VO TRANSLATIONSKONTROLLE

Helmut Dolznig, Teil 1

2017

Signaltransduktion zur Translation

Regulation der Zellgröße

Unterlagen unter:
http://www.meduniwien.ac.at/hp/medizinische-genetik/studium-lehre/externe-lehre/

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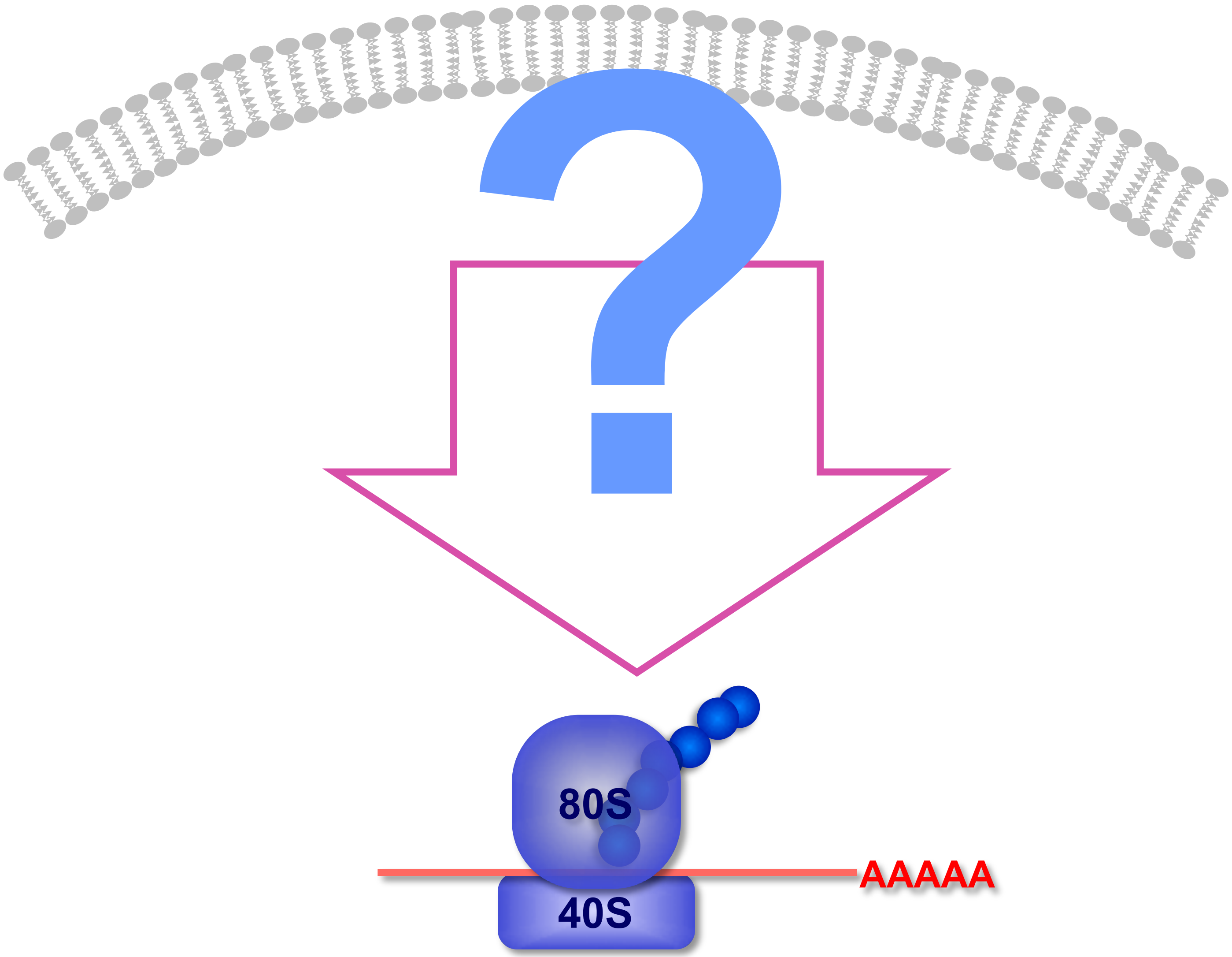
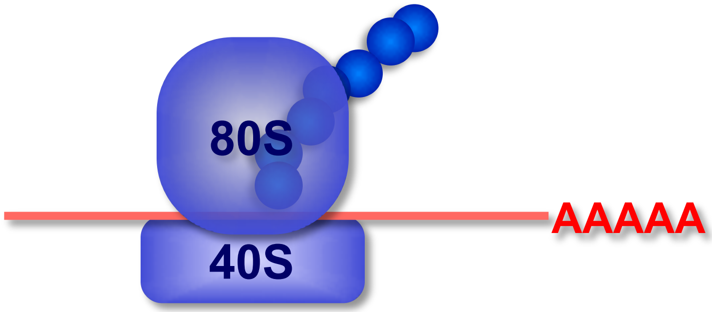
Upstream Signalling



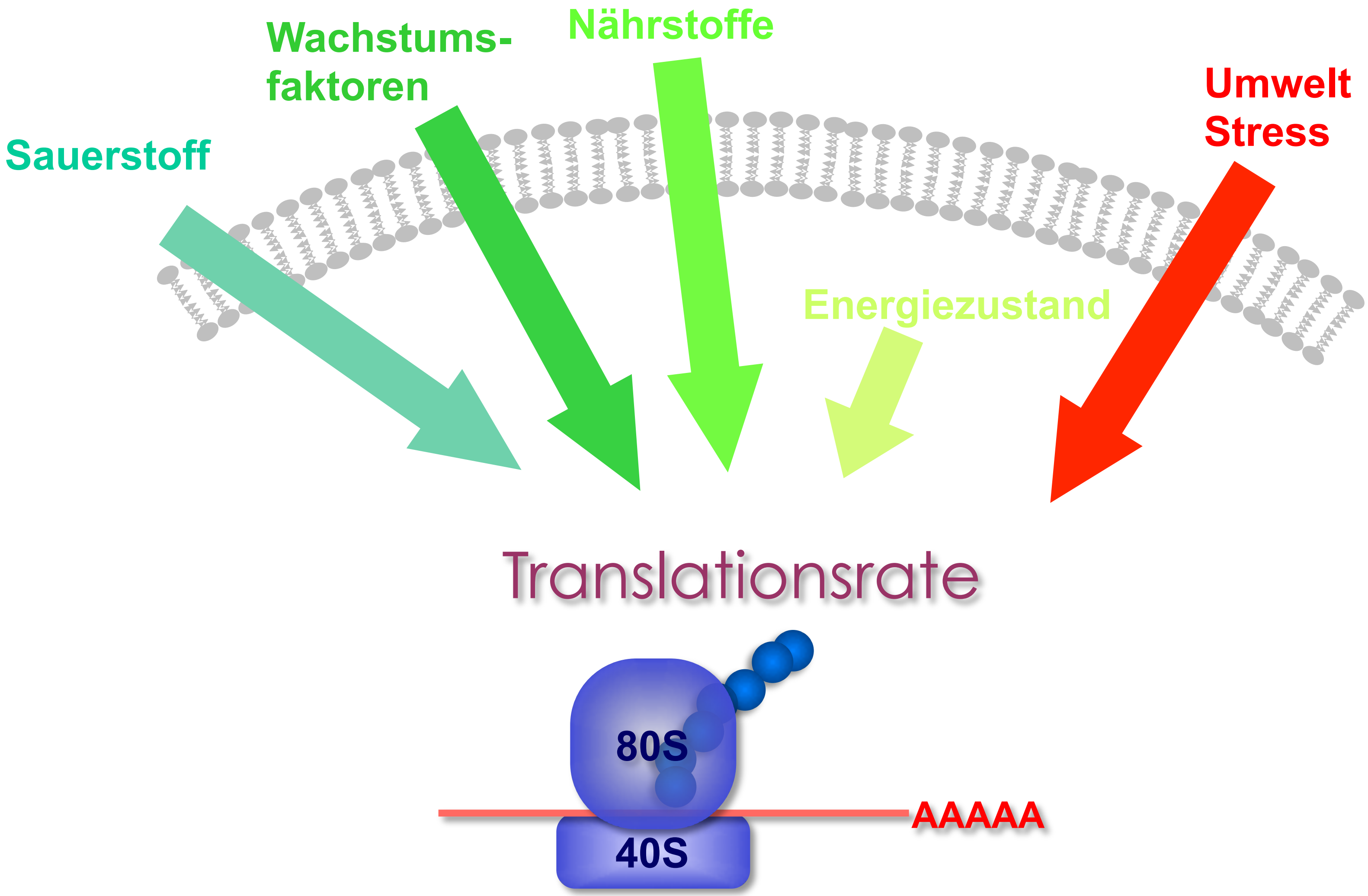
Translation verbraucht viel Energie

Translation



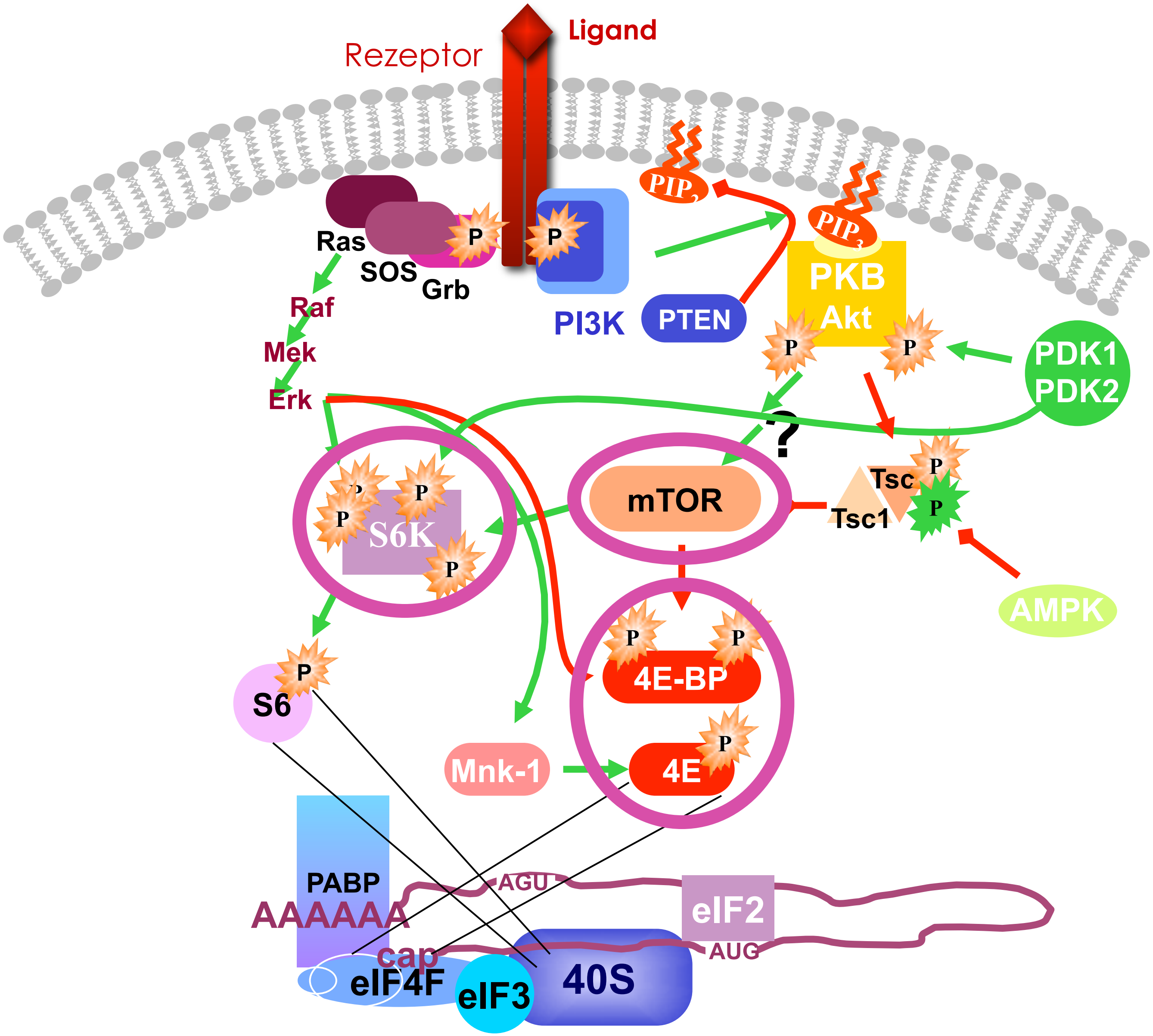
braucht 20-25% der gesamten zellulären Energie

Multiple Regulation der Translationsrate

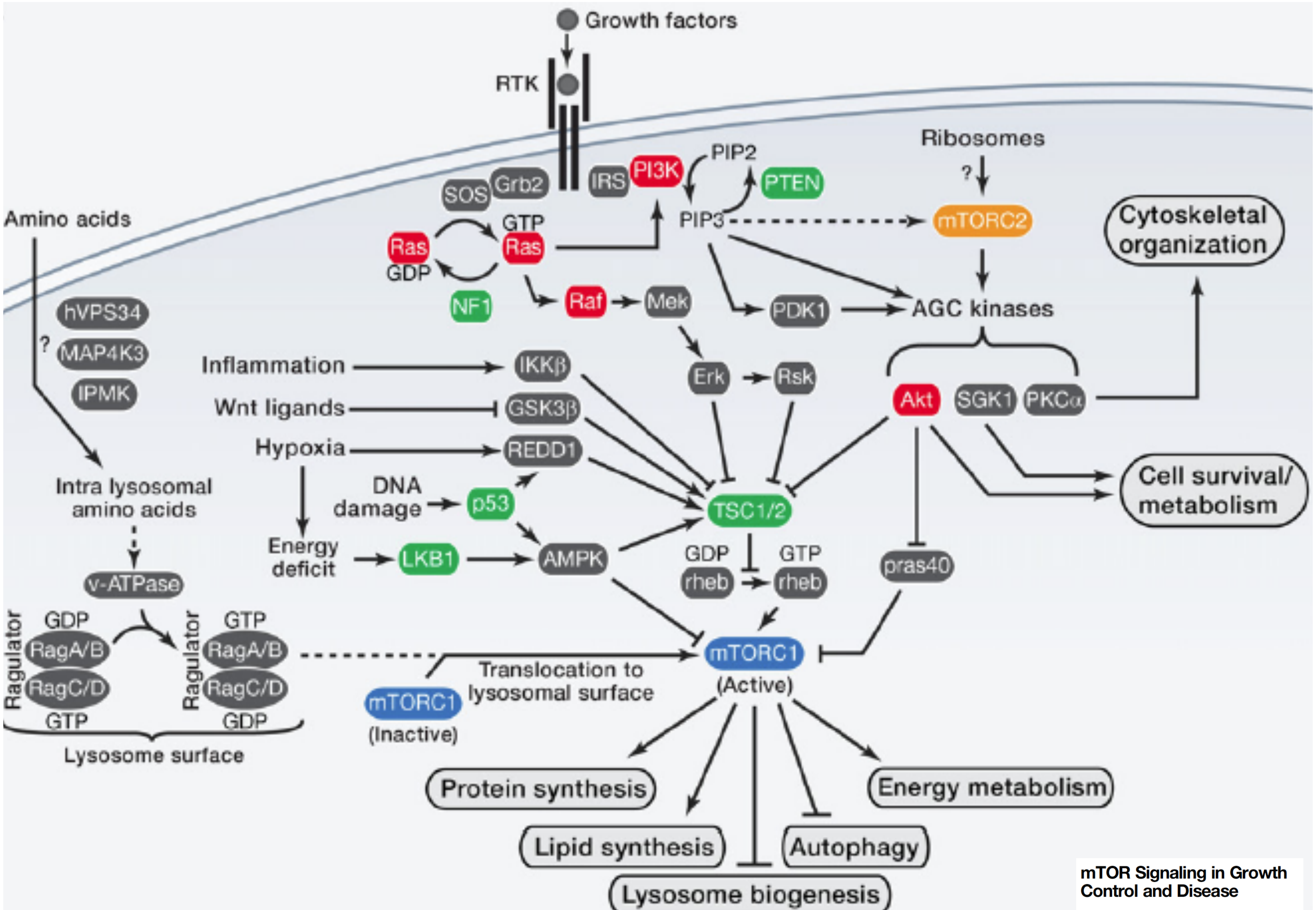


1. Regulation der Translation über Wachstumsfaktoren
2. Regulation über die Nährstoffverfügbarkeit
3. Regulation durch den Energiezustand der Zelle
4. Regulation durch Stress

Upstream Signalling of Translation: Players



"Complete" mTOR pathway



mTOR Signaling in Growth Control and Disease

Mathieu Laplante[1,2,3,4] and David M. Sabatini[1,2,3,*]

Cell *149*, April 13, 2012

“Complete” mTOR pathway

mTOR: from growth signal integration to cancer, diabetes and ageing

Roberto Zoncu*§, Aleio Efeuan*§ and David M. Sabatini*‡

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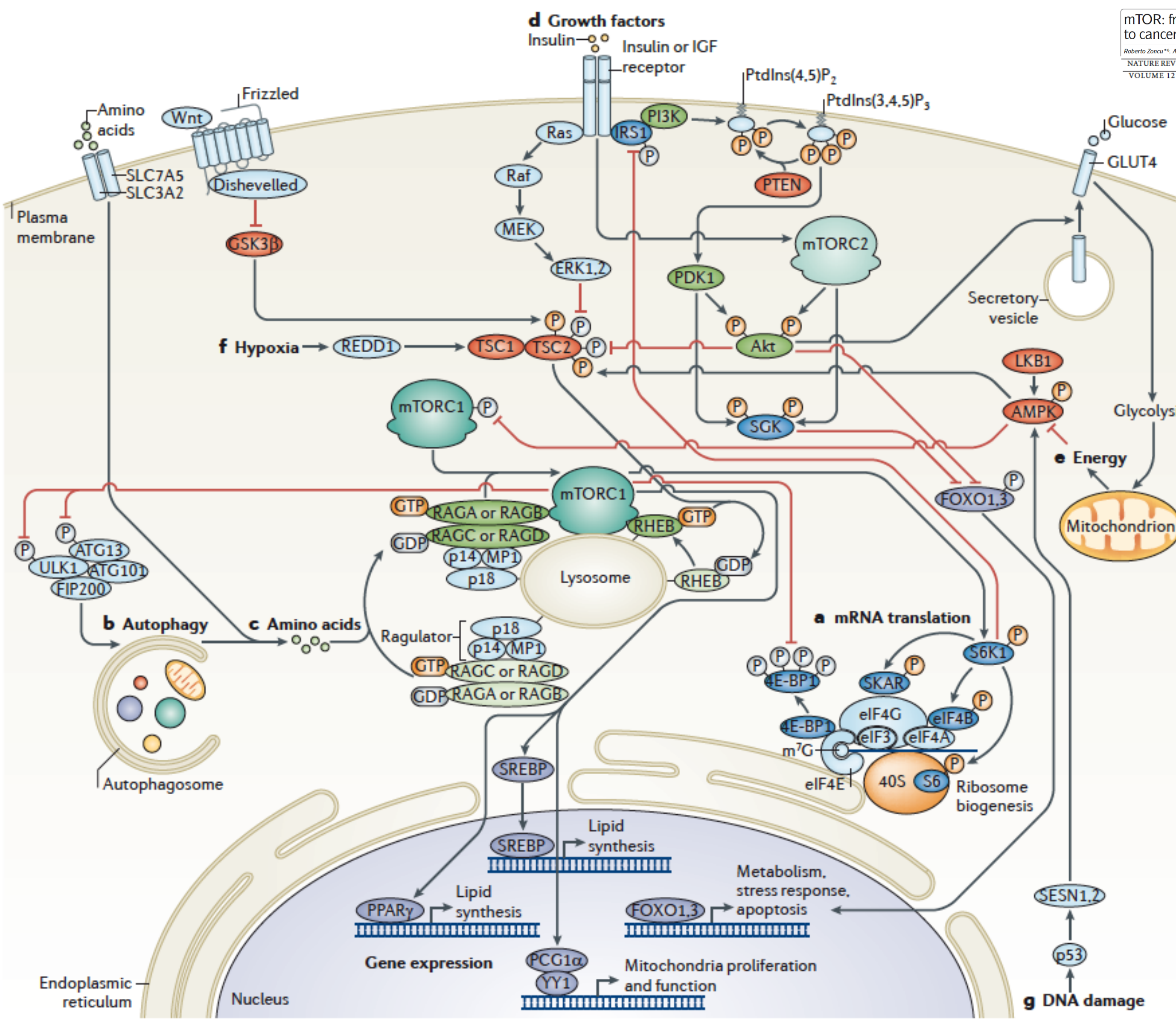
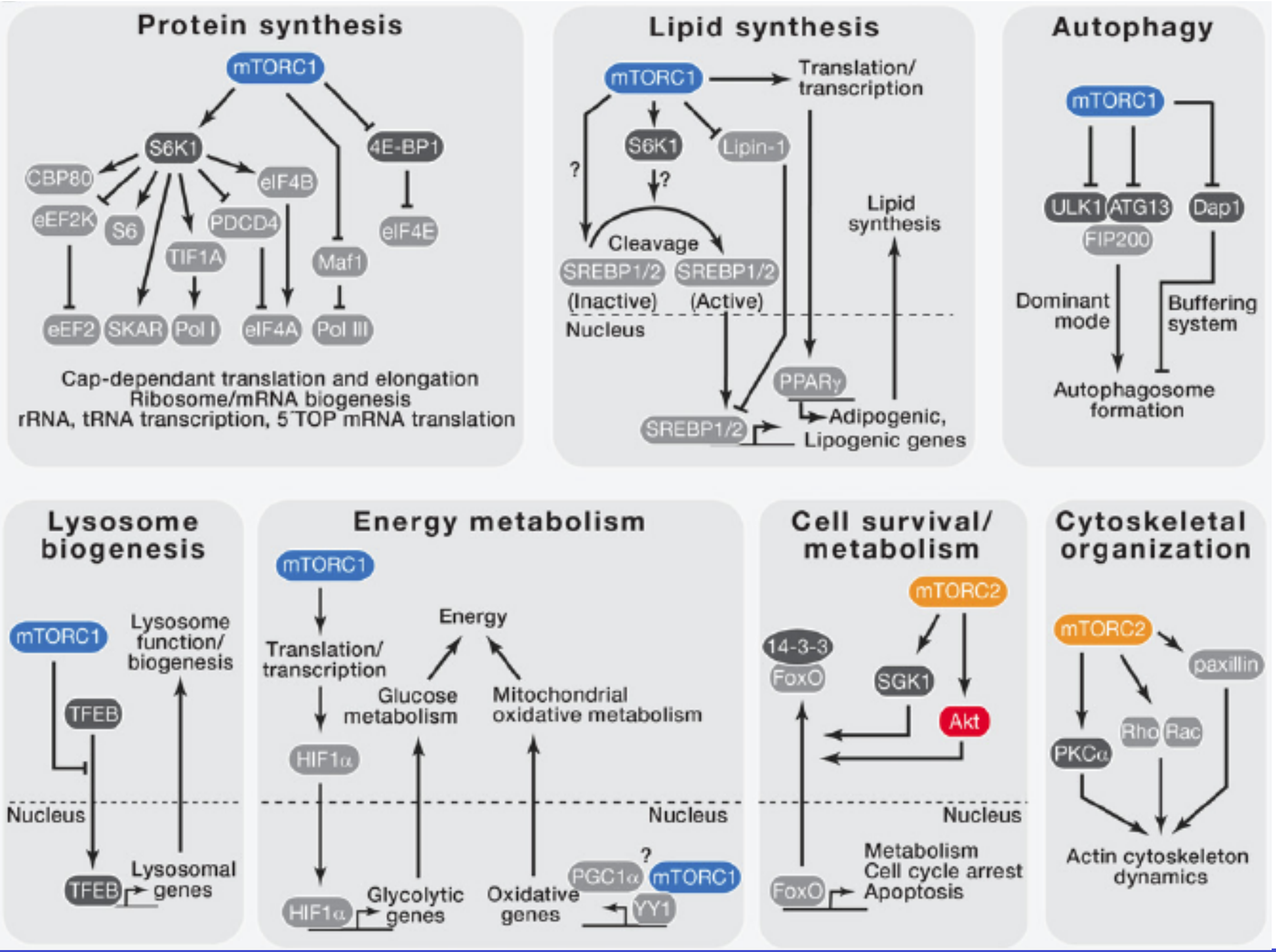
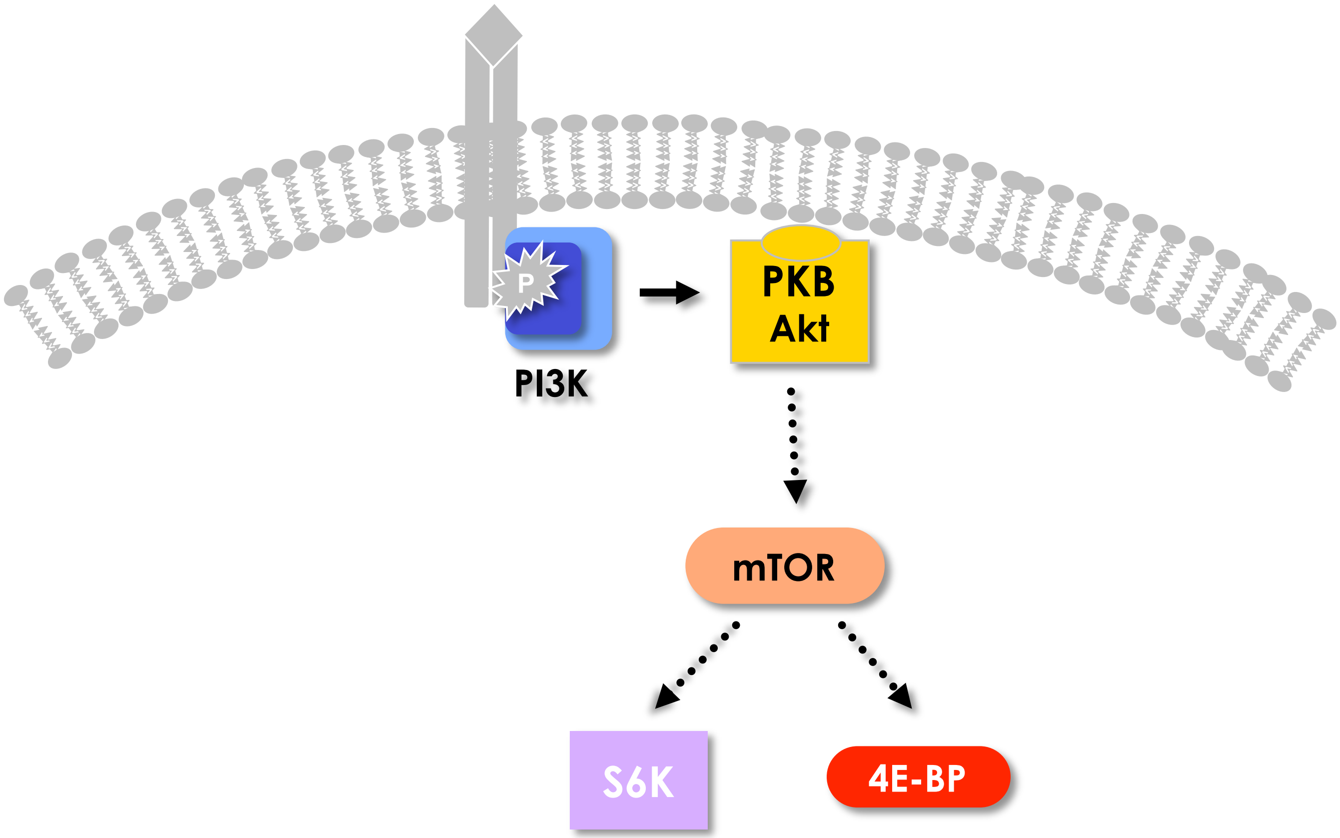


Biological Processes

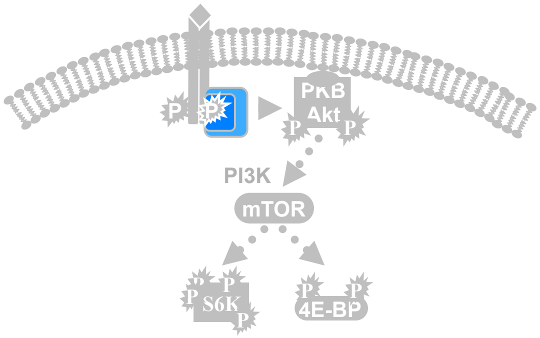


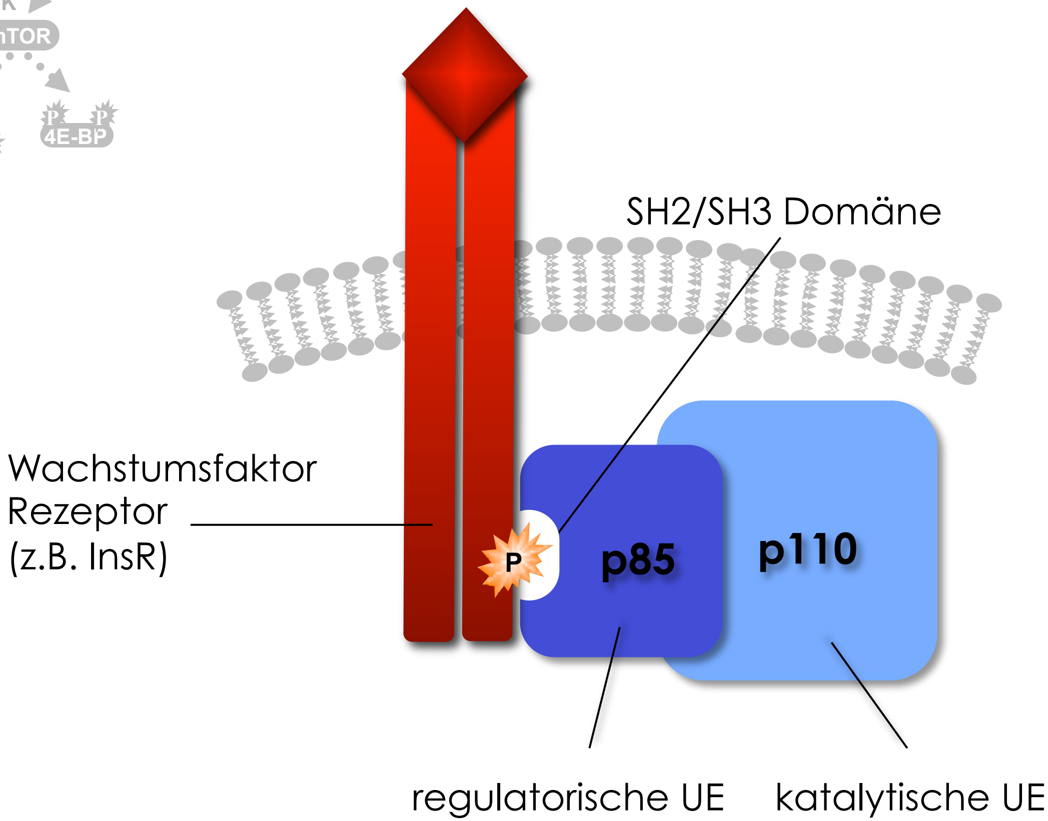
Upstream Signalling of Translation: Key Players

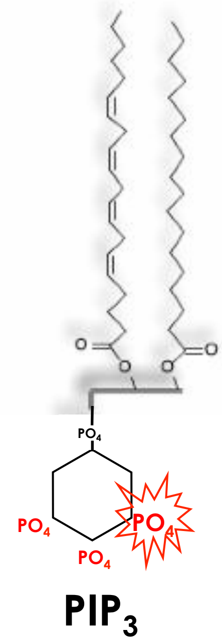


PI3K

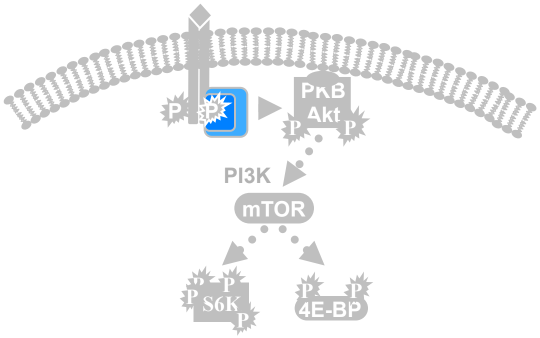
Phosphoinositid-3-Kinase = Lipidkinase



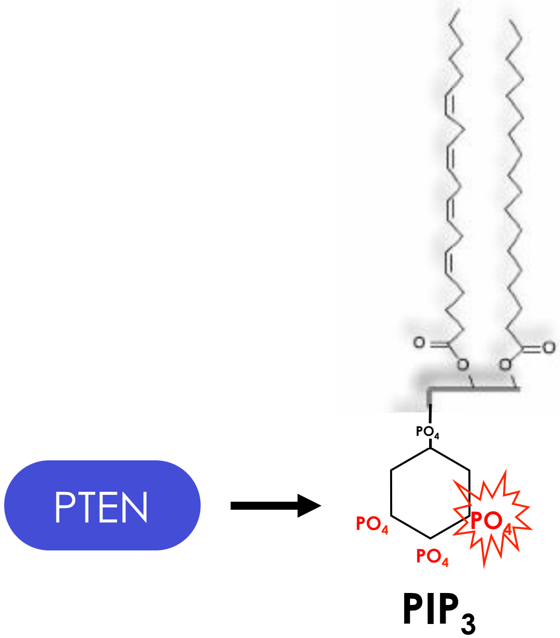




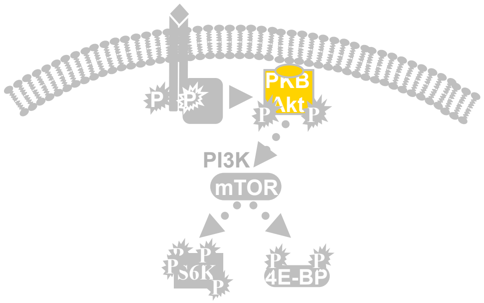
PTEN



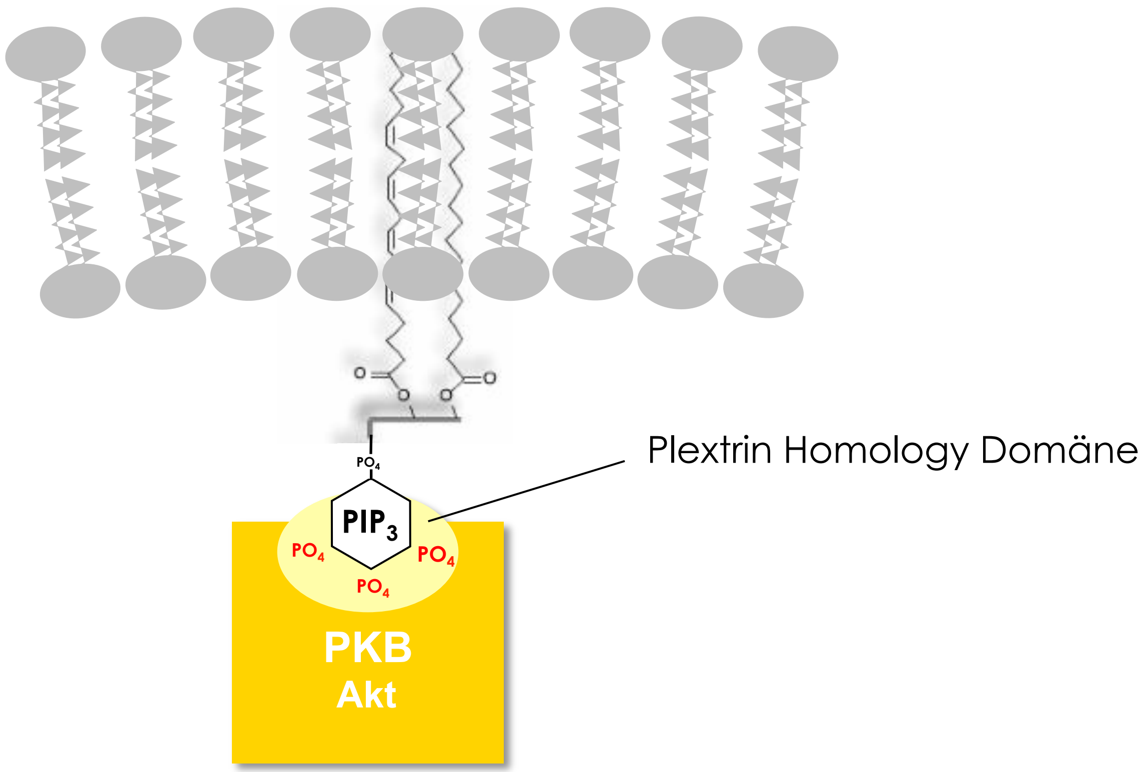
Phosphatase



PKB: PIP_3-PH domain Interaktion



Protein Kinase B / AKT



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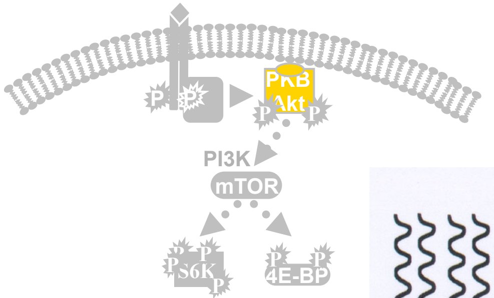
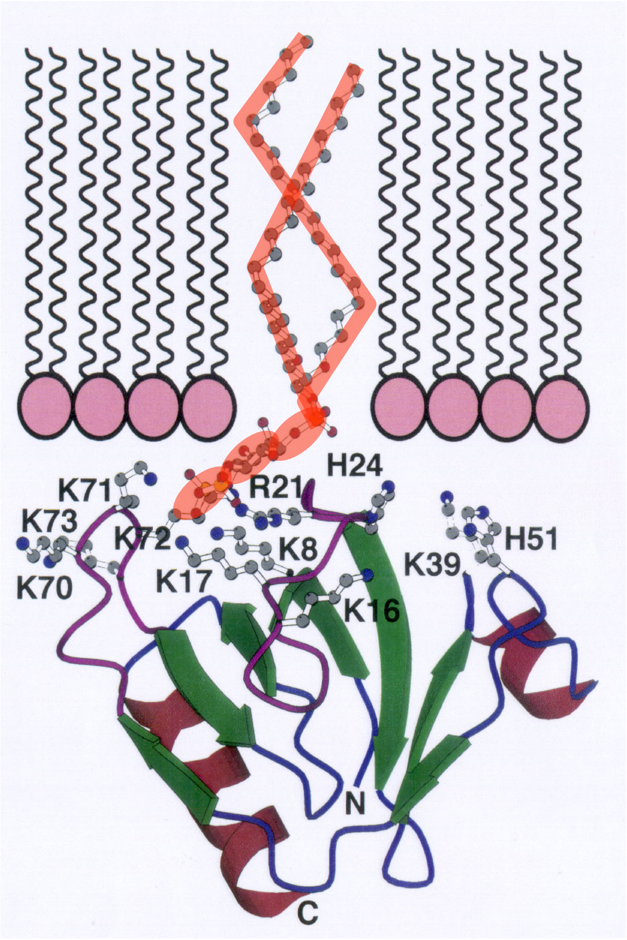
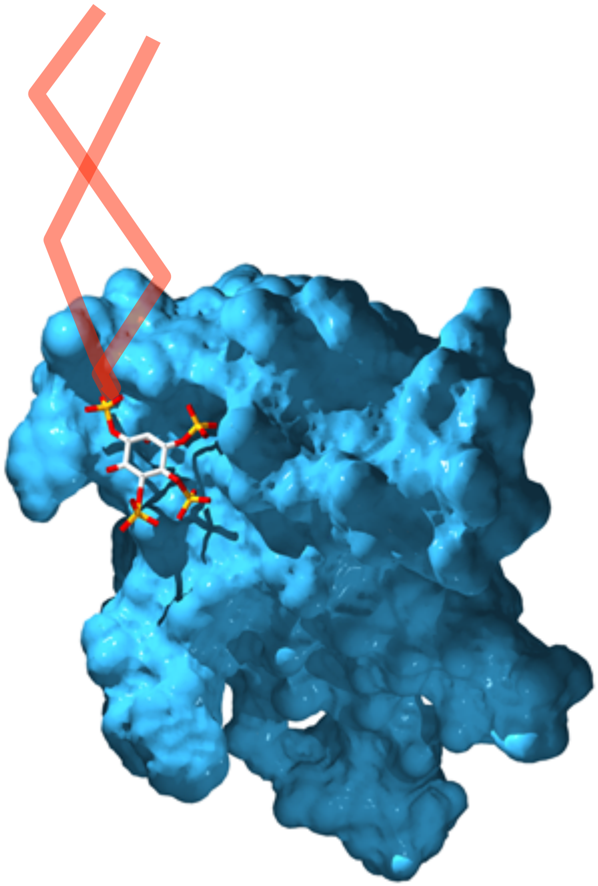
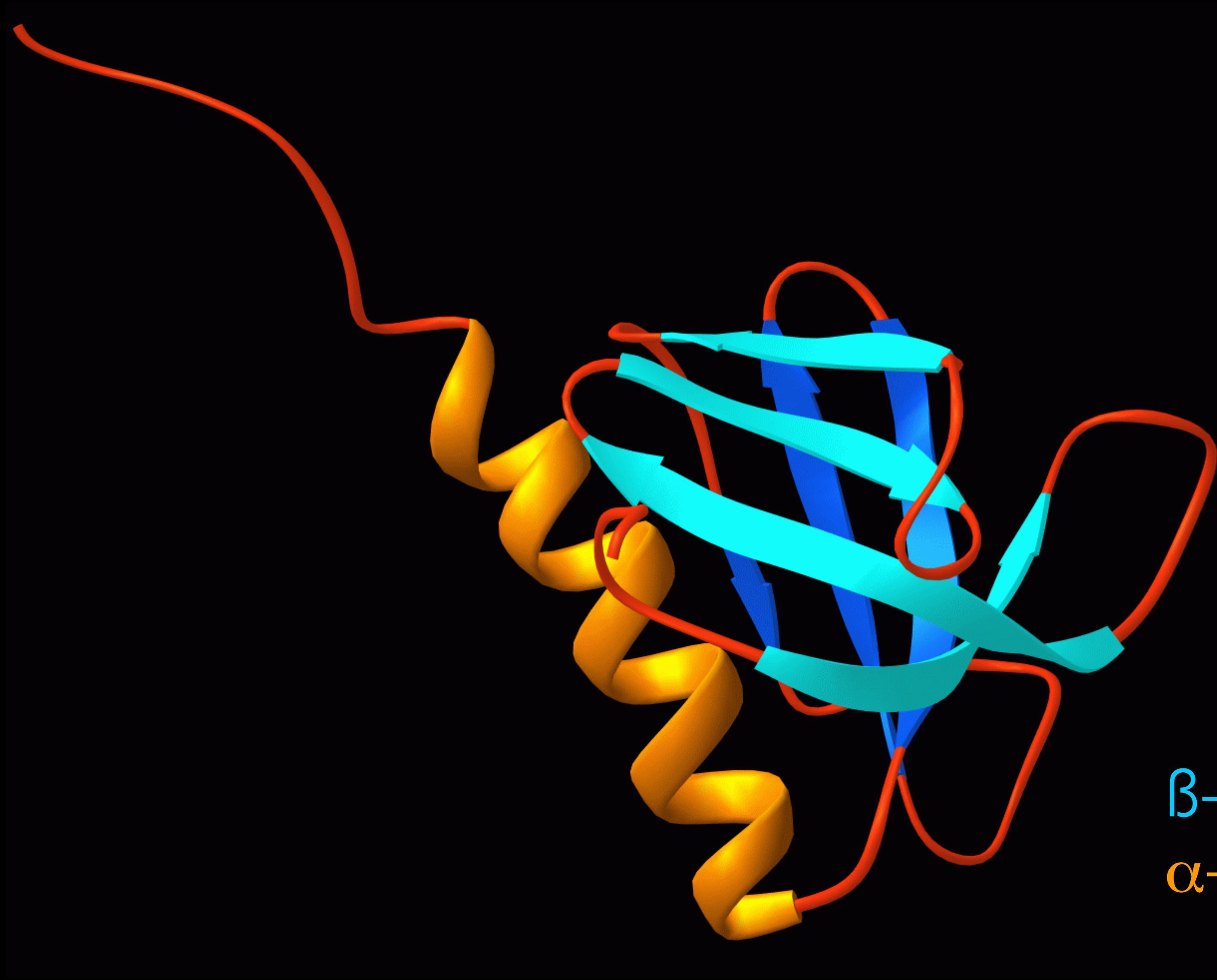


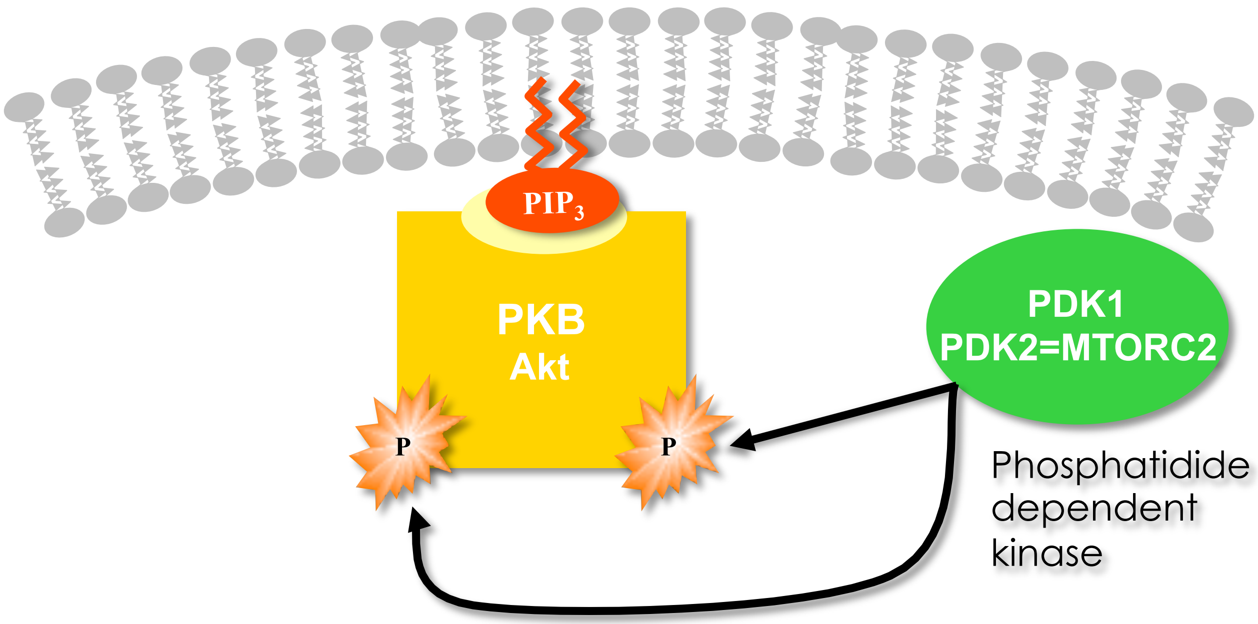




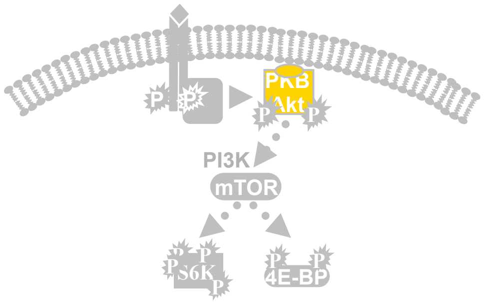
Plextrin homology (PH) domain



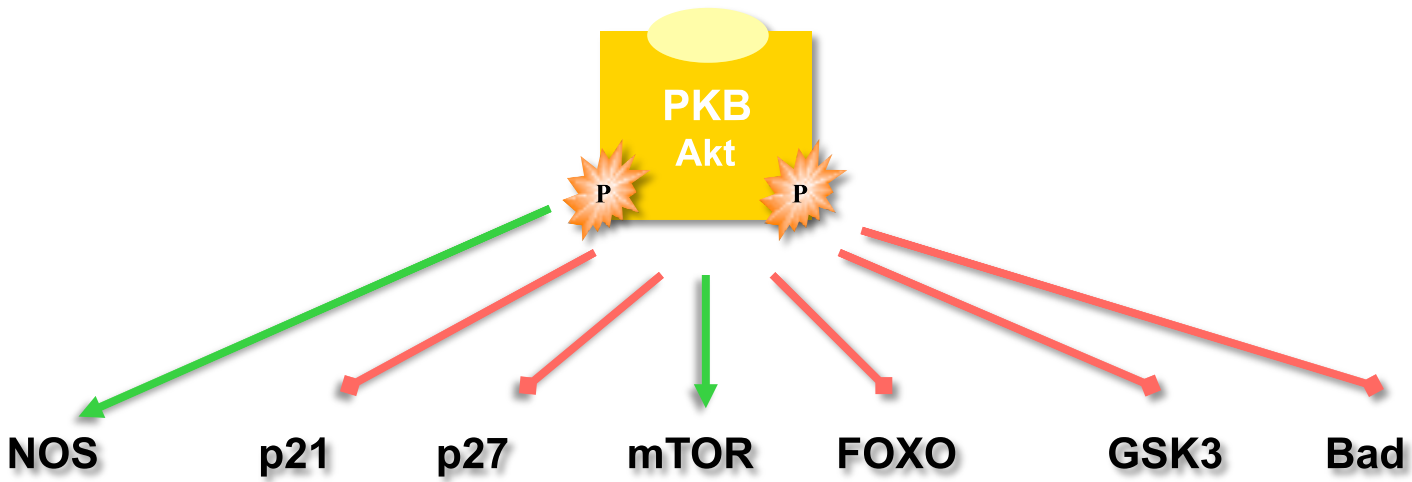
PKB Aktivierung



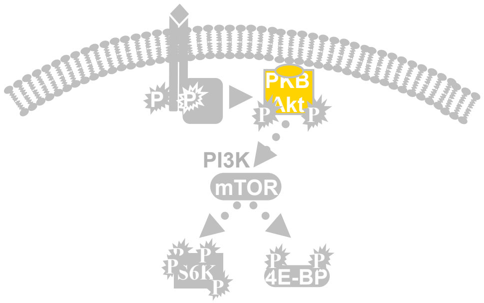
PKB/Akt Targets

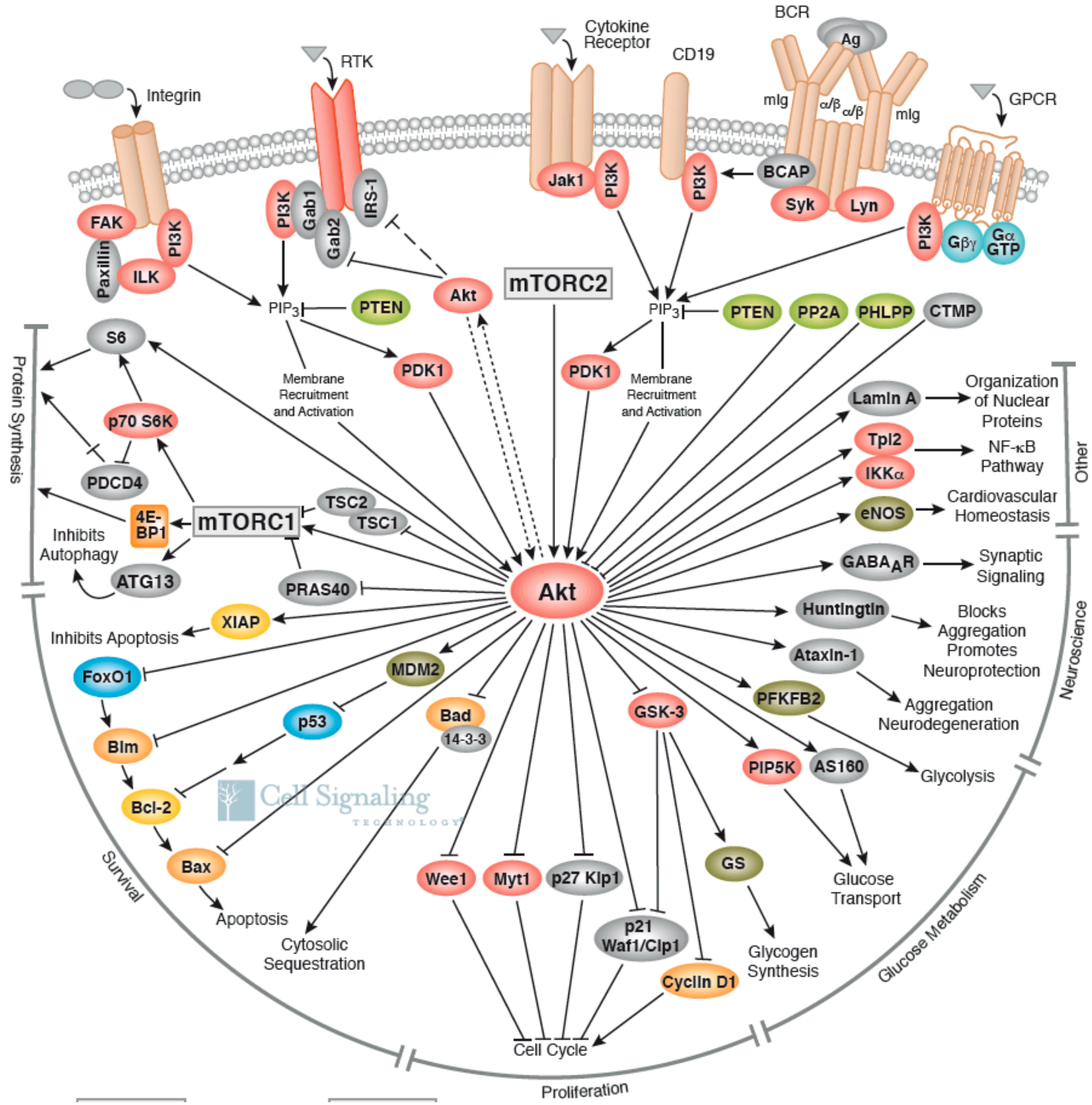


PKB = Ser/Thr Kinase

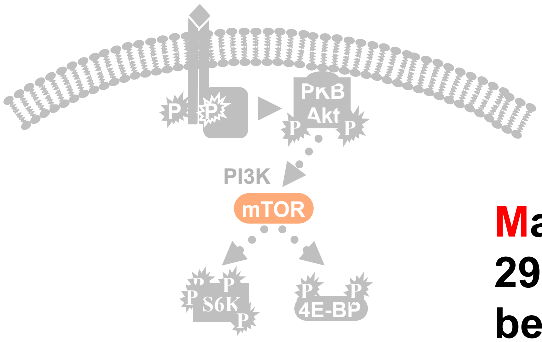


PKB/Akt Targets

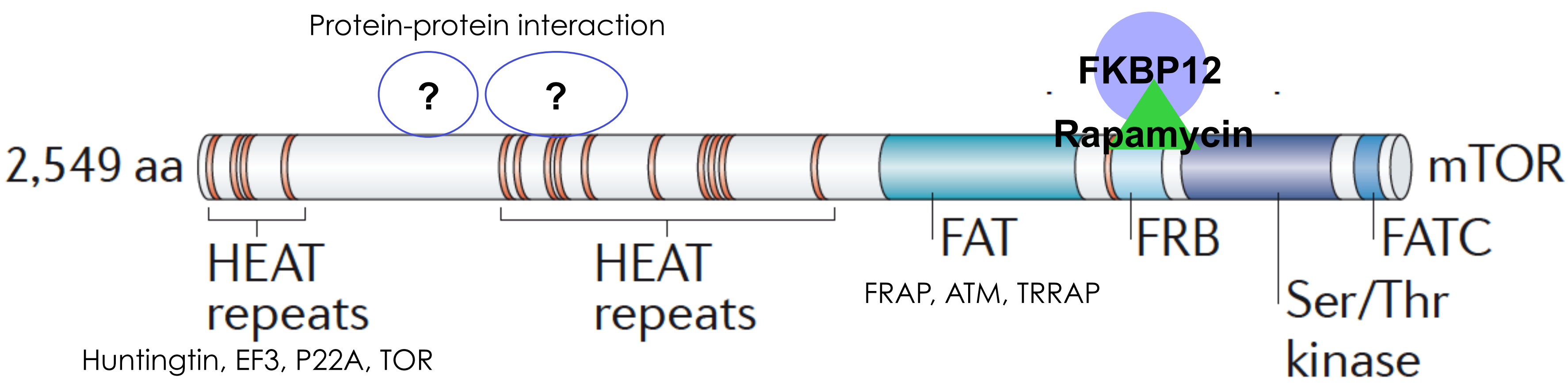




mTOR I



Mammalian (mechanistic) Target Of Rapamycin
290 kD protein
befindet sich in einem rießigen Multiproteinkomplex (1,5-2MDa)



Rapamycin bindet an FKBP12, ermöglicht erst die Bindung (an FRB) und Inhibierung von mTOR

Ser/Thr Kinase in vitro: P 4EBP, S6K
assoziierte Kinasen

Crystal structure: mTORΔN–mLST8–ATPγS-Mg complex

mTOR kinase structure, mechanism and regulation

Haijuan Yang[1], Derek G. Rudge[1], Joseph D. Koos[1], Bhamini Vaidialingam[1], Hyo J. Yang[1] & Nikola P. Pavletich[1,2]

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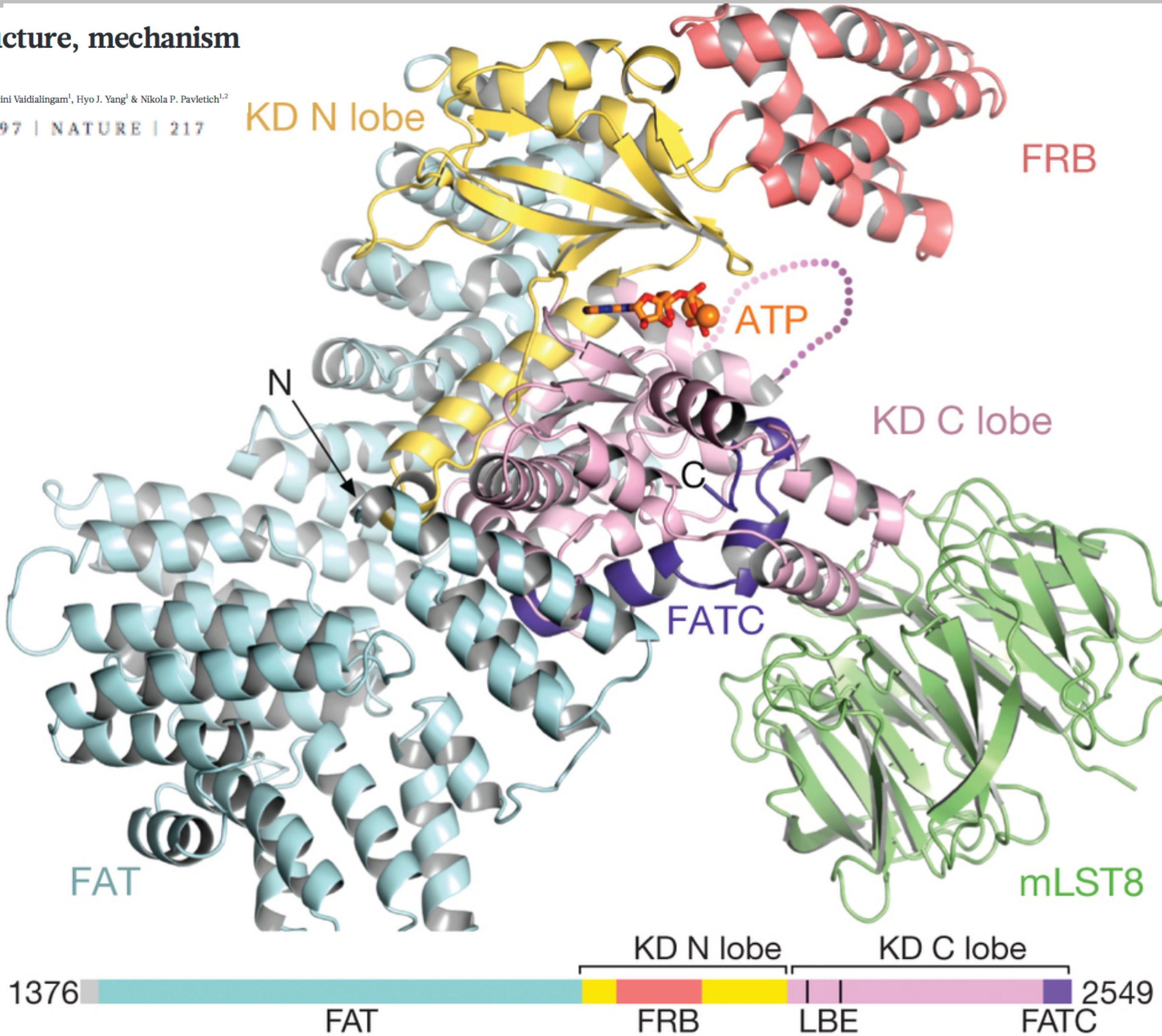
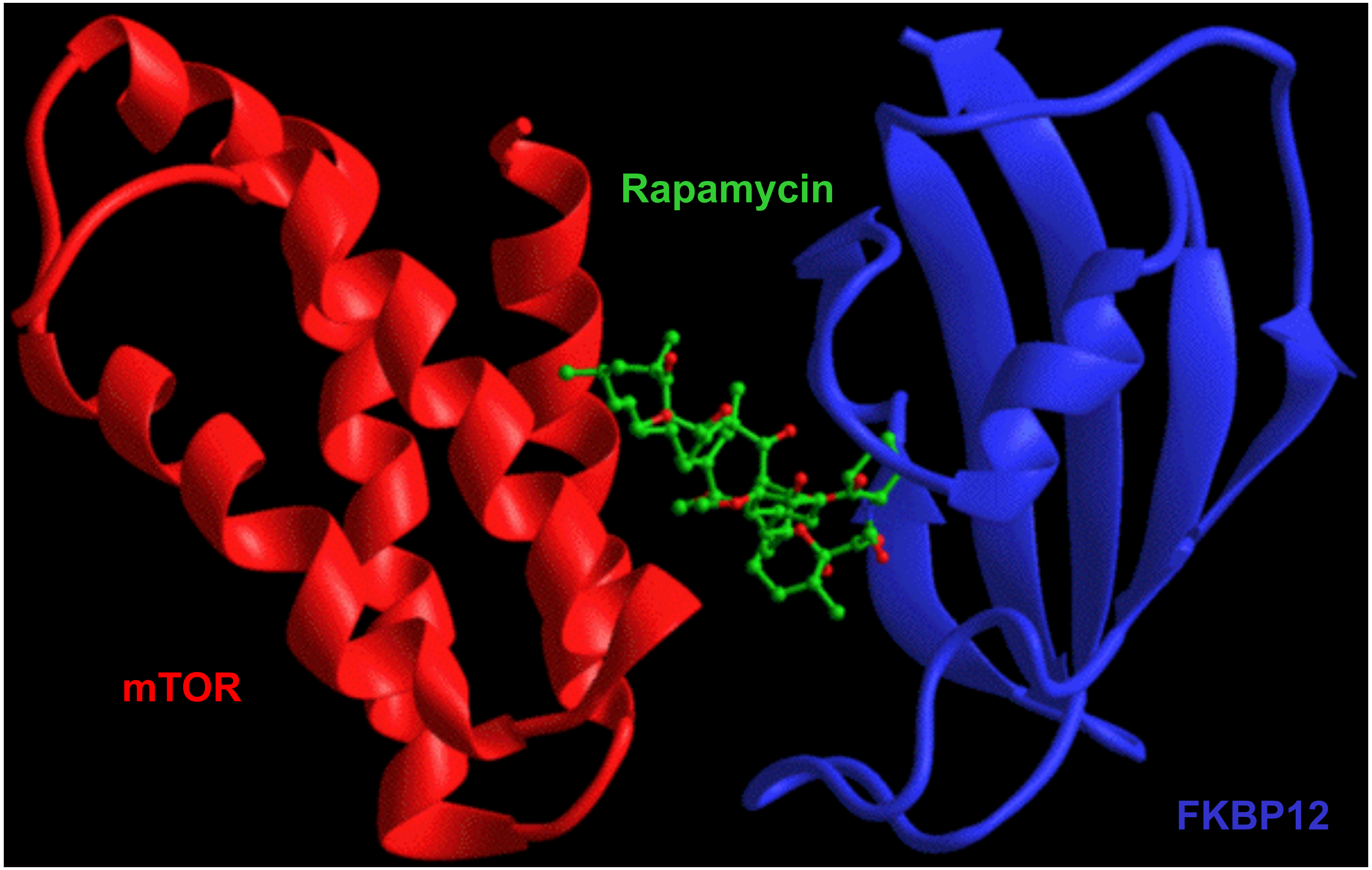


mTOR II

Rapamycin: **aus *Streptomyces hygroscopicus***
Antimycoticum, Immunosuppressiva, Tumormedikamente



mTOR III: physiologische Effekte

mTOR

Cell growth

Proliferation

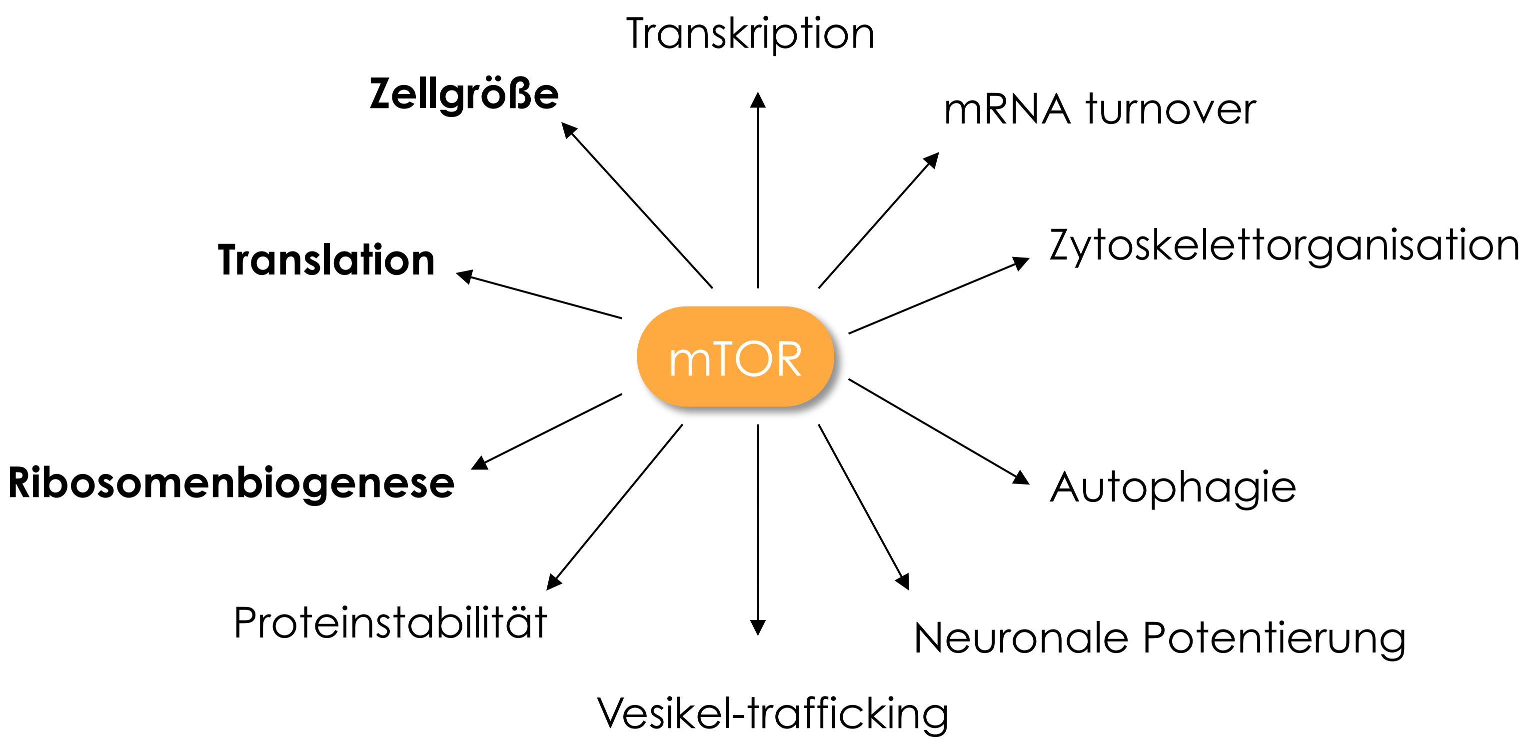
Differentiation

Migration

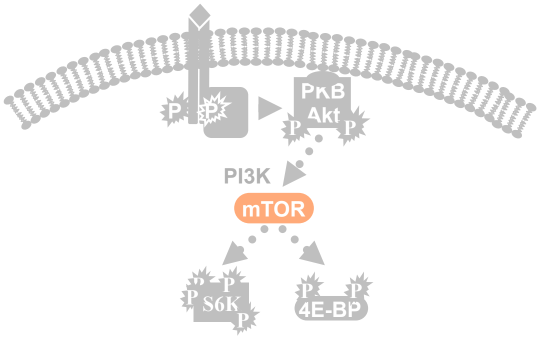
Survival

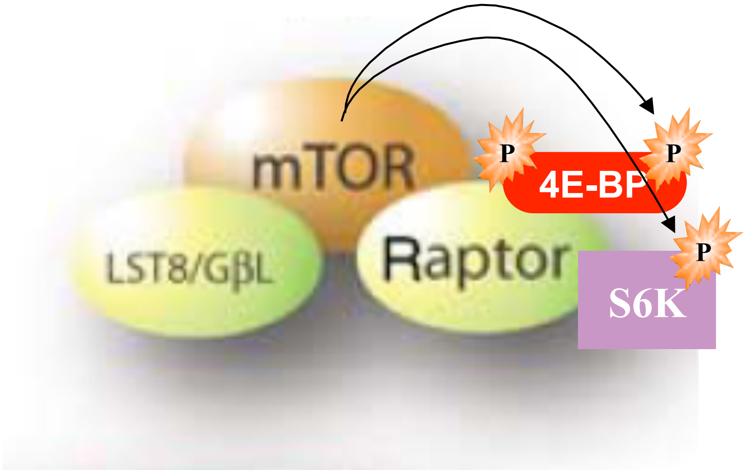
Autophagy

mTOR IV: Target pathways



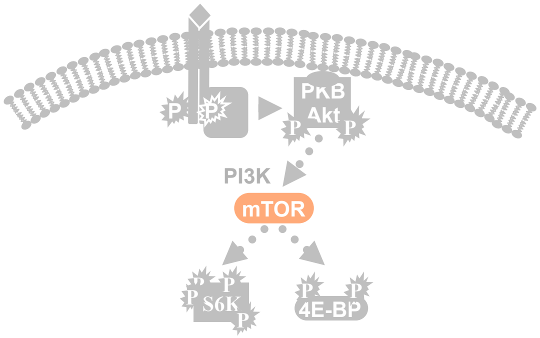
mTOR V: mTORC1 (**mTOR c**omplex 1)

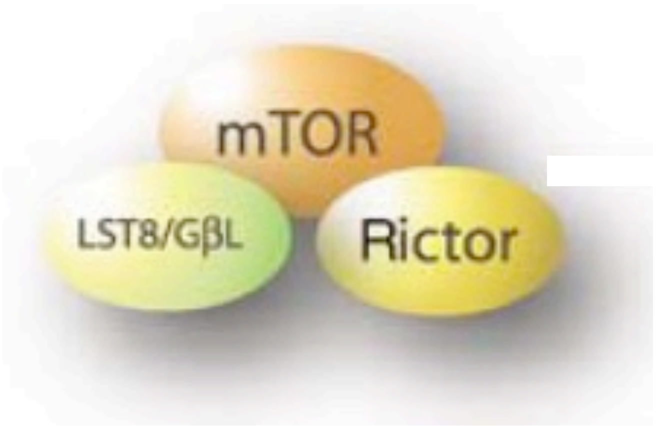




Raptor	**r**egulatory **a**ssociated **p**rotein of m**TOR**
mLST8	adaptor protein

mTOR VI: mTORC2 (**mTOR c**omplex **2**)

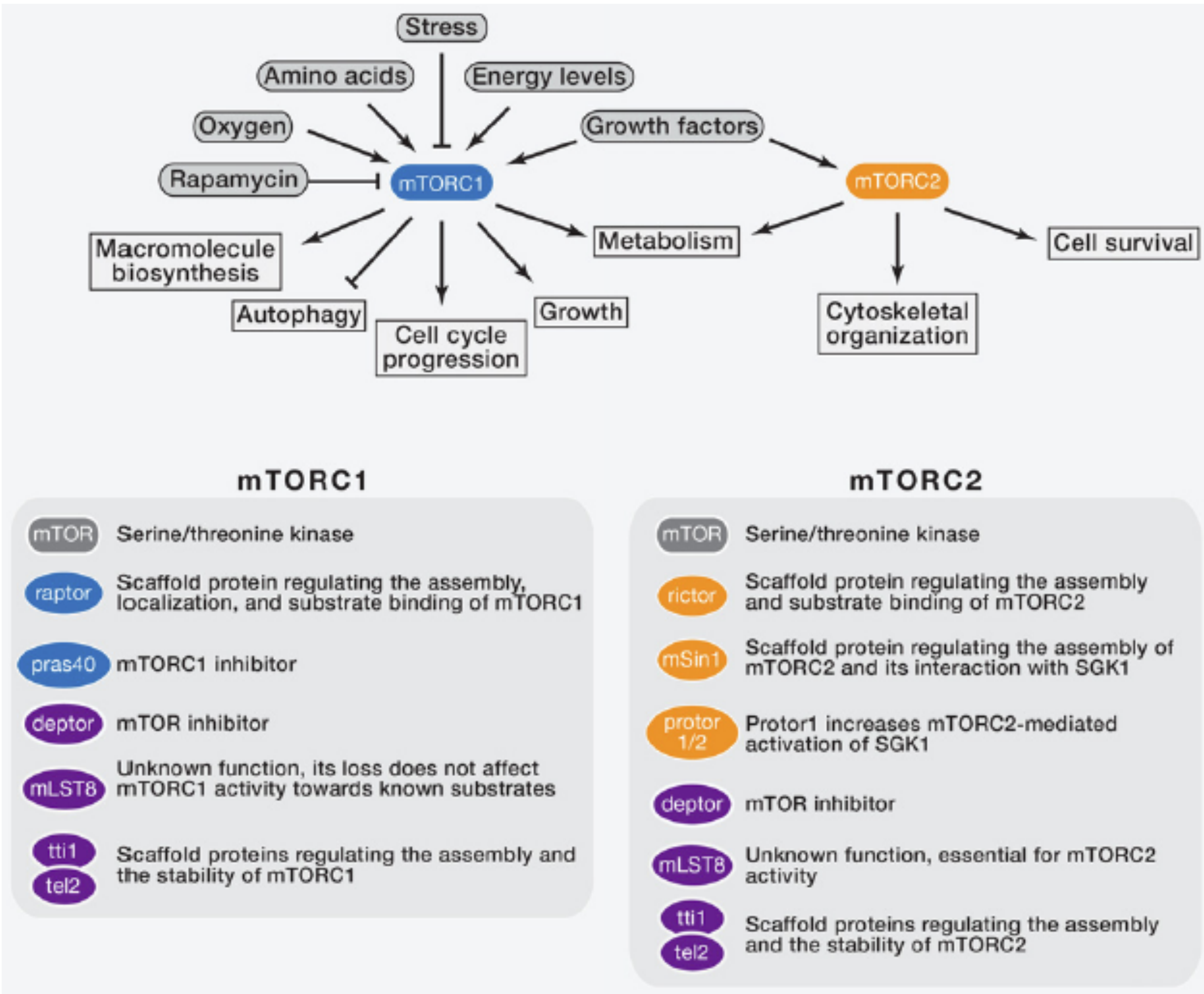




Rictor **r**apamycin **i**nsensitive **c**ompanion of m**TOR**

Kontrolliert Actin-Zytoskelett Dynamik, Metabolismus, Survival
Rapamycin UNABHÄNGIG

mTORC1 and mTORC2

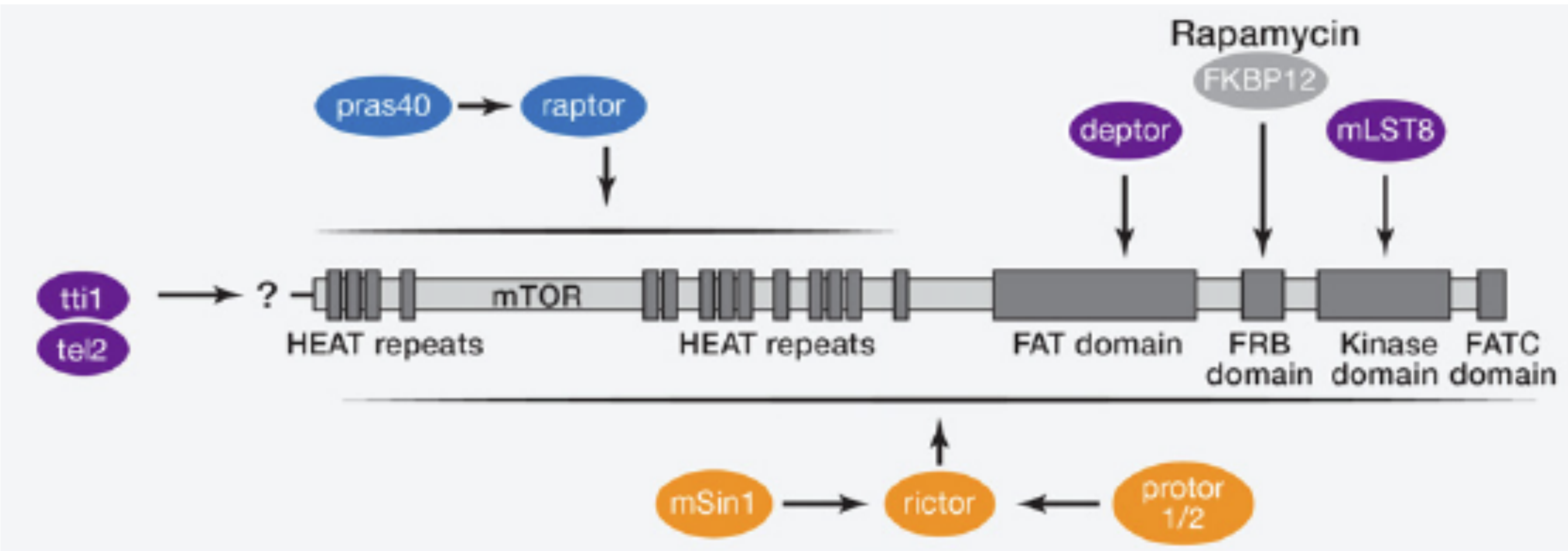


mTOR Signaling in Growth Control and Disease

Mathieu Laplante[1,2,3,4] and David M. Sabatini[1,2,3,*]

Cell *149*, April 13, 2012

mTORC1 and mTORC2



mTOR Signaling in Growth Control and Disease

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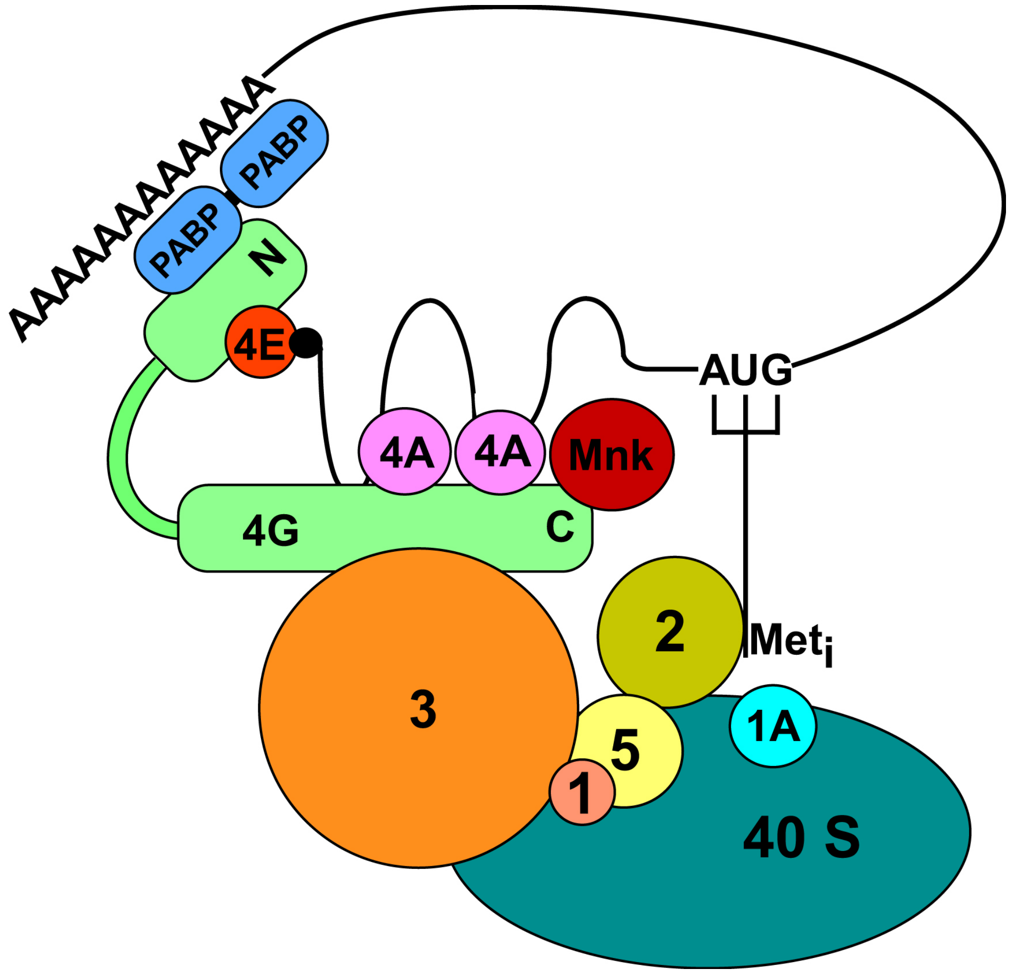
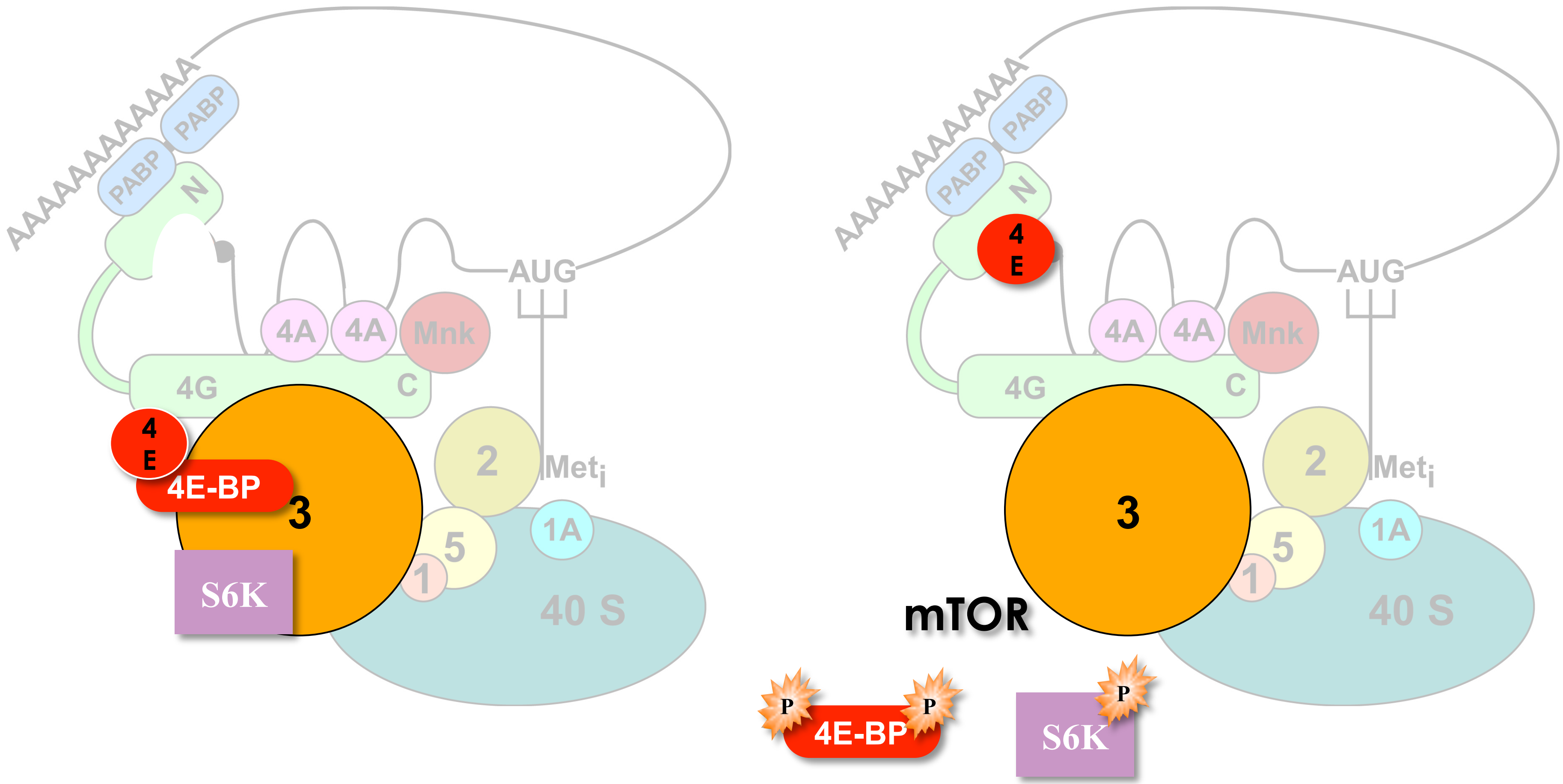
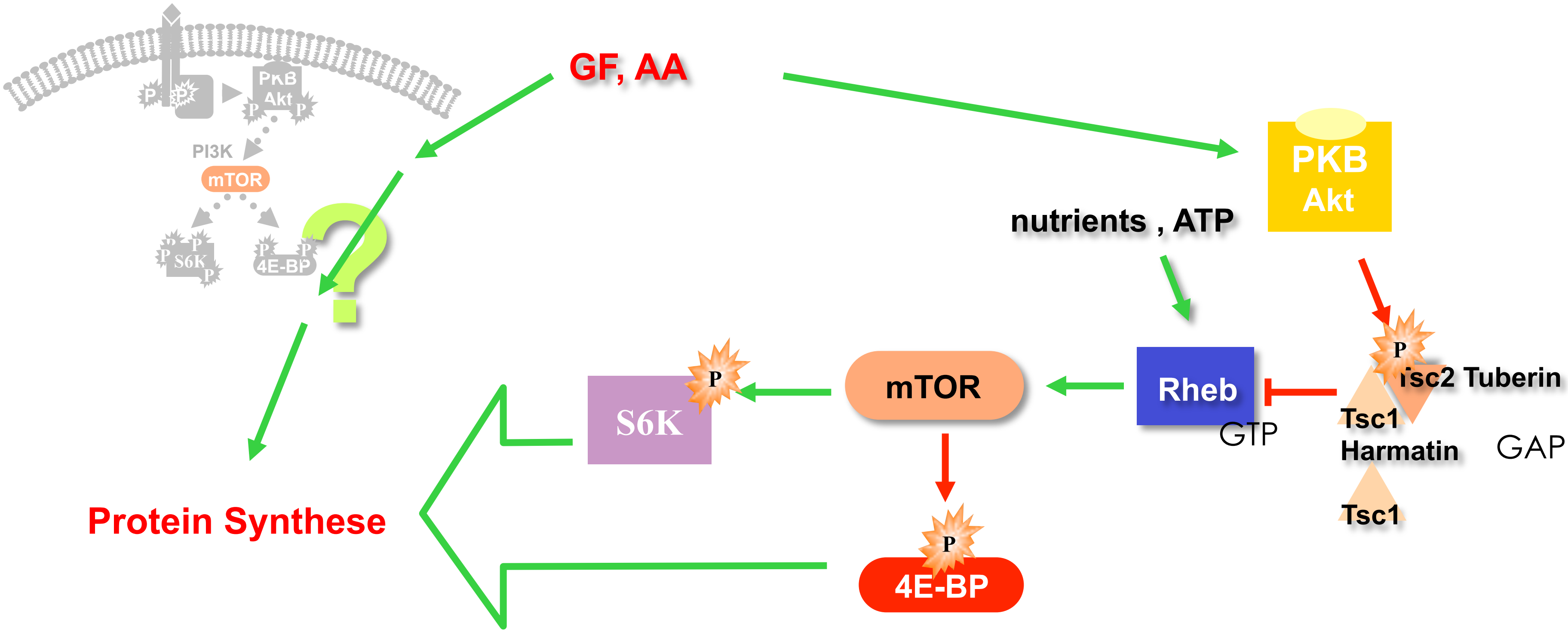
mTOR VII: 48S Translations-Initiations-Komplex



mTOR VIII: mTORC1 P von S6K und 4E-BP



mTOR IX: upstream regulation TSC1+2



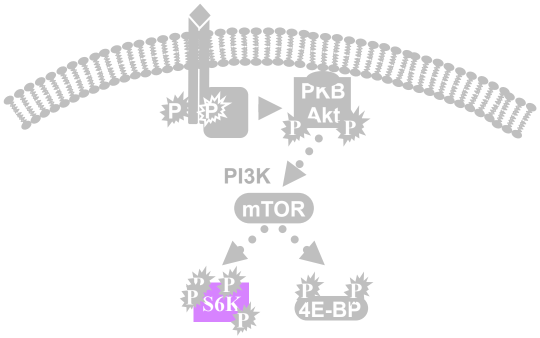
Tsc1+2: **Tuberosclerosis 1+2 (Hamartin+Tuberin)**
mutiert in bestimmten gutartigen Tumoren (Hamartome) im Menschen

TSC1/2 heterodimer, TSC2 ist ein GAP (GTPase activating protein) und aktiviert Rheb

Protein Synthese nicht "all oder none" reguliert durch mTOR

mTOR unabhängige pathways (rapamycin resistent),
in Herzmuskelzellen 50% d. Proteinsynthese aufrecht unter Rapamycin

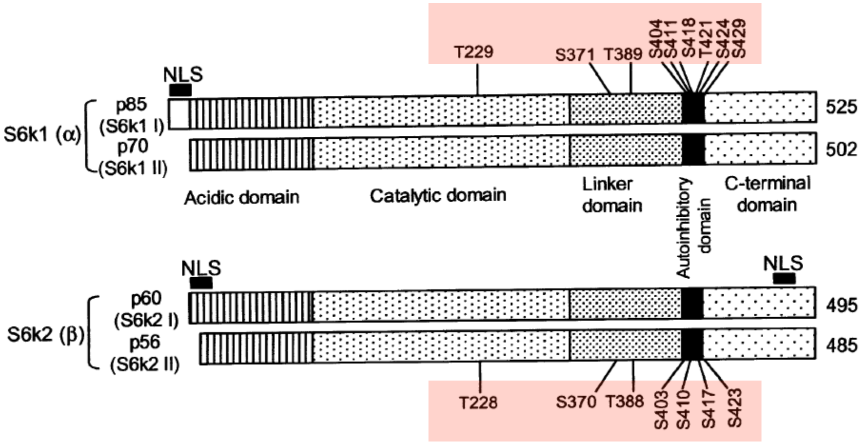
S6 Kinase I



2 Gene mit je 2 Splicevarianten (mammals)

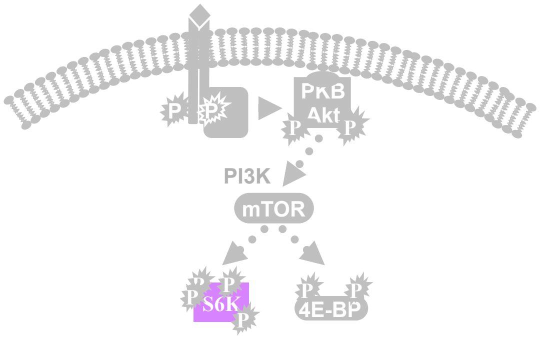
p70 S6K1(cs), **p85 S6K1**(n)
p54 S6K2(n), **p56 S6K2**(n)

multiple P sites



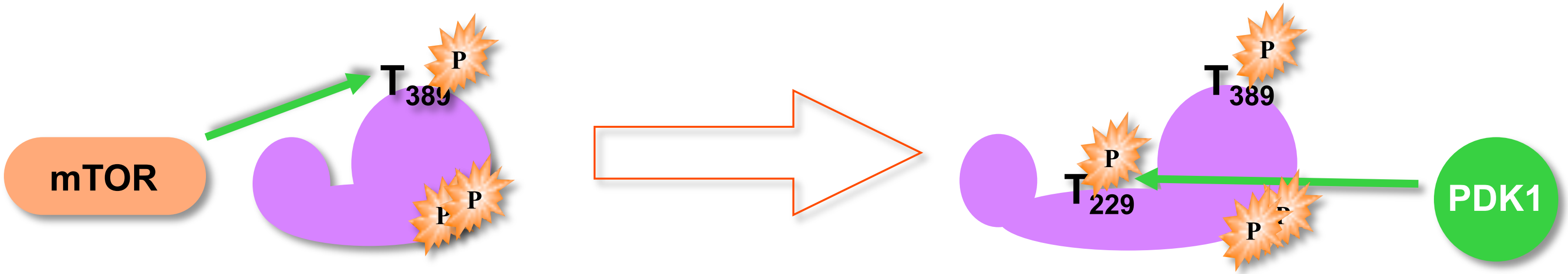
P S6 (ribosomales Protein, rpS6, mit 18S rRNA assoziiert 1x per 40S),
Hypothese: S6P aktiviert Translation
volle Aktivierung von S6K braucht AA+Glucose +Insulin (GF) (absolut mTOR abhängig)

S6 Kinase II



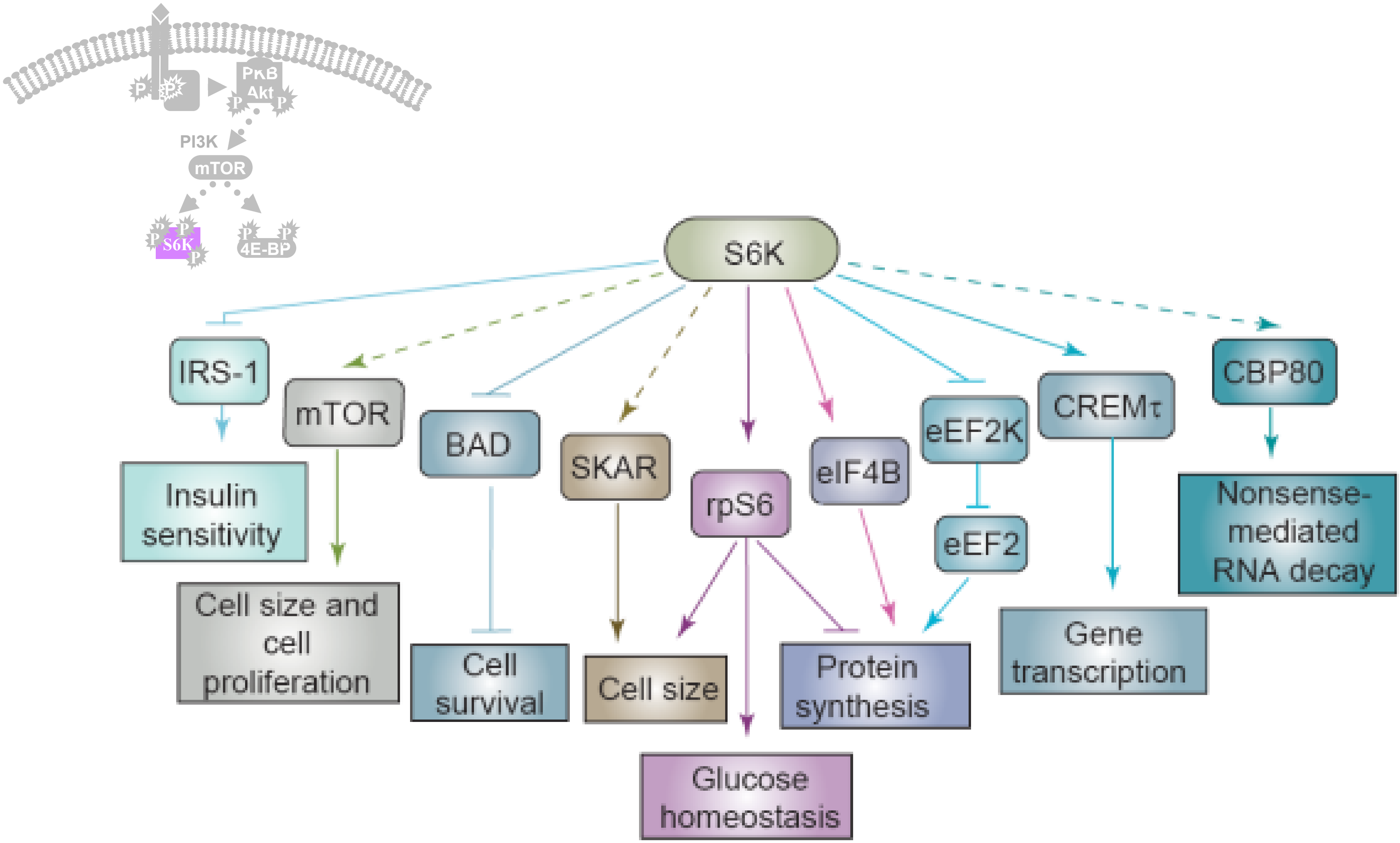
C-terminal residues: P (durch ERK) ermöglicht erst Zugang für folgende P:

- Thr389 durch mTOR
- Thr229 durch PDK1

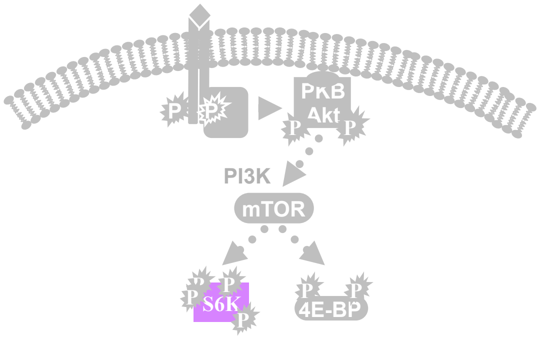


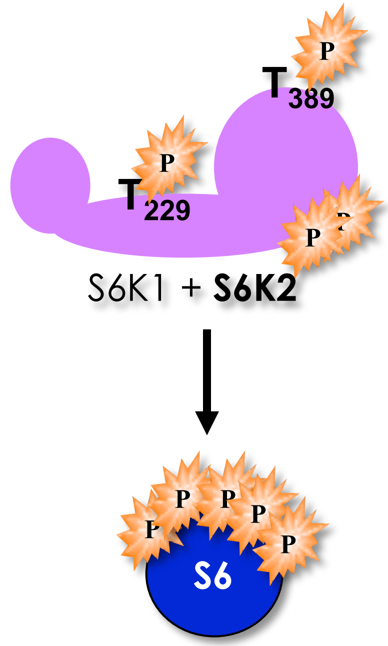
alle Aktivierungsstimuli Rapamycin sensitiv (d.h. mTORC1 abhängig)

S6 Kinase III: Substrate



S6 Kinase III: Substrat rpS6

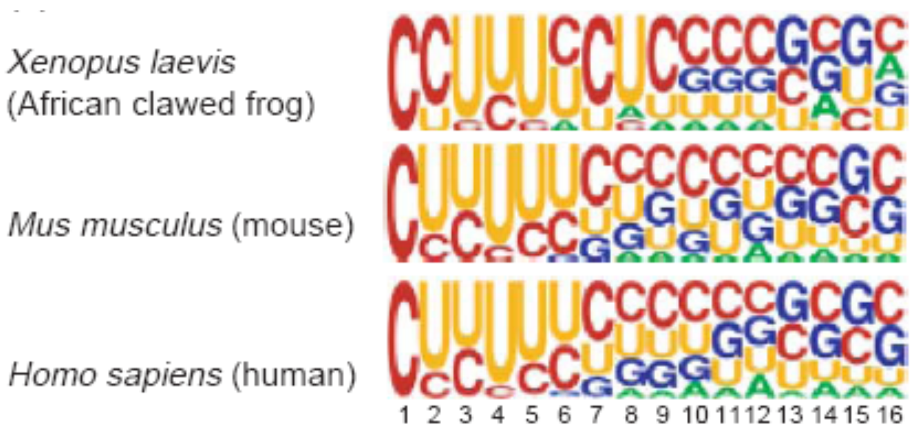




S235, S236, S240, S244, S247

S6 Kinase IV: Substrat rpS6

~~P-S6 aktiviert die Translation von 5‘ TOP-RNAs:~~ = FALSCH
ribosmale Proteine, Proteine der Translationsmaschinerie…

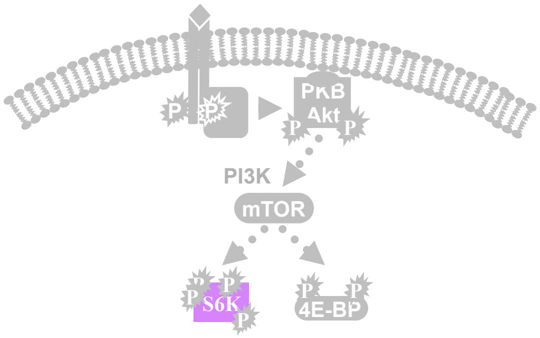


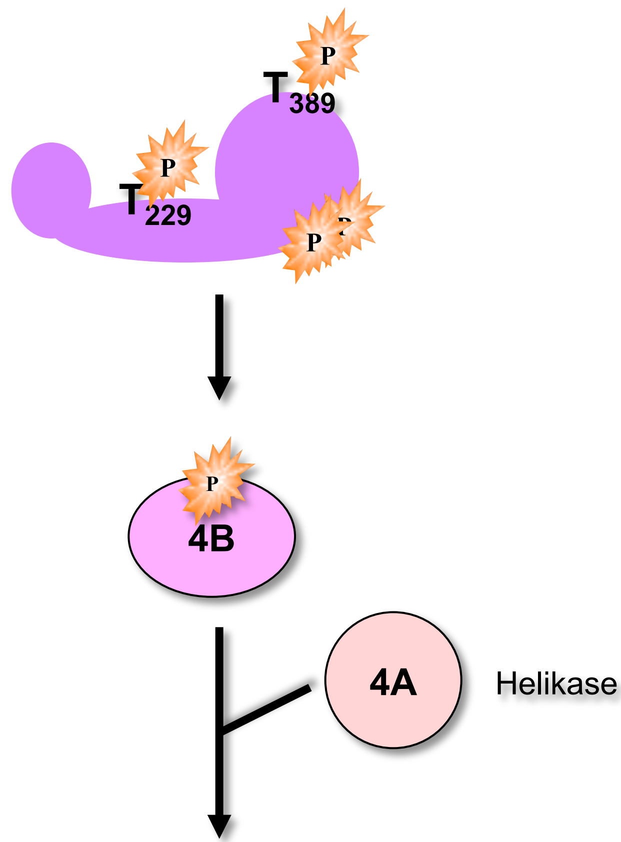
5‘ TOP-RNAs

= translationell reprimiert in Zellzyklus arrest, AS starvation
Translationell aktiviert bei Proliferationsinduktion und AS Zugabe

Experimente in Mäusen haben gezeigt, dass die P von S6 nichts an der Translation von 5‘ TOP RNAs ändert (rpS6$^{-/-}$ Mäuse mit S6$^{P-/-}$ knock in)

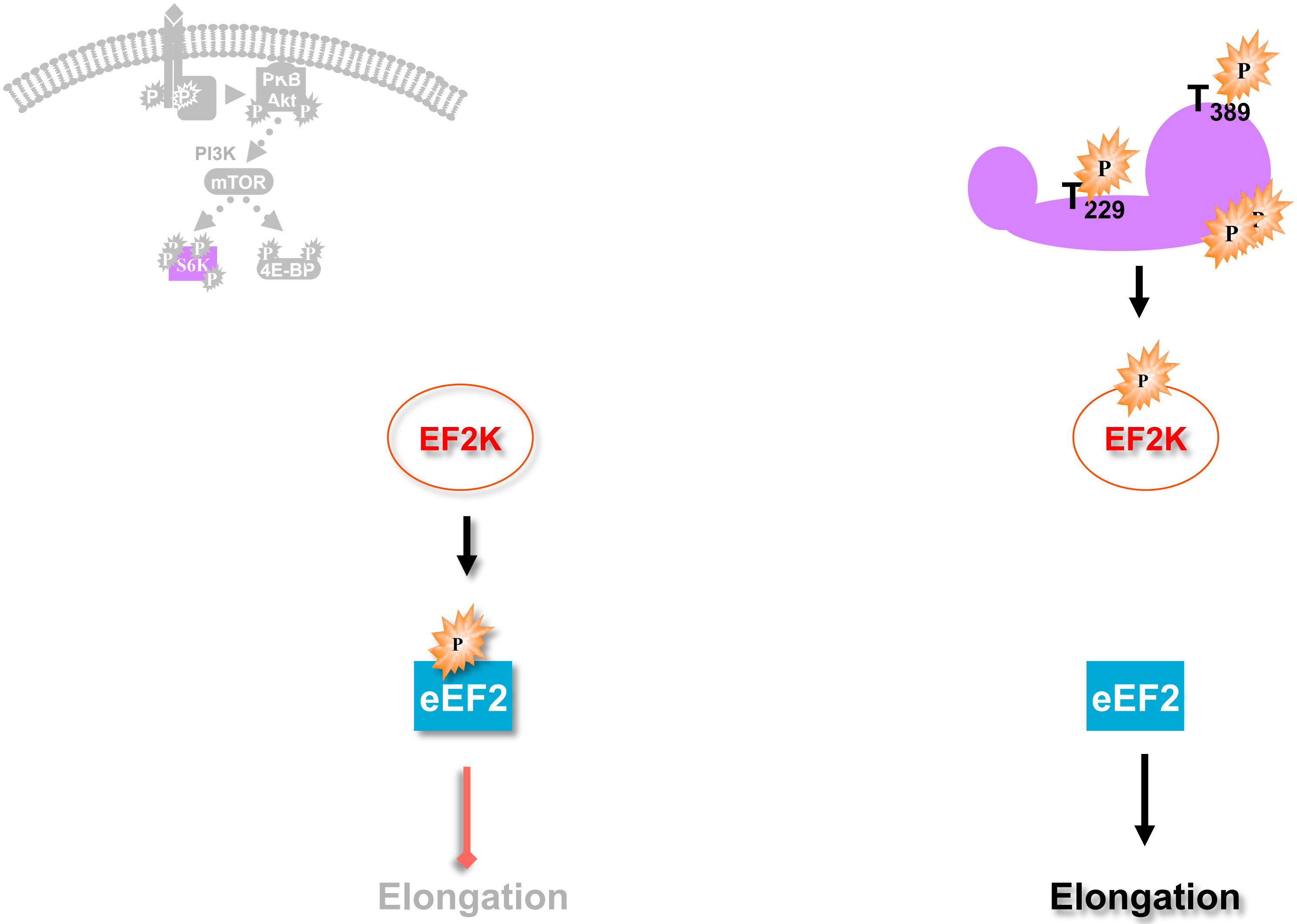
eIF4B als Target von S6K



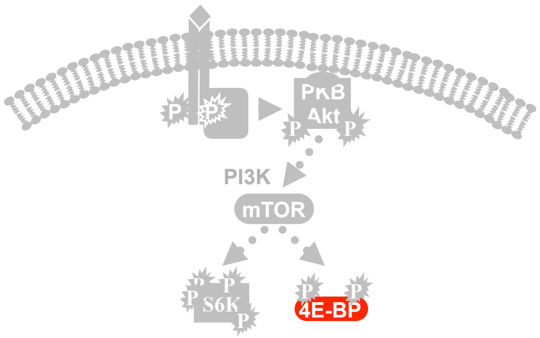


Verstärkt die Translation von mRNAs mit langen, strukturierten 5' UTRs (noch nicht 100% bewiesen)

eEF2 als Target von S6K



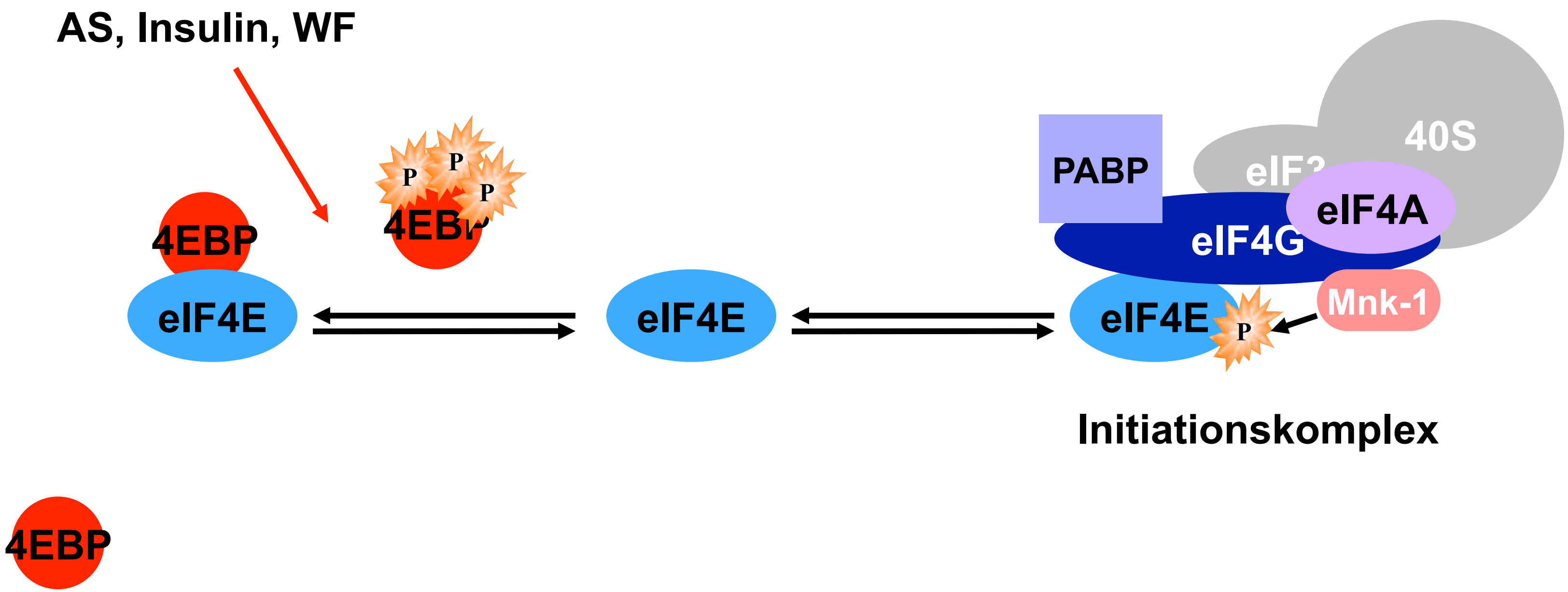
eIF4E-BP I



eIF4E binding protein, translationeller Repressor

3 Isoformen:
4E-BP1, 4E-BP2, 4E-BP3
multiple P (7x) an Thr, Ser streng hierarchisch
zuerst P am N-terminus, dann Mitte , zuletzt C-terminus
(durch mTOR und ERK)

eIF4E-BP II



P 2 Aufgaben: - Disssoziation von 4E
- Verhinderung der Wiederbindung

AA (v.a. Leucin) beeinflussen 4EBP P: keine im Medium: 4EBP deP
Zugabe von AA P von 4EBP
nur bei bestimmten Zelltypen: ovary, embryonic kidney cells

AA (precursor für Proteinsynthese) + Energiequelle (Prot.synthese: ~25% cell. Energie)

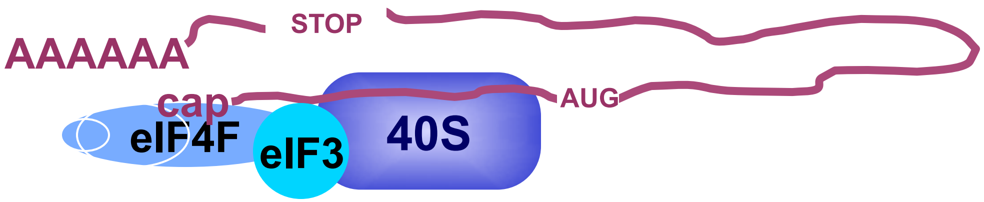
1. Regulation der Translation über Wachstumsfaktoren
2. Regulation über die Nährstoffverfügbarkeit
3. Regulation durch den Energiezustand der Zelle
4. Regulation durch Stress

Nutrients

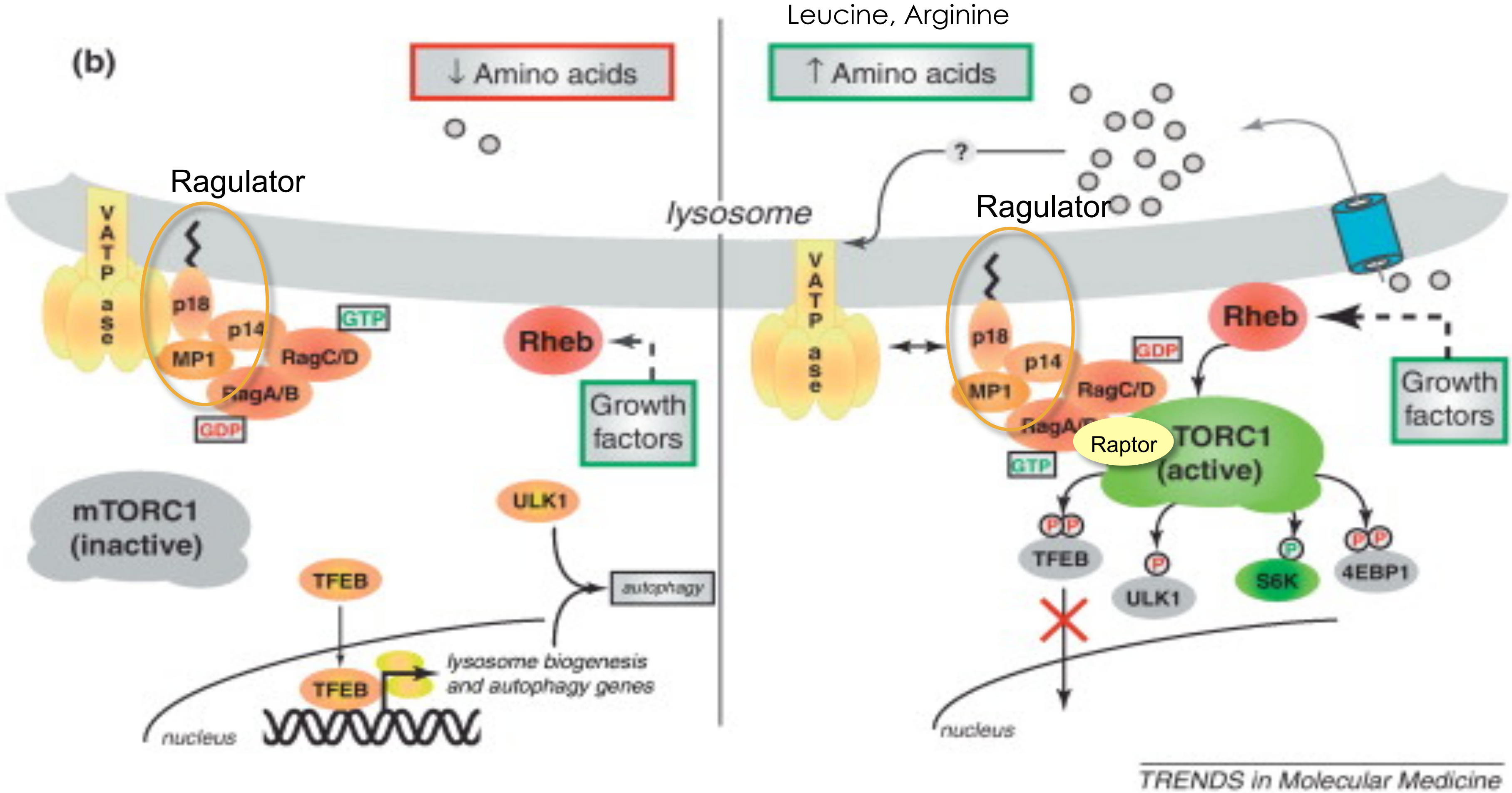
Starvation beeinflußt Translation
Protein Synthese: -AA als precursor
-metabolische Energie

einige Komponenten der Translationsmaschinerie direkt beeinflußt von Nährstoffstatus
(erst seit einigen Jahren bekannt)

Initiation
Elongation
Phosphorylierung der kleinen ribosomalen Untereinheit



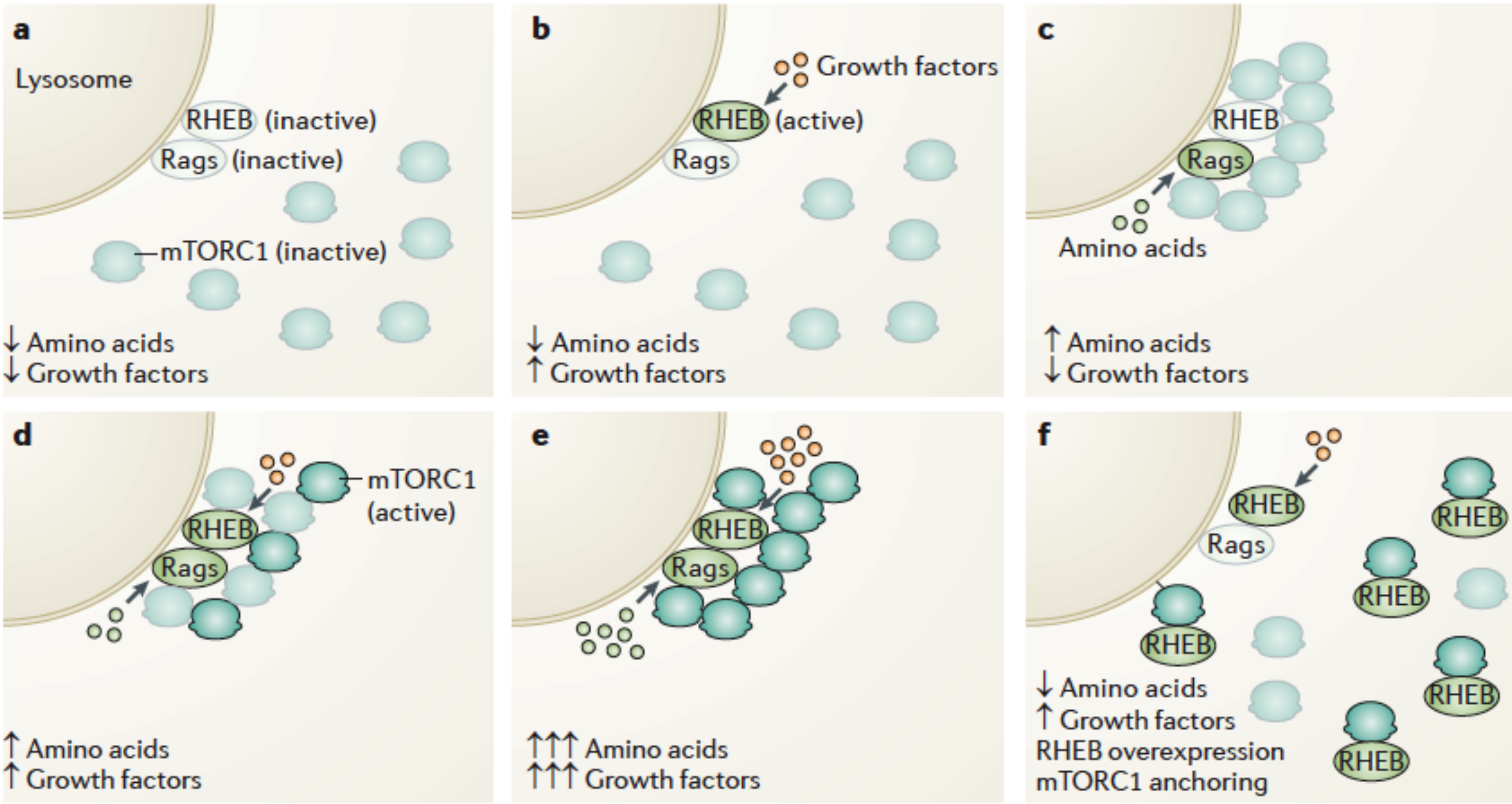
Nutrients



RAG GTPasen notwendig für mTORC1 Aktivierung

Molekularer UND Schalter: wenn Growth Factor signaling an **UND** AS vorhanden dann mTORC Aktivierung

Nutrients

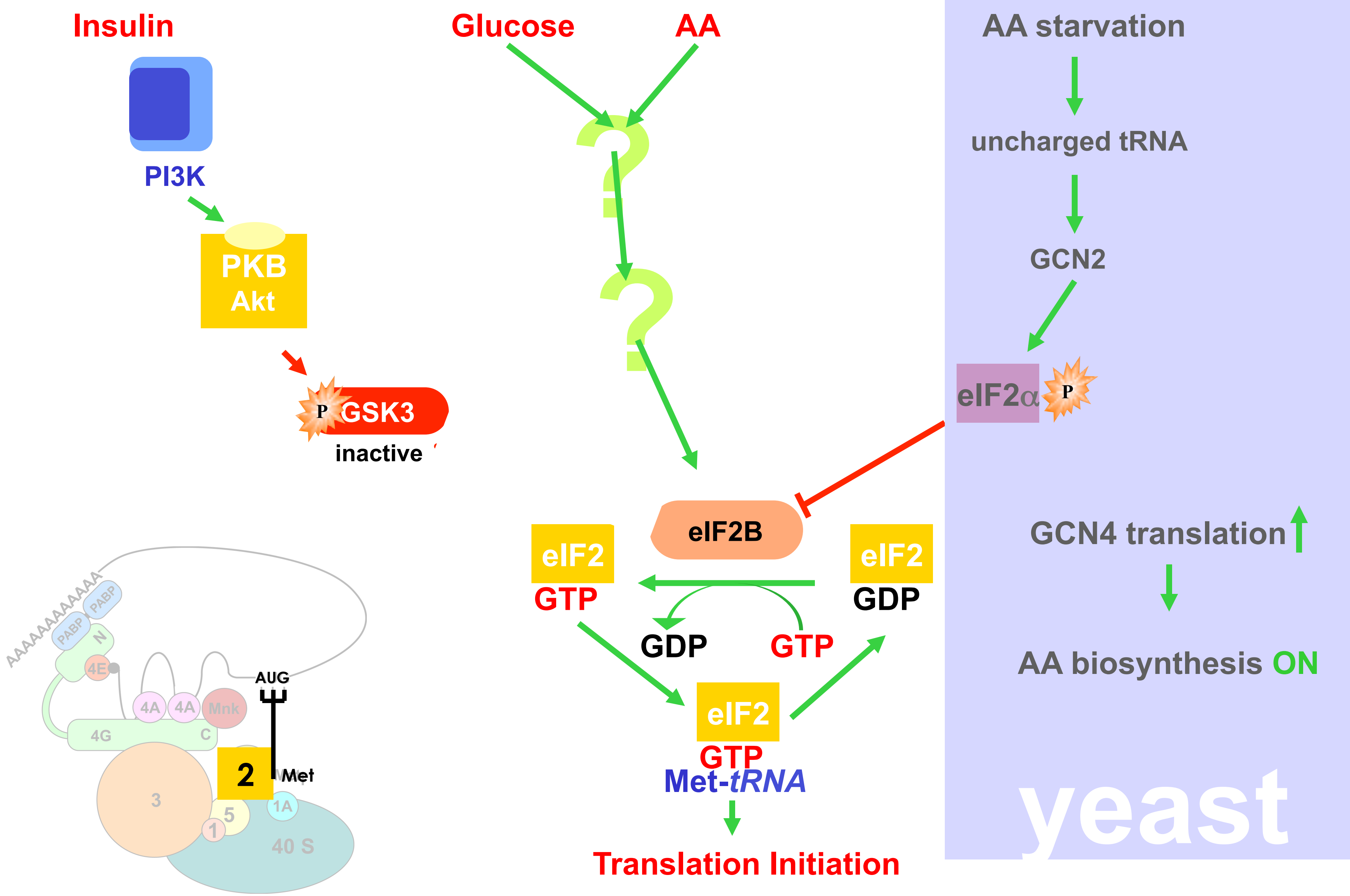


RAG GTPasen notwendig für mTORC1 Aktivierung

mTOR: from growth signal integration to cancer, diabetes and ageing
Roberto Zoncu, Alejo Efeyan and David M. Sabatini
NATURE REVIEWS | MOLECULAR CELL BIOLOGY
VOLUME 12 | JANUARY 2011 | 21

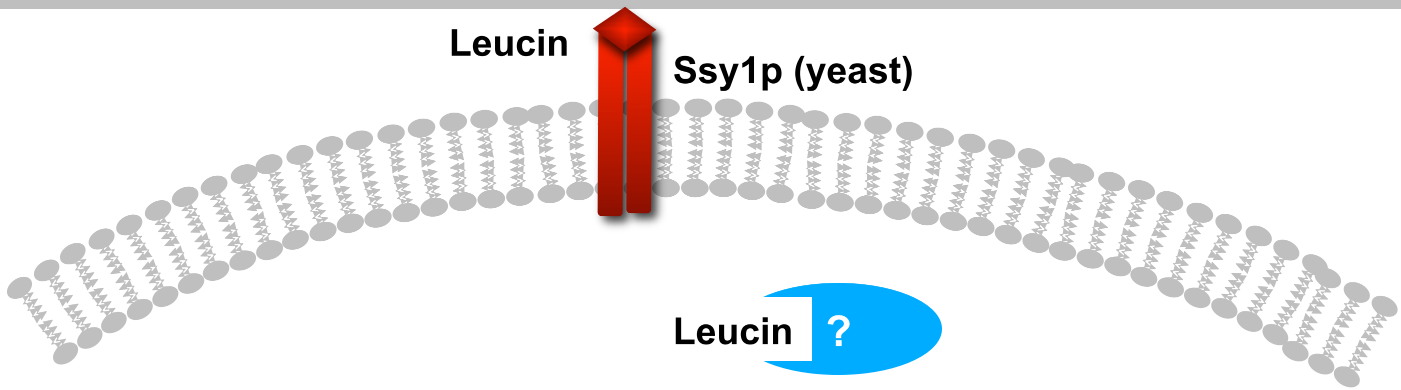
Molekularer UND Schalter: wenn Growth Factor signaling an **UND** AS vorhanden dann mTORC Aktivierung

eIF2



Insulin
PI3K
PKB
Akt
P
GSK3
inactive
Glucose
AA
eIF2B
eIF2
GTP
eIF2
GDP
GDP
GTP
eIF2
GTP
Met-tRNA
Translation Initiation
AA starvation
uncharged tRNA
GCN2
eIF2α
P
GCN4 translation
AA biosynthesis ON
yeast
AAAAAAAAAAAA
PABP
PABP
N
4E
4A
4A
Mnk
4G
C
AUG
2
Met
3
5
1
1A
40 S

AS Sensing



Extracellular Sensor

AA also regulate (repress) autophagy, e.g. in the liver
a non-cell-permeant leucine analogue could still inhibit autophagy
->extracellular leucine

plasma membrane amino acid sensor (Ssy1p) in yeast no homolog in mammals so far

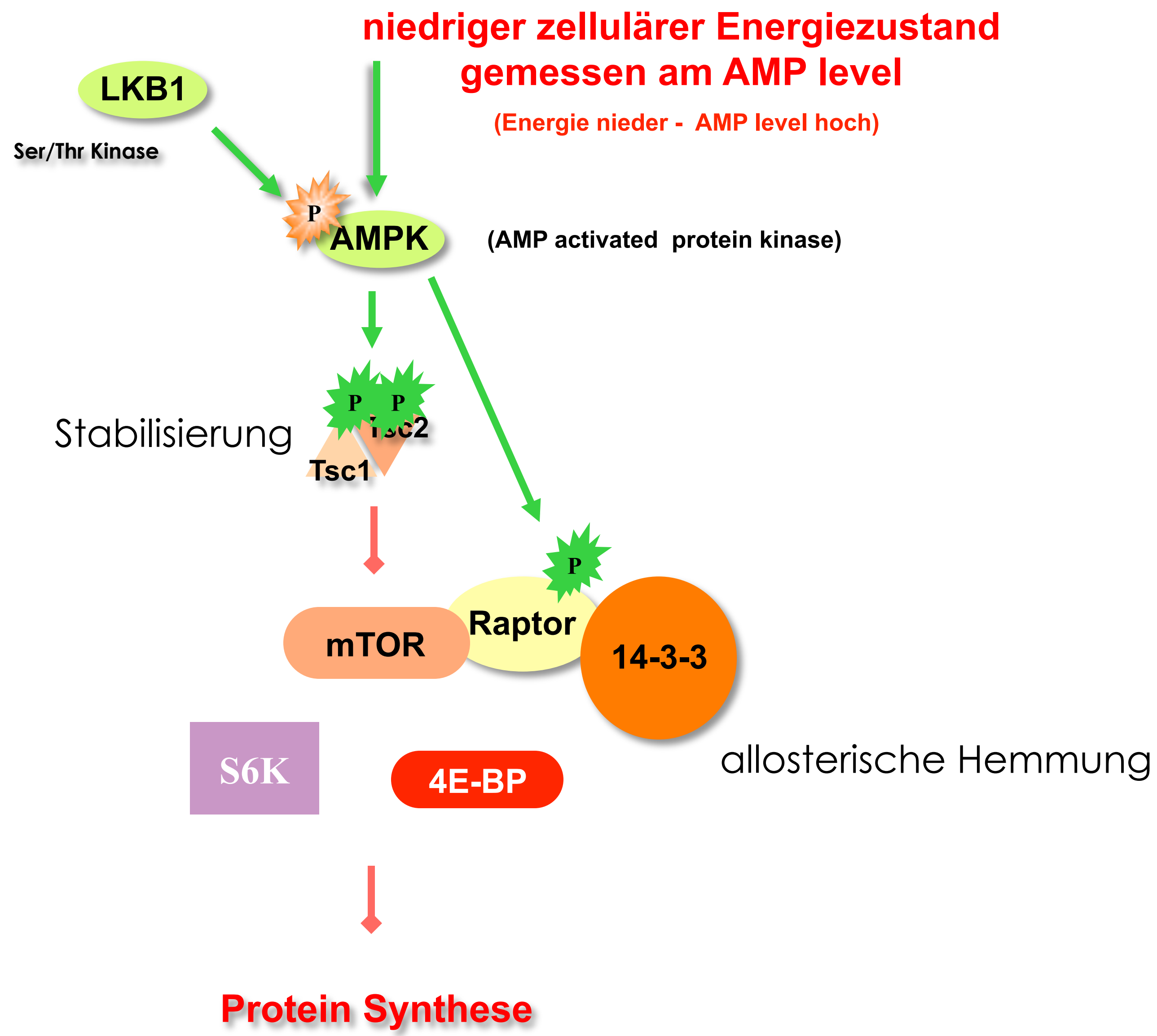
Intracellular Sensor

mTOR may be regulated by intracellular amino acid levels
(C.G. Proud unpublished data)

(a) injection of leucine into Xenopus oocytes activated TOR signalling, P S6K
(b) alteration of intracellular amino acid levels affect mTOR signalling

1. Regulation der Translation über Wachstumsfaktoren
2. Regulation über die Nährstoffverfügbarkeit
3. Regulation durch den Energiezustand der Zelle
4. Regulation durch Stress

Energiezustand



1. Regulation der Translation über Wachstumsfaktoren
2. Regulation über die Nährstoffverfügbarkeit
3. Regulation durch den Energiezustand der Zelle
4. Regulation durch Stress

Stress

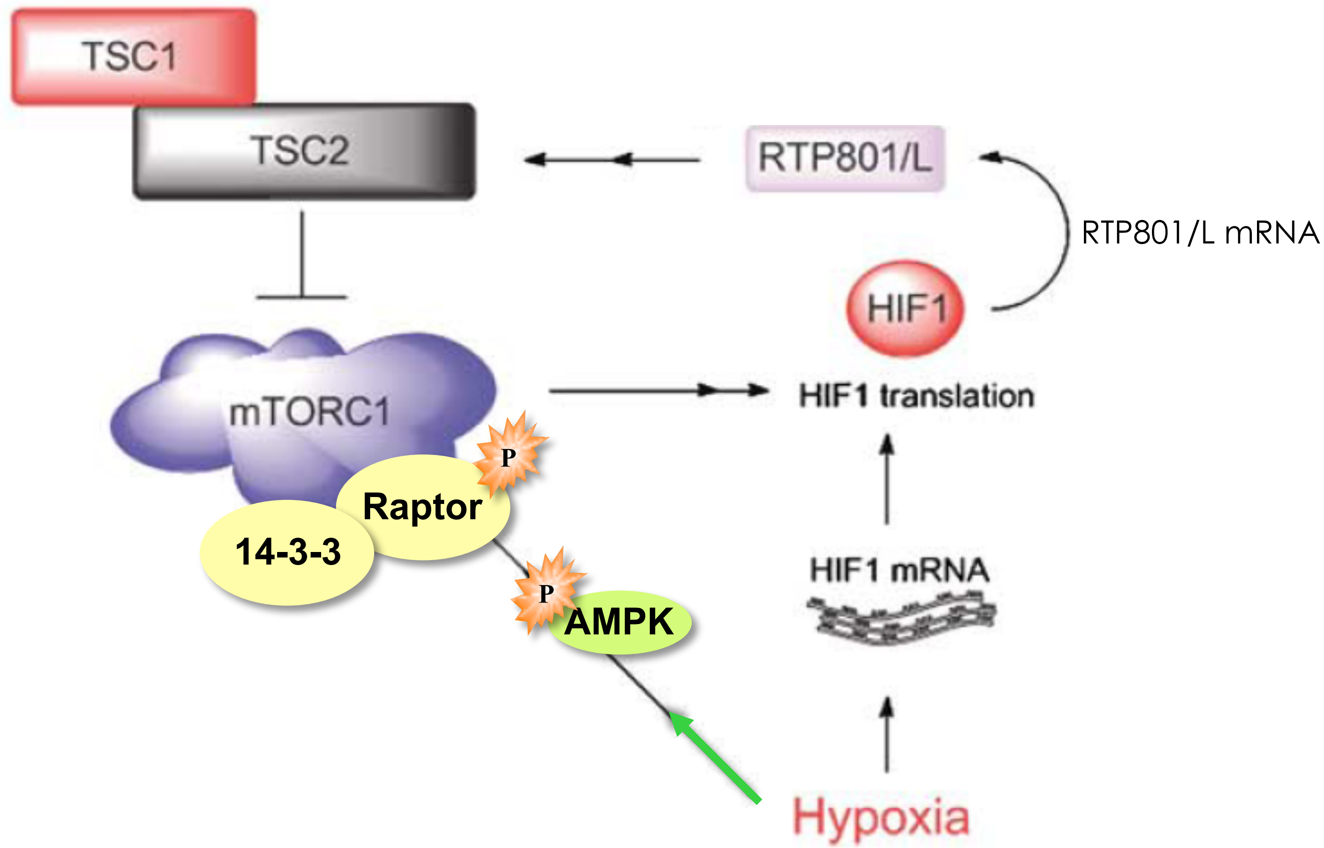
- Hypoxie
- Osmotischer Stress
- ROS
- DNA damage
- Strahlung (UV+Ionisierende)
- Viren

schalten Translation ab

DNA damage

- p53 dependent
- TSC2 and PTEN upregulation (PI3K Akt pathway deactivation)
- AMPK activation

Hypoxia


TSC1
TSC2
RTP801/L
RTP801/L mRNA
HIF1
mTORC1
HIF1 translation
P
Raptor
14-3-3
P
AMPK
HIF1 mRNA
Hypoxia

Zusammenfassung

- **Translation ist ein stark energieverbrauchender Vorgang**
  1/4 der gesamten zellulären Energie gebraucht

- **Translationsrate ist von Wachstumsfaktoren, Energiezustand der Zell, Nährstoffverfügbarkeit (AS) und zellulärem Stress abhängig**

  Wachstumsfaktoren induzieren Translation
  Stress, niedriger Energiegehalt und Nährstoffengpass reduzieren Translation

- **Zentraler Pathway: PI3K – Akt/PKB – mTOR - S6K / 4E-BP**

- **Die Regulation der Translationsrate erfolgt vorwiegend über die Translationsinitiation**
  eIF4E – 4E-BP; eIF4B; eIF2

- **Aber auch über Elongation**
  eEF2

- **mTOR: zentrales Molekül, Integration von vielen extrazellulären und intrazellulären Inputs**
  Sensor für AS Status, Energiestatus, Wachstumsfaktoren , Stress

Literatur

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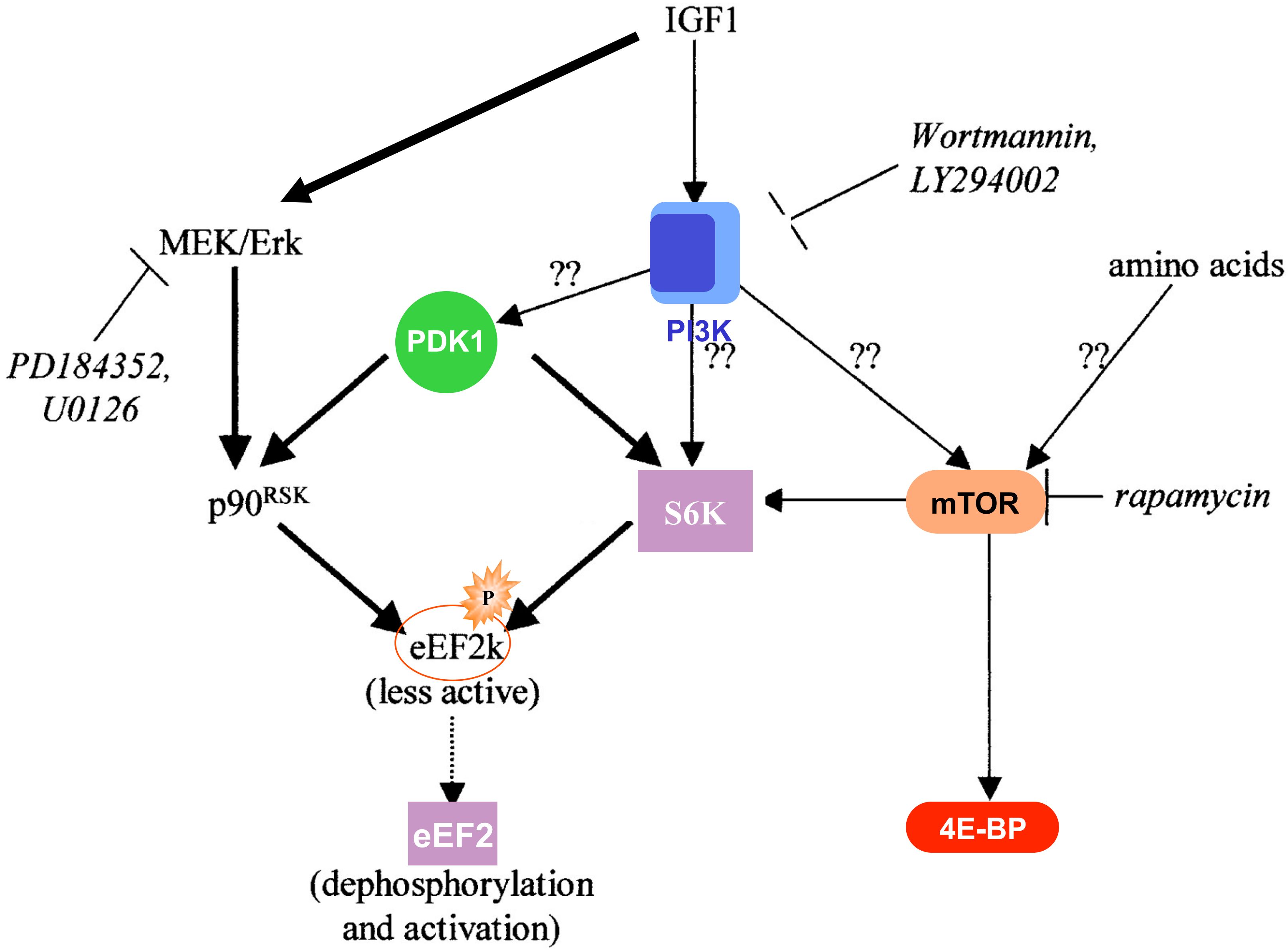
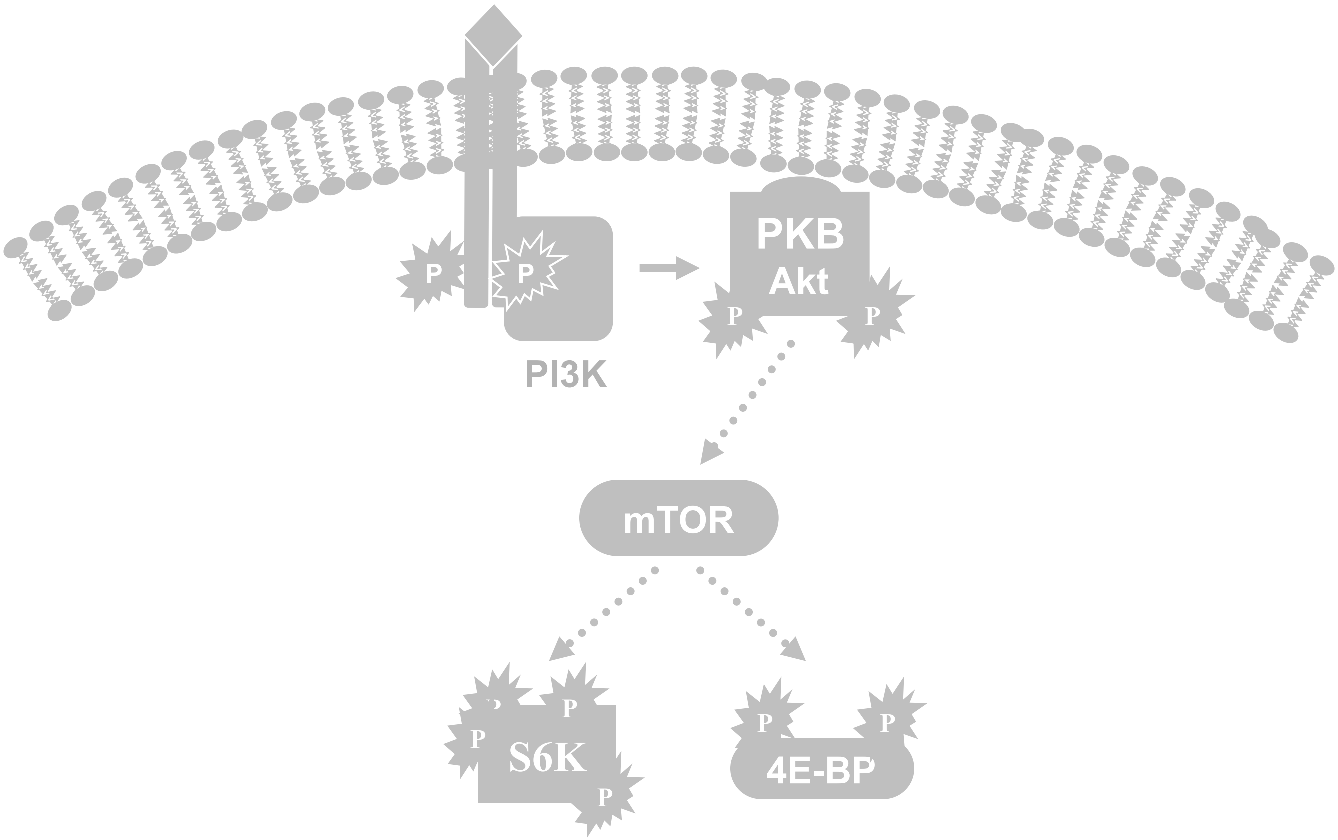
Supplement

eEF2



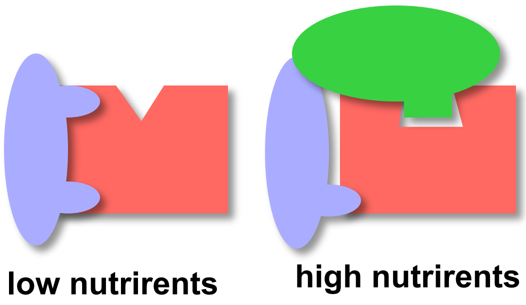
Wang et al, EMBOJ 20, 4370-79 (2001)

Upstream Signalling of Translation: Players

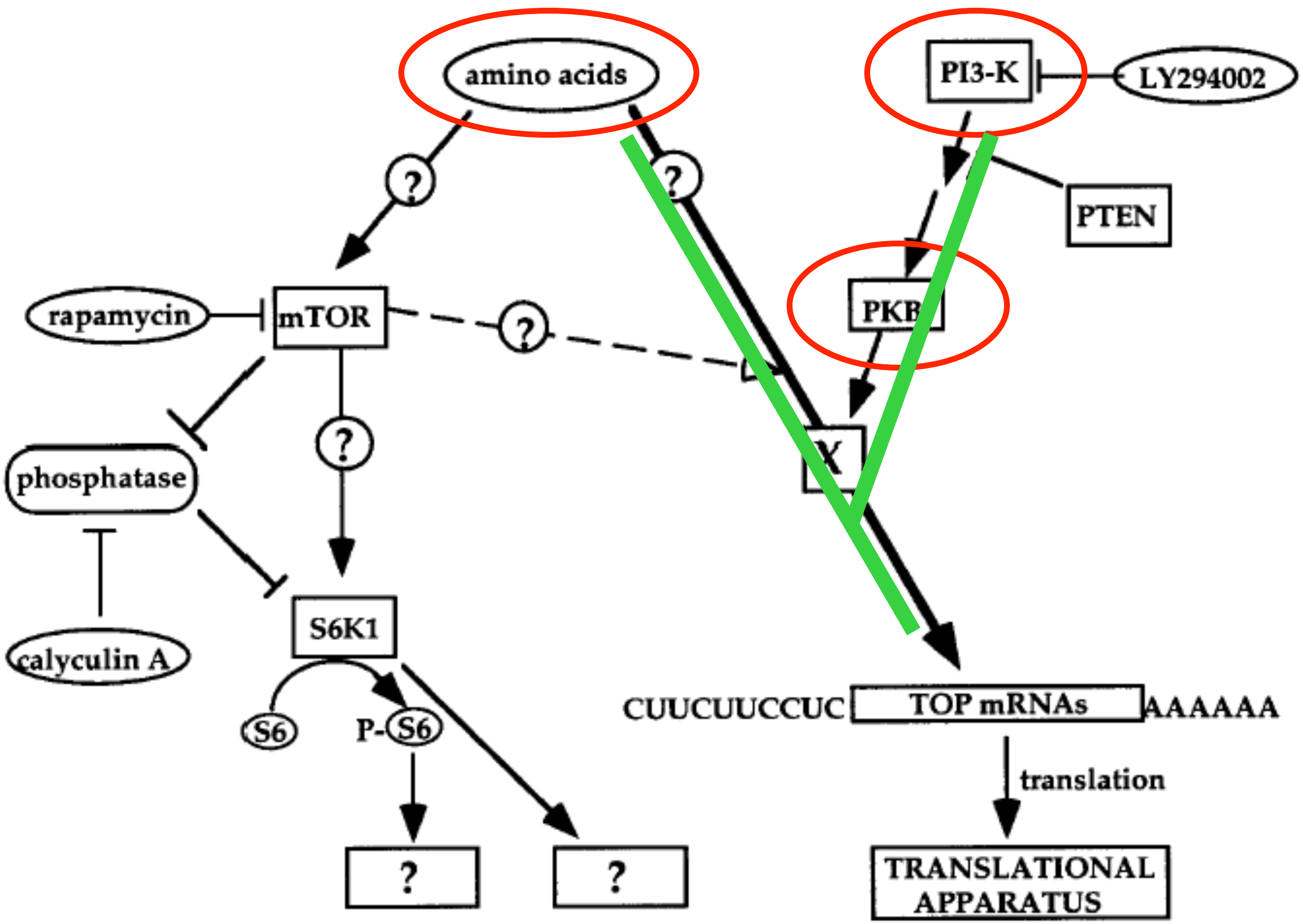


Raptor

Raptor (regulatory associated protein of tor) 150kD,
negativer Regulator von mTOR Kinase Aktivität
aber wird gebraucht für 4EBP, S6K P, scaffold protein



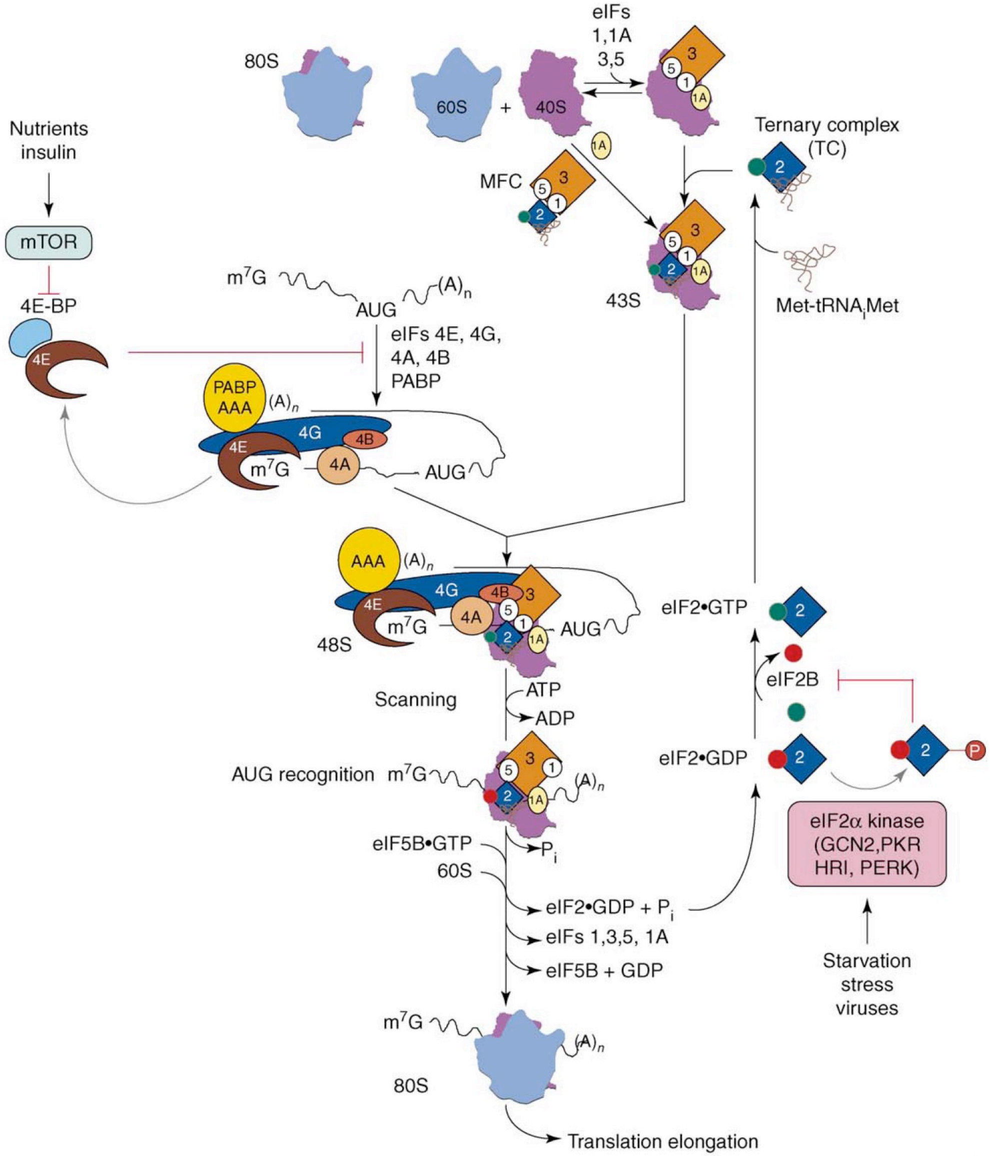
5' TOPs S6 Kinase Independence4



TOP mRNA translation abhängig von: AA, PI3K u. PKB
unabängig S6K or S6P (S6K KO, S6K overexpression)

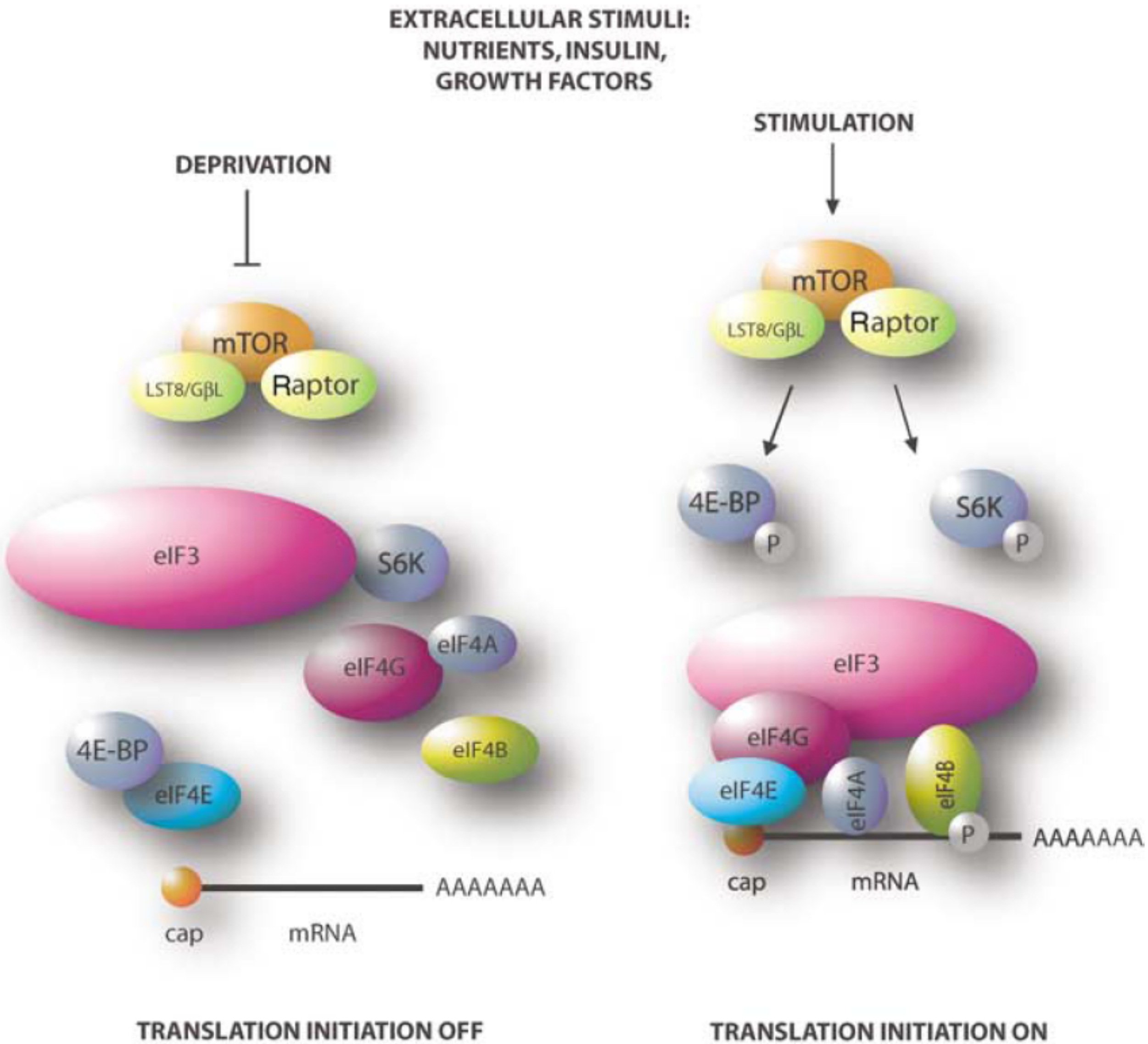
Tang et al, MCB 21, 8671-83 (2001)

5' TOPs S6 Kinase Independence



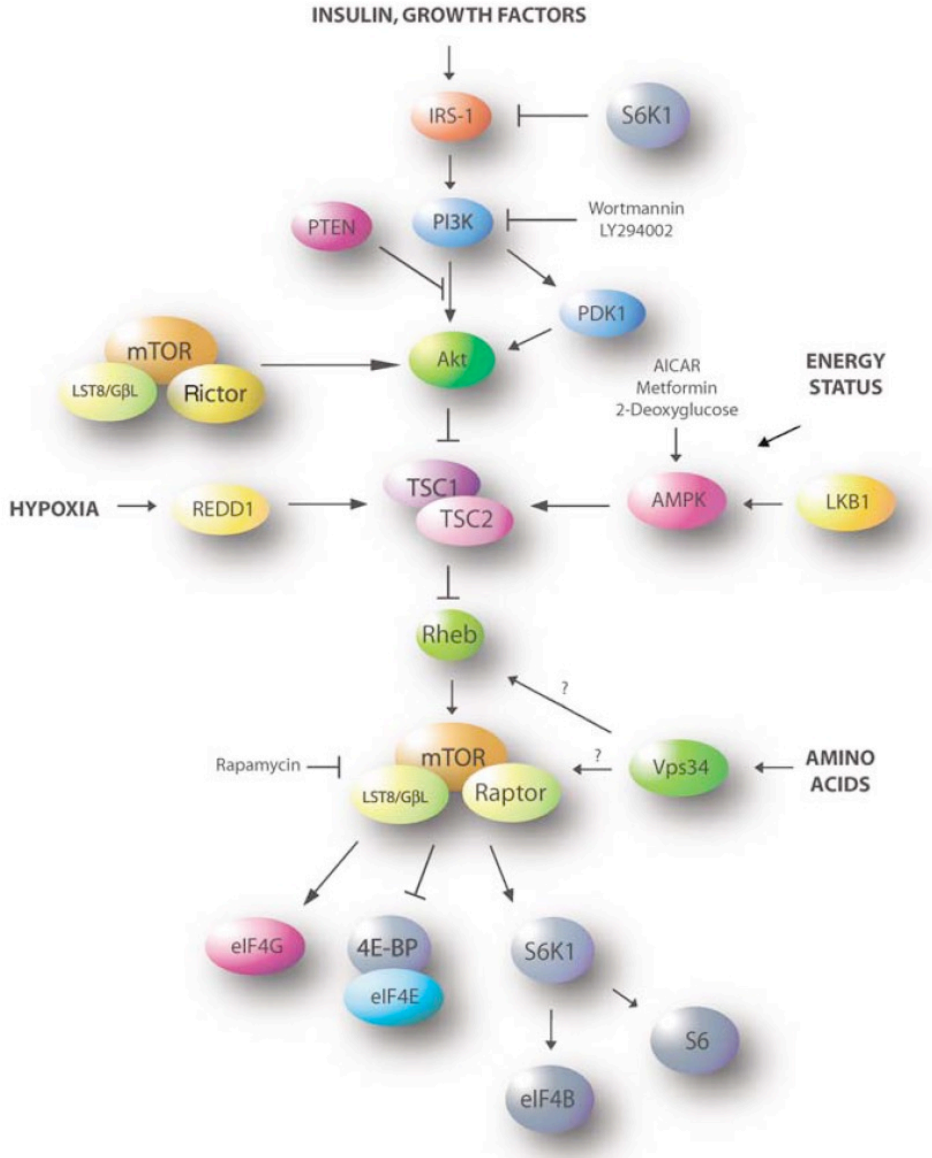
Tang et al, MCB 21, 8671-83 (2001)

mTOR II



EXTRACELLULAR STIMULI:
NUTRIENTS, INSULIN,
GROWTH FACTORS
DEPRIVATION
STIMULATION
mTOR
LST8/GβL
Raptor
mTOR
LST8/GβL
Raptor
4E-BP
P
S6K
P
eIF3
S6K
eIF4G
eIF4A
4E-BP
eIF4E
eIF4B
cap
AAAAAAA
mRNA
eIF3
eIF4G
eIF4E
eIF4A
eIF4B
P
AAAAAAA
cap
mRNA
TRANSLATION INITIATION OFF
TRANSLATION INITIATION ON

mTOR II


INSULIN, GROWTH FACTORS
IRS-1
S6K1
PTEN
PI3K
Wortmannin
LY294002
PDK1
mTOR
LST8/GβL
Rictor
Akt
AICAR
Metformin
2-Deoxyglucose
ENERGY
STATUS
HYPOXIA
REDD1
TSC1
TSC2
AMPK
LKB1
Rheb
?
?
Rapamycin
mTOR
LST8/GβL
Raptor
Vps34
AMINO
ACIDS
eIF4G
4E-BP
eIF4E
S6K1
S6
eIF4B