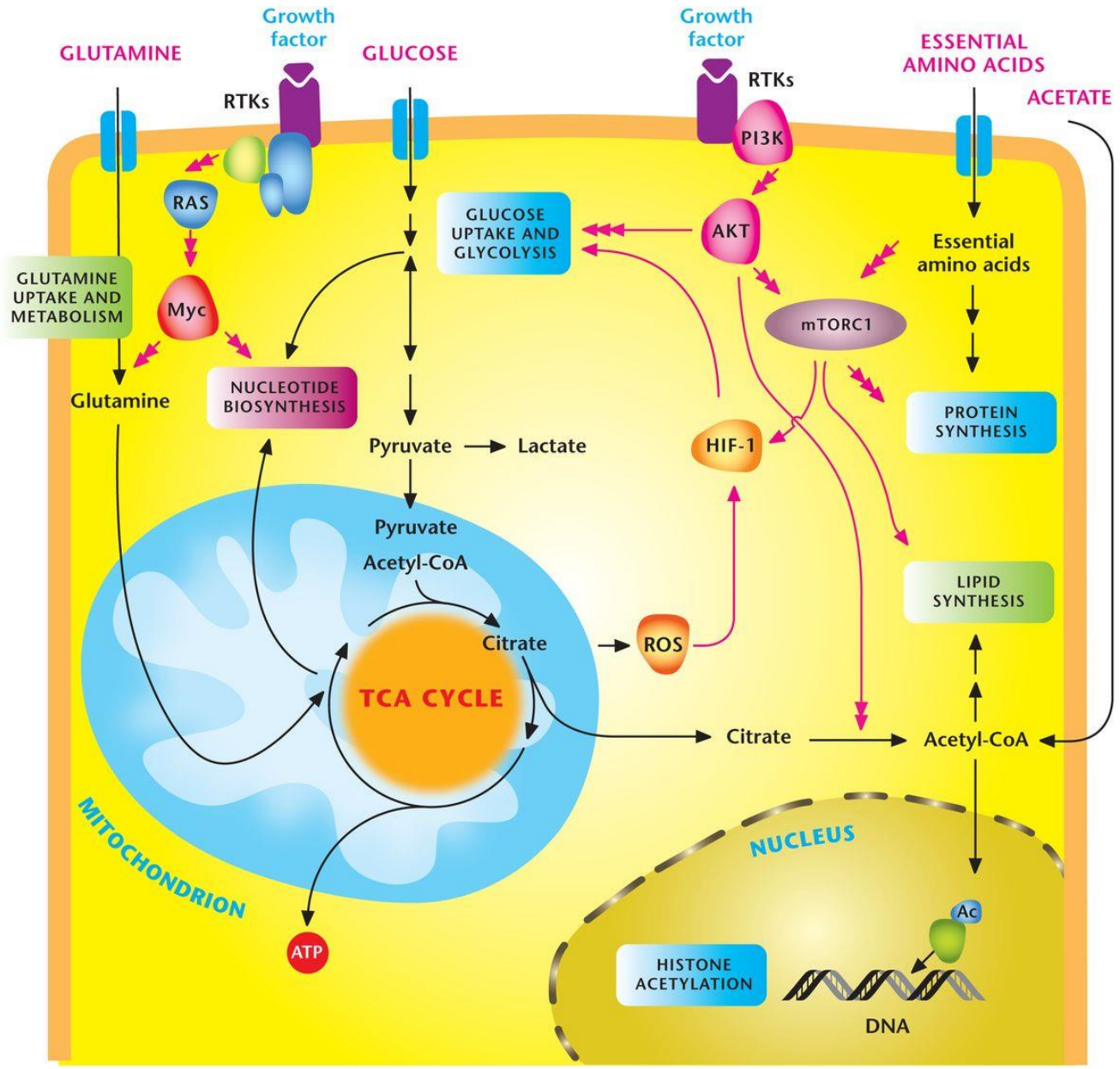
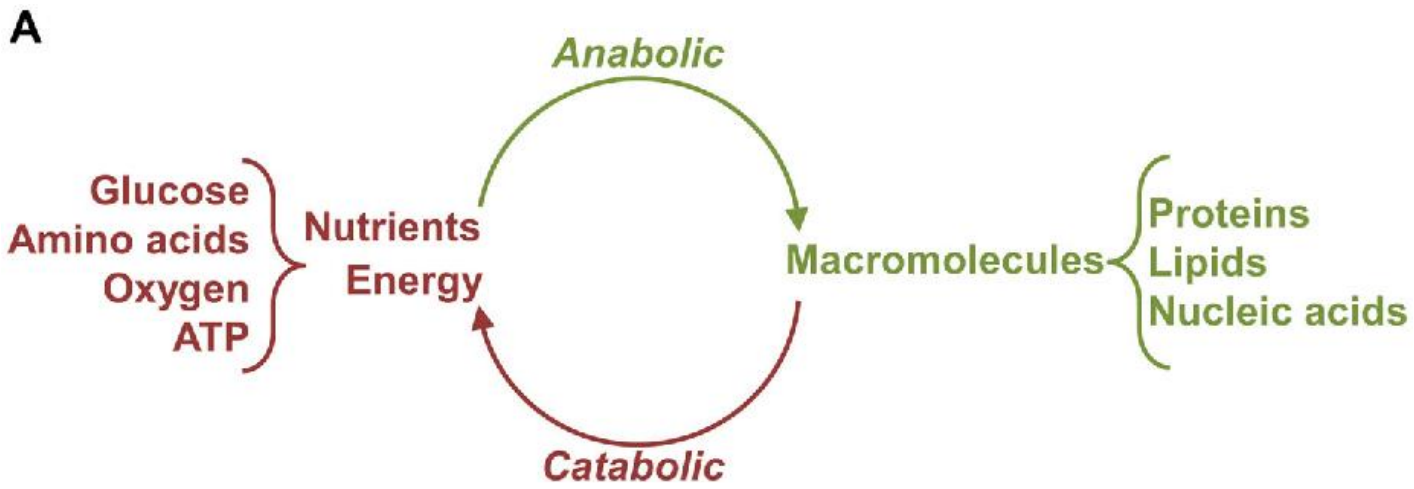
Signaling and metabolism

Signal transduction pathways regulate metabolism.



mTORC1 is a molecular switch between catabolic and anabolic processes.



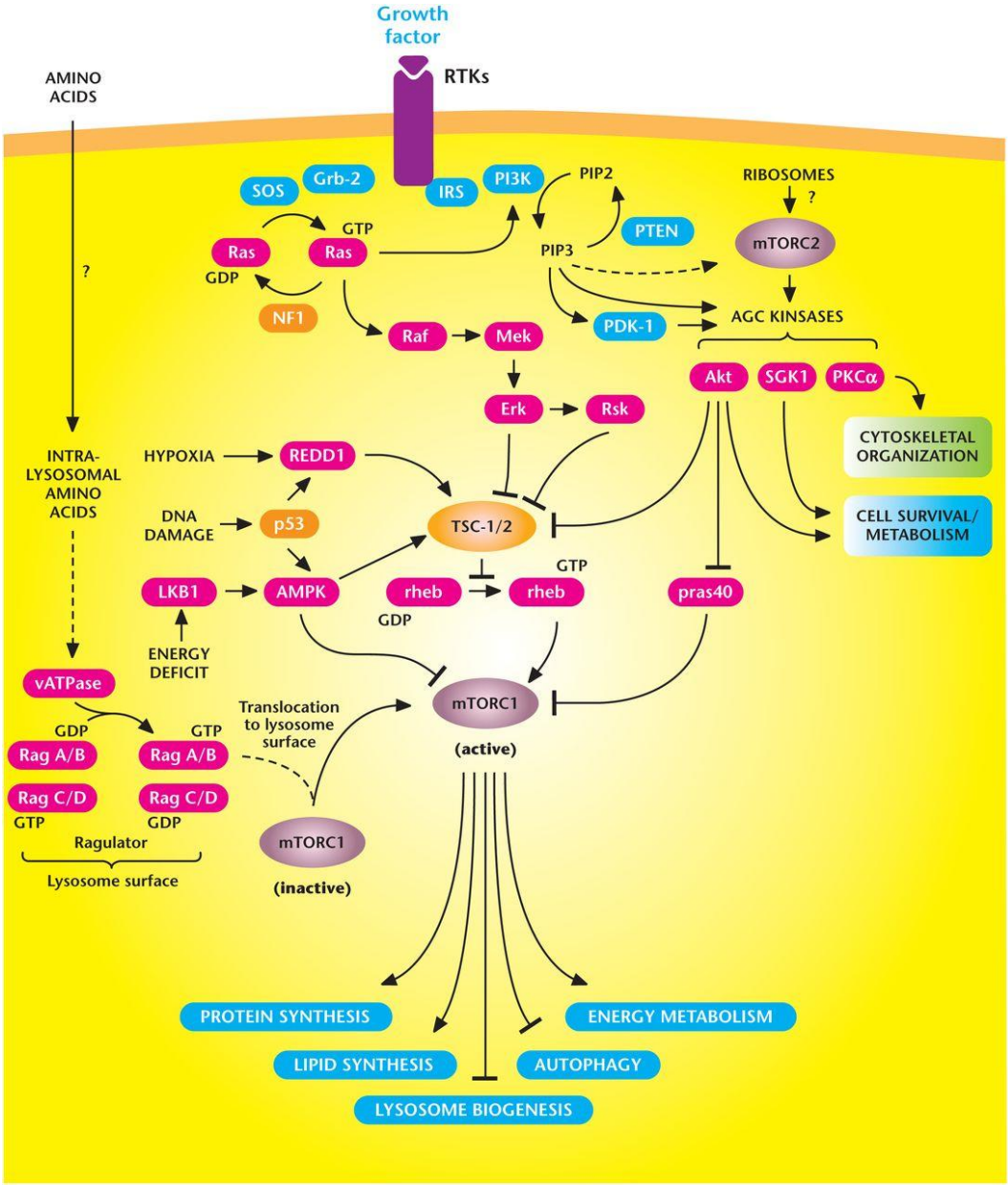
mTOR characteristics

mammalian Target Of Rapamycin

- ✓ High conserved
- ✓ Serine/threonine kinase protein (289 kDa)
- ✓ Belonging to PIKK (phosphatidylinositol 3-kinase-related kinase)
- ✓ In mammalian cells → 2 distinct complexes

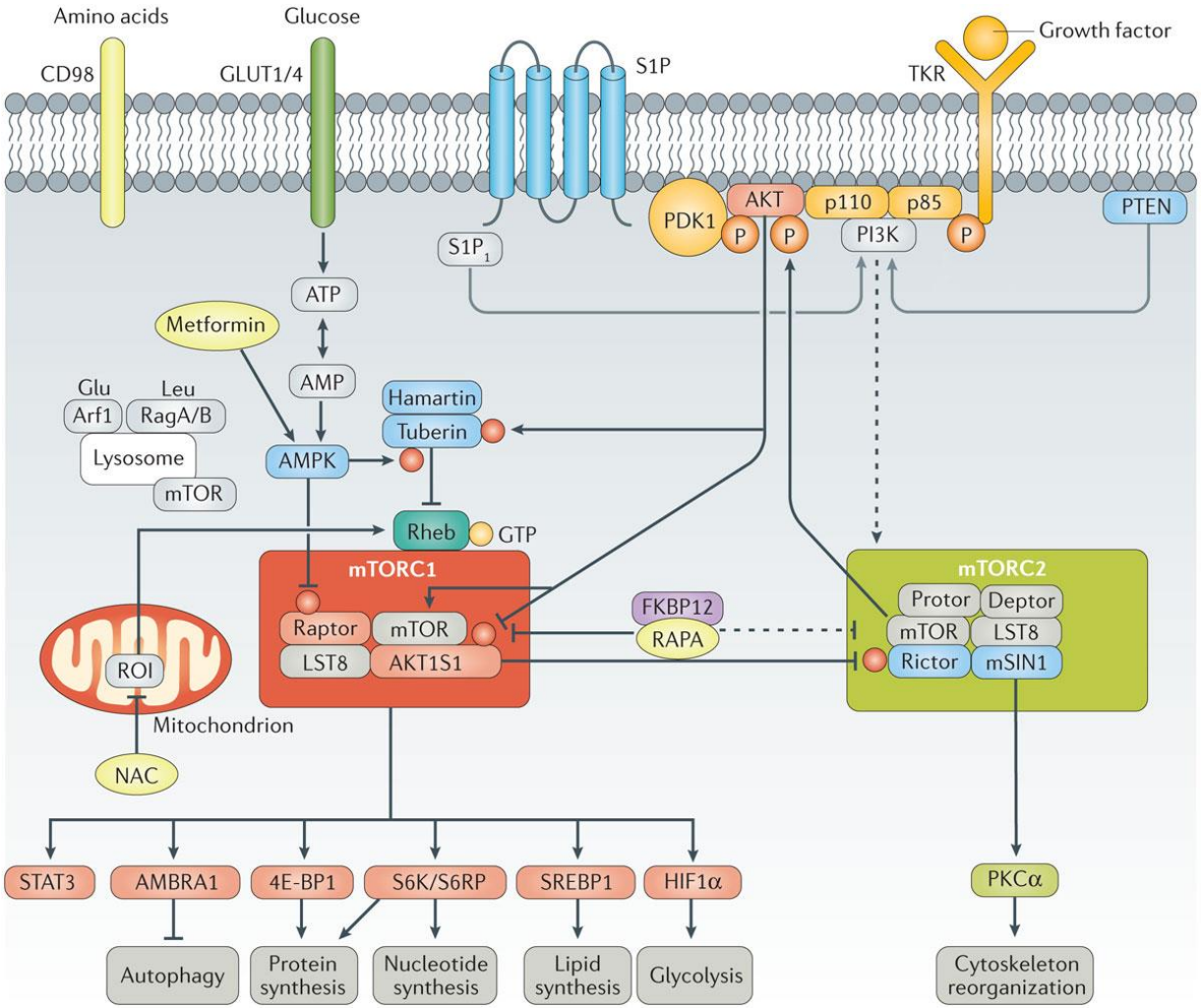
The mechanistic target of rapamycin (mTOR) signaling pathway controls metabolism and cell survival.

mTORC1 stimulates multiple anabolic metabolic pathways (lipid and protein synthesis) in the presence of nutrients and growth factors. Conversely, limiting nutrient or growth factor availability promotes catabolic pathways (autophagy).



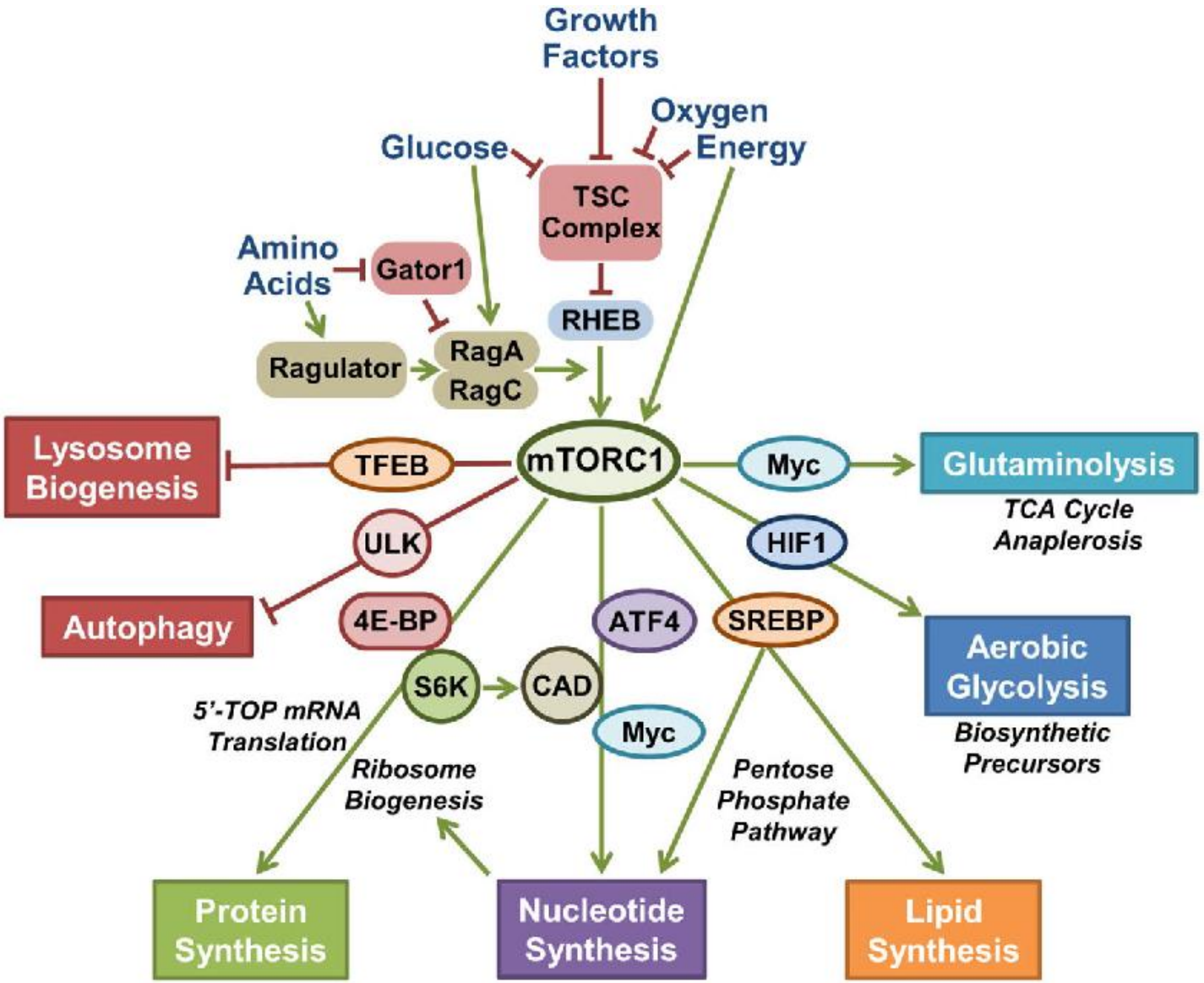
mTORC2 promotes cell survival through activation of AKT.

mTORC1/2 pathway downstream effectors

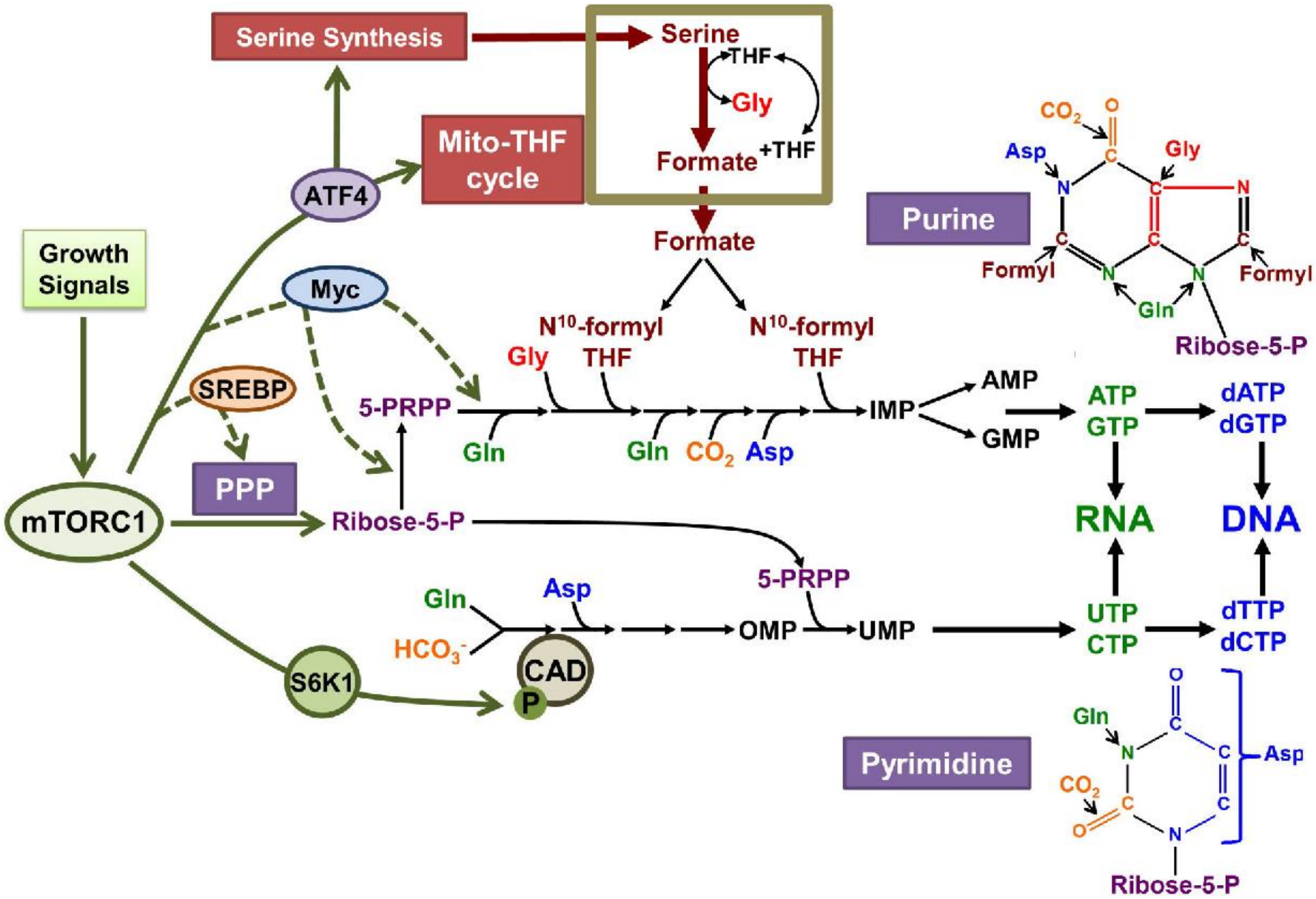


Nature Reviews | Rheumatology

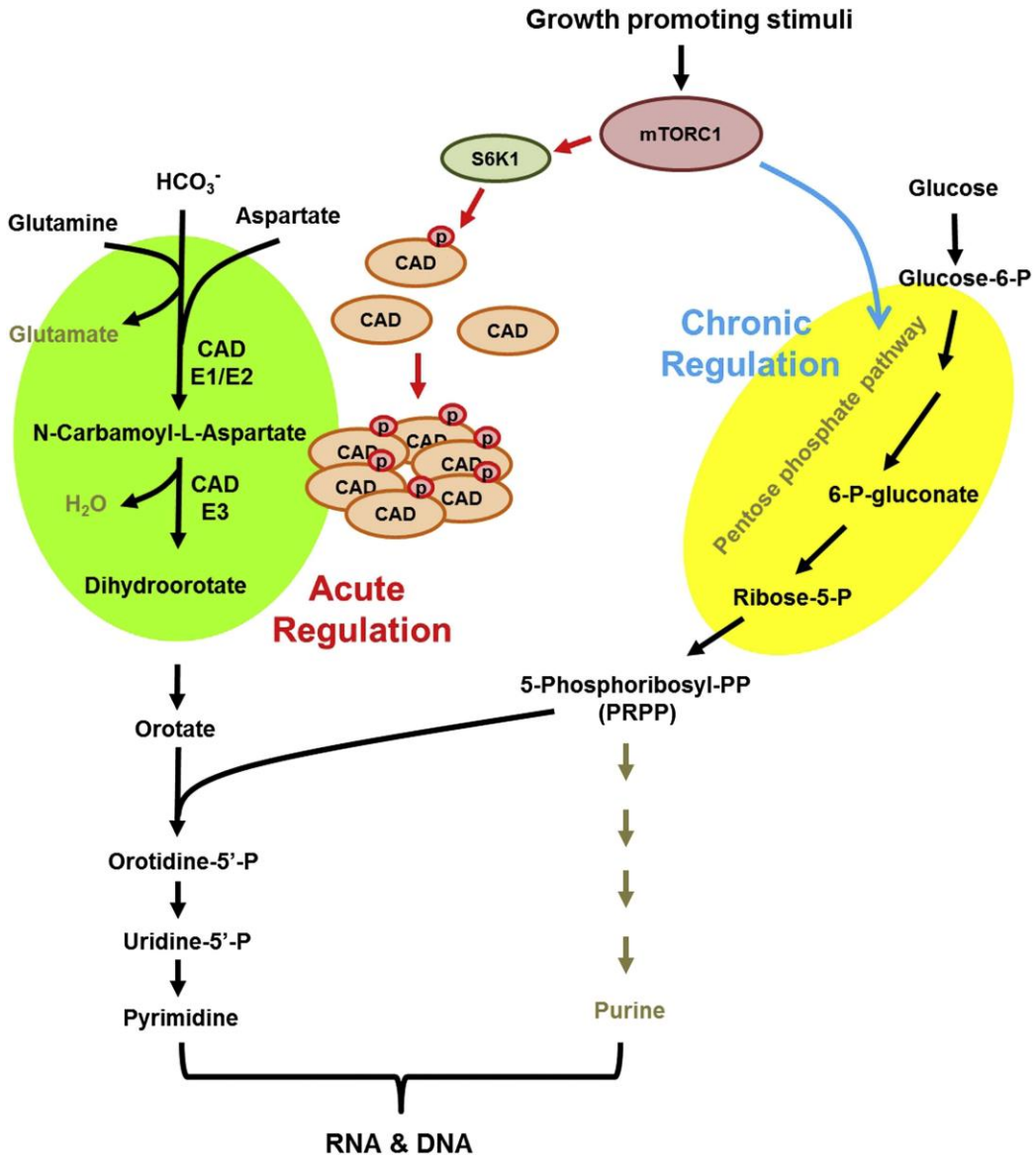
The mTORC1 network senses cellular growth signals and relays their status to an array of downstream metabolic processes.



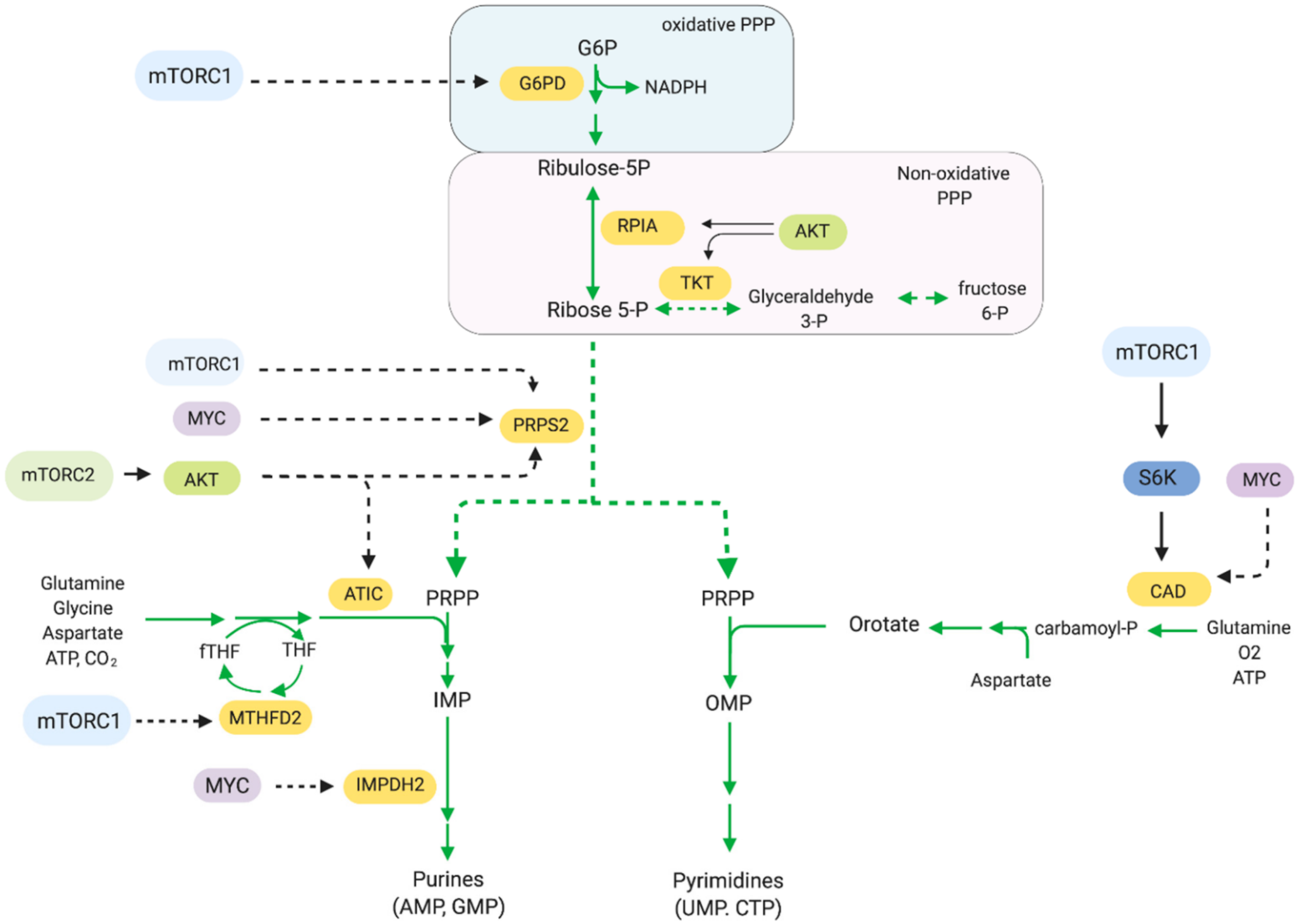
mTORC1 signaling stimulates de novo nucleotide synthesis through multiple downstream mechanisms



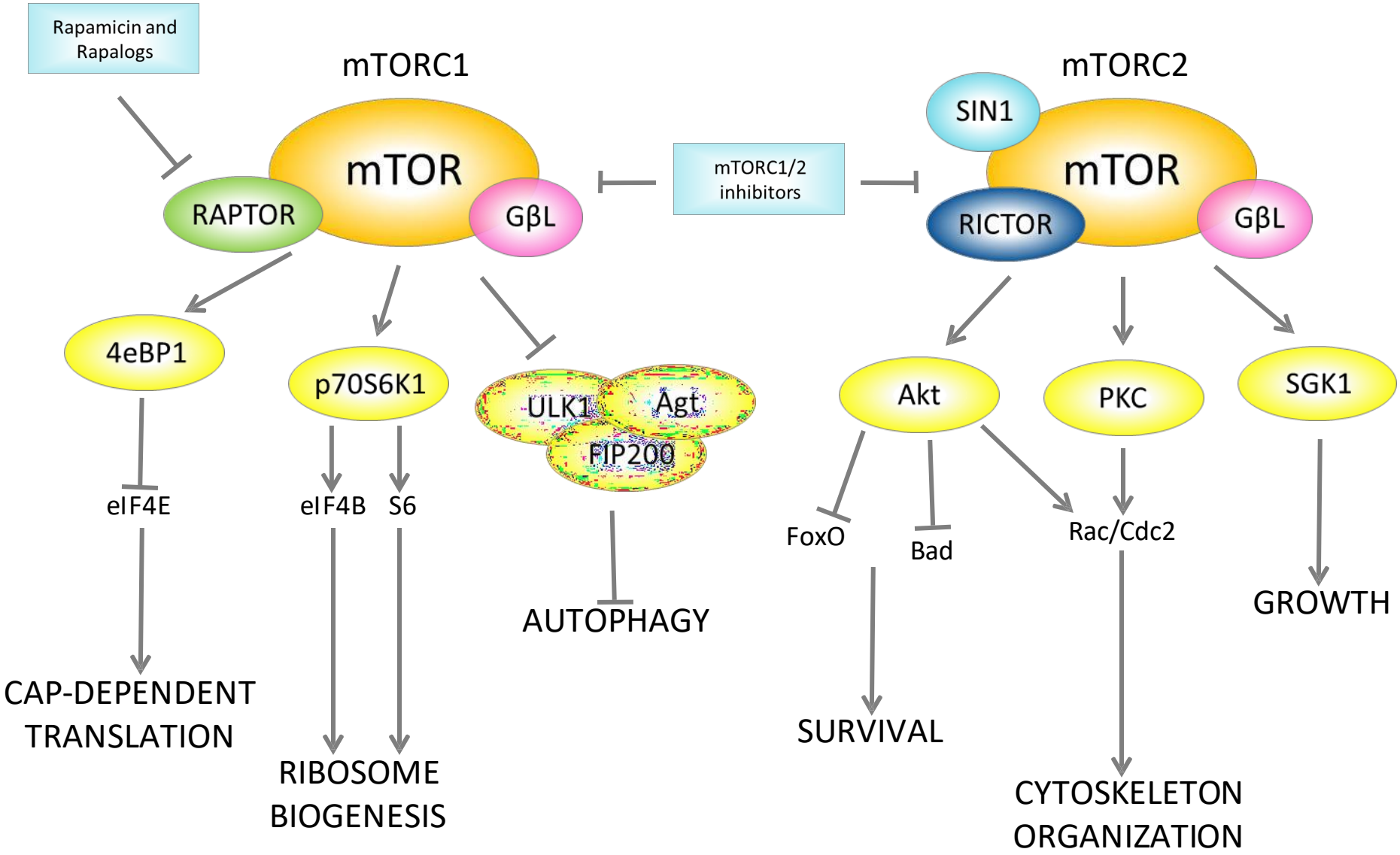
De Novo Pyrimidine Synthesis Is Regulated by mTORC1 Signaling via S6K1 Phosphorylation of CAD



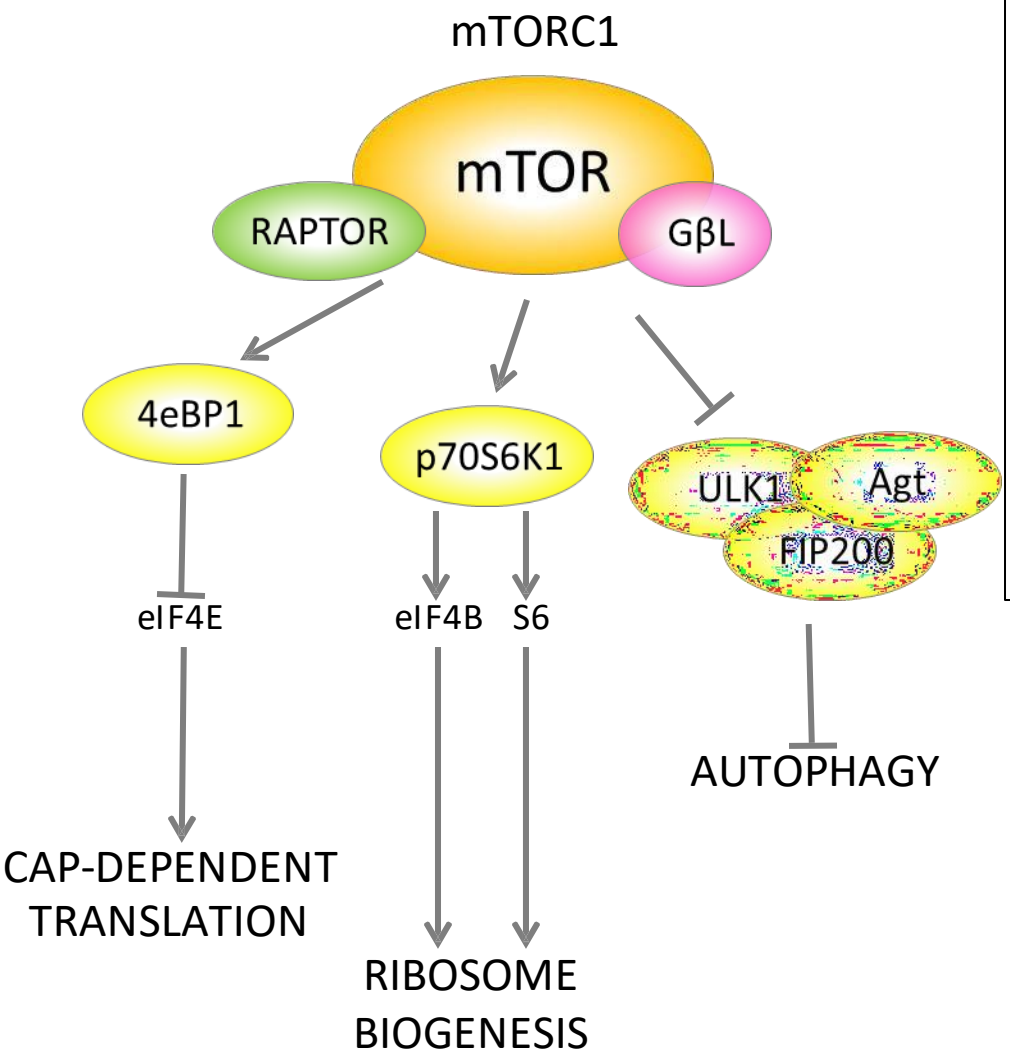
The role of mTOR in the pentose phosphate pathway and nucleotide synthesis. mTOR modulates the expression of the enzymes involved in the pentose phosphate pathway, purine and pyrimidine synthesis.



mTOR in mammalian cells



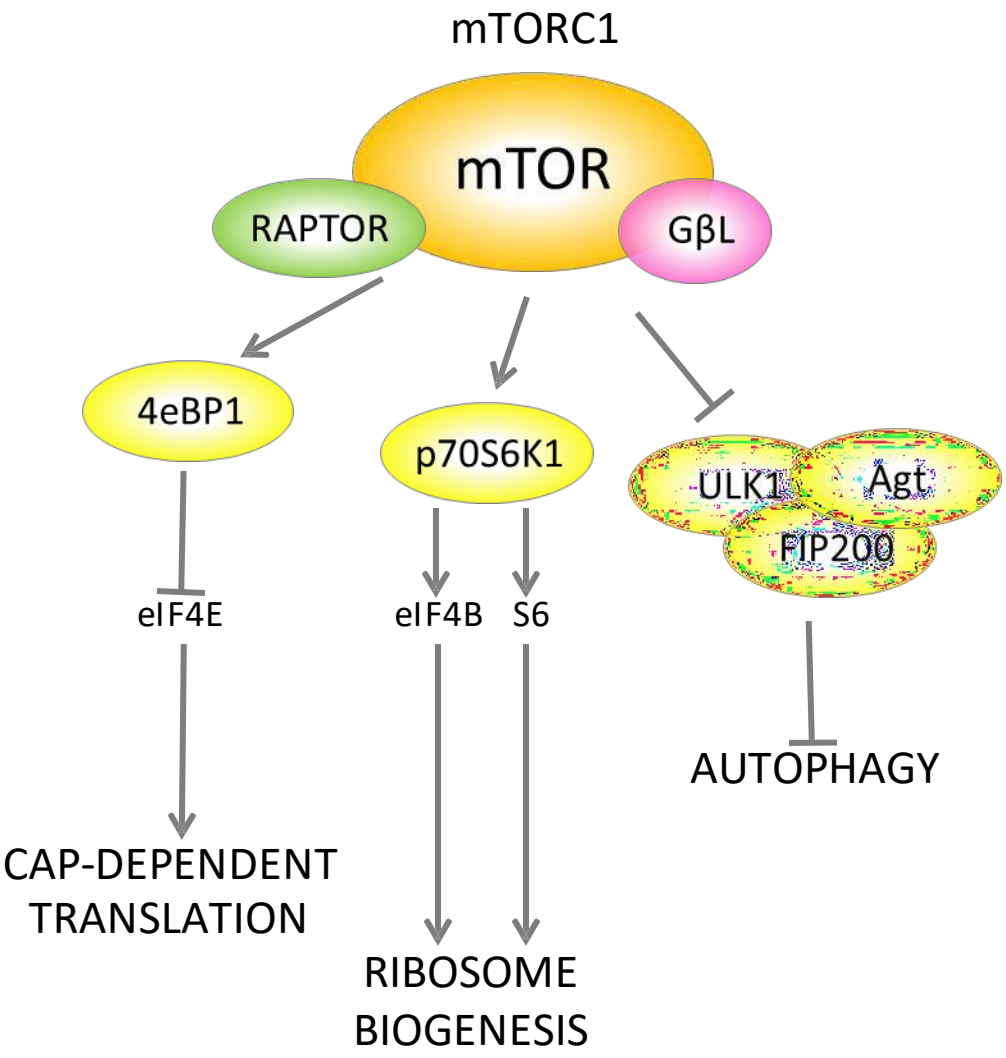
mTOR complex 1



RAPTOR (Regulatory-associated protein of mTOR)

- ✓ facilitates the association between mTORC1 and downstream substrates
- ✓ binds in a rapamycin-sensitive or nutrient-responsive manner
- ✓ mTOR activity could be modulated by RAPTOR phosphorylation

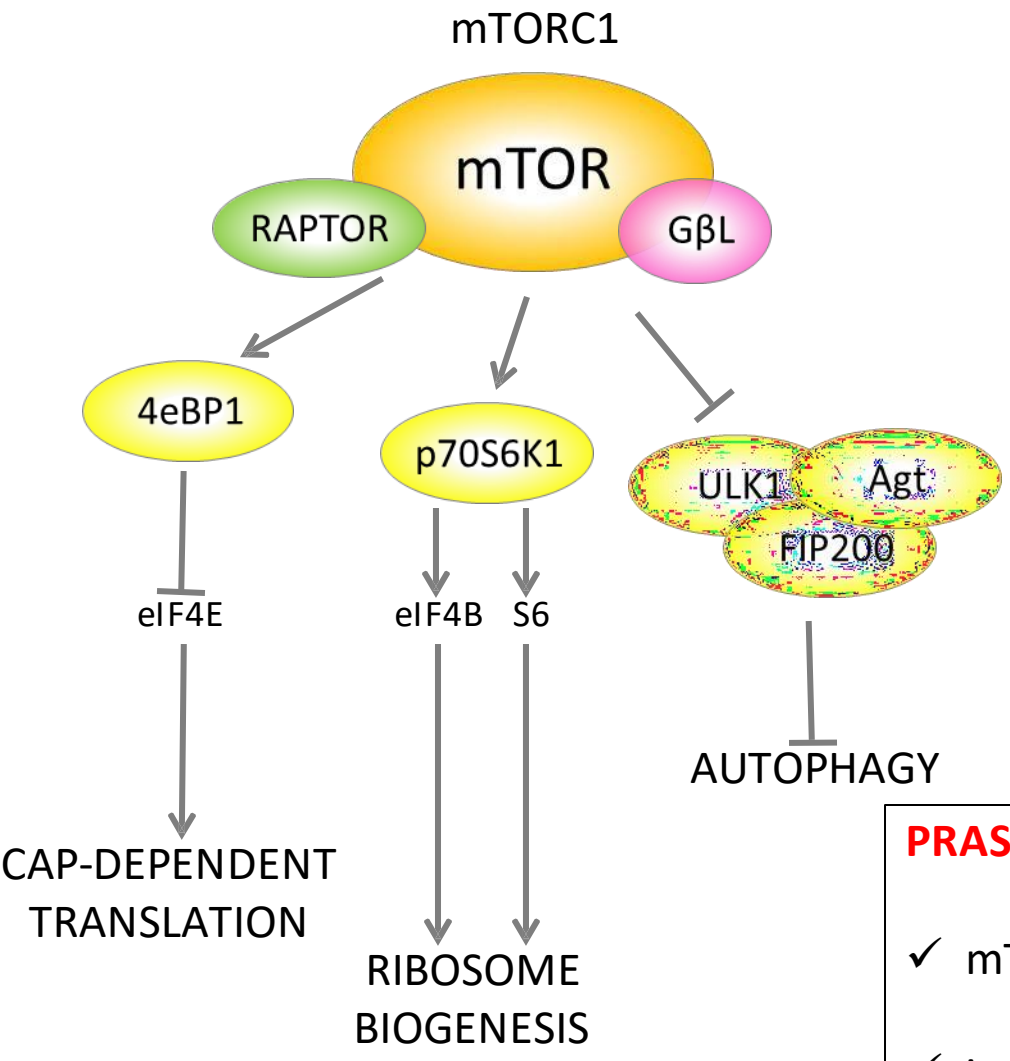
mTOR complex 1



mLST8/GβL

- ✓ interacts with RAPTOR and mTOR in a nutrient- and rapamycin-sensitive manner
- ✓ Positivity regulates mTORC1 activation

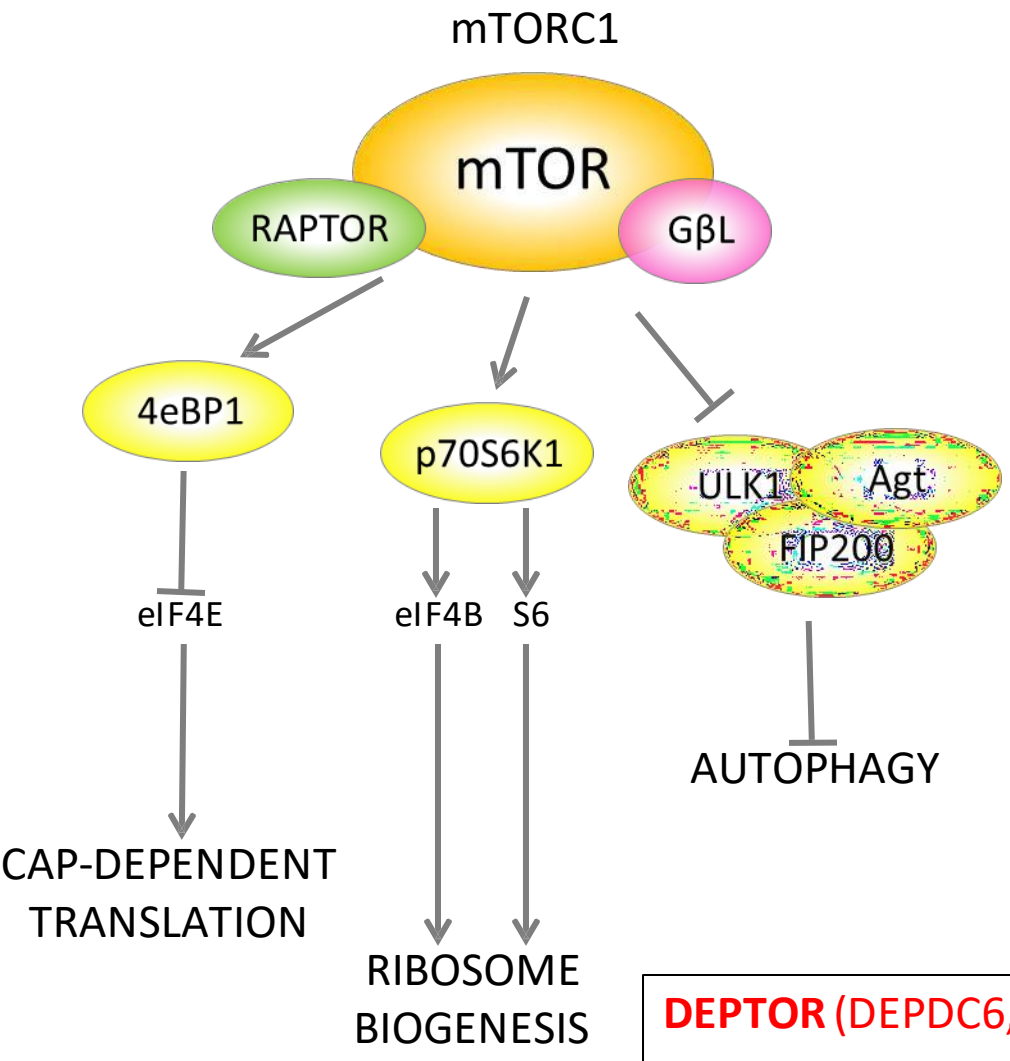
mTOR complex 1



PRAS40

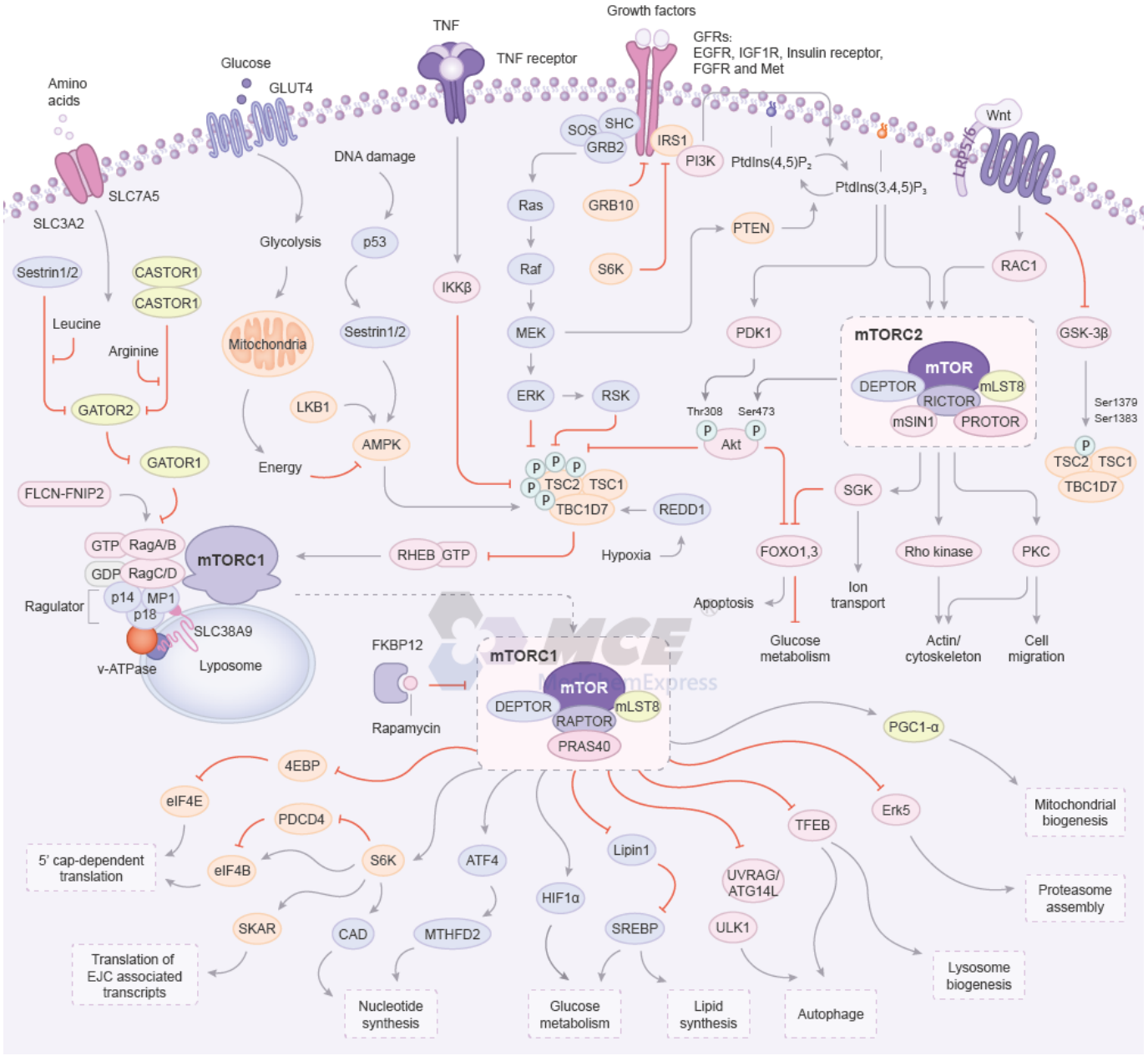
- ✓ mTORC2 negative feedback
- ✓ interacts with RAPTOR to prevent mTORC1 assembly

mTOR complex 1

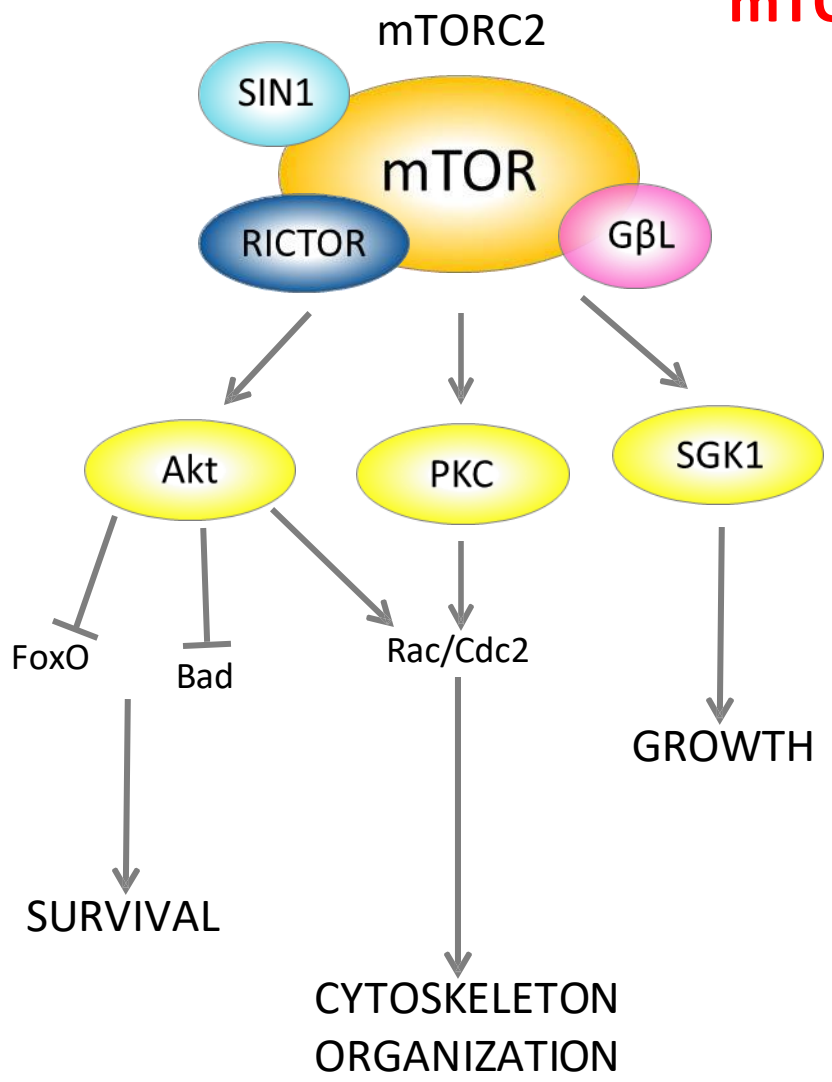


DEPTOR (DEPDC6, DEP domain-containing protein 6)

✓ negative feedback loop


Growth factors
TNF
GFRs:
EGFR, IGF1R, Insulin receptor,
FGFR and Met
TNF receptor
Glucose
GLUT4
Amino
acids
SLC7A5
SLC3A2
DNA damage
Wnt
LRP5/6
SOS
SHC
GRB2
IRS1
PI3K
PtdIns(4,5)P2
PtdIns(3,4,5)P3
Ras
GRB10
S6K
PTEN
RAC1
Raf
MEK
PDK1
mTORC2
mTOR
DEPTOR
mLST8
RICTOR
mSIN1
PROTOR
GSK-3β
Ser1379
Ser1383
TSC2
TSC1
TBC1D7
Sestrin1/2
CASTOR1
CASTOR1
Leucine
Arginine
GATOR2
GATOR1
Glycolysis
p53
Mitochondria
Sestrin1/2
IKKβ
ERK
RSK
LKB1
AMPK
Energy
Thr308
Ser473
Akt
SGK
FLCN-FNIP2
GTP
RagA/B
GDP
RagC/D
p14
MP1
p18
Ragulator
mTORC1
SLC38A9
v-ATPase
Lyposome
RHEB
GTP
REDD1
Hypoxia
FOXO1,3
Rho kinase
PKC
Apoptosis
Ion
transport
Glucose
metabolism
Actin/
cytoskeleton
Cell
migration
FKBP12
Rapamycin
mTORC1
mTOR
DEPTOR
mLST8
RAPTOR
PRAS40
PGC1-α
4EBP
eIF4E
PDCD4
5' cap-dependent
translation
eIF4B
S6K
ATF4
Lipin1
TFEB
Erk5
Mitochondrial
biogenesis
UVRAG/
ATG14L
Proteasome
assembly
HIF1α
SKAR
CAD
MTHFD2
SREBP
ULK1
Translation of
EJC associated
transcripts
Lysosome
biogenesis
Nucleotide
synthesis
Glucose
metabolism
Lipid
synthesis
Autophage

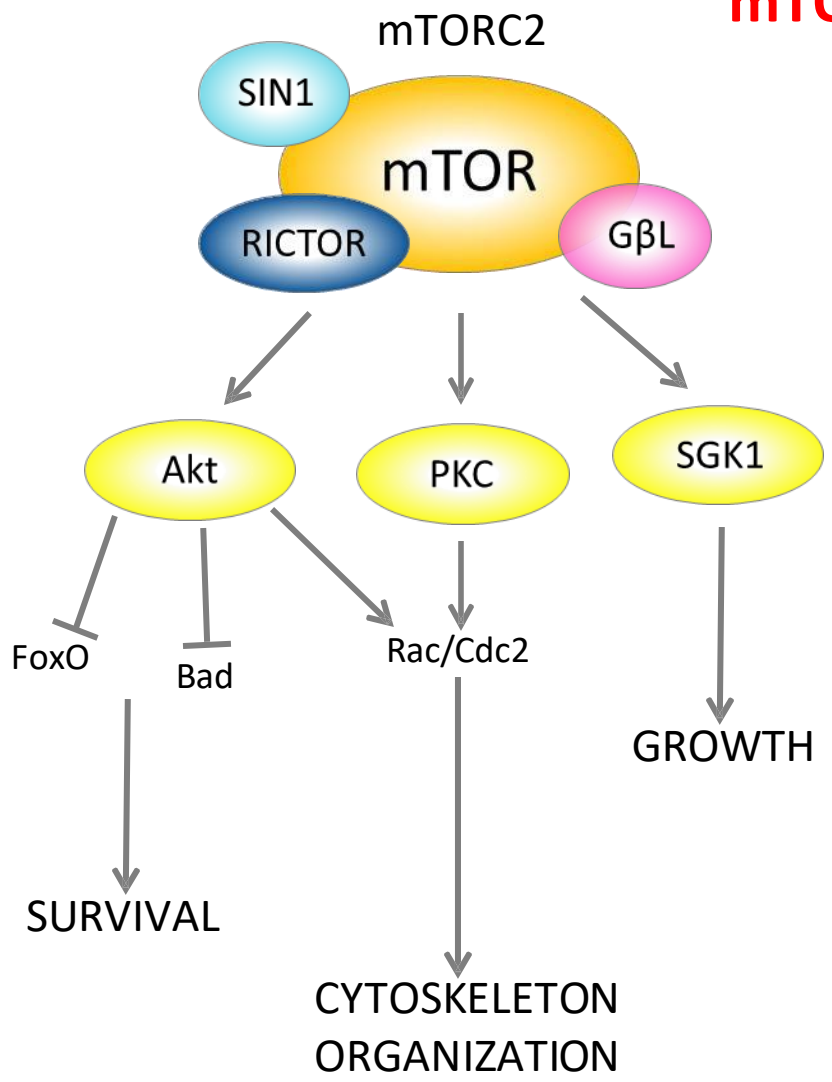
mTOR complex 2



RICTOR (Rapamycin-insensitive companion of mTOR)

- ✓ Required for mTORC2 activation
- ✓ gains integrity of mTORC2
- ✓ exclusive of mTORC2

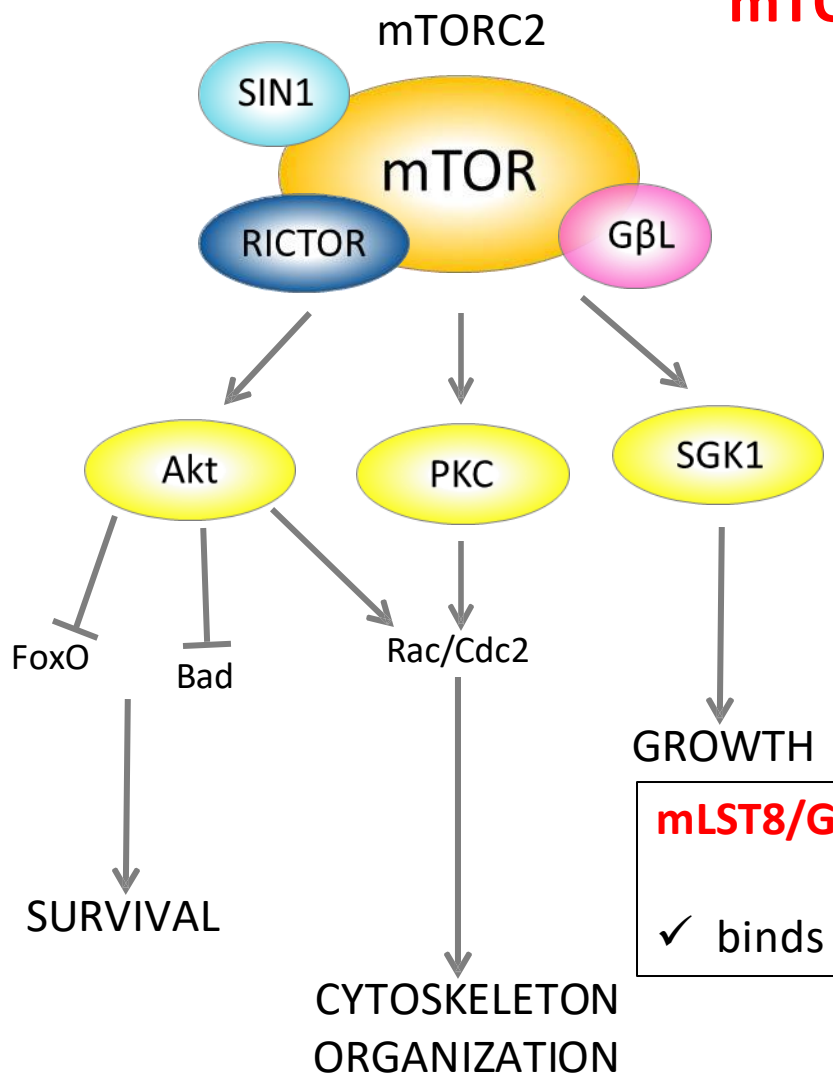
mTOR complex 2



SIN1 (stress-responsive protein)

- ✓ implicated in the JNK (c-Jun N-terminal kinase) and MAPK (mitogen-activated protein kinase)/ERK (extracellular-regulated-protein kinase) pathways
- ✓ essential for mTORC2 activity and AKT phosphorilation

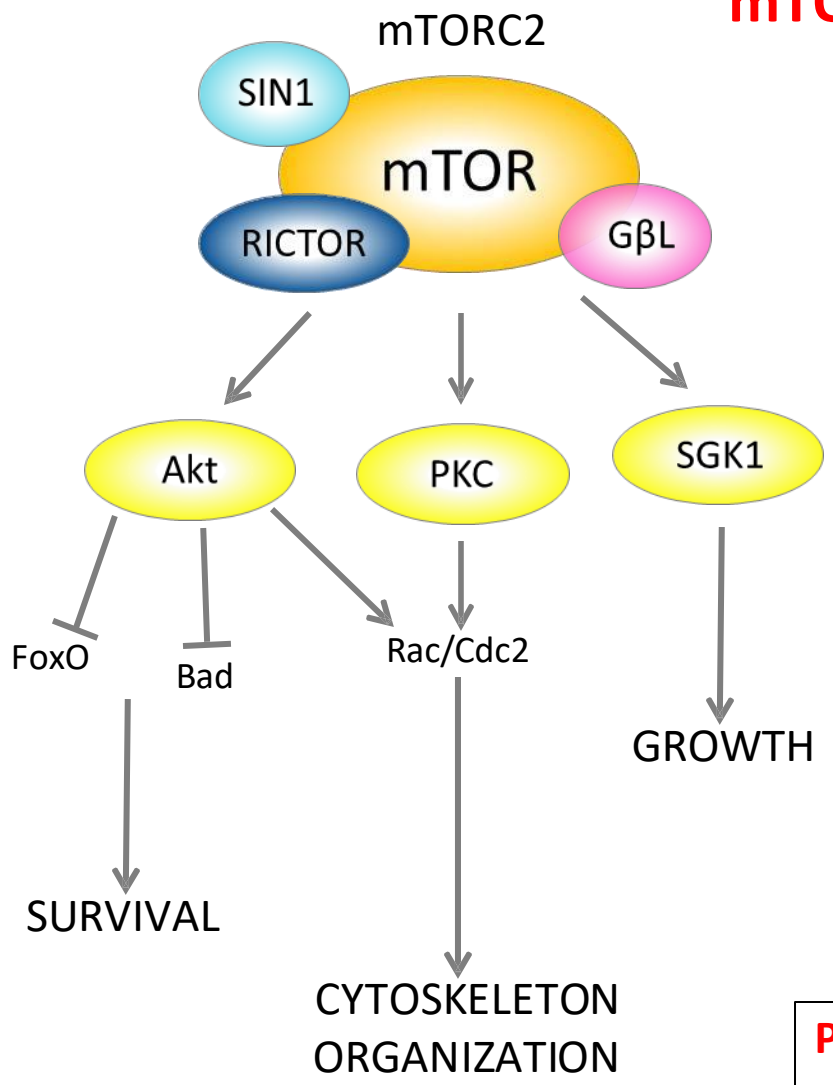
mTOR complex 2



mLST8/GβL

✓ binds C-term mTOR favoring mTORC2 kinase activity

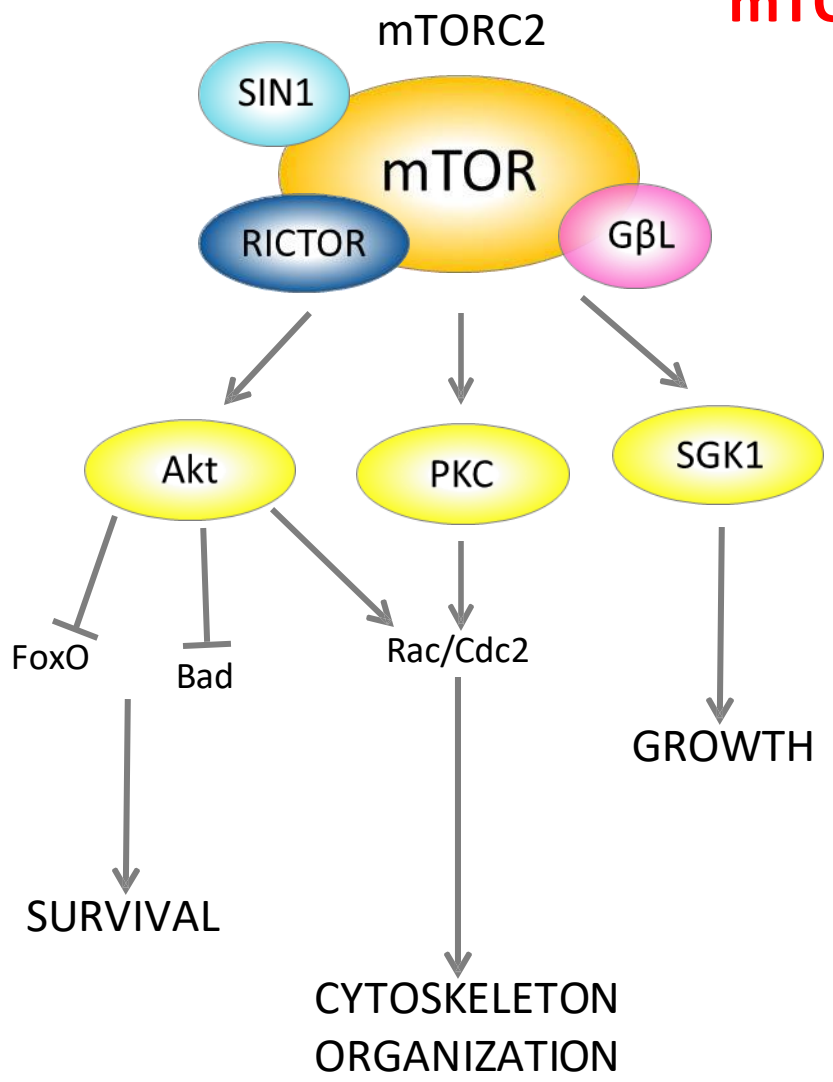
mTOR complex 2



PROTOR (protein observed with rictor)

- ✓ non-essential subunits
- ✓ required for the phosphorylation of SGK1, but not of Akt and PKCα

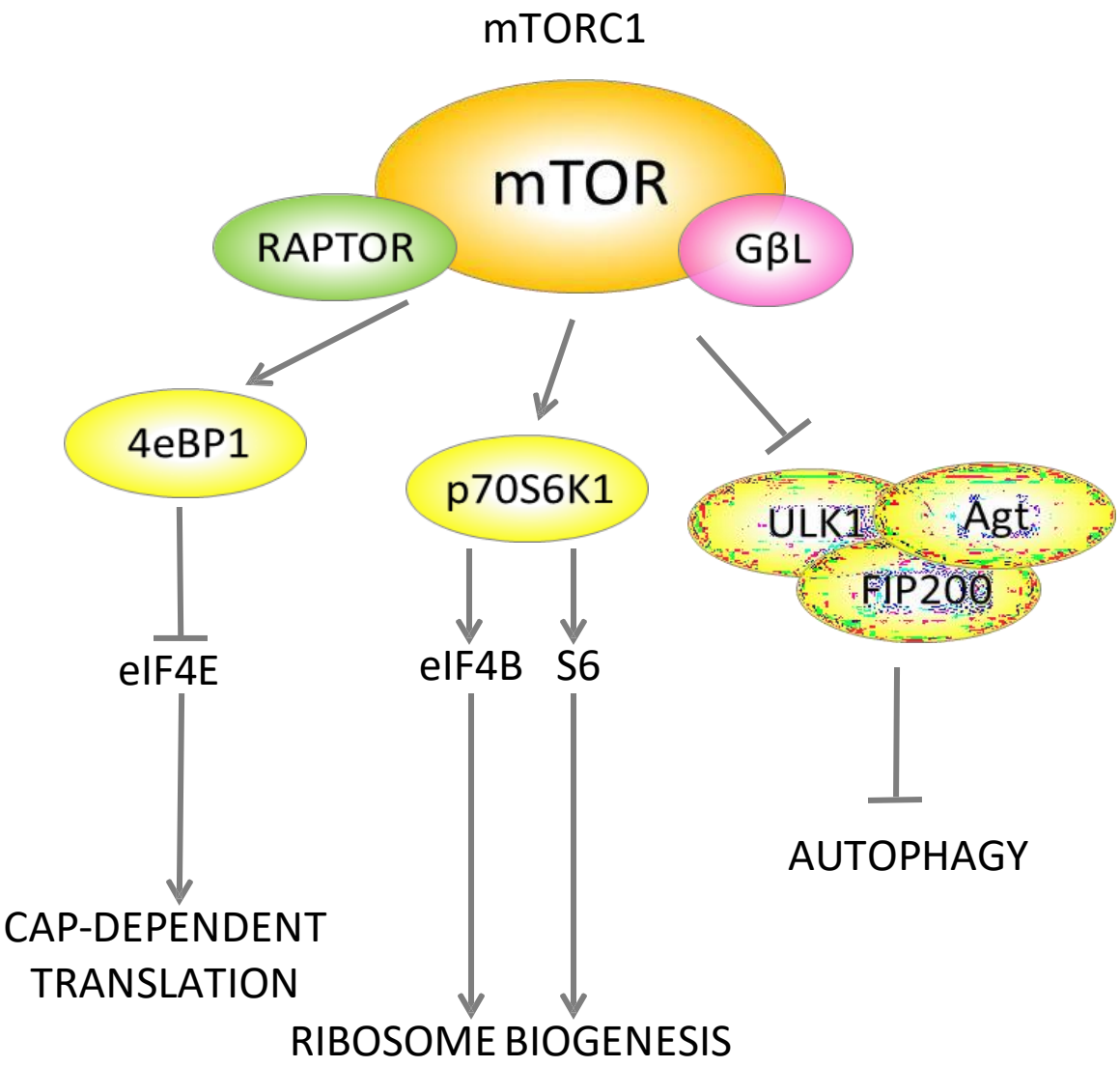
mTOR complex 2



DEPTOR (DEPDC6, DEP domain-containing protein 6)

✓ negative feedback loop

mTORC1 downstream



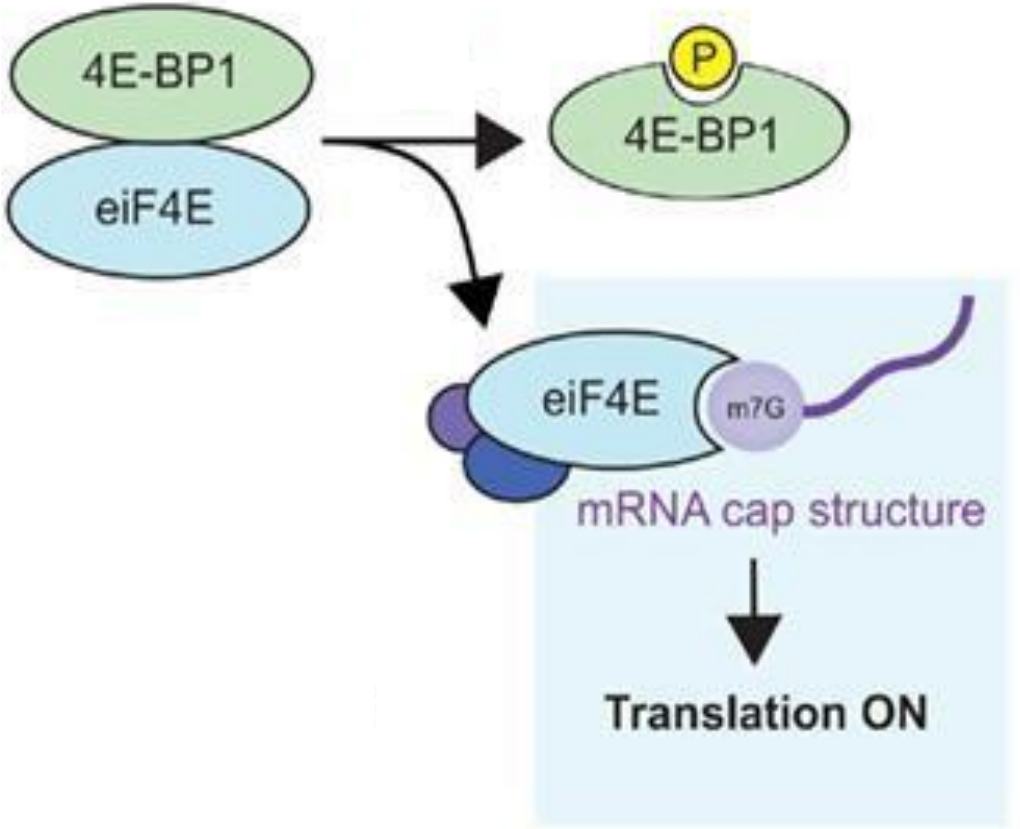
mTORC1 downstream

4eBP1

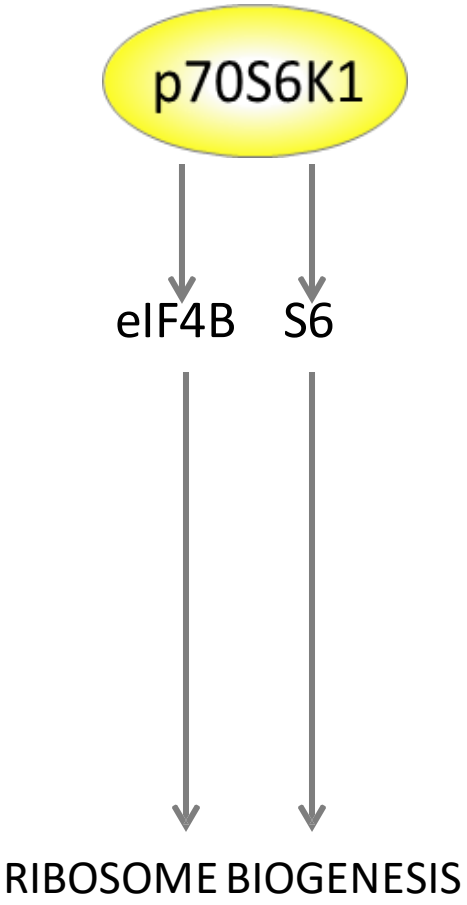
eIF4E

CAP-DEPENDENT TRANSLATION

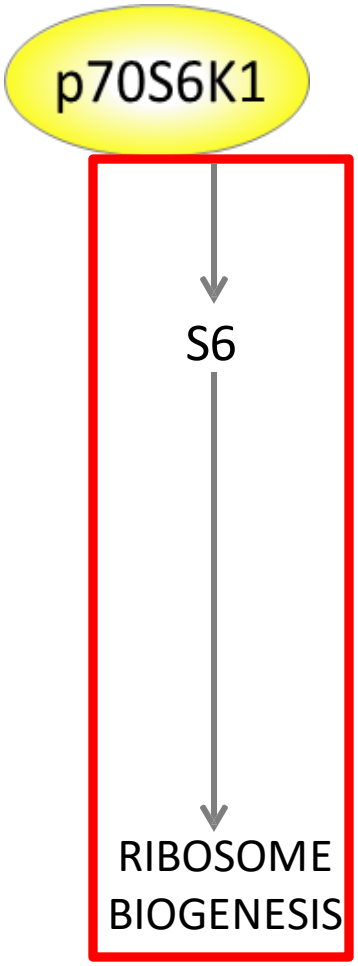
✓ Prevents cap-binding eIF4E → NO eIF4E complex



mTORC1 downstream



mTORC1 downstream

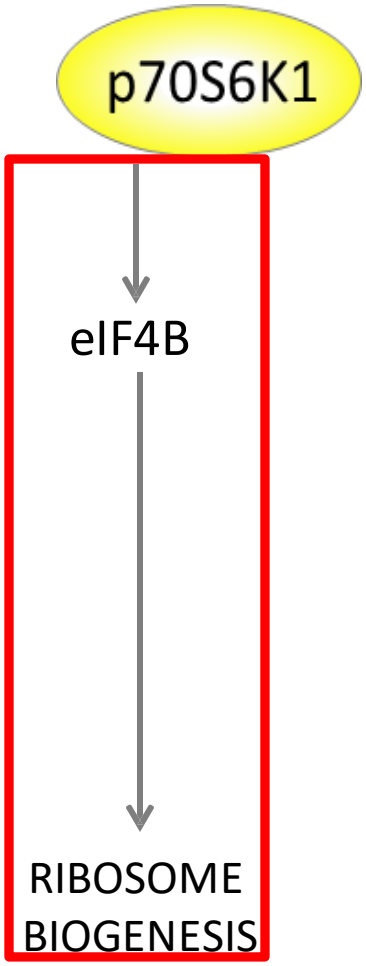


p70S6K1 promotes multi stage mRNA translation

Phosphorilation **S6**:

- ✓ binding SKAR → initiation/elongation through EJC (exon junction complex)
- ✓ phosphorylates the 40S ribosomal protein S6 (rpS6) → translation of mRNA with 5' terminal oligopyrimidine tract (TOP mRNAs)
- ✓ phosphorylates PDCD4 (programmed cell death 4), an inhibitor of RNA helicase eIF4A → enhances eIF4A helicase activity and facilitates 40S ribosomal subunit scanning to the initiation codon

mTORC1 downstream

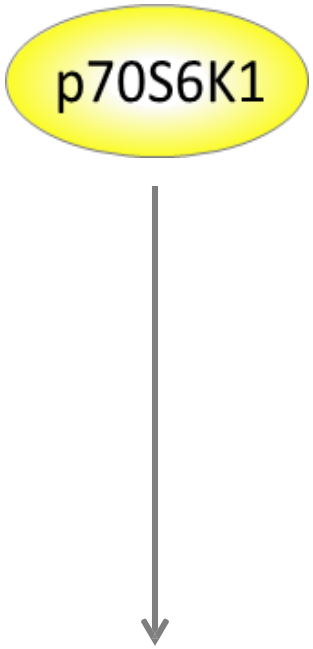


p70S6K1 promotes multistage mRNA translation

Phosphorilation **eIF4B**:

- ✓ eIF4A and eIF-4B increasing levels → eIF3 complex (largest scaffolding initiation factor; controls the assembly of 40S ribosomal subunit on mRNA)

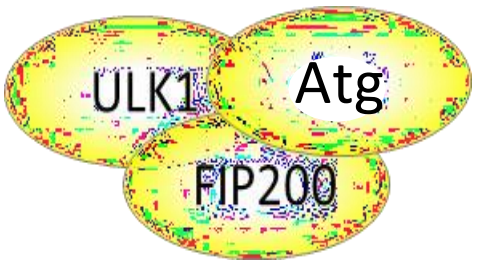
mTORC1 downstream



p70S6K1 regulates ribosome biogenesis at different levels

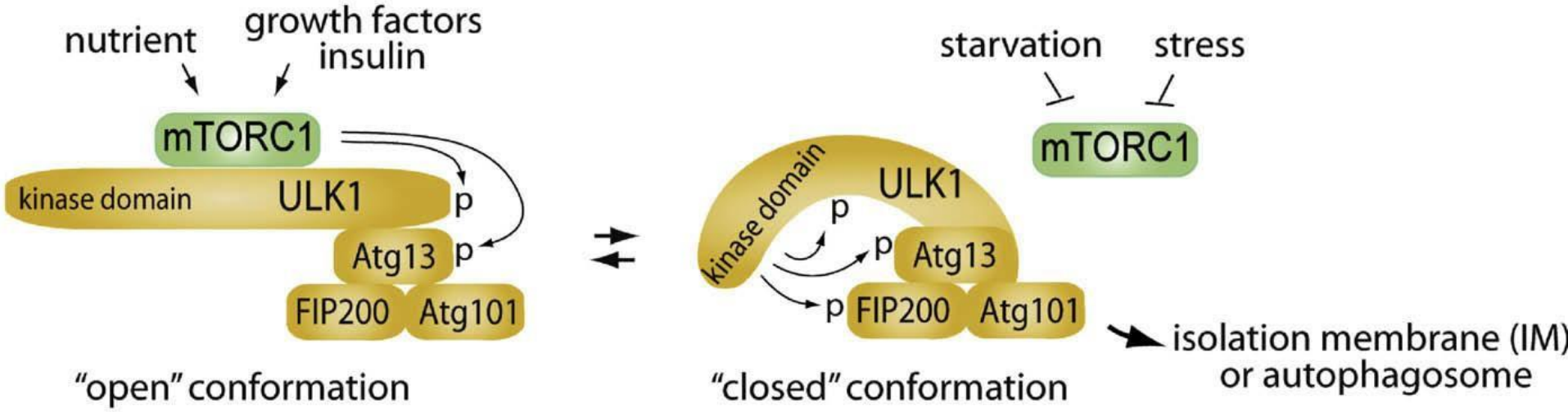
- ✓ biosynthesis of the translational machinery
- ✓ production of ribosomal proteins, pre-rRNA processing and rRNA synthesis
- ✓ coordinates nuclear RNA polymerases, Pol I, Pol II and Pol III
- ✓ interacts with rDNA (ribosomal DNA) promoters to promote Pol I and Pol III transcription
- ✓ regulates the nuclear translocation of TIF1A, a transcription factor (essential for Pol I-associated transcription initiation)
- ✓ inhibits Maf1, a Pol III repressor, and so induces 5S rRNA and transfer RNA (tRNA) transcription

mTORC1 downstream

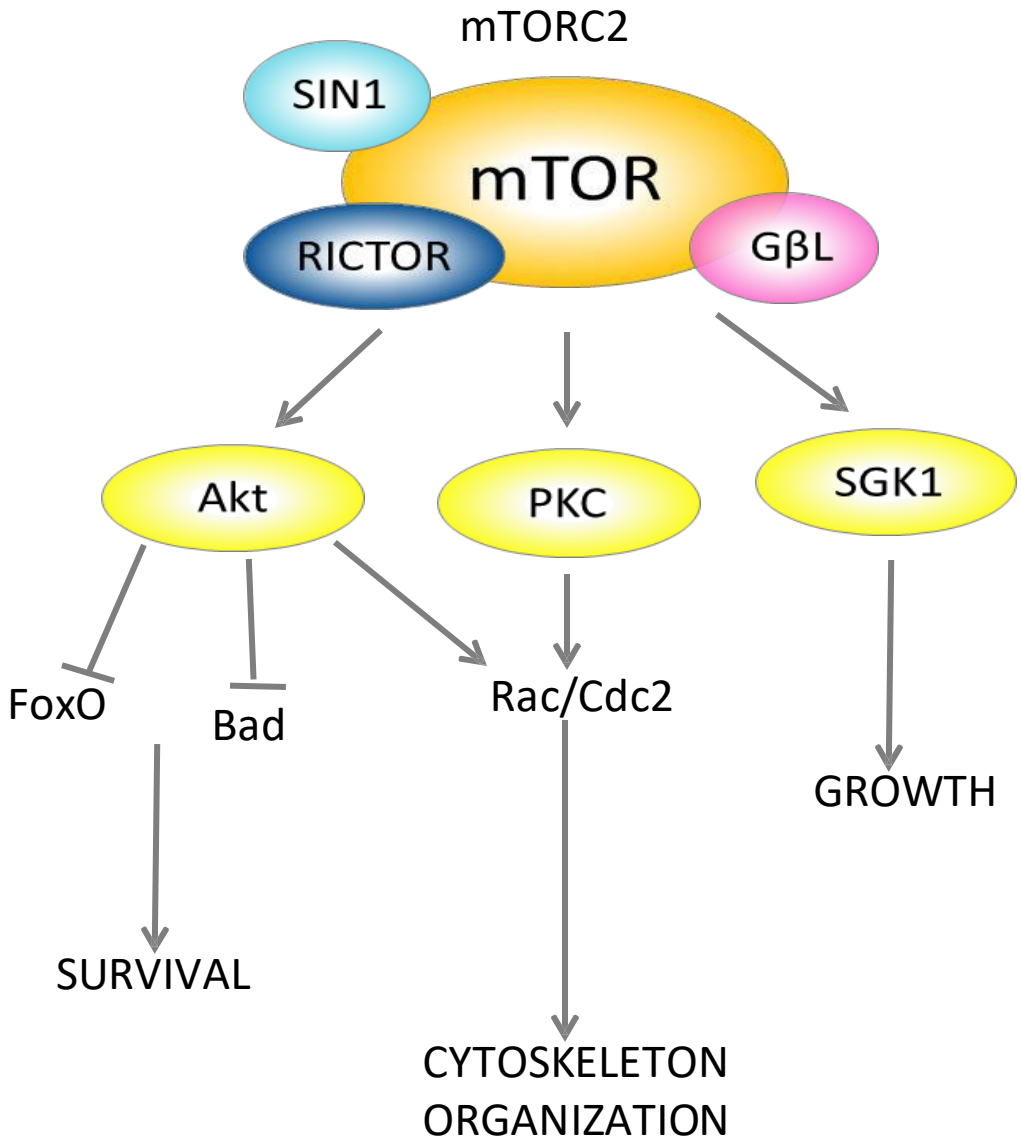


AUTOPHAGY

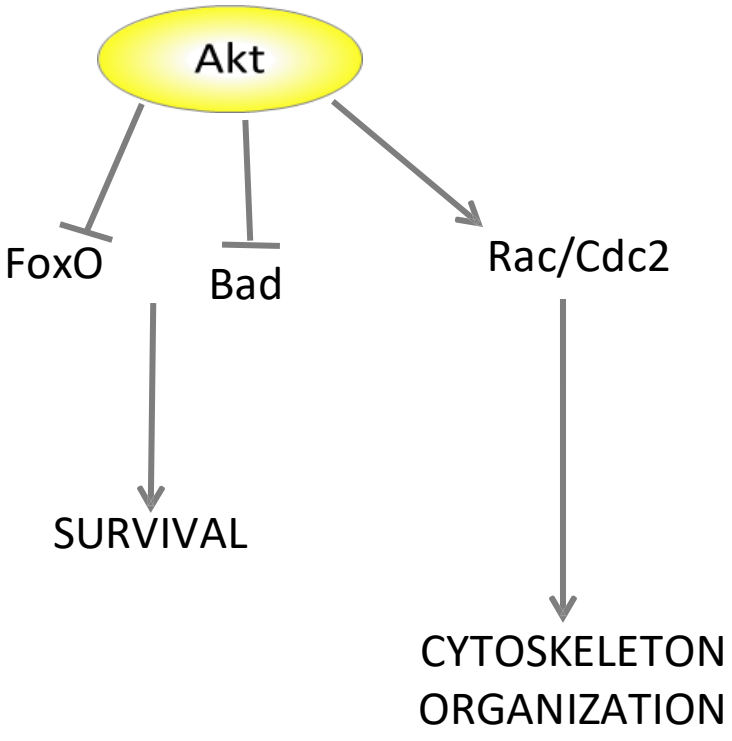
- ✓ recycling of damaged organelles and for the organismal and cellular adaptation to nutrient starvation
- ✓ phosphorylates and suppresses ULK1/Atg13/FIP200 (unc-51-like kinase 1/mammalian autophagy-related gene 13/focal adhesion kinase family-interacting protein of 200 kDa)
- ✓ «open» and «closed» conformations → regulate autophagosome activity



mTORC2 downstream



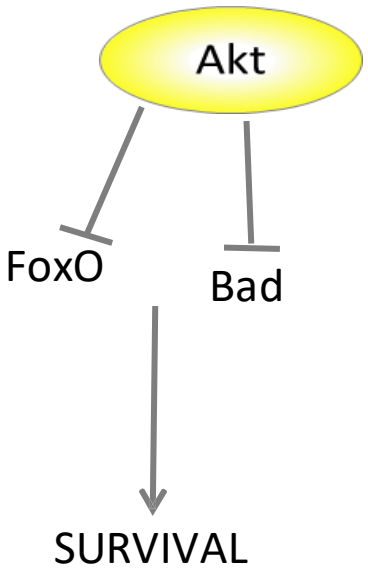
mTORC2 downstream



Protein kinase B (PKB)

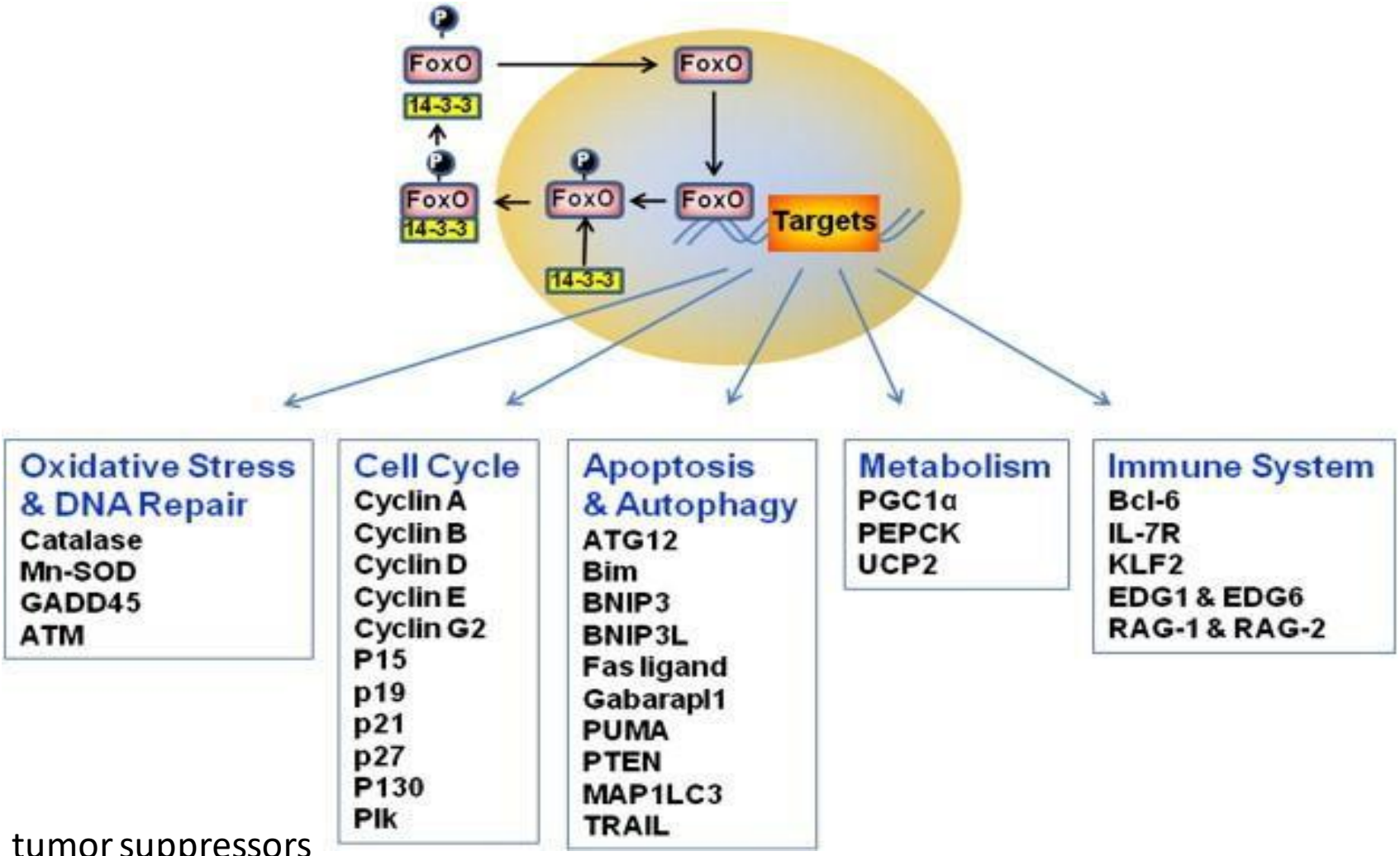
- ✓ serine-threonine kinase
- ✓ three isoforms are encoded by distinct loci → functional redundancy among Akt isoforms → lethal to development
- ✓ activation by receptor tyrosine kinases, cytokine receptors, G-protein coupled receptors, B and T cell receptors and integrins
- ✓ insulin metabolism and cancer progression
- ✓ drugs resistance

mTORC2 downstream



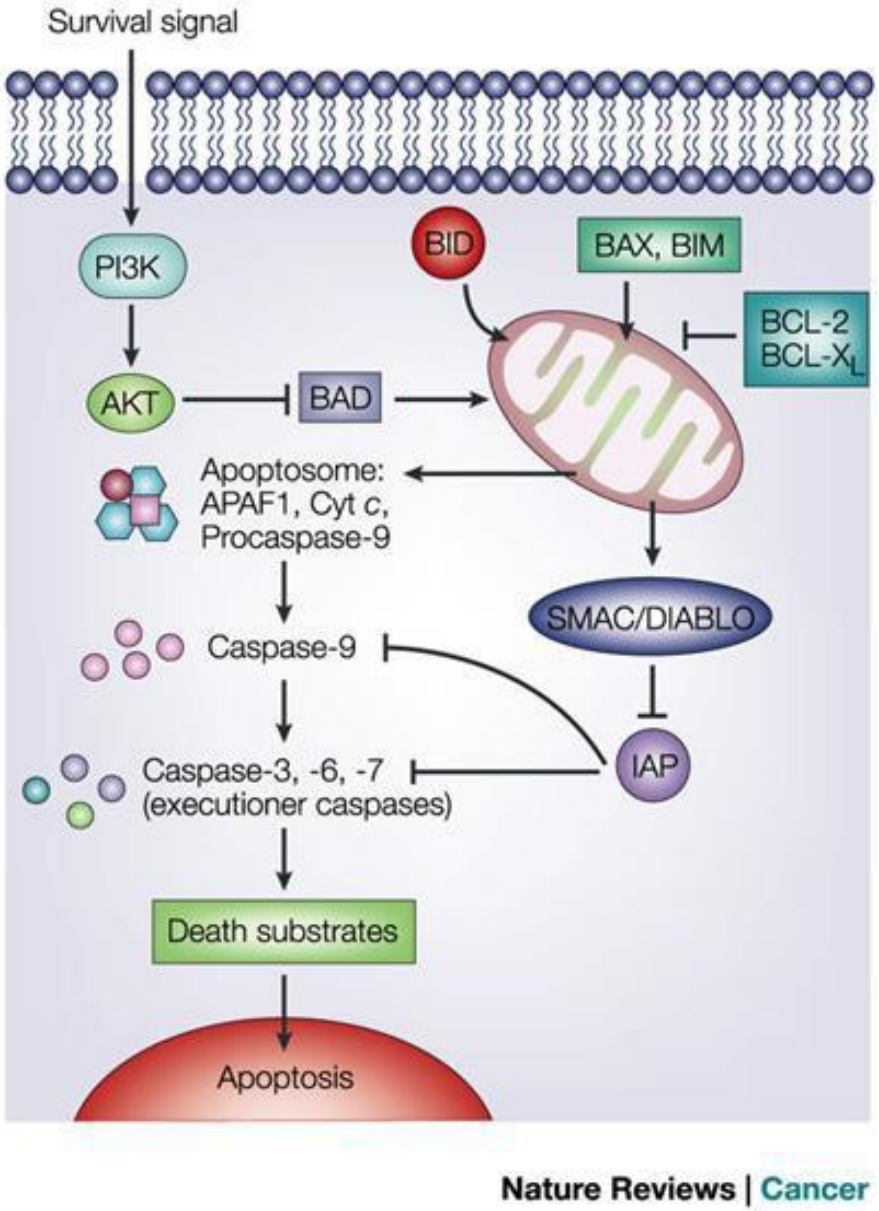
- ✓ promotes cytoplasmic localization of CKIs ($p21^{WAF1/CIP1}$ and $p27^{KIP1}$) → inhibiting their function
- ✓ stabilizes cyclin D1 e D3 levels→ progression through the G1 phase of the cell cycle
- ✓ facilitates MDM2 nuclear localization → inhibitory action on p53
- ✓ inhibits (phosphorylation) of pro-apoptotic signals (Bad and FoxO) → anti-apoptotic effets

FoxO functions



- ✓ tumor suppressors
- ✓ nuclear localization of FoxOs suspends cell cycle progression, promotes apoptosis and negatively regulates angiogenesis

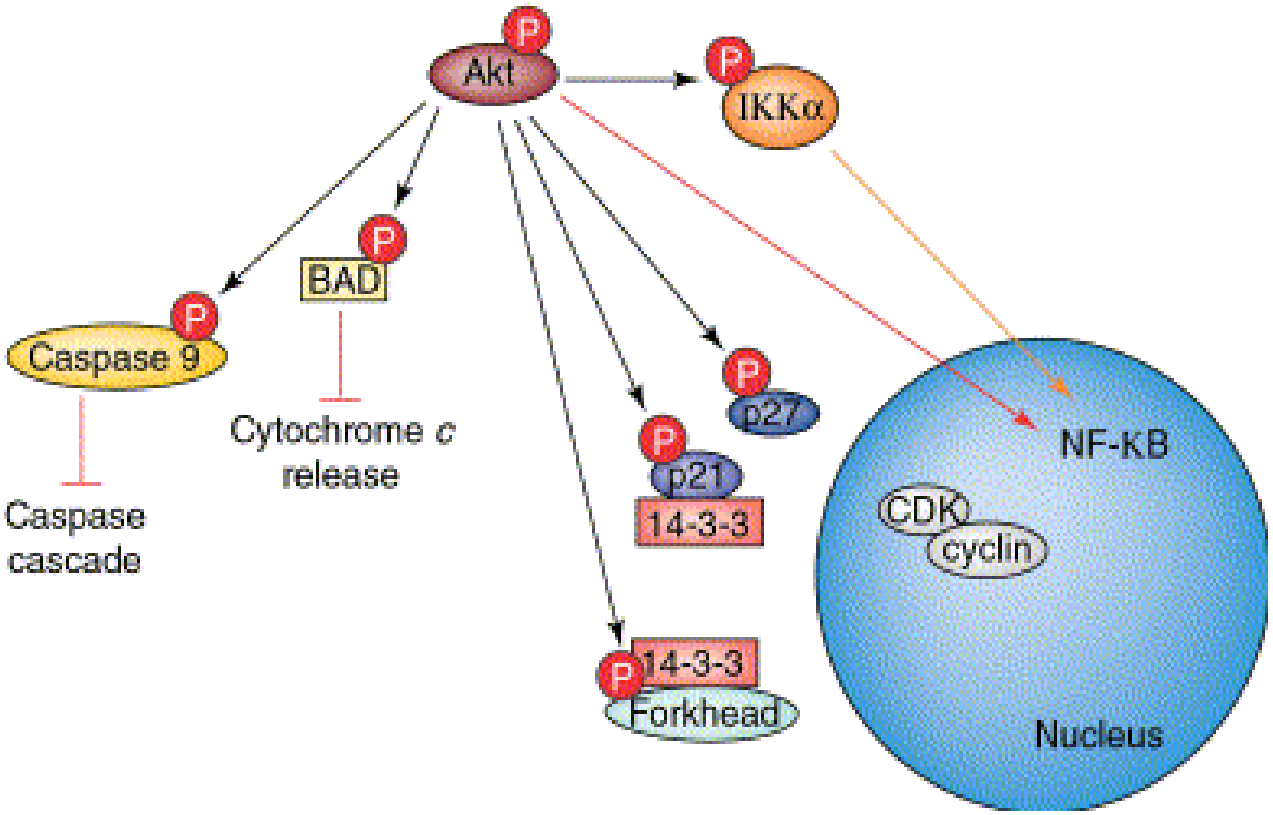
Bad functions



Bcl-2-associated death promoter

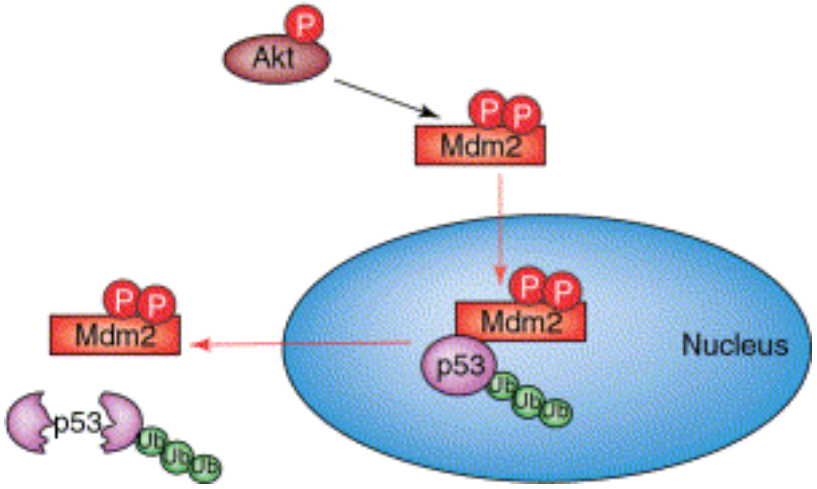
- ✓ member of BCL-2 family
- ✓ inhibits anti-apoptotic BCL-2-family members (BCL-x and Bcl-2)
- ✓ allows two pro-apoptotic proteins (BAK and BAX)
- ✓ induce release of cytochrome c → caspase activation → apoptosis

Akt functions



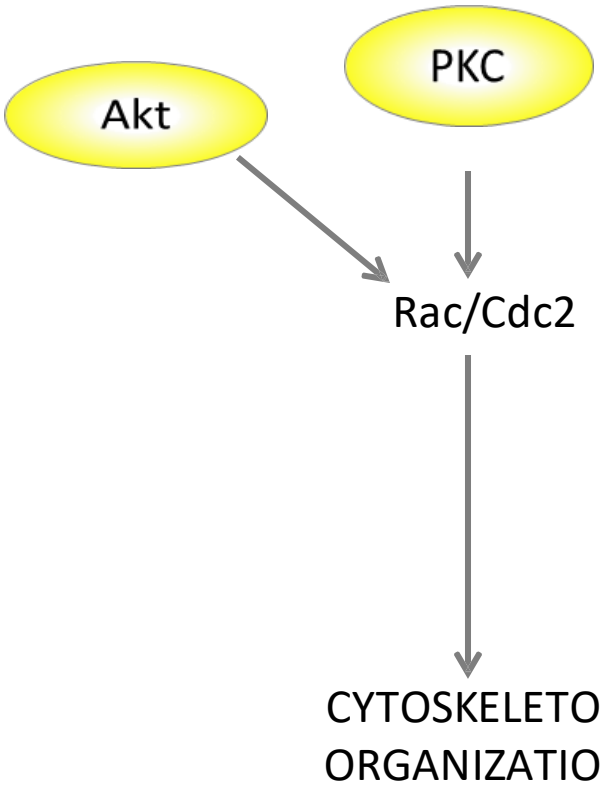
- ✓ **Anti apoptotic effects and proliferation effects through inhibition of BAD and FoxO and activation of ciclins involved in cell cicle progression**

Akt functions



✓ **Anti apoptotic effects through activation of Mdm2**

mTORC2 downstream



Protein kinase C

- ✓ involved in controlling of other proteins
- ✓ phosphorylation of hydroxyl groups of serine and threonine amino acid residues on proteins

Rac/Cdc2

- ✓ Rho family of small G proteins (21-25 kDa) belong to the Ras superfamily
- ✓ regulators of actin reorganization
- ✓ involved in chemoattractant gradient sensing

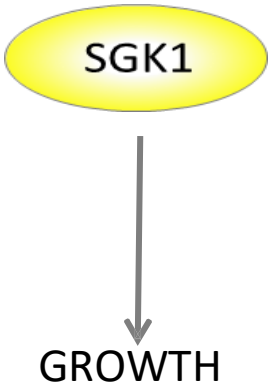
Rac

- ✓ induces membrane ruffling and extension of lamellipodia

Cdc42

- ✓ induces the extension of membrane protrusions

mTORC2 downstream



Serum/glucocorticoid regulated kinase 1

- ✓ serine/threonine kinases
- ✓ role in cellular stress response
- ✓ promoting cell survival by phosphorylating and inactivating FOXO3a → inhibits apoptosis

Proto-oncogenes linked to mTOR pathway

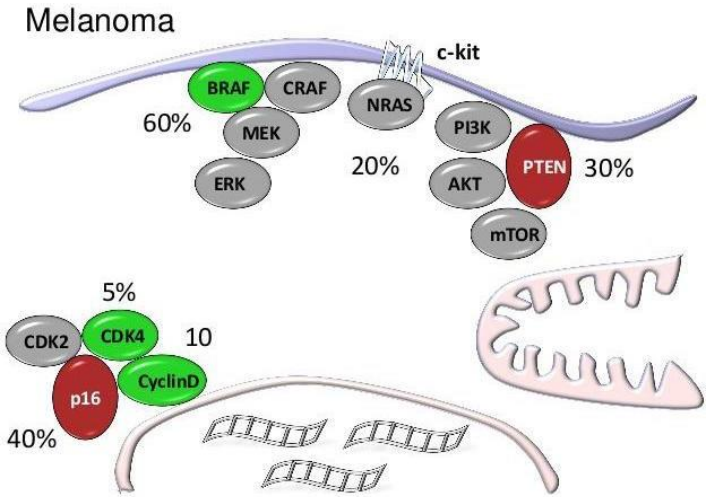
Proto-oncogenes	Alterations described
AKT	*AKT* is amplified in a subset of human cancers, such as breast and ovarian cancers.
4EBP1	4EBP1 expression was found to be associated with poor prognosis in several human tumours, such as breast, colon, ovarian and prostate cancers. The phosphorylation of 4EBP1 was also found to be associated with chemoresistance in ovarian cancer.
eIF4E	Ectopic overexpression of eIF4E can transform cells *ex vivo* and *in vivo*. eIF4E is overexpressed in many human tumours, such as breast, colon, and head and neck cancers, non-Hodgkin's lymphomas, and chronic and acute myelogenous leukemias.
PI3K	High *PI3K* activity was implicated in cell transformation and tumour progression and described in several human cancers, such as ovarian, gastrointestinal, breast and prostate cancers.
Rheb	Rheb overexpression is described in many tumour cells, and Rheb upregulation is critical for squamous carcinoma and associates with poor prognosis in breast and head and neck cancers.
S6K1	S6K1 is overexpressed in in lung and ovary cancers and its expression correlates with poor prognosis in breast, kidney and hepatocellular carcinomas.

Tumor suppressor genes linked to mTOR pathway

Tumour suppressor genes	
LKB1	Individuals with mutations in *LKB1* develop Peutz-Jeghers syndrome, which includes the occurrence of gastrointestinal tract hamartomas.
PTEN	Loss of *PTEN* function has been described in a large proportion of advanced human cancers, such as melanoma, breast, prostate and renal cancers. Individuals with inherited mutations in *PTEN* develop hamartoma tumour syndromes (Cowden disease, Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, Lhermitte-Duclos disease) and are at higher risk for developing several cancers.
TSC1/TSC2	Patients with mutations in *TSC1* or *TSC2* develop tuberous sclerosis complex (TSC), a syndrome that includes the development of hamartomas in many organs. Mutations in *TSC2* may also lead to the development of Lymphangioleiomyomatosis (LAM).

mTOR and melanoma

- ✓ Loss of PTEN in 30-50% of melanomas
- ✓ Akt amplification in 60%
- ✓ In association with BRAF and NRAS mutations



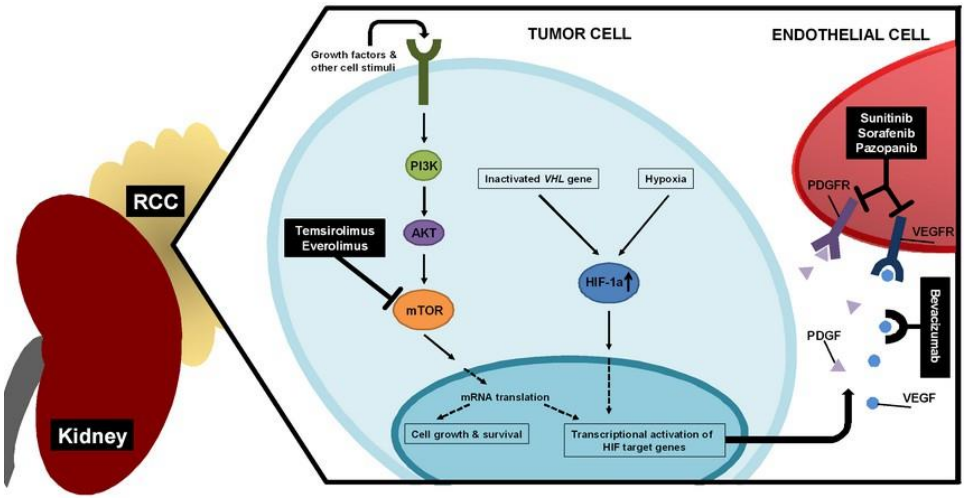
Increased pAKT, loss of PTEN, MAPK mutations correlates with **tumour progression** , **chemoresistance** and **shorter survival**

- ✓ Use of rapamycin increases apoptosis and chemosensitivity in melanoma cells
- ✓ anti-tumour effects enhanced in association with MAPK and PI3K inhibitor

mTOR and RCC

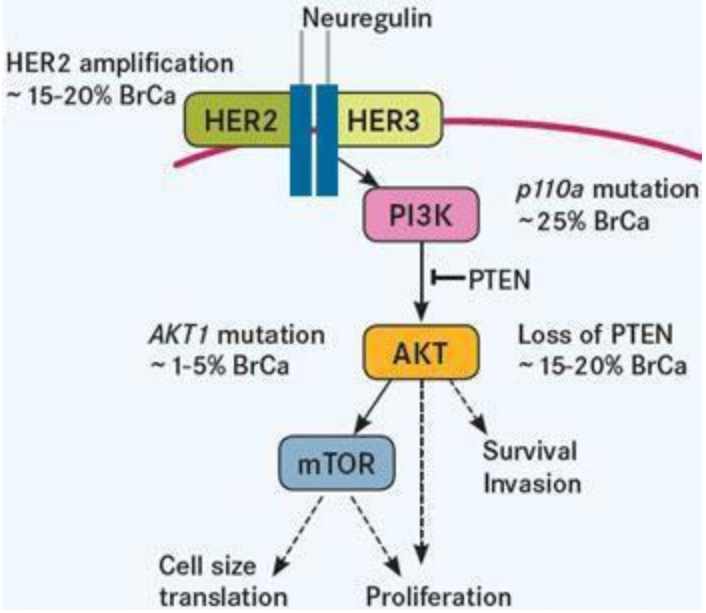
Iperactivation of mTOR pathway:

- ✓ overexpression or activation of growth factor receptors
- ✓ activation of mutations in PI3K/AKT
- ✓ decresed expression of tuberous sclerosis tumor suppressor genes TCS1/2 or Von Hippel-Lindau (VHL) tumor suppressore gene



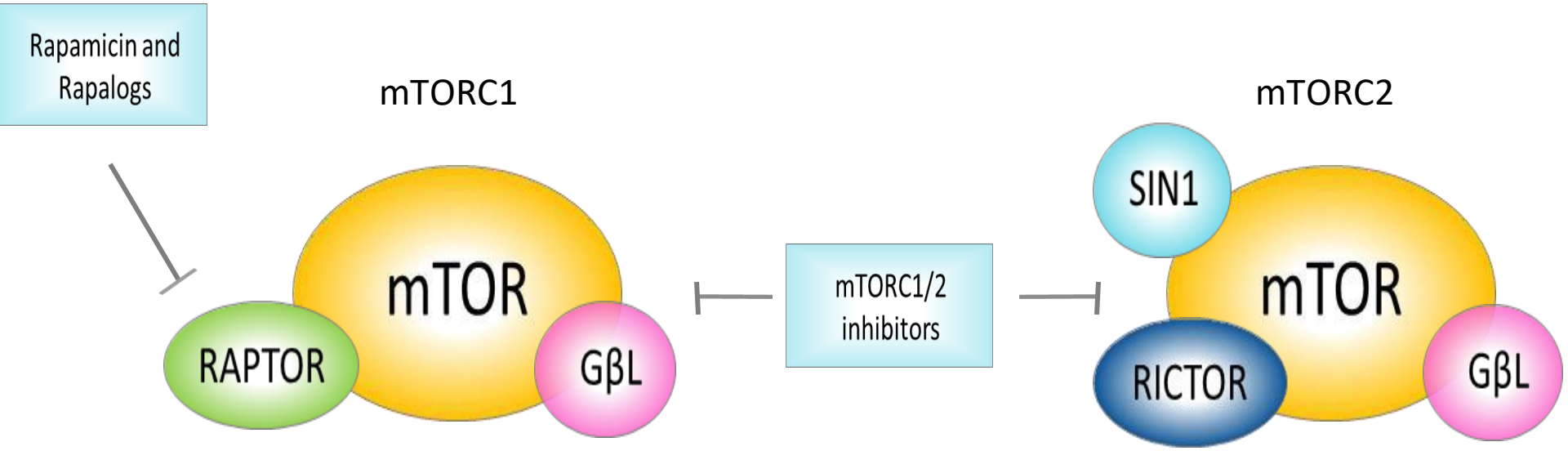
mTOR and breast cancer

- ✓ Associated with overexpression or activation of HER2
- ✓ Involved in resistance to endocrine therapy, human epidermal growth factor receptor 2 (HER2)-directed therapy (trastuzumab) and cytotoxic therapy
- ✓ Preclinical studies indicate that inhibitors of the pathway can act synergically with trastuzumab in resistance cells



Everolimus (rapamycin analog) is currently the only compound approved for the treatment of hormone receptor (HR)-positive, HER2-negative metastatic or locally advanced breast cancer

mTOR inhibitors



mTOR inhibitors

Type	Drug	IC_{50} mTORC1	IC_{50} PI3K
Rapalog	Rapamycin/sirolimus	0.4–0.9 nM	N/A
	Everolimus	1.8–2.6 nM	N/A
mTOR kinase inhibitors	Torin 1	0.29 nM	250 nM
	Torin 2	2.1 nM	4.68 nM
	PP30	80 nM	3 uM
	PP242	8 nM	1.96 μM
	OSI-027	22 nM	1.3 μM
	AZD8055	0.8 nM	3,590 nM
	KU-0063794	10 nM	>10 μM
	WYE-125132	0.19 nM	1,179 nM
Dual mTOR/PI3K	NVP-BEZ235	20.7 nM	4 nM
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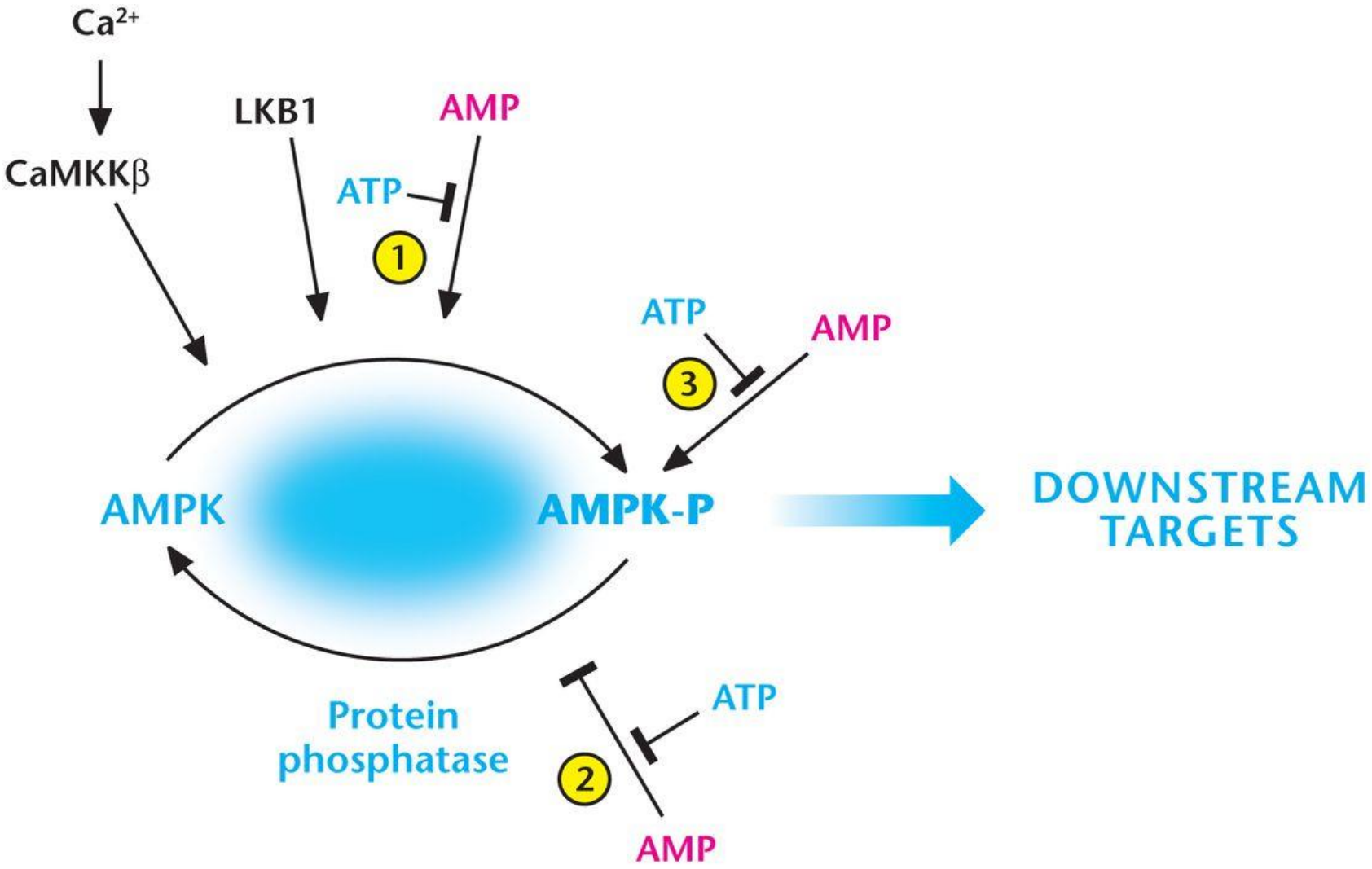
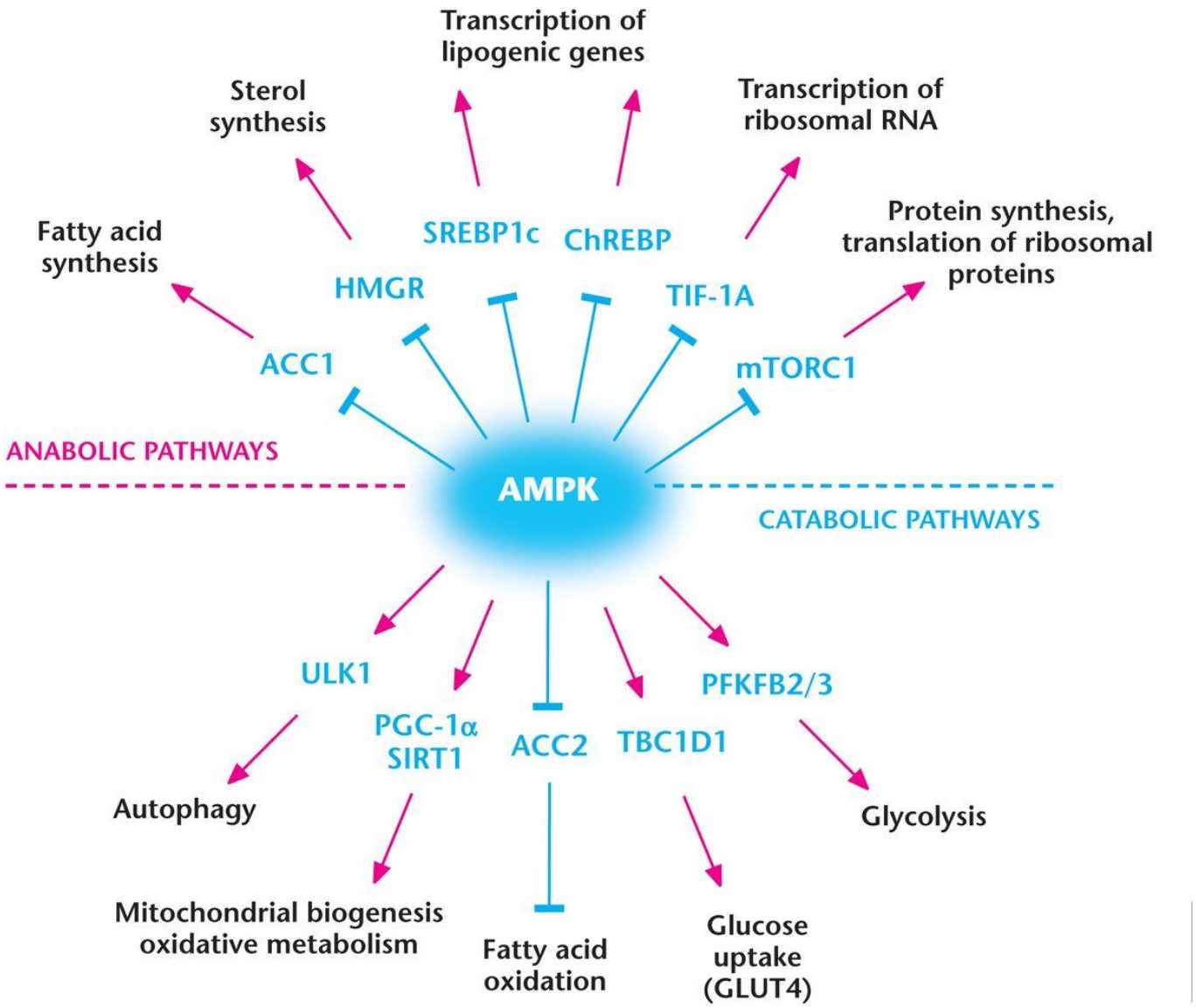
mTOR inhibitors

mTOR inhibitors	Origination	Development status	Potential use for the tumor types	Action mechanism
First generation of mTOR inhibitors				
Rapamycin	Wyeth, USA	FDA approved	(Renal transplantation)	Bind to the intracellular receptor FKBP12, and the rapamycin/FKBP12 complex then binds to the FKBP-rapamycin binding (FRB) domain of mTOR kinase
Temsirolimus (CCI-779)	Wyeth, USA	FDA approved	Renal cell carcinoma	
Everolimus (RAD001)	Novartis, Switzerland	FDA approved	Advanced kidney cancer and progressive or metastatic pancreatic neuroendocrine tumors	
Deforolimus (AP23573)	ARIAD, USA	FDA approved	Designated by the FDA as an orphan drug for treatment of soft-tissue and bone sarcomas	
Nab-rapamycin (ABI 009)	Abraxis BioScience, USA	Phase I	Breast cancer, colon cancer	
Second generation of mTOR inhibitors				
mTOR and PI3K dual-specificity inhibitors				
PI-103	Merck, Germany	Preclinical	Acute myeloid leukemia, glioblastoma, melaloma	Target the ATP binding sites of mTOR and PI3K
NVP-BEZ235	Novartis, Switzerland	Phase I/II	Breast cancer, multiple myeloma, glioblastoma, sarcoma, pancreatic cancer	
WJD008	Chinese Academy of Sciences, China	Preclinical	Breast cancer, colon cancer, prostate cancer, glioblastoma, lung cancer	
XL765	Exelixis, USA	Phase I/II	Breast cancer, lung cancer, ovarian cancer, prostate cancer, gliomas	
SF-1126	Semafore, USA	Phase I	Gastrointestinal stromal tumor, colorectal cancer, ovarian cancer, breast cancer, prostate cancer, haematological cancer	
Selective mTORC1/2 inhibitors				
Torin1	Gray Laboratory, Harvard, USA	Preclinical	–	Target the active site of mTOR in both mTORC1 and mTORC2
PP242	University of California, USA	Preclinical	Multiple myeloma, leukemia, breast cancer	
PP30	University of California, USA	Preclinical	–	
Ku-0063794	Kudos, UK	Preclinical	–	
WYE-354	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
WAY-600	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
WYE-687	Wyeth, USA	Preclinical	Breast cancer, prostate cancer, glioblastoma, colon cancer, renal cell carcinoma	
INK128	Intellikine, USA	Phase I	Multiple myeloma, breast cancer, prostate cancer, non-Hodgkin's lymphoma,	
AZD8055	AstraZeneca, UK	Phase I	Gliomas, breast cancer, renal cell carcinoma	
OSI-027	OSI, USA	Phase I	Lymphoma, colorectal cancer, melanoma, neuroendocrine tumors, endometrial cancer, renal cell carcinoma, cervical cancer	

AMP and calcium activate AMP-activated protein kinase (AMPK).



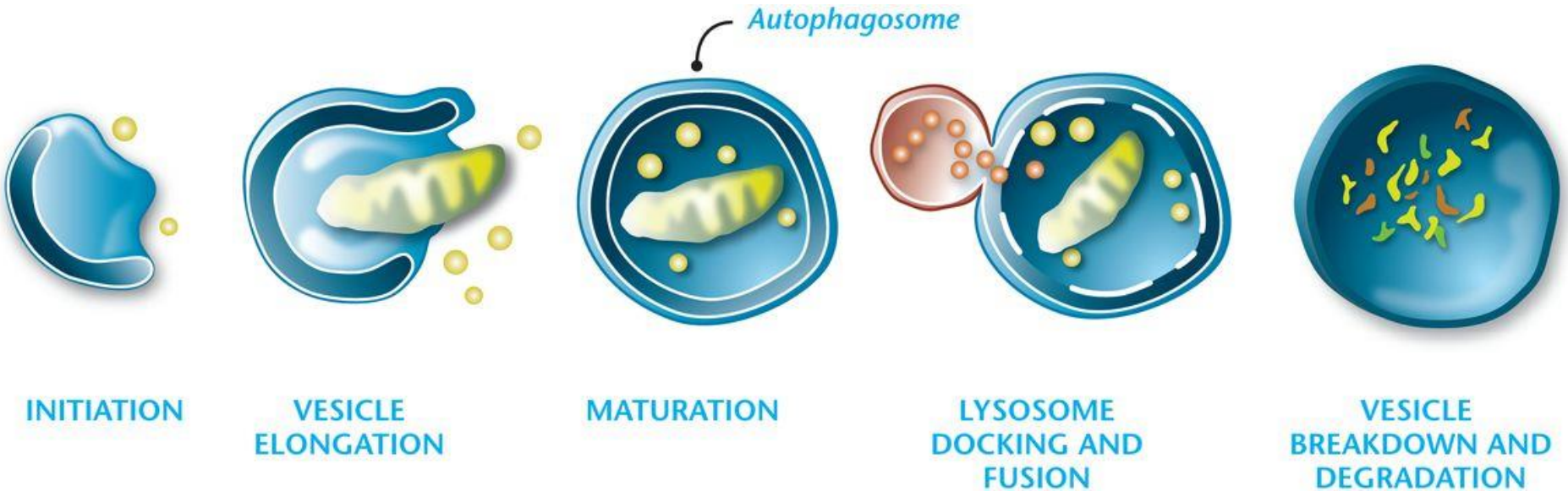
AMP-activated protein kinase (AMPK) regulates metabolism.



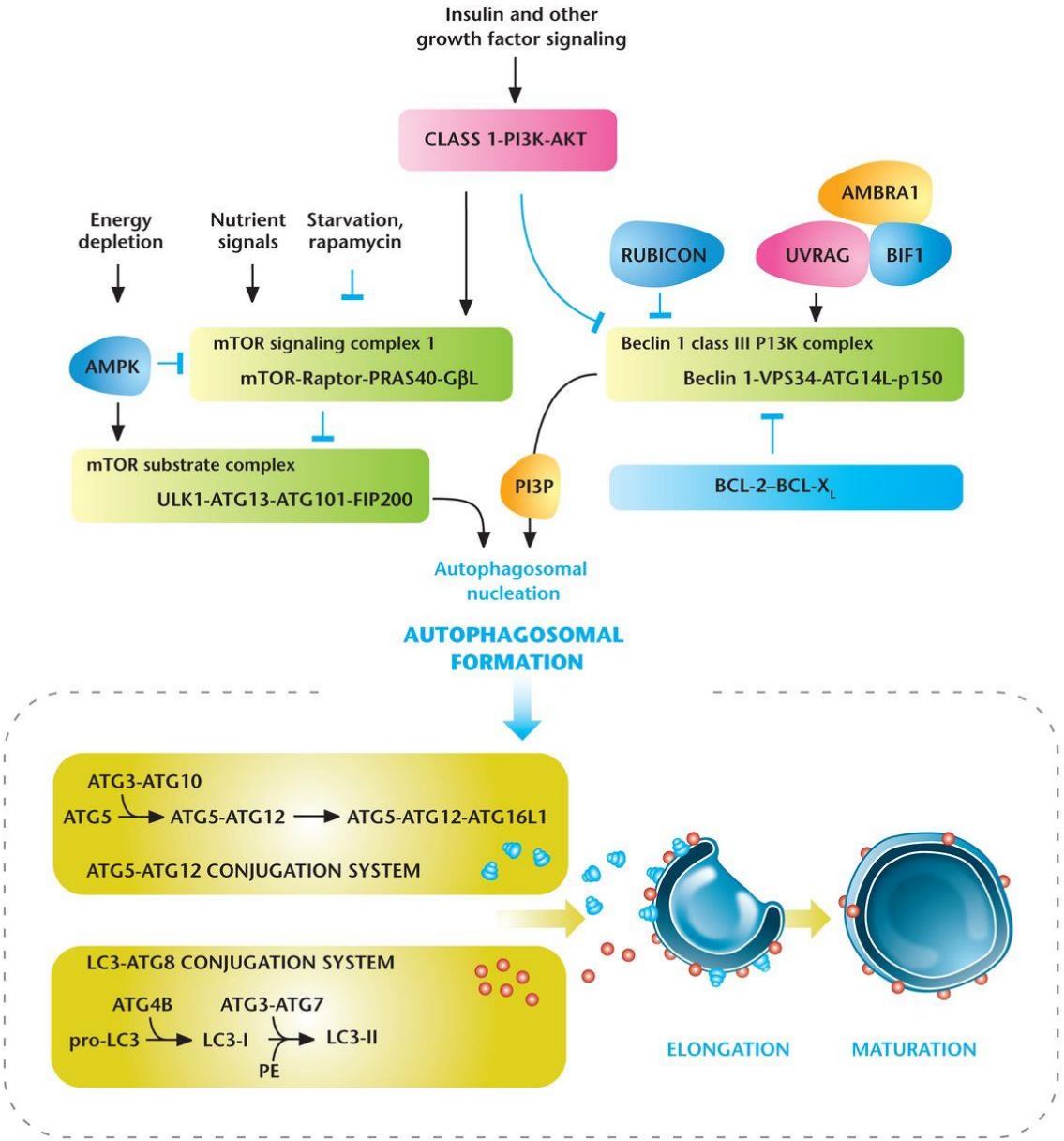
Overview of autophagy

There are three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA).

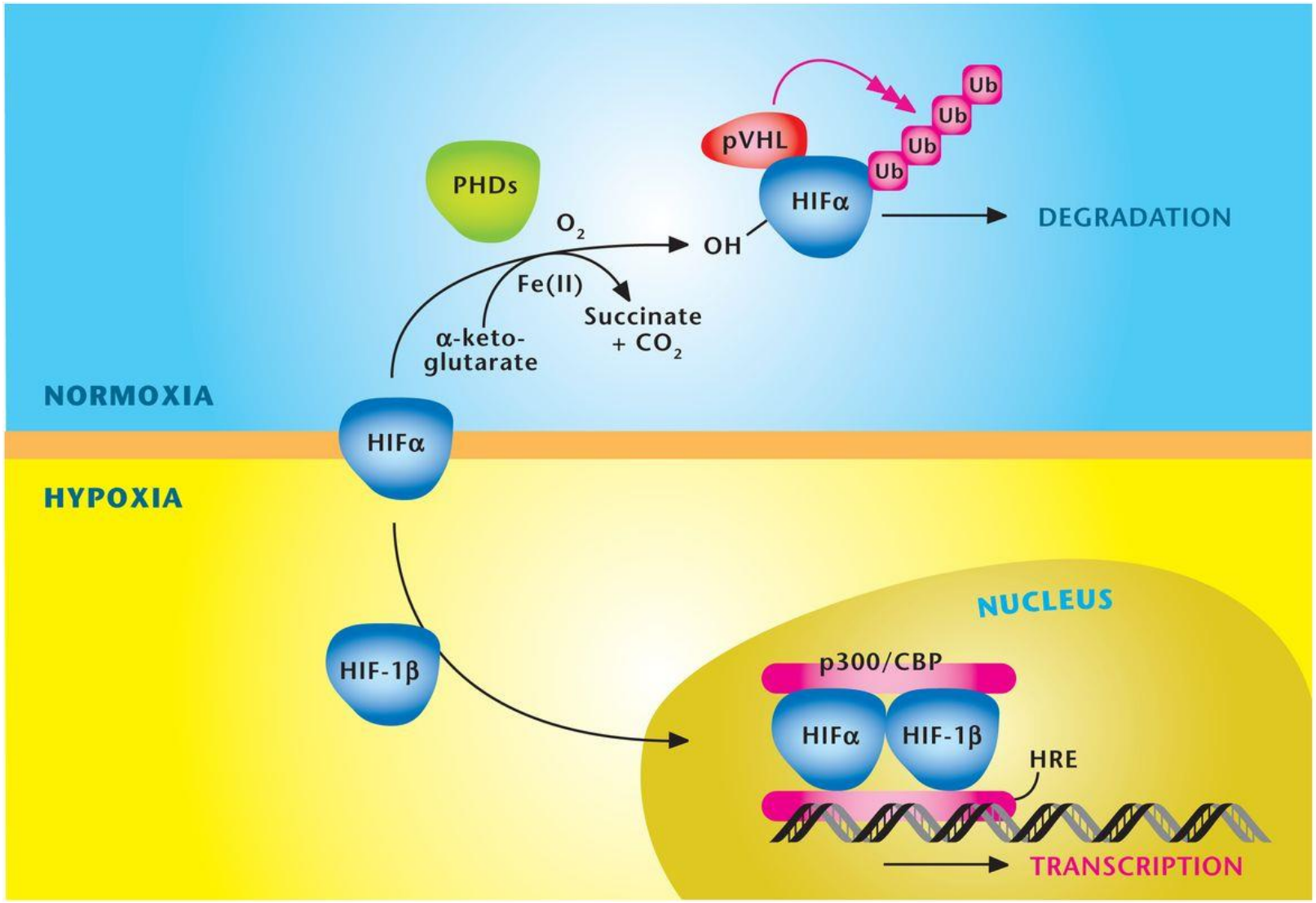
Macroautophagy pathway can be broken down into several discrete phases:



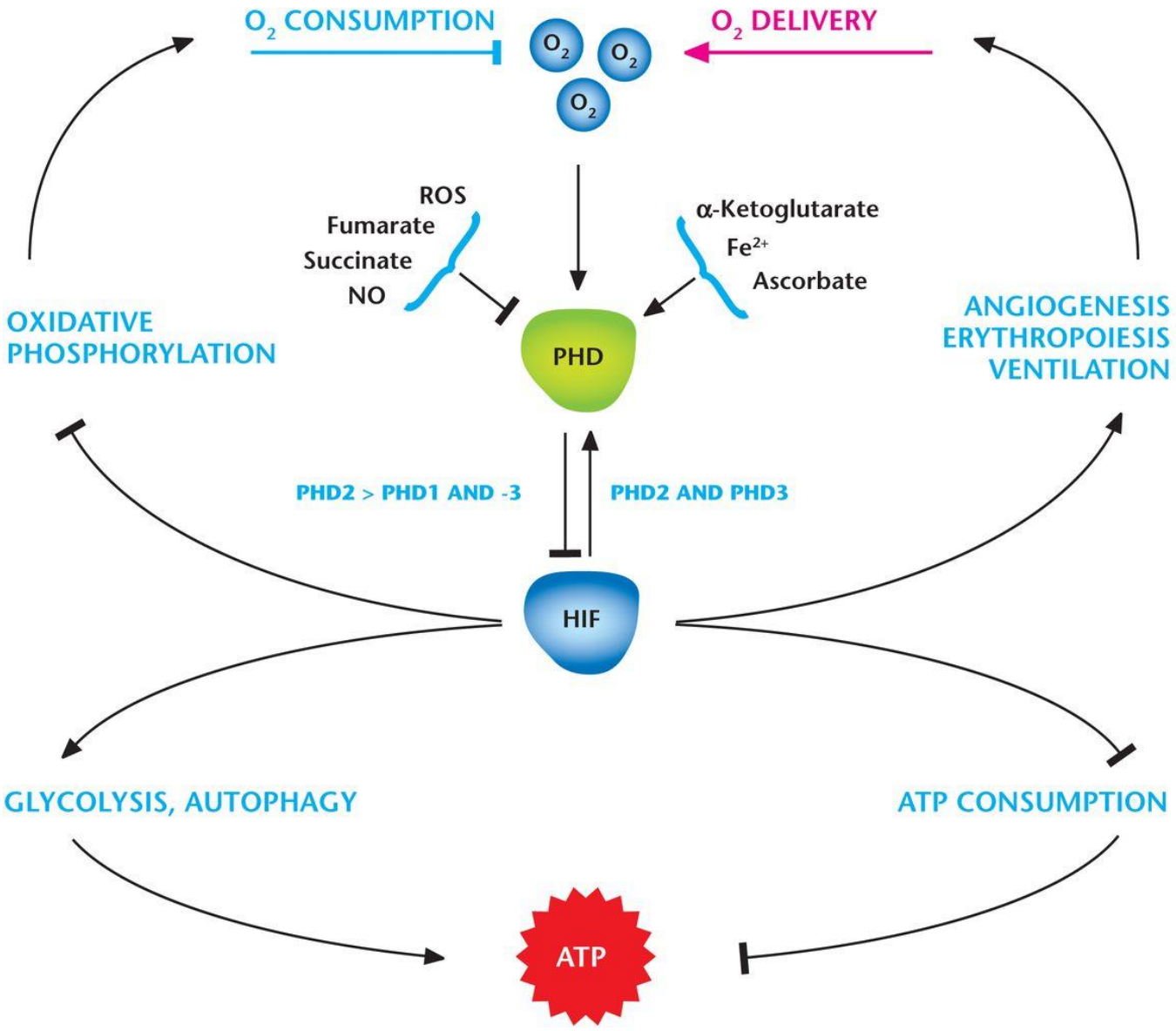
Regulators of autophagy.



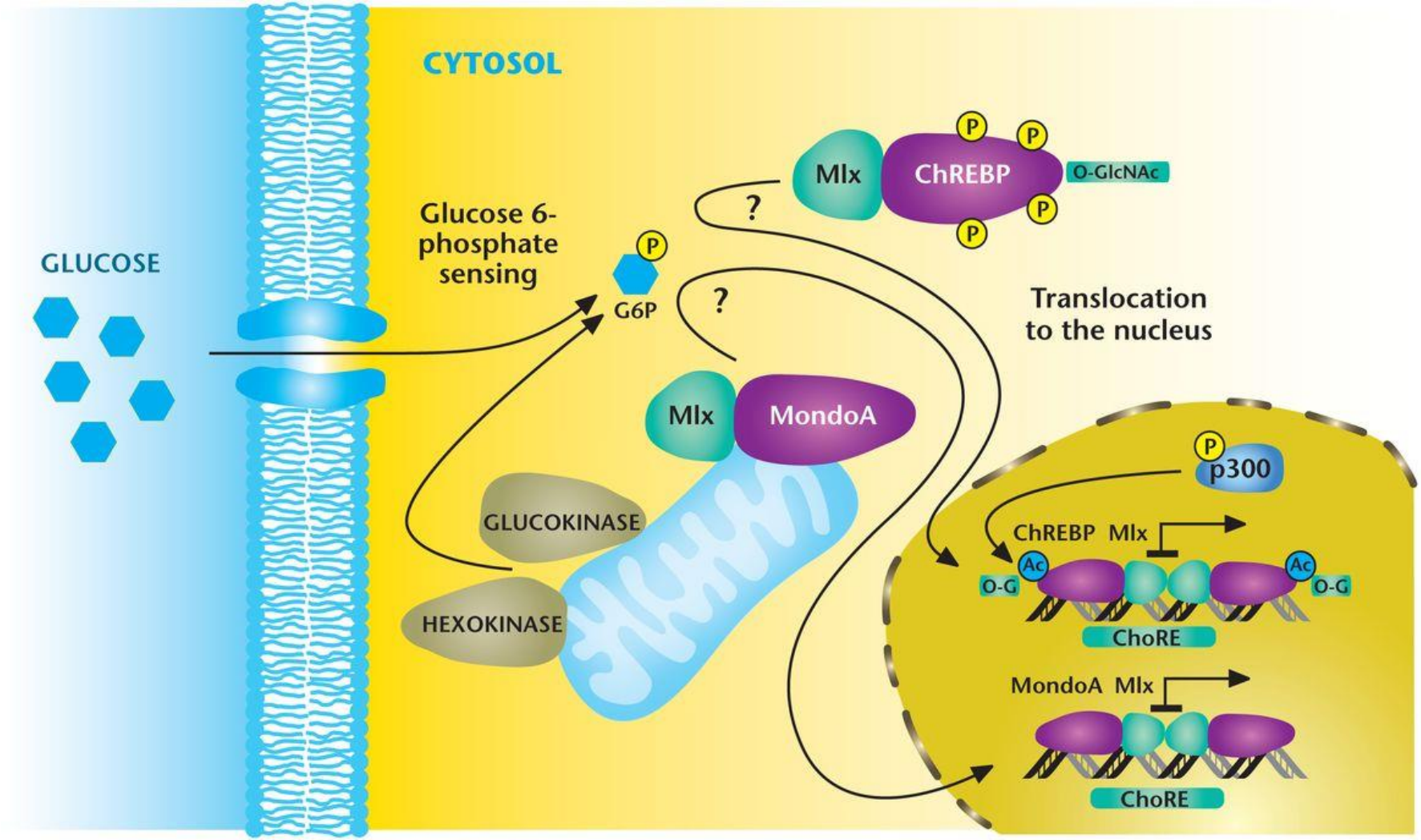
Oxygen levels control stabilization of HIFs. HIFs are a heterodimer, consisting of a constitutively stable HIF-1β and an oxygen-sensitive HIFα subunit.



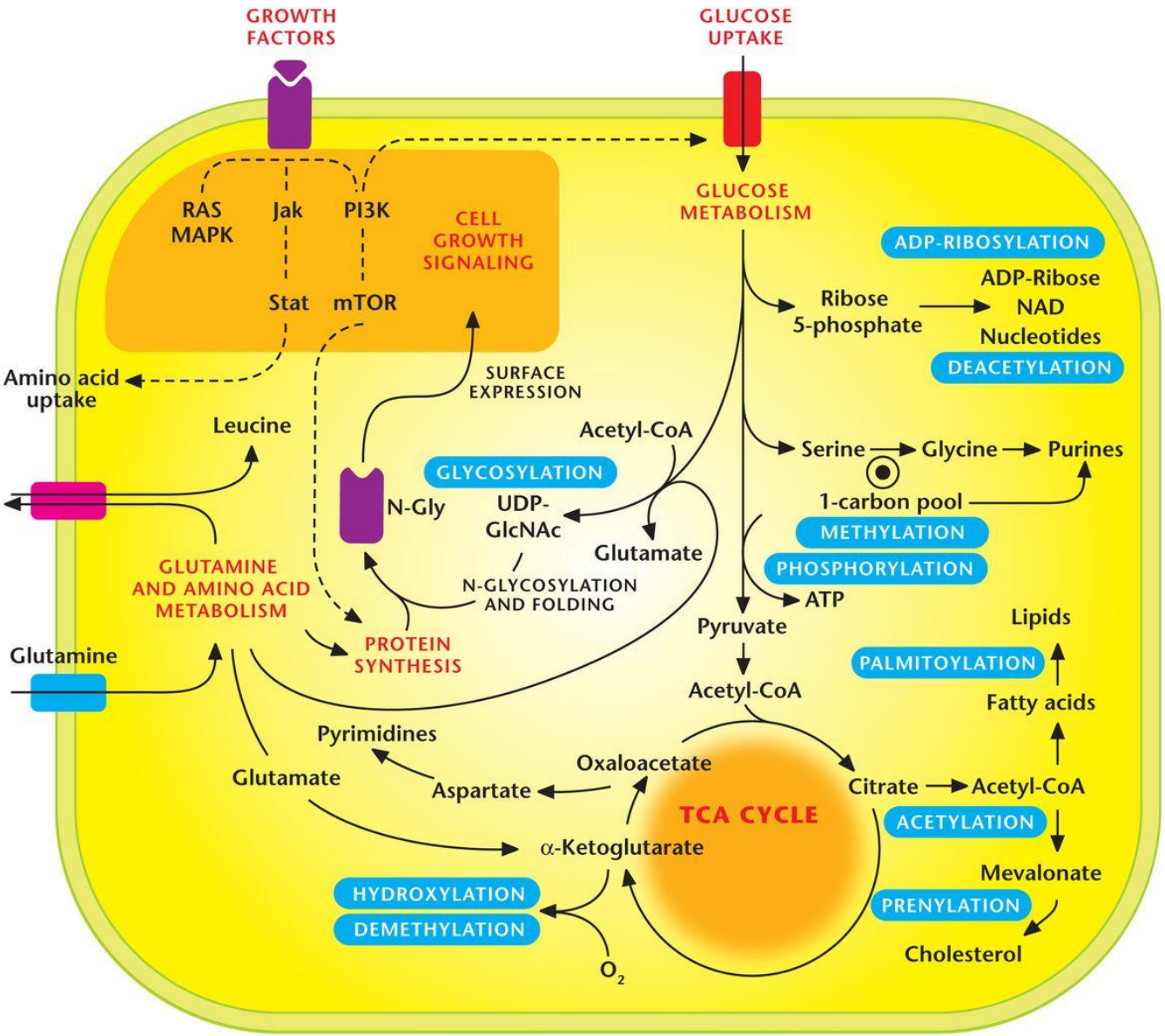
HIFs regulate adaptation to hypoxia.



Mondo transcription factors respond to glucose flux.

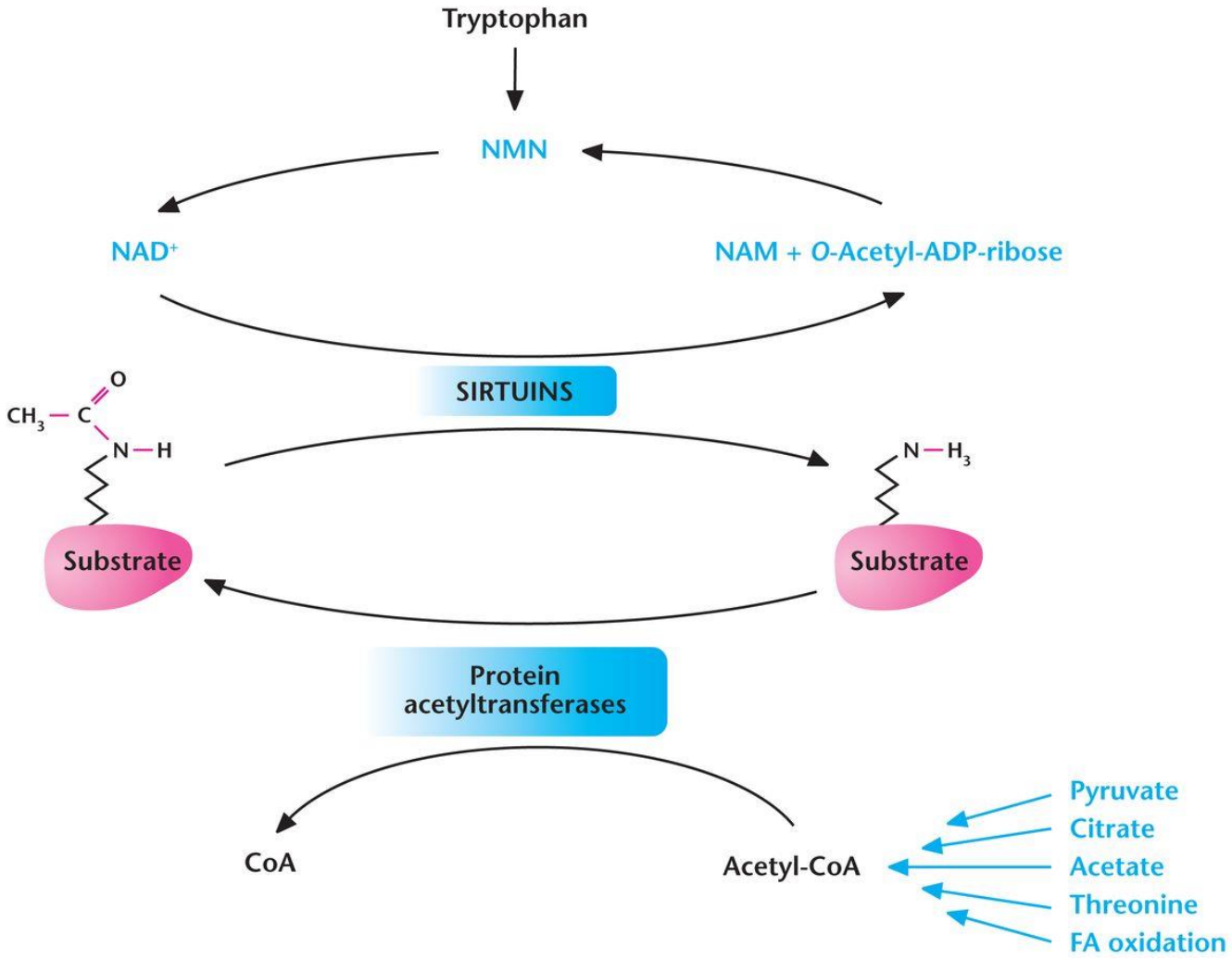


Metabolic pathways regulate signaling pathways.

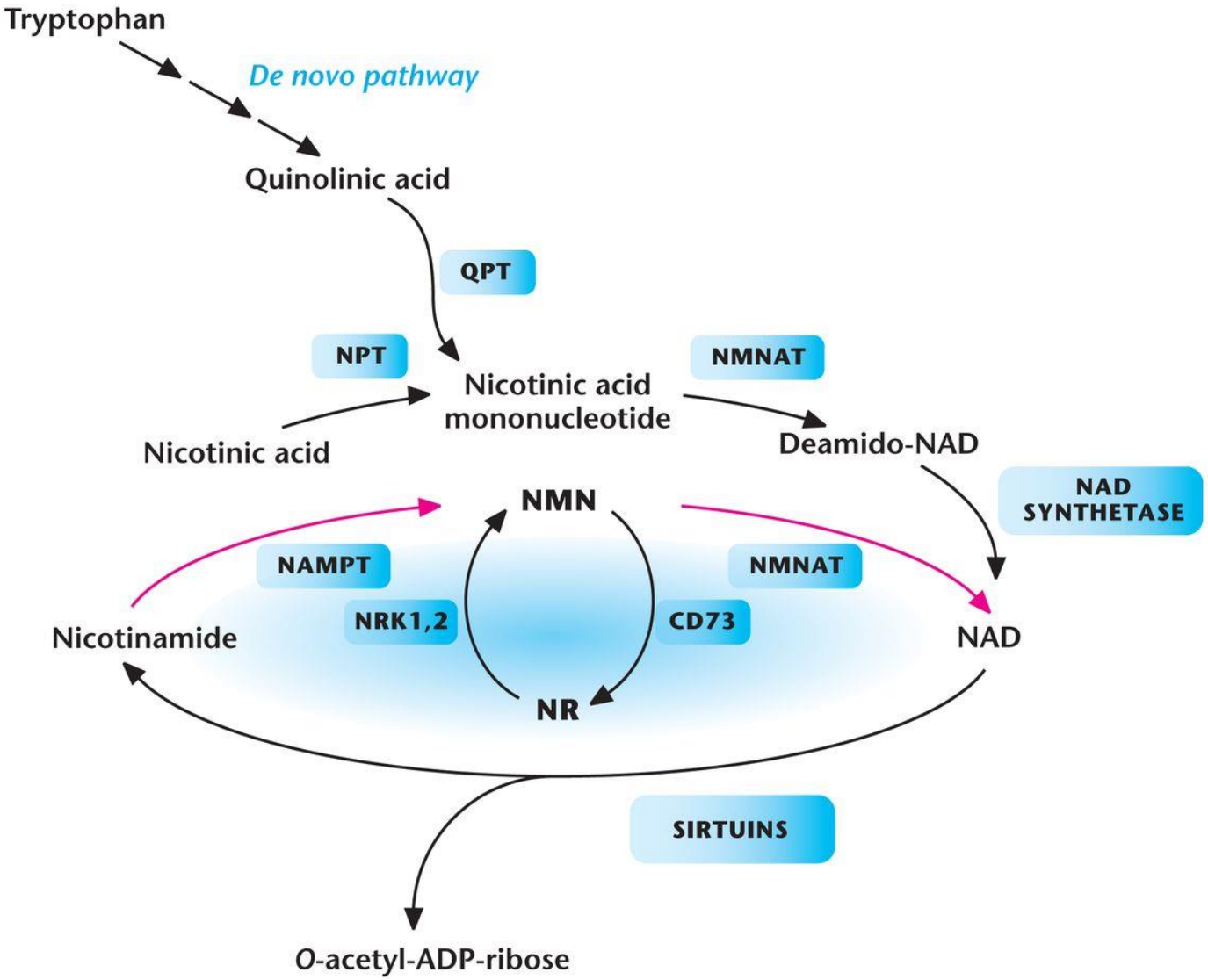


Metabolism regulates acetylation and deacetylation.

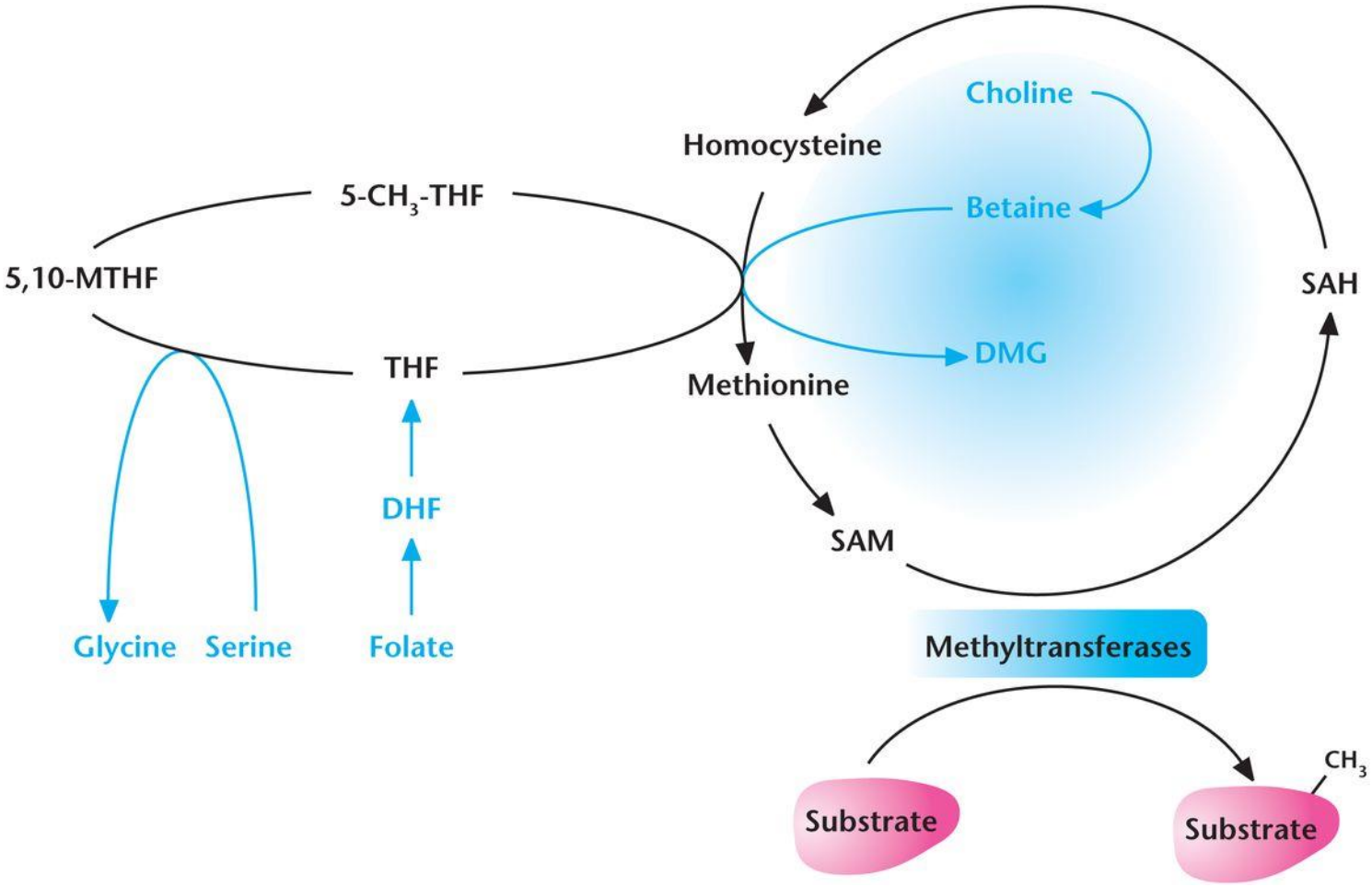
A key metabolite that governs many of these PTMs is acetyl-CoA.



Metabolic pathways that generate NAD+ in mammals.



Metabolism regulates methylation.



Metabolism regulates demethylation.