E-content

M.Sc. Zoology (Semester IV)
Elective Paper: Cell and Molecular biology

Unit: 3.7

Signal transduction pathways and their regulation

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An overview of signal transduction

Signal transduction pathways convert extracellular stimuli into specific cellular responses . Typically, signal transduction begins with a signal to a receptor and ends with a change in cell function.

Sometimes, there is a cascade of signals within the cell. With each step of the cascade, the signal can be amplified, so a small signal can result in a large response.

Eventually, the signal creates a change in the cell, either in the expression of the DNA in the nucleus or in the activity of enzymes in the cytoplasm.

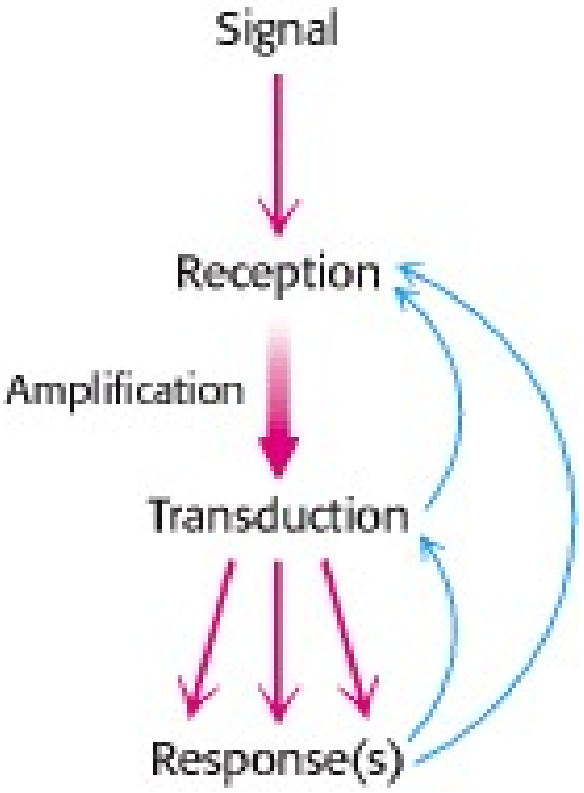


Figure 1: An environmental signal, such as a hormone, is first received by interaction with a cellular component, most often a cell-surface receptor. The information that the signal has arrived is then converted into other chemical forms, or *transduced*. The signal is often amplified before evoking a response. Feedback pathways regulate the entire signaling process.

An overview of signal transduction

Most signal transduction involves the binding of extracellular signalling molecules (and ligands) to cell-surface receptors.

Intracellular signal transduction is largely carried out by second messenger molecules such as cyclic-AMP (cAMP) and -GMP (cGMP), calcium (Ca^{2+}), nitric oxide and lipophilic second messenger molecules (diacylglycerol, ceramide and the eicosanoids).

Here, we will discuss some major signal transduction pathways

1. **MAP kinase pathways**
2. **JAK-STAT Pathway**
3. **TGF-beta/Smad pathways**
4. **NF-kB Pathway**
5. **The hedgehog Pathway**
6. **Notch pathways**
7. **Wnt Pathway**
8. **PKB/AKT signaling pathway**
9. **mTOR pathways**

1. Mitogen-activated protein kinases (MAPK) pathways

Mitogen-activated protein kinases (MAPK) are proteins that are serine/ threonine specific kinases which are activated by a wide range of stimuli including pro-inflammatory cytokines, growth factors, mitogens, osmotic stress, heat shock etc.

These proteins function in a signaling cascade are activated upon ligand binding to a cell surface receptor activating several MAP/ER kinases, which in turn phosphorylate their respective substrates.

These events thereby regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis.

Activation of MAPK signaling

Mitogen-activated protein kinase (MAPK) modules containing three sequentially activated protein kinases are key components of a series of vital signal transduction pathways.

Activation of MAPK signaling

Each cascade is initiated by specific extracellular cues and leads to activation of a particular MAPK following the successive activation of a MAPK kinase kinase (MAPKKK) and a MAPK kinase (MAPKK).

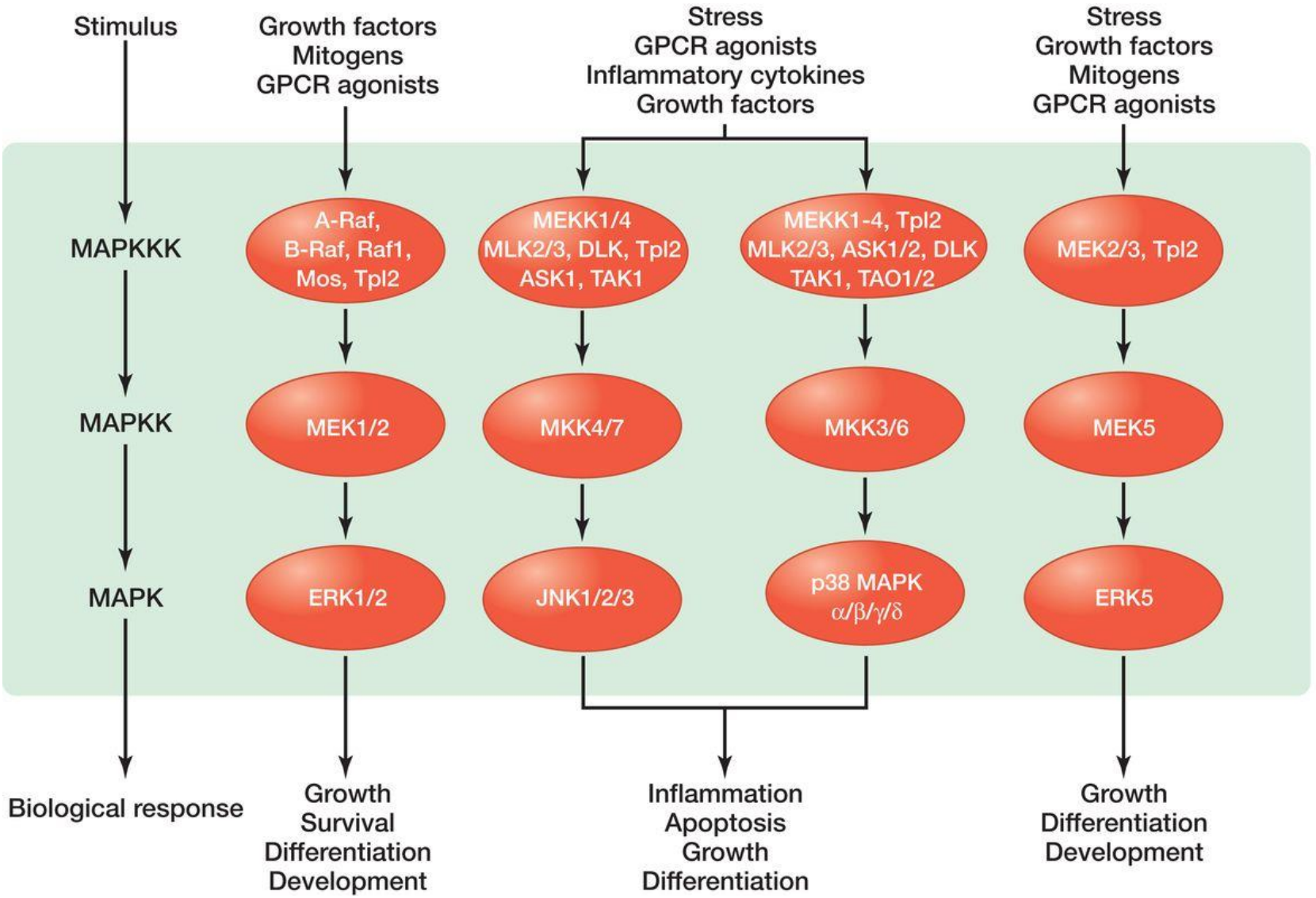
The MAPKKK is typically activated by interactions with a small GTPase and/or phosphorylation by protein kinases downstream from cell surface receptors.

The MAPKKK directly phosphorylates and activates the MAPKK, which, in turn, activates the MAPK by phosphorylation of a conserved motif.

Once activated, the MAPK phosphorylates diverse substrates in the cytosol and nucleus to bring about changes in protein function and gene expression that execute the appropriate biological response.

MAPKs generally contain docking sites for MAPKKs and substrates to ensure that they are activated by a particular upstream MAPKK and that they recognize specific downstream targets

An overview of MAPK pathways



Cold Spring Harb Perspect Biol 2012;4:a011254

MAPK signaling

The MAP kinases can be grouped into three main families.

In mammals, these are

A) ERKs (extracellular-signal-regulated kinases)
B) JNKs (Jun amino-terminal kinases)
C) p38/SAPKs (stress-activated protein kinases)

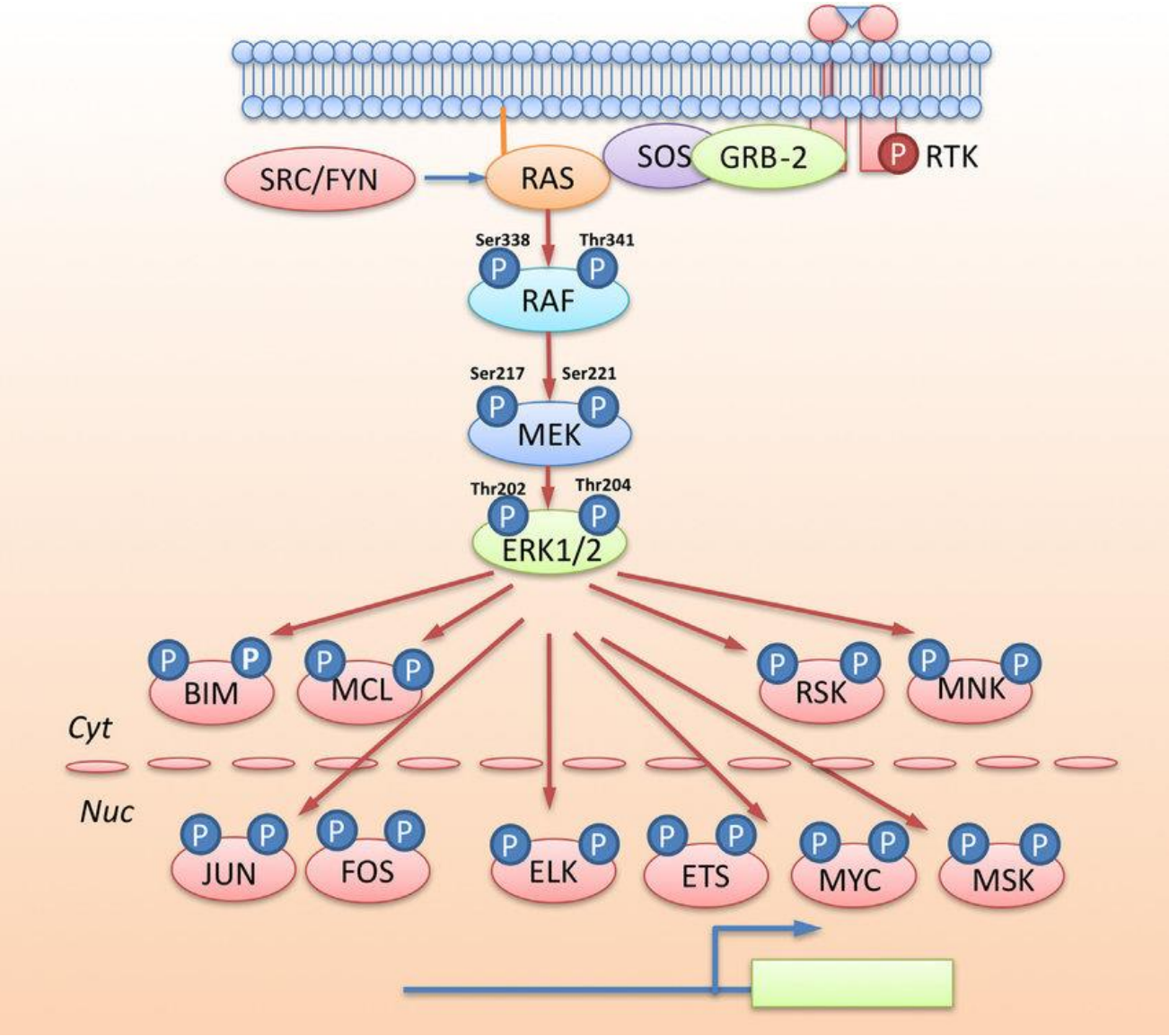
A) ERKs (extracellular-signal-regulated kinases)

ERK family members (ERK 1, ERK2 and ERK5)possess a TEY motif in the activation segment.

Important upstream regulators of this module include cell surface receptors, such as receptor tyrosine kinases (RTKs), G-protein-coupled receptors (GPCRs), and integrins, as well as the small GTPases Ras and Rap.

MAPKKs for the classic ERK1/2 module are MEK1 and MEK2, and the MAPKKKs include members of the Raf family, Mos, and Tpl2.

The ERK MAPK pathway.



Acta Pharmaceutica Sinica B, Vol 8, Issue 4, July 2018, Pages 552-562

B) JNKs (Jun amino-terminal kinases)

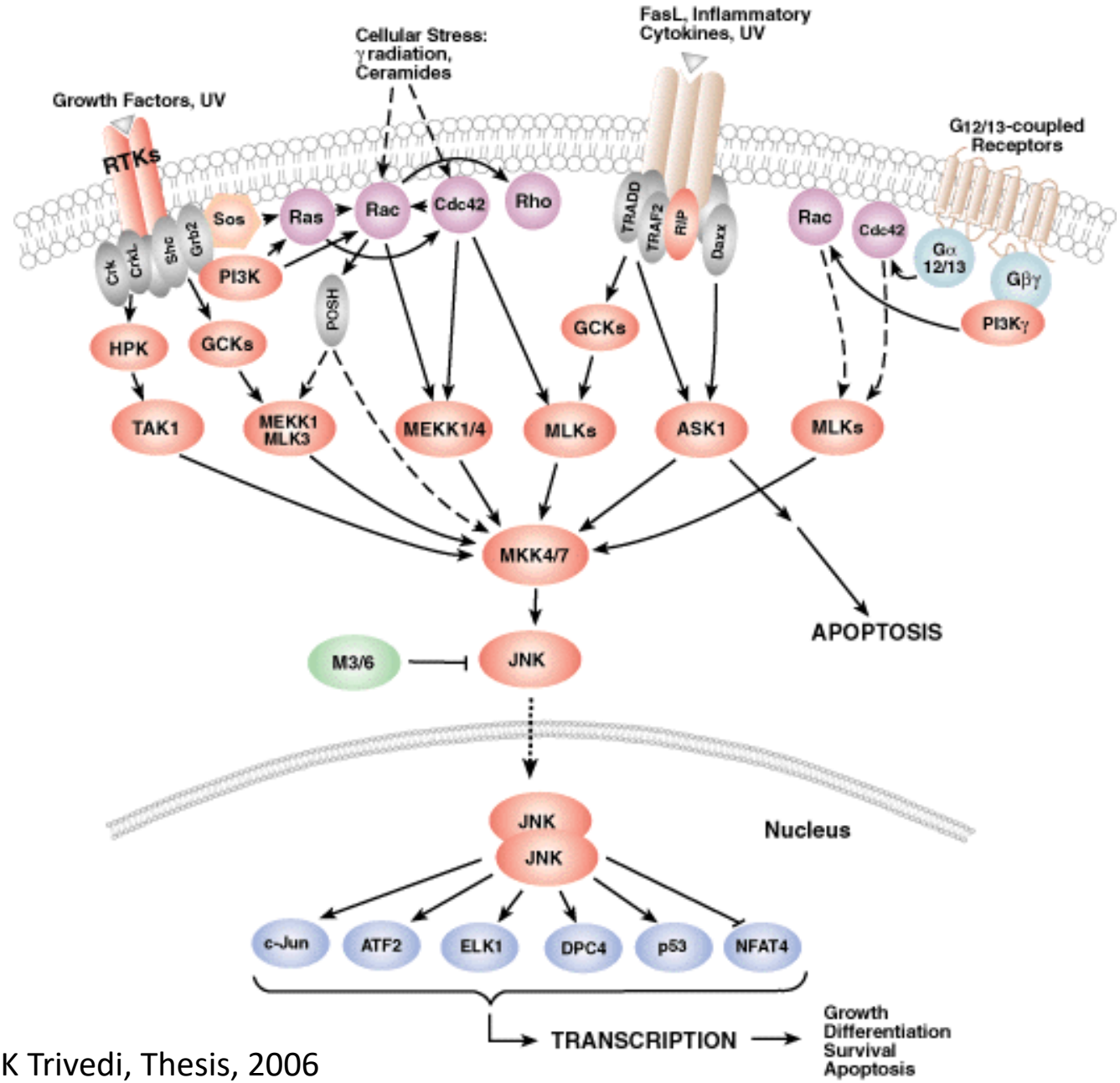
JNK family members contain a TPY motif in the activation segment and include JNK1, JNK2, and JNK3.

The JNK module is activated by environmental stresses (ionizing radiation, heat, oxidative stress, and DNA damage) and inflammatory cytokines, as well as growth factors, and signaling to the JNK module often involves the Rho family GTPases Cdc42 and Rac.

The JNK module plays an important role in apoptosis, inflammation, cytokine production, and metabolism.

MAPKKs for the JNK module are MKK4 and MKK7, and the MAPKKKs include MEKK1 and MEKK4, MLK2 and MLK3, ASK1, TAK1, and Tpl2.

The JNK MAPK pathway



AK Trivedi, Thesis, 2006

C) p38/SAPKs (stress-activated protein kinases)

p38 family members possess a TGY motif in the activation segment and include p38α, p38β, p38γ, and p38δ.

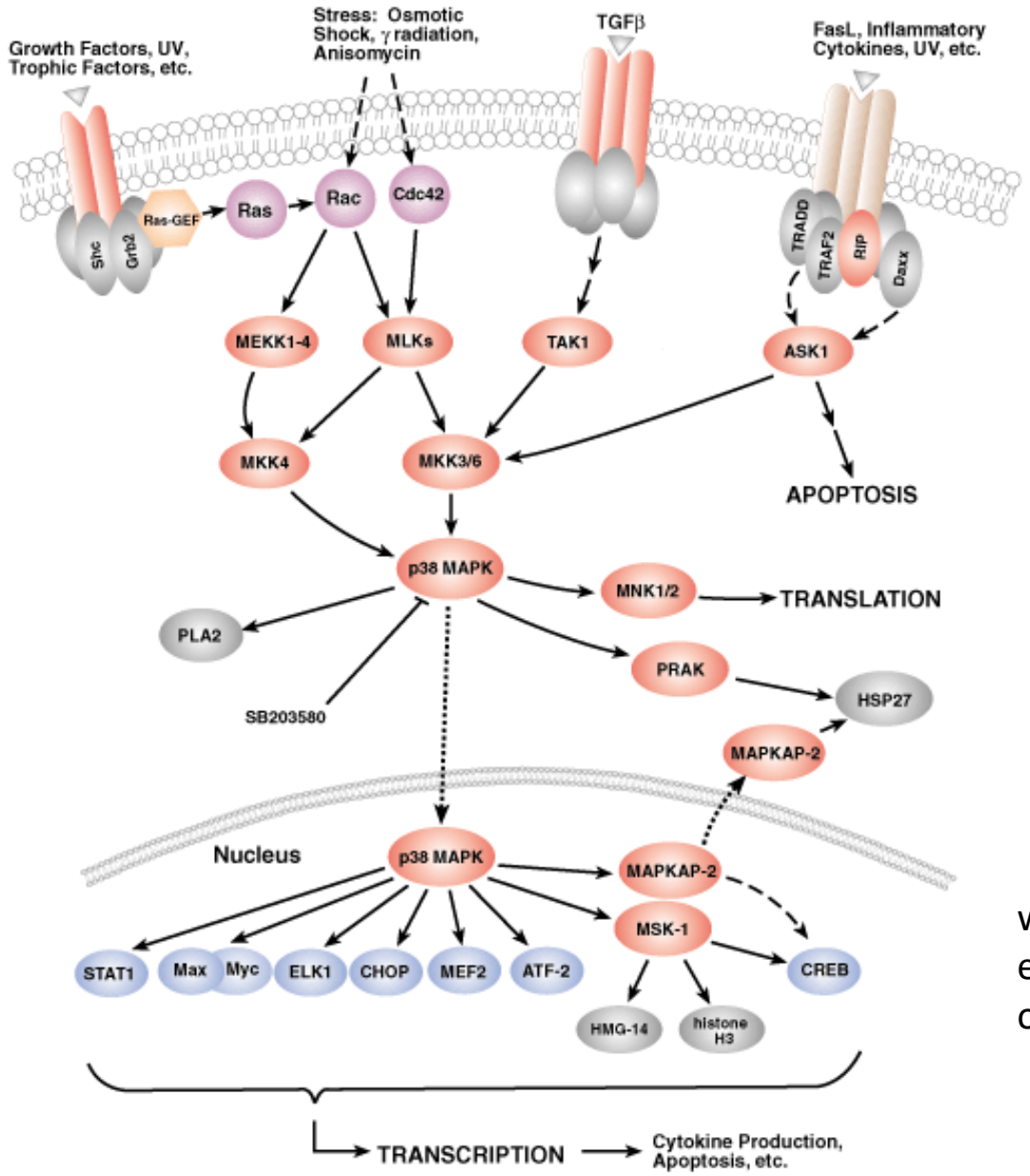
Like JNK modules, p38 modules are strongly activated by environmental stresses and inflammatory cytokines.

p38 activation contributes to inflammation, apoptosis, cell differentiation, and cell cycle regulation.

The primary MAPKKs for p38 modules are MKK3 and MKK6, and the MAPKKKs include MLK2 and MLK3, MEKKs, ASKs, TAK1, and TAO1 and TAO2.

Important substrates in p38 signaling include the downstream kinases MK2/3, PRAK, and MSK1 and MSK2, as well as various transcription factors.

The p38 MAPK pathway.



www.ufrgs.br/imunovet/molecular_immunology/p38map.html

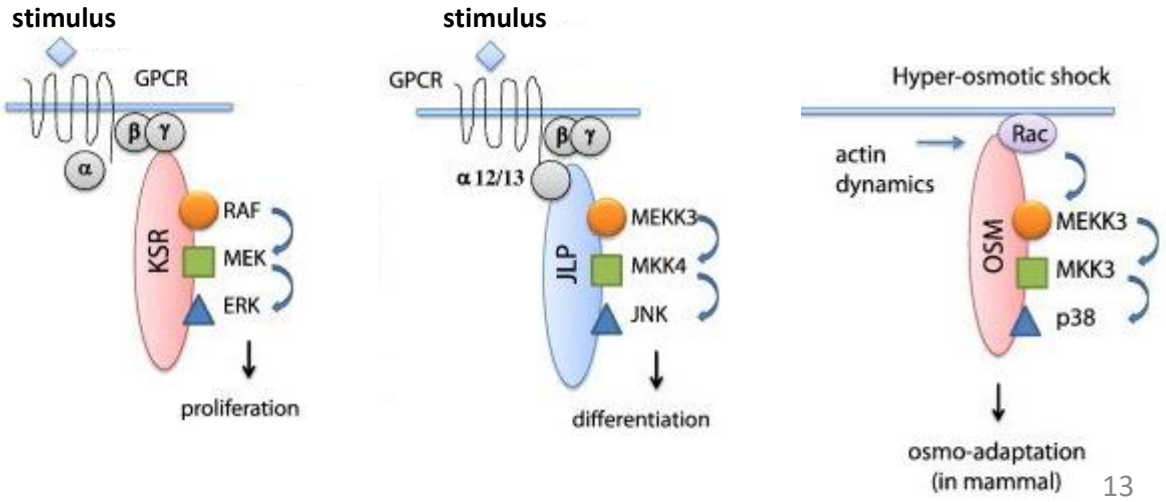
Regulation of MAP kinase signaling

A) Scaffold proteins:

These scaffolds contribute to MAPK signaling by increasing the local concentration of the components, providing spatial temporal regulation of cascade activation, and/or localizing the module to specific cellular sites or substrates.

For all of the MAPK modules, specific scaffold proteins have been identified that dock at least two of the core kinases of the module.

Scaffold proteins involved in MAPK cascade signaling include KSR and MP1 for the ERK module; JIP1, JIP2, JIP3, JIP4, and POSH for the JNK module; and JIP2, JIP4, and OSM for the p38 module



Cellular Signaling, Vol 24, Issue 11, Nov 2012, Pages 2143-2165

B) Subcellular localization:

Spatial heterogeneity and compartmentalization within cells permits pathways and molecular components to be regulated on the basis of their subcellular localization and molecular accessibility.

In mammalian cells, the MAPK ERK2 is localized in the cytosol when inactive and enters the nucleus after activation.

The upstream MAPKKs, MEK1 and MEK2, have a high-affinity docking site for ERK2, but also contain a potent nuclear export signal (NES), thus ensuring that prior to activation, the complex is in the cytoplasm.

Upon activation, dually-phosphorylated ERK2 dissociates from MEK1 and MRK2 and enters the nucleus.

Further, MEK1 and MEK2, which undergoes nucleocytoplasmic shuttling, can retrieve ERK2 from the nucleus once it has undergone inactivation/ dephosphorylation.

In this way the various components of MAPK signaling are regulated by their specific subcellular localisation patterns

C) Temporal characteristics and pathway inactivation

The responses of cells to external signals and acute stresses, while necessary for viability or developmental transitions in the short term, often involve behaviors that can impair growth or viability in the long term.

MAPKs, particularly those that either arrest or substantially divert resources away from cellular proliferation, generally are kept at low activation levels in the absence of their appropriate signals and are rapidly inactivated following the course of a relatively brief response.

Inactivation of MAPK pathways occurs through both constitutive and induced (negative feedback) mechanisms.

The phosphorylated MAPK members are deactivated by dephosphorylation by the phosphatases.

Besides dephosphorylation of MAPKs, MAPK pathway activity are also be attenuated in a timed manner by the ubiquitin-mediated degradation of pathway components.

2. JAK/STAT pathway

The **Janus kinase-signal transducer and activator of transcription (JAK-STAT)** pathway plays a major role in transferring of signals from cell-membrane receptors to the nucleus.

The JAK-STAT pathway is essential for a wide range of cytokines and growth factors, leading to critical cellular events, such as hematopoiesis, lactation and development of the immune system and mammary glands.

A variety of ligands and their receptors stimulate the JAK/STAT pathway. Intracellular activation occurs when ligand binding induces the multimerization of receptor subunits.

For some ligands, such as erythropoietin and growth hormone, the receptor subunits are bound as homodimers while, for others, such as interferons and interleukins, the receptor subunits are heteromultimers.

For signal propagation through either homodimers or heteromultimers, the cytoplasmic domains of two receptor subunits must be associated with JAK tyrosine kinases.

JAK/STAT pathway

In mammals, the JAK family comprises four members: JAK1, JAK2, JAK3 and Tyk2.

JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowing trans-phosphorylation.

The activated JAKs subsequently phosphorylate additional targets, including both the receptors and the major substrates, STATs.

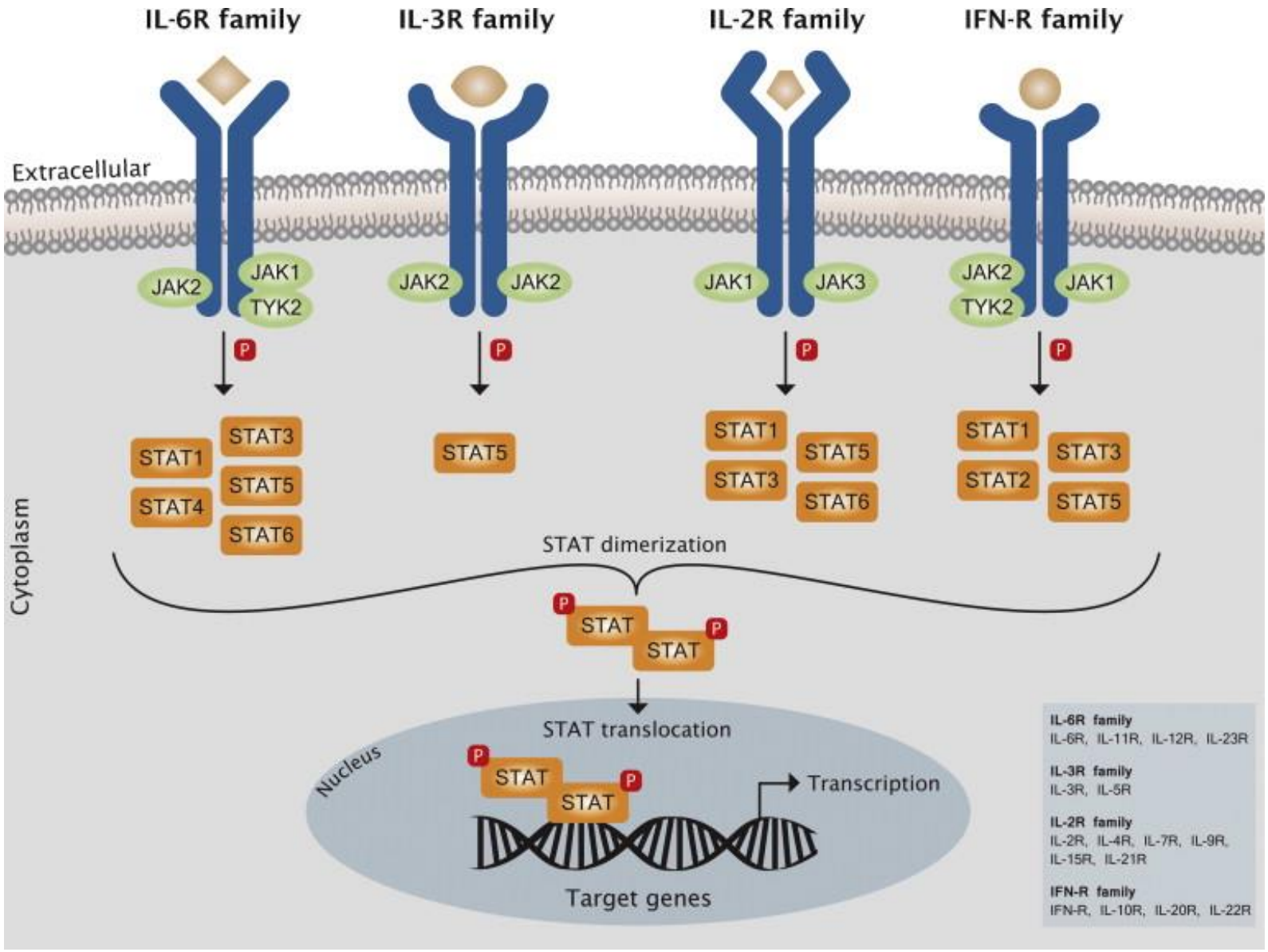
STATs are latent transcription factors that reside in the cytoplasm until activated. The seven mammalian STATs bear a conserved tyrosine residue near the C-terminus that is phosphorylated by JAKs.

This phosphotyrosine permits the dimerization of STATs through interaction with a conserved SH2 domain.

Phosphorylated dimerised STATs enter the nucleus and bind specific regulatory sequences to activate or repress transcription of target genes.

Thus, the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response.

JAK/STAT pathway



Pharmacological Research, Volume 76, October 2013, Pages 1-8

Regulation of JAK-STAT signaling

Signaling through the JAK-STAT pathway is tightly controlled by a number of distinct mechanisms. Key regulators of this pathway include:

1. Suppressors of cytokine signaling (SOCS):
SOCS proteins form a classic negative-feedback loop of cytokine signaling. They are typically expressed at low levels and become rapidly induced following cytokine activation of the JAK-STAT pathway. SOCS proteins can interact with phosphotyrosine phosphorylated proteins through their SH2 domain.

2. Protein inhibitors of activated STATs (PIASs):
The mammalian PIAS family consists of four members: PIAS1, PIAS3, PIASX and PIASY84. After cytokine stimulation, PIAS1, PIAS3 and PIASX interact with STAT1, STAT3 and STAT4, respectively to inhibit their activity.

3. Protein tyrosine phosphatases (PTPs):
STATs can also be inactivated by PTPs in both the cytoplasm and the nucleus. SHP2 is involved in the dephosphorylation of STAT5 in the cytoplasm.

3. TGF-beta signaling

TGF-beta signaling is involved in the regulation of proliferation, differentiation and survival/or apoptosis of many cells, including glioma cells.

TGF-beta acts via specific receptors activating multiple intracellular pathways resulting in phosphorylation of receptor-regulated Smad2/3 proteins that associate with the common mediator, Smad4.

Such complex translocates to the nucleus, binds to DNA and regulates transcription of many genes.

TGF-beta superfamily of cytokines bind to receptors at the cell surface, and recruit two type I receptors and two type II receptors forming a tetrameric complex.

Activated TGF-beta superfamily receptors induce a series of phosphorylation cascade, from receptor phosphorylation to subsequent phosphorylation and activation of downstream signal transducer R-Smads (receptor-activated Smads).

TGF-beta signaling

Phosphorylated R-Smads form a heteroligomeric (often trimeric) complex with Smad4 (Co-Smad).

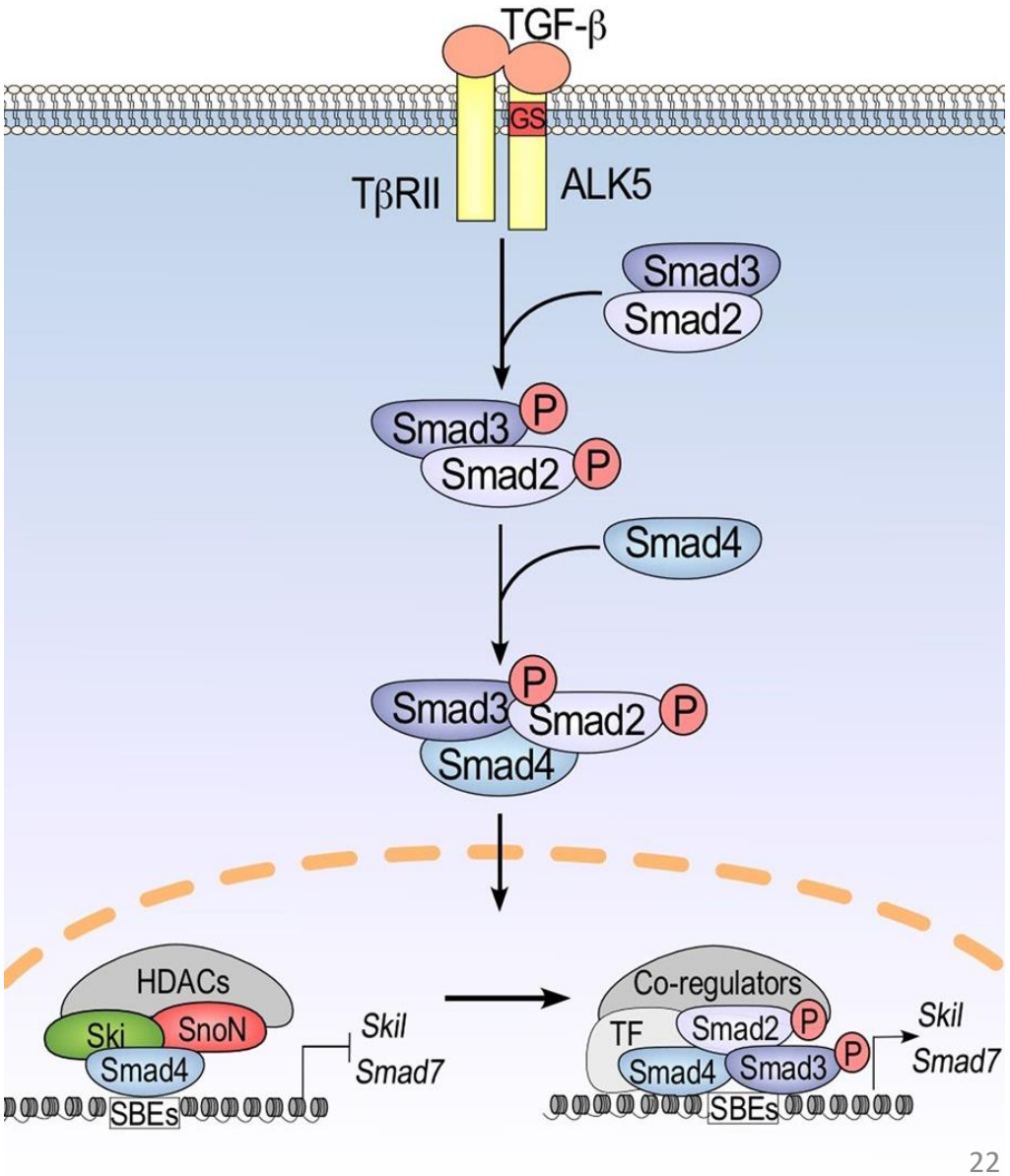
The Smad complex is imported into the nucleus and regulates the expression of target genes by direct binding to the target gene promoter and/or through the interaction with transcriptional cofactors in a cell-type-specific manner.

TGF-β superfamily ligand	Type II Receptor	Type I receptor	R-SMADs	coSMAD
Activin A	ACVR2A	ACVR1B (ALK4)	SMAD2 , SMAD3	SMAD4
GDF1	ACVR2A	ACVR1B (ALK4)	SMAD2 , SMAD3	SMAD4
GDF11	ACVR2B	ACVR1B (ALK4), TGFβRI (ALK5)	SMAD2 , SMAD3	SMAD4
BMP	BMPR2	BMPR1A (ALK3), BMPR1B (ALK6)	SMAD1 , SMAD5, SMAD9	SMAD4
Nodal	ACVR2B	ACVR1B (ALK4), ACVR1C (ALK7)	SMAD2 , SMAD3	SMAD4
TGFβs	TGFβRII	TGFβRI (ALK5)	SMAD2 , SMAD3	SMAD4

Table showing various members of TGF β signaling

There are a variety of mechanisms that the TGF-Beta pathway is modulated both positively and negatively: There are agonists for ligands and R-SMADs; there are decoy receptors; and R-SMADs and receptors are ubiquitinated.

TGF-beta Pathway



Signal Transduct Target Ther. 2018; 3: 15

4. NF-kB signaling pathway

The master molecule of this pathway comprises the NF-κB family of proteins. In mammals, the NF-κB family is composed of five related transcription factors: p50, p52, RelA (p65), c-Rel and RelB.

These transcription factors share homology through a 300 amino acid N-terminal DNA binding/dimerization domain, called the Rel homology domain (RHD).

The RHD is a platform where family members can form homodimers and heterodimers, enabling them to bind promoters and enhancer regions of genes to modulate their expression.

NF-κB proteins are inhibited by IκB proteins present in the cytoplasm. There are currently seven identified IκB family members: IκBα, IκBβ, Bcl-3, IκBε, IκBγ and the precursor proteins p100 and p105.

NF-kB signaling pathway

NF-kB protein dimmers are nuclear transcription factor, they need to migrate to the nucleus, combined with DNA to have function.

In most types of the normal cells under resting state, NF-KB remains inactive and retained in the cytoplasm. They binds to a specific inhibitors called IK-B protein and interfere with its nuclear localization sequence (NLS) function.

In order to activate the NF-kB molecular, the cells first need to separate the NF-kB protein from their inhibitors.

There are two major signaling pathways lead to the IK-B protein inhibitor dissociation from NF-kB dimmer and let the translocation of NF-kB dimers from the cytoplasm into the nucleus.

1. Canonical pathway
2. Non-canonical pathway

1. Canonical/Classical Cascade

Canonical or classical cascade signaling starting from the cell surface receptor of pro-inflammatory cytokines and pathogen-associated molecular patterns (PAMPs) such as the tumor necrosis factor receptor (TNFR), toll-like receptor (TLR) and T/B cell receptor.

These receptors binding with their ligand molecules and transfer the signal across the cell membrane, cause the activation of the IkB kinase (IKK) complex.

The most common form of this complex consist heterodimer of IKKα and IKKβ catalytic subunits and an IKKγ regulatory subunit. The IKKγ unit also called NEMO for NF-kB essential modulator.

The activated IKK complex, predominantly acting through IKKβ in an IKKγ-dependent manner, catalyzes the phosphorylation and polyubiquitination of IkB and subsequent degradation by the 26S proteasome.

The released NF-kB dimers such as p50 and RelA, translocate to the nucleus, bind DNA and activate the down-stream gene transcription.

2. Non-canonical/Alternative pathway Cascade

This pathway is independent of IKKβ and IKKγ, but dependent on IKKα dimmer instead.

The signaling transfer into the cytoplasm through the LT-β or BAFF receptor.

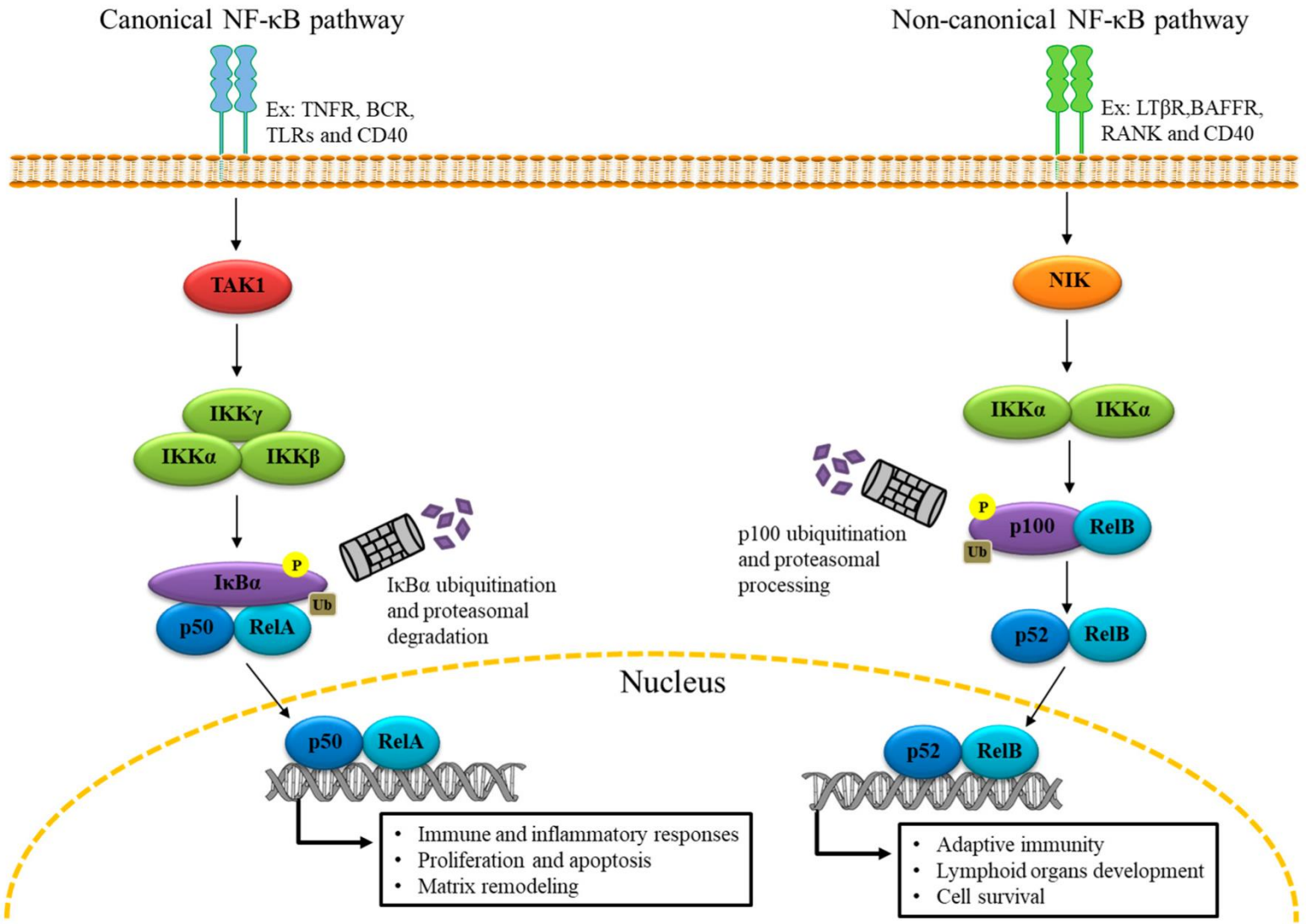
Ligand-induced activation results triggers NF-κB inducing kinase (NIK) to phosphorylate and activate the IKKα complex.

In turn, the IKKα complex phosphorylates p100 leading to the processing and liberation of the p52/RelB active heterodimer.

However, instead of leading to complete p100 degradation, as seen with IkBs, the phosphorylation-dependent ubiquitination of p100 results only in degradation of its inhibitory C-terminal half parts.

Once the C-terminal half is degraded, the N-terminal portion of NF-kB is released leading to the nuclear translocation of p52–RelB dimers. The dimer finally bind to DNA and active the down-stream gene transcription.

NF-kB signaling pathway



Int. J. Mol. Sci. **2019**, *20*(17), 4185

NF-κB Pathway regulation

Ubiquitination plays an essential role in the regulation of NF-kB pathways. In unstimulated cells, NF-kB binds to inhibitory proteins of kB family (IkB) and is sequestered in the cytoplasm.

Upon stimulation, IkB is phosphorylated by the IkB kinase (IKK) complex, Phosphorylated IkB is subsequently ubiquitinated and degraded by 26S proteasome, thus allowing NF-kB to translocate to the nucleus, where it regulates the expression of a plethora of genes.

As the regulatory subunit of the IKK complex, NEMO (IKKγ) is the key factor for transducing ubiquitination signal to IKK activation.

Several deubiquitnases (DUBs) function as key negative regulators of IKK to allow a tight control of NF-kB activation.

One of the best studied DUBs is A20, It has been proposed that A20 suppress hyperactivation of NFkB by deubiquitinaton.

5. Hedgehog (Hh) pathway

The evolutionarily conserved Hedgehog (Hh) pathway is essential for normal embryonic development and plays critical roles in adult tissue maintenance, renewal and regeneration.

Secreted Hh proteins act in a concentration- and time-dependent manner to initiate a series of cellular responses that range from survival and proliferation to cell fate specification and differentiation.

Proper levels of Hh signaling require the regulated production, processing, secretion and trafficking of Hh ligands– in mammals this includes Sonic (Shh), Indian (Ihh) and Desert (Dhh).

All Hh ligands are synthesized as precursor proteins that undergo autocatalytic cleavage and concomitant cholesterol modification at the carboxy terminus and palmitoylation at the amino terminus, resulting in a secreted, dually-lipidated protein.

Hh ligands are released from the cell surface through the combined actions of Dispatched and Scube2, and subsequently trafficked over multiple cells through interactions with the cell surface proteins LRP2 and the Glypican family of heparan sulfate proteoglycans (GPC1-6).

Hedgehog (Hh) pathway

Hh proteins initiate signaling through binding to the canonical receptor Patched (PTCH1) and to the co-receptors GAS1, CDON and BOC.

Hh binding to PTCH1 results in derepression of the GPCR-like protein Smoothened (SMO) that results in SMO accumulation in cilia and phosphorylation of its cytoplasmic tail.

SMO mediates downstream signal transduction that includes dissociation of GLI proteins (the transcriptional effectors of the Hh pathway) from kinesin-family protein, Kif7, and the key intracellular Hh pathway regulator SUFU.

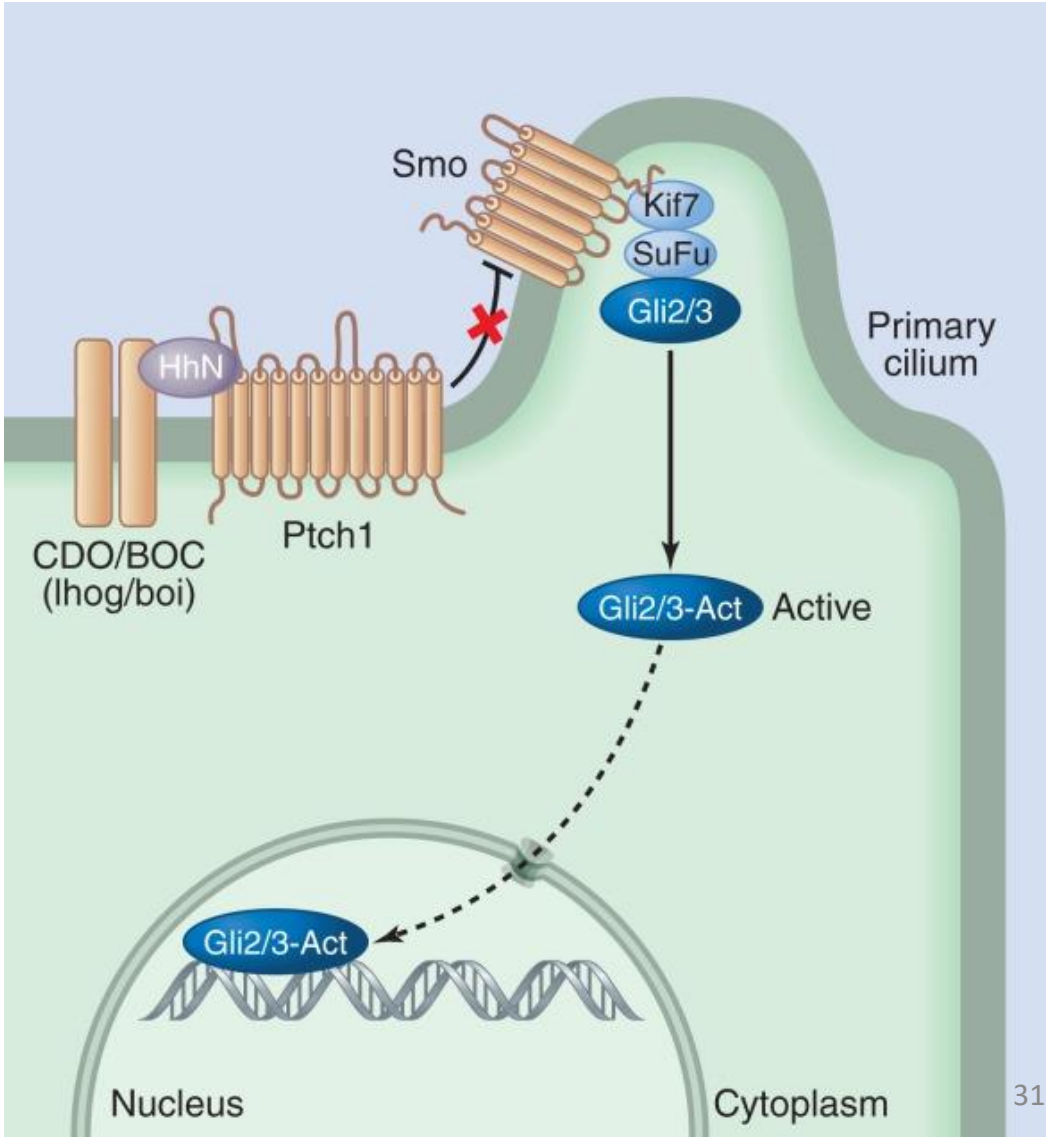
GLI proteins also traffic through cilia and in the absence of Hh signaling are sequestered by SUFU and Kif7, allowing for GLI phosphorylation by PKA, GSK3β and CK1, and subsequent processing into transcriptional repressors (through cleavage of the carboxy-terminus) or targeting for degradation (mediated by the E3 ubiquitin ligase β-TrCP).

In response to activation of Hh signaling, GLI proteins are differentially phopshorylated and processed into transcriptional activators that induce expression of Hh target genes, many of which are components of the pathway (e.g. PTCH1 and GLI1).

Hedgehog (Hh) pathway

Simplified view

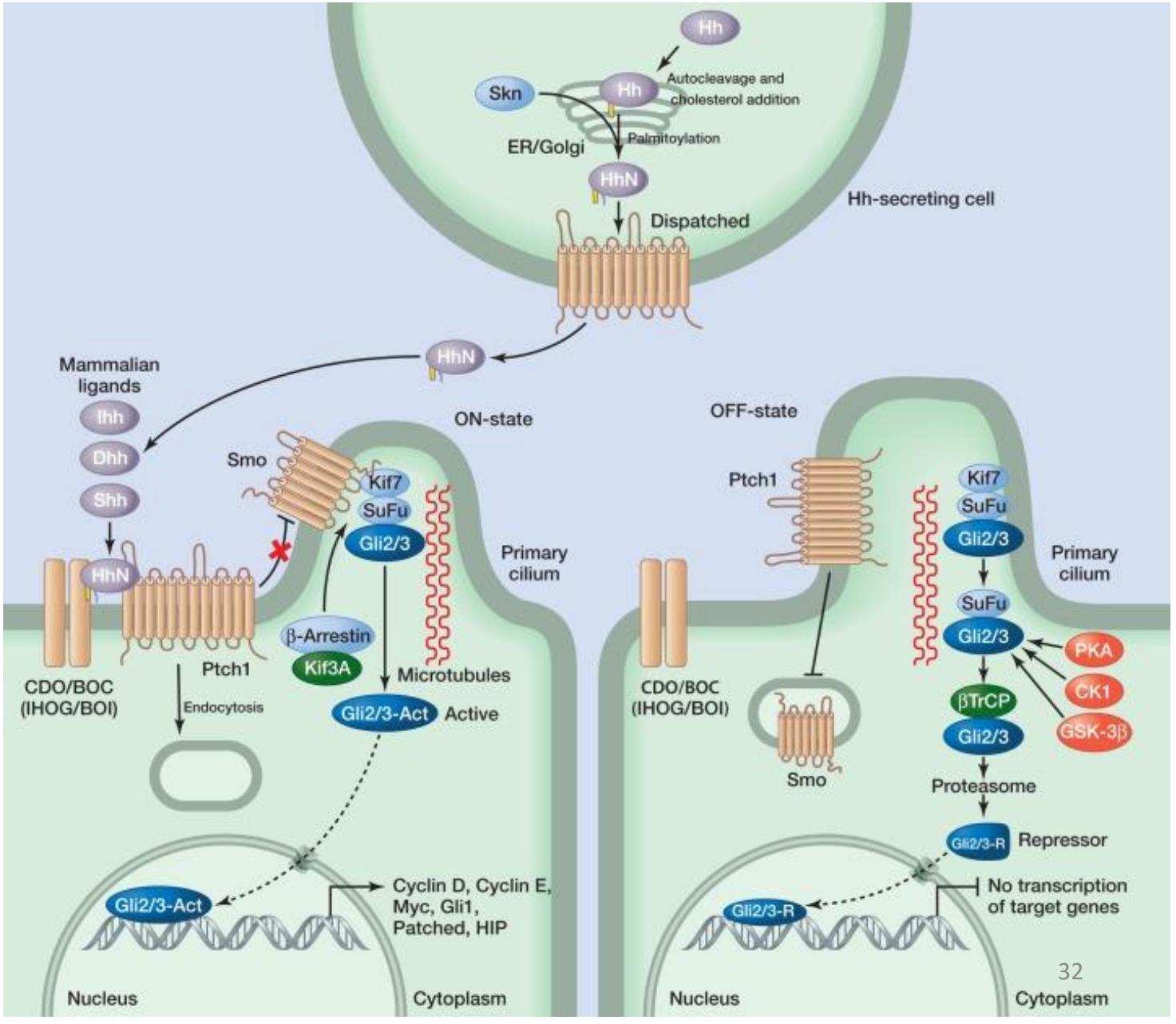
Cold Spring Harb Perspect Biol. 2012 Jun; 4(6): a011221



Hedgehog (Hh) pathway

Detailed view

Cold Spring Harb Perspect Biol. 2012 Jun; 4(6): a011221



Regulation of Hh pathway

SULF1 plays a significant role in regulating the establishment of a gradient of Hedgehog ligand.

In vertebrates, the membrane proteins CDO, BOC, and GAS1 interact with SHH and positively regulate signaling , while the co-receptor HHIP binds to SHH to negatively regulate signaling.

Feedback mechanisms include the induction of Hh pathway antagonists (PTCH1, PTCH2 and Hhip1) that interfere with Hh ligand function, and GLI protein degradation mediated by the E3 ubiquitin ligase adaptor protein, SPOP.

6. Notch Pathway

Notch signaling is an evolutionarily conserved pathway in multicellular organisms that regulates cell-fate determination during development and maintains adult tissue homeostasis.

The Notch pathway mediates juxtacrine cellular signaling wherein both the signal sending and receiving cells are affected through ligand-receptor crosstalk by which an array of cell fate decisions in neuronal, cardiac, immune, and endocrine development are regulated.

Notch receptors are single-pass transmembrane proteins composed of functional extracellular (NECD), transmembrane (TM), and intracellular (NICD) domains.

Notch receptors are processed in the ER and Golgi within the signal-receiving cell through cleavage and glycosylation, generating a Ca2+-stabilized heterodimer composed of NECD non-covalently attached to the TM-NICD inserted in the membrane (S1 cleavage).

The processed receptor is then endosome-transported to the plasma membrane to enable ligand binding in a manner regulated by Deltex and inhibited by NUMB.

Notch Pathway

In mammalian signal-sending cells, members of the Delta-like (DLL1, DLL3, DLL4) and the Jagged (JAG1, JAG2) families serve as ligands for Notch signaling receptors.

Upon ligand binding, the NECD is cleaved away (S2 cleavage) from the TM-NICD domain by TACE (TNF-α ADAM metalloprotease converting enzyme).

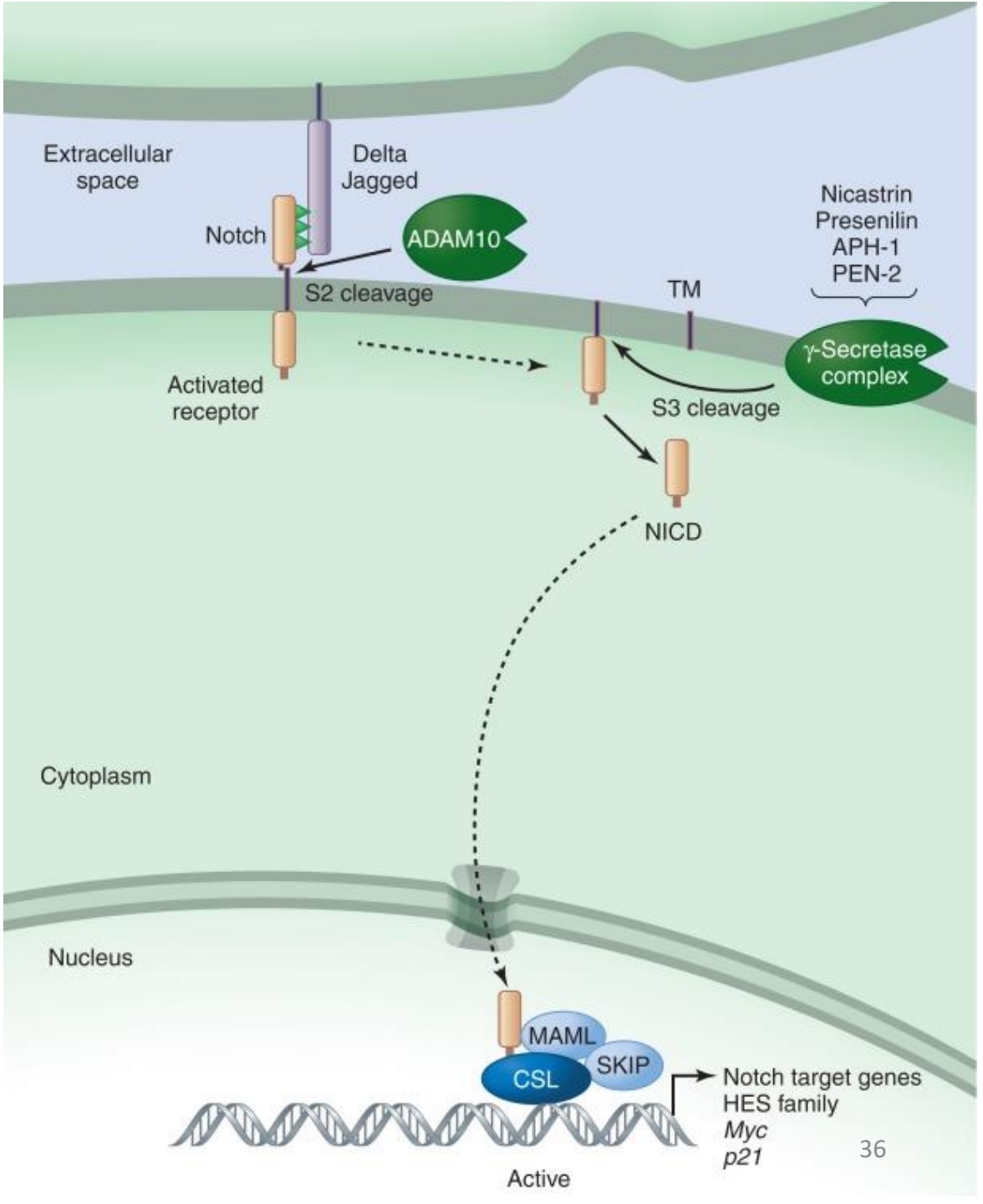
The NECD remains bound to the ligand and this complex undergoes endocytosis/recycling within the signal-sending cell in a manner dependent on ubiquitination by Mib.

In the signal-receiving cell, γ-secretase releases the NICD from the TM (S3 cleavage), which allows for nuclear translocation where it associates with the CSL (CBF1/Su(H)/Lag-1) transcription factor complex, resulting in subsequent activation of the canonical Notch target genes: Myc, p21, and the HES-family members.

Notch Pathway

Simplified View

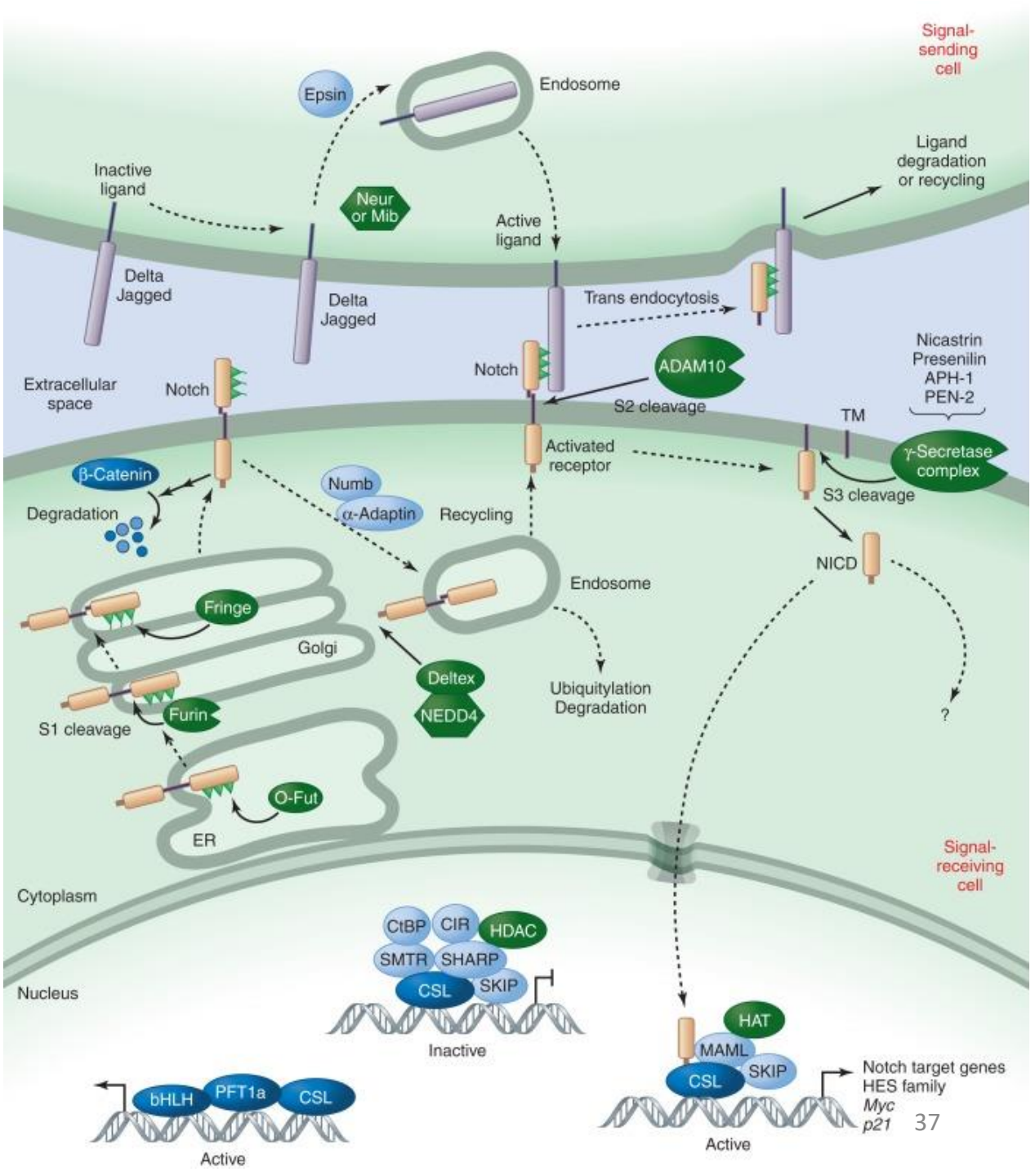
Cold Spring Harb Perspect Biol. 2012 Oct; 4(10): a011213



Notch Pathway

Detailed View

Cold Spring Harb Perspect Biol. 2012 Oct; 4(10): a011213



7. Wnt Signaling pathway

Members of the Wnt family are secreted ligands that regulate numerous developmental pathways .

Wnt binds to members of the Frizzled family, activating a canonical signaling pathway that targets members of the LEF/TCF transcription factor family.

These control gene expression programs that regulate cell fate and morphogenesis.

Wnt also activates so-called non-canonical pathways, which regulate planar cell polarity by stimulating cytoskeletal reorganization and can also lead to calcium mobilization.

Canonical Wnt signaling pathway

In cells not exposed to Wnt signals, the major signaling components including the receptors and the β-catenin protein are kept in an off state.

Active Wnt signaling rearranges these complexes.

In the inactive state, β-catenin levels are kept low through interactions with the protein kinases GSK-3b and CK1, the adenomatous polyposis coli tumor suppressor protein (APC), and the scaffolding protein axin.

β-Catenin is degraded, after phosphorylation by GSK-3 and CK1 through the ubiquitin pathway, which involves interactions with β-TrCP.

β-Catenin is also regulated by adhesion complexes containing cadherins and α-catenin.

During signaling, Wnt proteins interact with Frizzled receptors; the transmembrane protein LRP is also required for Wnt signaling.

When Wnt proteins bind, the receptors presumably rearrange, leading to the activation of β-catenin.

Canonical Wnt signaling pathway

The cytoplasmic tail of LRP binds to axin in a Wnt- and phosphorylation-dependent manner. Phosphorylation of the tail of LRP is regulated by CK1, and Dishevelled (Dvl) and Frizzled also have roles in this process.

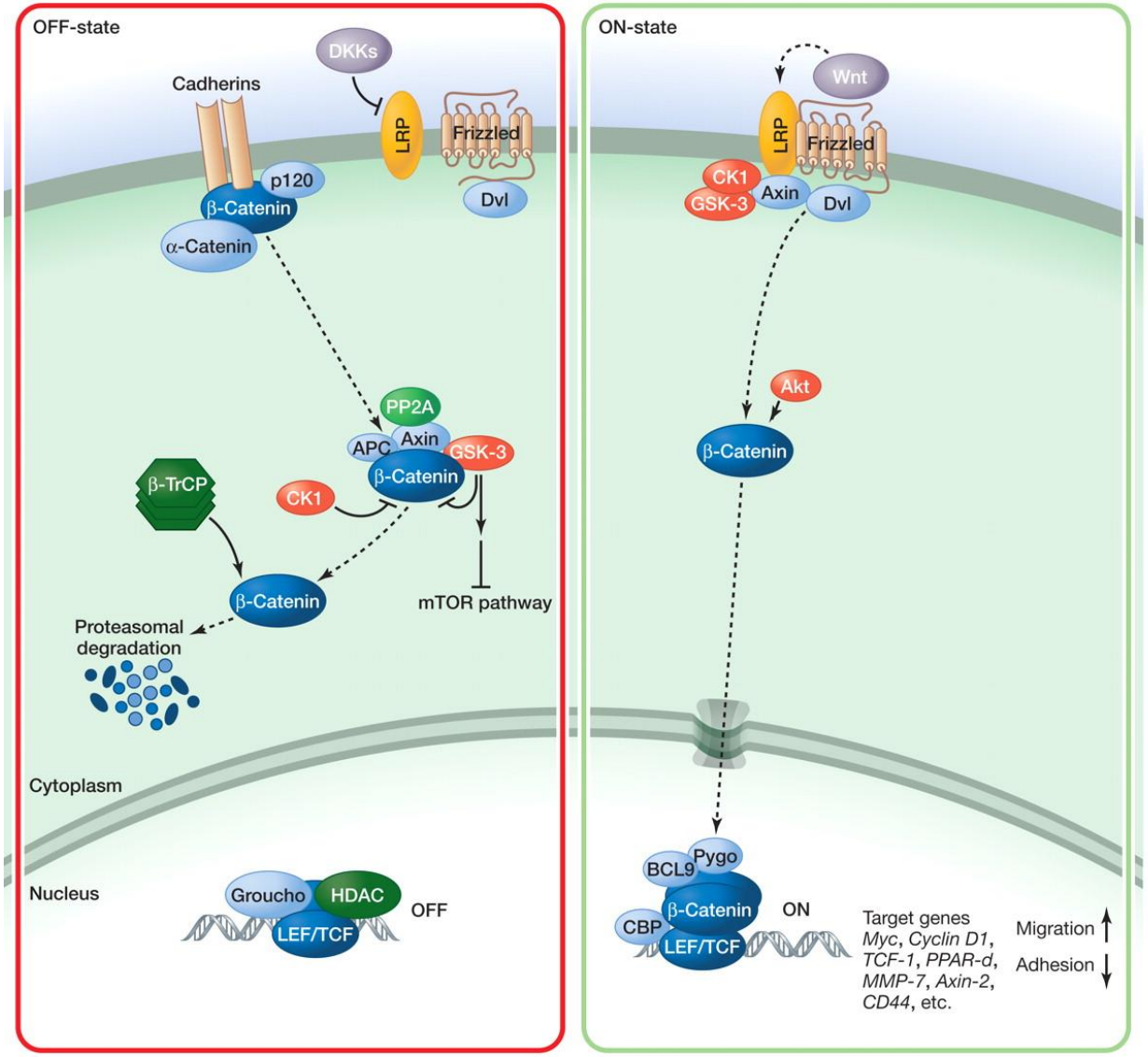
Wnt signaling initially leads to formation of a complex involving Dvl, axin, and GSK3. As a consequence, GSK does not phosphorylate β-catenin anymore, releasing it from the axin complex and allowing it to accumulate.

The stabilized β-catenin then enters the nucleus to interact with TCF/LEF transcription factors.

In the nucleus, in the absence of the Wnt signal, TCF/LEF acts as a repressor of Wnt target genes, in a complex with Groucho. β-Catenin can convert TCF/LEF into a transcriptional activator of the same genes that are repressed by TCF/LEF alone.

There are many target genes for the canonical Wnt pathway. Most of these genes are cell type specific, with the possible exception of axin 2, which acts as a negative feedback regulator .

Canonical Wnt signaling.



Cold Spring Harb Perspect Biol. 2012 May; 4(5): a011163

Non-canonical Wnt signaling

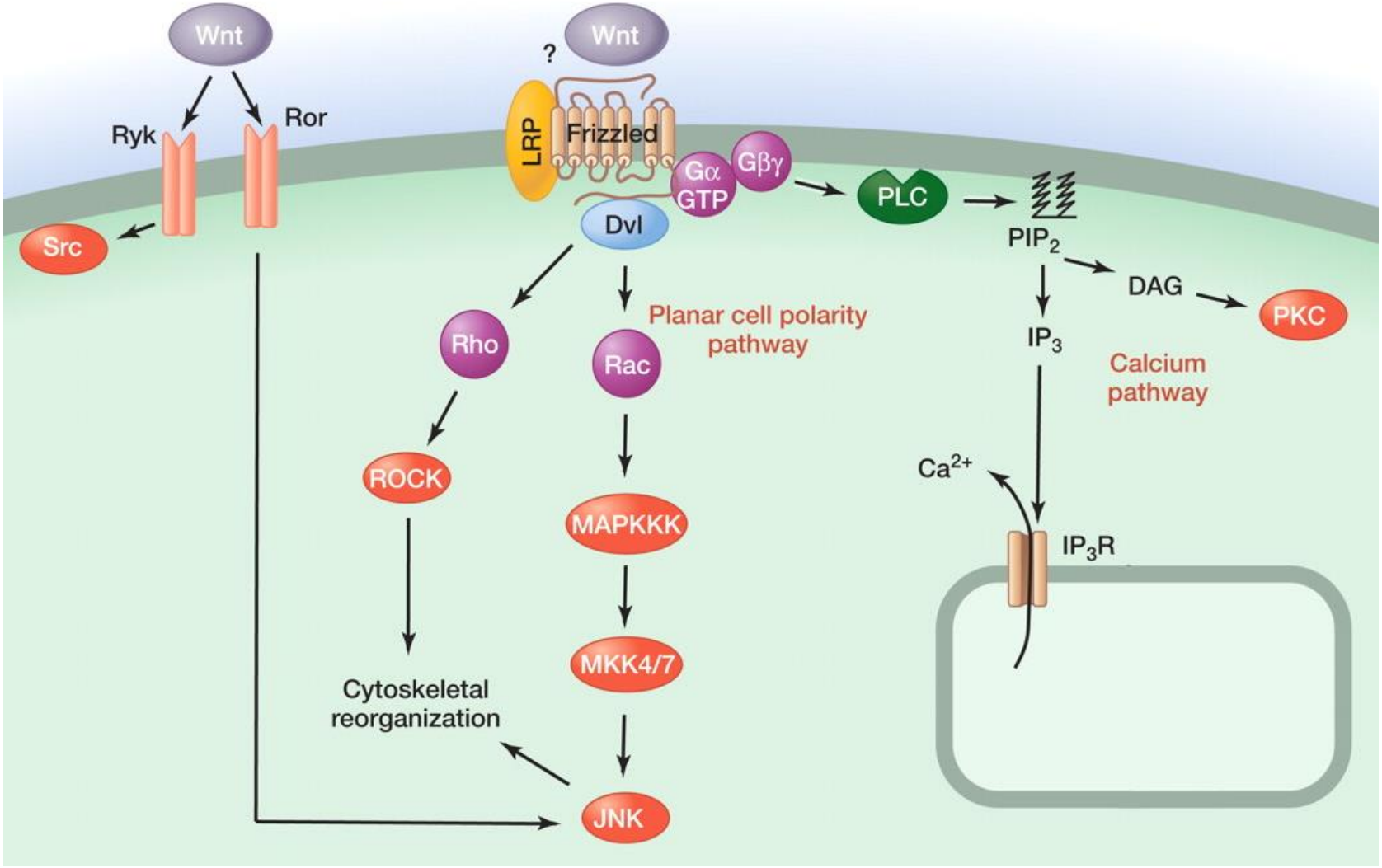
Different non-canonical Wnt signals are transduced through FZD receptors and coreceptors.

Depending on the major intracellular mediators used, those are called the Wnt/JNK or Wnt/calcium pathway.

The core element of the Wnt/JNK pathway includes the activation of small GTPases of the rho family, such as rac, cdc42, and rhoA. The GTPases can activate more downstream mediators like JNK or rho kinase (ROK).

The Wnt/calcium pathway ,the Wnt induces the release of intracellular calcium that in turn activates different intracellular calcium-sensitive enzymes such as protein kinase C, PKC, calcium–calmodulindependent kinase II, CamKII and the calcium-sensitive phosphatase calcineurin.

Non-canonical Wnt signaling pathways.



Cold Spring Harb Perspect Biol. 2012 May; 4(5): a011163

8. PKB/AKT signaling pathway

Protein kinase B (PKB, or Akt) plays a role in cell metabolism, growth, proliferation, and survival. Its activation is controlled by a multi-step process that involves phosphoinositide-3-kinase (PI3K).

The PI3K-PKB/Akt pathway is highly conserved, and its activation is tightly controlled via a multistep process .

Activated receptors directly stimulate class 1A PI3Ks bound via their regulatory subunit or adapter molecules such as the insulin receptor substrate (IRS) proteins.

This triggers activation of PI3K and conversion by its catalytic domain of phosphatidylinositol (3,4)-bisphosphate (PIP_2) lipids to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3).

PKB/Akt binds to PIP_3 at the plasma membrane, allowing PDK1 to access and phosphorylate T308 in the “activation loop,” leading to partial PKB/Akt activation.

This PKB/Akt modification is sufficient to activate mTORC1 (target) by directly phosphorylating and inactivating proline-rich AKT substrate, PRAS40 and TSC2.

PKB/AKT signaling pathway

Phosphorylation of Akt at S473 in the carboxy-terminal hydrophobic motif, either by mTOR or by DNA-PK, stimulates full AKT activity.

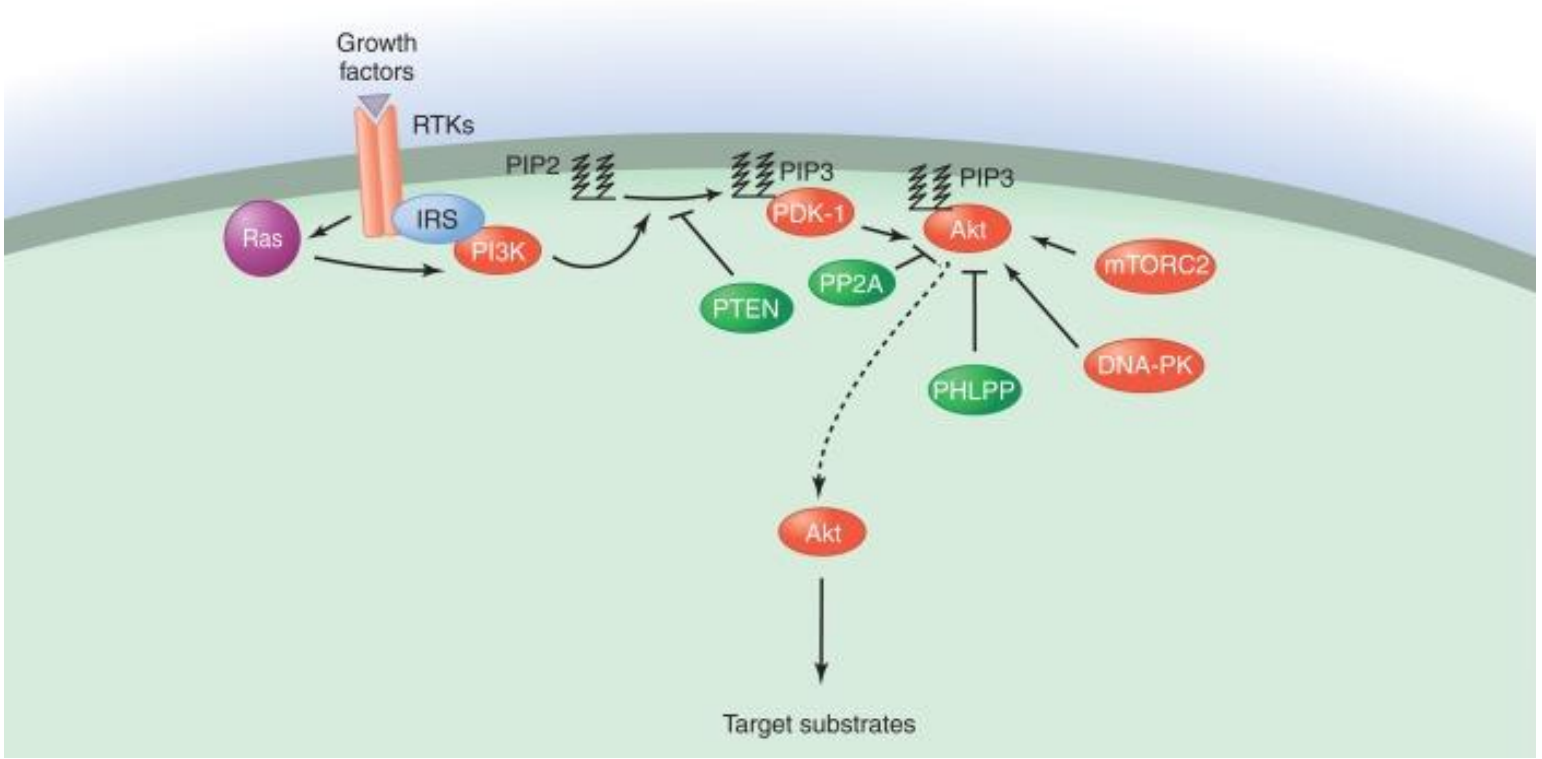
Full activation of AKT leads to additional substrate-specific phosphorylation events in both the cytoplasm and nucleus, including inhibitory phosphorylation of the pro-apoptotic FOXO proteins.

Fully active PKB/AKT mediates numerous cellular functions including angiogenesis, metabolism, growth, proliferation, survival, protein synthesis, transcription, and apoptosis.

Dephosphorylation of T308 by PP2A, and S473 by PHLPP1/2, and the conversion of PIP_3 to PIP_2 by PTEN antagonize AKT signaling.

PKB/AKT signaling pathway

Simplified view



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PKB/AKT signaling pathway

Detailed view

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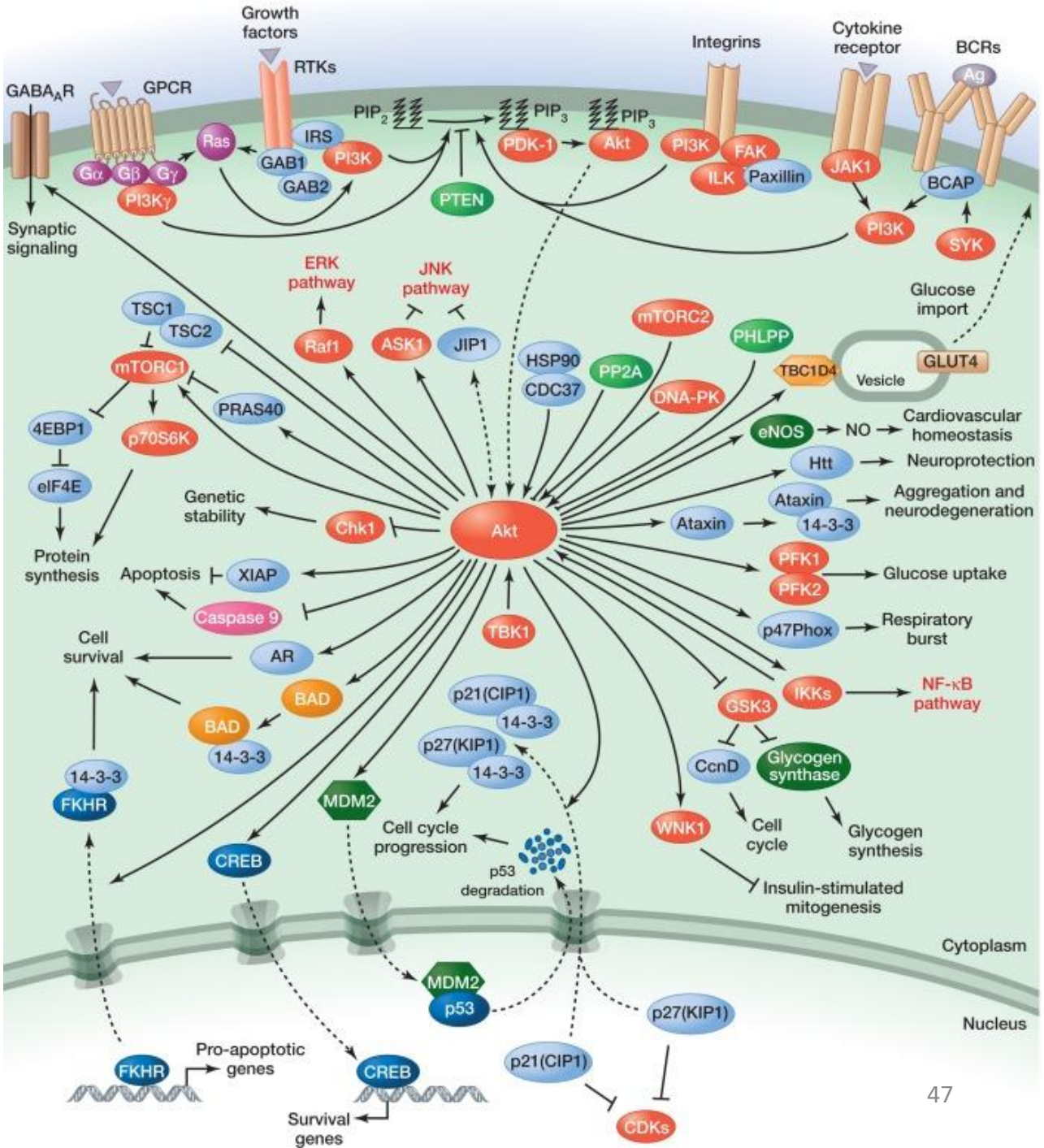


Regulation of PI3K-AKT signaling

The PI3K-Akt pathway has many downstream effects and must be carefully regulated.

Negatively regulation of PI3K-AKT pathway can be achieve at to target: the PIP3 level and the inactivation of AKT protein. Phosphatase and tensin homolog (PTEN) is a main down regulation protein which can converting PIP3 into PIP2.

Protein phosphatase 2A (PP2A), which dephosphorylates AKT at Thr308 and phosphatase PHLPP dephosphorylates AKT at Ser473 are also two negative regulation proteins.

Addition to these regulation protein, the pathway itself also have feedback mechanisms:

1. Transcription factor NF-κB, activated by AKT, regulates peroxisome proliferator-activated receptor delta (PPARβ/δ) agonists and tumor necrosis factor α (TNFα), which in turn repress PTEN expression as a positive feedback.

2. Negative feedback loop is functioned by mTORC1 and S6K1 activation. S6K1 is able to phosphorylate IRS-1 at multiple serine residues, preventing binding to RTKs, resulting the suppression of PI3K activation.

9. mTOR pathway

The mammalian target of rapamycin (mTOR) signaling pathway integrates both intracellular and extracellular signals and serves as a central regulator of cell metabolism, growth, proliferation and survival.

The mTOR protein is a 289-kDa serine-threonine kinase that belongs to the phospho-inositide 3-kinase (PI3K)-related kinase family and is conserved throughout evolution. mTOR is a part of at least two distinct multi-protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)

The mTORC1 complex is made up of mTOR, Raptor, mLST8, and PRAS40. It is extremely sensitive to rapamycin and thus represents the target of first-generation mTOR inhibitors. It activates S6K and inactivates 4EBP1, leading to protein translation and cell growth.

The mTORC2 complex is composed of mTOR, Rictor, Sin1, and mLST8. It is less sensitive to rapamycin and its role in normal cell function has not been well clarified.

mTOR pathway

The mTOR pathway activation depends on mitogen-driven signaling through PI3K/Akt as discussed earlier, although alternative non-Akt dependent activation through the Ras/MEK/ERK pathway is also known.

When activated, mTORC1 promotes protein synthesis mainly by phosphorylating the kinase S6K and the translation regulator 4E-BP1.

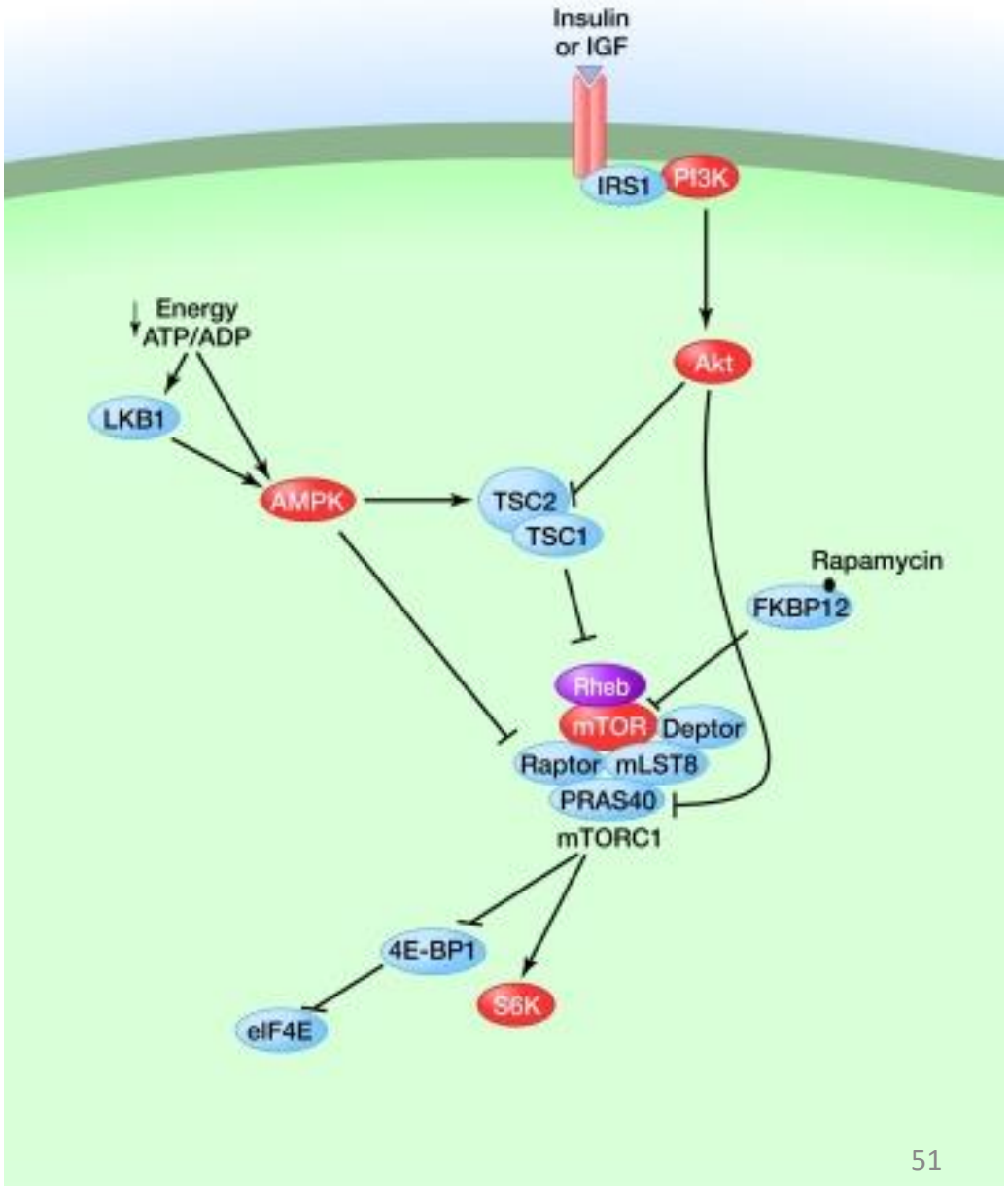
mTORC1 complex also induces lipid biogenesis by activating SREBP1 and PPARγ transcription factors.

In addition to promoting anabolism, mTORC1 also inhibits catabolism by blocking autophagy through the phosphorylation of the ULK1–Atg13–FIP200 complex.

Compared with mTORC1, mTORC2 biology is not well understood. mTORC2 is also activated by growth factors and regulates cell survival, metabolism, and cytoskeletal organization by phosphorylating many AGC kinases, including Akt, SGK1, and PKCα.

mTOR pathway

Simplified view



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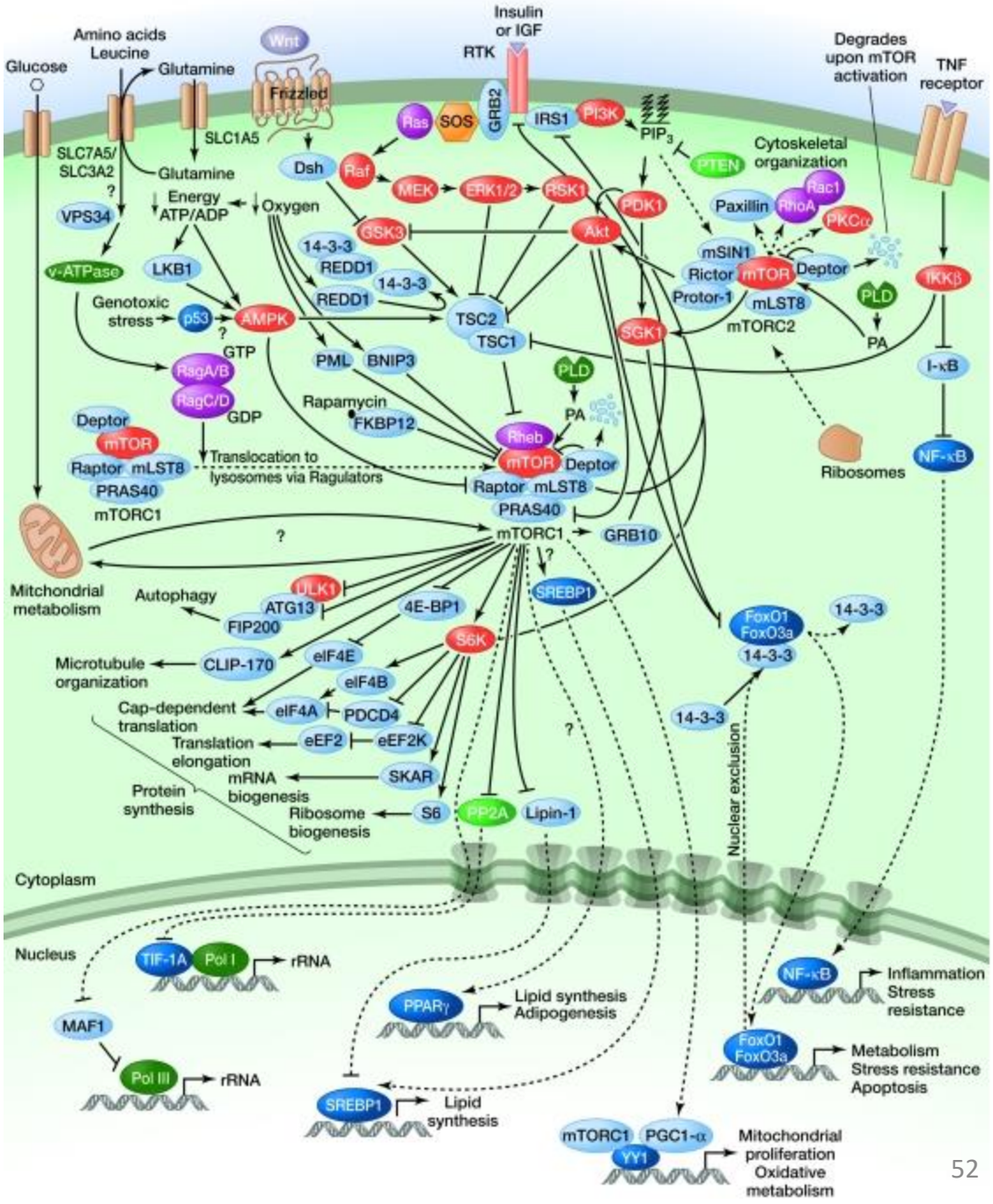
mTOR pathway

Detailed view



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Regulation of mTOR pathway

In normal cells, mTOR activity is controlled by positive and negative upstream regulators.

Positive regulators include growth factors and their receptors, such as insulin-like growth factor-1 (IGF-1) and its cognate receptor IFGR-1, members of the human epidermal growth factor receptor (HER) family and associated ligands, and vascular endothelial growth factor receptors (VEGFRs) and their ligands, which transmit signals to mTOR through the PI3K-Akt.

Negative regulators of mTOR activity include phosphatase and tensin homolog (PTEN) that inhibits signaling through the PI3K-Akt pathway, and tuberous sclerosis complex (TSC) 1 (hamartin) and TSC2 (tuberin).

Phosphorylation of TSC2 by Akt releases its inhibitory effect on mTOR and up-regulates mTOR activity. Another negative regulator, LKB1, is in an energy-sensing pathway upstream of TSC .

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