Sinalização via PI3K-Akt-mTOR

Obrigado à Profa. Bettina Malnic por fornecer o arquivo de Powerpoint que usei como base para esta aula

Five parallel intracellular pathways activated by GPCRs, RTKs, or both

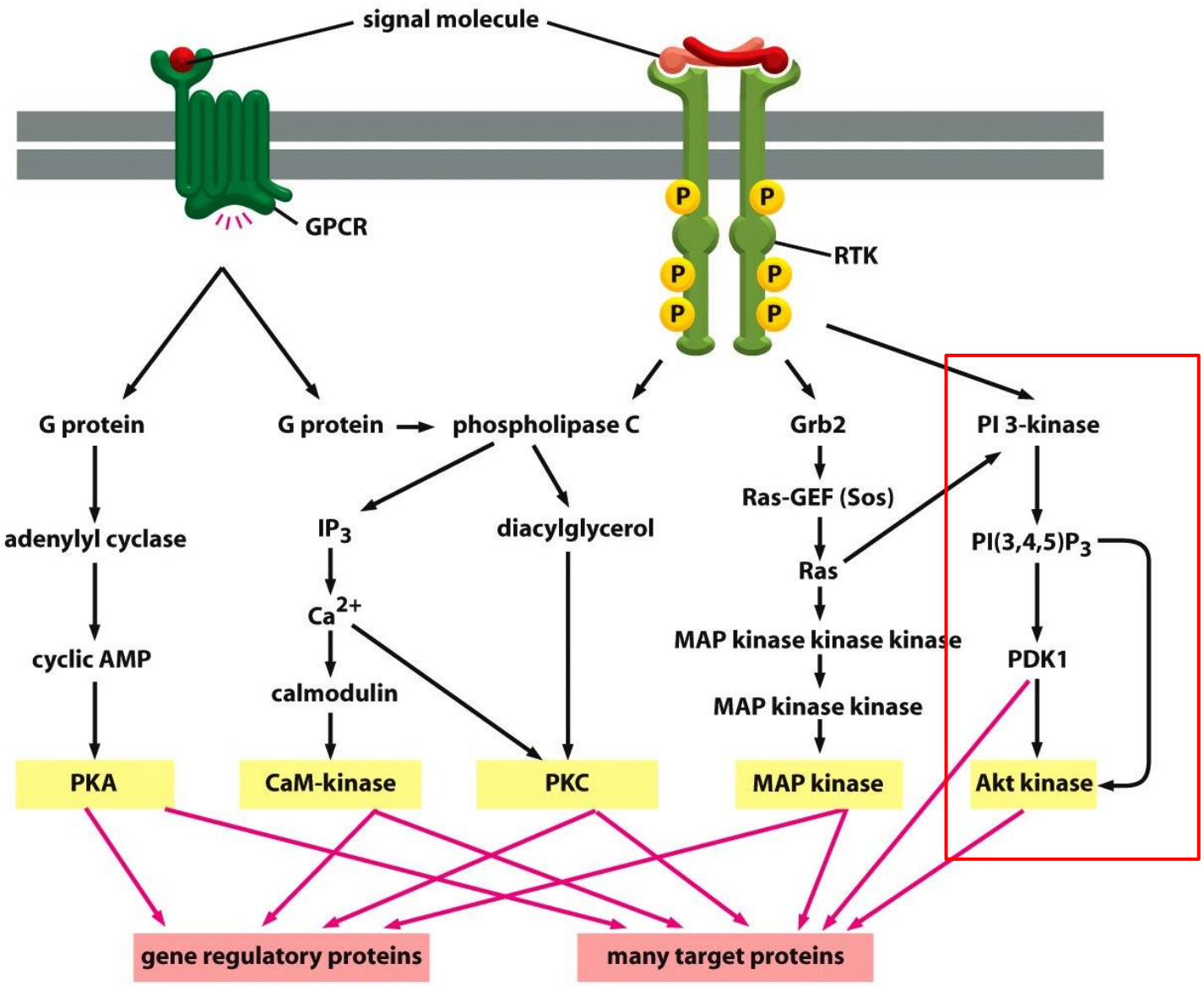
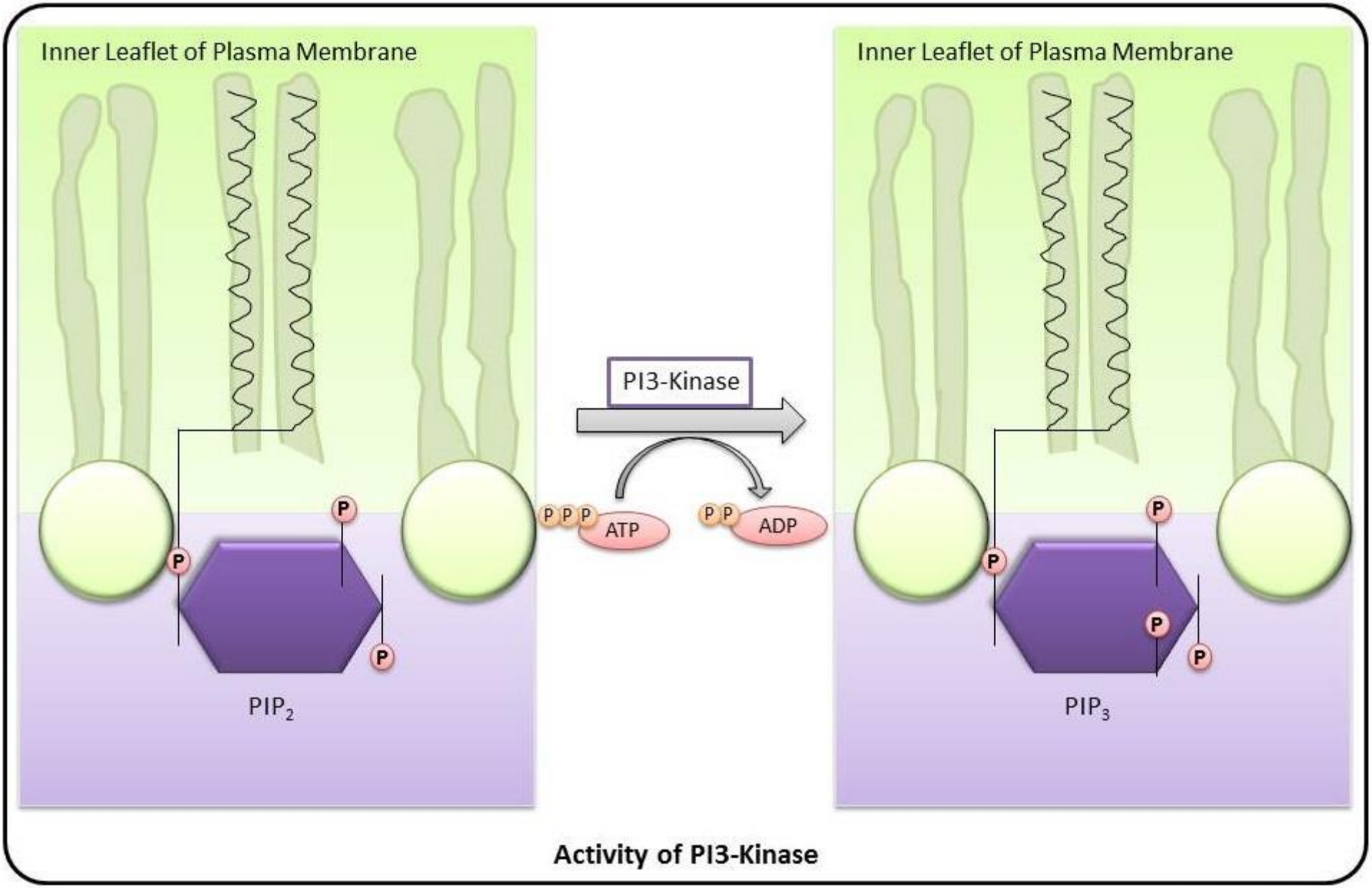


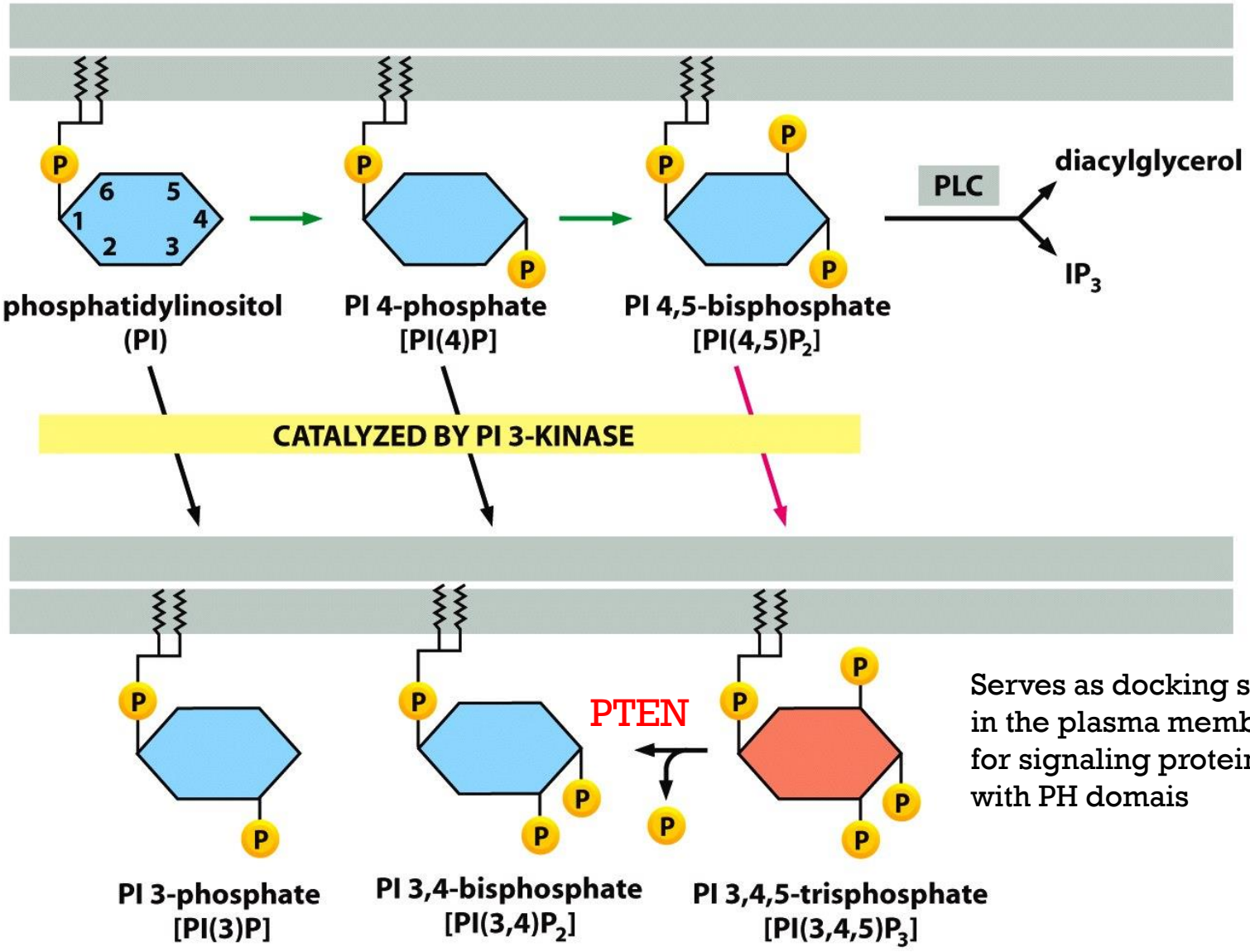
Figure 15-66 *Molecular Biology of the Cell* (© Garland Science 2008)



Activity of PI3-Kinase

Namrata Heda

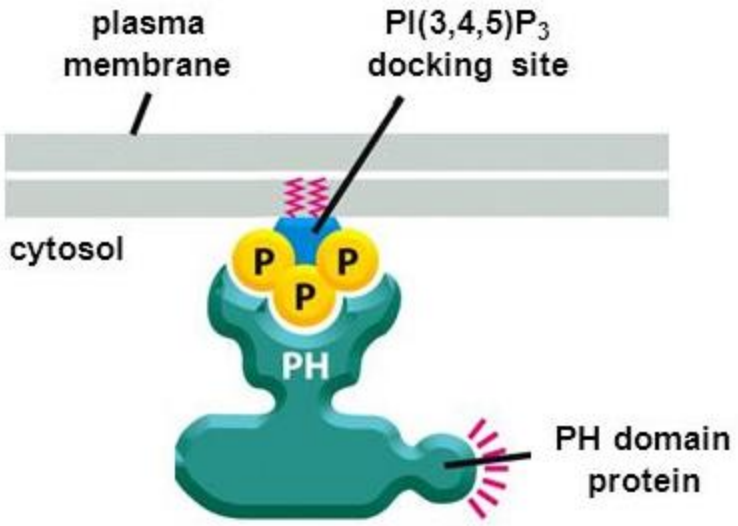
The generation of inositol phospholipid docking sites by PI 3-kinase



Serves as docking sites in the plasma membrane for signaling proteins with PH domais

Figure 15-63 *Molecular Biology of the Cell* (© Garland Science 2008)

Pleckstrin homology (PH) domains can mediate binding to $PI(3,4,5)P_3$



PH domains are found in about 200 human proteins.
First identified in Pleckstrin, a protein found in platelets.
PH domains function mainly as protein-protein interaction domains, and it is only a small subset of them that bind to PIP3, at least some of these also recognize a specific membrane-bound protein as well as PIP3, which greatly increases specificity and explains why not all PH containing proteins dock at PIP3 sites.

There are various types of PI3 kinases.

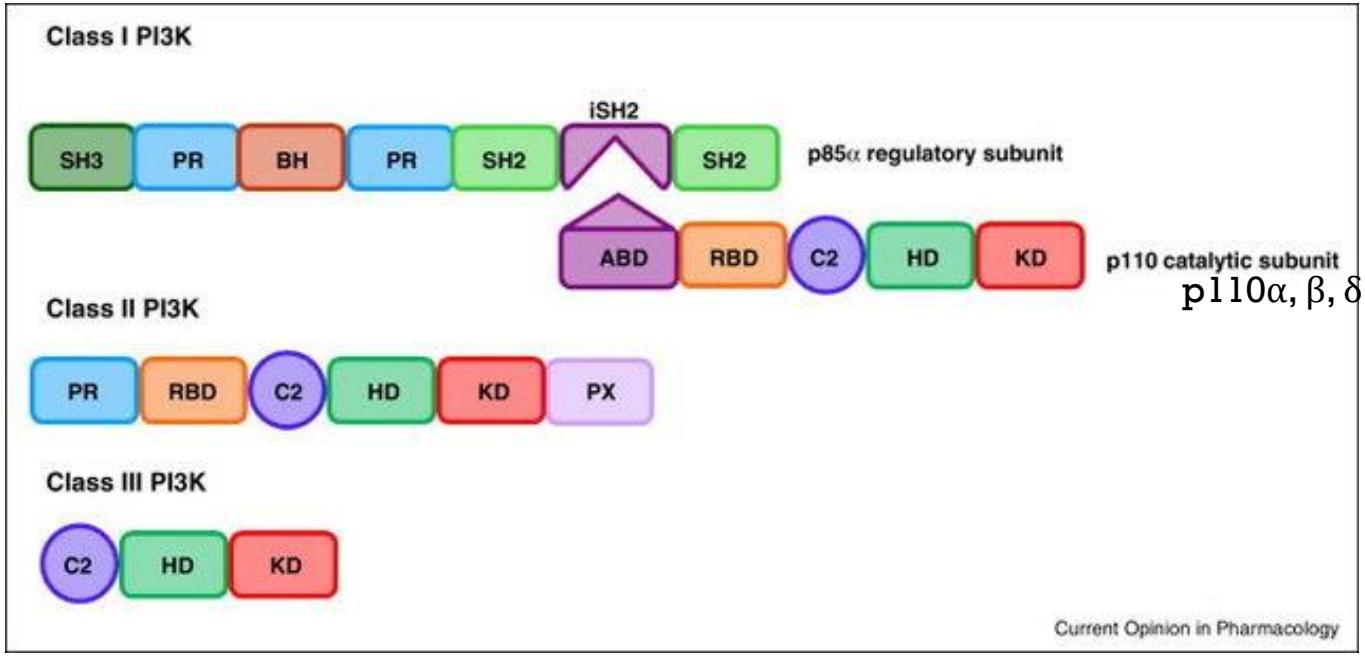
Class 1 is activated by RTKs and GPCRs. Class I PI3Ks function as heterodimers consisting of one of four catalytic p110 subunits (p110α, β, δ or γ) and a regulatory subunit. Class I PI3Ks also harbor a Ras-binding domain (RBD) in the N-terminal extension, and p110α, p110δ and p110γ are each stimulus-dependent Ras effectors.
Class Ia are activated by RTKs and have regulatory subunit with SH2 domains
Class Ib are activated by GPCRs, and have regulatory subunit that binds to Gbg subunits.
Activated ras can bindo to the catalytic subunit through the RBD domain.

Class II have no known obligatory regulatory subunit, but the class II enzymes interact with proteins that could serve adaptor functions.

A single class III PI3K is conserved in all eukaryotes. This enzyme was first identified as vacuolar protein sorting 34 (Vps34), the sole PI3K in yeast.

Schematic representation of class I-III PI3K structures

Schematic representation of class I-III PI3K structures ABD: adaptor binding domain; RBD: RAS binding domain; C2: C2 domain; HD: helical domain; KD: kinase domain; PR: proline rich domain; PX: phox homology domain; BH: breakpoint cluster region homology domain (Rho-Gap-like domain); iSH2: inter-SH2 domain (p110 binding domain). Complexes between p110α, p110β, p110δ and p110γ and their respective regulatory subunits, such asPI3Kα, PI3Kβ, PI3Kδ and PI3Kγ.



p110α, β, δ or γ

All PI3Ks possess a 'PI3K signature motif' that is composed of a C2 domain, which likely binds membranes, a helical domain and the catalytic kinase domain

Stark et al., PI3K inhibitors in inflammation, autoimmunity and cancer. Current Opinion in Pharmacology 2015, 23:82–91

1.GRB2-Associated-Binding Protein (GAB):

1. **Function:** GAB proteins, including GAB1 and GAB2, are adaptor proteins that play a role in linking receptor tyrosine kinases (RTKs) to PI3K activation. They contain multiple binding sites for various signaling proteins, including PI3K.

2.Insulin Receptor Substrate (IRS) Proteins:

1. **Function:** IRS proteins (e.g., IRS-1, IRS-2) are adaptor proteins that mediate the signaling of insulin and other growth factors. They have tyrosine phosphorylation sites that serve as docking sites for PI3K, among other signaling molecules.

3.Adaptor Protein with Pleckstrin Homology and Src Homology 2 Domains (APS):

1. **Function:** APS is an adaptor protein that contains both pleckstrin homology (PH) and Src homology 2 (SH2) domains. It is involved in insulin signaling and can interact with both insulin receptor and PI3K.

4.p85 Regulatory Subunit of PI3K:

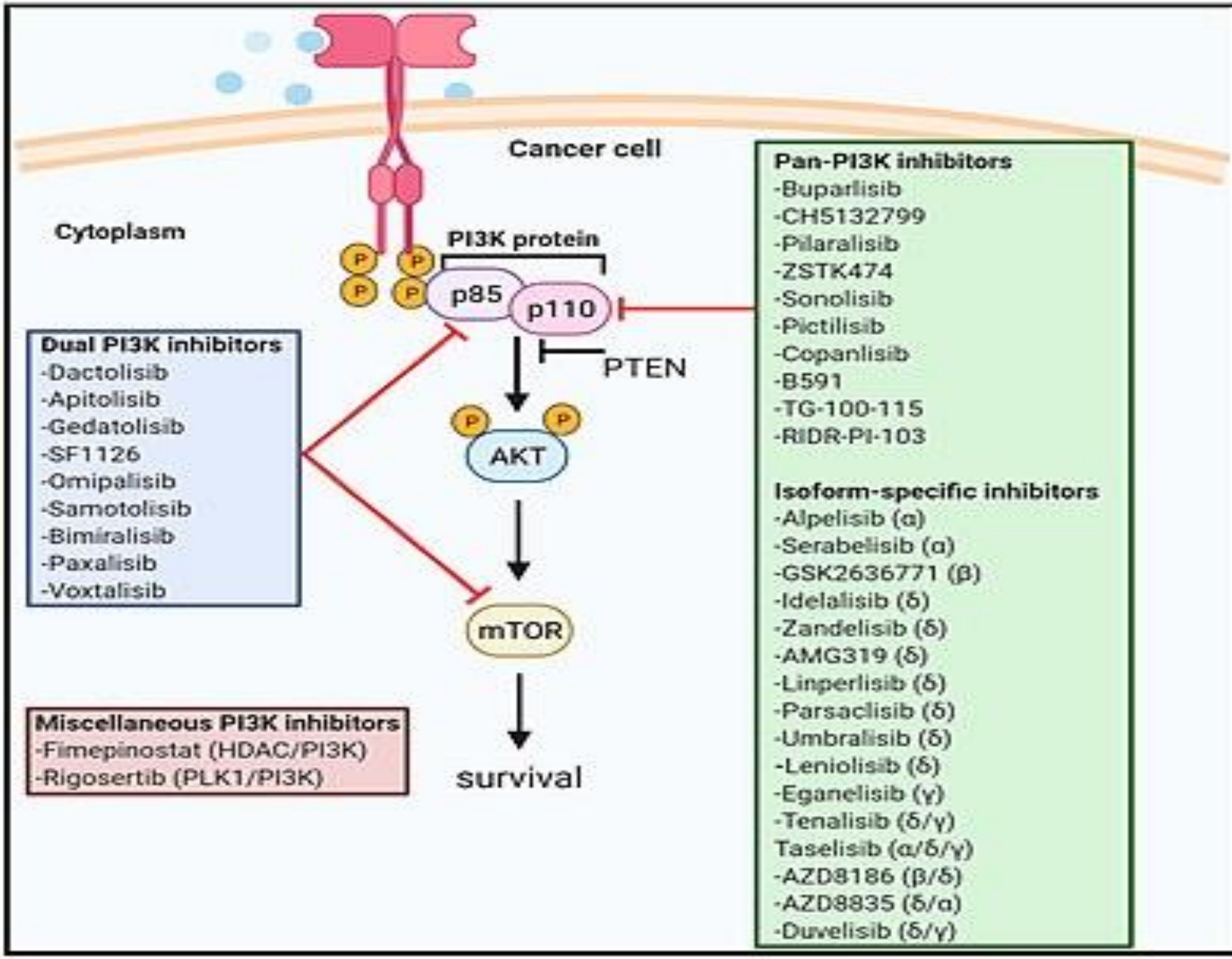
1. **Function:** The regulatory subunit of Class IA PI3K (p85) itself acts as an adaptor. It contains SH2 domains that can bind to phosphorylated tyrosine residues on activated receptors, initiating the recruitment of the catalytic subunit (p110) to the plasma membrane.

5.BCR (Breakpoint Cluster Region) Proteins:

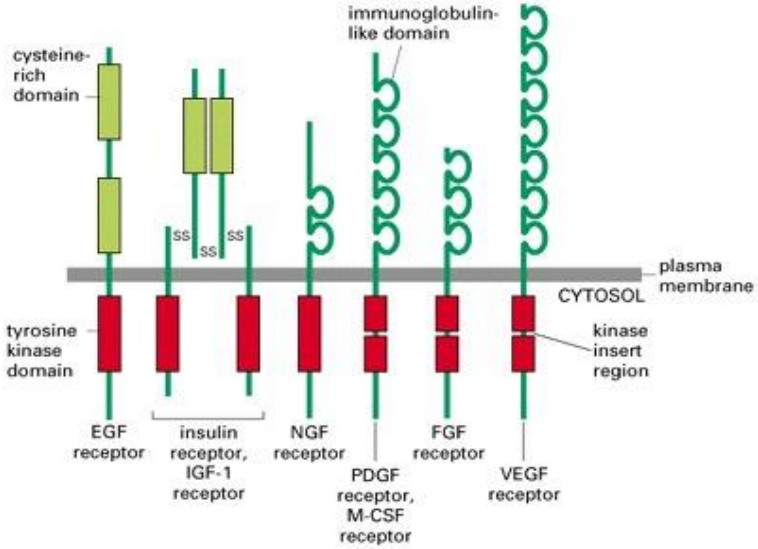
1. **Function:** BCR proteins, such as BCR and ABL, can interact with the p85 regulatory subunit of PI3K. This interaction is implicated in BCR-ABL-mediated activation of PI3K in the context of certain leukemias.

6.SH2-Containing Inositol 5'-Phosphatase (SHIP):

1. **Function:** SHIP is an inositol phosphatase that negatively regulates PI3K signaling by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate (PIP3). It has an SH2 domain that can interact with phosphorylated tyrosine residues on proteins, influencing its recruitment.


Cancer cell
Cytoplasm
PI3K protein
p85
p110
PTEN
AKT
mTOR
survival
Pan-PI3K inhibitors
-Buparlisib
-CH5132799
-Pilaralisib
-ZSTK474
-Sonolisib
-Pictilisib
-Copanlisib
-B591
-TG-100-115
-RIDR-PI-103
Isoform-specific inhibitors
-Alpelisib (α)
-Serabelisib (α)
-GSK2636771 (β)
-Idelalisib (δ)
-Zandelisib (δ)
-AMG319 (δ)
-Linperlisib (δ)
-Parsaclisib (δ)
-Umbralisib (δ)
-Leniolisib (δ)
-Eganelisib (γ)
-Tenalisib (δ/γ)
Taselisib (α/δ/γ)
-AZD8186 (β/δ)
-AZD8835 (δ/α)
-Duvelisib (δ/γ)
Dual PI3K inhibitors
-Dactolisib
-Apitolisib
-Gedatolisib
-SF1126
-Omipalisib
-Samotolisib
-Bimiralisib
-Paxalisib
-Voxtalisib
Miscellaneous PI3K inhibitors
-Fimepinostat (HDAC/PI3K)
-Rigosertib (PLK1/PI3K)

IR and IGF-I persistantly dimerise and do not provide docking sites for phosphotyrosine adaptor proteins.

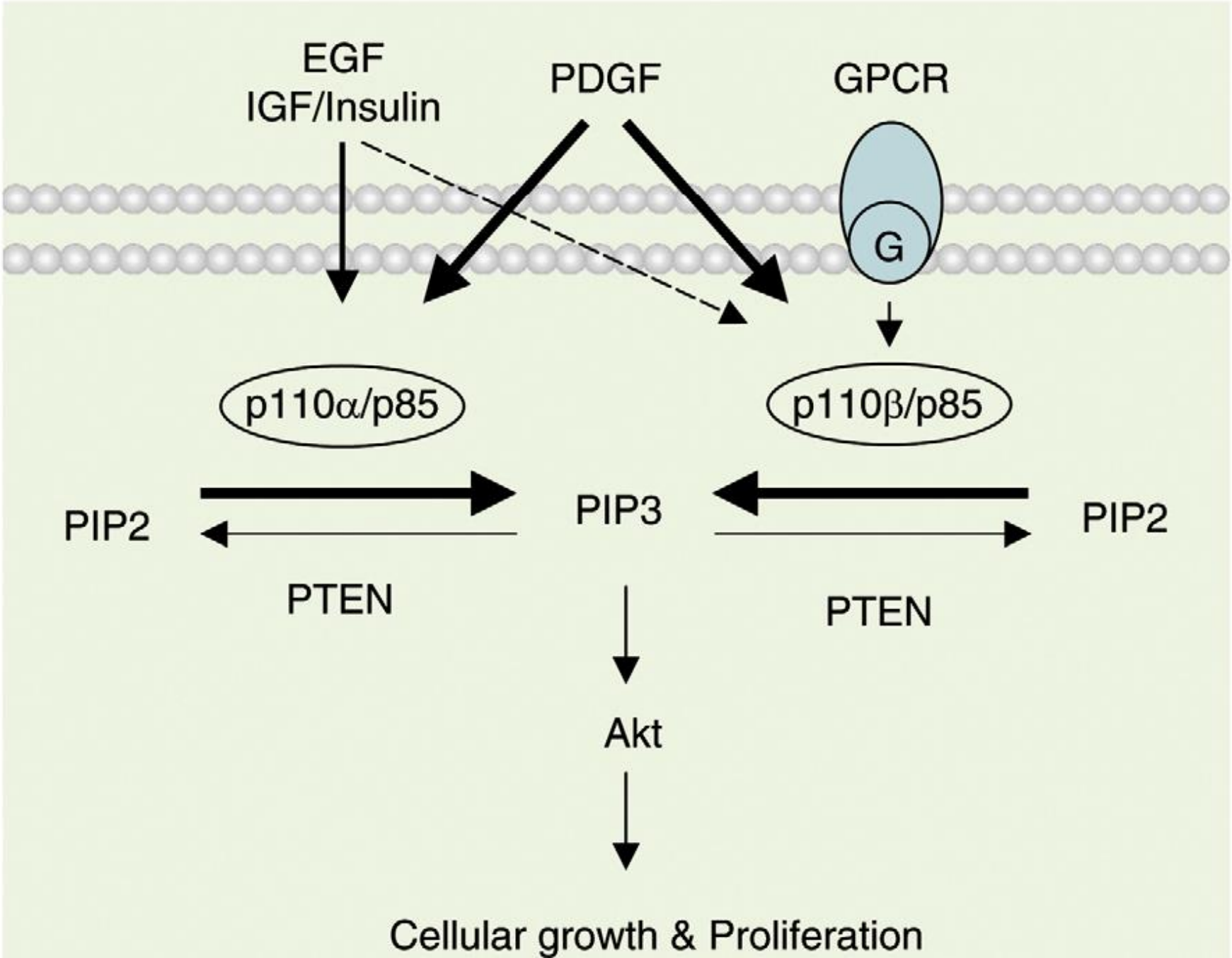


Seven subfamilies of receptor tyrosine kinases

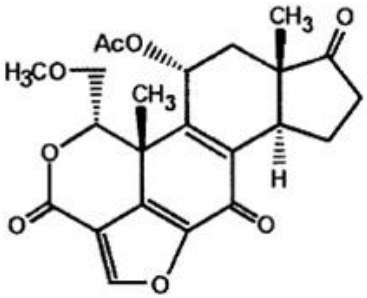
Only one or two members of each subfamily are indicated. Note that the tyrosine kinase domain is interrupted by a "kinase insert region" in some of the subfamilies. The functional roles of most of the cysteine-rich, immunoglobulin-like, and fibronectin-type III-like domains are not known.

The receptors for insulin and IGF-1 act in a slightly different way. They are tetramers to start with, and ligand binding is thought to induce a rearrangement of the transmembrane receptor chains, so that the two kinase domains come close together.

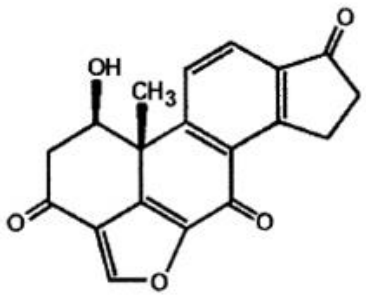
The insulin receptor is unusual in being a dimer of two pairs of polypeptide chains (designated α and β).


EGF
IGF/Insulin
PDGF
GPCR
G
p110α/p85
p110β/p85
PIP2
PIP3
PIP2
PTEN
PTEN
Akt
Cellular growth & Proliferation

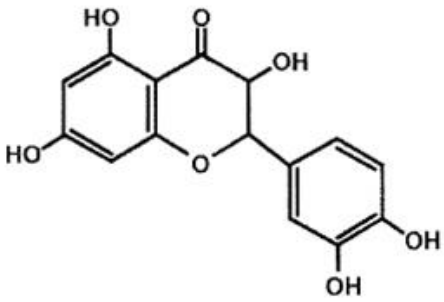
PI-3 kinase inhibitors



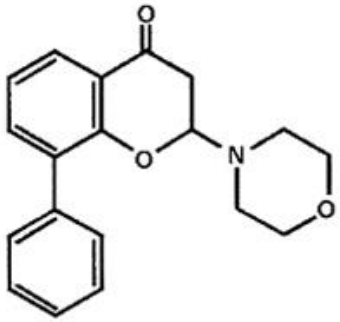
Wortmannin



Demethoxyviridin



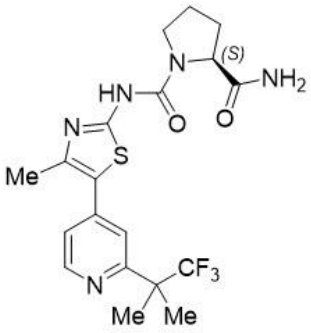
Quercetin



LY294002

Endocrine-Related Cancer (2001) **8** 237–248

First PI3K Inhibitor Approved for Metastatic, Advanced Breast Cancer
May, 2019



PIQRAY (alpelisib) is a potent and selective inhibitor of PI3KCA (mutação em PI3KCA é frequente em certos tipos de cancer de mama)

PIP3 can no longer propagate its signal downstream. Which In combination with other factors, could lead to increased cell growth and possible tumor development.

PIQRAY is a potent and selective inhibitor of PI3Kα (PI3CA=catalytic subunit alfa (and not b, g and d).

The PIK3CA mutation is present in approximately 40% of cases of HR+/HER2- advanced breast cancer and is the most common in this patient population. It leads to increased pi3K activity.

The PI3-kinase Akt signaling pathway is the major pathway activated by the hormone insulin. It also plays a key part in promoting the survival and growth of many cell types.

Table 15–4 Some Signal Proteins That Act Via RTKs

SIGNAL PROTEIN	RECEPTORS	SOME REPRESENTATIVE RESPONSES
Epidermal growth factor (EGF)	EGF receptors	stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF1 and IGF2)	IGF receptor-1	stimulate cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factors (FGF1 to FGF24)	FGF receptors (FGFR1–FGFR4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; act as inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

Table 15-4 *Molecular Biology of the Cell* (© Garland Science 2008)

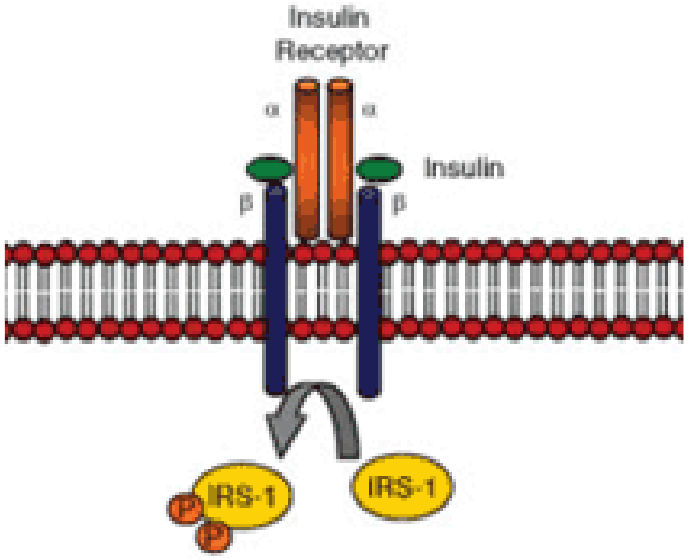
Insulin receptor substrate (IRS)

Intermediate docking protein phosphorylated by the receptor kinase on multiple tyrosines then leaves the receptor.

Acts as an anchorage point for SH2 domains.

Several IRS are phosphorylated amplifying the signal.

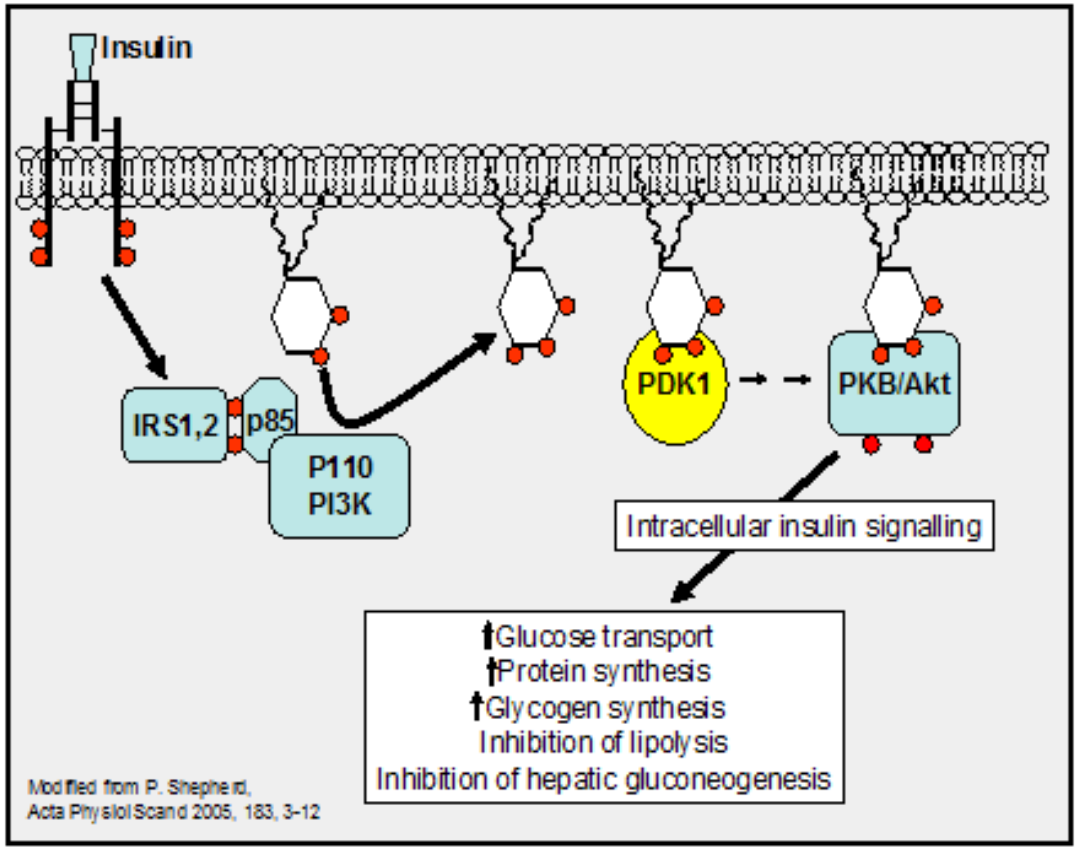
Binds to several IRS interacting proteins.



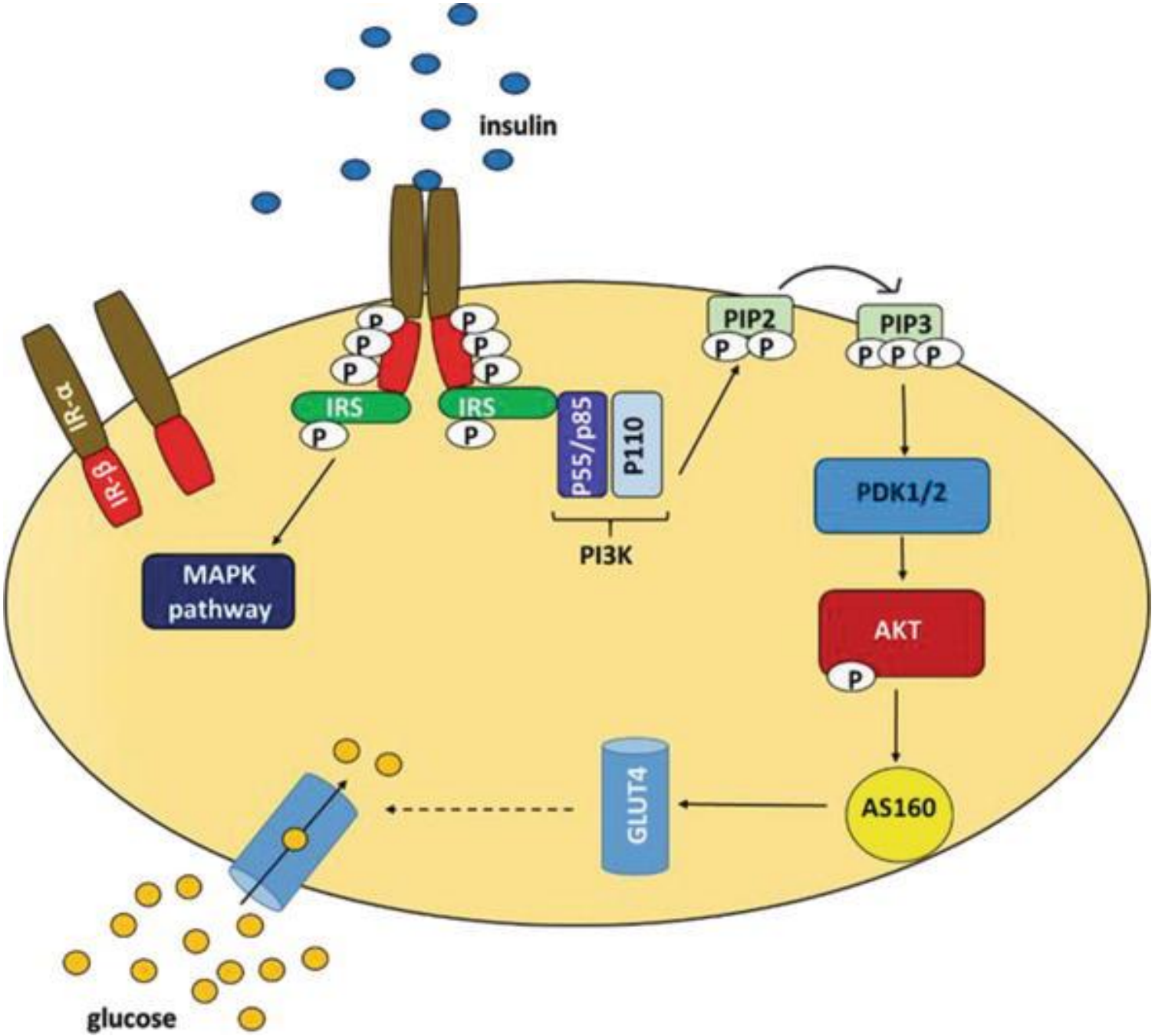
Most of the phosphotyrosine docking sites generated by ligand binding are not on the receptor itself, but on a specialized docking protein called *insulin receptor substrate-1 (IRS-1)*. The activated receptor first autophosphorylates its kinase domains, which then phosphorylate IRS-1 on multiple tyrosines, thereby creating many more docking sites than could be accommodated on the receptor alone. Other docking proteins are used in a similar way by some other receptor tyrosine kinases to enlarge the size of the signaling complex.

At least four IRS isoforms occur in mammals: IRS-1 and IRS-2 are widely expressed, whereas IRS-3 is restricted to adipose tissue and IRS-4 is expressed in the thymus, brain, and kidney.

InsR downstream pathway through Akt



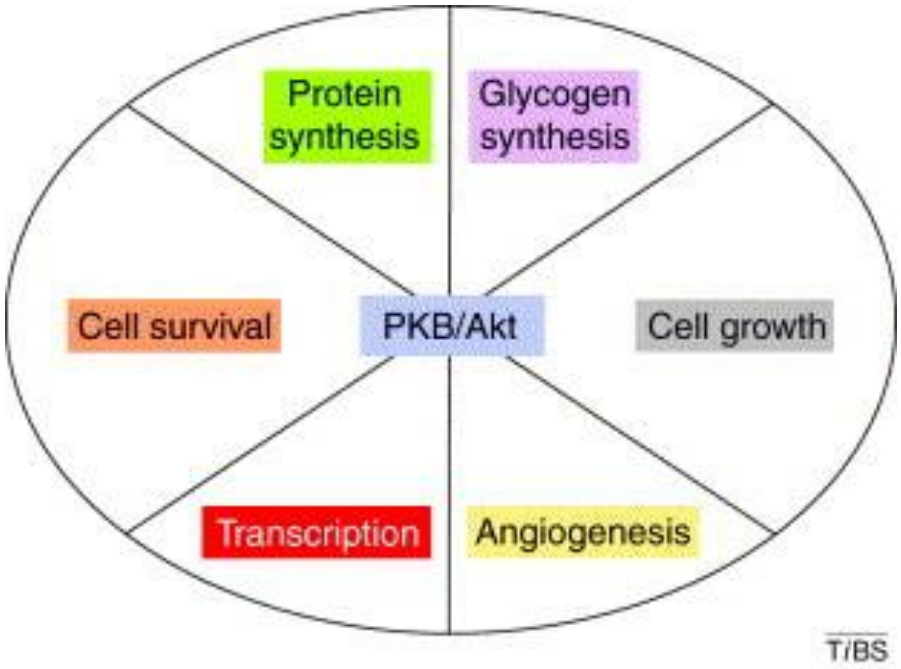
PDK1 = phosphoinositol-dependent kinase
PDK1 e Akt possuem PH domains


insulin
IR-α
IR-β
P
IRS
IRS
P55/p85
P110
PI3K
PIP2
PIP3
PDK1/2
AKT
MAPK
pathway
GLUT4
AS160
glucose

After Tyr phosphorylation IRS-1 serves as a docking protein for a number of effector molecules bearing the SH2 domain, such as Grb2, Syp, Nck, and the regulatory subunit of PI3K, p85. p85 binding to IRS-1 stimulates the PI-3K activity of the p110 catalytic subunit, as well as downstream signaling proteins. Phosphorylated Shc and IRS-1 bind to Grb2 and mediate p21ras GTP-loading via the guanyl nucleotide exchange factor, SOS. Active p21ras associates with and activates the Raf-1 kinase (a MAPK kinase kinase, MAPKKK), which phosphorylates and activates MEK-1 (a MAPK kinase, MAPKK), which in turn phosphorylates and activates ERK, a member of the MAPK family of signaling enzymes. Activation of the MAPK cascade is associated mostly with transcriptional and mitogenic effects of insulin, while the PI3K pathway is generally engaged with the hormone's metabolic effects

IRS proteins do not have kinase or other intrinsic enzymatic activity;

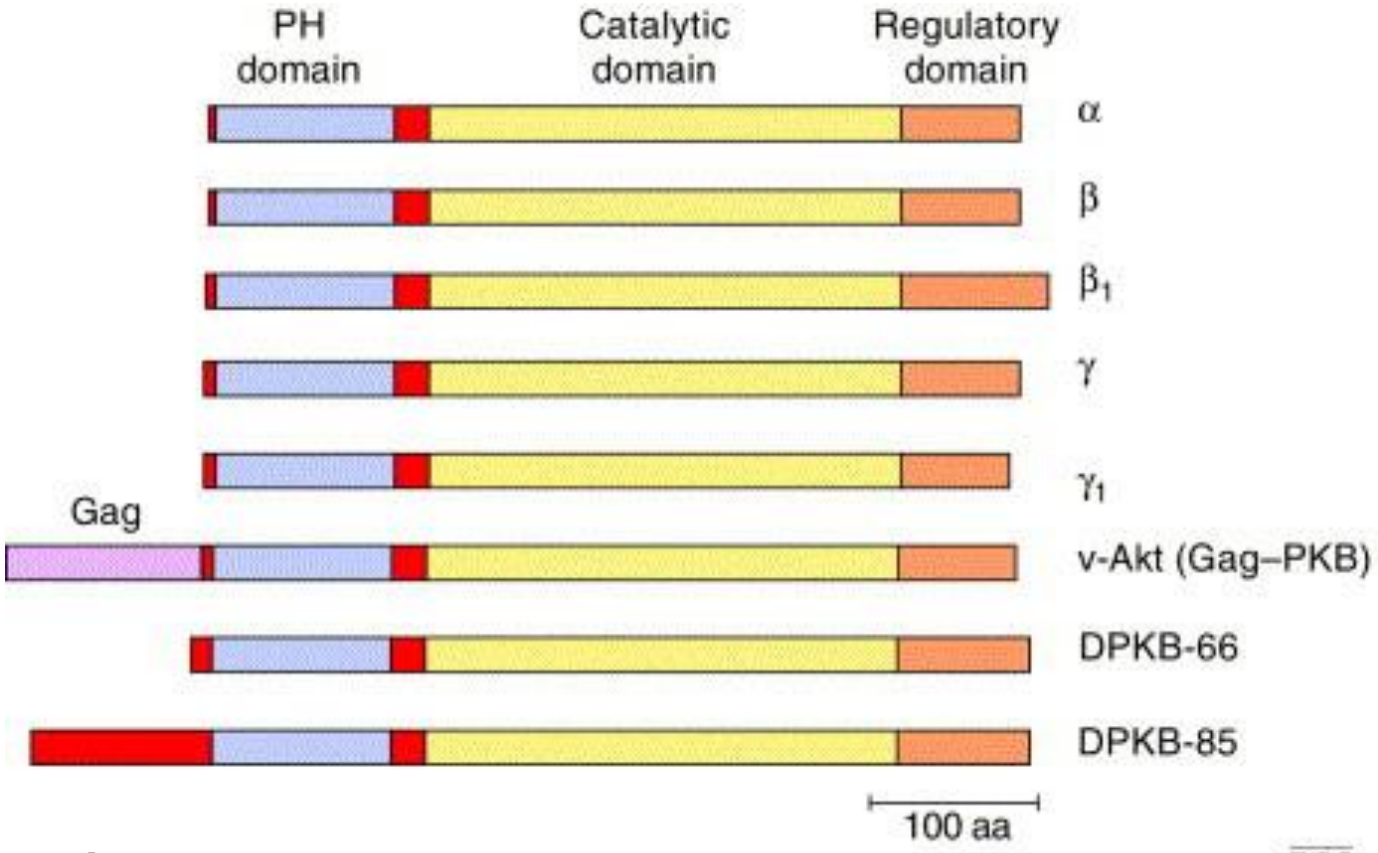
Akt, also known as Protein Kinase B (PKB), is a serine/threonine protein kinase



Brazil & Hemmings

TRENDS in Biochemical Sciences Vol.26 No.11 November 2001

The domain structure of the protein kinase B (PKB)/Akt family



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TRENDS in Biochemical Sciences Vol.26 No.11 November 2001

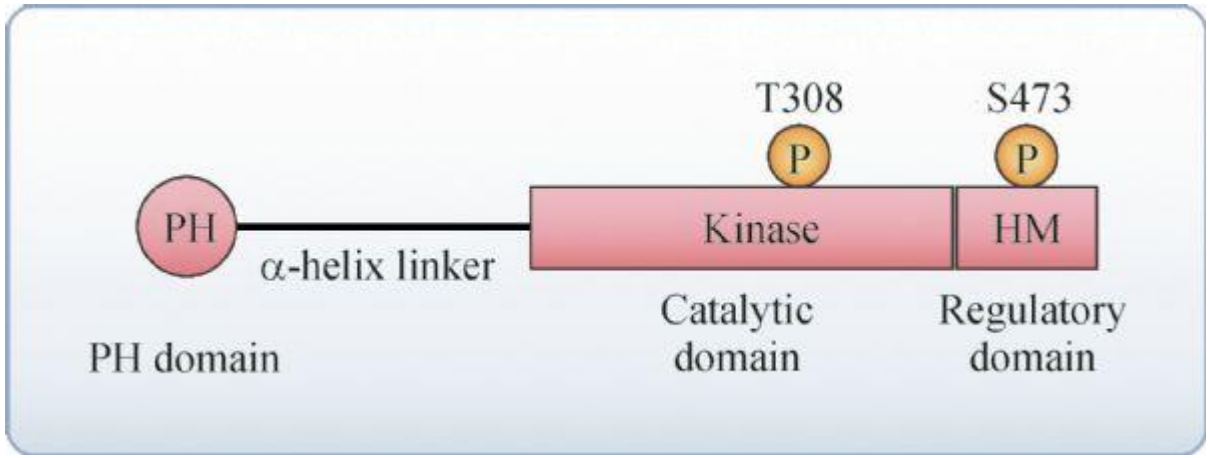
Proteus syndrome

Somatic activating mutation (Glu17Lys) in the AKT1 oncogene

The domain structure of the protein kinase B (PKB)/Akt family. All family members contain an N-terminal pleckstrin homology (PH) domain, a catalytic domain and a C-terminal regulatory domain. PKBβ_1 contains an ~40 amino acid (aa) extension at the end of the C-terminal regulatory domain. PKBγ_1 has a unique C-terminal sequence of 14 amino acids compared to 28 amino acids for PKBγ, which also lacks the Ser473 regulatory site. v-Akt is the viral form of PKB/Akt and is a fusion between the viral Gag and mouse PKBα/Akt1. DPKB-66 and DPKB-85 are forms of PKB/Akt identified from *Drosophila melanogaster*.

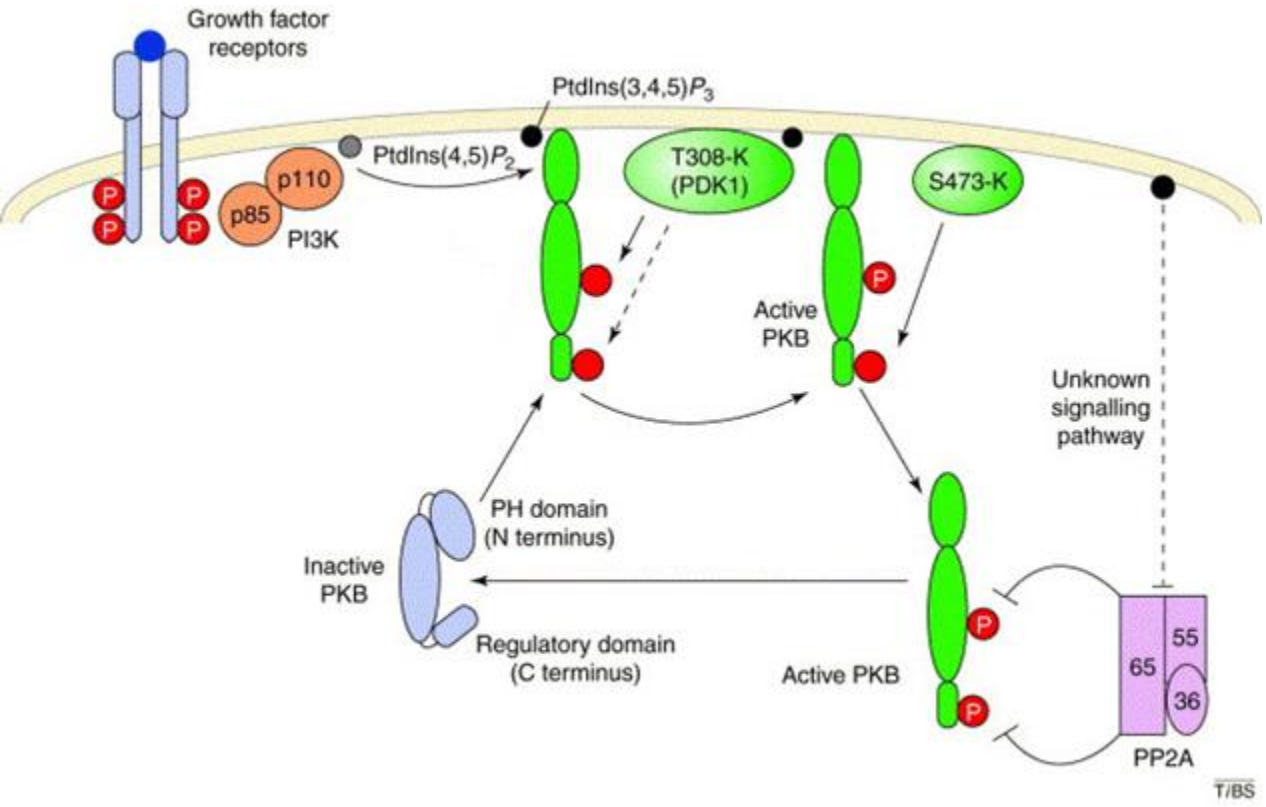
AKT was originally identified as the oncogene in the transforming retrovirus, AKT8 isolated from a spontaneous thymoma cell line derived from AKR mice The transforming cellular sequences, v-akt, were cloned and these sequences were used to identify Akt1 and Akt2 in a human clone library.

Structure of AKT



AKT consists of three domains: the PH domain, the kinase domain and the C-terminal regulatory domain with a hydrophobic motif HM. The kinase domain contains T308, one of two residues that need to be phosphorylated in order for AKT to become active. The second residue, S473, is located in the hydrophobic motif of AKT.

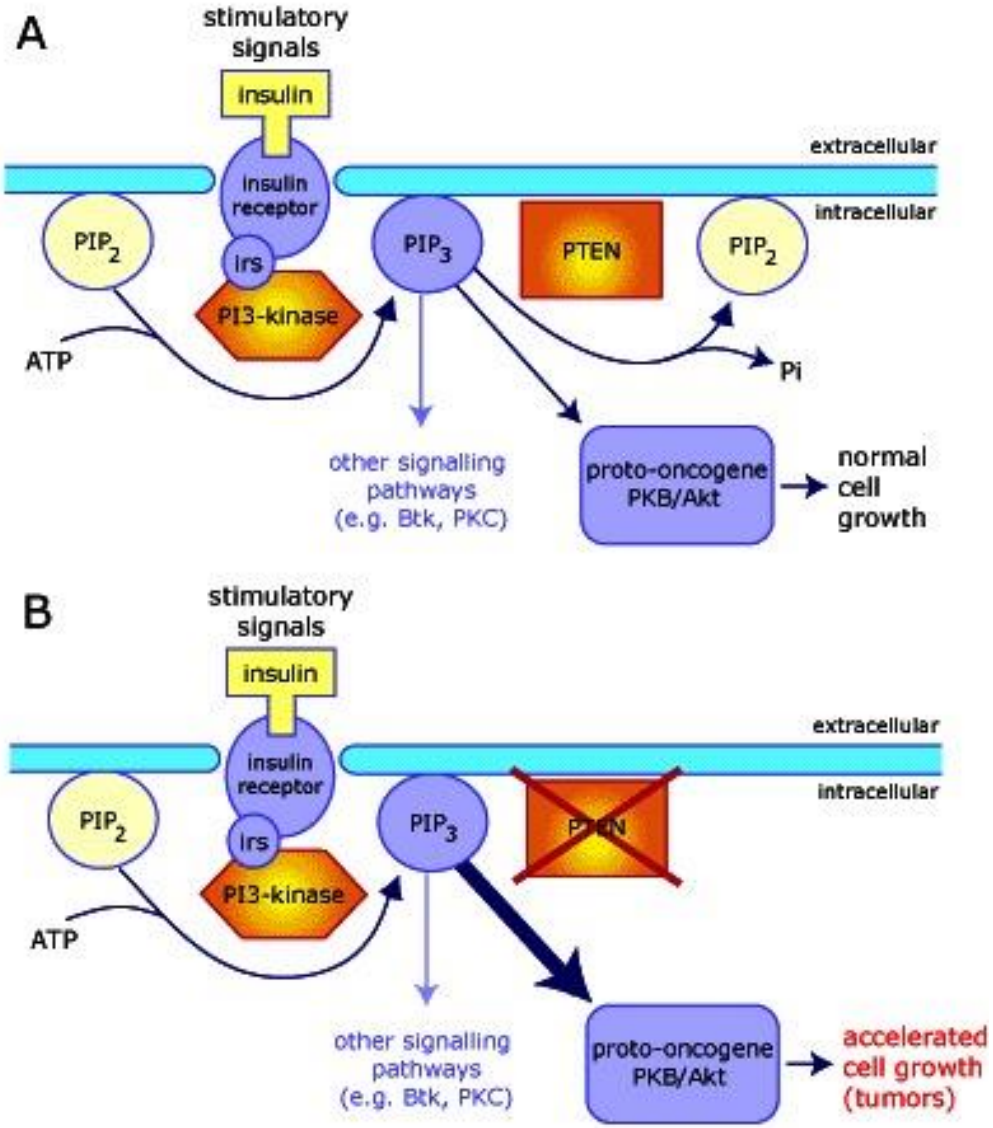
Proposed model for PKB/Akt regulation by receptor tyrosine kinases.



Brazil & Hemmings TRENDS in Biochemical Sciences Vol.26 No.11 November 2001

Once correctly positioned at the membrane via binding of PIP3, Akt can then be phosphorylated by its activating kinases, phosphoinositide dependent kinase 1 (PDK1) and mTORC2 (the long-sought PDK2 molecule).

The PTEN tumor suppressor gene acts as a phospholipid phosphatase



PTEN (Phosphatase and TENsin homolog deleted on chromosome ten) as a phosphatase to dephosphorylate PtdIns(3,4,5)P3 b ack to PtdIns(4,5)P2.

This removes the membrane-localization factor from the Akt signaling pathway. Without this localization, the rate of Akt activation decreases significantly, as do all of the downstream pathways that depend on Akt for activation.

***PTEN* acts as a tumor suppressor *gene* through the action of its phosphatase protein product.**

PTEN function is commonly lost in a large proportion of human cancers through somatic mutations, gene silencing, or epigenetic mechanisms.

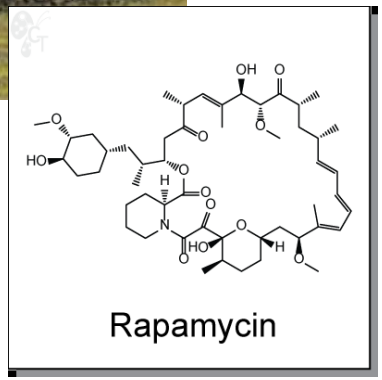
Frequent genetic inactivation of PTEN occurs in glioblastoma, endometrial cancer, and prostate cancer; and reduced expression is found in many other tumor types such as lung and breast cancer. Furthermore, *PTEN* mutation also causes a variety of inherited predispositions to cancer.

mTOR (mammalian Target Of Rapamycin) is a serine/threonine kinase

Rapa Nui

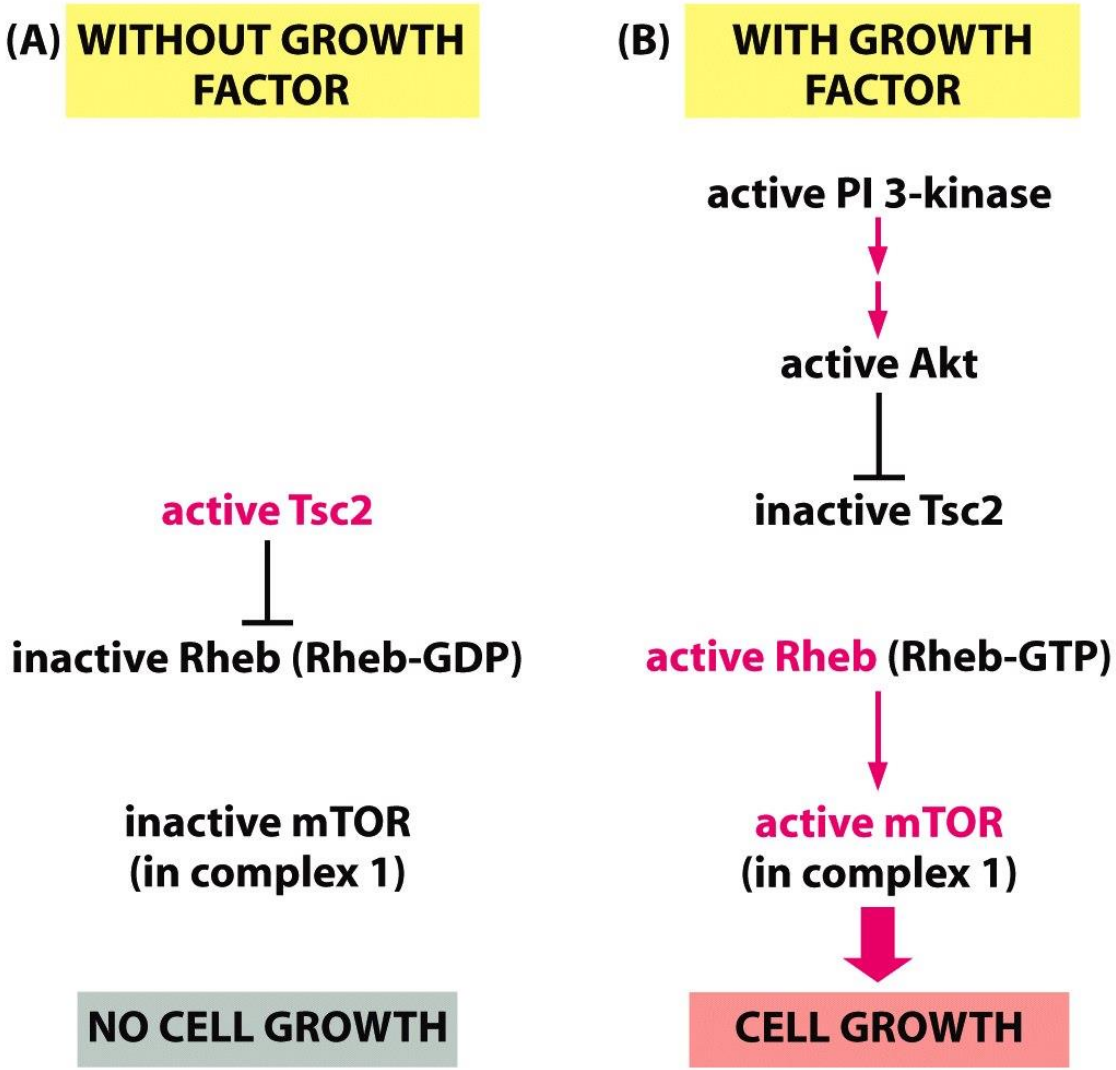
Streptomyces hygroscopicus → rapamycin

Immunosuppressive and anti-proliferative properties



Rapamycin

Activation of mTOR by the PI-3 kinase-Akt signaling pathway



TSC1/TSC2= tuberous sclerosis complex (hamartin and tuberin) GAP

Small GTPase (Ras homolog enriched in the brain)

Rapamycin

Figure 15-65 *Molecular Biology of the Cell* (© Garland Science 2008)

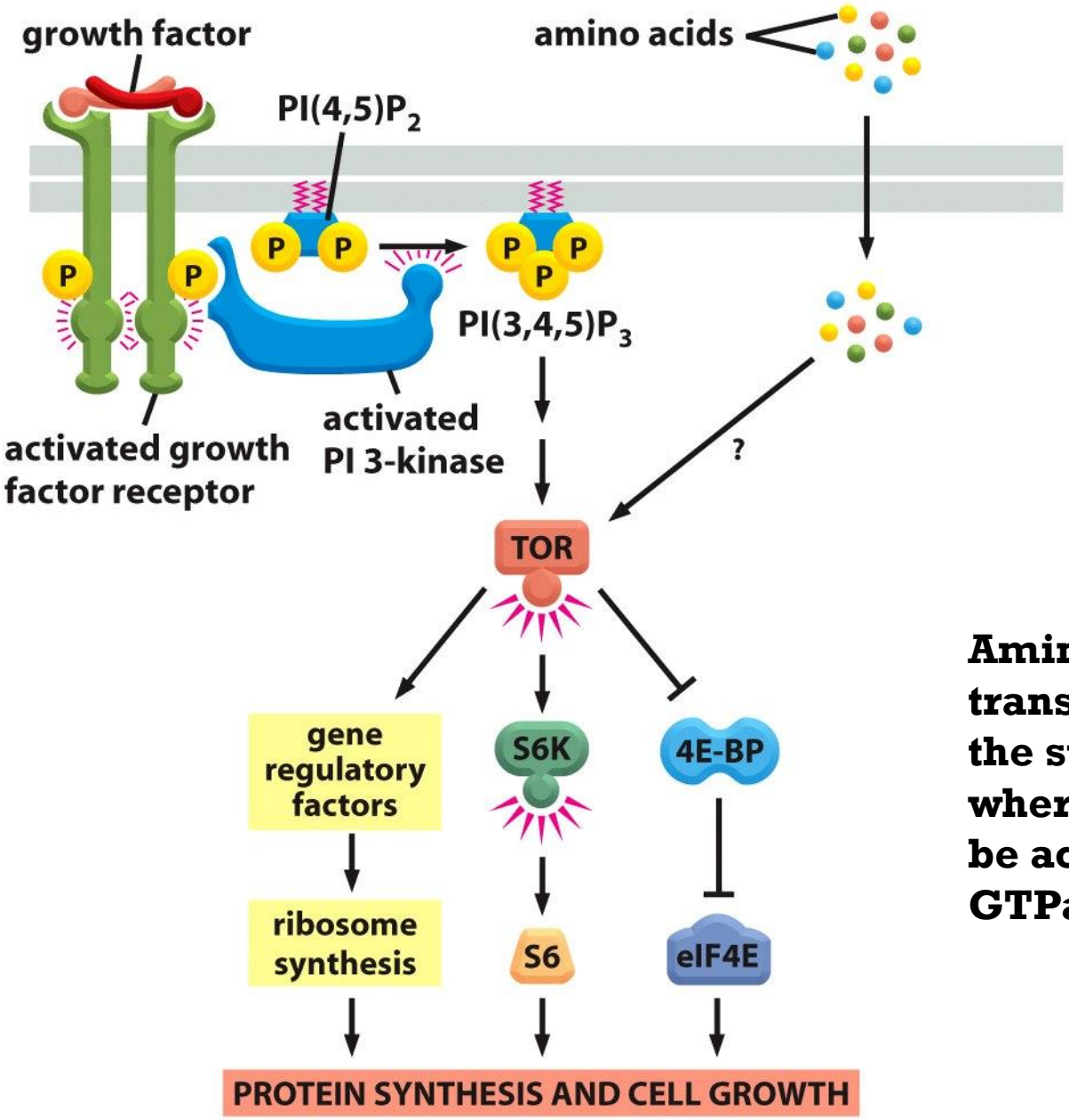
TSC= tuberous sclerosis complex, hamartin and tuberin, work as GAPs. Rheb= ras homolog enriched in the brain.
Rapamycin+FKBP12 inhibit mtorc1

Tuberous sclerosis complex (**TSC**) is a rare multisystem autosomal dominant genetic disease that causes non-cancerous tumours.

TSC is caused by a mutation of either of two genes, *TSC1* and *TSC2*, which code for the proteins hamartin and tuberin, respectively, with *TSC2* mutations accounting for the majority and tending to cause more severe symptoms.

Rheb/GTP directly interacts with TORC1 and activates its kinase activity.
TSC1/TSC2 is a Rheb GAP, (converts Theb into the GDP bound state, and inactivates it.

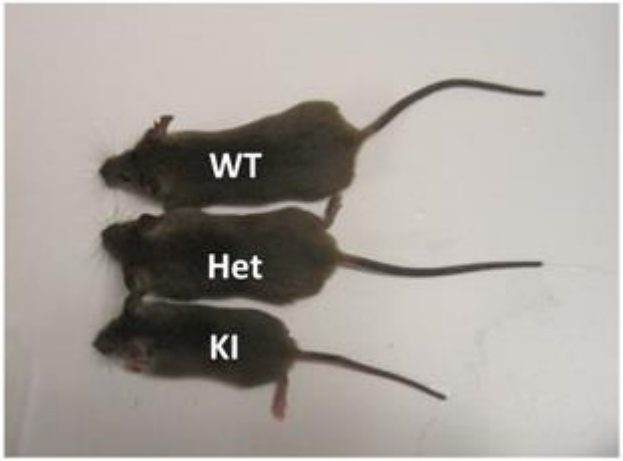
Stimulation of cell growth by extracellular growth factors and nutrients



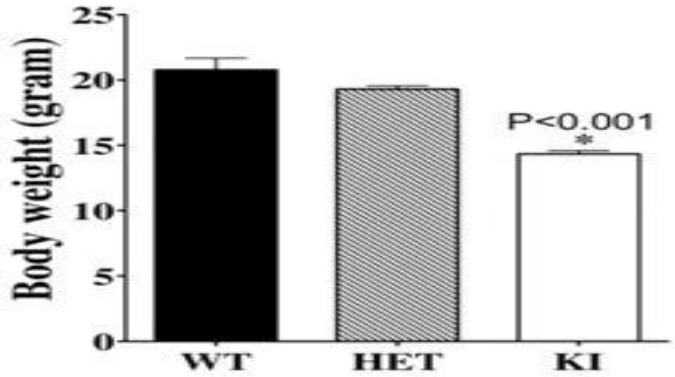
Amino acids promote the translocation of mTORC1 to the surface of the lysosome, where it can interact with and be activated by the Rheb GTPase

Figure 17-65 *Molecular Biology of the Cell* (© Garland Science 2008)

Viable hypomorphic mTOR mouse mutants exhibit reduced body, organ, and cell sizes.



a viable hypomorphic mouse by neo-insertion that partially disrupts mTOR transcription and creates a potential physiologic model of mTORC1/TORC2 inhibition.



Body sizes and weights of KI mice are less than that observed in age-matched WT and HET mice (N = 7 each).

Zhang et al 2011. Constitutive reductions in mTOR alter cell size, immune cell development, and antibody production. Blood 117:1228-1238

One way in which signaling through PI3-kinase promotes cell survival

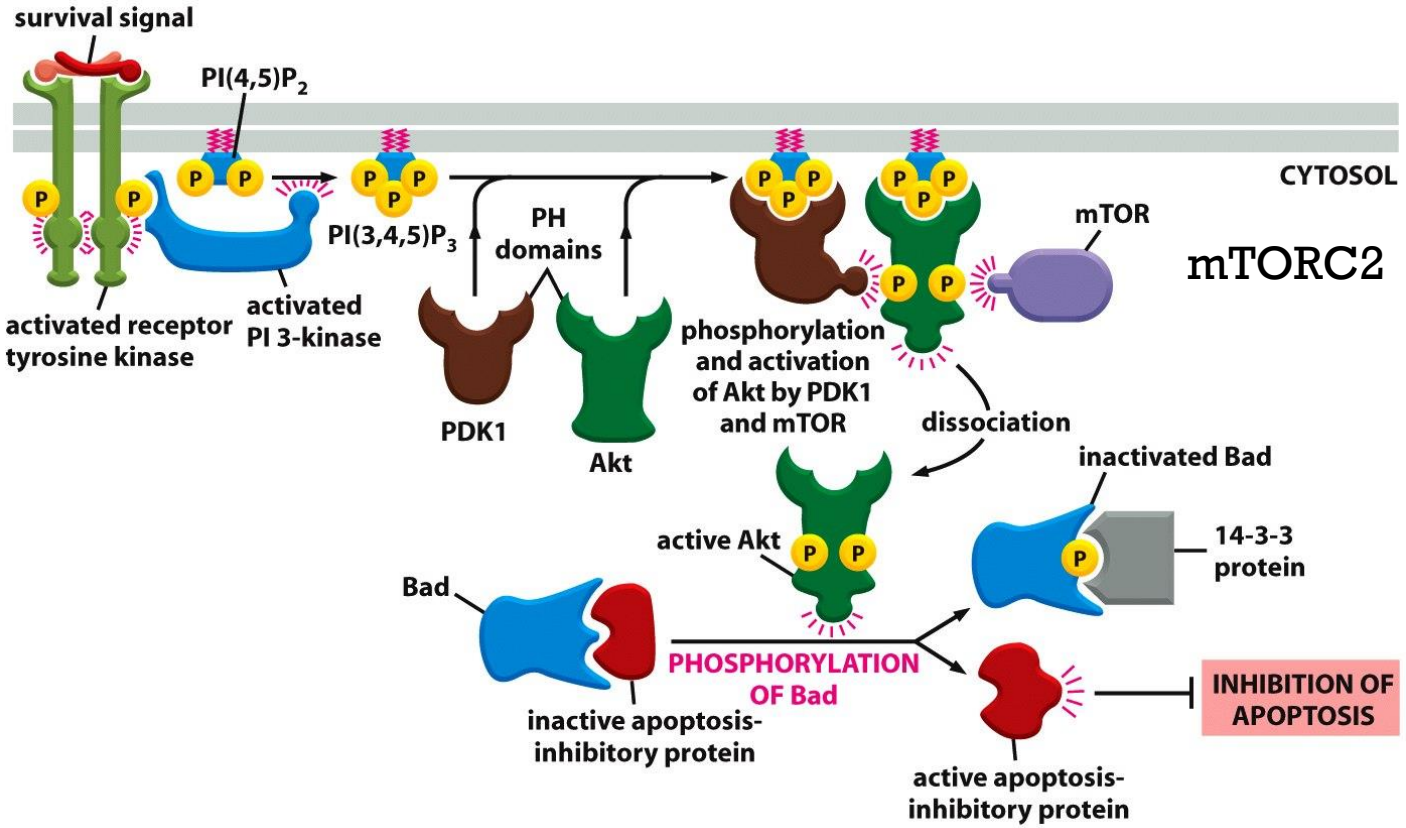


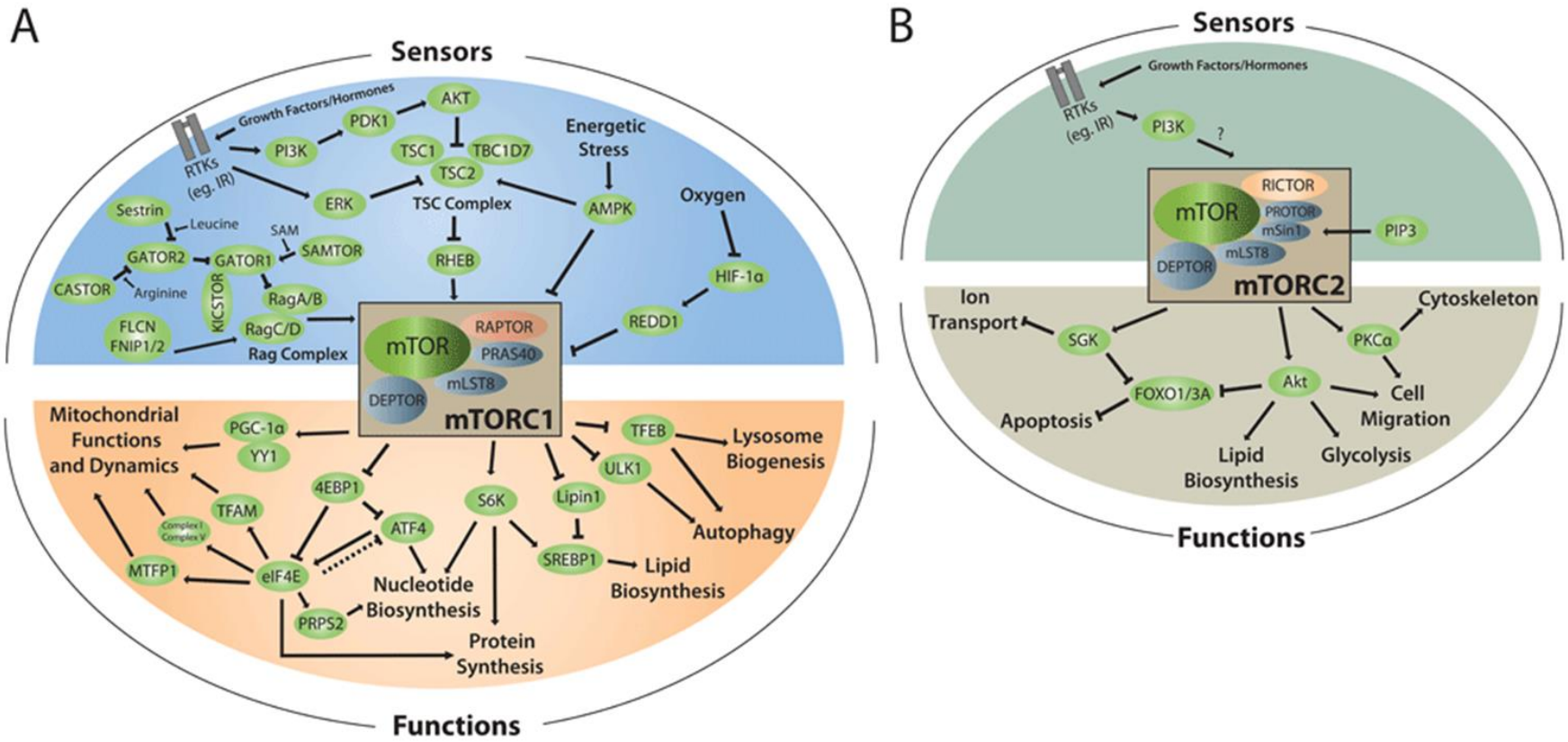
Figure 15-64 *Molecular Biology of the Cell* (© Garland Science 2008)

Extracellular survival signals include many growth factors, cytokines, hormones, cell-cell interactions and adhesion to the extra- cellular matrix.

The activated PKB now dissociates from the plasma membrane and phosphorylates the BAD protein, which, when unphosphorylated, holds one or more death-inhibitory proteins in an inactive state. Once phosphorylated, BAD releases the inhibitory proteins, bcl2 proteins, which now can block programmed cell death (apoptosis) and thereby promote cell survival.

As shown, once phosphorylated, BAD binds to a ubiquitous cytosolic protein called 14-3-3, which keeps BAD out of action. There are about 20 14-3-3 proteins in human cells, all of which bind to specific phosphoserine-containing motifs in proteins. The activation of other signaling pathways can also lead to BAD phosphorylation and the promotion of cell survival.

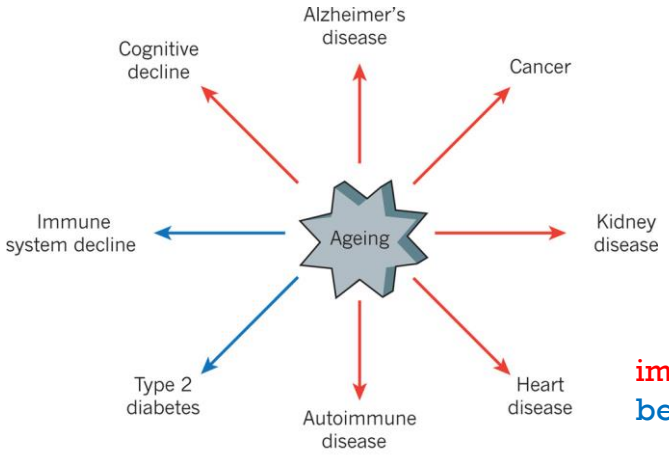
Figure 1. mTOR acts as a nutrient sensor coordinating cellular functions linked to proliferation, growth,...



Papadopoli D, Boulay K, Kazak L et al. mTOR as a central regulator of lifespan and aging [version 1]. F1000Research 2019, 8:998 (doi: 10.12688/f1000research.17196.1)

F1000Research

mTOR and ageing



improved by rapamycins

beneficial and detrimental consequences of mTORC1 inhibition

Table 1 | Comparison of species in which genetic or pharmacological inhibition of an mTORC1-pathway component extends lifespan

	*Saccharomyces cerevisiae**	*Caenorhabditis elegans*	*Drosophila melanogaster*	*Mus musculus*
Rapamycin	Yes[9,10]	Yes[11]	Yes[12]	Yes[13–15]
mTOR gene mutation and knockdown	Yes[8,9]	Yes[6]	Yes[7]	Yes[3]
Raptor gene mutation and knockdown	Not reported	Yes[5]	Not reported	Not reported
Tsc1 and *Tsc2* activation	Not applicable†	Not applicable	Yes[7]	Not reported
S6K gene mutation and knockdown	Yes[4,8]	Yes[24]	Yes[7]	Yes[37]
4E-BP activation	Not applicable	Not applicable	Yes[18]	Not reported
Translation initiation factor mutation and knockdown	Yes[44]	Yes[17,24,74,75]	Not reported	Not reported
Ribosomal protein mutation and knockdown	Yes[44,47,76]	Yes[17,75]	Not reported	Not reported

*Includes both replicative and chronological lifespan; †Not applicable is used in cases for which homologues have not yet been identified.

From: mTOR is a key modulator of ageing and age-related disease. Simon C. Johnson, et al. Nature 493, 338–345 (2013)

Rapamycin extends lifespan (‘duração da vida’) in yeast, nematodes, fruitflies and mice, firmly establishing mTORC1 as a central, evolutionarily conserved regulator of longevity .

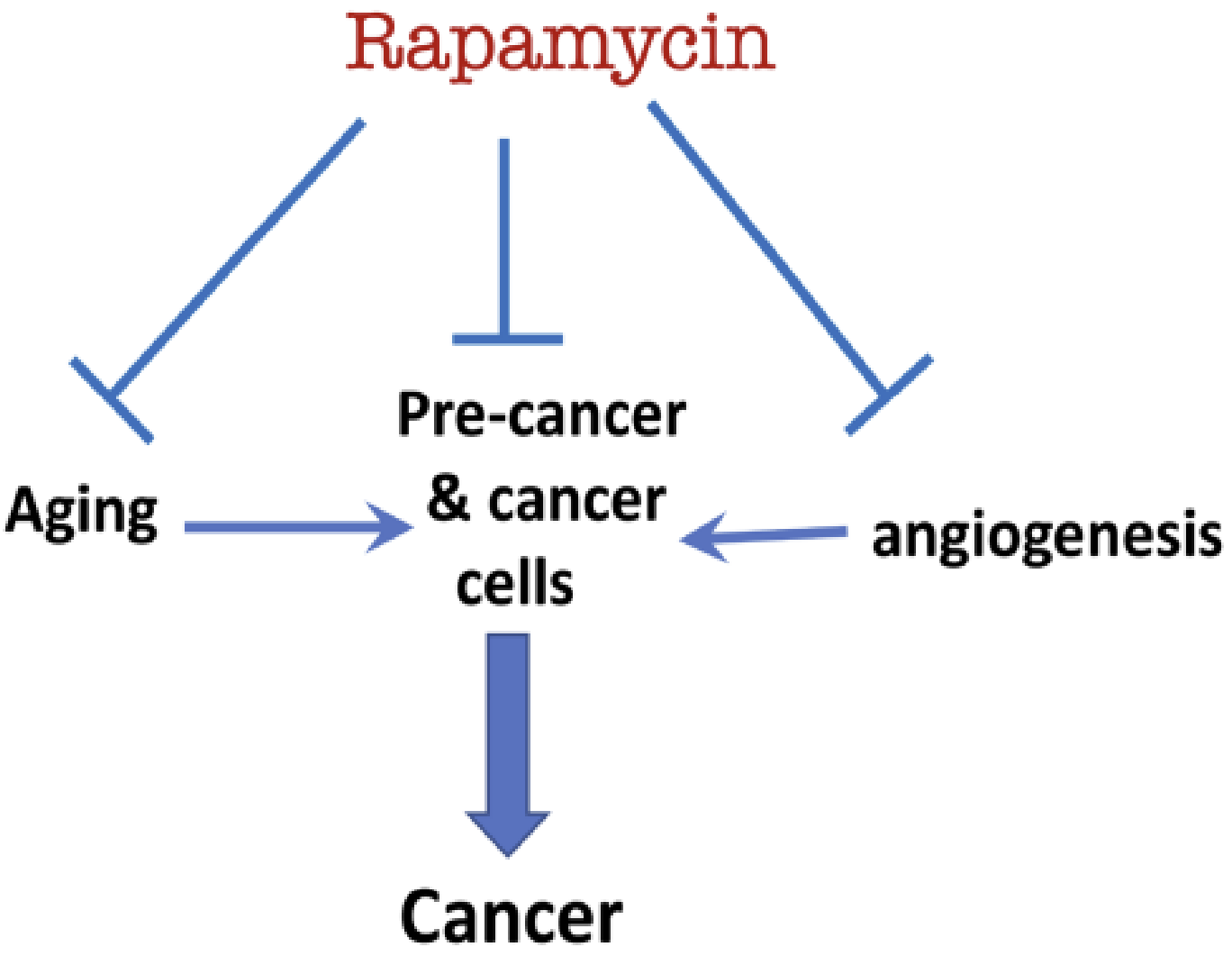
Ageing drives the onset and progression of multiple disorders that are modulated by mTORC1 signalling. Data from animal and human studies indicate that some disorders (red arrows) are improved by rapamycins. However, for others (blue arrows), although influenced by rapamycins, evidence suggests there are both beneficial and detrimental consequences of mTORC1 inhibition.

BOX 1

Key events for rapamycins in ageing

- **1970** Soil samples containing rapamycin-producing *Streptomyces hygroscopicus* taken from the Polynesian island of Rapa Nui
- **1975** Purification of rapamycin and identification of fungicidal activity
- **1977** Immunosuppressive activity discovered
- **1984** Antitumour activity discovered
- **1991** *TOR1* and *TOR2* genes identified in yeast
- **1994** mTOR gene *FRAP1*, now *MTOR*, identified in mammals
- **1995** Mechanism of action of rapamycin discovered
- **1999** Approved by US Food and Drug Administration (FDA) for use in preventing host-rejection in patients undergoing kidney transplantation
- **2003** Approved by FDA for use in drug-eluting stents
- **2006** Shown to extend lifespan in budding yeast
- **2007** Approved by FDA for treatment of renal-cell carcinoma
- **2008** Approved by FDA for treatment of mantle cell lymphoma
- **2009** Shown to extend lifespan in mice
- **2010** Shown to extend lifespan in fruitflies
- **2010** Approved by FDA for treatment of tuberous sclerosis
- **2011** Shown to improve outcome in mouse models of Alzheimer's disease
- **2011** Approved by FDA for treatment of pancreatic cancer
- **2012** Shown to extend lifespan in nematodes
- **2012** More than 1,300 clinical trials under way or completed

From: mTOR is a key modulator of ageing and age-related disease. Simon C. Johnson, et al. Nature 493, 338–345 (2013)


Rapamycin
Pre-cancer
& cancer
cells
Aging
angiogenesis
Cancer

Phosphoinositide 3-kinase (PI3 kinase) and pleckstrin are both proteins involved in cellular signaling pathways.

1.Phosphoinositide 3-Kinase (PI3 Kinase):

1. **Function:** PI3 kinase is an enzyme that phosphorylates the 3-position hydroxyl group of the inositol ring of phosphatidylinositol. This phosphorylation event leads to the generation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), a key secondary messenger involved in intracellular signaling.
2. **Signaling Pathways:** PI3 kinase is a crucial component of various signaling pathways, including the insulin signaling pathway, growth factor signaling, and other pathways that regulate cell growth, survival, and metabolism.
3. **Downstream Targets:** One of the major downstream effectors of PIP3 is the protein kinase Akt (also known as protein kinase B), which plays a central role in mediating many of the cellular responses to PI3 kinase activation.

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