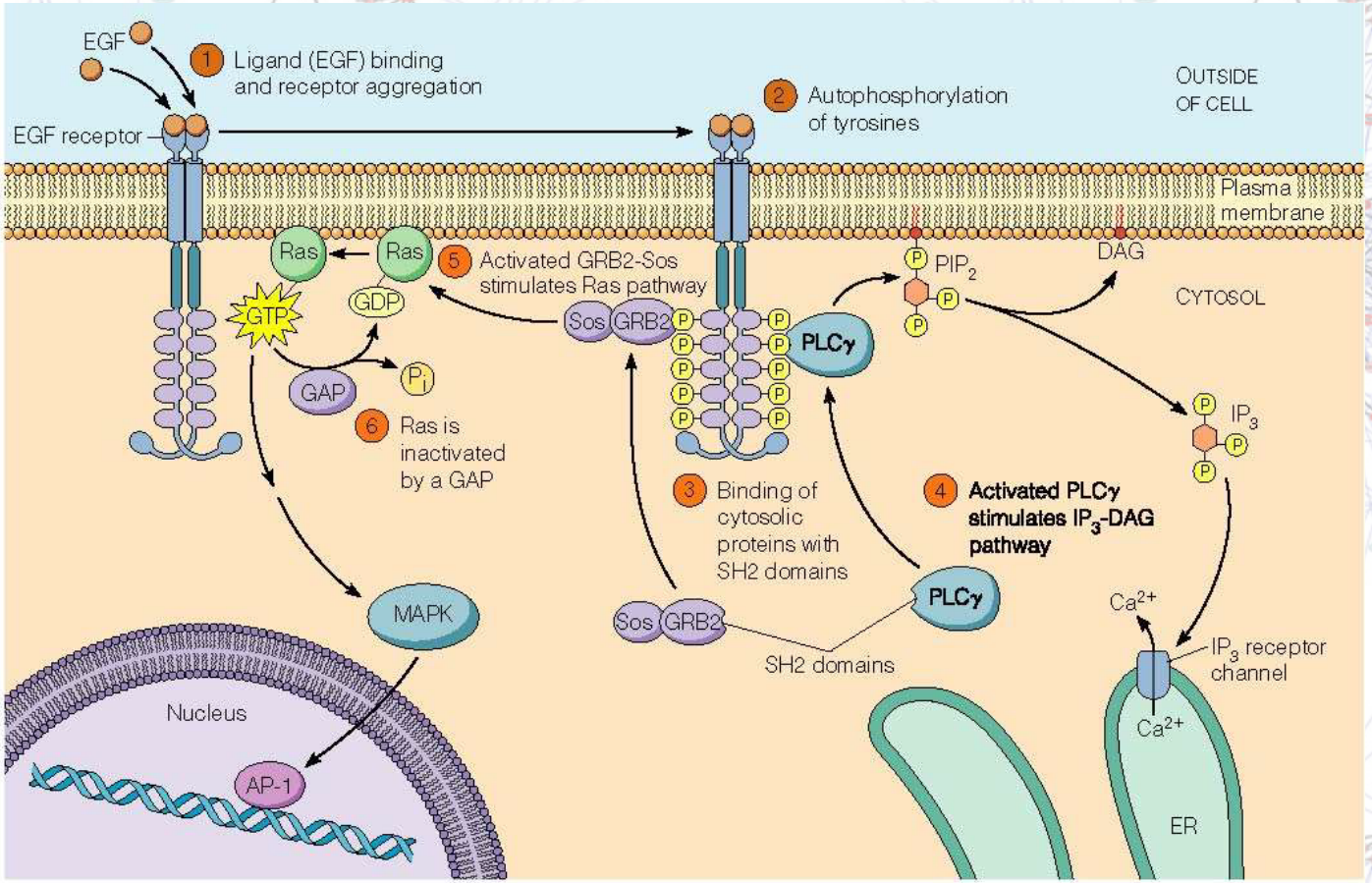
Kinase cascade

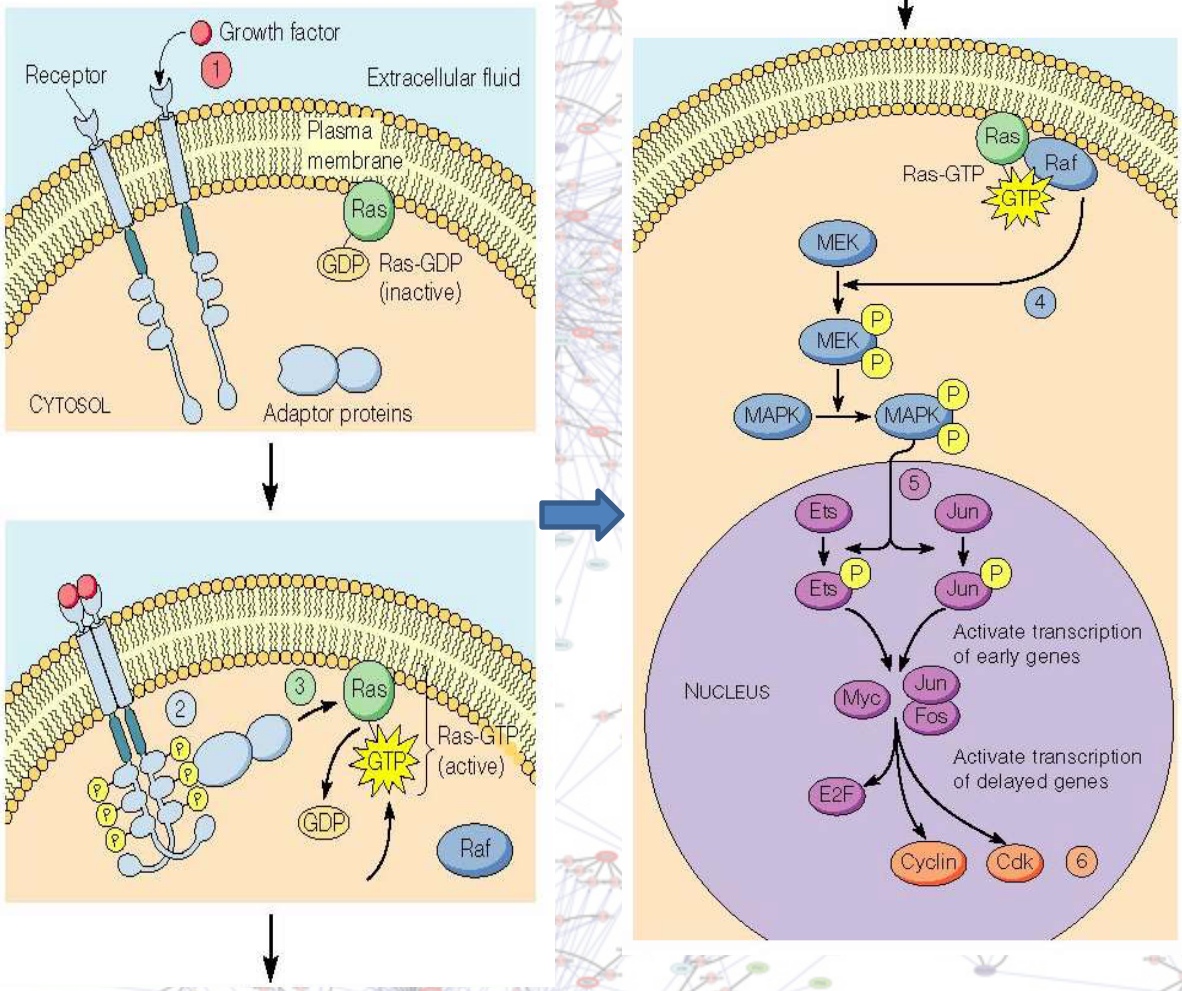
- Activated Ras activates the protein kinase activity of RAF kinase. RAF kinase phosphorylates and activates MEK (MEK1 and MEK2). MEK phosphorylates and activates a mitogen-activated protein kinase (MAPK).
- RAF, and ERK (also known as MAPK) are both serine/threonine-selective protein kinases. MEK is a serine/tyrosine/threonine kinase.
- MAPK was originally called "extracellular signal-regulated kinases" (ERKs) and "microtubule associated protein kinase" (MAPK).

- ***SOS*** = ***Son of Sevenless*** (***SOS***) refers to a set of genes encoding guanine nucleotide exchange factors that act on the Ras subfamily of small GTPases.

- ***RAS*** = First two *ras* genes, *HRAS* and *KRAS*, were identified from studies of two cancer-causing viruses, the Harvey sarcoma virus and Kirsten sarcoma virus, by Edward M. Scolnick and colleagues at the National Institutes of Health (NIH).These viruses were discovered originally in rats during the 1960s by Jennifer Harvey and Werner H. Kirsten, respectively, hence the name **Ra**t **S**arcoma.

- ***RAF*** = It was isolated from the murine retrovirus so this gene was given the name Virus-induced Rapidly Accelerated Fibrosarcoma (V-RAF)

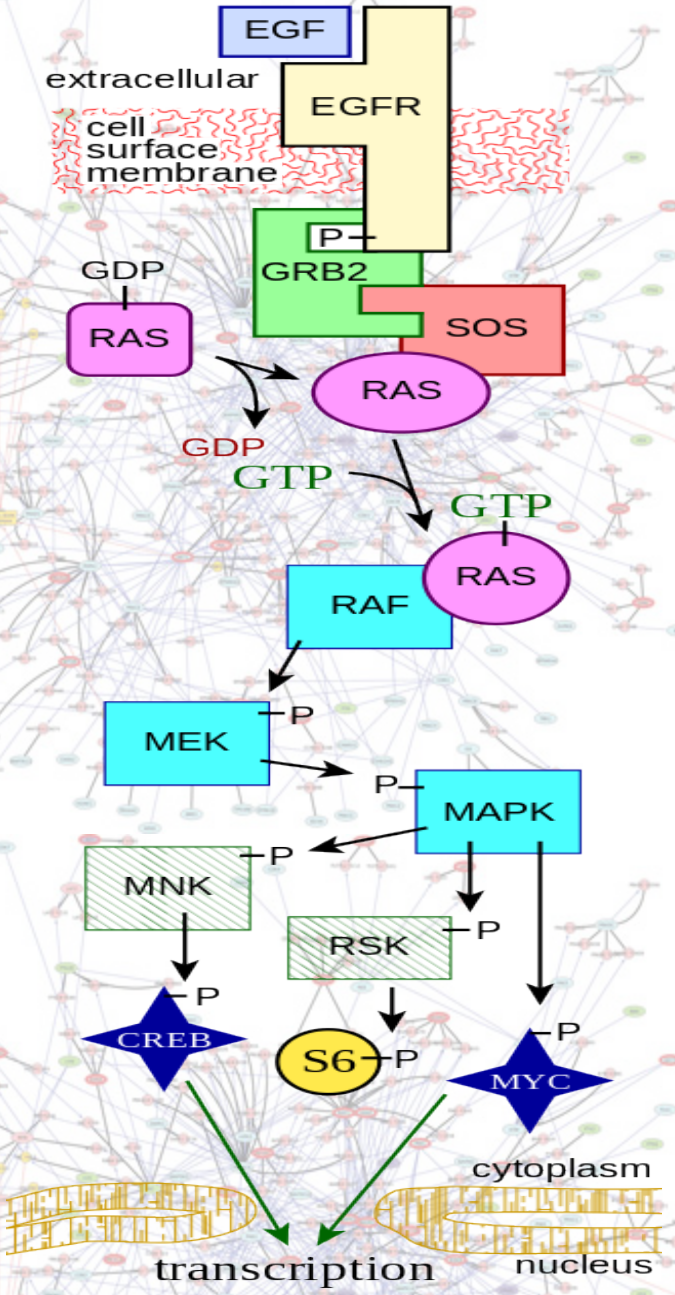




This is a kinase cascade:

- Raf turns on MEK by putting phosphates on it, MEK turns on map kinase by putting phosphates on it (end of kinase cascade).

- Once on, map kinase puts phosphates on transcription factors like Jun, which combine to form AP-1, this turns on AP-1. AP-1 turns on genes for cell division (cyclin, cdk, etc)



"P" represents phosphate, which communicates the signal. Top, epidermal growth factor (EGF) binds to the EGF receptor (EGFR) in the cell membrane, starting the cascade of signals. Further downstream, phosphate signal activates MAPK (also known as ERK). Bottom, signal enters the cell nucleus and causes transcription of DNA, which is then expressed as protein.

- By altering the levels and activities of transcription factors, MAPK leads to altered transcription of genes that are important for the cell cycle.

Clinical significance

- Uncontrolled growth is a necessary step for the development of all cancers. In many cancers (e.g. melanoma), a defect in the MAP/ERK pathway leads to that uncontrolled growth.

SMAD (MAD+SMA)

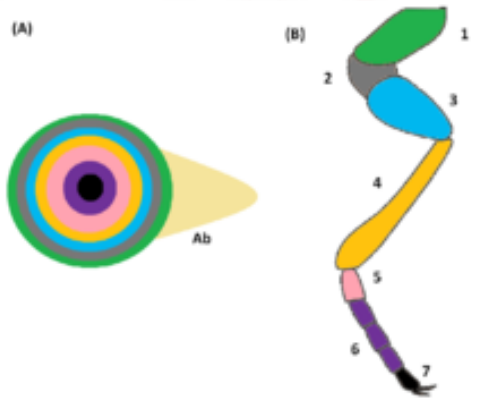
.....Pathway

SMAD.....Pathway

- Nomenclature
- The SMAD proteins are homologs of both the *Drosophila* protein, mothers against decapentaplegic (MAD) and the *Caenorhabditis elegans* protein SMA (from gene *sma* for small body size). The name is a portmanteau of the two. MAD mutations can be placed in an allelic series based on the relative severity of the maternal effect enhancement of weak dpp alleles, thus explaining the name Mothers against dpp.
- MAD+SMA=**SMAD**
- During *Drosophila* research, it was found that a mutation in the gene, *MAD*, in the mother, repressed the gene *decapentaplegic* in the embryo.

Decapentaplegic (Dpp) is a key morphogen which is known to be necessary for the correct patterning and development of the early *Drosophila* embryo and the fifteen imaginal discs, which are tissues that will become limbs and other organs and structures in the adult fly. It has also been suggested that Dpp plays a role in regulating the growth and size of tissues. Flies with mutations in decapentaplegic fail to form these structures correctly, hence the name **(*decapenta*-, fifteen, -*plegic*, paralysis)**. Dpp is the Drosophila homolog of the vertebrate bone morphogenetic proteins (BMPs), which are members of the TGF-β superfamily, a class of proteins that are often associated with their own specific signaling pathway.

- **Imaginal disk**

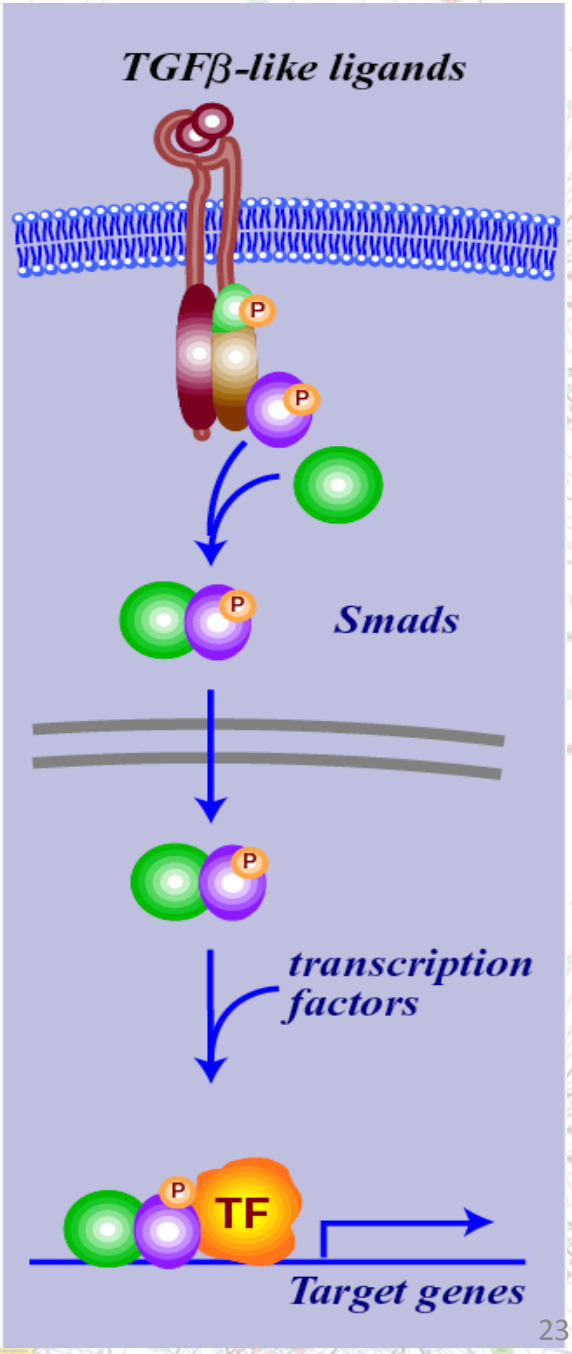


- **Smads** (or **SMADs**) comprise a family of structurally similar proteins that are the main signal transducers for receptors of bone morphogenetic proteins (BMPs), which are members of the the transforming growth factor beta (TGF-B) superfamily, which are critically important for regulating cell development and growth.

SMAD-family

- The Smad-factors mediate response to TGFb-related growth- and differentiation factors

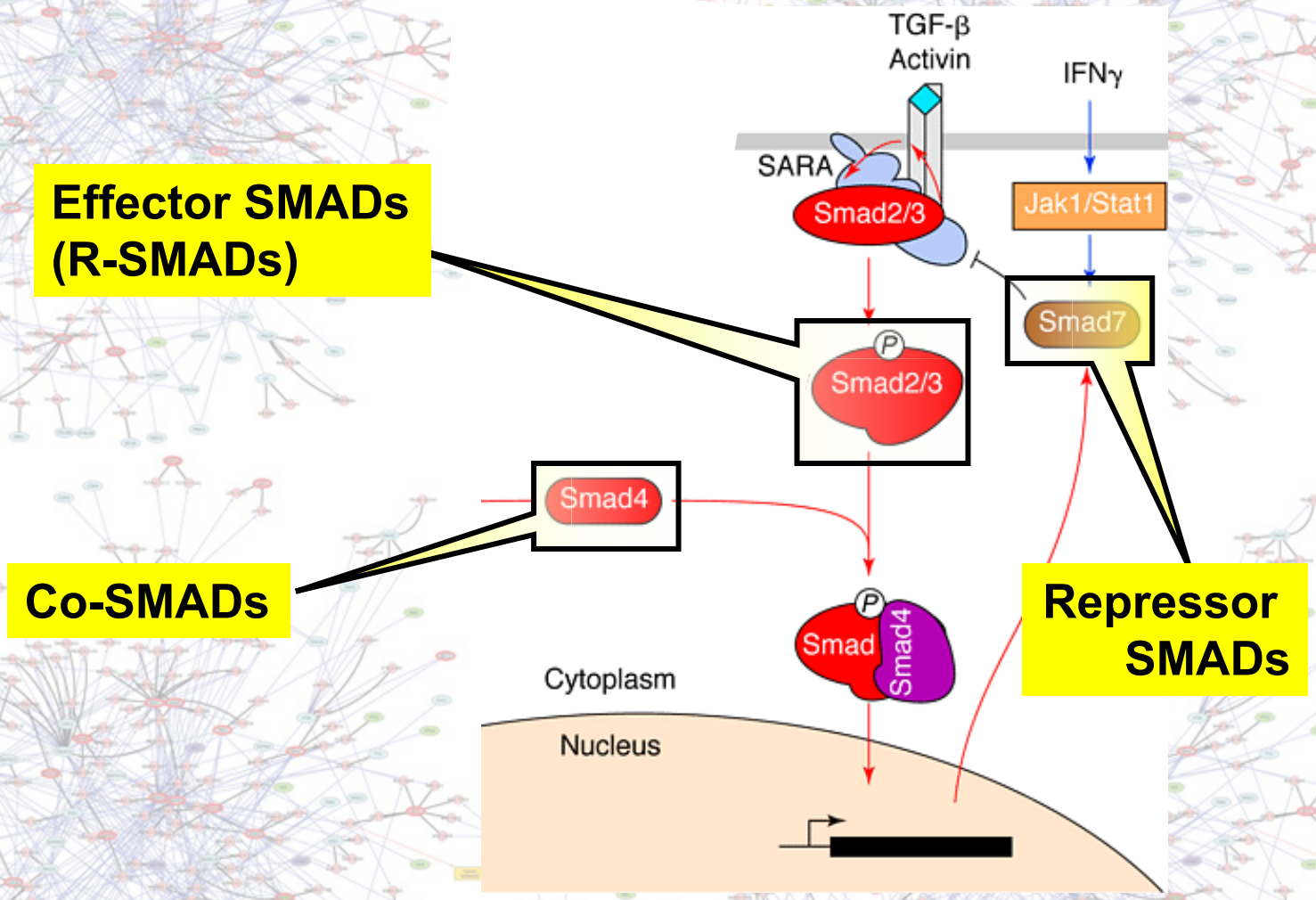
- STAT-related logic
  - Membrane-bound receptors (such as the TGFß-receptor) are activated by binding of ligand (TGFb). The receptors here are transmembrane serine/threonine-kinases
  - Activated kinases phosphorylate specific Smad-factors
  - phosphorylated Smad-factors associate with a common Smad-factor (Smad4)
  - The generated heteromeric complexes migrate to the nucleus as transcription factors



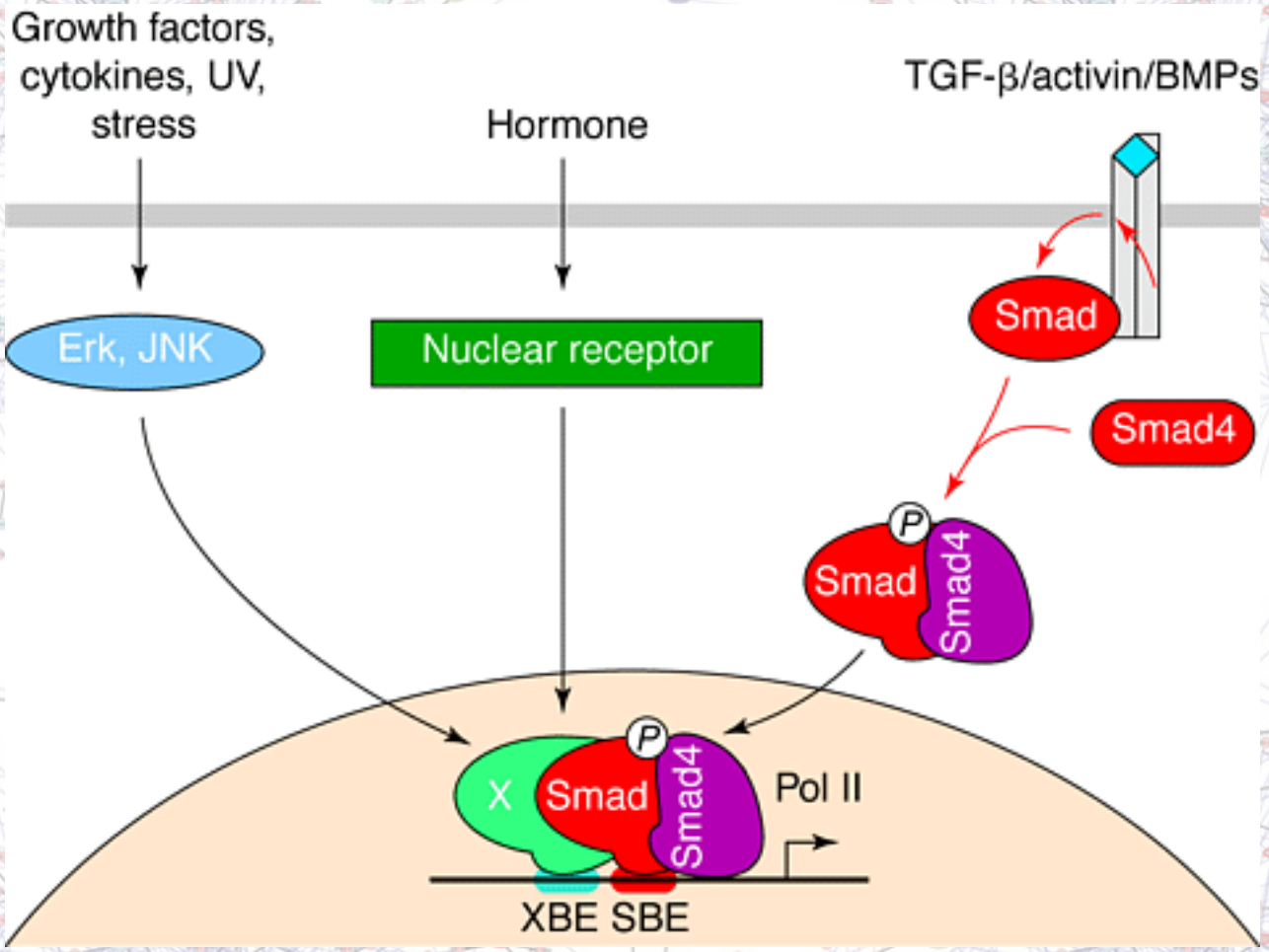
Three groups of SMADs

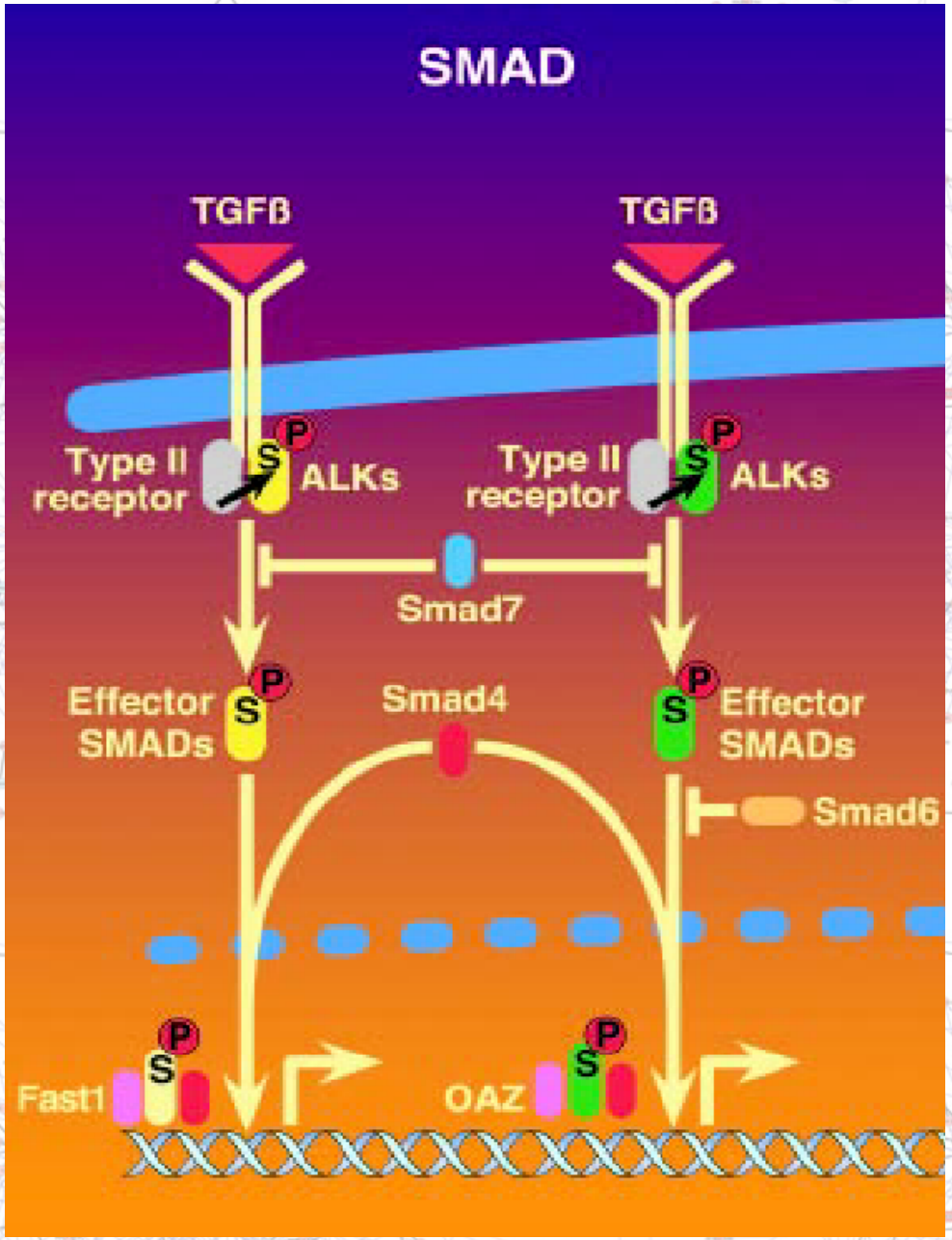
- First group: The effector SMADs (also called the R-SMADs) become serine-phosphorylated in the C-terminal domain by the activated receptor.
  - Smad1, Smad5, Smad8, and Smad9 become phosphorylated in response to bone morphogenetic morphogenetic protein (BMP) and growth and differentiation factor (GDF), and Smad2 and Smad3 become phosphorylated in response to the activin/nodal branch of the TGF-b pathway.
- Second group: regulatory or co-SMADs (common SMADs).
  - There are two regulatory SMADs: Smad4 and Smad4b (also called Smad10).
  - Smad4 binds to, and is essential for, the function of Smad1 and Smad2. The regulatory Smad4 binds to all effector SMADs in the formation of transcriptional complexes, but it does not appear to be required for nuclear translocation of the effector molecules.
- Third group: two inhibitory SMADs, Smad6 and Smad7.
  - provide negative regulation of the pathway by blocking Smad4 binding.

SMAD-signalling pathway



The Smad-factors activate their target genes in combination with other TFs




SMAD
TGFβ
TGFβ
Type II receptor
ALKs
Type II receptor
ALKs
P
S
Smad7
Effector SMADs
Smad4
Effector SMADs
Smad6
Fast1
OAZ

- **Bone morphogenetic proteins** (**BMPs**) are a group of growth factors also known as cytokines and as metabologens. Originally discovered by their ability to induce the formation of bone and cartilage, BMPs are now considered to constitute a group of pivotal morphogenetic signals, orchestrating tissue architecture throughout the Cancerous disease often involves misregulation of the BMP signalling system. Absence of BMP signalling is, for instance, an important factor in the progression of colon cancer, and conversely, overactivation of BMP signalling following reflux -induced esophagitis provokes Barrett's esophagus and is thus instrumental in the development of adenocarcinoma in the proximal portion of the gastrointestinal tract.

Role of Smad in cell cycle control

- In adult cells, TGF-B inhibits cell cycle progression, stopping cells from making the G1/S phase transition.This phenomenon is present in the epithelial cells of many organs, and is regulated in part by the Smad signaling pathway.

Role of Smad in cancer

- Defects in Smad signaling can result in TGF-B resistance, causing dysregulation of cell growth. Deregulation of TGF-B signaling has been implicated in many cancer types, including pancreatic, colon, breast, lung, and prostate cancer. Smad4 is most commonly mutated in human cancers, particularly pancreatic and colon cancer. Smad4 is inactivated in nearly half of all pancreatic cancers.

- https://en.wikipedia.org/wiki/JAK-STAT_signaling_pathway
- https://en.wikipedia.org/wiki/MAPK/ERK_pathway
- https://en.wikipedia.org/wiki/SMAD_(protein)