Organelle signaling

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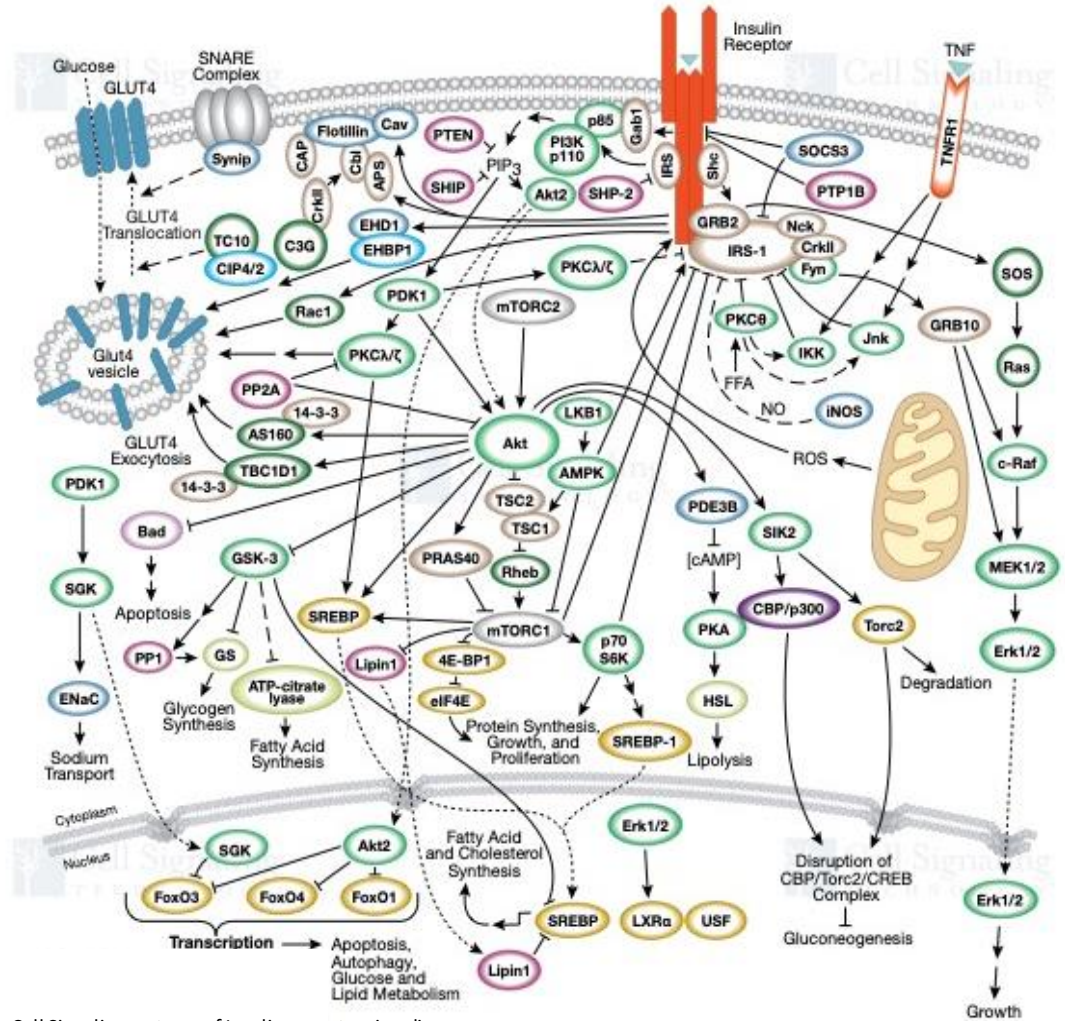
Lysosomal
membrane repair

Michael Ebner

The pervasive (and over-simplified) view of signal transduction

To gain biomass, cells scale protein synthesis and lipid synthesis to grow and enlarge organelles

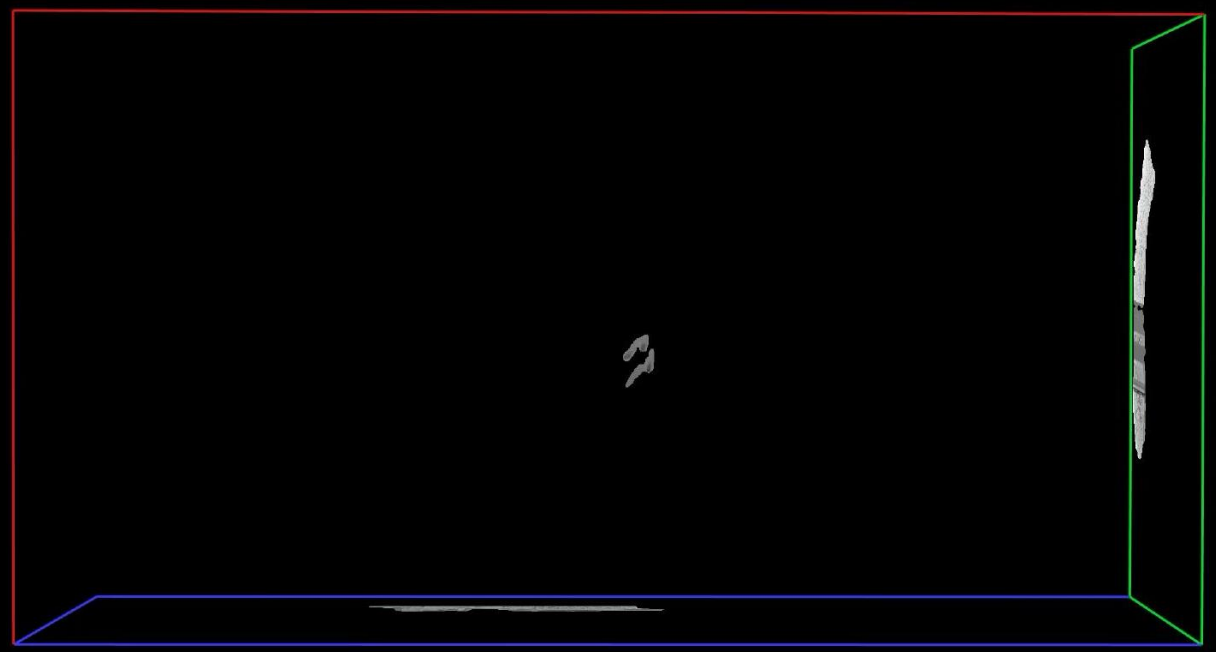
Insulin receptor signaling

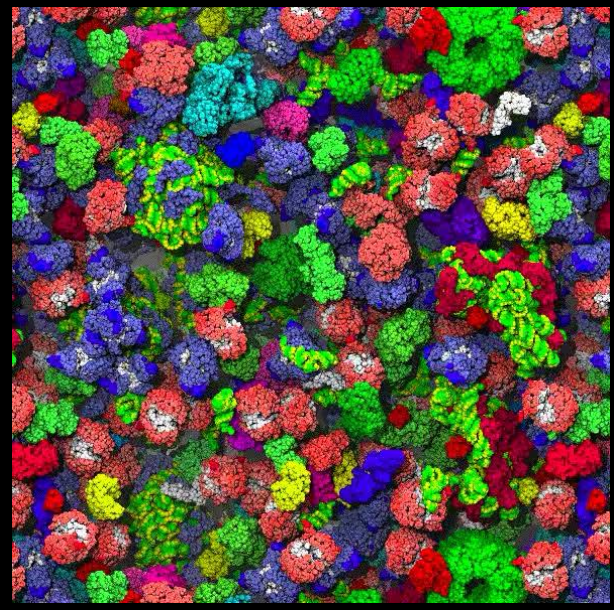


Cell Signaling cartoon of Insulin receptor signaling

For a long time, these models have not taken into consideration cellular architecture

Increasing understanding of cellular architecture





cytoplasm is very crowed

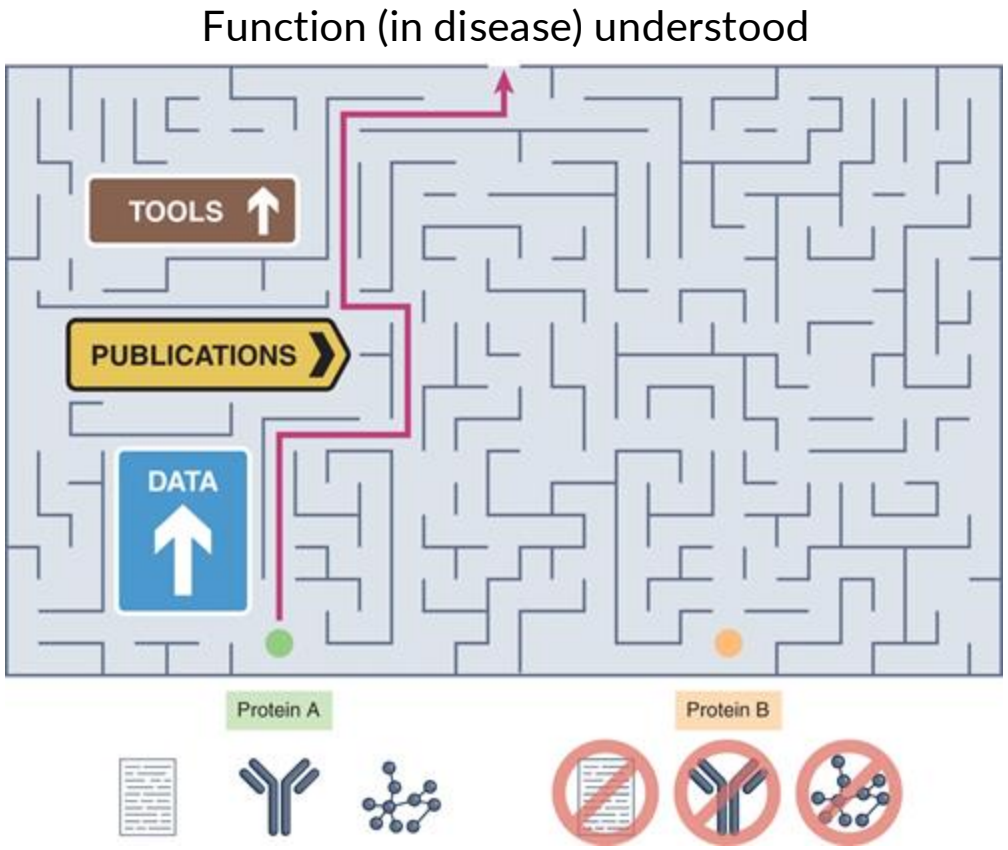
The cellular protein concentration is ~ 200 mg/ml.
That's a few 10^9 protein molecules / human cell
28 - 36 x 10^{12} cells in the human body

Heinrich et al Nature 2021

The understudied proteins challenge

Massive bias in the functional characterisation of the human proteome:

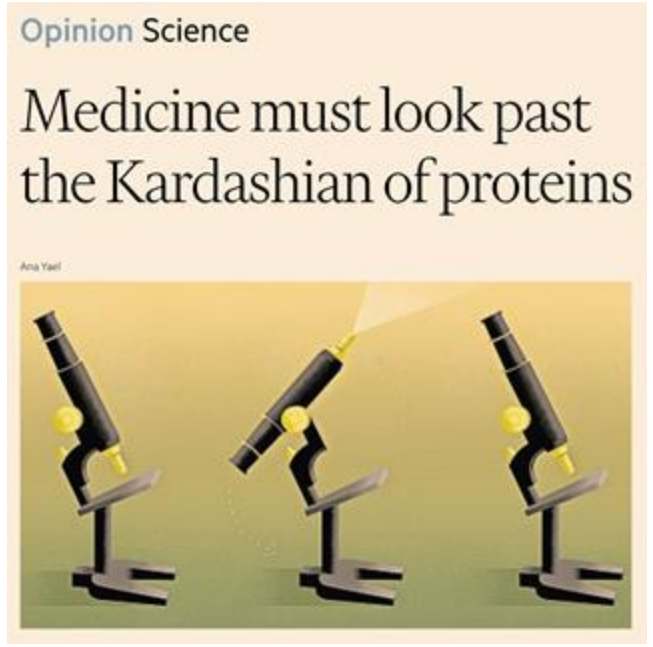
- 2 publications per day on p53 - nothing on 1,000s of others
- 95% of publications on 5,000 human proteins
- Much worse in non-model organisms



Kustatscher, Collins, Gingras, Guo, Hermjakob, Ideker, Lilley, Lundberg, Marcotte, Ralser, Rappsilber, *Nat Methods*, 2022

Are understudied proteins less important?

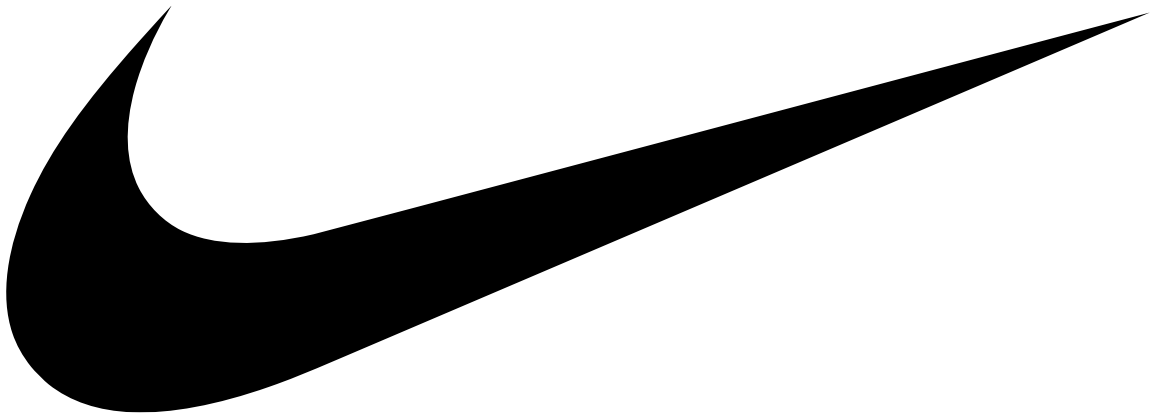
- 18% essential genes in human cell lines
- 31% essential genes in synthetic minimal bacterium
- Many understudied proteins in rare diseases
- Rare genetic variants drive common diseases, e.g. cancer
- 10% of druggable proteins targeted by FDA-approved drugs



Financial Times, 27.07.2022

Wang et al, Science, 2015; Hutchison, et al, Science, 2016; Stoeger & Amaral, eLife, 2020; Oprea et al, Nat Rev Drug Discov, 2018

There is so much to discover for you!



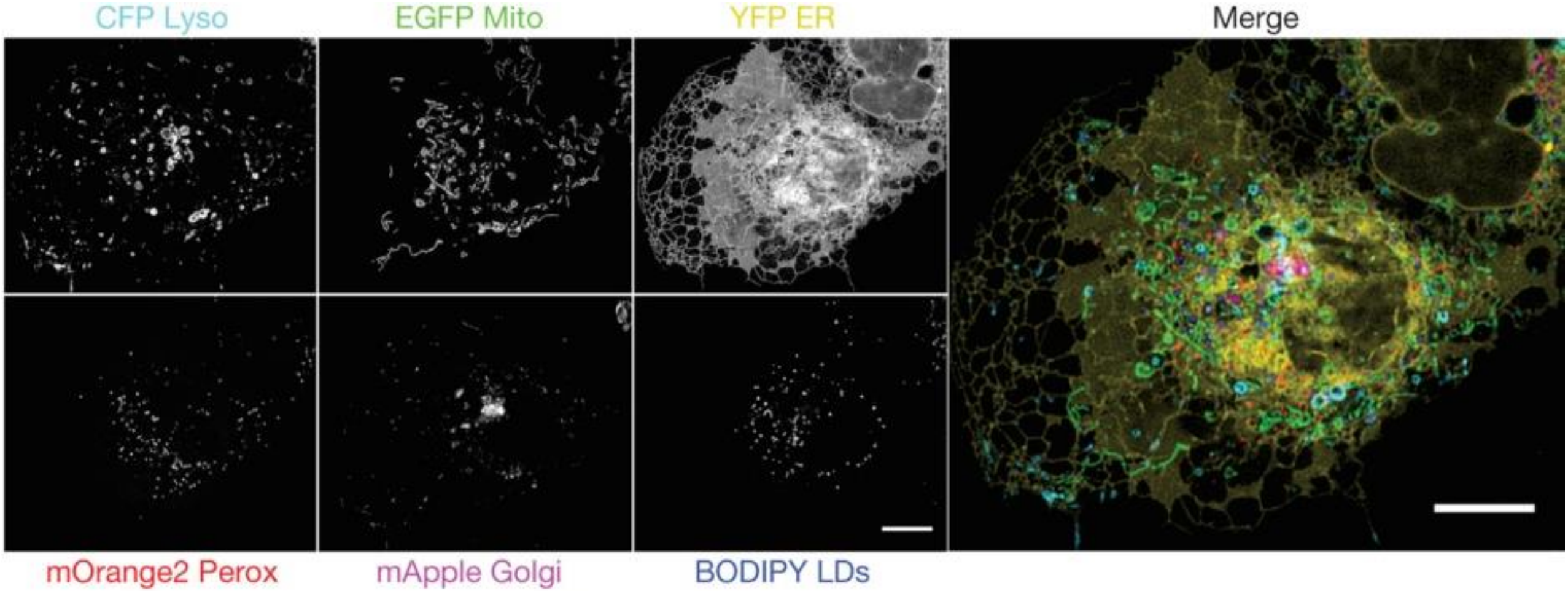
Cell signaling is initated at different organelles

- Plasma membrane
- Mitochondria
- Endoplasmic reticulum
- Lysosomes
- Other less well understood

How does organelle signaling contribute to cellular function?

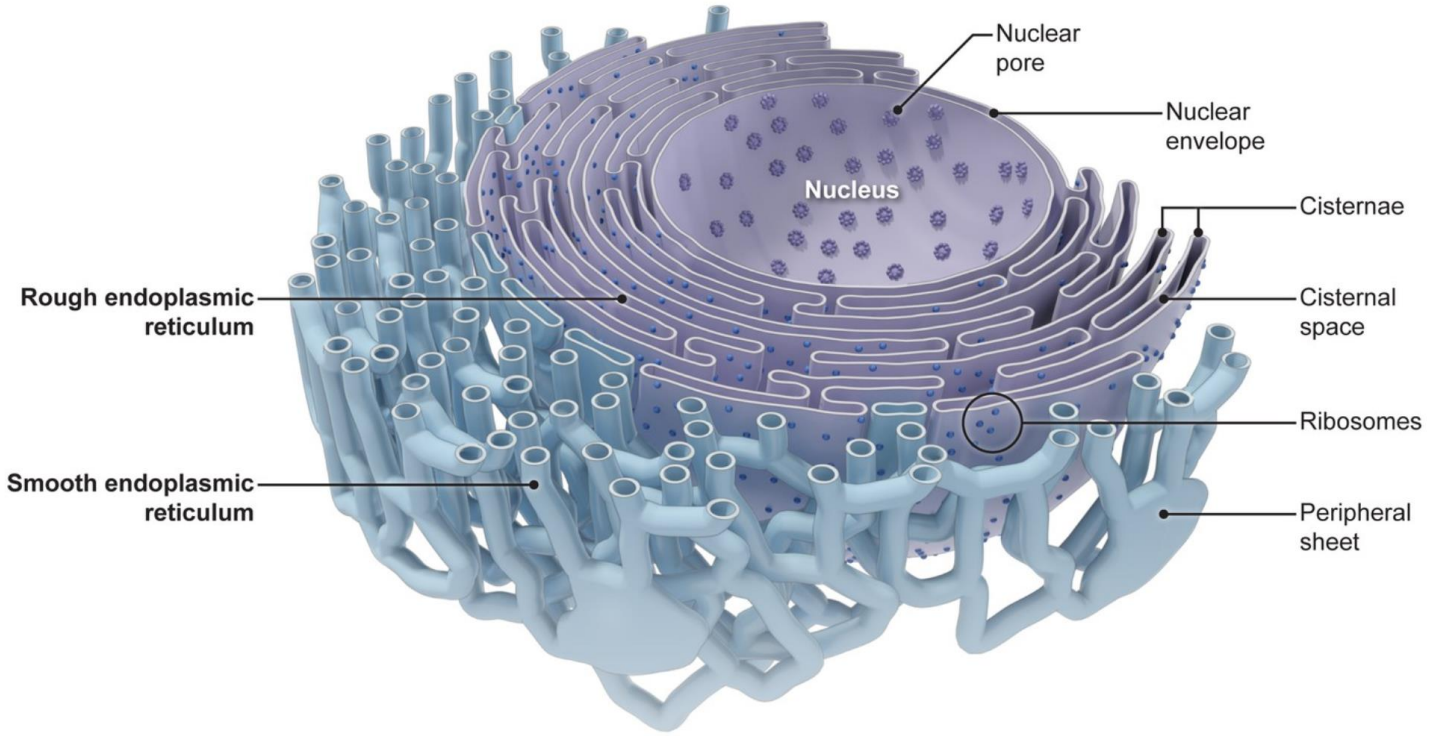
Signaling from two seeminlgy ‚antagonisitic‘ organelles

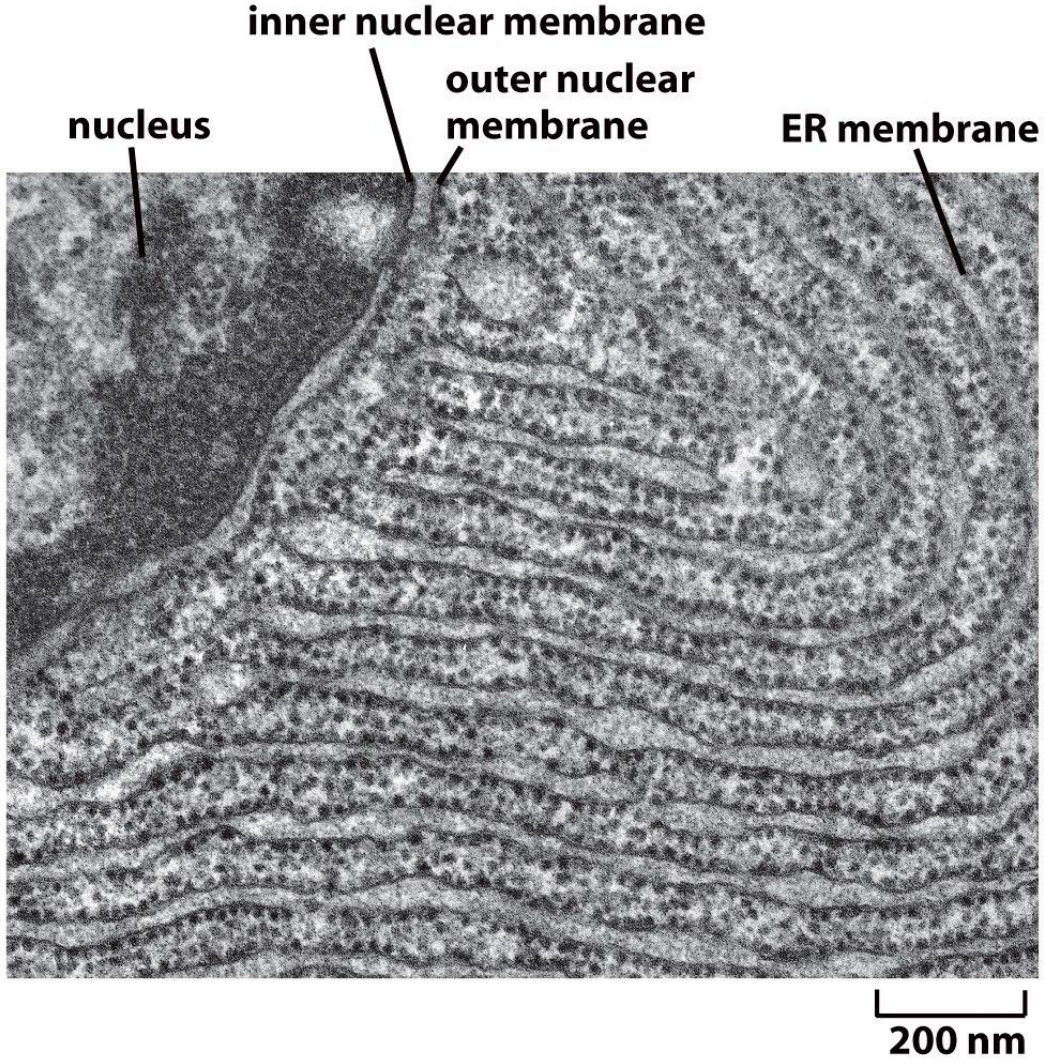
1. Endoplasmic reticulum (ER): Anabolic organelle -> protein & lipid synthesis
2. Lysosomes: Catabolic organelles -> protein & lipid degradation



ER signaling as a paradigm for organelle signaling

Biosynthetic organelle & a major site for protein and lipid synthesis





Sheets: translation (rER)
tubules: transport & lipid synthesis (smooth ER)

occupies approx. 10–20% of the cellular volume

ER signaling as a paradigm for organelle signaling

How do cells monitor the integrity of the ER?

How do cells adapt the protein & lipid content at the ER?

How do cells report the status of the ER to the nucleus?

The unfolded protein response (UPR) functionally links these processes

UPR and its role in diseases

UPR components

Table 1. **Physiological functions of UPR components in mouse models and their genetic association with human disease**

Gene	Factors that regulate expression	Phenotypes of knockout mouse model	Genetic association with human diseases	References
IRE1α	N.A.	(1) Embryonic lethality at E12.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia	(1) Human somatic cancers	Zhang et al., 2005, 2011; Greenman et al., 2007
XBP1s	XBP1s and ATF6α	(1) Embryonic lethality at E13.5 due to liver hypoplasia; (2) Liver deletion: hypolipidemia; (3) Intestinal epithelial cell deletion: enteritis; (4) Pancreatic acinar cell deletion: extensive pancreas regeneration; (5) Pancreatic β cell deletion: hyperglycemia; (6) Neuron deletion: leptin resistance	(1) Inflammatory bowel disease; (2) Schizophrenia in the Japanese population; (3) Bipolar disorder; (4) Ischemic stroke	Kakiuchi et al., 2003b, 2004; Kaser et al., 2008; Yilmaz et al., 2010
ATF6α	N.A.	(1) Susceptible to pharmacologically induced ER stress	(1) Type 2 diabetes and pre-diabetic traits; (2) Increased plasma cholesterol levels	Chu et al., 2007; Wu et al., 2007; Meex et al., 2009
CREBH	PPARα, HNF4α, and ATF6α	(1) Hypoferremia and spleen iron sequestration; (2) Hyperlipidemia; (3) Liver knockdown: fasting hyperglycemia	(1) Extreme hypertriglyceridemia	Zhang et al., 2006; Vecchi et al., 2009; J.H. Lee et al., 2011
PERK	N.A.	(1) Neonatal hyperglycemia	(1) Wolcott-Rallison syndrome; (2) Supranuclear palsy	Delépine et al., 2000; Höglinger et al., 2011
ATF4	CHOP	(1) Delayed bone formation; (2) Severe fetal anemia; (3) Increased insulin sensitivity; (4) Defects in long-term memory	N.A.	Elefteriou et al., 2006; Costa-Mattioli et al., 2007; Yamaguchi et al., 2008
CHOP	ATF4 and ATF6α	(1) Protected from pharmacologically induced ER stress; (2) Protected from type 2 diabetes; (3) Protected from atherosclerosis; (4) Protected from leukodystrophy	(1) Early-onset type 2 diabetes in Italians	Oyadomari et al., 2002; Marciniak et al., 2004; Silva et al., 2005; Gragnoli, 2008; Song et al., 2008

Unfolded protein response - UPR

The UPR monitors the proteome and lipidome of the ER and prevents defects that jeoparidize ER integrity.

To do so the UPR sends a signal from the lumen of the ER to

(I) the nucleus to change the transcriptional program
(II) ribosomes to change/dampen translation (and in turn change transcription)

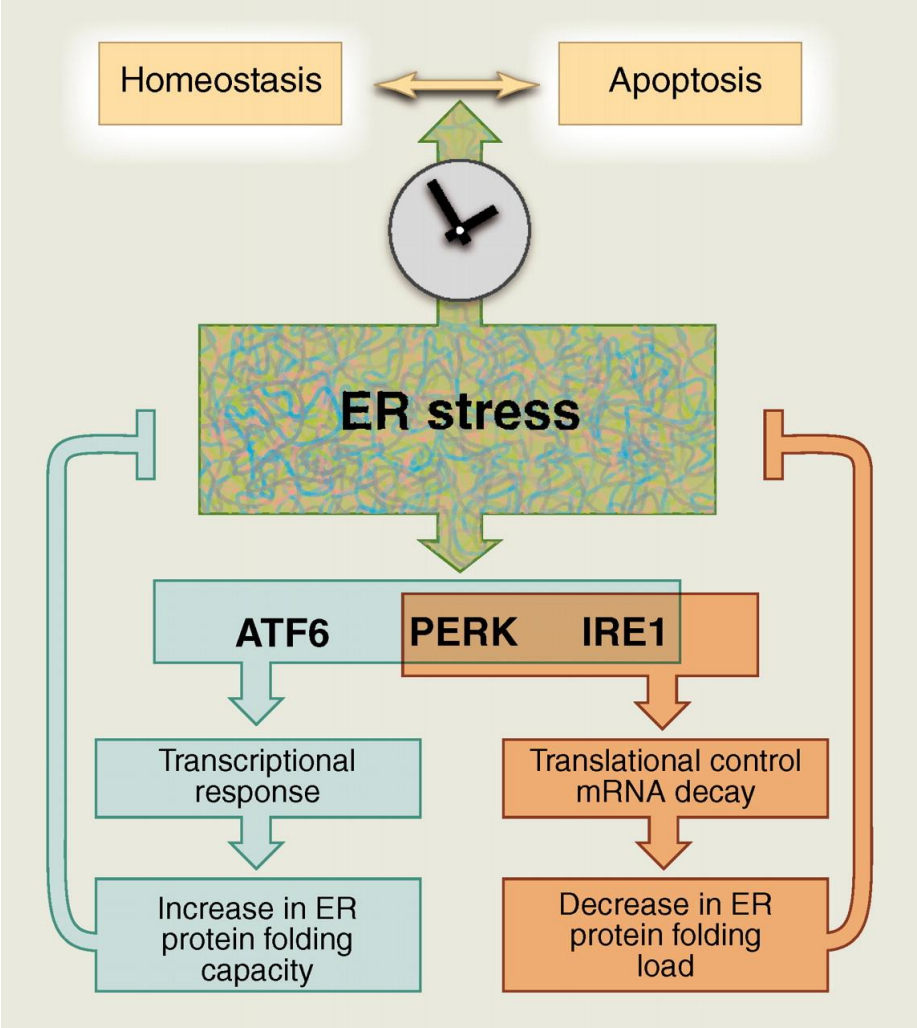
Very generally speaking the UPR has two possible outcomes:

1. Homeostatic UPR activation implements adaptive programs that modulate, augment and finally resolve ER stress.
2. Maladaptive and/or chronic UPR outputs triggers pro-inflammatory and pro-death signals

How can the UPR transmit a signal from the lumen of ER to other organelles?

There are three distinict UPR branches in human cells

(1) ATF6, (2) PERK, (3) IRE1

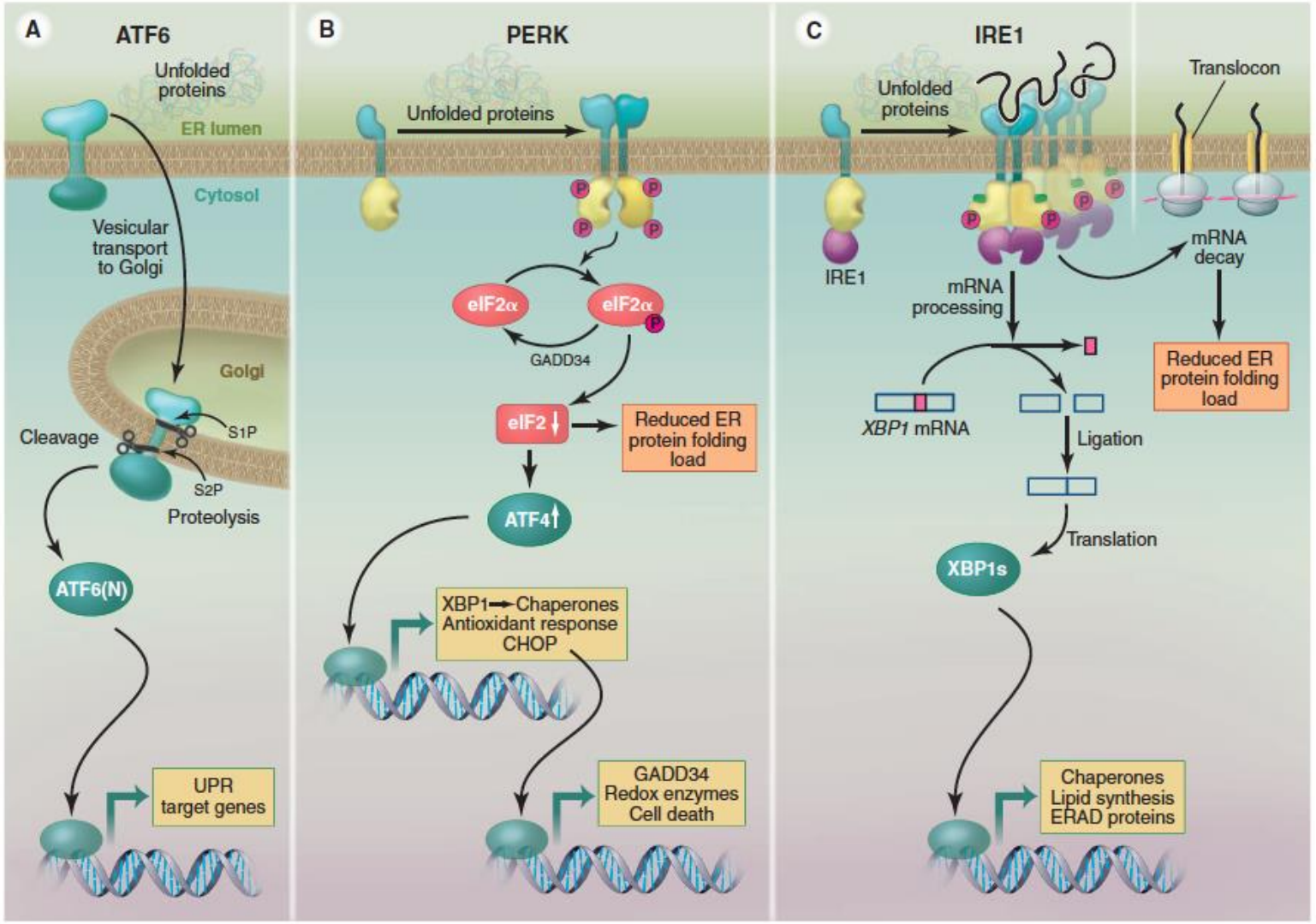


ATF6 (Activating transcription factor 6)
PERK (Protein Kinase RNA-Like ER Kinase)
Ire1 (Inositol-requiring Enzyme 1)

DOI: 10.1126/science.1209038

The three UPR pathways

regulated Ire1-dependent decay (RIDD)



sensor component

signal relay

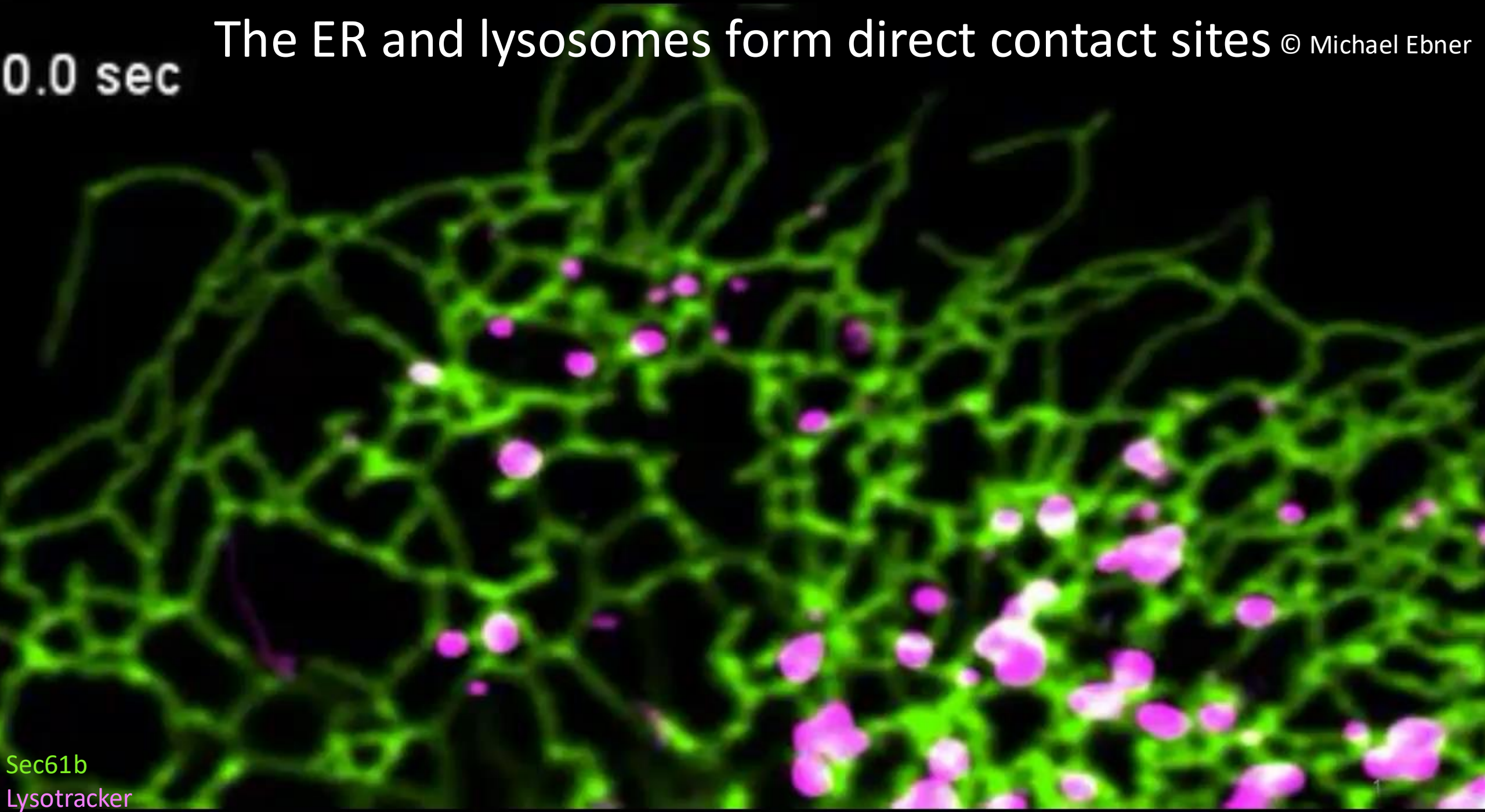
effector activation

translational adaptation

transcriptional adaptation

Fig. 2. (**A** to **C**) The three branches of the UPR. Three families of signal transducers (ATF6, PERK, and IRE1) sense the protein-folding conditions in the ER lumen and transmit that information, resulting in production of bZIP transcription regulators that enter the nucleus to drive transcription of UPR target genes. Each pathway uses a different mechanism of signal transduction: ATF6 by regulated proteolysis, PERK by translational control, and IRE1 by nonconventional mRNA splicing. In addition to the transcriptional responses that largely serve to increase the protein-folding capacity in the ER, both PERK and IRE1 reduce the ER folding load by down-tuning translation and degrading ER-bound mRNAs, respectively.

The ER and lysosomes form direct contact sites



Lysosome signaling

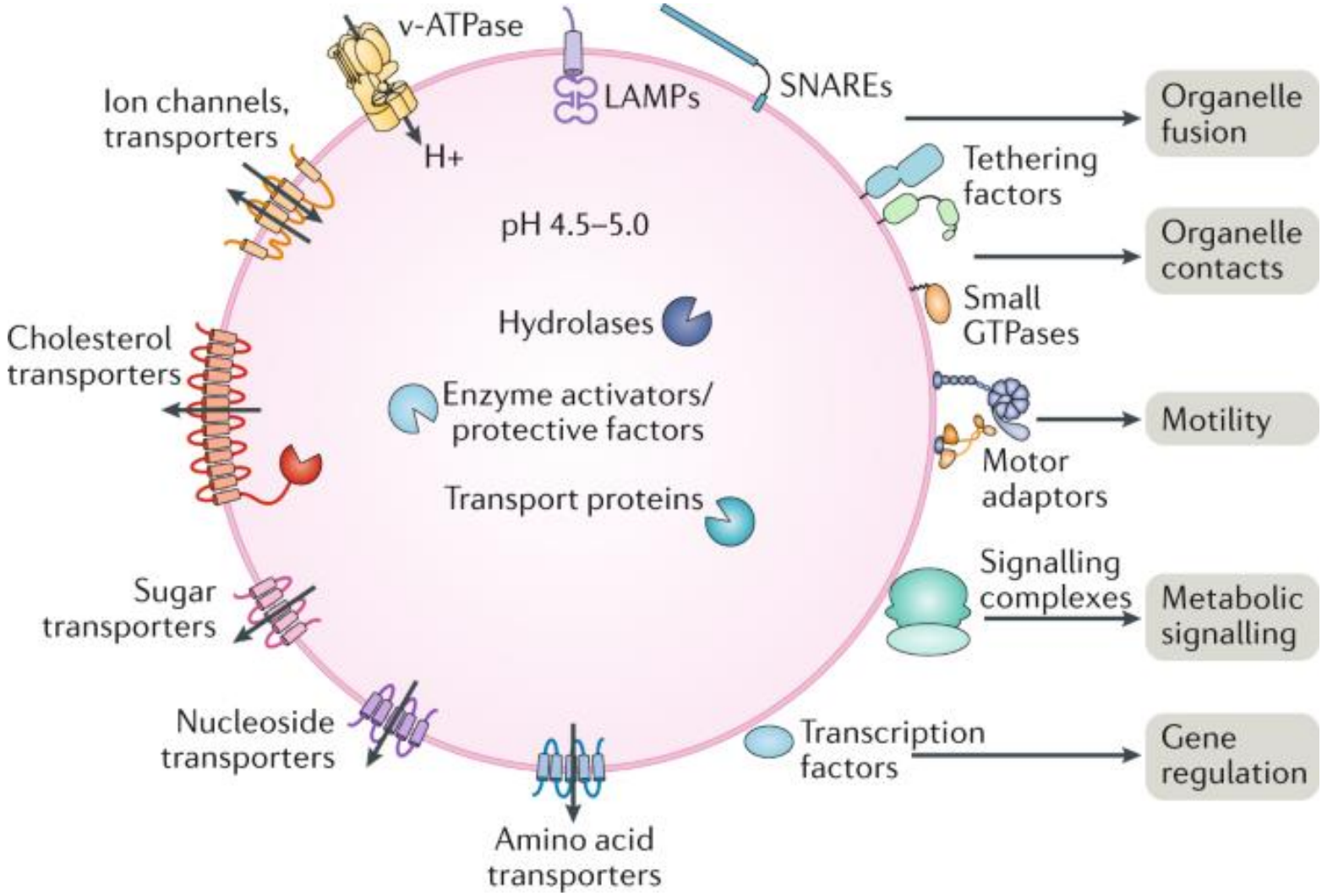
How do cells monitor the integrity of the lysosomes?

How do cells adapt the protein & lipid content of lysosomes and control the number of lysosomes?

How do cells signal from the lysosomes into the nucleus?

The answers to these questions are only partially clear!

Lysosome function



Defects in lysosome function cause neurodegeneration

doi.org/10.1038/s41580-019-0185-4

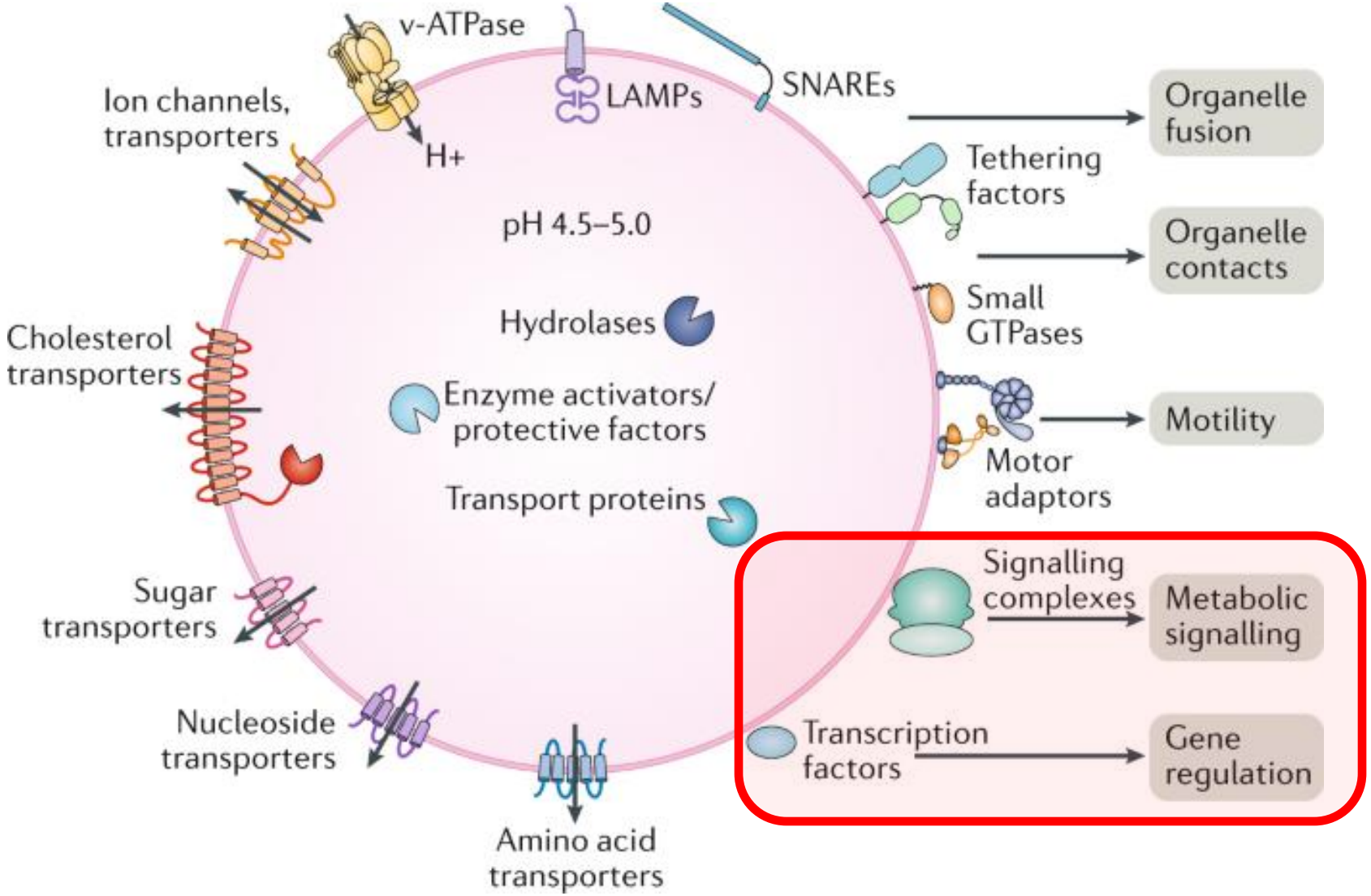
The awesome lysosome

Andrea Ballabio[1,2,3,4]

In the early 50s, Christian De Duve identified a new cellular structure, the lysosome, defined as the cell's "suicide bag" (de Duve, 2005). Sixty years later, it is clear that the lysosome greatly exceeded the expectations of its discoverer. Over 50 different types of lysosomal storage diseases have been identified, each due to the deficiency or malfunction of a specific lysosomal protein. In addition, an important role of the lysosome has been unveiled in several common human diseases, such as cancer, obesity, neurode- generative diseases, and infection. Recent studies have led to the identification of a lysosome-to-nucleus signaling pathway and a lysosomal gene network that regulate cellular clearance and energy metabolism. These observations have opened a completely new field of research and changed our traditional view of the lysosome from a dead-end organelle to a control center of cell metabolism.

DOI 10.15252/emmm.201505966

Lysosomes are major signaling organelles



major site of TOR (target of rapamycin) signaling & hence cell growth regulation

Discovery of Rapamycin

Georges Nógrády conducted bio-prospecting of soil: he wanted to understand why inhabitants of Rapa Nui did not get tetanus -> did not find an answer but gave the soil sample to a company called Ayerst Pharmaceuticals - now Pfizer. They isolated from a fungus, Rapamycin (a macrolide) that was used as an immunosuppressant

14 March 1991; accepted 25 June 1991

Targets for Cell Cycle Arrest by the Immunosuppressant Rapamycin in Yeast

JOSEPH HEITMAN,* N. RAO MOVVA, MICHAEL N. HALL†

FK506 and rapamycin are related immunosuppressive compounds that block helper T cell activation by interfering with signal transduction. In vitro, both drugs bind and inhibit the FK506-binding protein (FKBP) proline rotamase. *Saccharomyces cerevisiae* cells treated with rapamycin irreversibly arrested in the G1 phase of the cell cycle. An FKBP-rapamycin complex is concluded to be the toxic agent because (i) strains that lack FKBP proline rotamase, encoded by *FPR1*, were viable and fully resistant to rapamycin and (ii) FK506 antagonized rapamycin toxicity in vivo. Mutations that conferred rapamycin resistance altered conserved residues in FKBP that are critical for drug binding. Two genes other than *FPR1*, named *TOR1* and *TOR2*, that participate in rapamycin toxicity were identified. Nonallelic noncomplementation between *FPR1*, *TOR1*, and *TOR2* alleles suggests that the products of these genes may interact as subunits of a protein complex. Such a complex may mediate nuclear entry of signals required for progression through the cell cycle.

proline rotamase activity but is not immunosuppressive (*15*). Our studies investigate the action of rapamycin and FK506 in yeast.

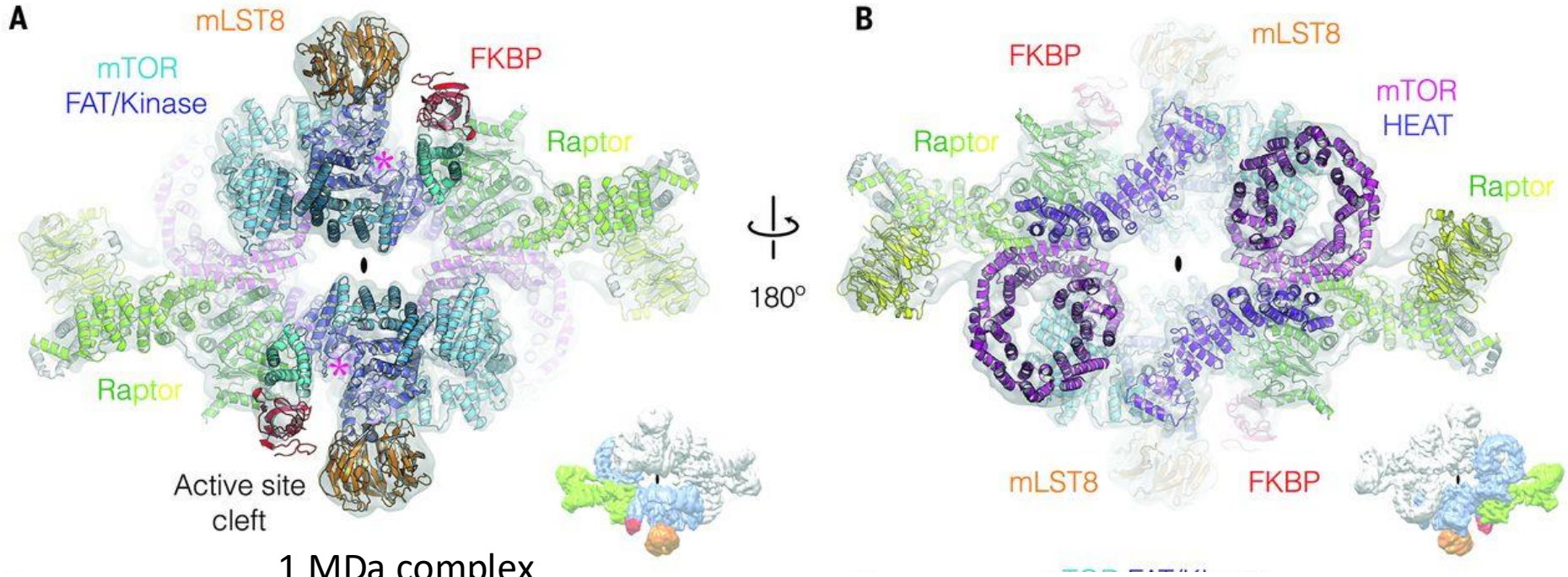
Growth of isogenic haploid (Fig. 1) and diploid derivatives of *S. cerevisiae* strain JK9-3d (*16, 17*) was sensitive to the immunosuppressant rapamycin (*18*) with a minimum inhibitory concentration (MIC) of

To study the interaction of FKBP with rapamycin and identify other proteins that contribute to rapamycin toxicity, we isolated rapamycin-resistant yeast mutants. Spontaneous independent mutants resistant to rapamycin (0.1 μg/ml) were isolated from a

mTOR structure + Rapamycin

Sirolimus, Everolimus and other rapalogues

Immunosupressive drugs & anti-cancer drugs



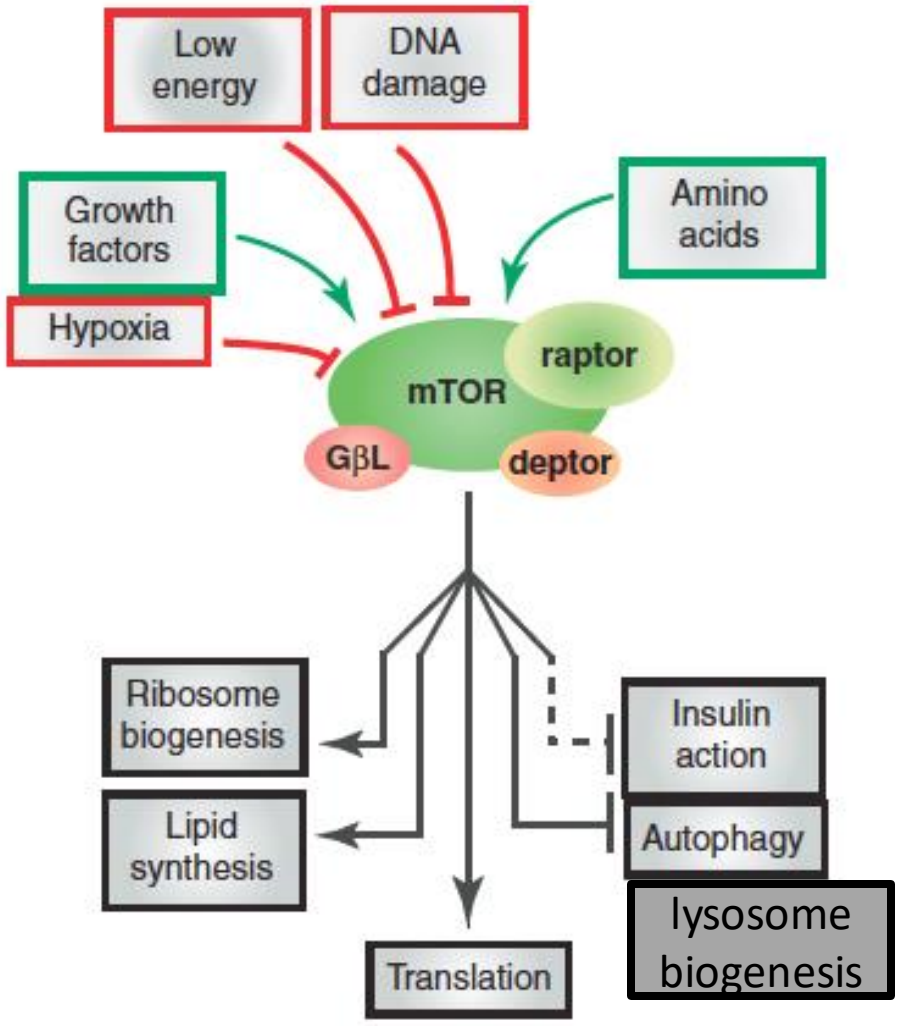
1 MDa complex

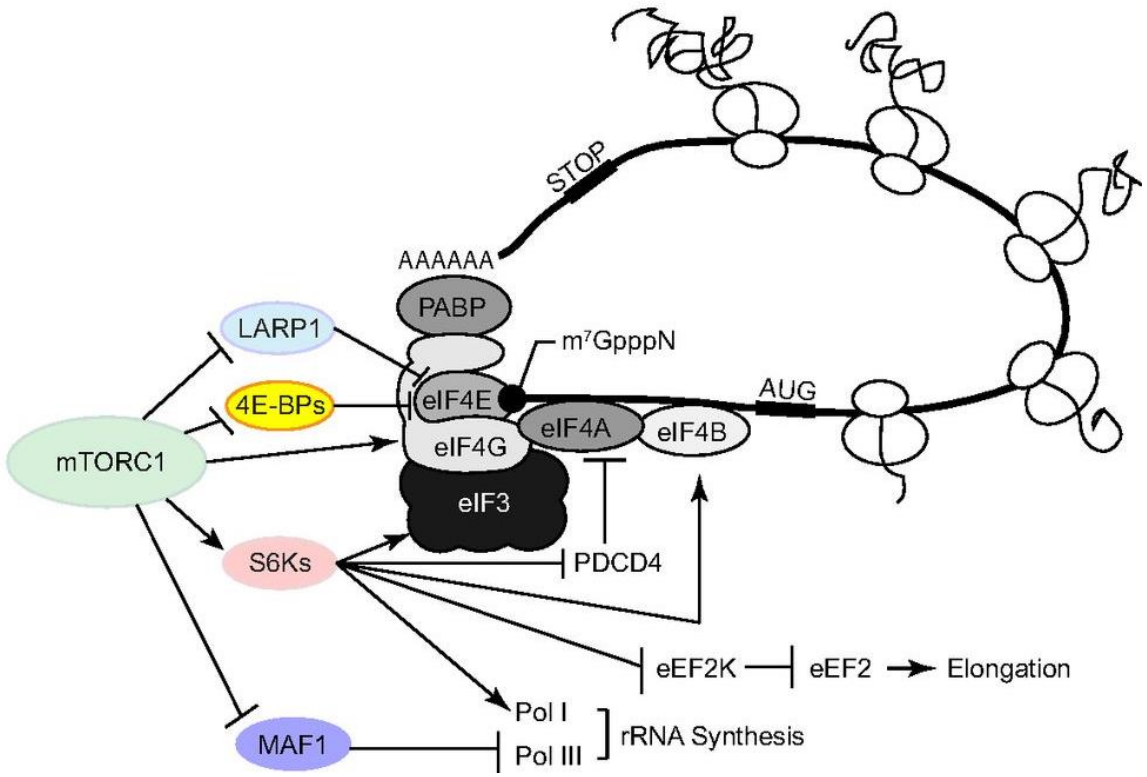
FK506 binding protein (FKBP) and Rapamycin form a complex (an aduct)
This complex binds to the FKBP-rapamycin complex binding (FRB) domain
at the N-terminus of the TOR Kinase domain

Aylett et al., Science 2016

mTORC1 is the central regulator of cell growth

Integration of cell intrinsic signals (amino acids) & extracellular signals (growth factors)





mTORC1 ⊣ Ulk1 (autophagy regulator)

mTORC1 ⊣ TFEB (transcription factor for lysosome biogenesis)

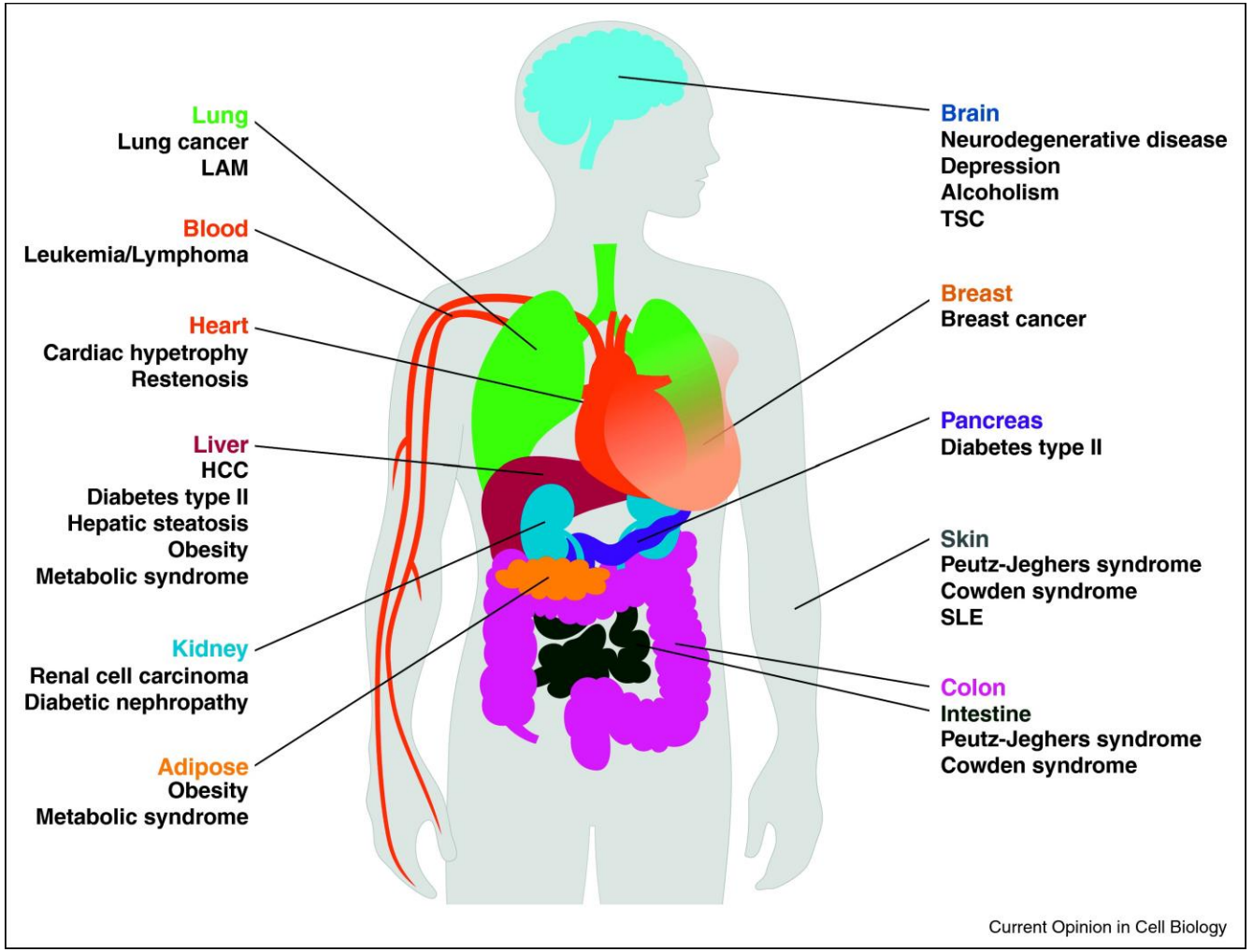
mTOR is hyperactive in Tumors, metabolic and neurological disorders, and inflammation

mTOR pathway hyperactivity causes a group 14 of rare diseases (TORopathies)

e.g. LoF in Tsc1/2

Tuberous Sclerosis (TSC)

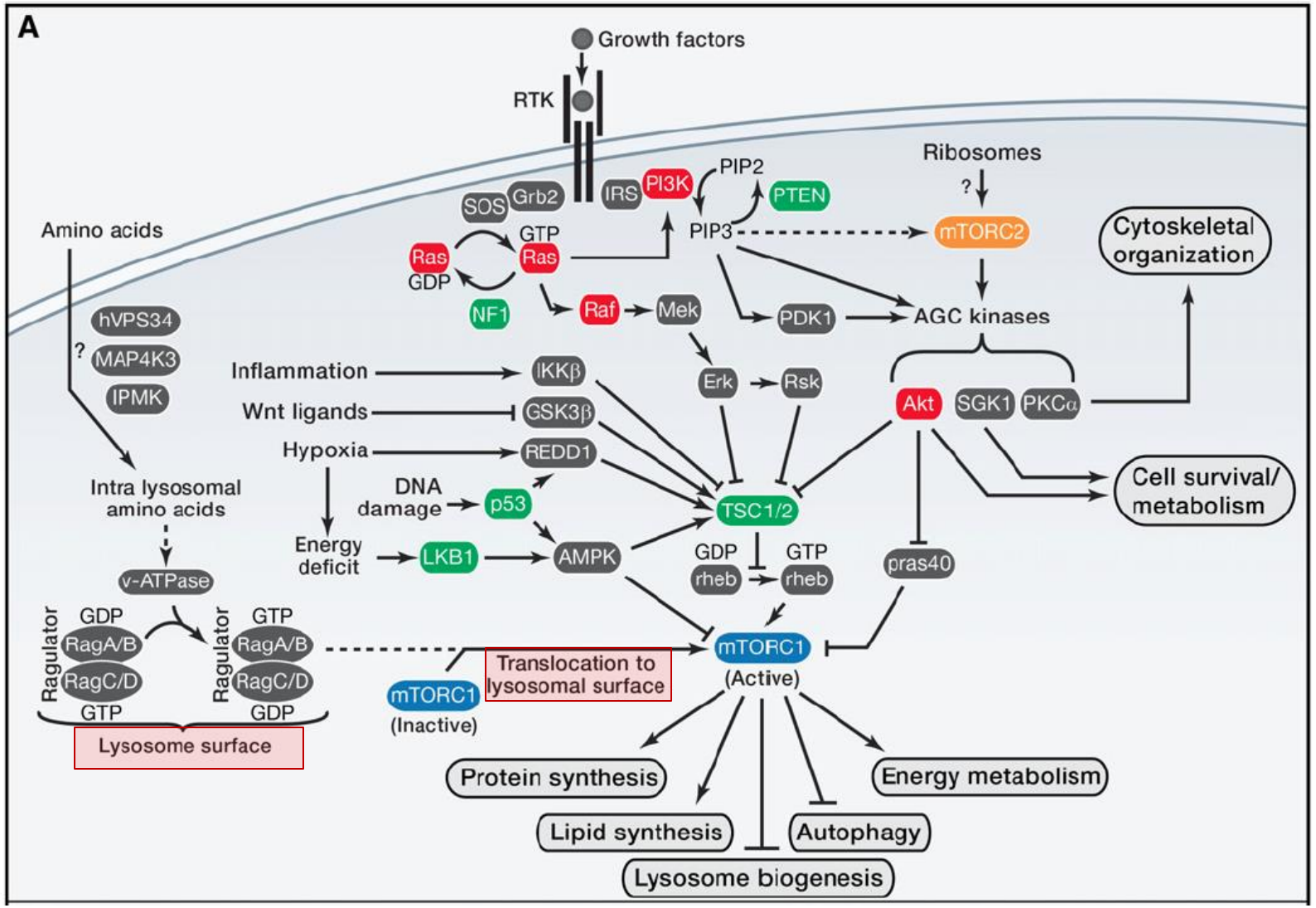
Tuberous sclerosis complex (TSC), also known as tuberous sclerosis, is a rare genetic disease that causes non-cancerous tumors (Hamartome) to grow in the brain and several areas of the body, including the spinal cord, nerves, eyes, lung, heart, kidneys, and skin. In addition many patients develop epilepsy, autism and learning difficulties from birth



https://www.sciencedirect.com/science/article/pii/S0955067411001116

How does mTORC1 signaling control cell growth?

Integration of cell intrinsic signals (e.g.: amino acid) & extracellular signals (growth factors)

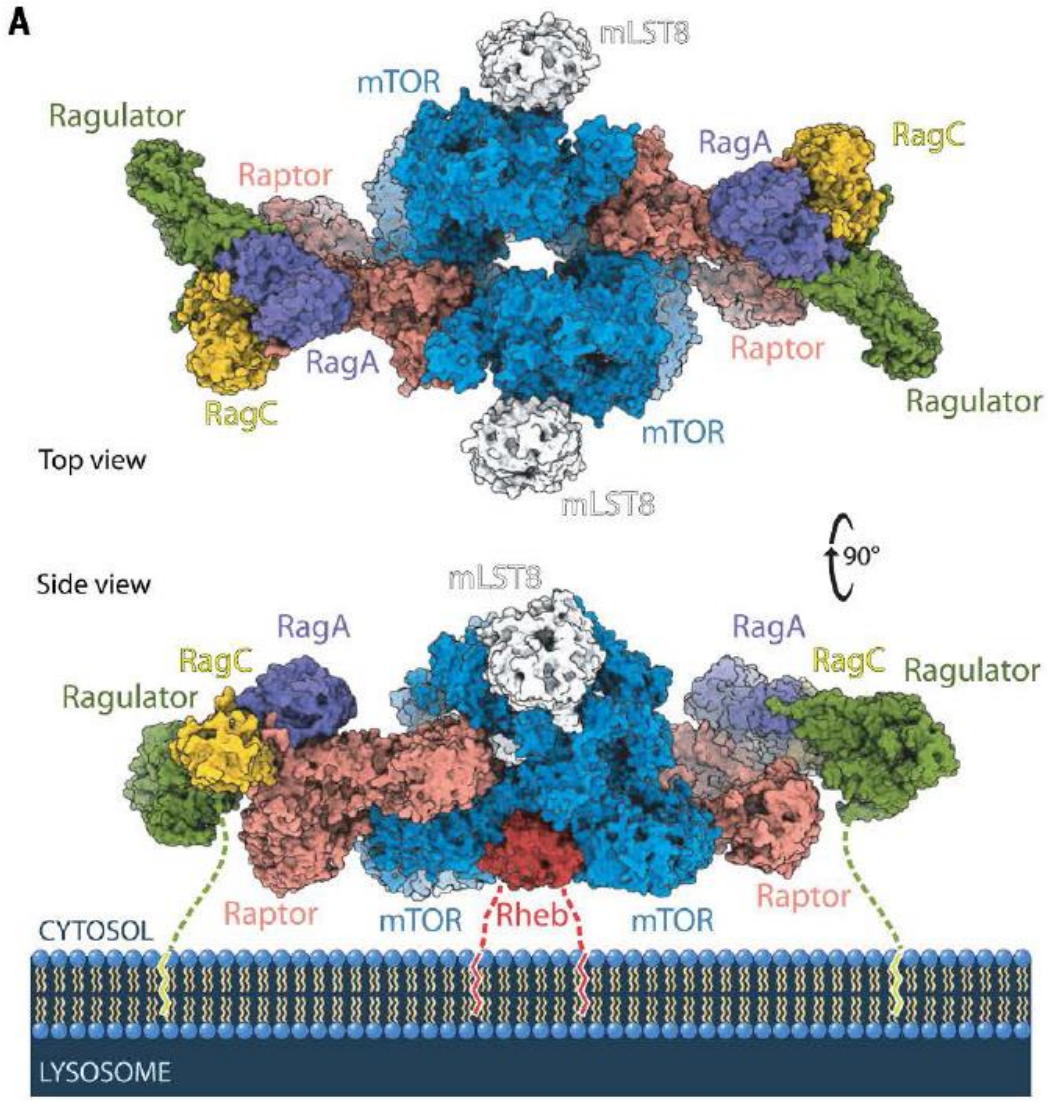


TORC1 stimulates directly:
Protein translation
Ribosome biogenesis
Lipid biosynthesis

TORC1 inhibits directly:
Autophagy
Lysosome biogenesis

Sabatini D 2012 Cell

Activation of mTORC1 on the lysosomal surface



Rheb – GTP: under control of growth factor signaling recruits mTORC1 to lysosomes.

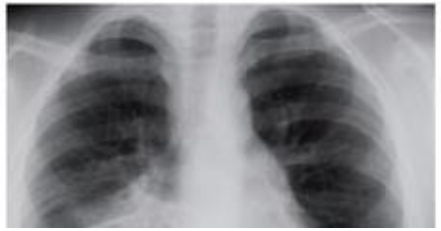
RagA(GTP)/C(GDP): under control of amino acids recruits mTORC1 to lysosomes.

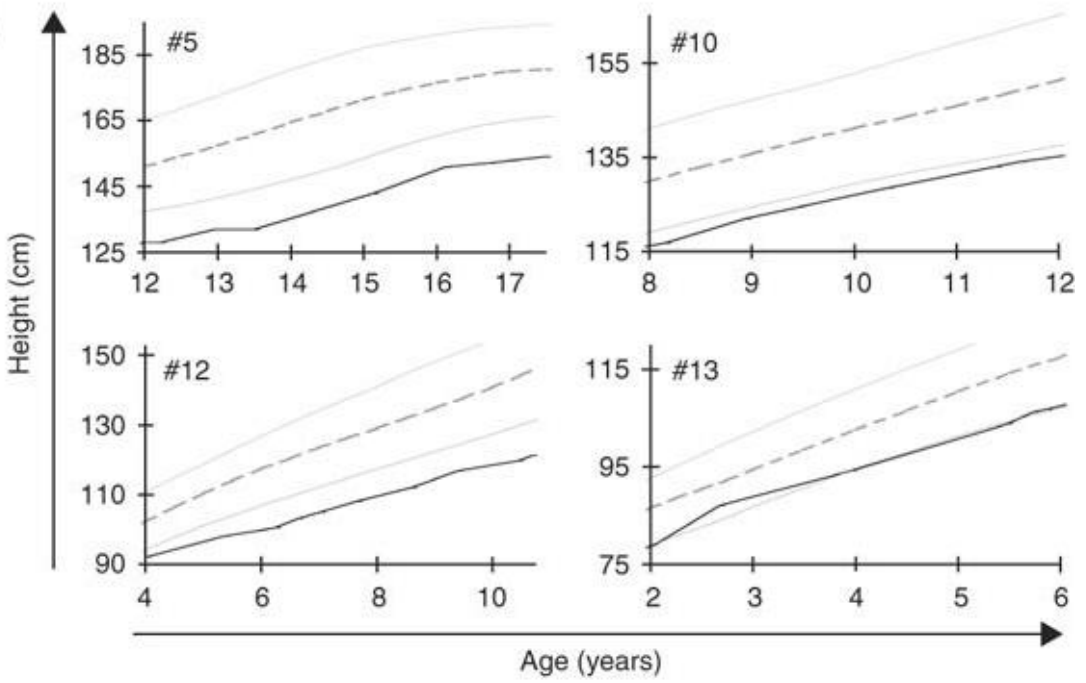
Kacper B. Rogala et al Science 10.10.2019

Lysosomal mTORC1 signaling controls growth

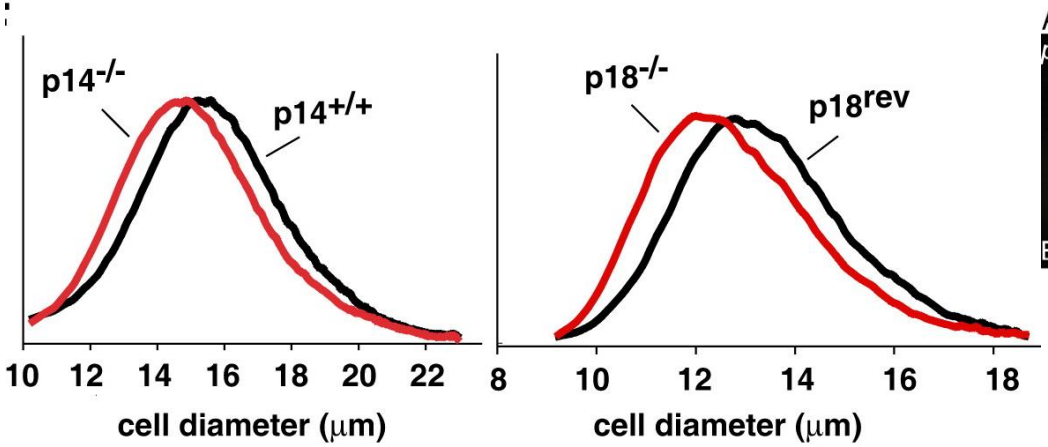
of organisms

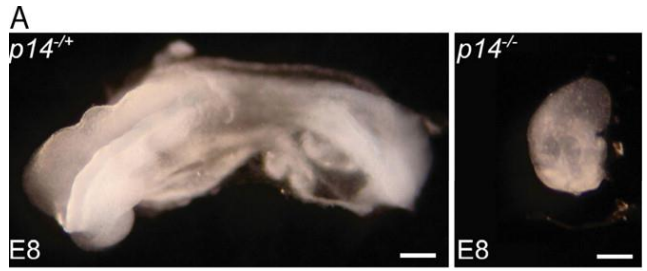
mutations in the 3‘UTR of LAMTOR2





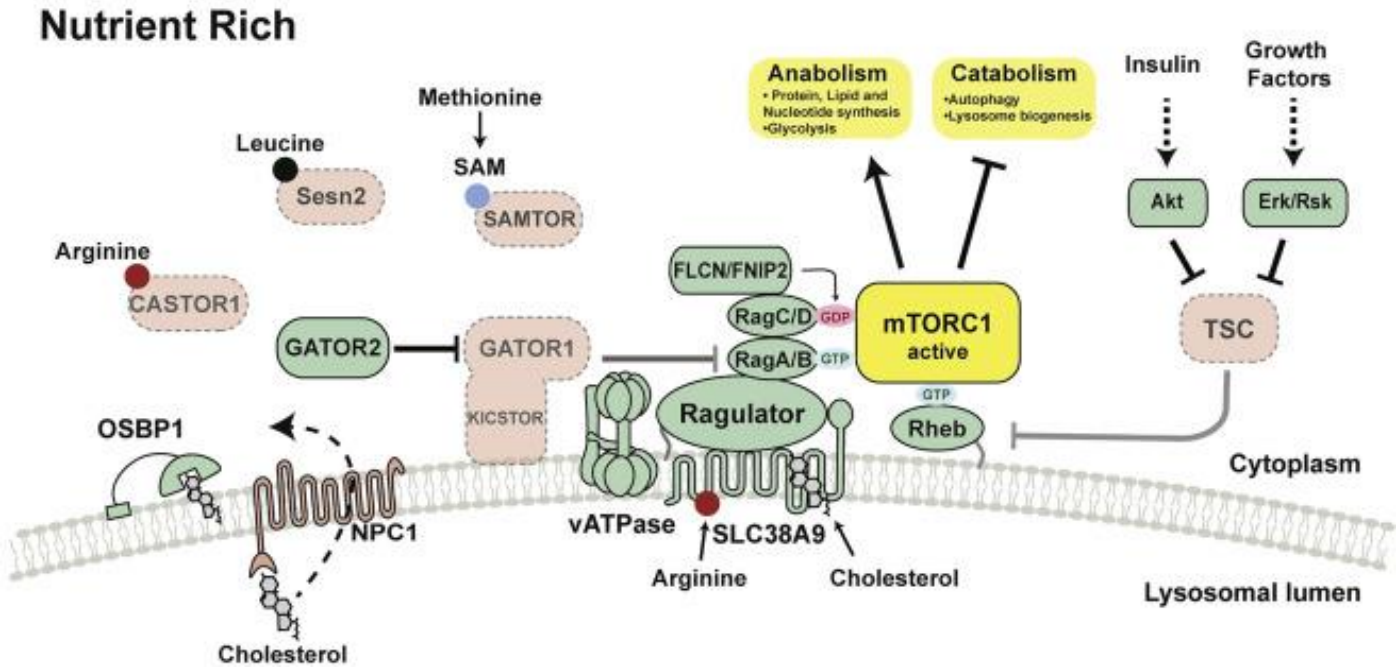
of individual cells





Cell size of LAMTOR1 (p18) & 2 (p14) deficient MEFs

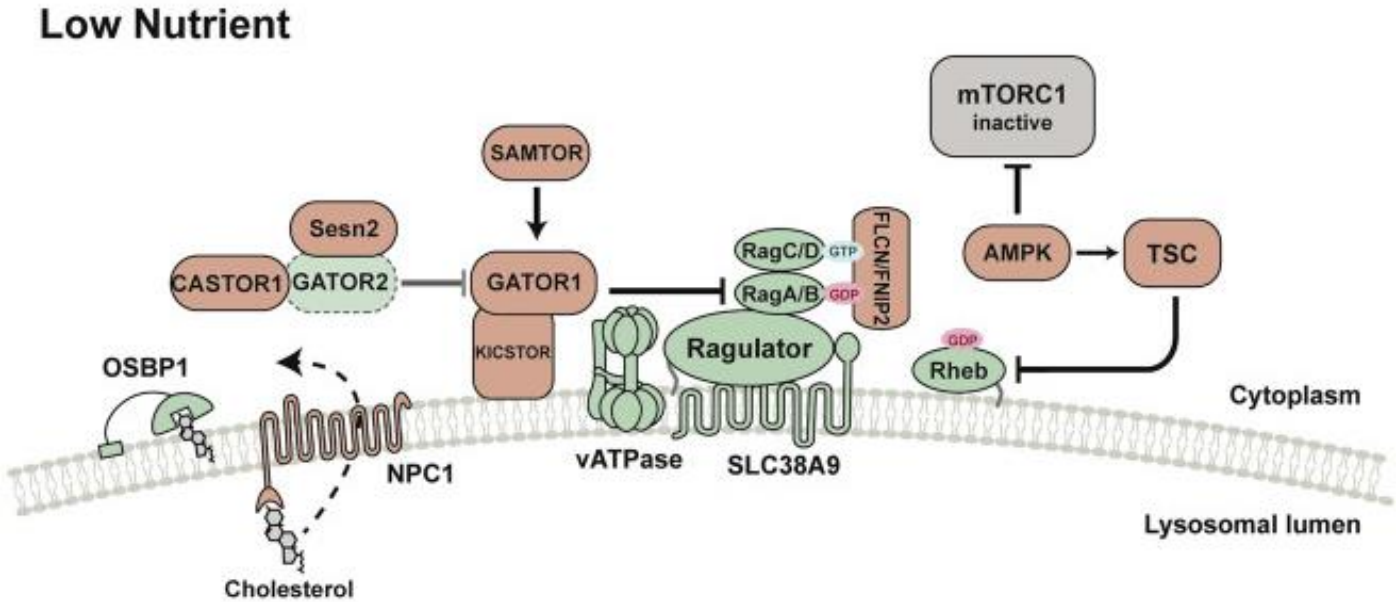
https://doi.org/10.1083/jcb.200607025
https://doi.org/10.1038/nm1528
https://doi.org/10.1016/j.cell.2010.02.024



off **TSC1/2** -> GAP for Rheb

off **GATOR1** -> GAP for RagA/B

on **FLCN/FNIP2** -> GAP for RagC/D

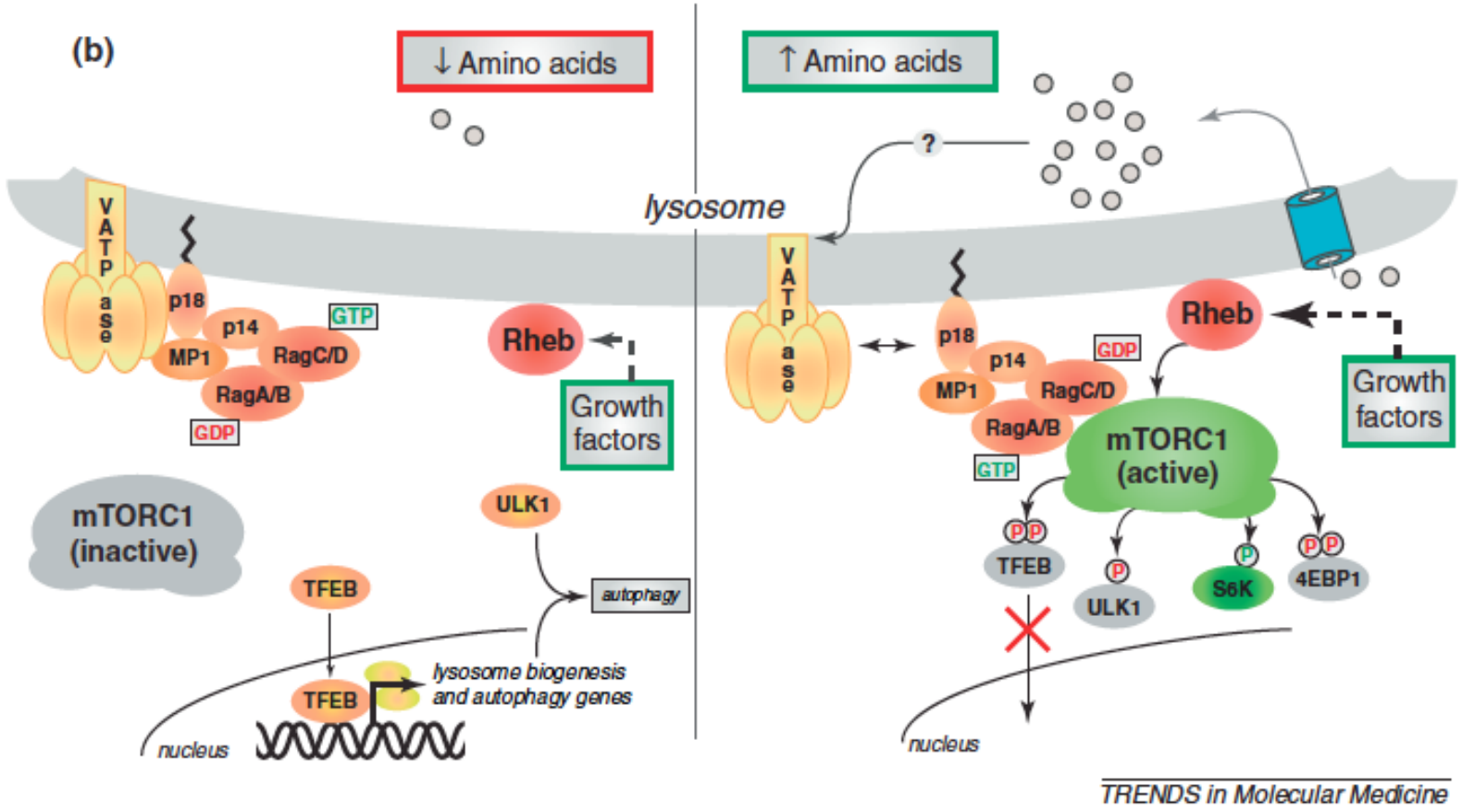


on **TSC1/2** -> GAP for Rheb

on **GATOR1** -> GAP for RagA/B

off **FLCN/FNIP2** -> GAP for RagC/D

Based on these models mTORC1 is either on or off



How is spatio-temporal substrate specificity of mTOR defined?

Are there scenarios were growth factor signaling can be uncoupled from nutrients sensing and vice versa?

Efeyan, A

Article

A substrate-specific mTORC1 pathway underlies Birt–Hogg–Dubé syndrome

https://doi.org/10.1038/s41586-020-2444-0

Received: 3 December 2019

Accepted: 27 April 2020

Published online: 1 July 2020

Check for updates

Gennaro Napolitano[1,2,10], Chiara Di Malta[1,10], Alessandra Esposito[1], Mariana E. G. de Araujo[3], Salvatore Pece[4,5], Giovanni Bertalot[4], Maria Matarese[1], Valerio Benedetti[1], Angela Zampelli[1], Taras Stasyk[3], Diletta Siciliano[1], Alessandro Venuta[1], Marcella Cesana[1], Claudia Vilardo[1], Edoardo Nusco[1], Jlenia Monfregola[1], Alessia Calcagnì[6,7], Pier Paolo Di Fiore[4,5], Lukas A. Huber[3,8] & Andrea Ballabio[1,2,6,7,9] ✉

The mechanistic target of rapamycin complex 1 (mTORC1) is a key metabolic hub that controls the cellular response to environmental cues by exerting its kinase activity on multiple substrates[1–3]. However, whether mTORC1 responds to diverse stimuli by differentially phosphorylating specific substrates is poorly understood. Here we show that transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagy[4,5], is phosphorylated by mTORC1 via a substrate-specific mechanism that is mediated by Rag GTPases. Owing to this mechanism, the phosphorylation of TFEB—unlike other substrates of mTORC1, such as S6K and 4E-BP1— is strictly dependent on the amino-acid-mediated activation of RagC and RagD GTPases, but is insensitive to RHEB activity induced by growth factors. This mechanism has a crucial role in Birt–Hogg–Dubé syndrome, a disorder that is caused by mutations in the RagC and RagD activator folliculin (*FLCN*) and is characterized by benign skin tumours, lung and kidney cysts and renal cell carcinoma[6,7]. We found that constitutive activation of TFEB is the main driver of the kidney abnormalities and mTORC1 hyperactivity in a mouse model of Birt–Hogg–Dubé syndrome. Accordingly, depletion of TFEB in kidneys of these mice fully rescued the disease phenotype and associated lethality, and normalized mTORC1 activity. Our findings identify a mechanism that enables differential phosphorylation of mTORC1 substrates, the dysregulation of which leads to kidney cysts and cancer.

TFEB phosphorylation does not require RHEB

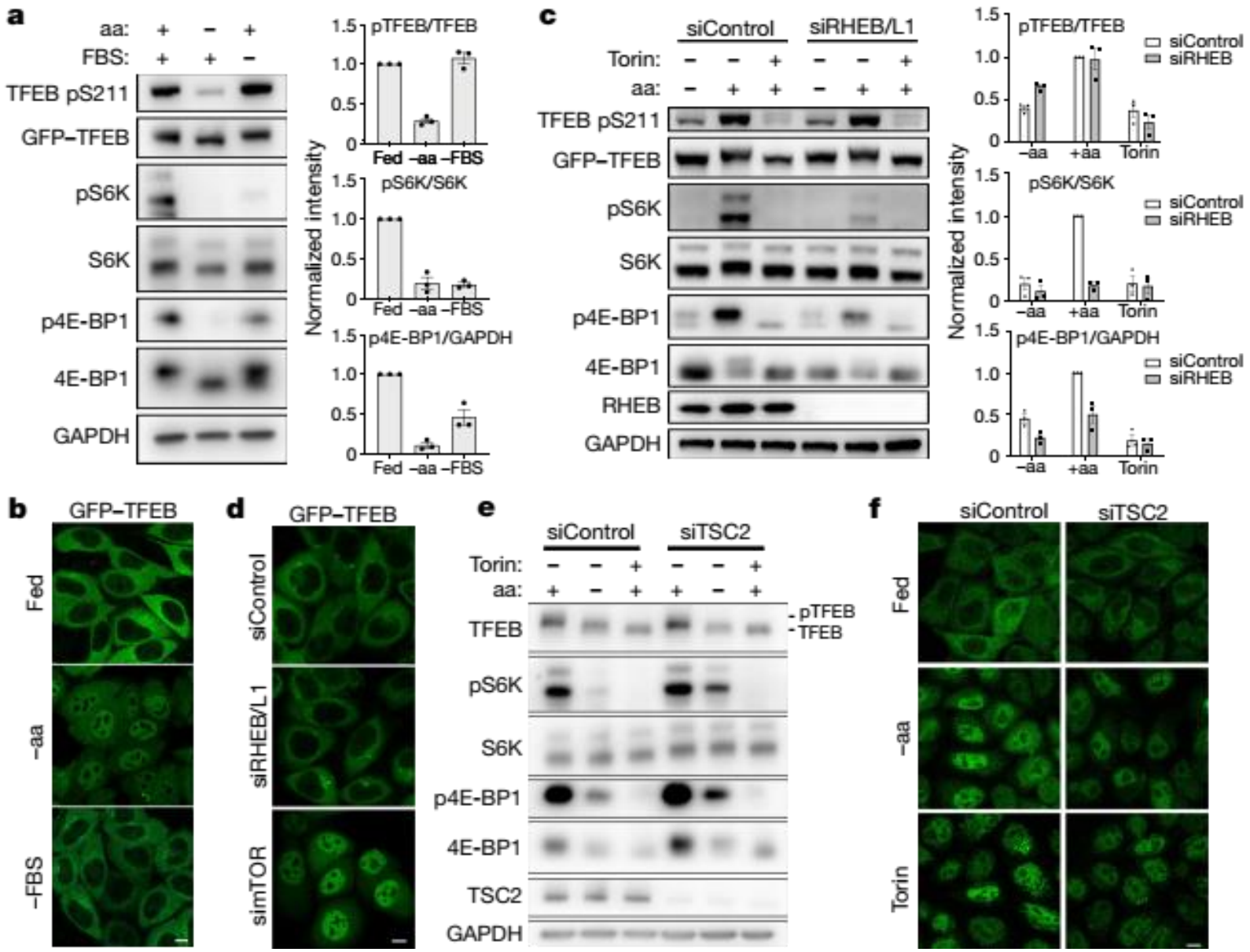
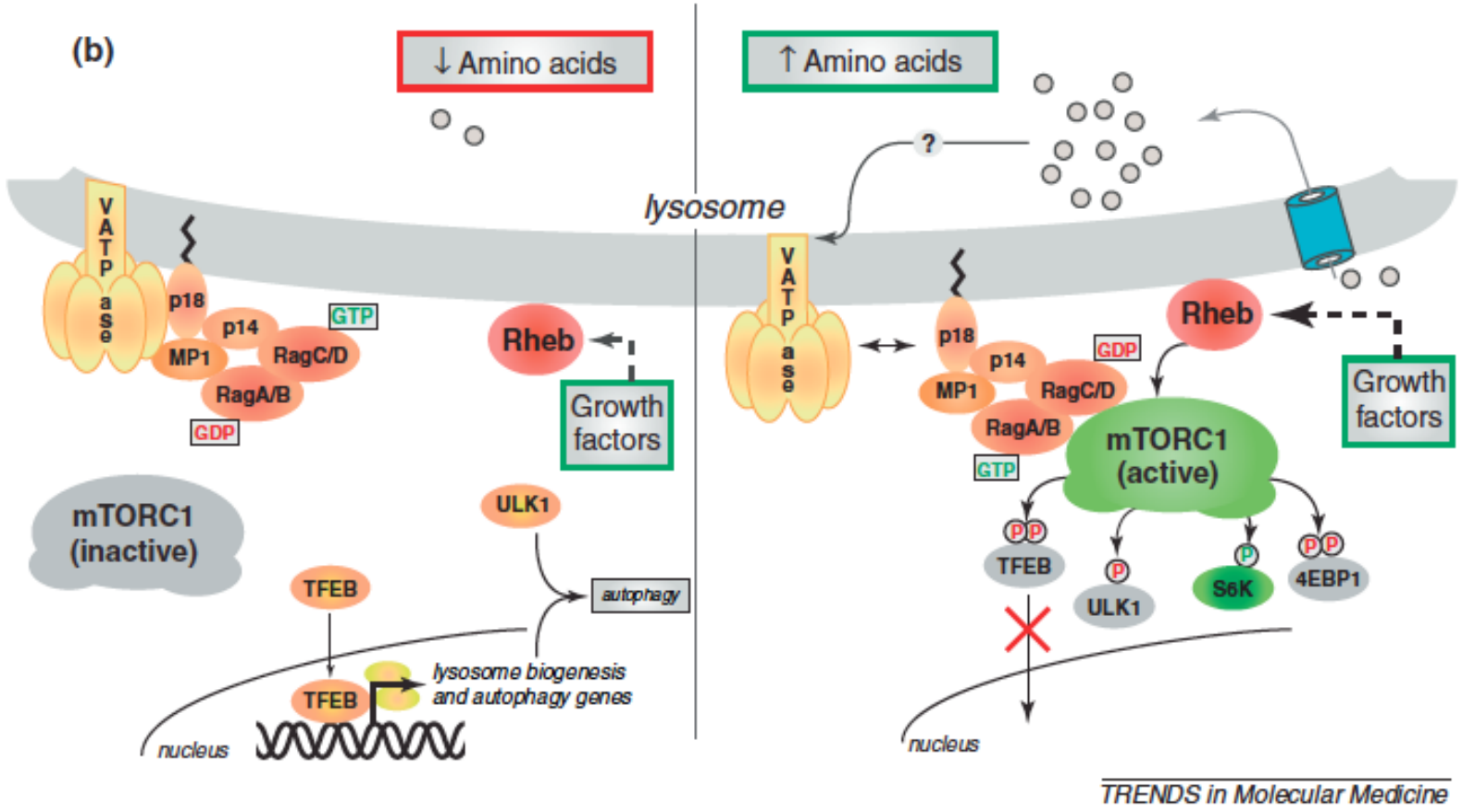


Figure 1

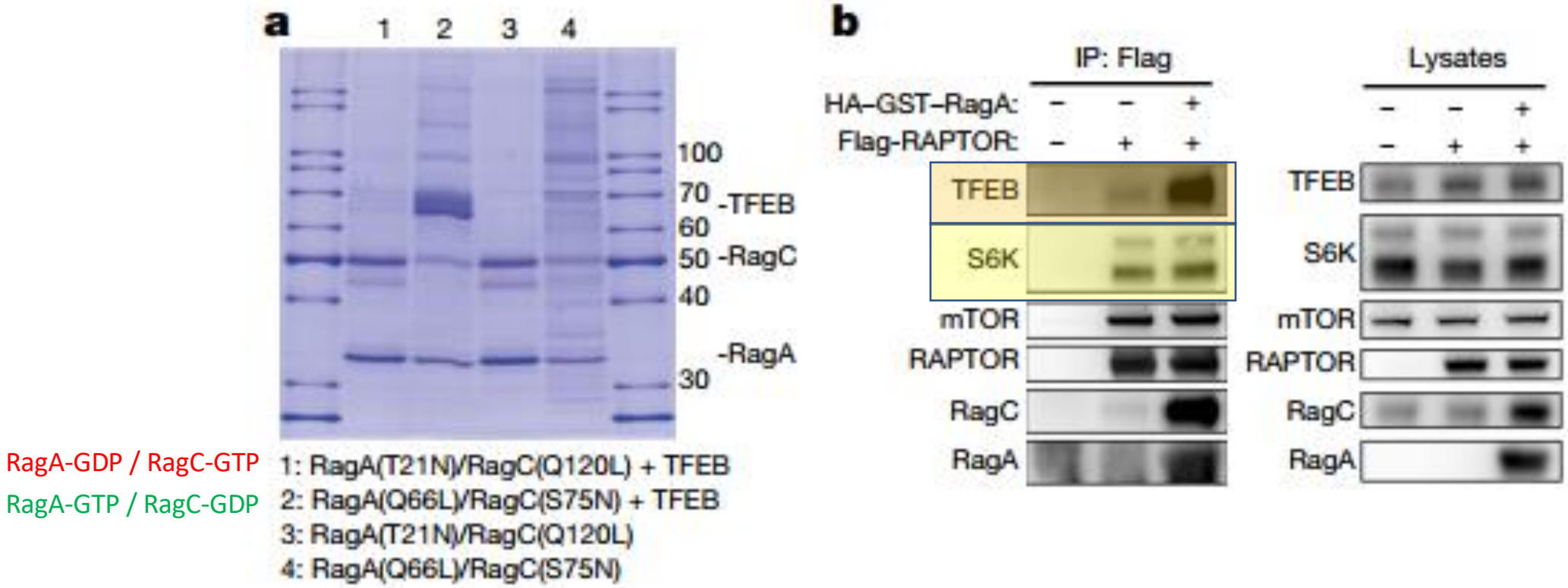
mTORC1 can be on for TFEB and off for S6K



According to the data we just discussed, this model is too simple....

Efeyan, A

Rag GTPases mediate mTORC1–TFEB interaction, but not S6K interaction



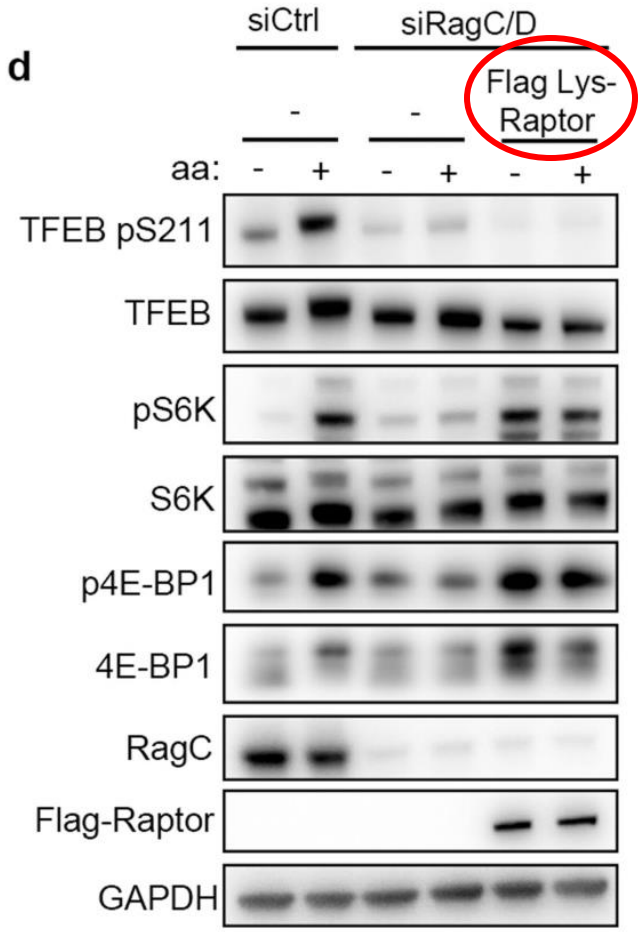
***in vitro* binding**

***in vivo* binding**

Why bother and do two experiments that show RagA/C – TFEB interaction?

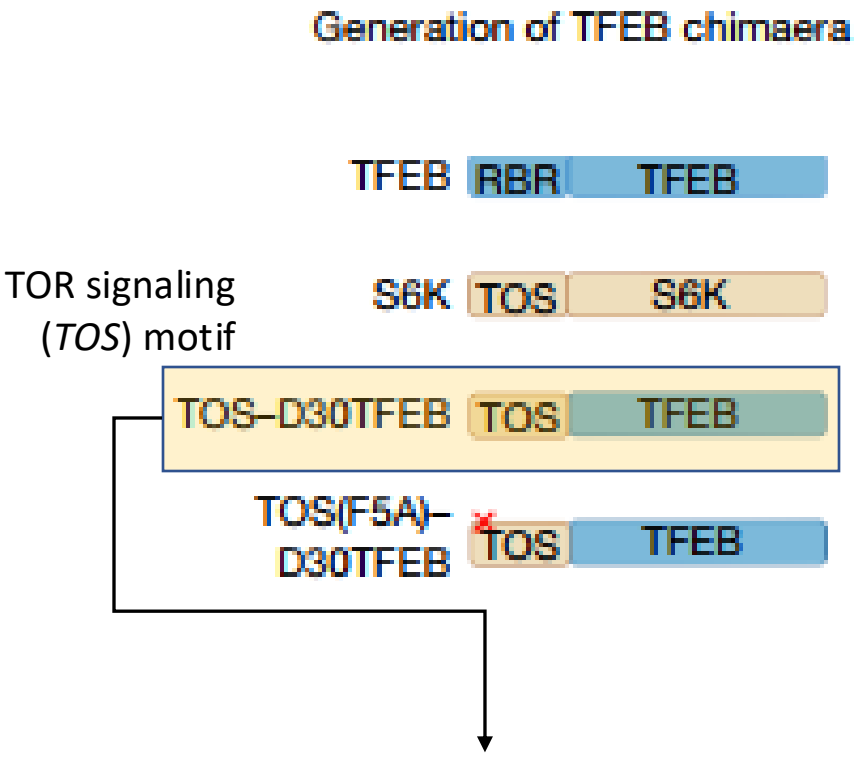
Figure 2

Rag GTPase are required for TFEB phosphorylation



Lys–RAPTOR (last 15 amino acids of RHEB1) anchors mTORC1 to lysosomes, independent of Rag‘s

mTORC1 specificity towards TFEB is mediated by Rag‘s



TOR signaling (*TOS*) motif

able to interact with both mTOR and RAPTOR

but not with Rag GTPases

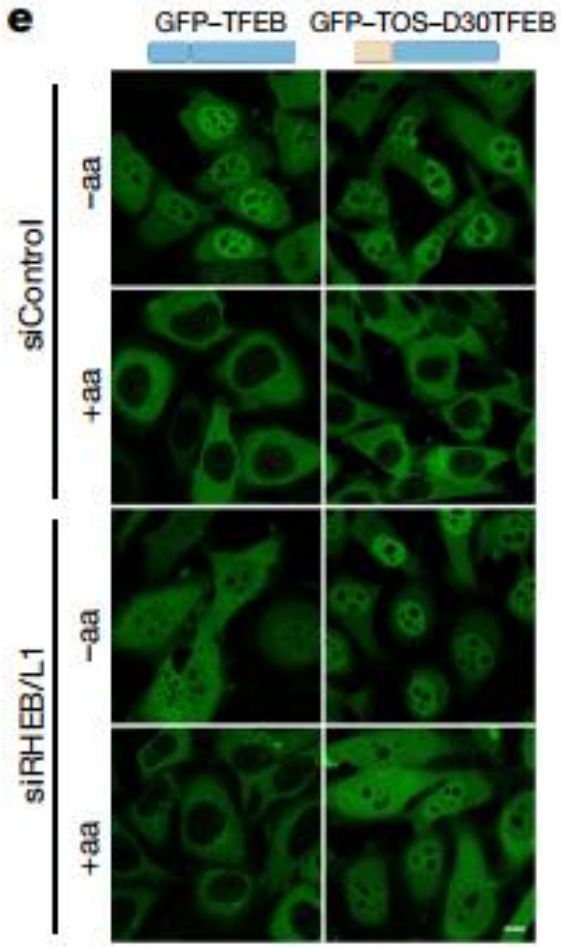


Figure 2

TFEB is recruited by active Rag-GTPases

S6K is recruited by Raptor, and active Rheb and active Rag-GTPases

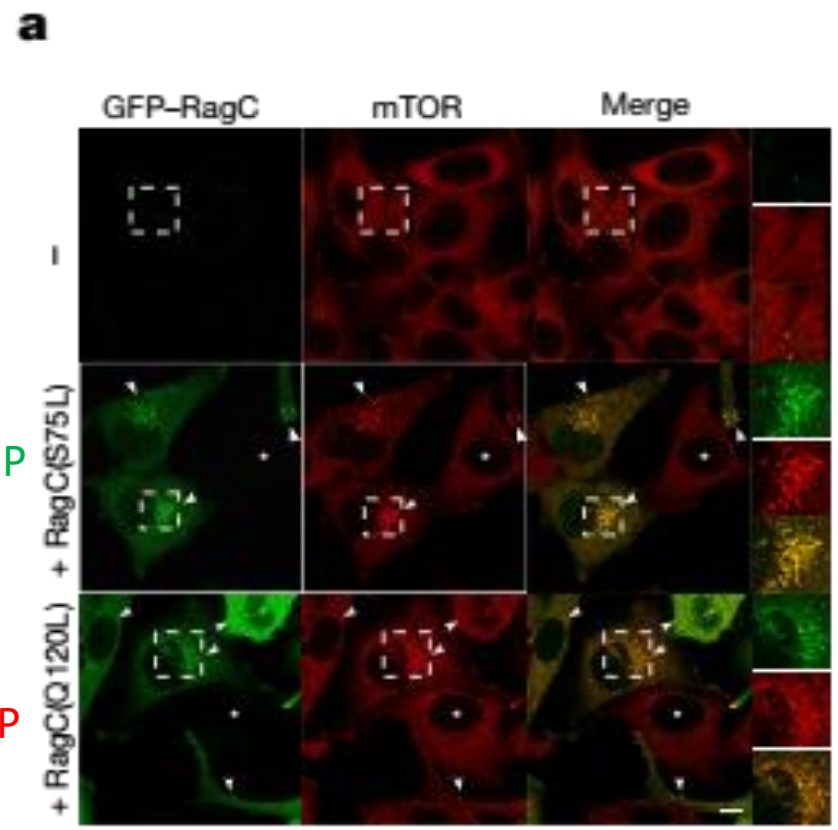
mTORC1 dependent TFEB phopshorylation is controlled by amino acids and not by growth factors

TFEB and S6K have different substrate recruitment to mTORC1

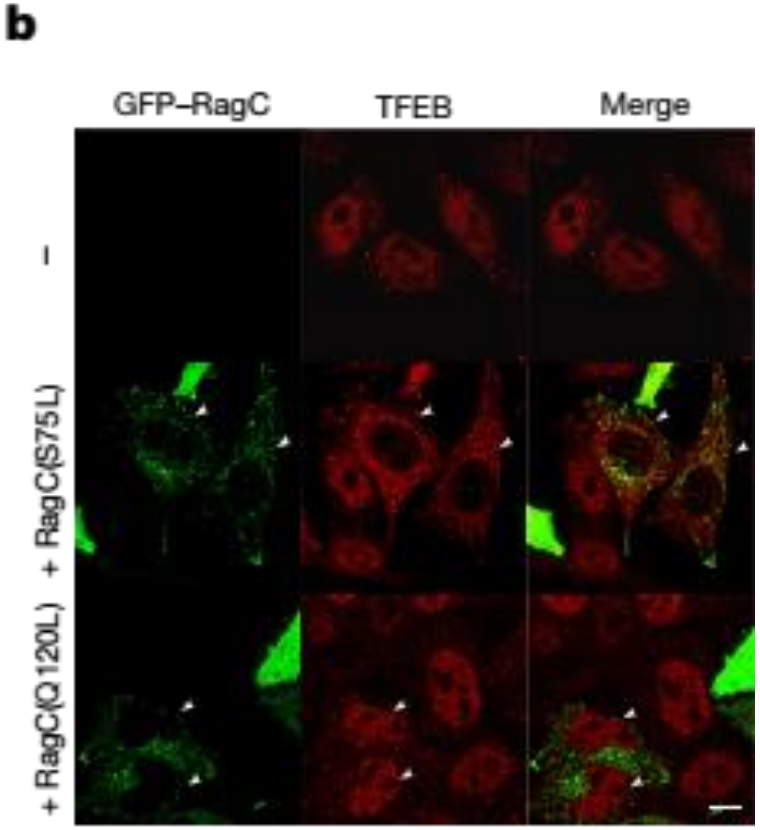
TFEB phosphorylation requires active RagC (RagC-GDP)

RagC-GDP

RagC-GTP



mTORC1 on lysosomes
regardless of RagC status



TFEB inhibition by lysosomal mTORC1
requires active RagC-GDP

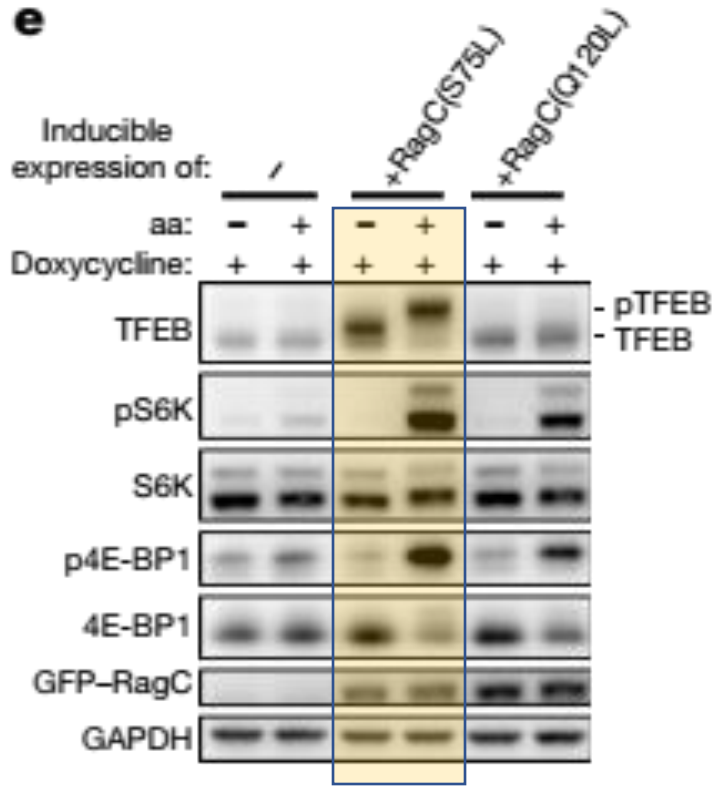
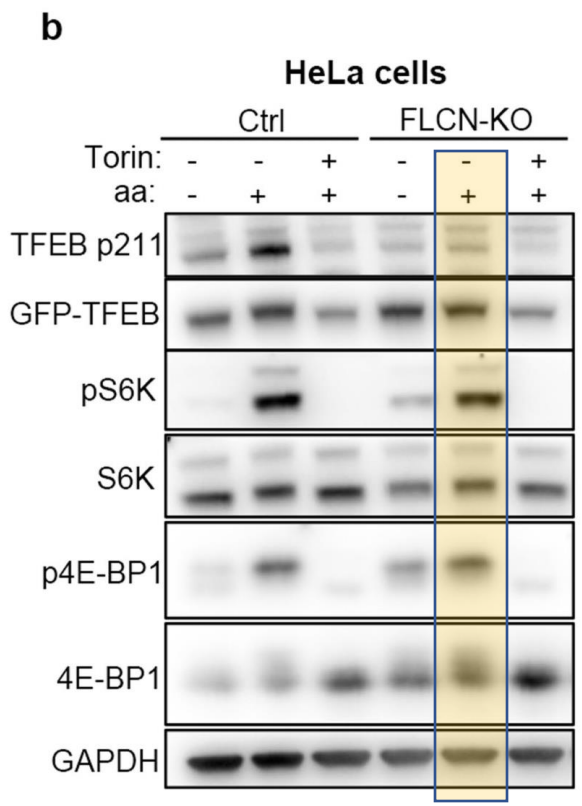
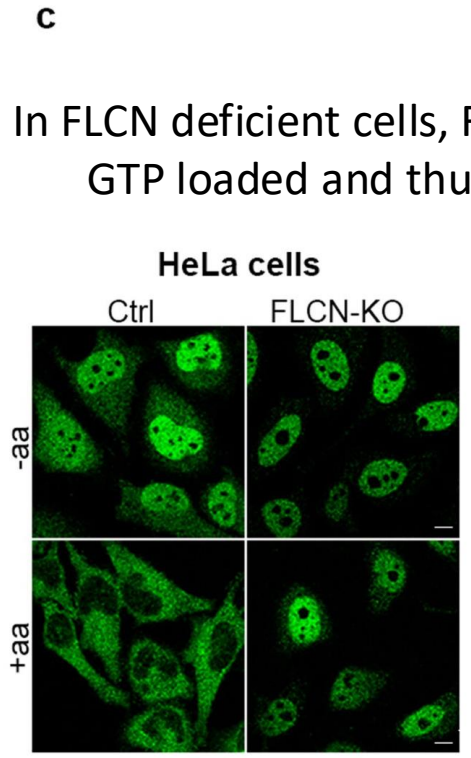


Figure 3

Folliculin (FLCN, the GAP for RagC) is essential for TFEB phosphorylation





In FLCN deficient cells, RagC is always GTP loaded and thus inactive

Together, these data suggest that a dimer of RagA and inactive RagC-GTP is unable to promote mTORC1 activity towards TFEB, whereas it retains—to a large extent—its ability to promote mTORC1 lysosomal recruitment and consequent phosphorylation of S6K and 4E-BP1.

Extended Data Fig. 8 |

Mutations in FLCN cause Birt-Hogg-Dubé-Syndrom (BHDS)

Prof. Ivan Tancevski, Pneumologische Ambulanz, Univ.-Klinik für Innere Medizin II

frequency: 1:200.000 - 1:250.000 worldwide

genetics: Mutation in **FLCN-gene**

clinical manifestation:

- Lung-cysts & recurrent pneumothorax.

- fibrofollikulomas (bengine tumors in the face, head, neck)

- high risk for renal carcinoma.

A 45-year-old woman presents and requests referral to the Department of Pulmonology at the Medical University of Innsbruck.

Medical history:
The patient reports that she had led a normal life and worked as a nurse until her mid-30s.

1.She suddenly experienced a **spontaneous pneumothorax** on one side.

2.In the following years, she had **more than 10 pneumothoraces**, including **simultaneous bilateral** episodes.

3.She underwent **partial lung resection** and **several pleurodeses**.

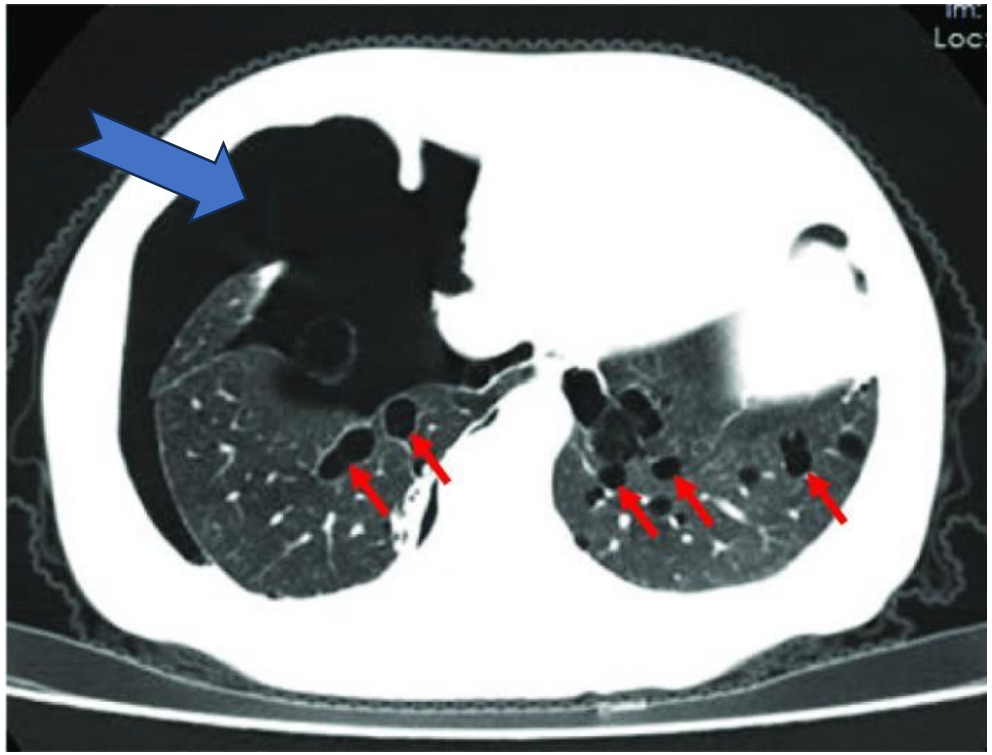
4.(Pleurodesis: a procedure in which the pleural layers are fused—e.g., by talc instillation—to prevent further pneumothoraces. During the partial lung resection, a large cyst was surgically removed to reduce the risk of recurrence.)

5.Over time, **fibrofolliculomas** have gradually developed on her face.

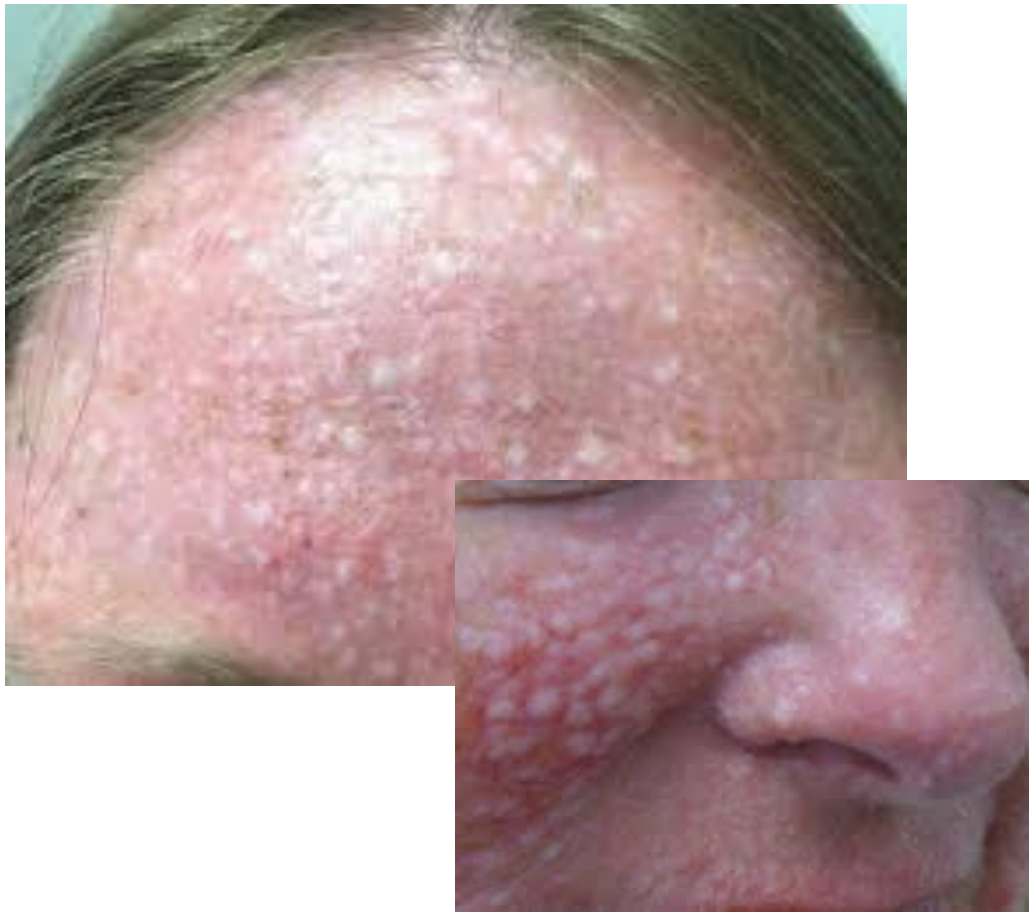
Alter der Patientin

35 | 45 | 55

Pulmonale Zysten (rote Pfeile) und **Pneumothorax** (blau)



Fibrofollikulome



Nierentumore?

TFEB drives the kidney phenotype of BHD mice

Birt–Hogg–Dubé syndrome is caused by Flcn LOF mutations

BHD rare inherited cancer-predisposing syndrome characterized by skin lesions, kidney tumors, and pulmonary cysts that may be associated with pneumothorax.

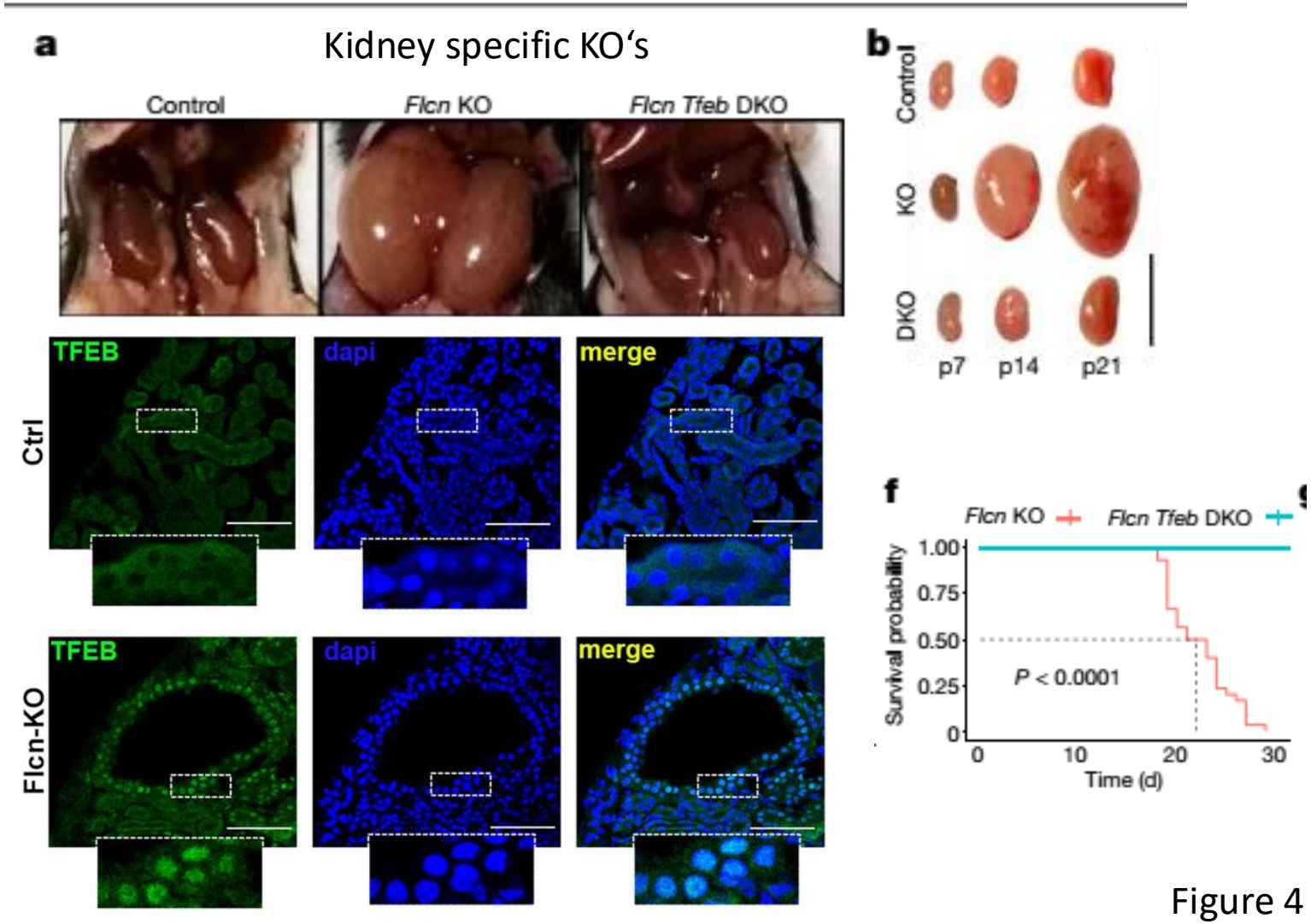


Figure 4

These results suggest that the constitutive activation of TFEB as a result of the loss of function of FLCN is a crucial determinant of the kidney phenotype associated with BHD syndrome.

The new model