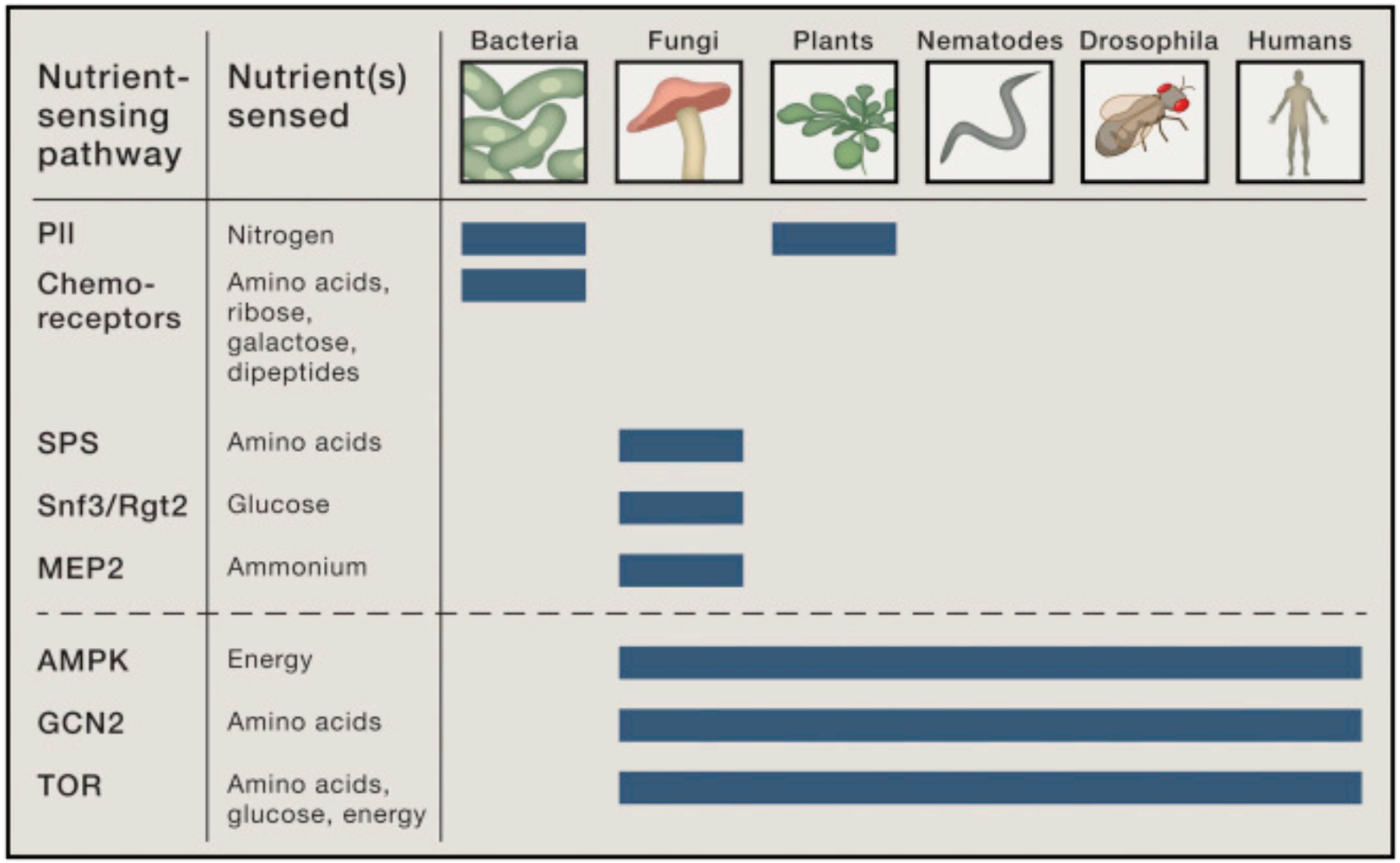
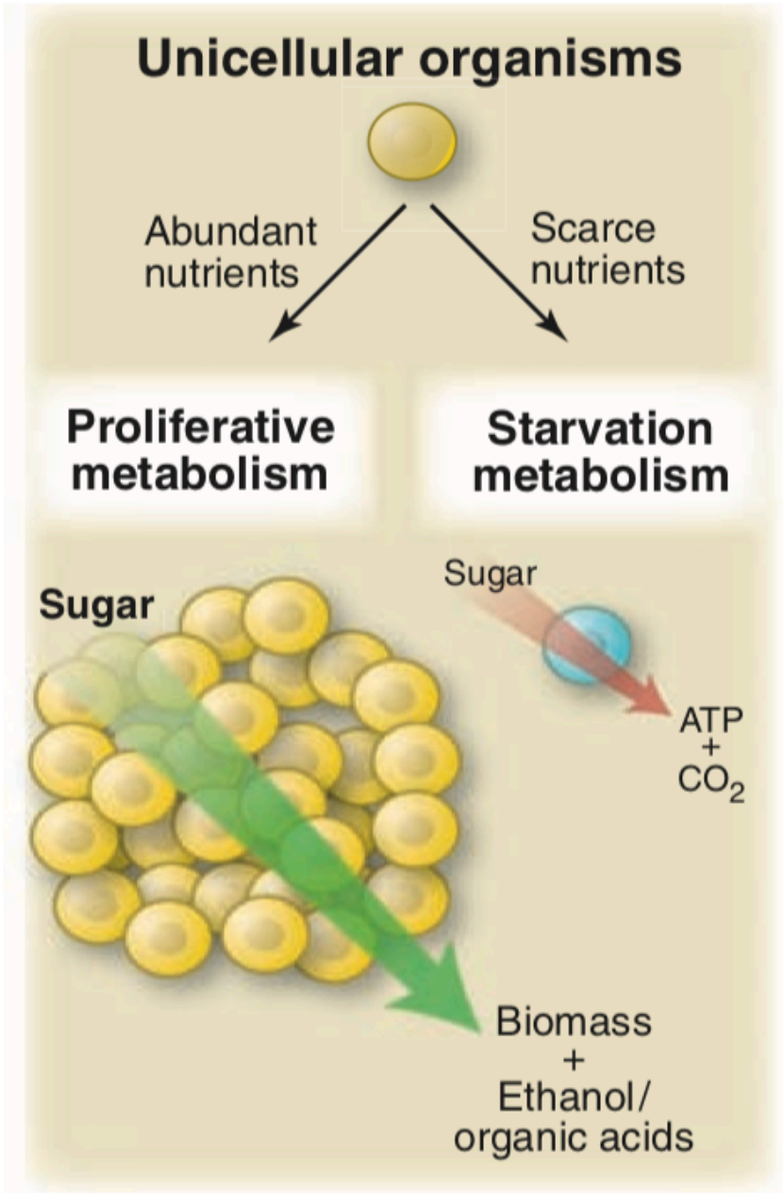
What is Nutrient Sensing?

All organisms have the capacity to sense the presence and absence of the nutrients required to generate energy and the building blocks of cells



Chantranupong et al, ***Cell****, 2015*

Organisms gauge environmental conditions to decide cell fate



Nutrient sensing regulates growth in unicellular organisms

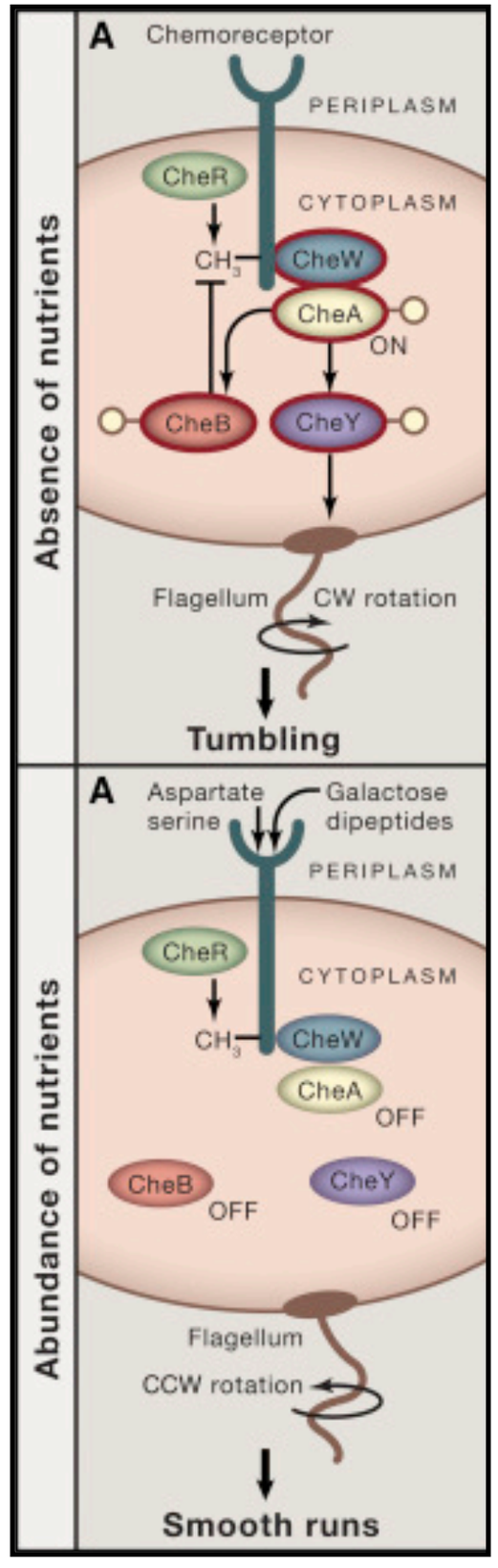
Bacteria have evolved many interesting mechanisms for sensing diverse nutrients, undoubtedly an adaptation to living in environments where the concentrations and types of nutrients can vary unpredictably.

E. coli express five dimeric, single-pass transmembrane chemoreceptors—Tar, Tsr, Tap, Trg, and Aer—which function as distinct nutrient sensors.

In aggregate, they allow *E. coli* to detect and respond to a broad spectrum of extracellular molecules, with aspartate, maltose, Co2+, and Ni2+ binding to Tar; ribose and galactose to Trg; flavin adenine dinucleotide to Aer; serine to Tsr; and dipeptides to Tap.

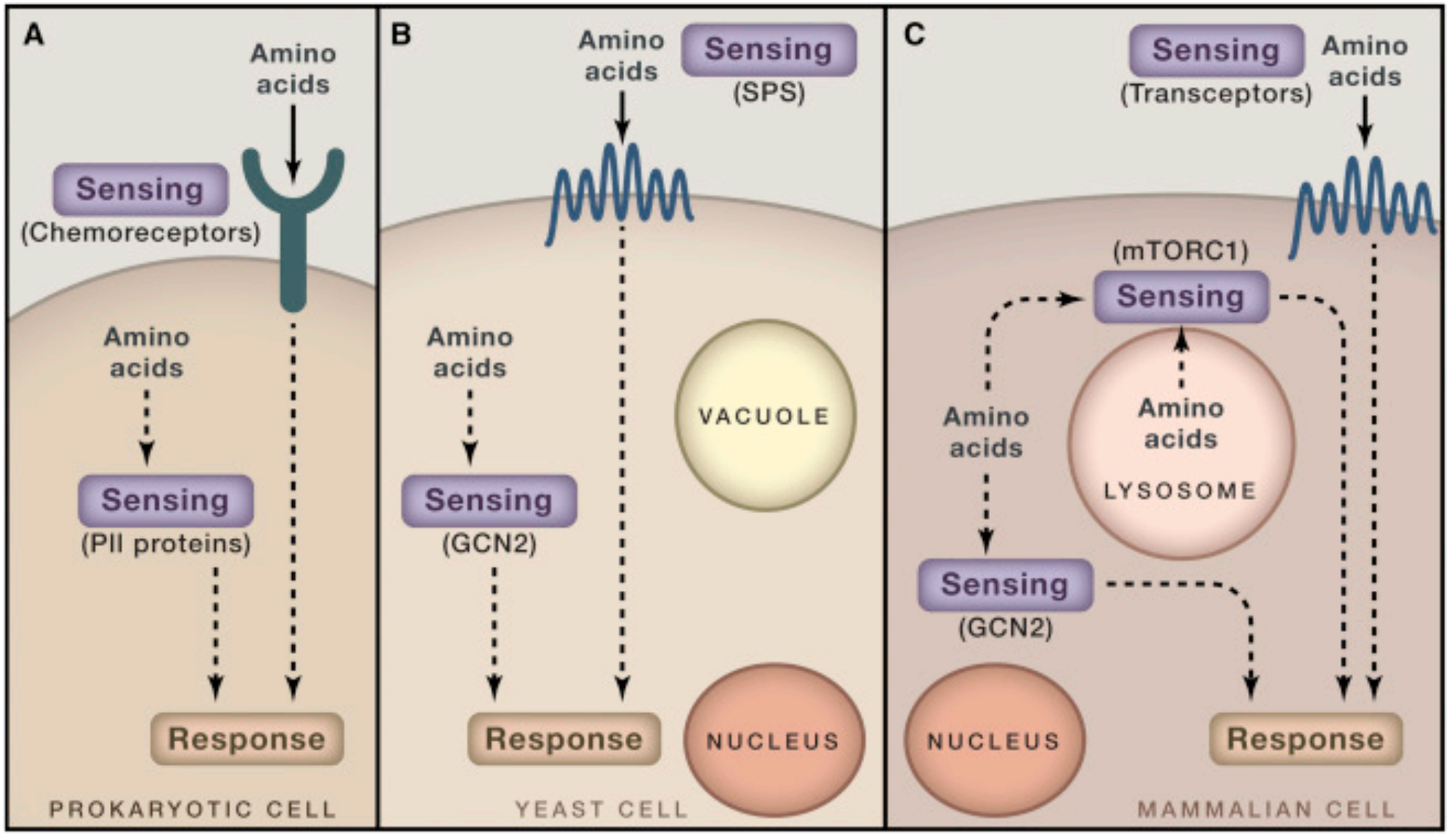
Chemoreceptors sense ligand concentrations as low as 3 nM and function over a concentration range of five orders of magnitude.

This high sensitivity stems from the clustering at the cell pole of the receptors into higher-order arrays, enabling one ligand-binding event to affect multiple neighboring receptors and effectors.



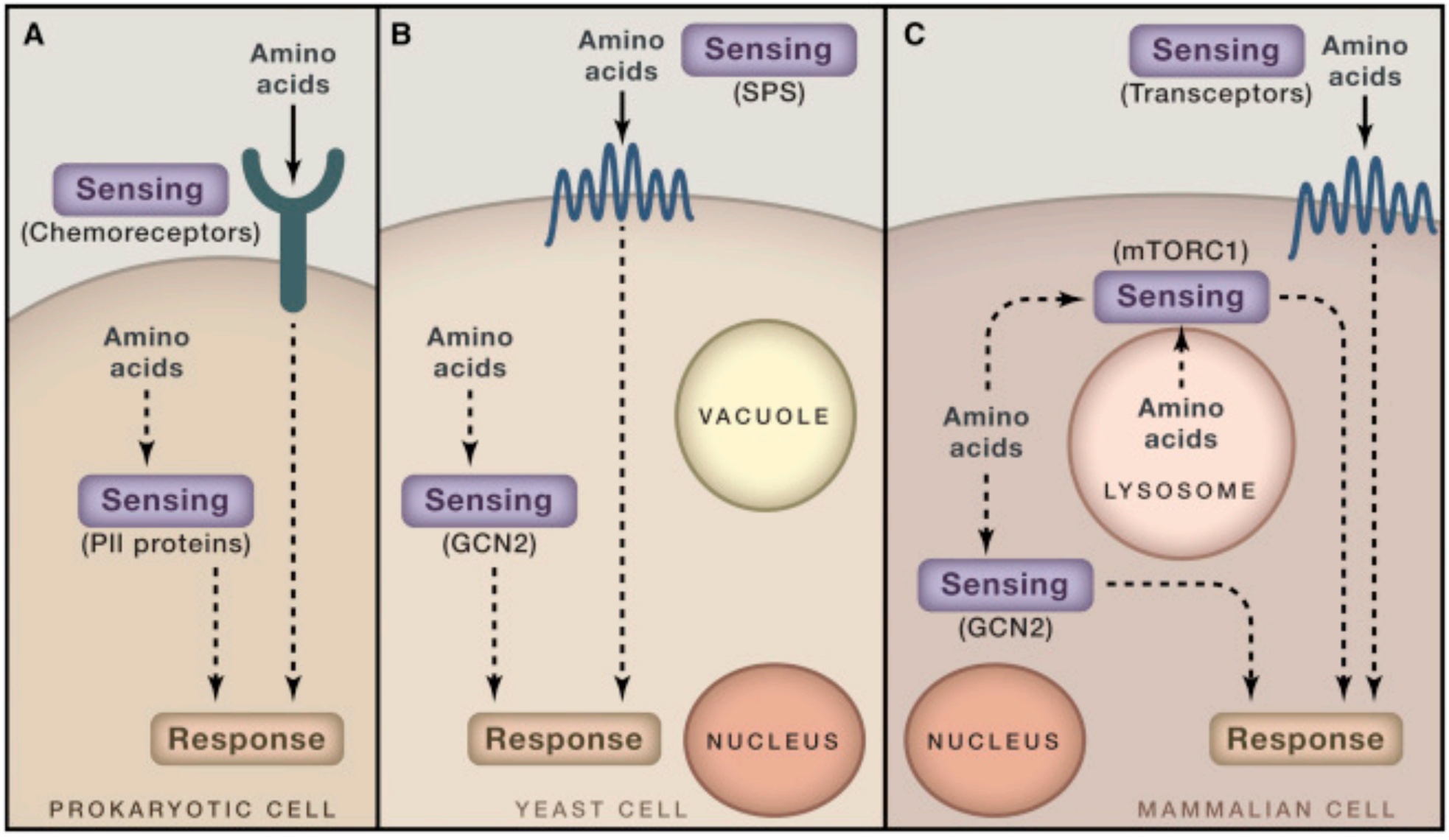
Chantranupong et al, ***Cell****, 2015*

Multicellular organisms adapted ancient nutrient sensing mechanisms



Chantranupong et al, ***Cell****, 2015*

Multicellular organisms adapted ancient nutrient sensing mechanisms



…and integrated hormonal regulation!!

Chantranupong et al, ***Cell****, 2015*

Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly

Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly

HOMEOSTASIS and ADAPTATION

Why Nutrient Sensing?

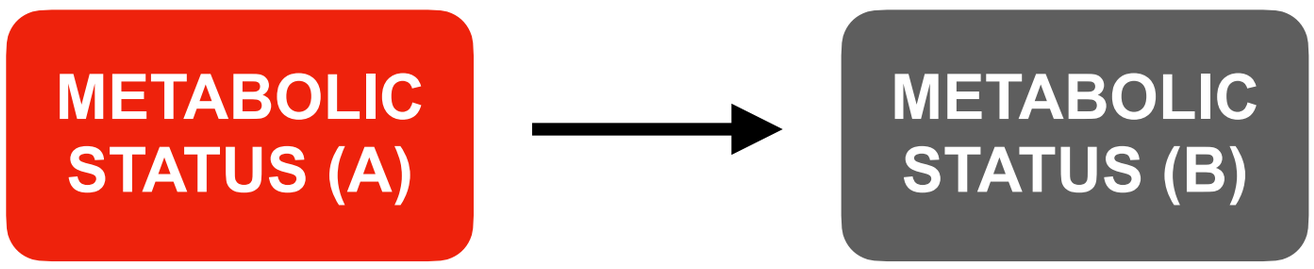
Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly

HOMEOSTASIS and ADAPTATION

FEEDBACK or FEEDFORWARD

Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly

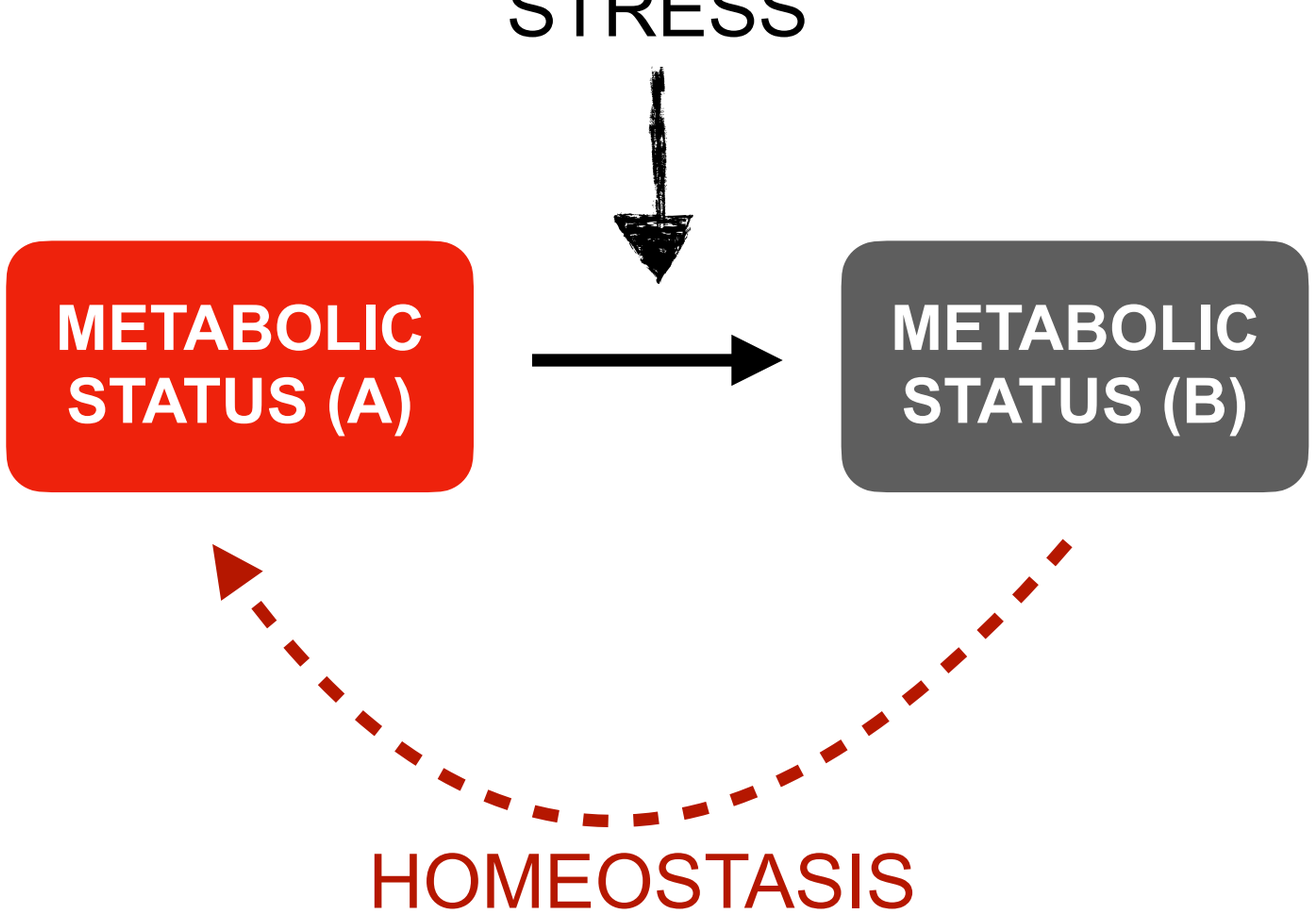


Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly

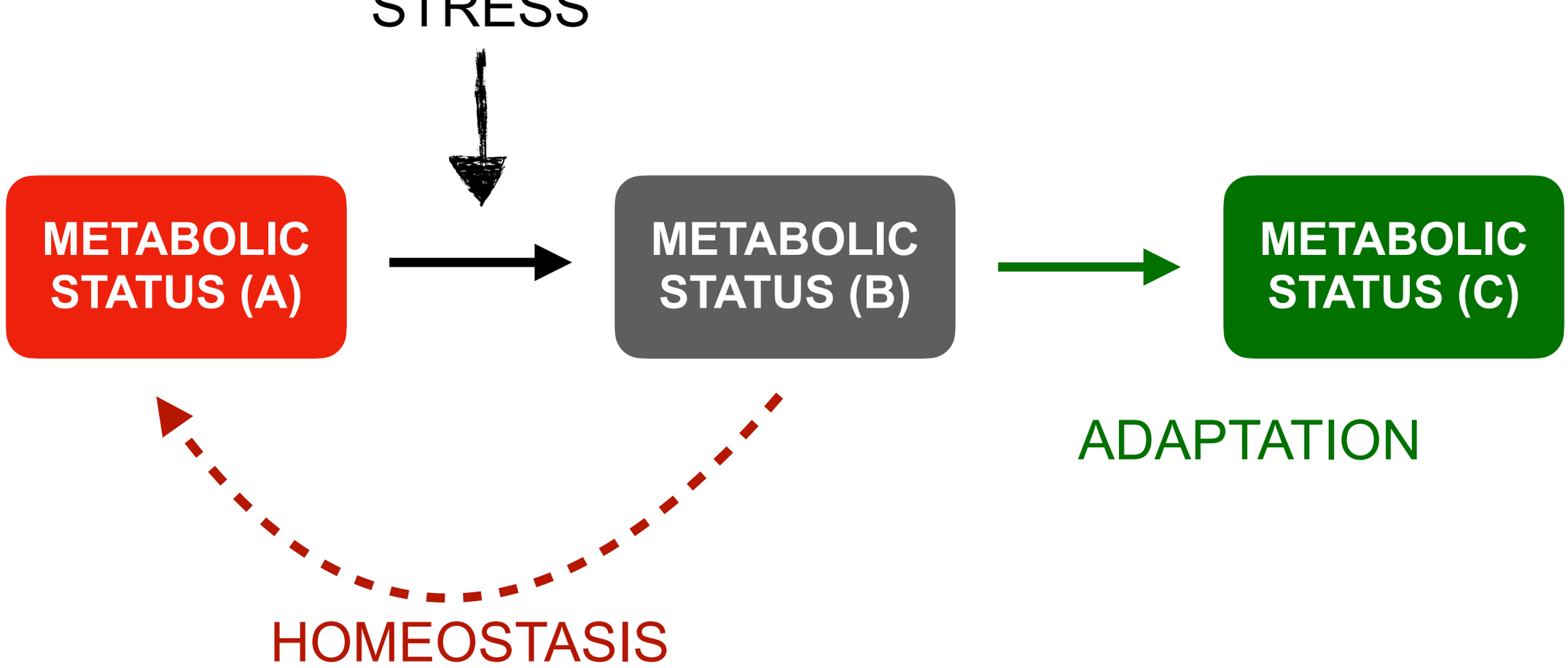
Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly



Why Nutrient Sensing?

Like all biological systems, cells must respond to changes in resources and adjust their metabolism accordingly



Why Nutrient Sensing?

1. Restrain toxicity
2. Enable metabolic conservation
3. Ensure stable levels of key metabolites

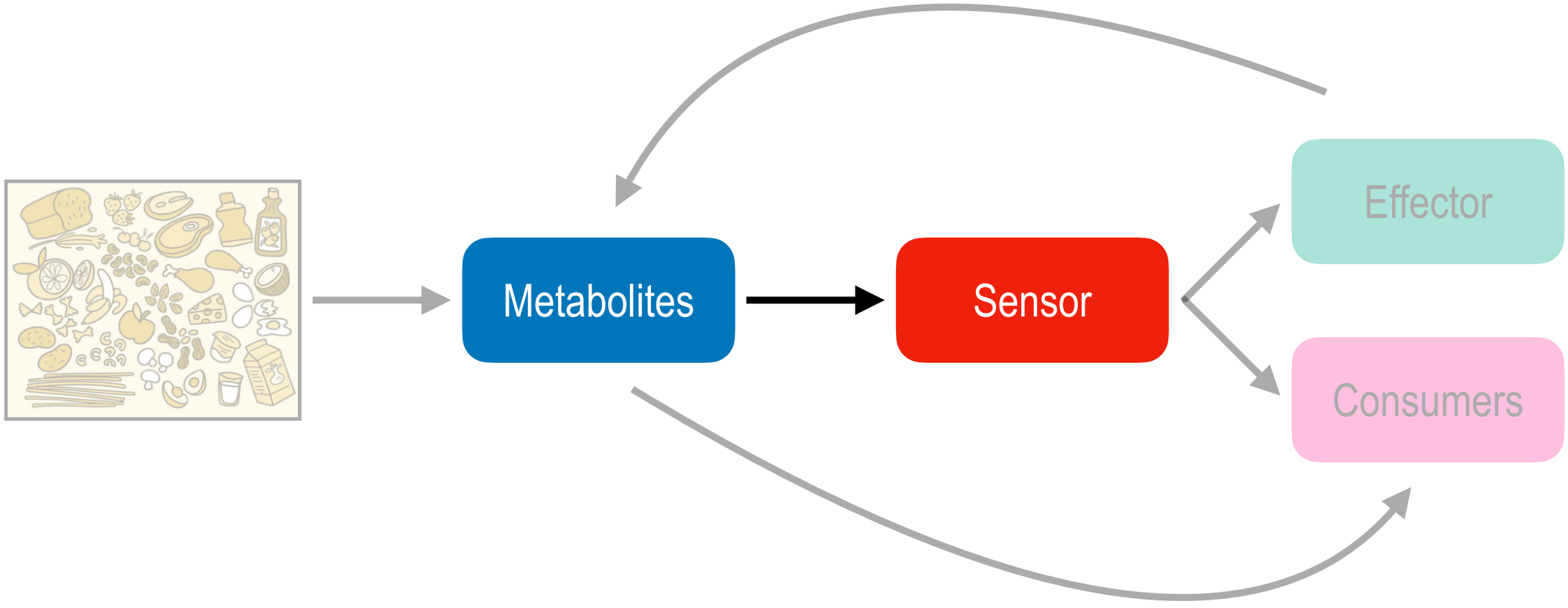
FEEDBACK

4. Allow metabolic plasticity
5. Protect against stress

FEEDFORWARD

Molecular mechanisms of nutrient sensing

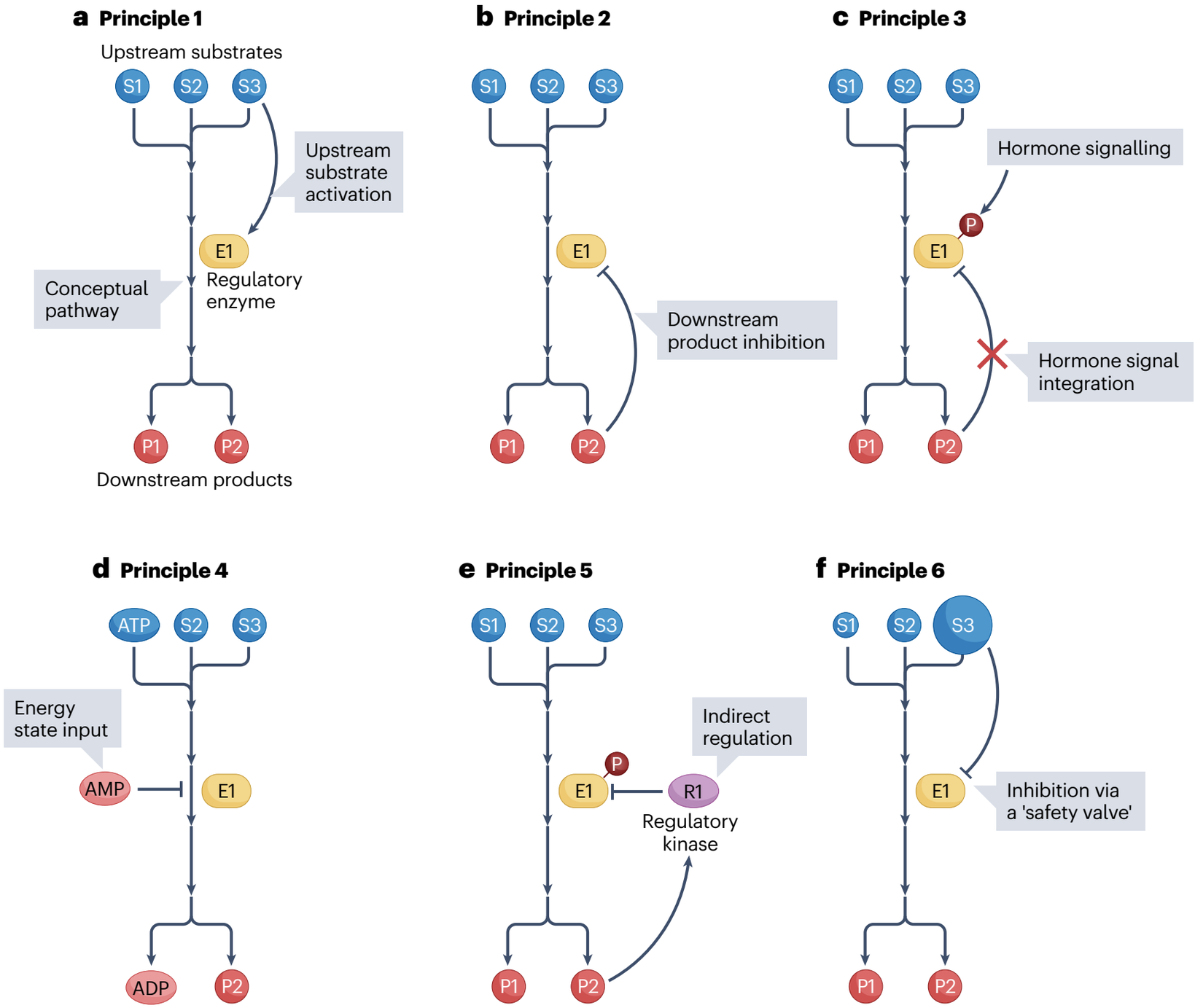
Metabolites are sensed by proteins



Regardless of the manner in which nutrient sensing occurs, for a protein to be considered a sensor, its affinity must be within the range of physiological fluctuations of the concentration of the nutrient or its surrogate.

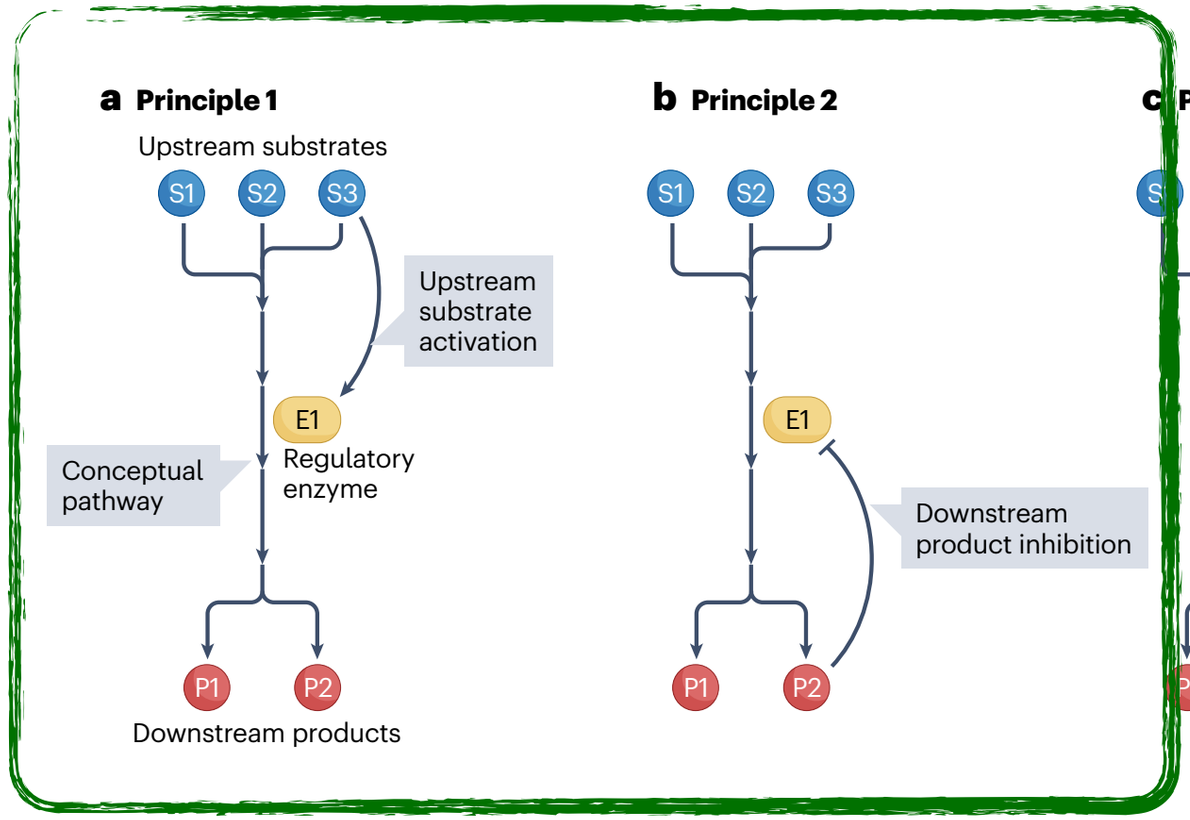
Adapted from: Ye & Medzhitov, ***Nat Metab****, 2019*

Different biochemical logics can mediate feedback or feedforward signals



Baker & Rutter, ***NRMCB****, 2023*

Different biochemical logics can mediate feedback or feedforward signals



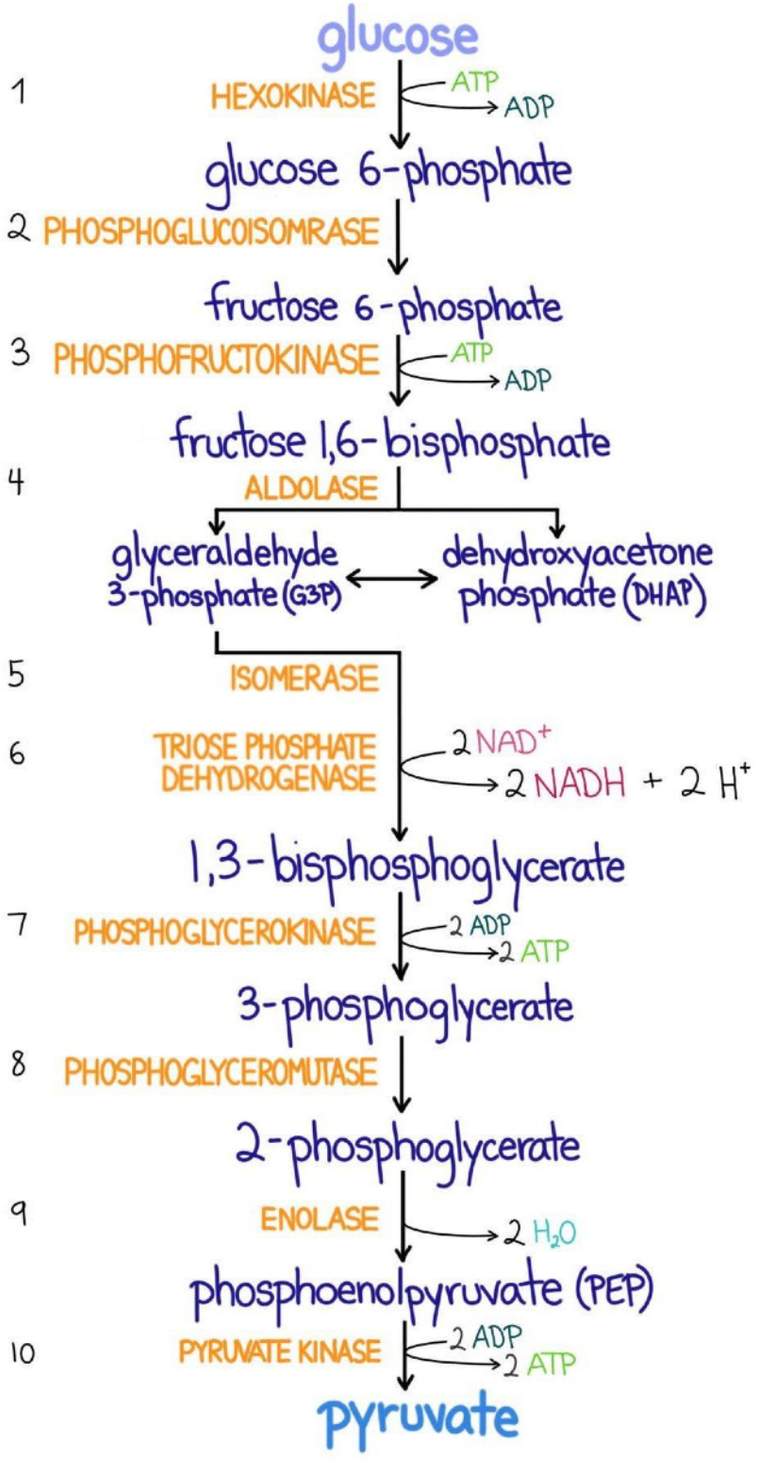
To accomplish metabolite homeostasis, two clear strategies have evolved.

First, the hyper-accumulation of upstream substrates often activates downstream regulatory steps in a pathway. This serves to increase the flux through the pathway, thereby returning metabolite concentrations to within the desired window.

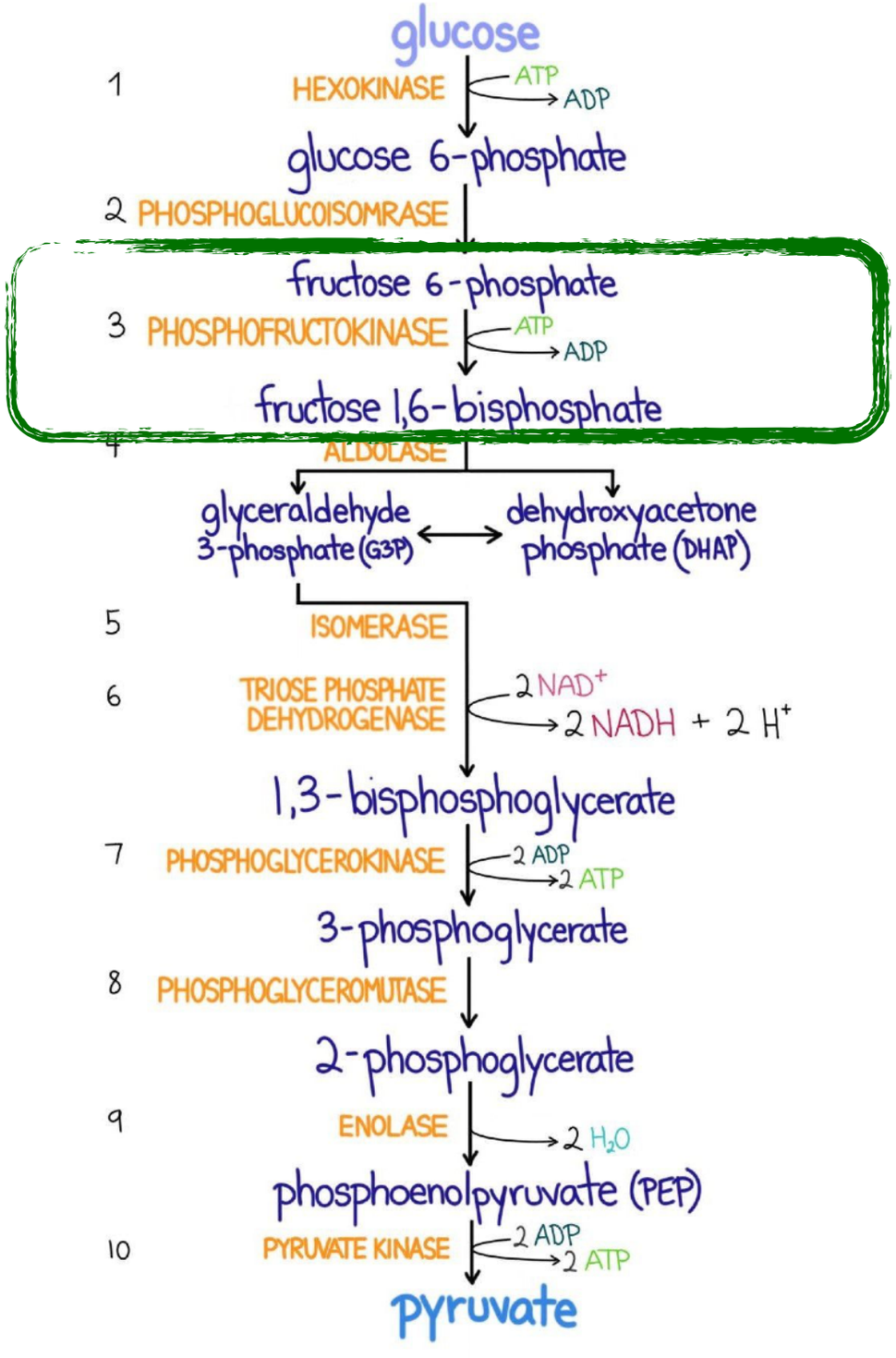
Second, the hyper-accumulation of downstream products often inhibits upstream steps in a pathway. This mechanism slows the synthesis of overly abundant intermediates to modulate a pathway based on the physiologic state.

Baker & Rutter, ***NRMCB****, 2023*

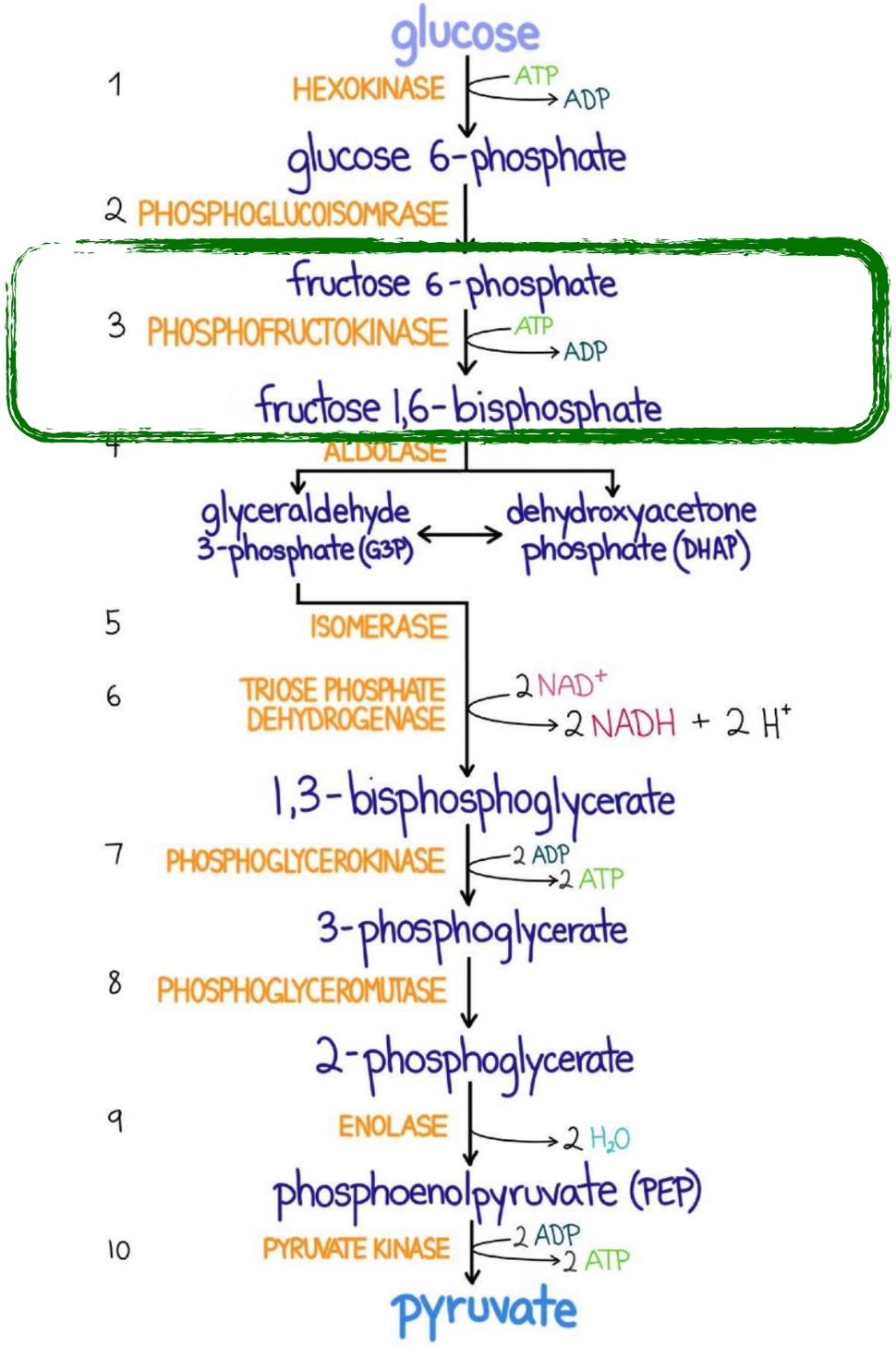
Example: Phospho-Fructose Kinase 1 (PFK1)



Example: Phospho-Fructose Kinase 1 (PFK1)

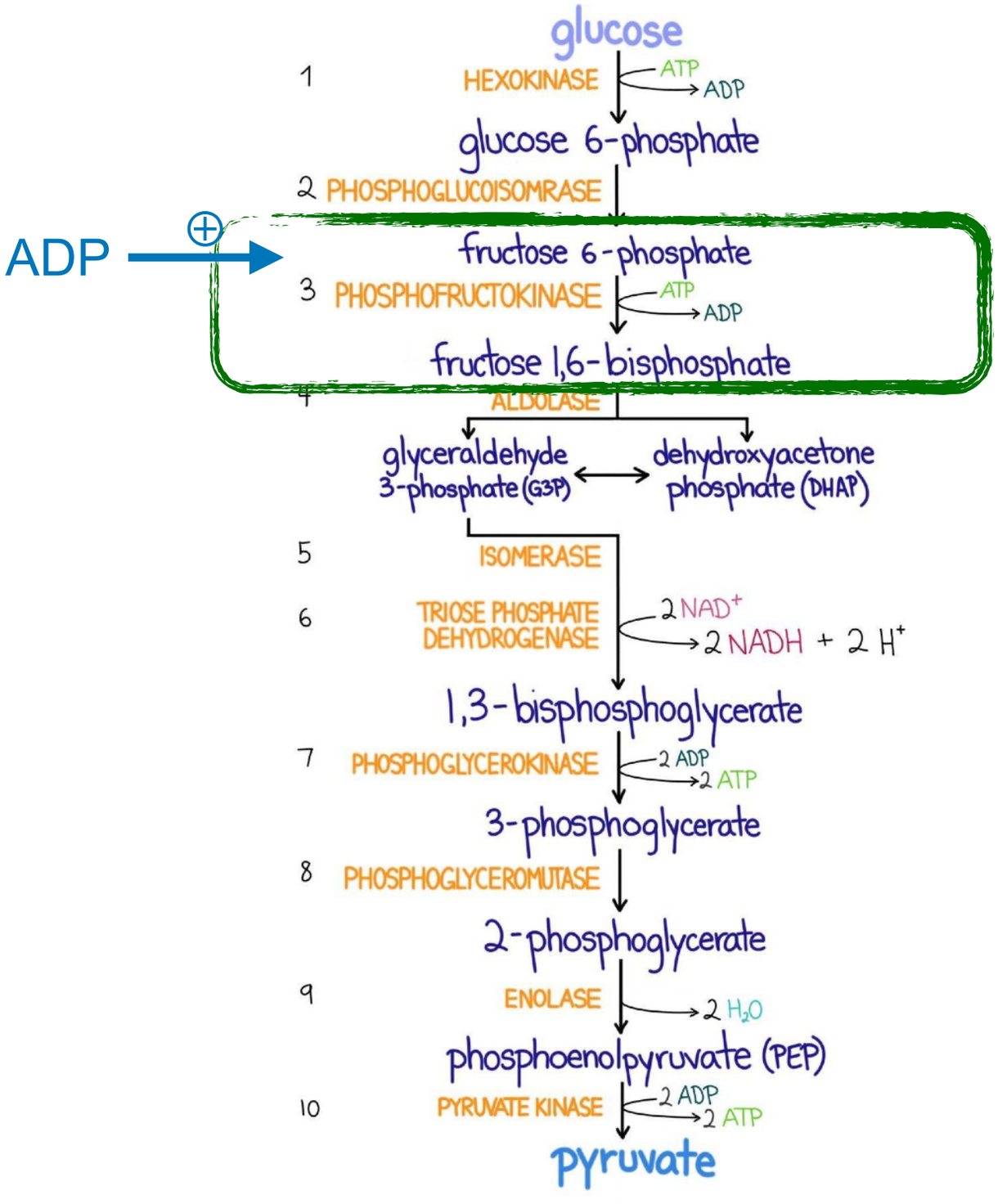


Example: Phospho-Fructose Kinase 1 (PFK1)



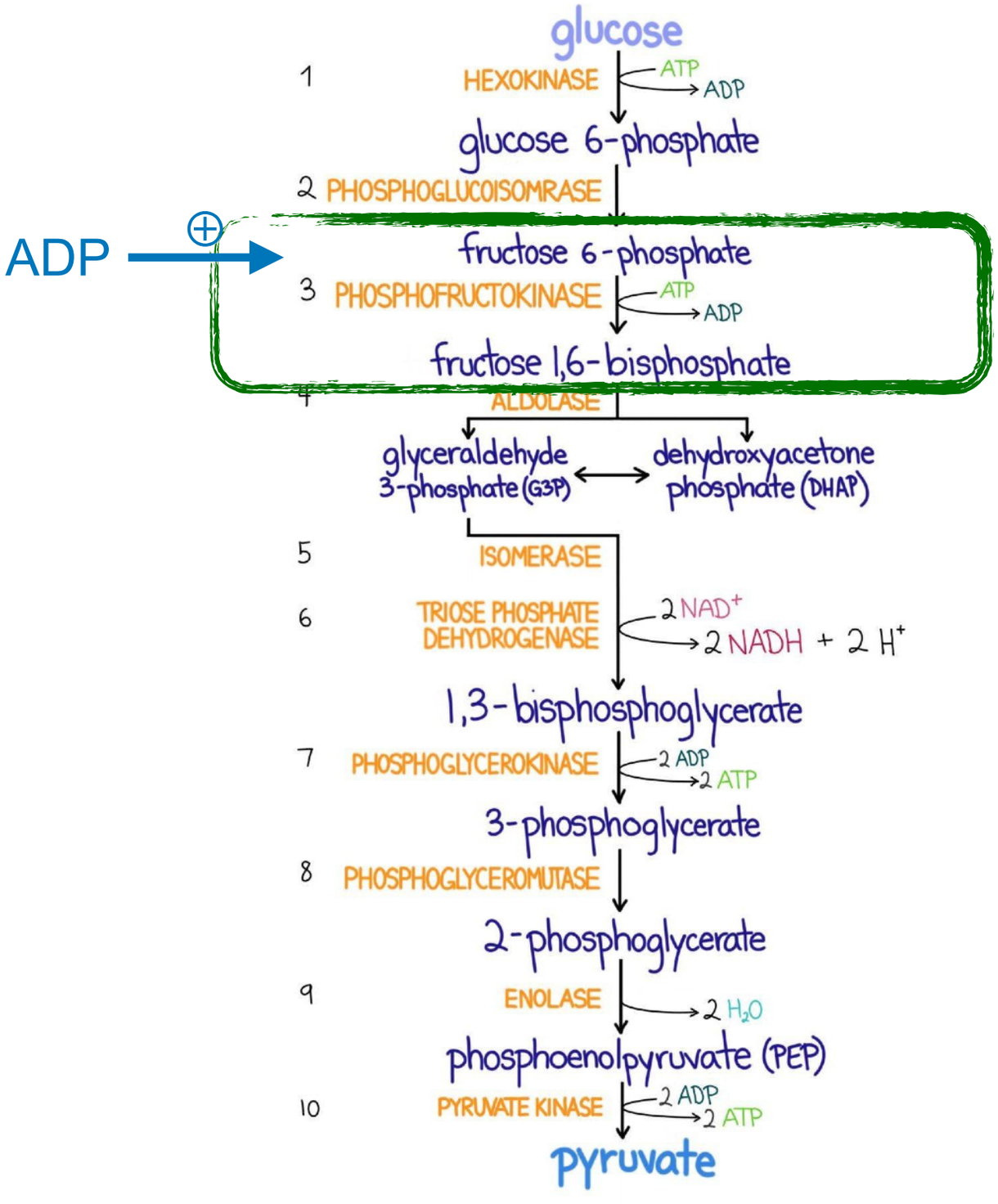
Even though ADP is produced directly by PFK1 in the process of phosphate transfer, the overall result of glycolysis is to produce two net ATPs from ADP per consumed glucose.

Example: Phospho-Fructose Kinase 1 (PFK1)



Even though ADP is produced directly by PFK1 in the process of phosphate transfer, the overall result of glycolysis is to produce two net ATPs from ADP per consumed glucose.

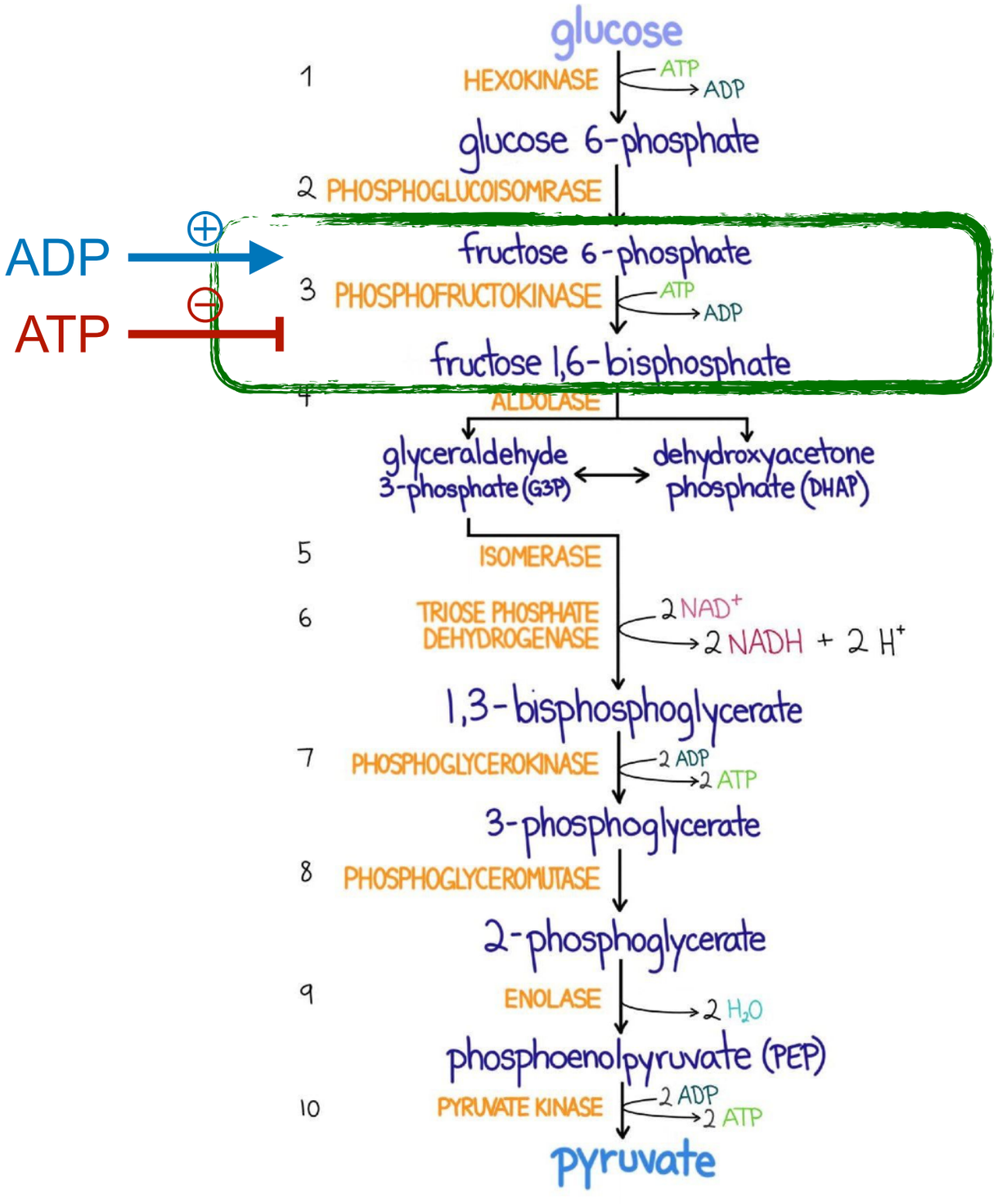
Example: Phospho-Fructose Kinase 1 (PFK1)



Even though ADP is produced directly by PFK1 in the process of phosphate transfer, the overall result of glycolysis is to produce two net ATPs from ADP per consumed glucose.

Activation of PFK1 by ADP illustrates the first principle of metabolic regulation in that ADP is an upstream ‘pathway substrate’ of glycolysis and, when accumulated, stimulates PFK1 to facilitate net ADP to ATP conversion.

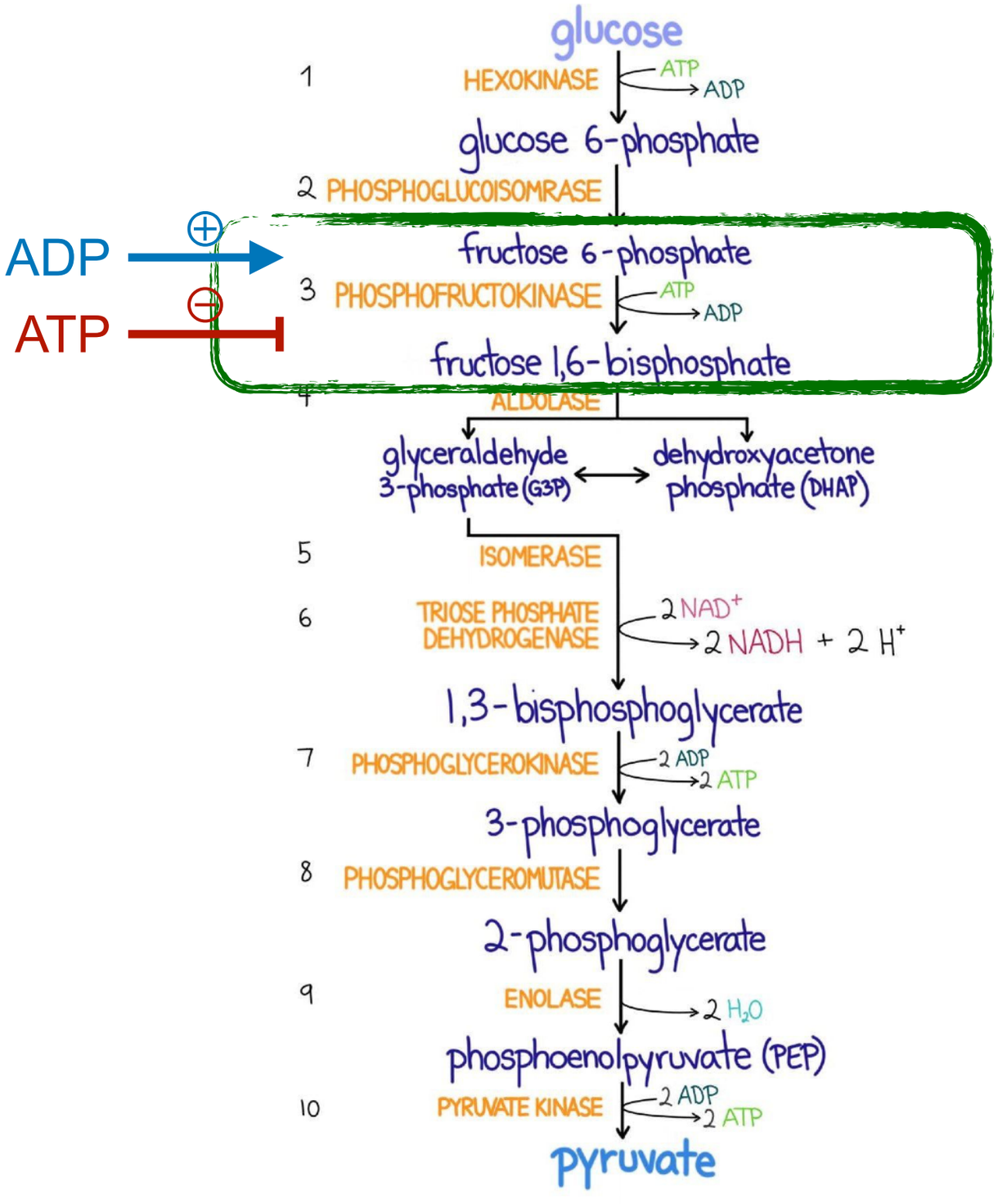
Example: Phospho-Fructose Kinase 1 (PFK1)



Even though ADP is produced directly by PFK1 in the process of phosphate transfer, the overall result of glycolysis is to produce two net ATPs from ADP per consumed glucose.

Activation of PFK1 by ADP illustrates the first principle of metabolic regulation in that ADP is an upstream ‘pathway substrate’ of glycolysis and, when accumulated, stimulates PFK1 to facilitate net ADP to ATP conversion.

Example: Phospho-Fructose Kinase 1 (PFK1)



Even though ADP is produced directly by PFK1 in the process of phosphate transfer, the overall result of glycolysis is to produce two net ATPs from ADP per consumed glucose.

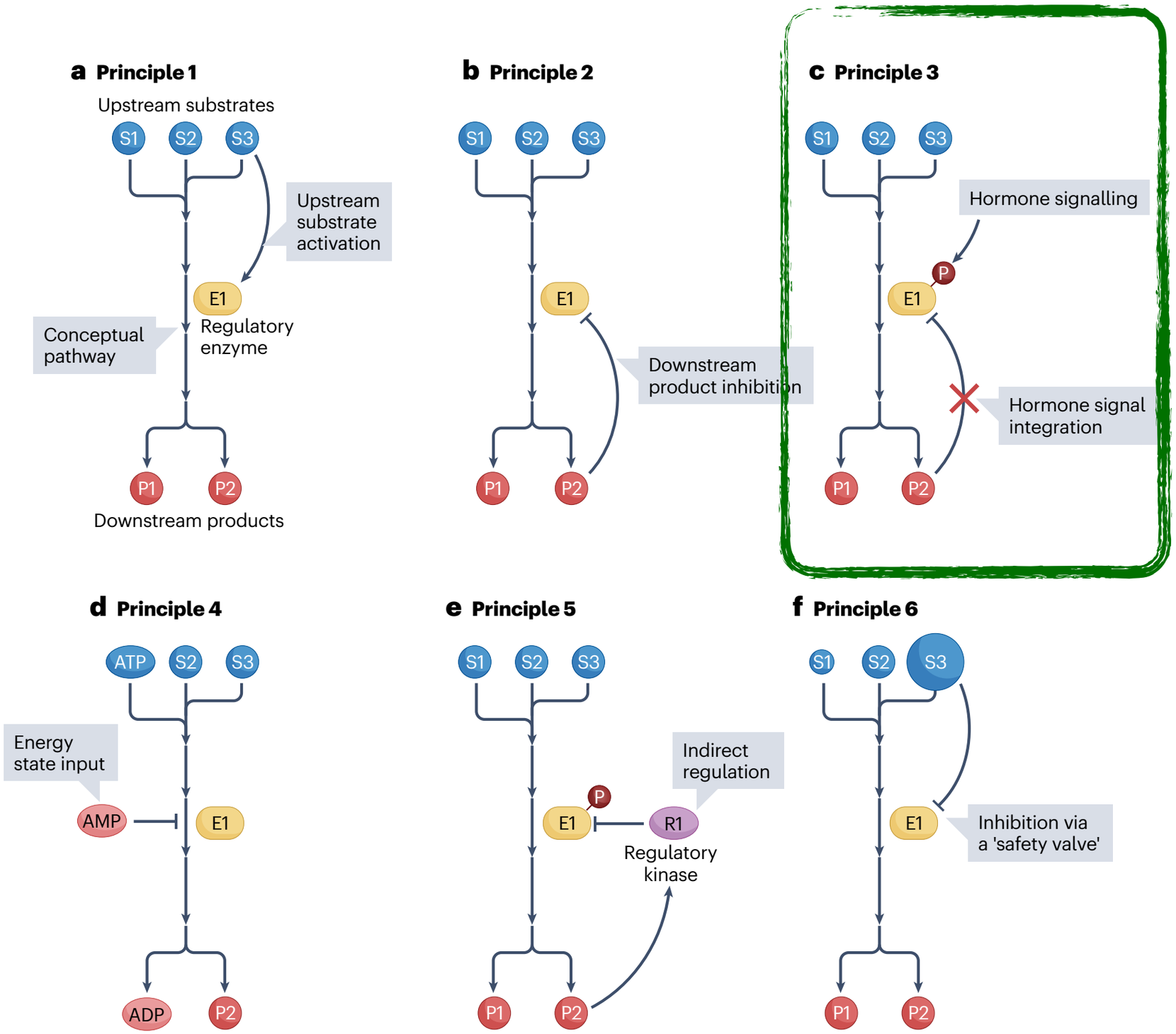
Activation of PFK1 by ADP illustrates the first principle of metabolic regulation in that ADP is an upstream ‘pathway substrate’ of glycolysis and, when accumulated, stimulates PFK1 to facilitate net ADP to ATP conversion.

PFK1 is also negatively regulated by ATP, as well as multiple downstream products from glycolysis including phosphoenolpyruvate (PEP), 3-phosphoglycerate (3PG) and citrate.

This regulation highlights the second principle of metabolic regulation.

Importantly, the accumulation of downstream products, but not their formation per se, inhibits upstream reactions.

Different biochemical logics can mediate feedback or feedforward signals

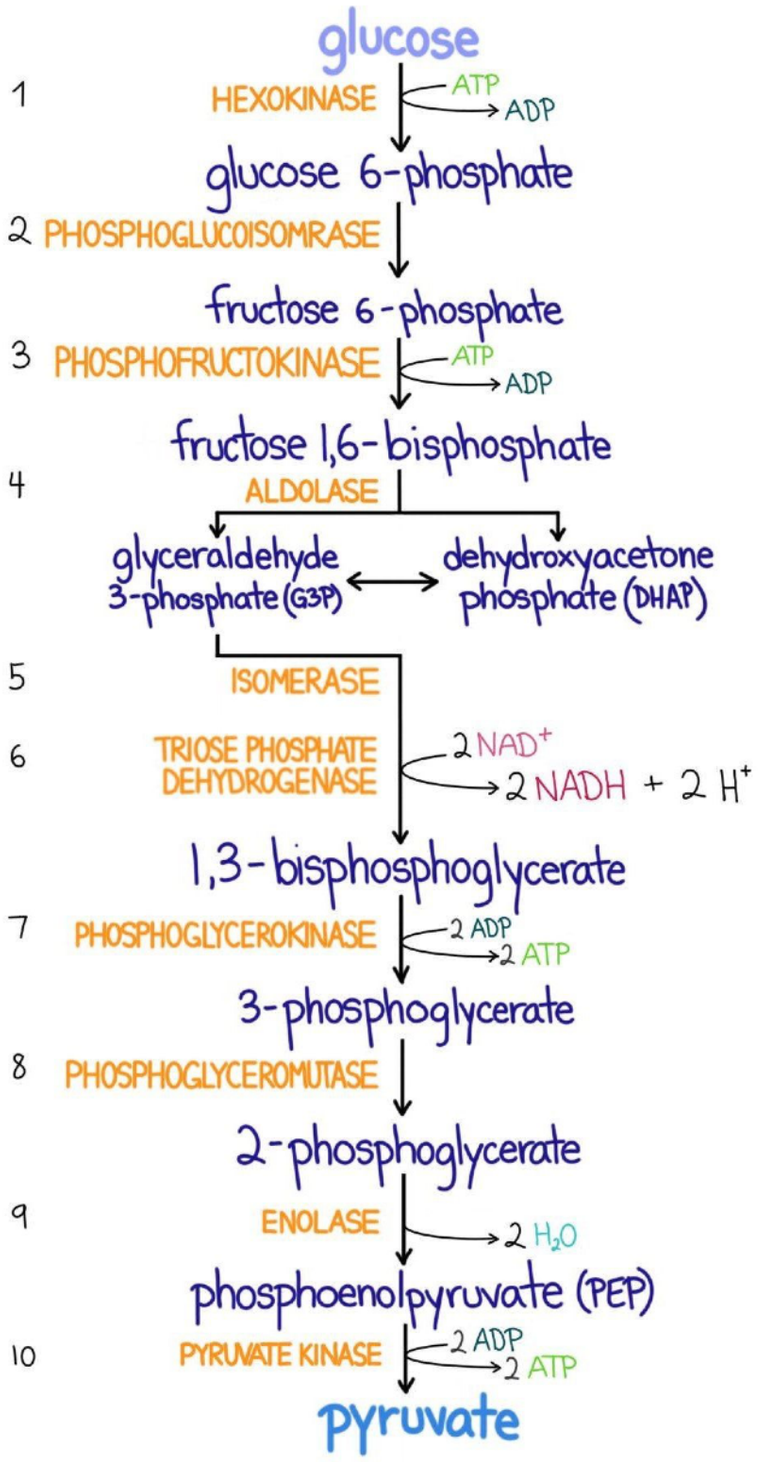


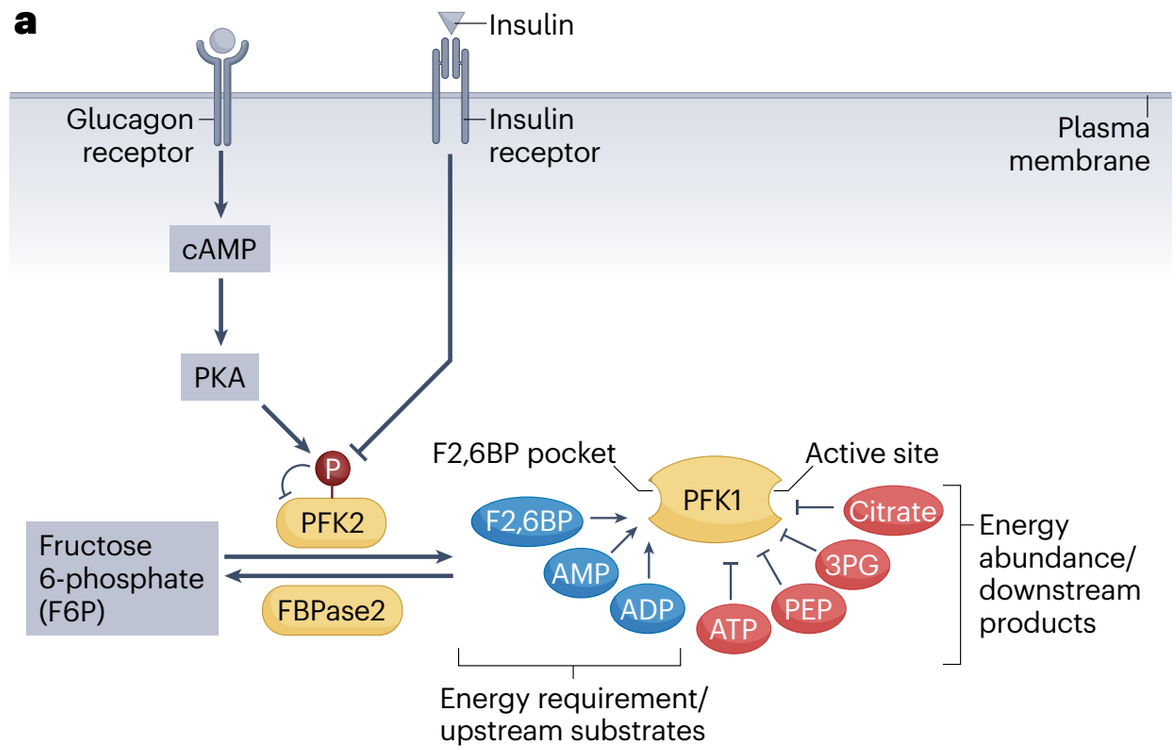
First two principles are very simple. Multicellular organisms require more sophisticated systems.

Inputs from additional pathways frequently feed into or even override intra-pathway homeostatic metabolite signals

*Baker & Rutter, **NRMCB**, 2023*

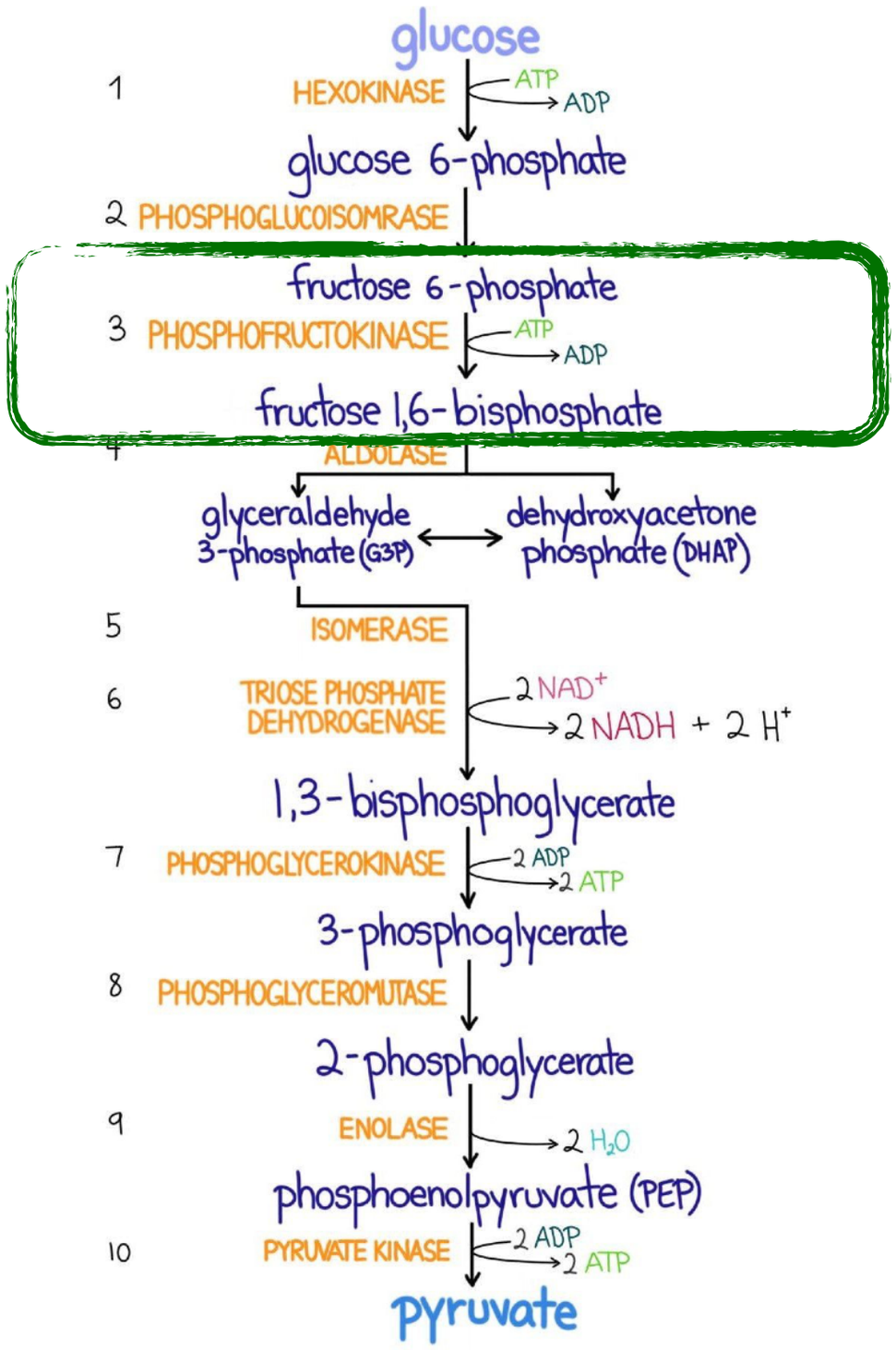
Example: Phospho-Fructose Kinase 1 (PFK1)

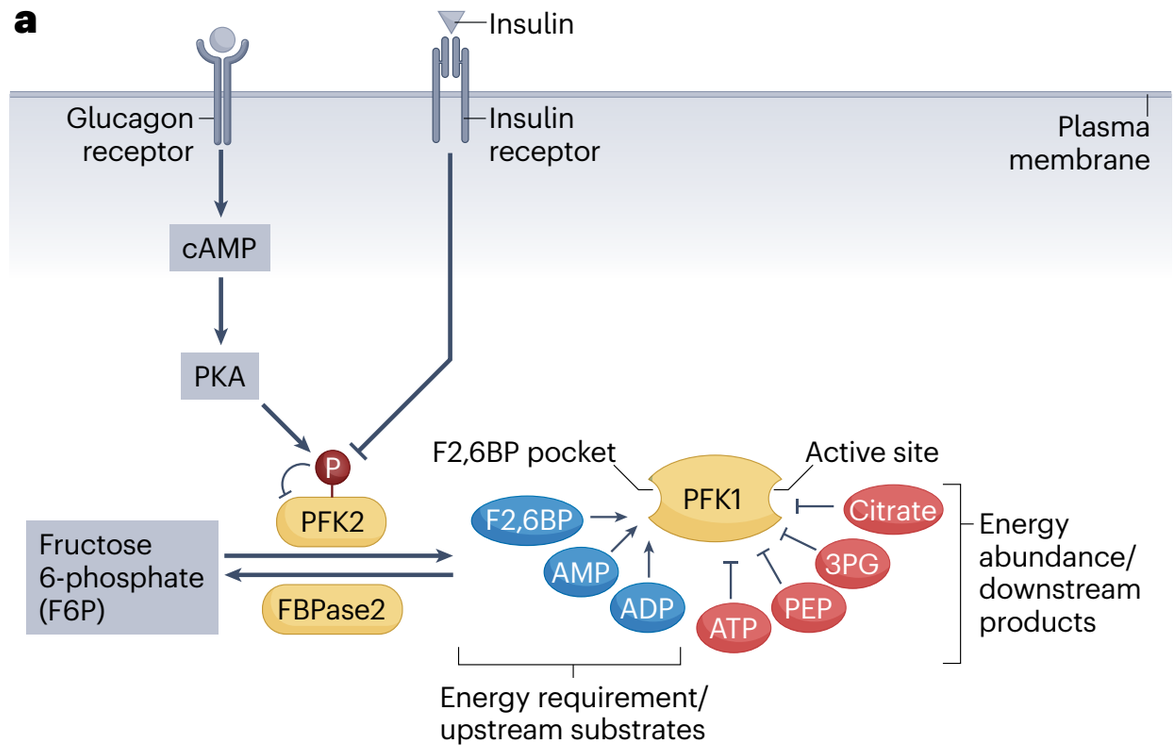




In the fasted state, glucagon activates protein kinase A (PKA) and induces PFK2 phosphorylation. This inactivates PFK2, thereby decreasing F2,6BP and inhibiting PFK1 and glycolytic flux. By contrast, insulin signalling in the fed state dephosphorylates and activates PFK2, thereby increasing F2,6BP to permit the flow of carbon through glycolysis even in a state of energy abundance (ATP accumulation).

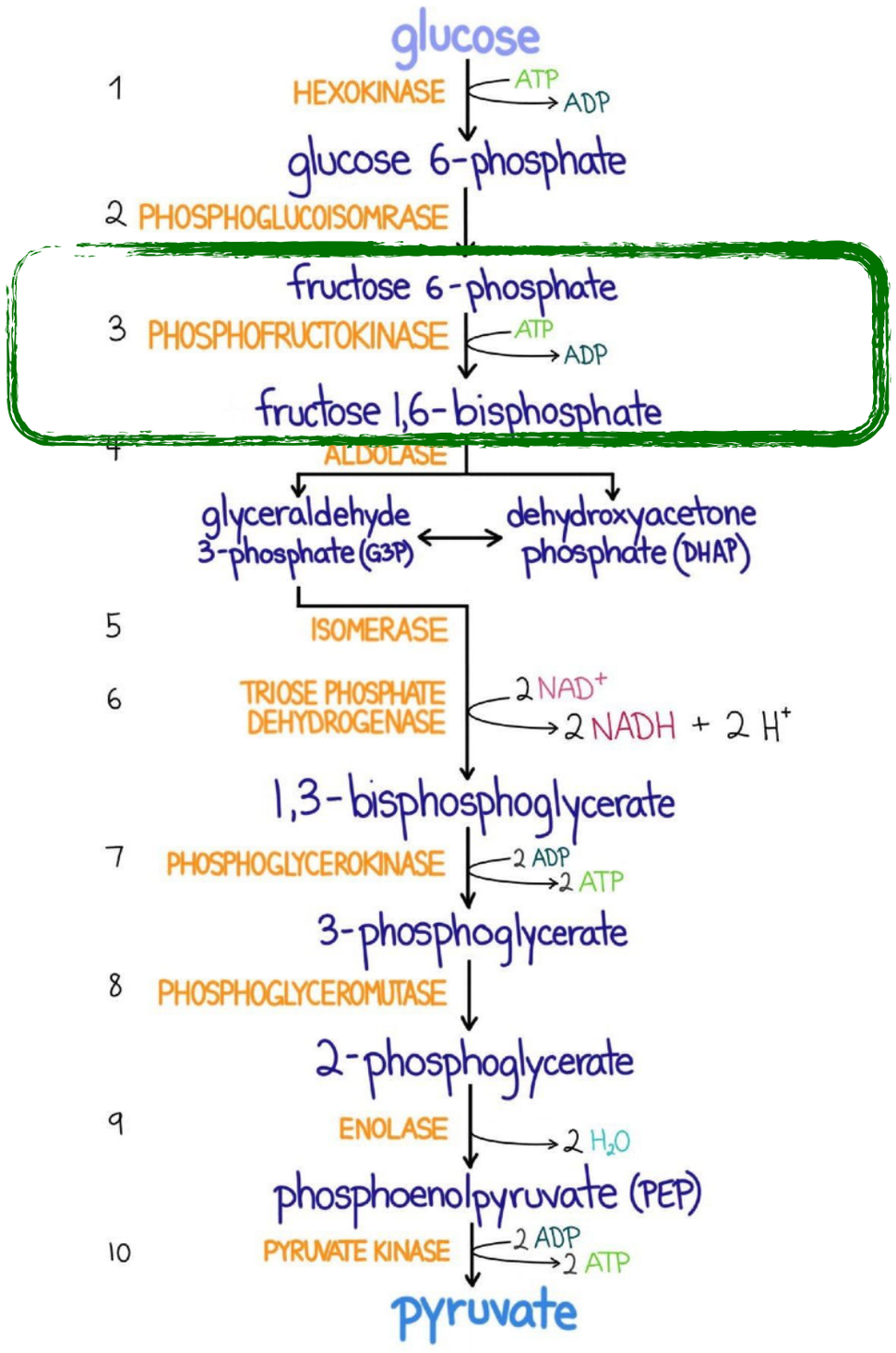
Example: Phospho-Fructose Kinase 1 (PFK1)



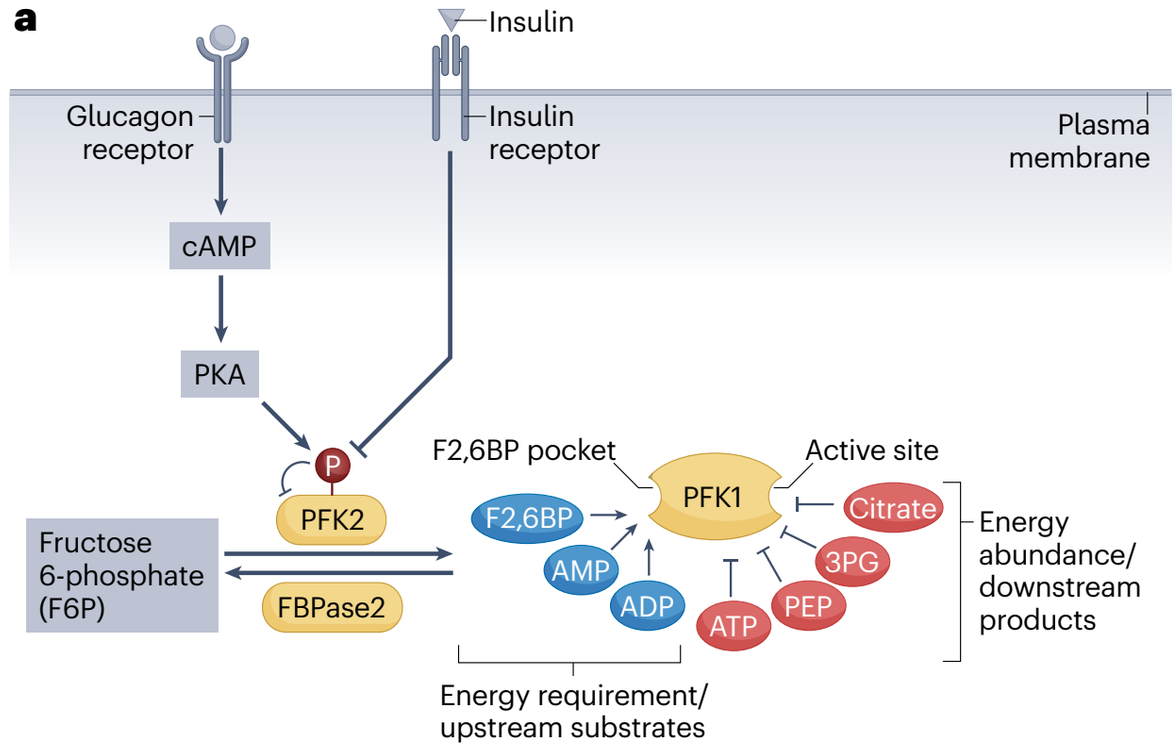


In the fasted state, glucagon activates protein kinase A (PKA) and induces PFK2 phosphorylation. This inactivates PFK2, thereby decreasing F2,6BP and inhibiting PFK1 and glycolytic flux. By contrast, insulin signalling in the fed state dephosphorylates and activates PFK2, thereby increasing F2,6BP to permit the flow of carbon through glycolysis even in a state of energy abundance (ATP accumulation).

Example: Phospho-Fructose Kinase 1 (PFK1)

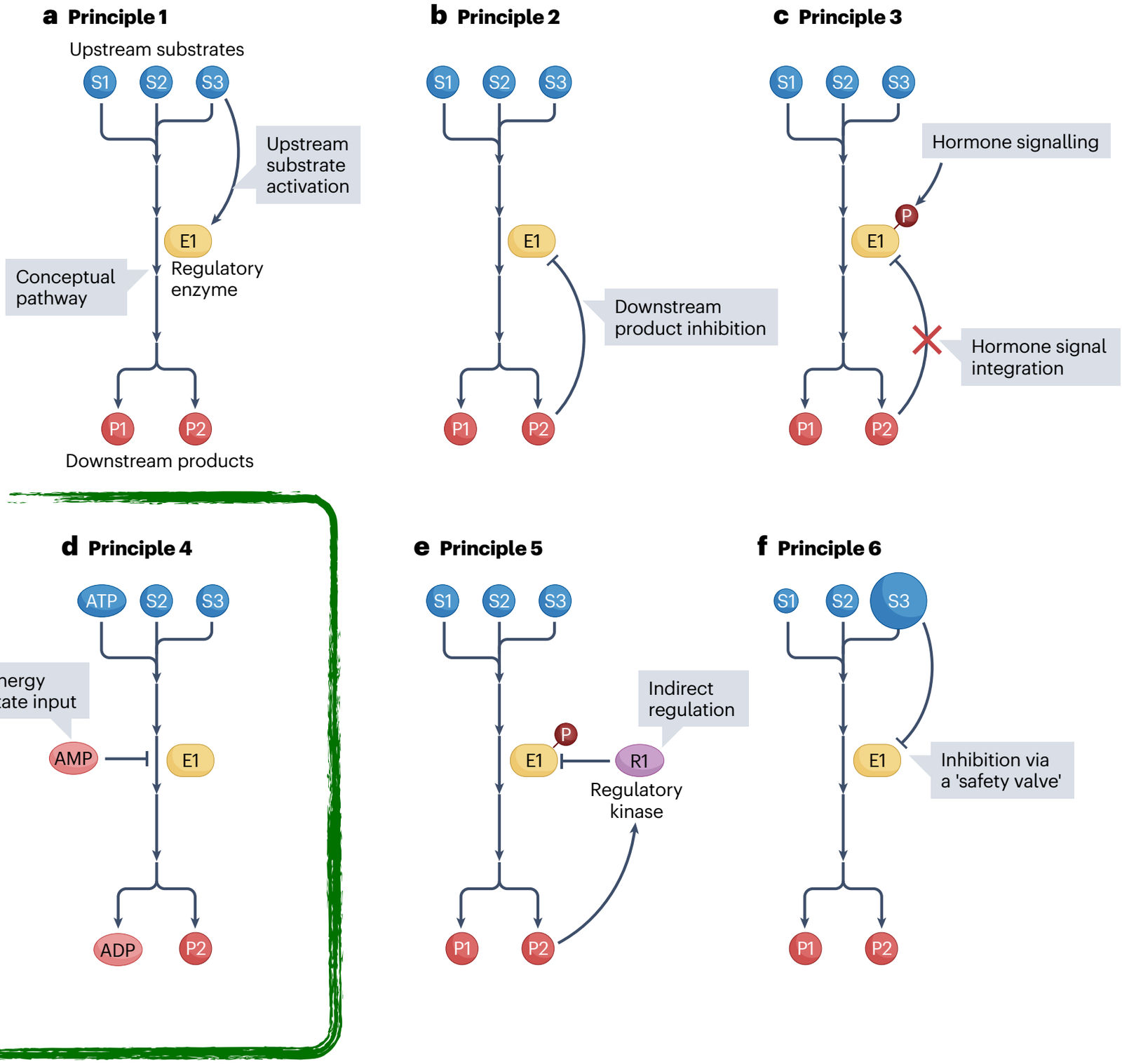


An example of this third principle, PFK1 is activated by the signaling metabolite fructose 2,6-bisphosphate (F2,6BP), which is synthesized by phosphofructokinase 2 (PFK2). PFK2 phosphorylates F6P to generate F2,6BP, and is regulated by the insulin and glucagon pathways in metazoans (to couple cell responses to systemic glucose levels).



In the fasted state, glucagon activates protein kinase A (PKA) and induces PFK2 phosphorylation. This inactivates PFK2, thereby decreasing F2,6BP and inhibiting PFK1 and glycolytic flux. By contrast, insulin signalling in the fed state dephosphorylates and activates PFK2, thereby increasing F2,6BP to permit the flow of carbon through glycolysis even in a state of energy abundance (ATP accumulation).

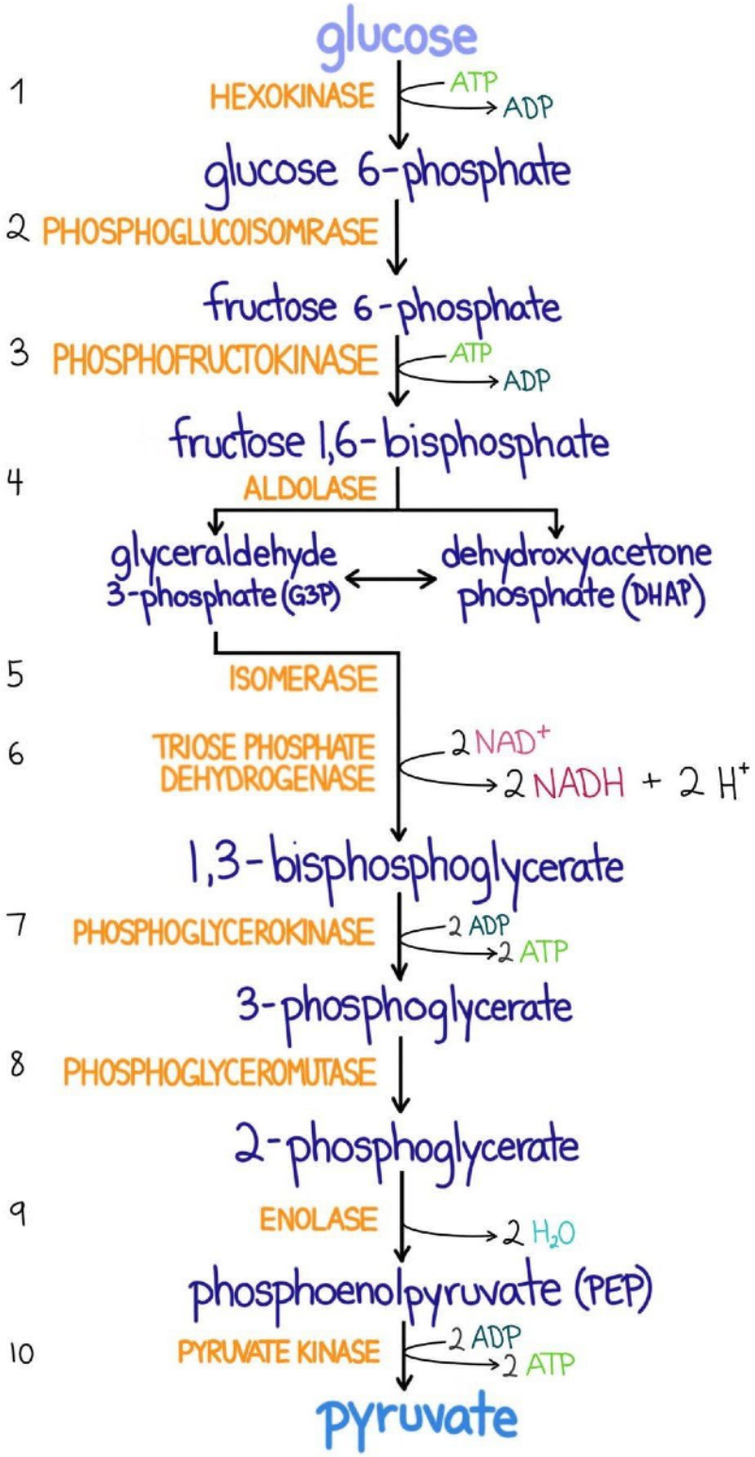
Different biochemical logics can mediate feedback or feedforward signals



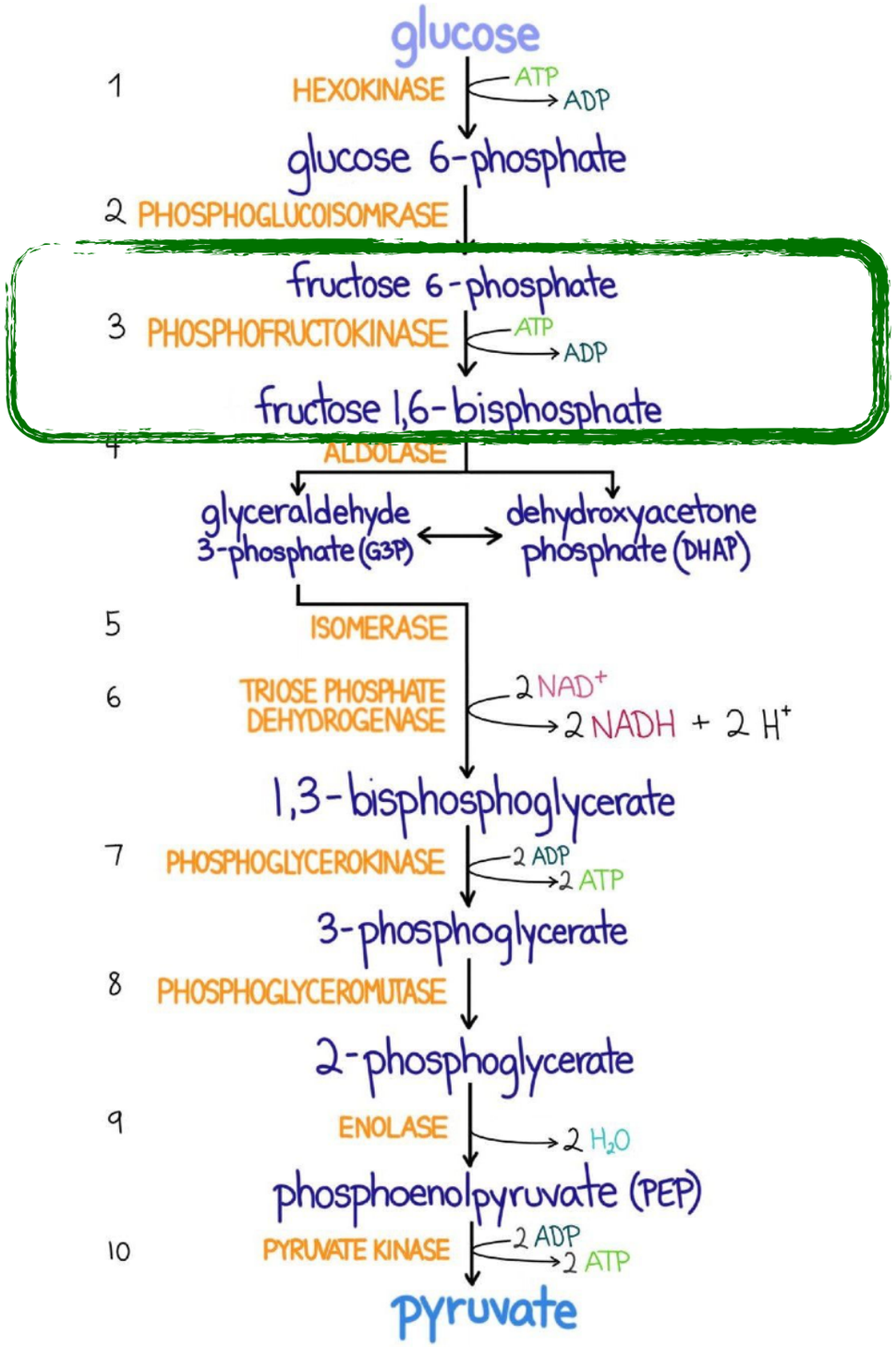
Reactions require energy equivalents (often, ATP). Pathways that consume a lot of ATP needs to account for the cell's energy status (ATP:ADP ratio)

Baker & Rutter, ***NRMCB****, 2023*

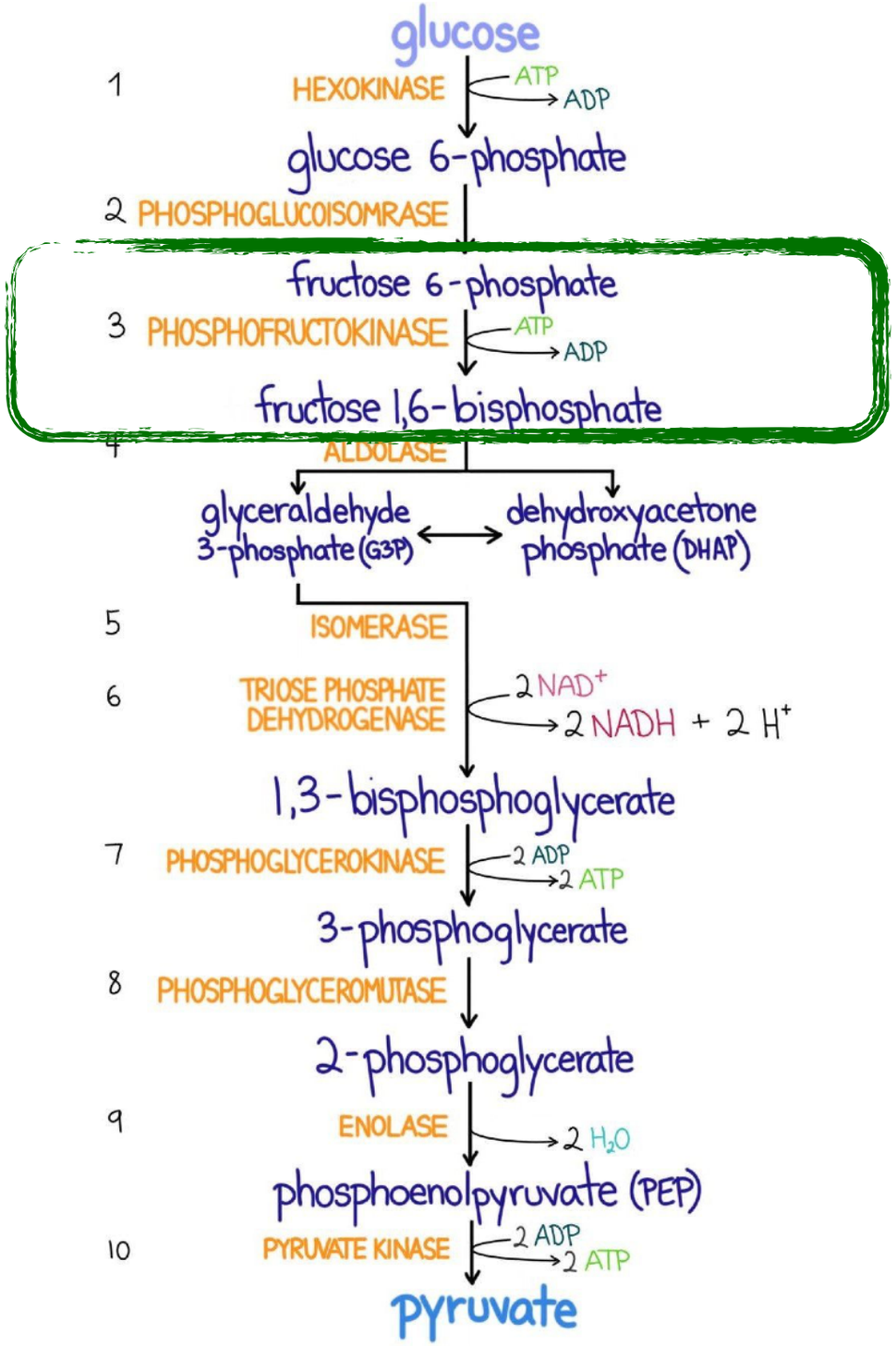
Example: Phospho-Fructose Kinase 1 (PFK1)



Example: Phospho-Fructose Kinase 1 (PFK1)



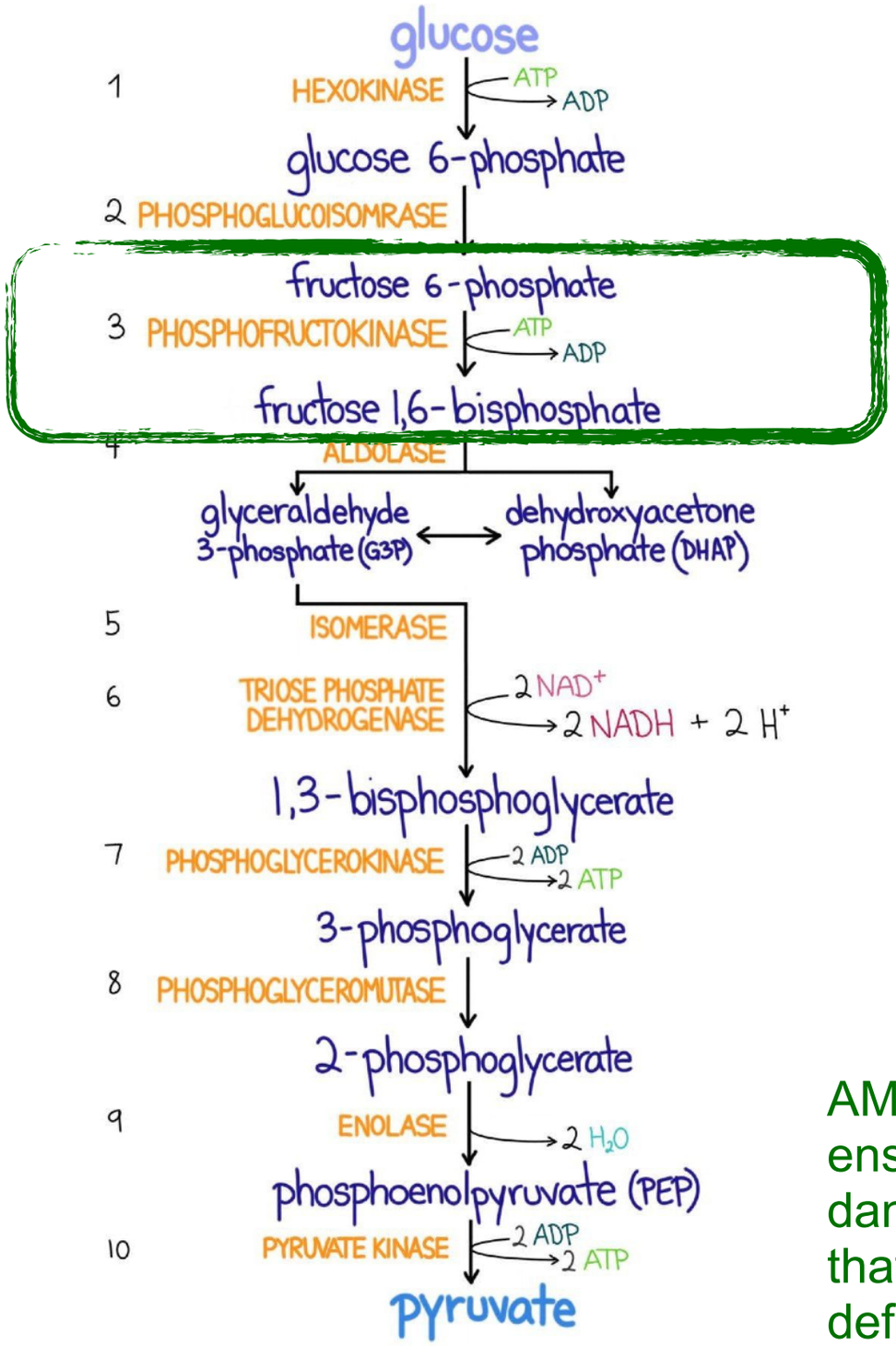
Example: Phospho-Fructose Kinase 1 (PFK1)



Glycolysis and gluconeogenesis are inversely regulated to prevent futile cycling.

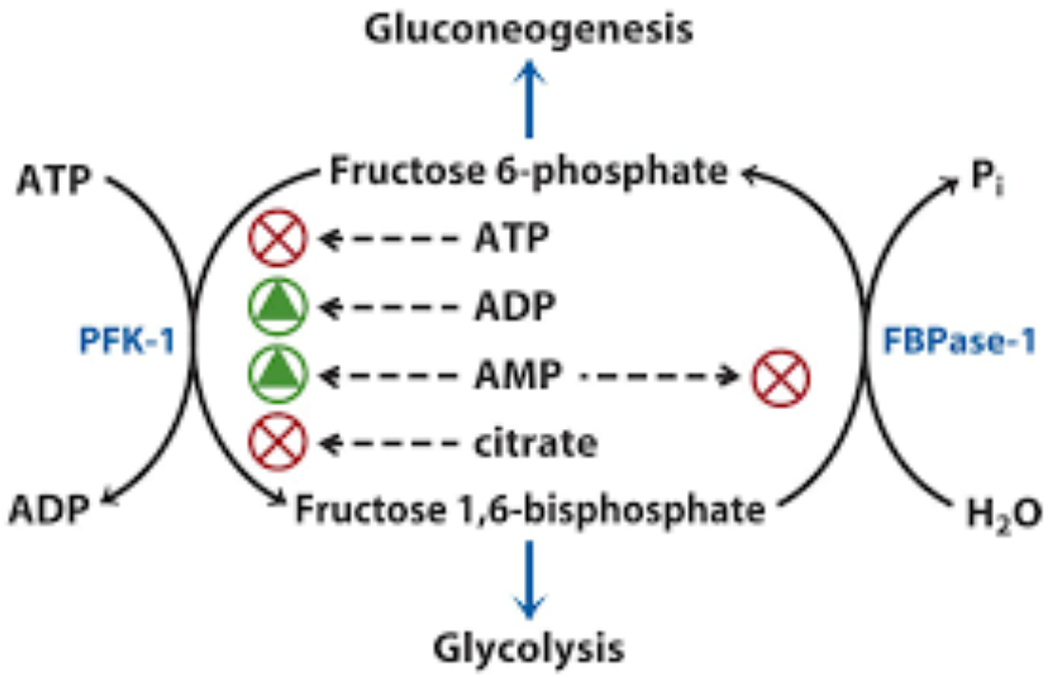
The counterpart of PFK1 for gluconeogenesis is the enzyme fructose 1,6-bisphosphatase (FBPase1). FBPase1 and PFK1 catalyze opposite reactions, albeit FBPase1 does not regenerate the ATP consumed by PFK1.

Example: Phospho-Fructose Kinase 1 (PFK1)



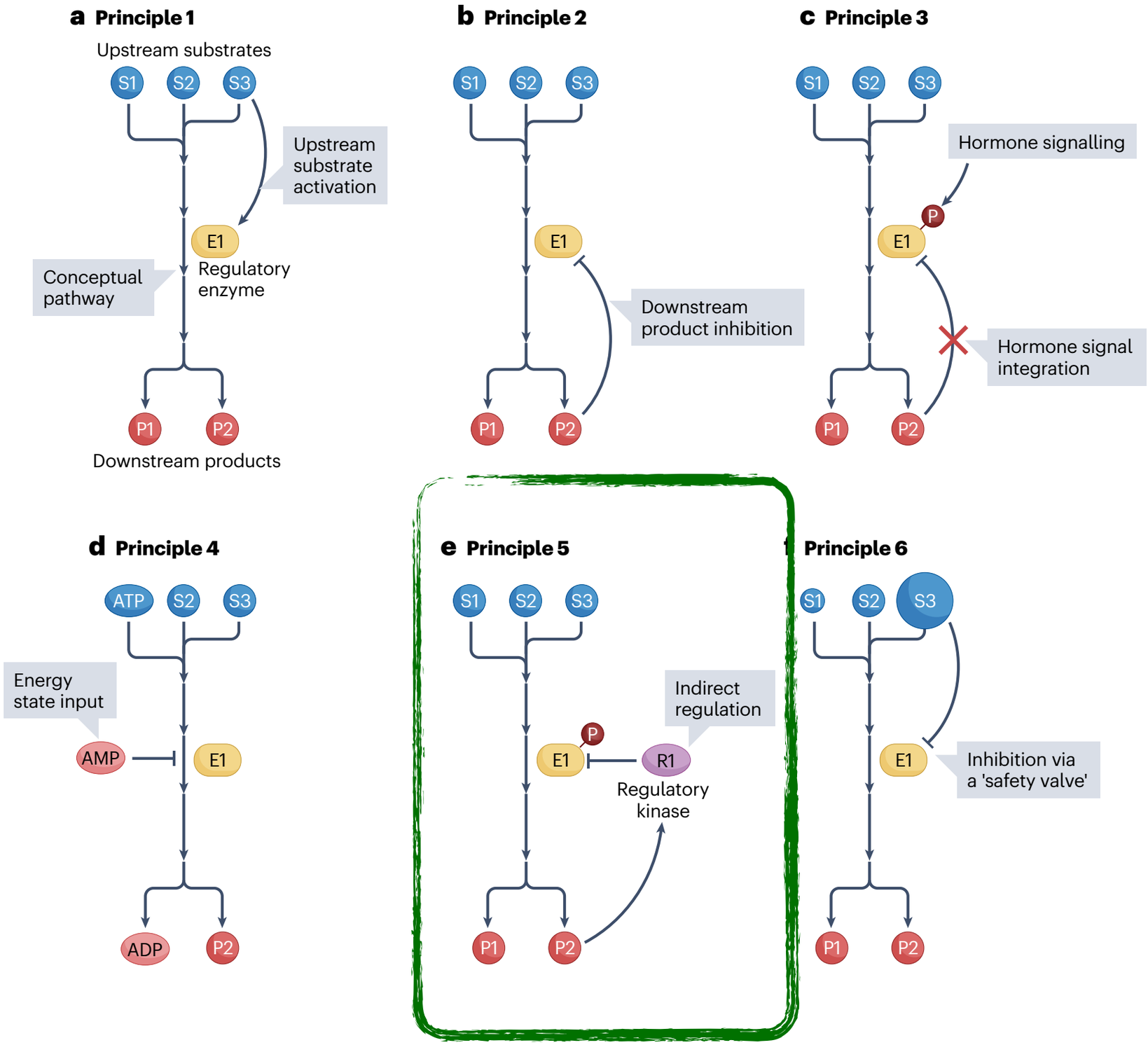
Glycolysis and gluconeogenesis are inversely regulated to prevent futile cycling.

The counterpart of PFK1 for gluconeogenesis is the enzyme fructose 1,6-bisphosphatase (FBPase1). FBPase1 and PFK1 catalyze opposite reactions, albeit FBPase1 does not regenerate the ATP consumed by PFK1.



AMP activates PFK1 and, conversely, inhibits FBPase1. This ensures cellular survival as the accumulation of AMP indicates dangerously low energy abundance in the form of ATP. In a cell that is undergoing gluconeogenesis but experiences energetic deficits, AMP can halt the production of glucose and propel glycolysis to restore the ATP concentration to a safe level.

Different biochemical logics can mediate feedback or feedforward signals

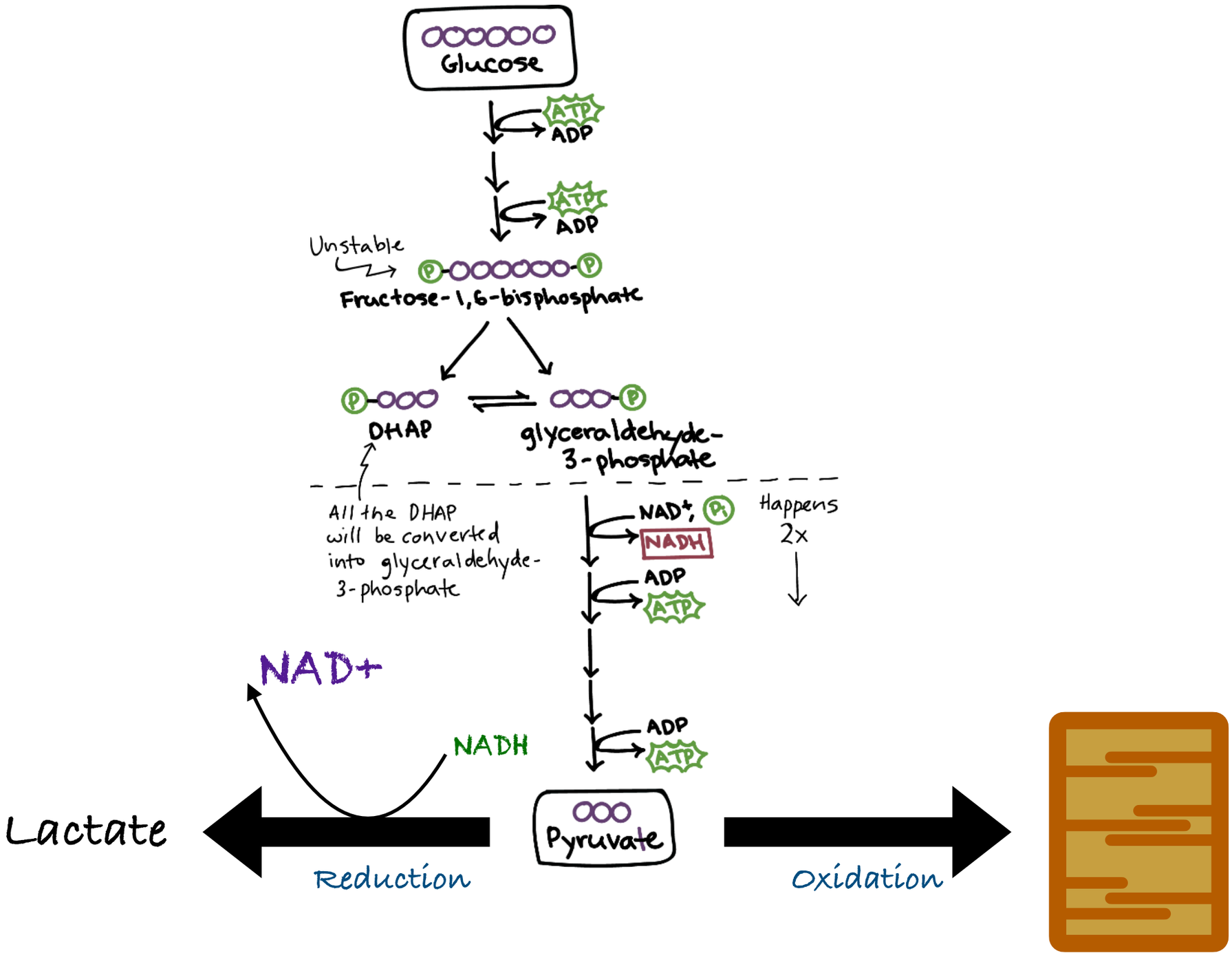


Regulation can occur indirectly.

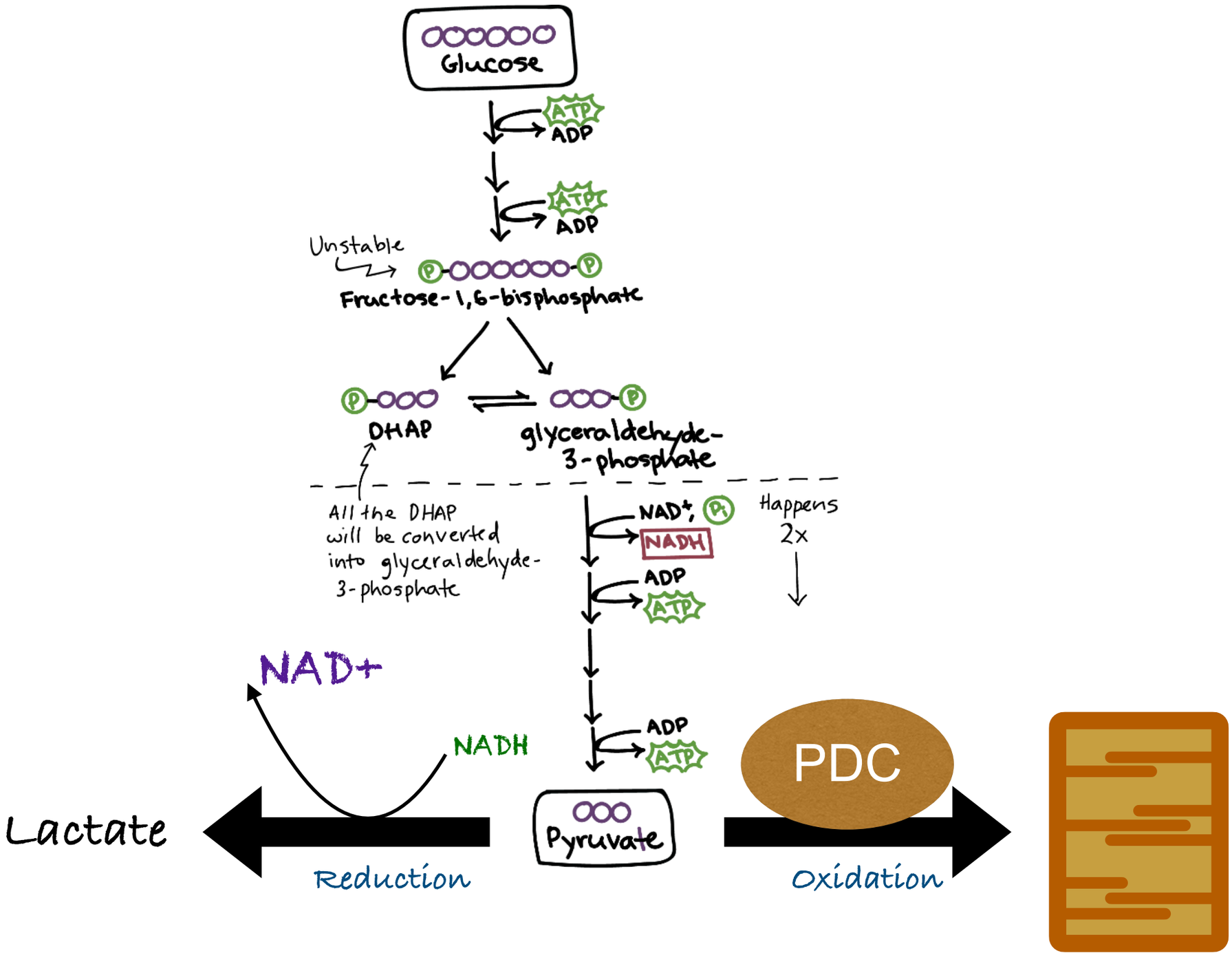
Pro: a regulatory kinase can control multiple pathways (outputs)

Baker & Rutter, ***NRMCB****, 2023*

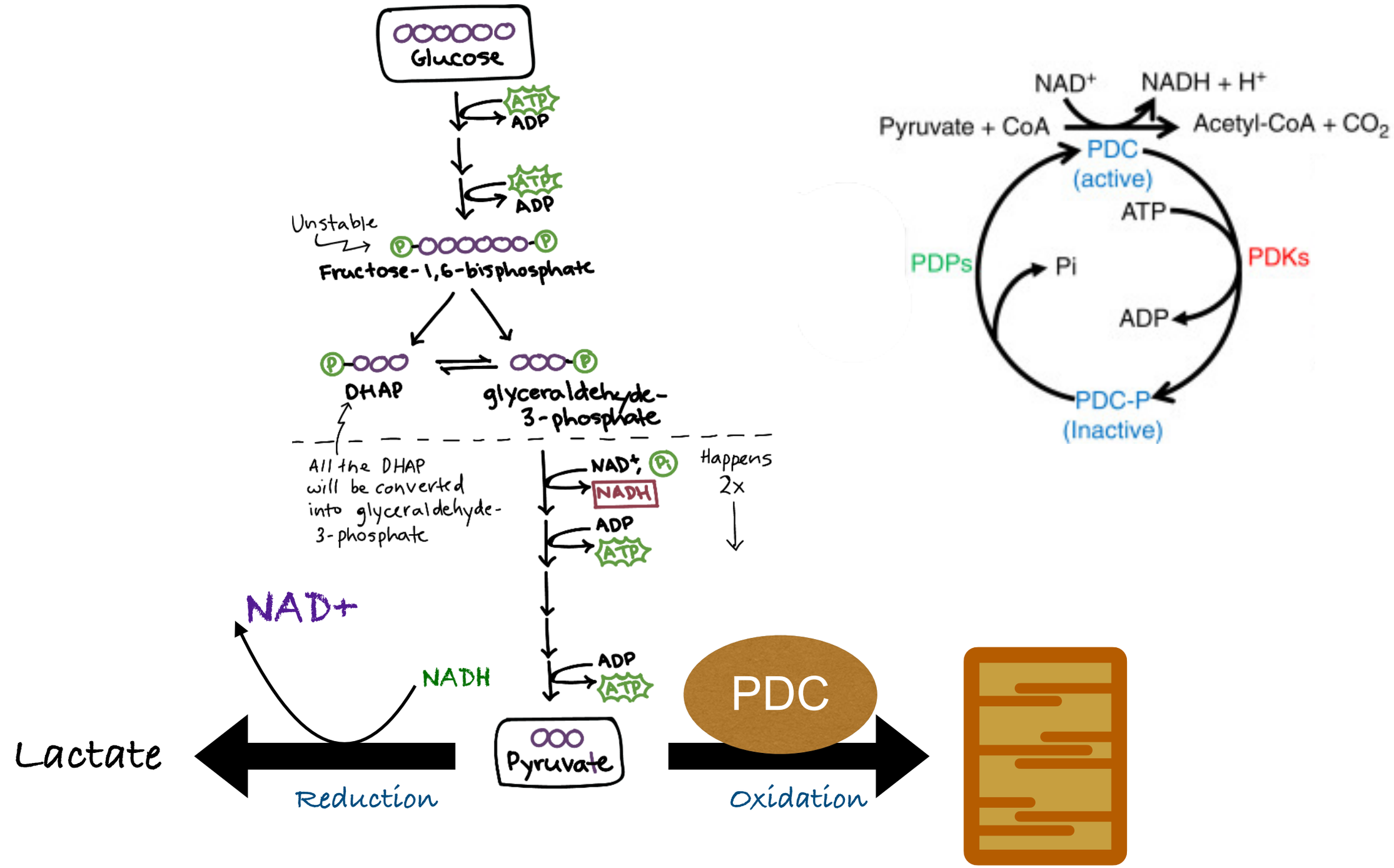
Example: Pyruvate metabolism (PDC-PDK)



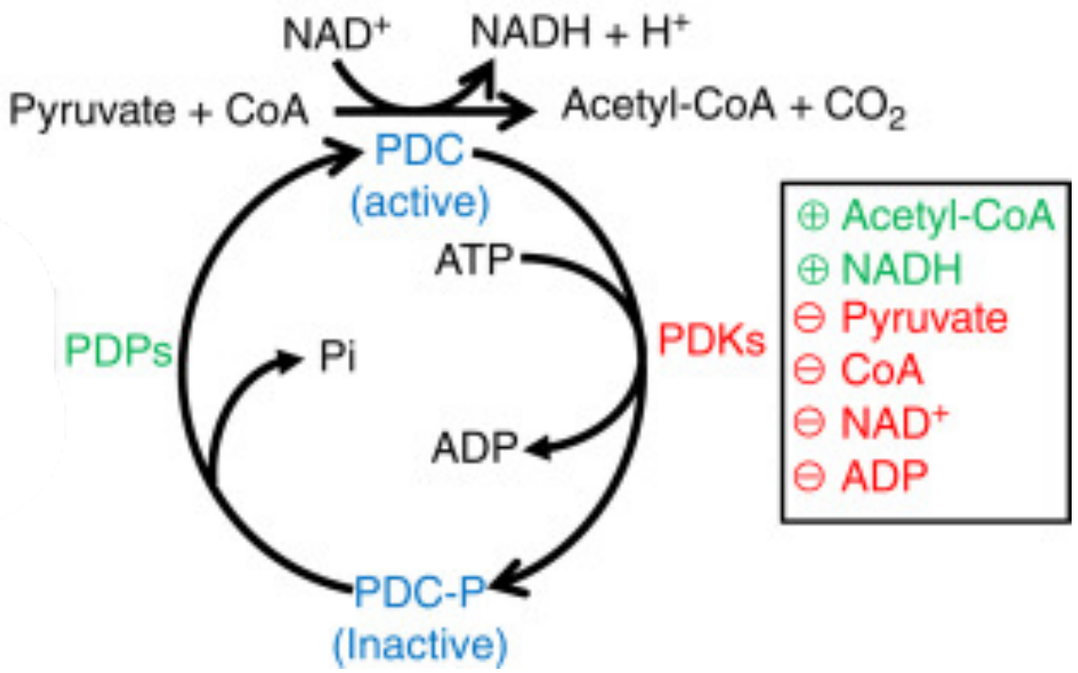
Example: Pyruvate metabolism (PDC-PDK)



Example: Pyruvate metabolism (PDC-PDK)



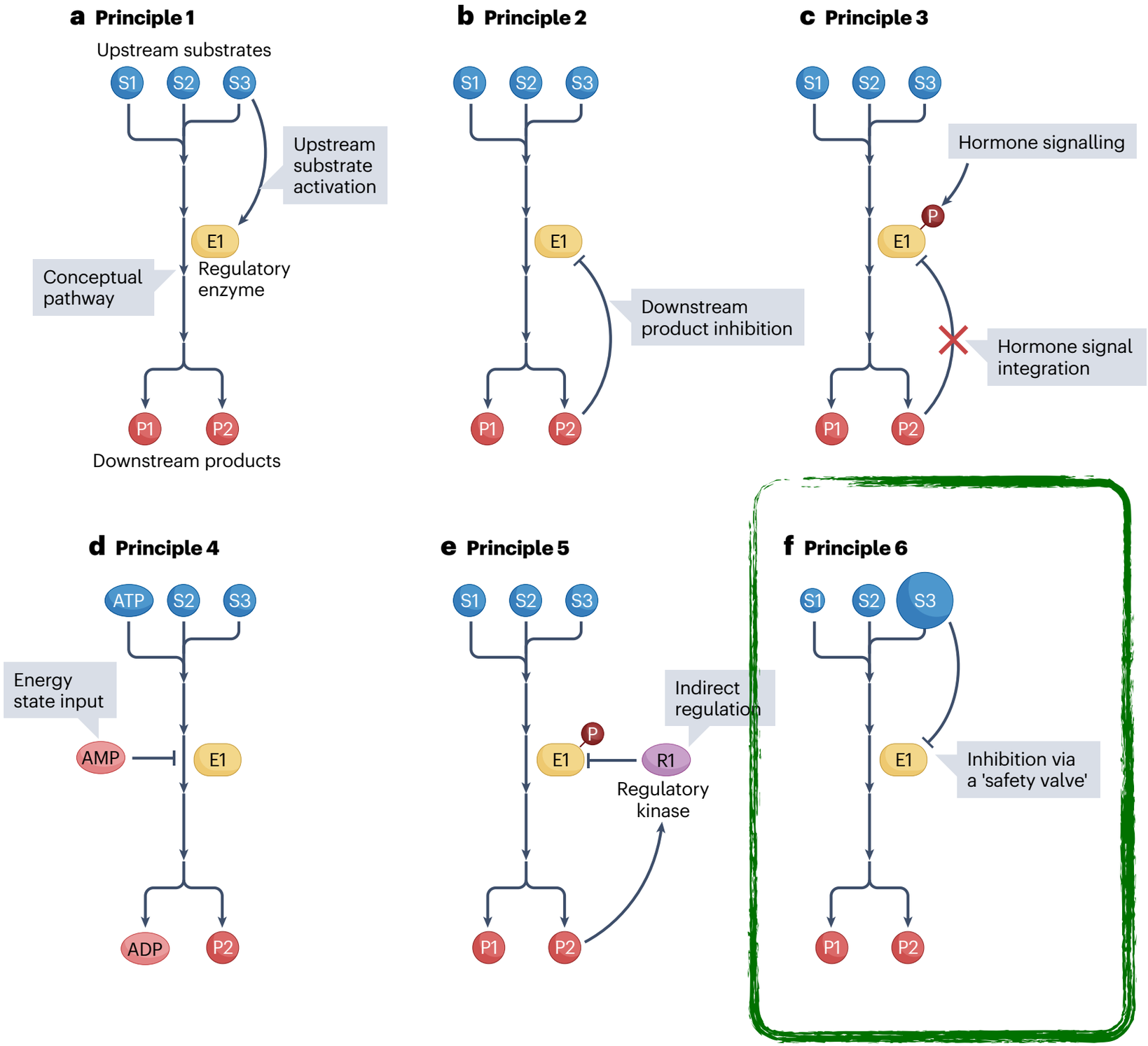
Example: Pyruvate metabolism (PDC-PDK)



PDC activity receives metabolite signaling indirectly through inhibitory phosphorylation by pyruvate dehydrogenase kinases (PDKs).

PDKs are allosterically activated by NADH and acetyl-CoA and inhibited by ADP, NAD+, coenzyme A (CoA-SH) and pyruvate

Different biochemical logics can mediate feedback or feedforward signals

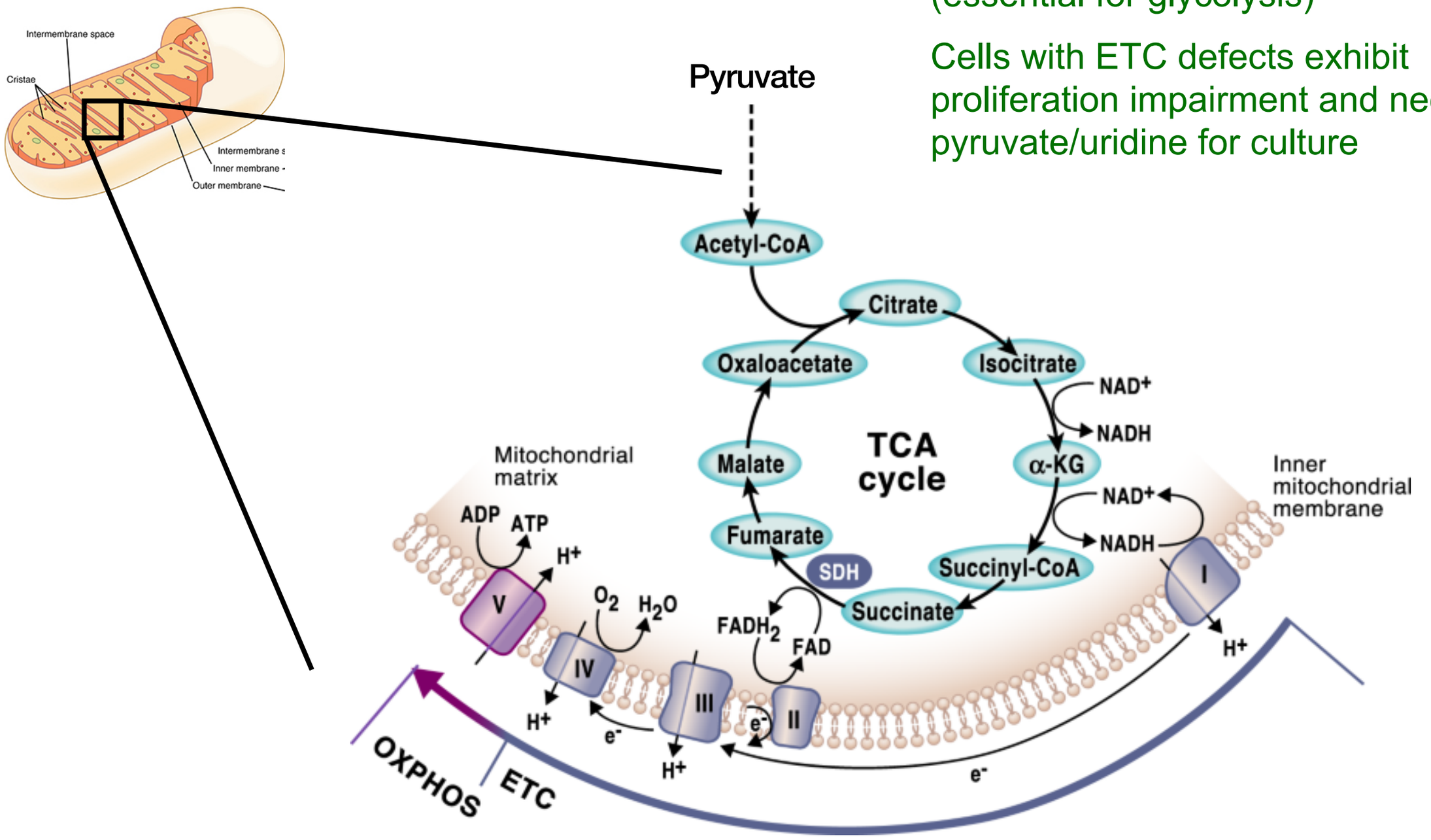


Metabolites can use a ‘safety valve’ measure to prevent the depletion of a key cellular resource (or detrimental accumulation)

Pro: rapid adjustment

Baker & Rutter, ***NRMCB****, 2023*

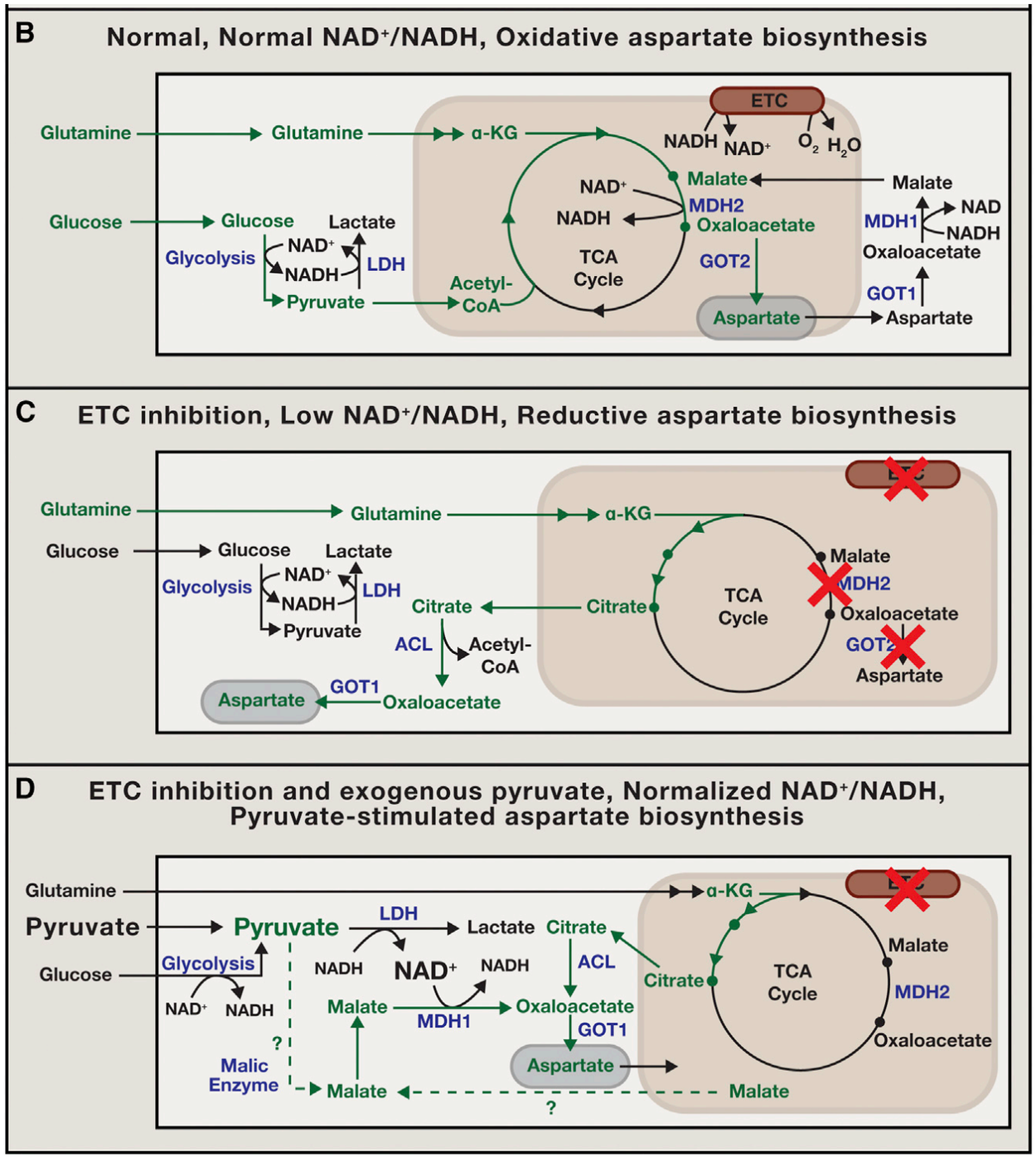
Example: Pyruvate metabolism (TCA/ETC)



The TCA cycle produces NADH, while the ETC regenerates NAD+ (essential for glycolysis)

Cells with ETC defects exhibit proliferation impairment and need pyruvate/uridine for culture

Example: Pyruvate metabolism (TCA/ETC)

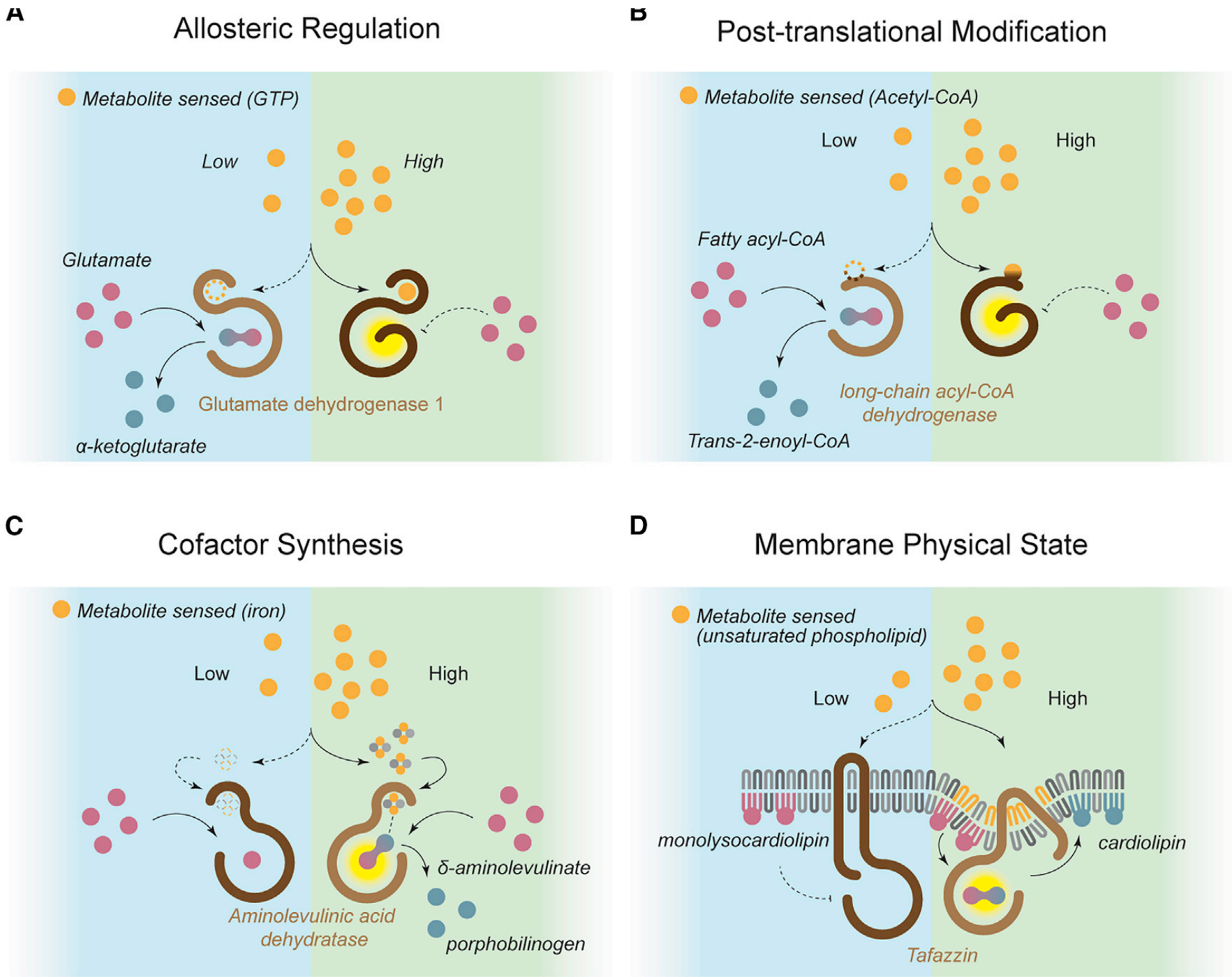


Both pyruvate and uridine provide cells with a mean to regenerate NAD+ independently of ETC

ETC-deficient cells increase their uptake of pyruvate to lower NADH:NAD+ ratio

Molecular mechanisms of nutrient sensing

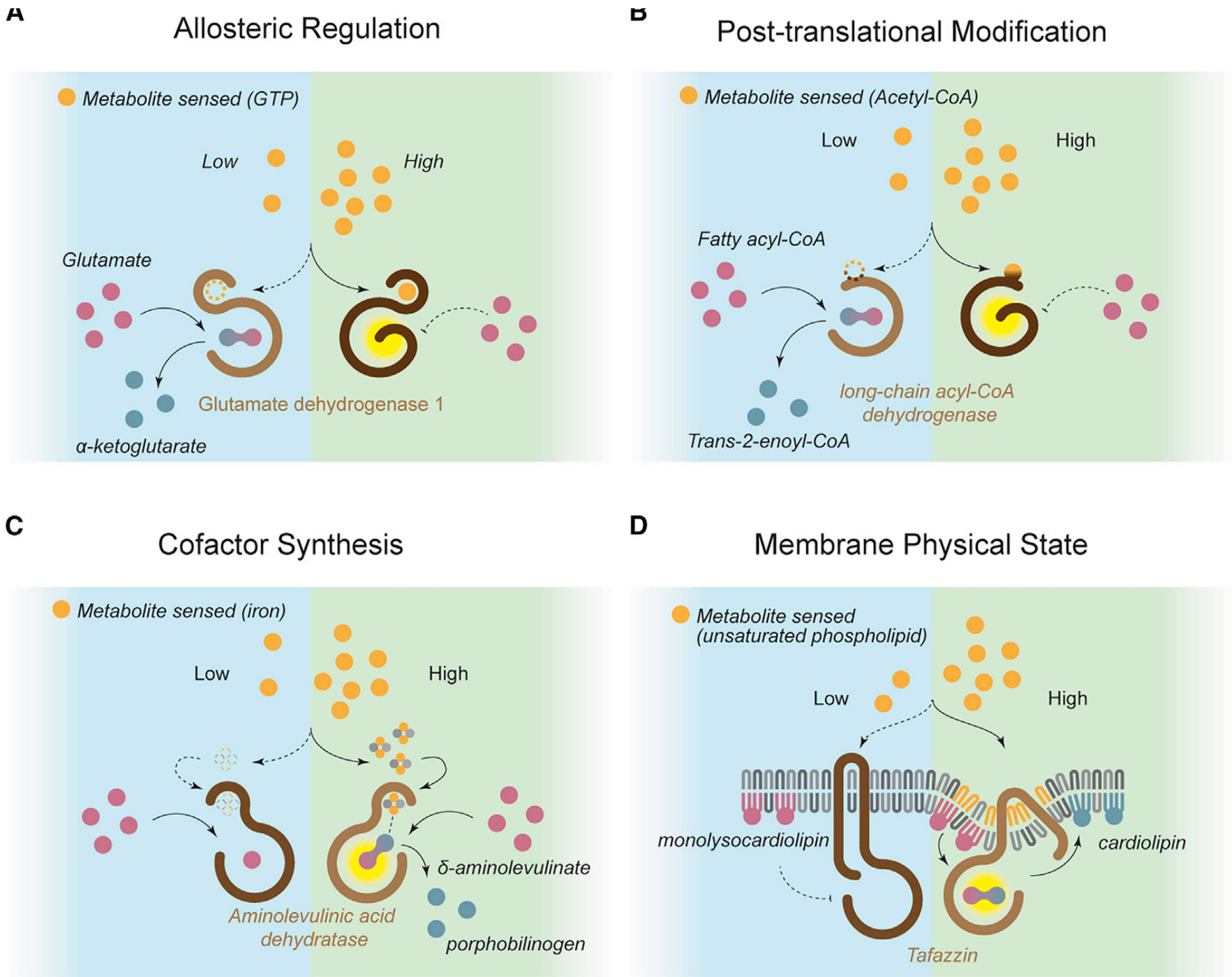
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

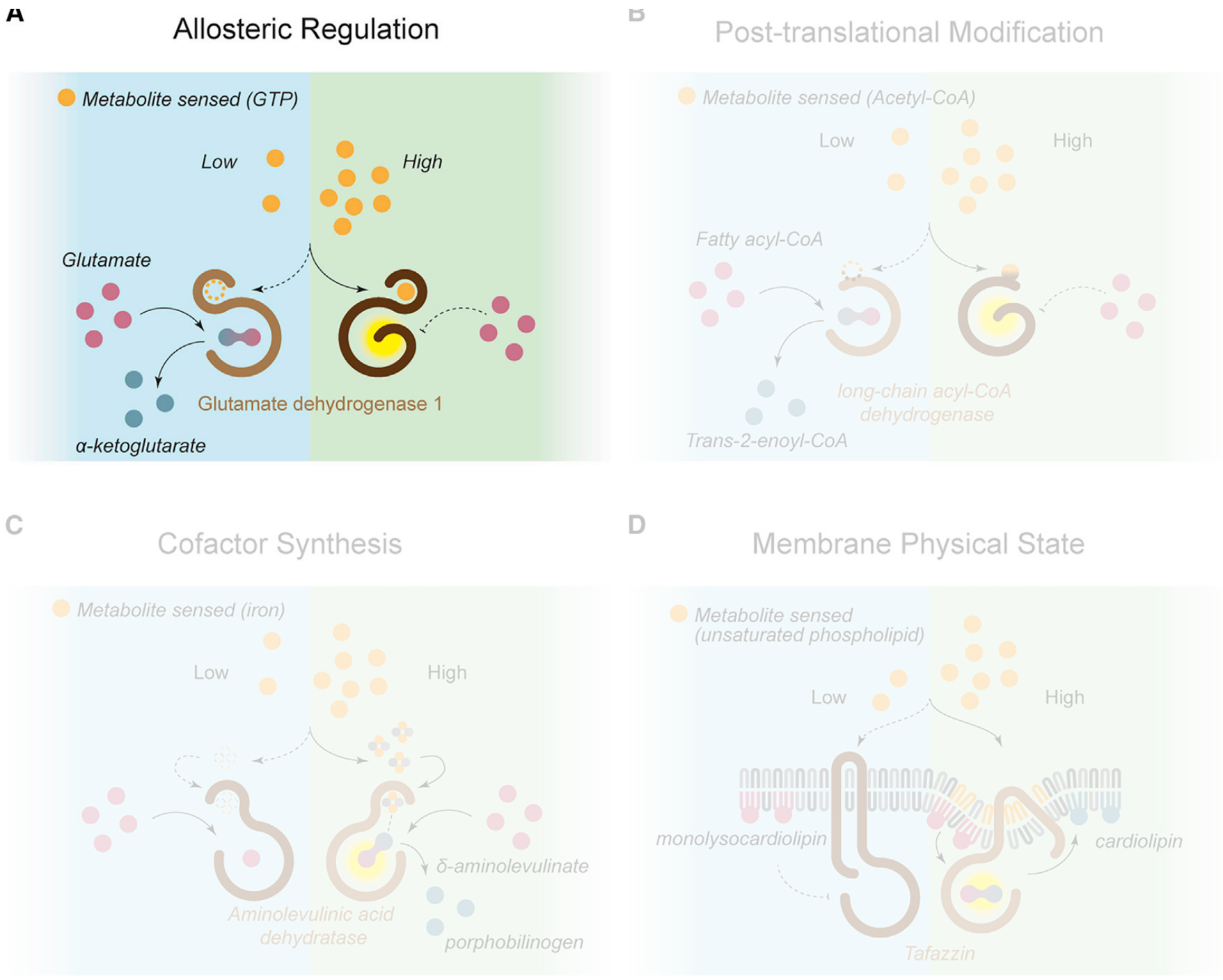
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

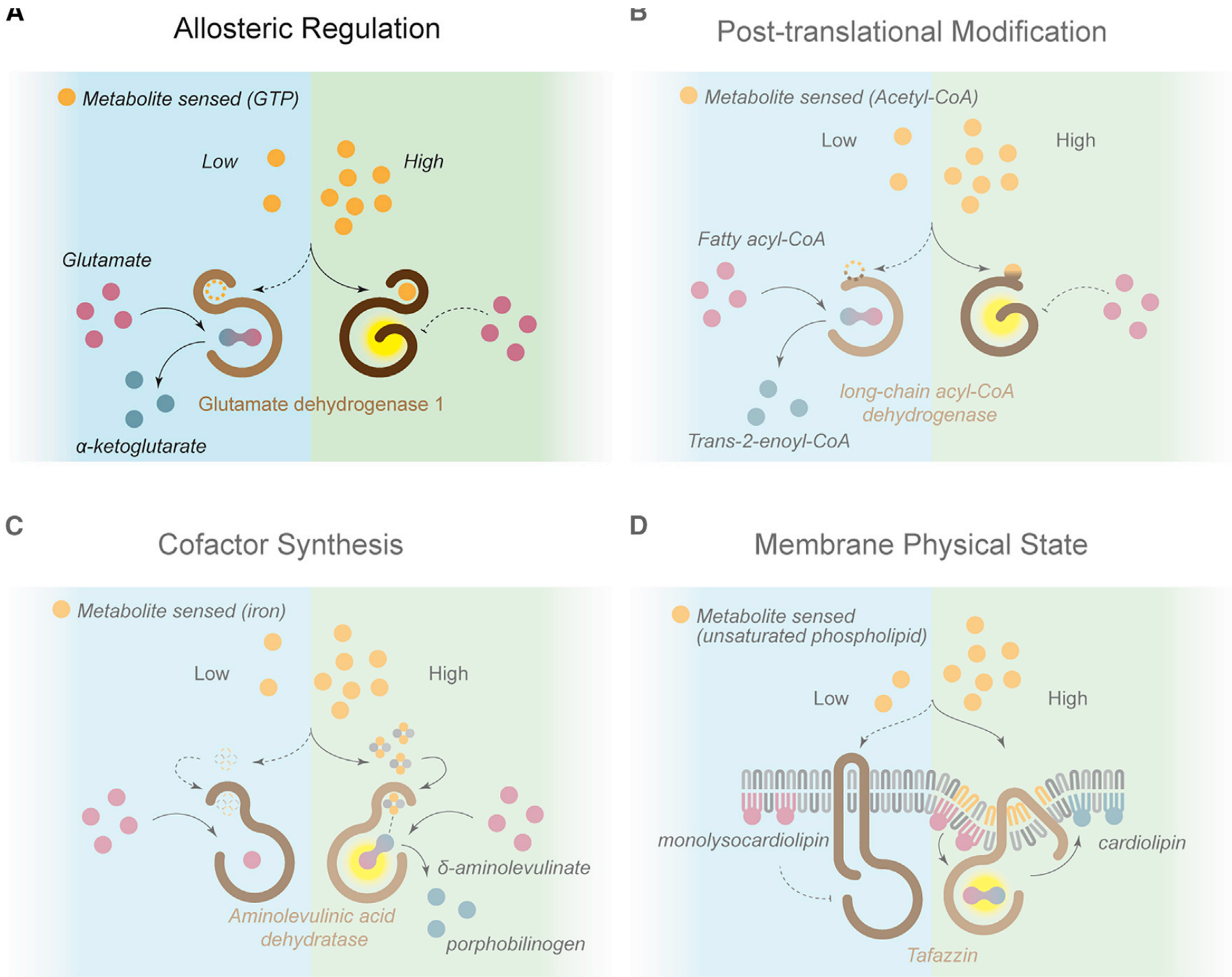
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

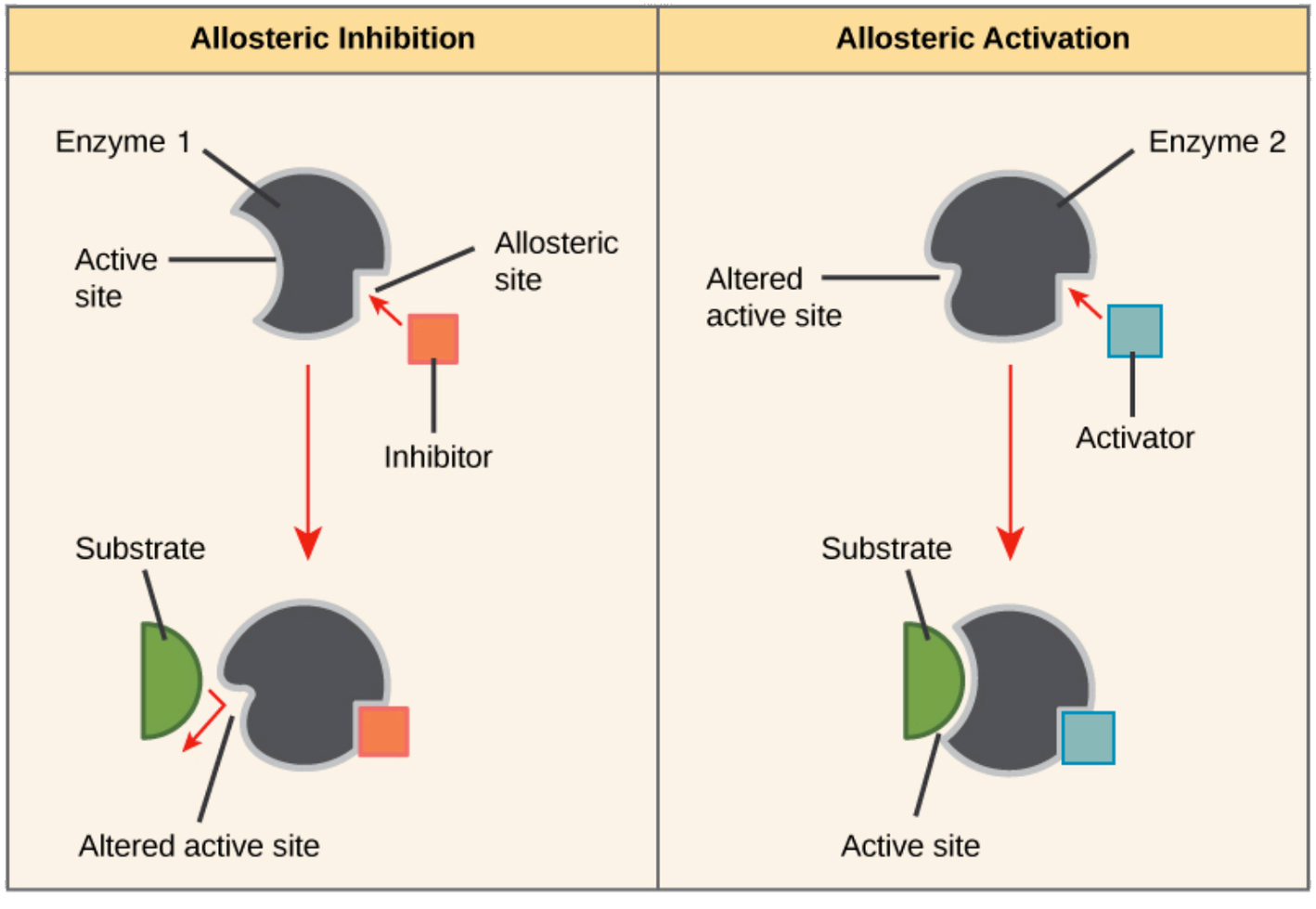
Liu & Birsoy, ***Mol Cell****, 2023*

Metabolites are sensed through allosteric regulation

One of the most common mechanisms for metabolite sensing is the allosteric regulation

Allows rapid tuning of biochemical fluxes in response to diverse metabolic cues

Factors sensed via allosteric regulation range from amino acids, lipids, carbohydrates, and metabolic intermediates to metals and cofactors; in many cases, it allows the integration of multiple metabolic signals via the activity of a single enzyme.



Metabolites are sensed through allosteric regulation

One of the most common mechanisms for metabolite sensing is the allosteric regulation

Allows rapid tuning of biochemical fluxes in response to diverse metabolic cues

Factors sensed via allosteric regulation range from amino acids, lipids, carbohydrates, and metabolic intermediates to metals and cofactors; in many cases, it allows the integration of multiple metabolic signals via the activity of a single enzyme.

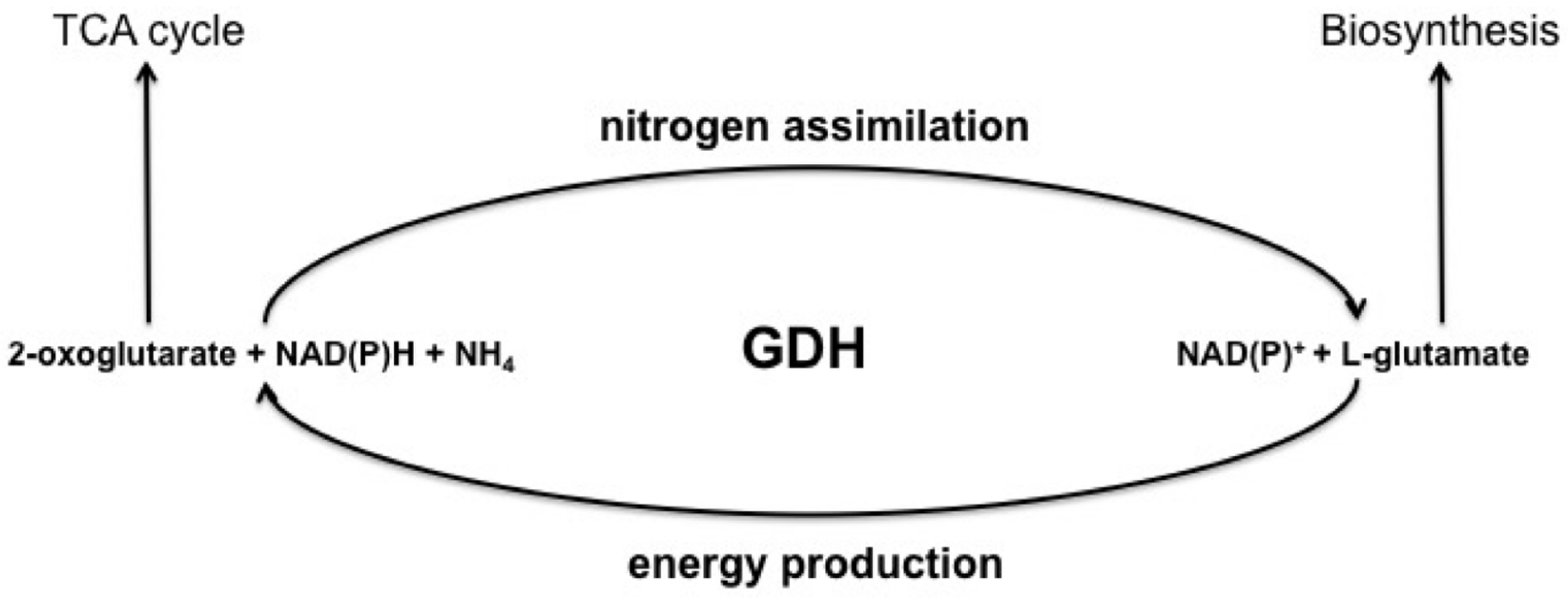
Metabolites are sensed through allosteric regulation

One of the most common mechanisms for metabolite sensing is the allosteric regulation

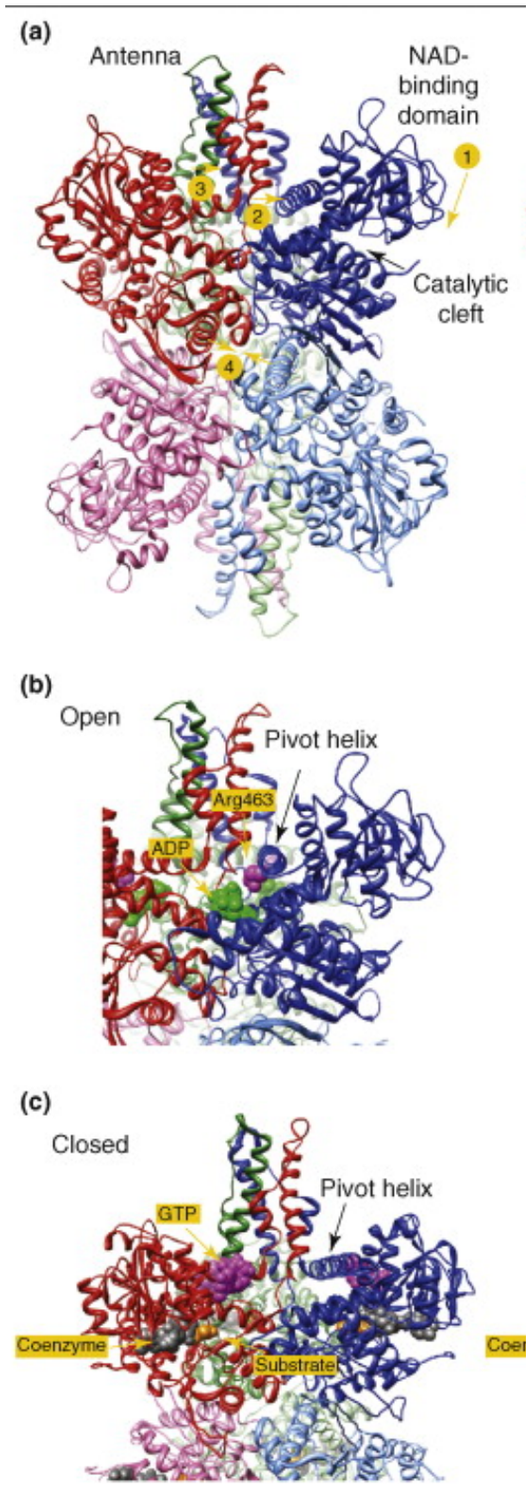
Allows rapid tuning of biochemical fluxes in response to diverse metabolic cues

Factors sensed via allosteric regulation range from amino acids, lipids, carbohydrates, and metabolic intermediates to metals and cofactors; in many cases, it allows the integration of multiple metabolic signals via the activity of a single enzyme.

A well-studied example is the regulation of glutamate dehydrogenase (GDH) by GTP, NADH, leucine, Mg^{2+}, and other metabolites.



Metabolites are sensed through allosteric regulation



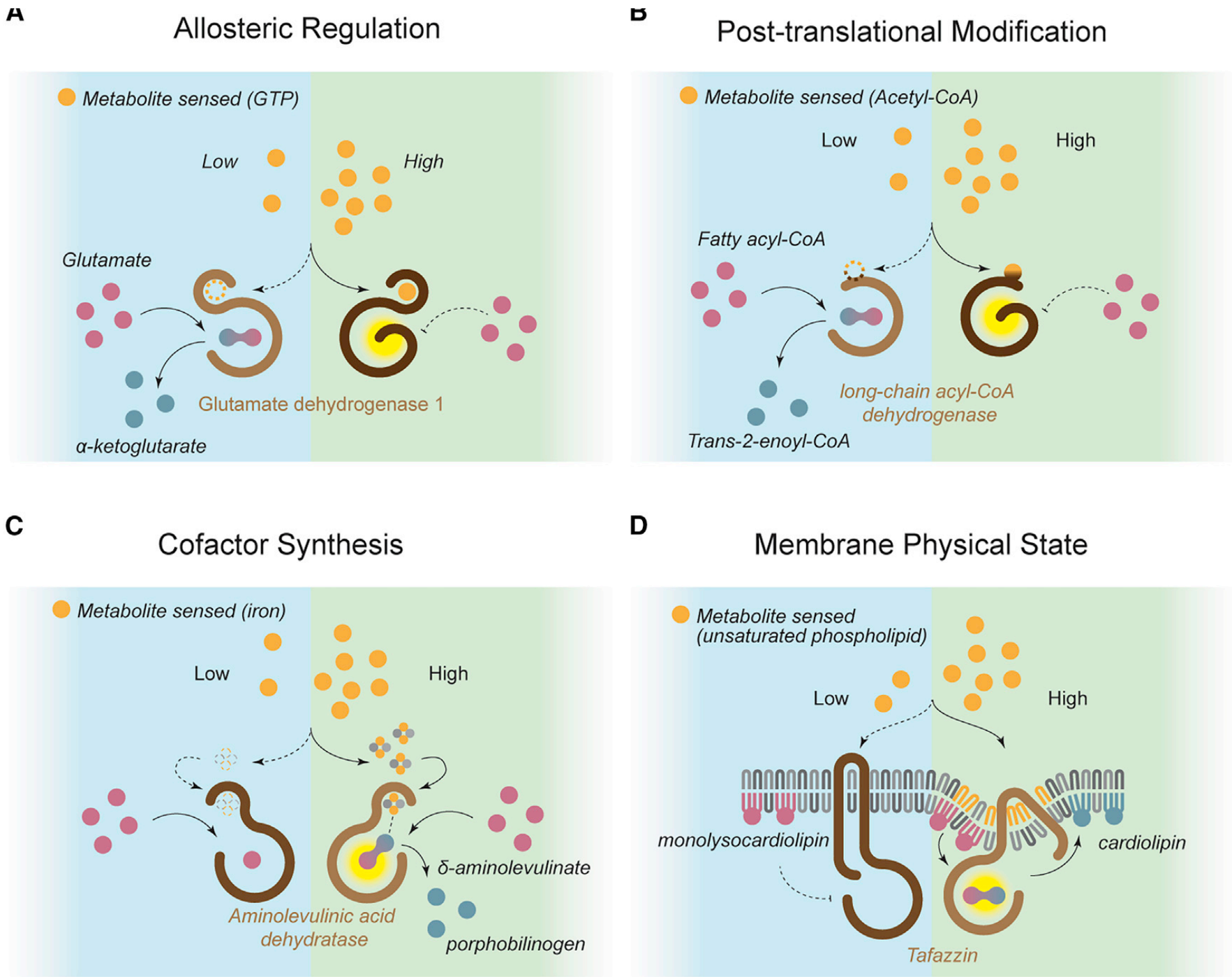
*Smith & Stanley, **TiBS**, 2008*

GTP inhibits the activity of the enzyme, while ADP exerts an activating effect

An increased ADP/GTP ratio signals a low-energy status in mitochondria that demands the replenishment of TCA cycle intermediates via the activity of GDH.

Allosteric activation of GDH enables insulin secretion. Pathogenic mutations have been identified in human GDH enzyme that specifically abolish the allosteric inhibition by GTP. These mutations lead to a gain-of-function effect on the GDH enzyme and hyperactive insulin secretion in beta cells.

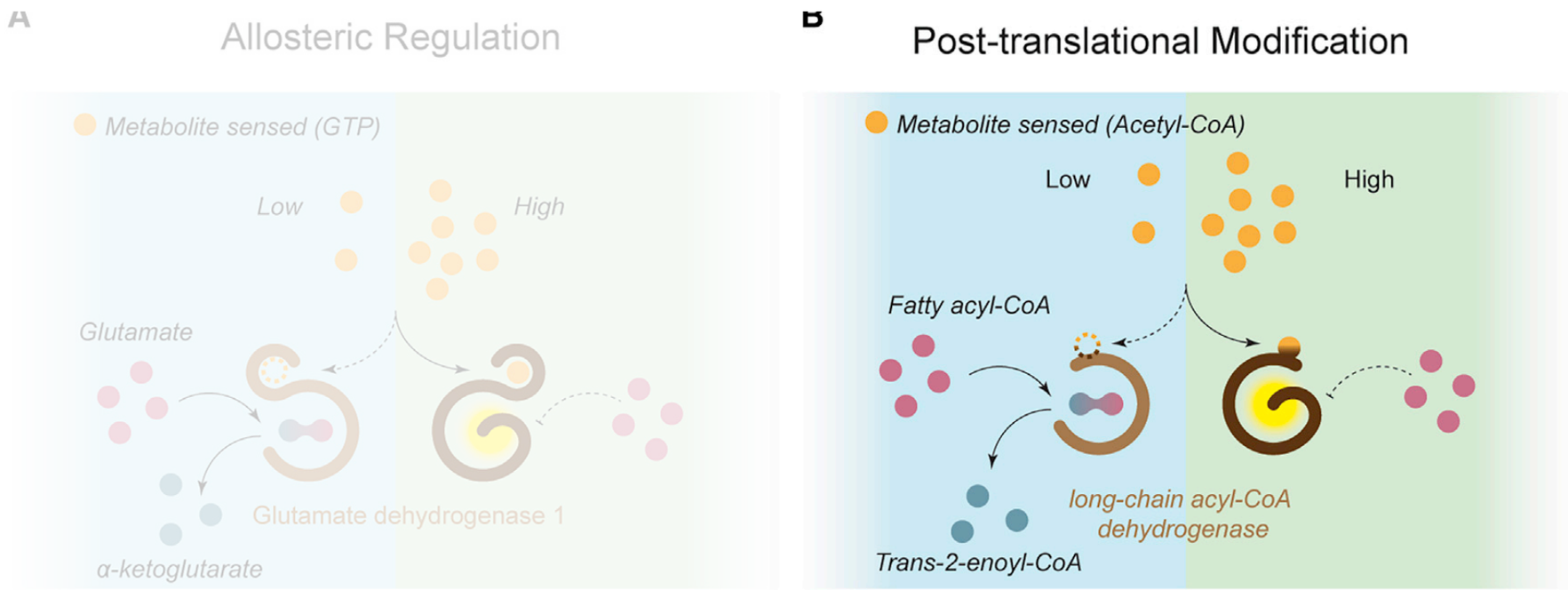
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

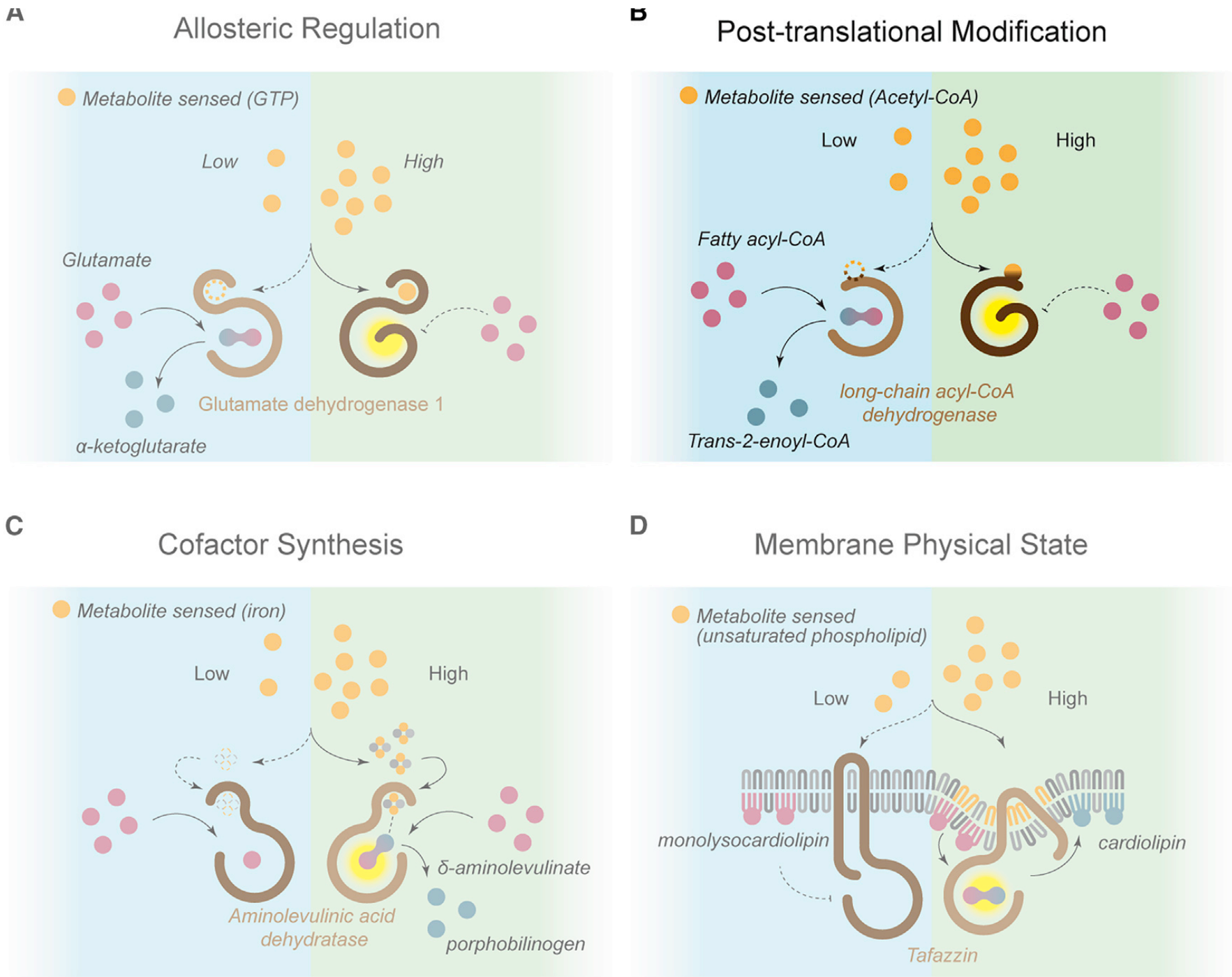
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

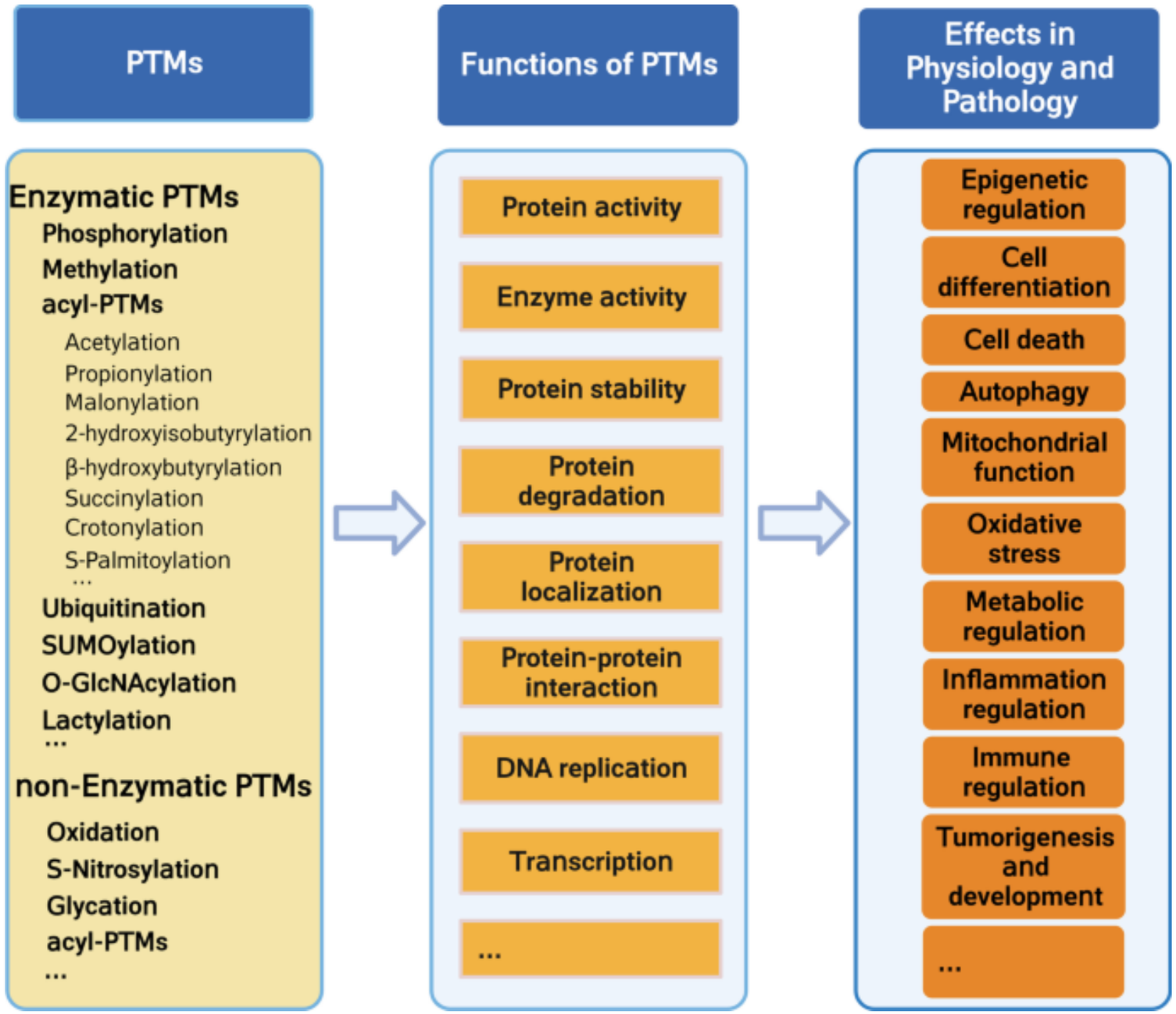
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

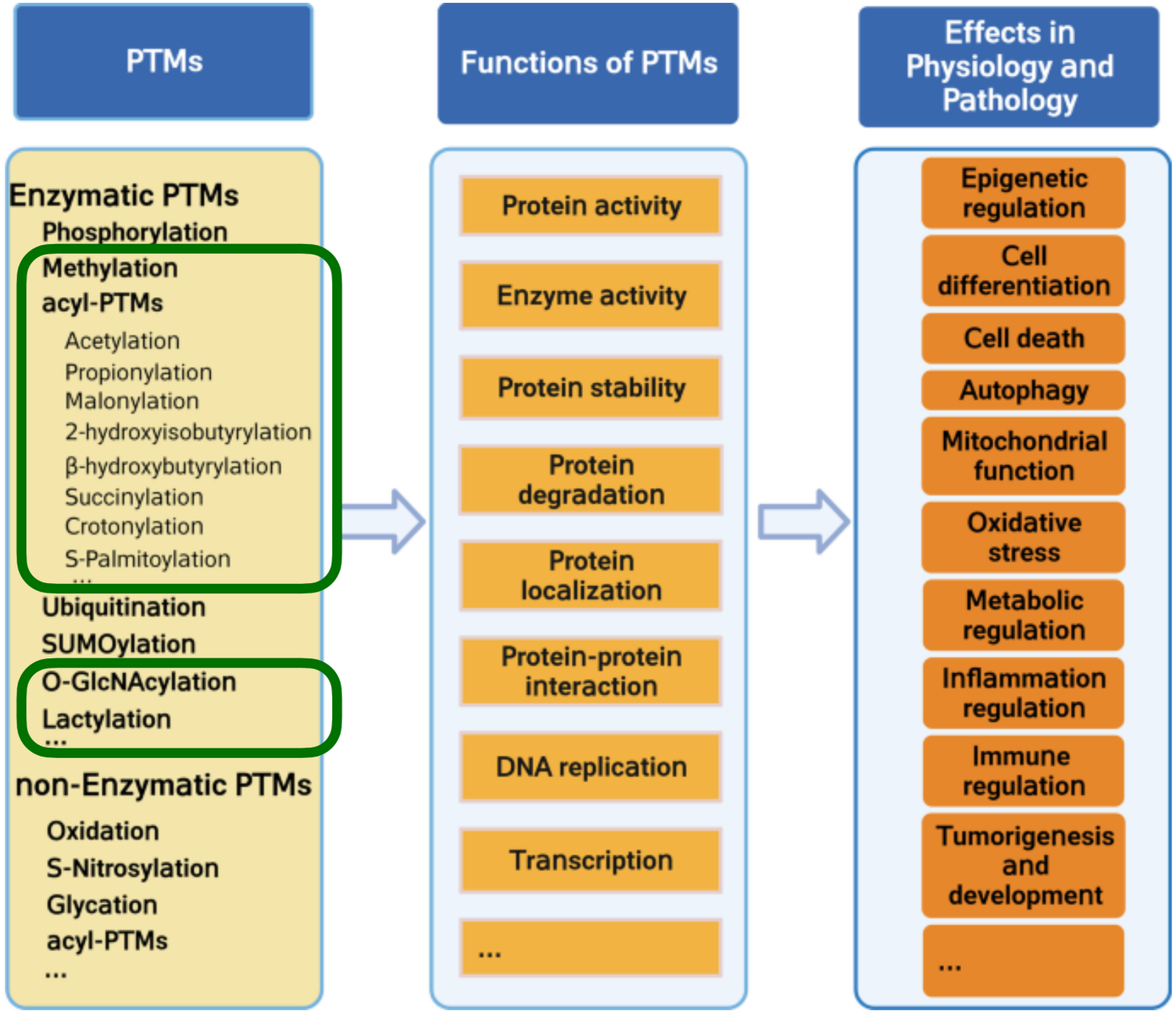
Metabolites are sensed through post-translational modifications



PTMs regulate the activity of many proteins and influence several cellular functions

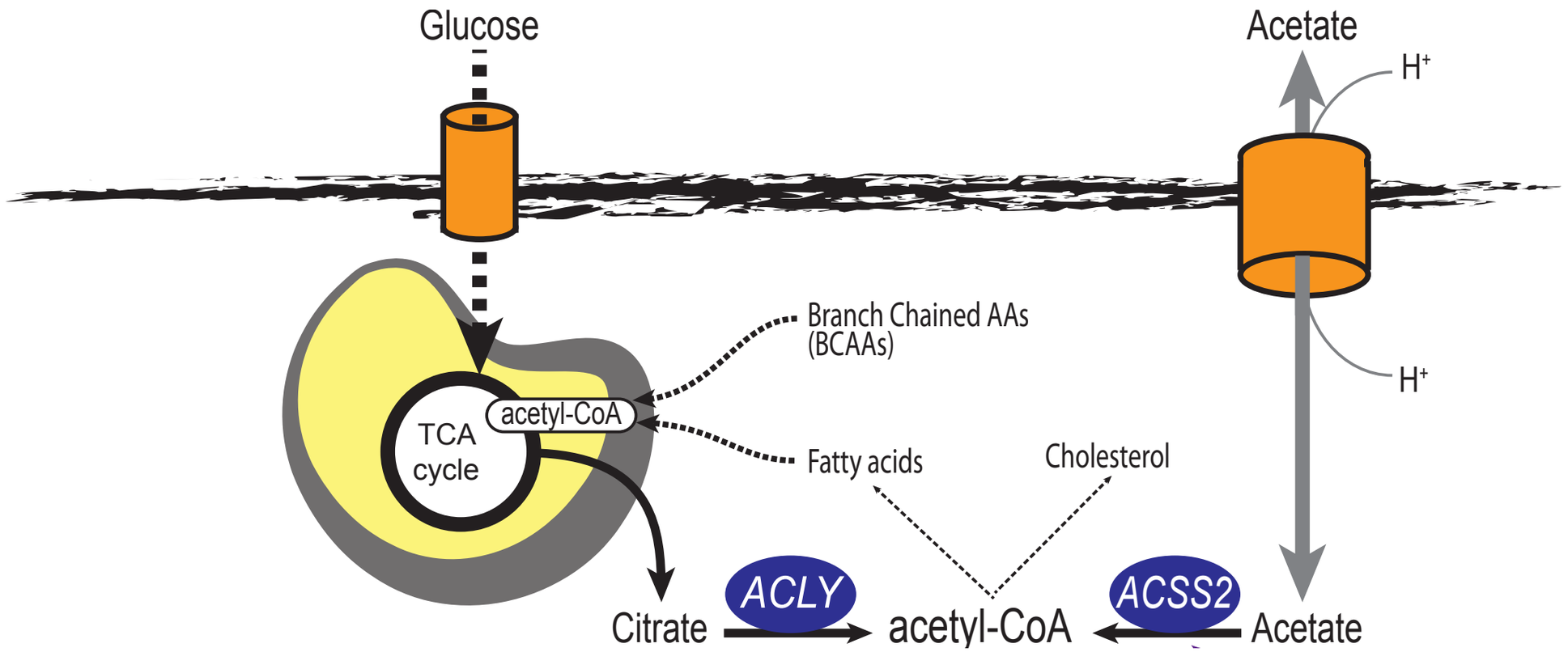
Metabolites are sensed through post-translational modifications

Metabolites are critical substrates



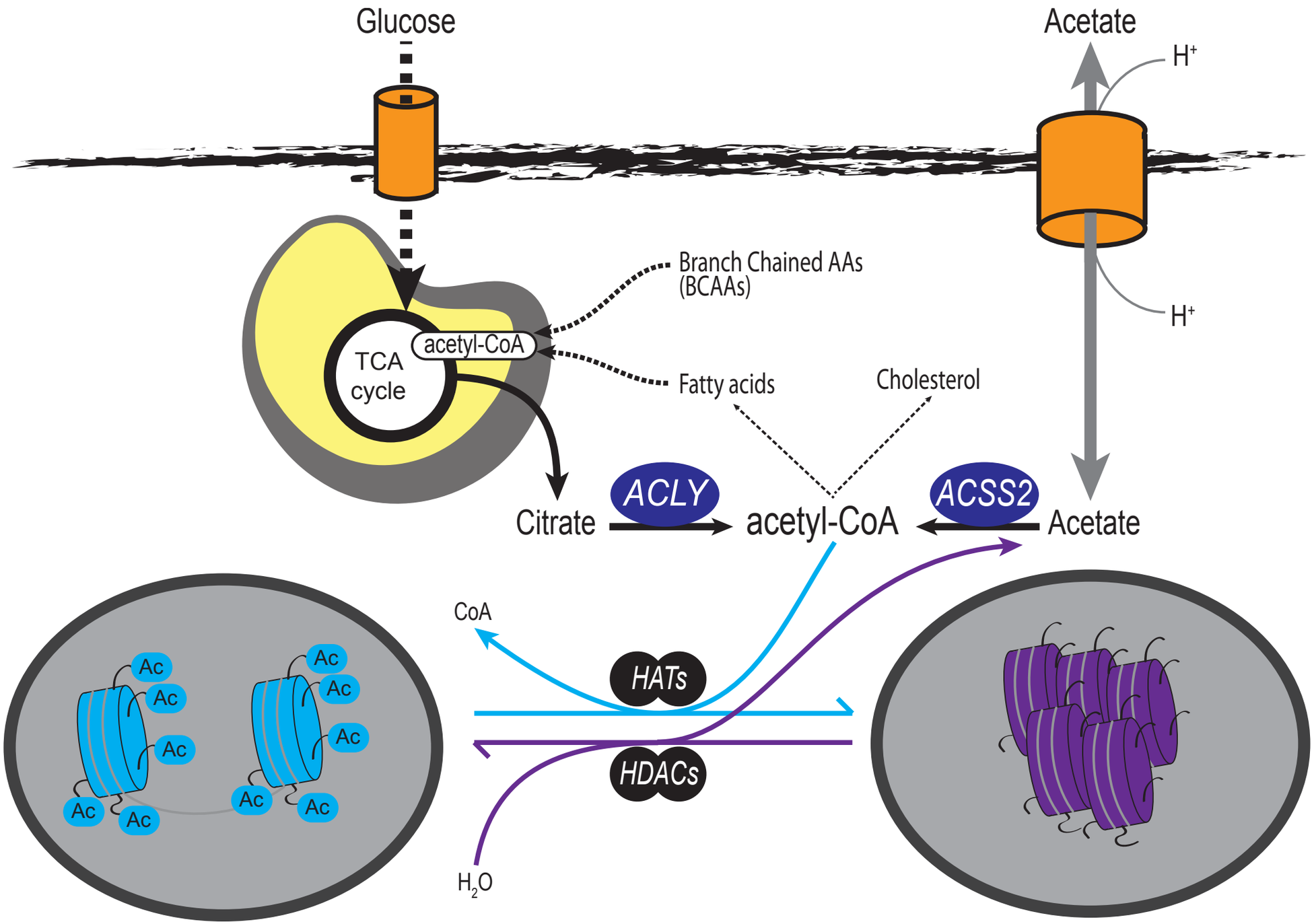
PTMs regulate the activity of many proteins and influence several cellular functions

Acetyl-CoA at the interface of metabolism and epigenome



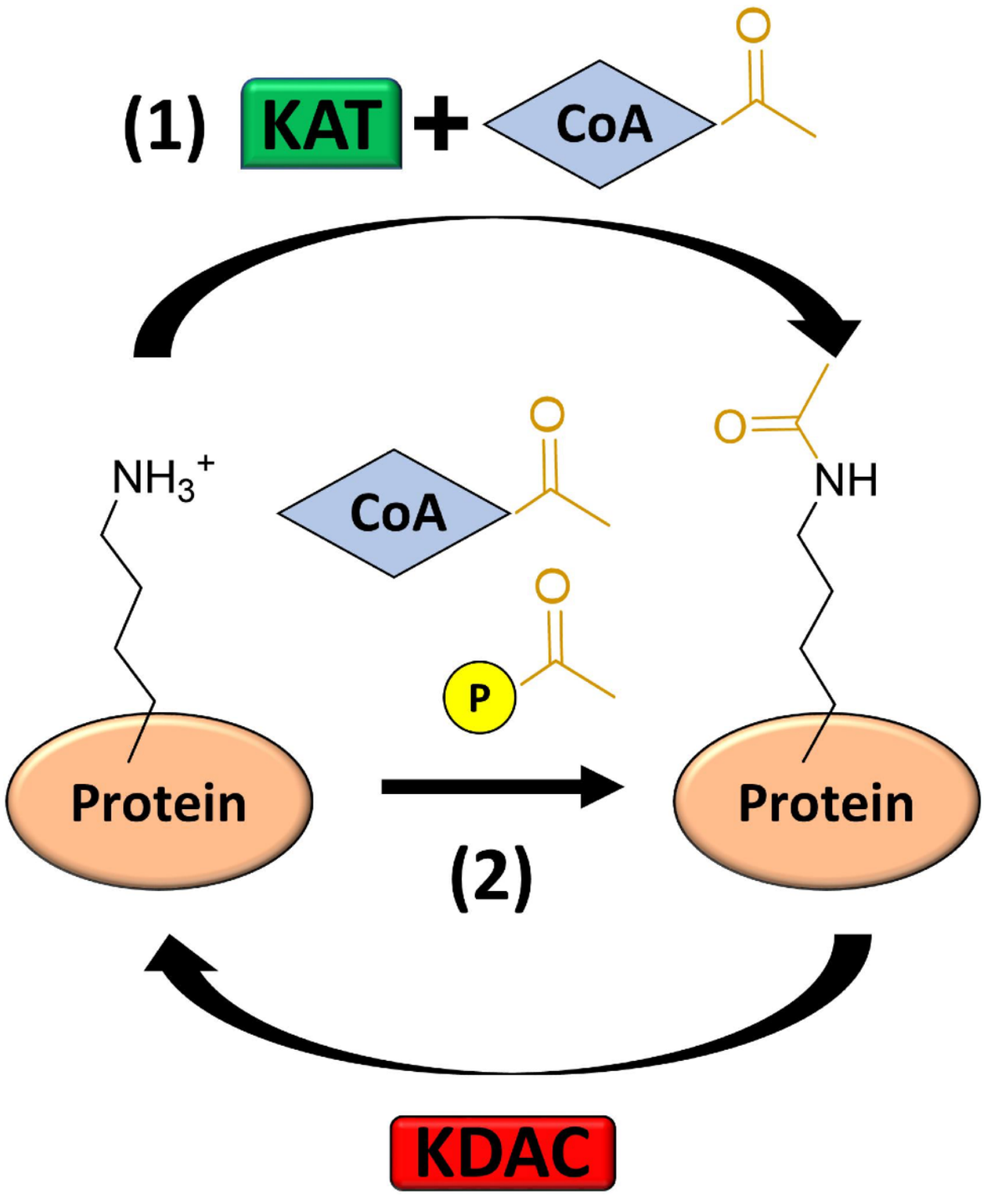
Carrer & Wellen, Curr Opin Biotechnol, 2015

Acetyl-CoA at the interface of metabolism and epigenome

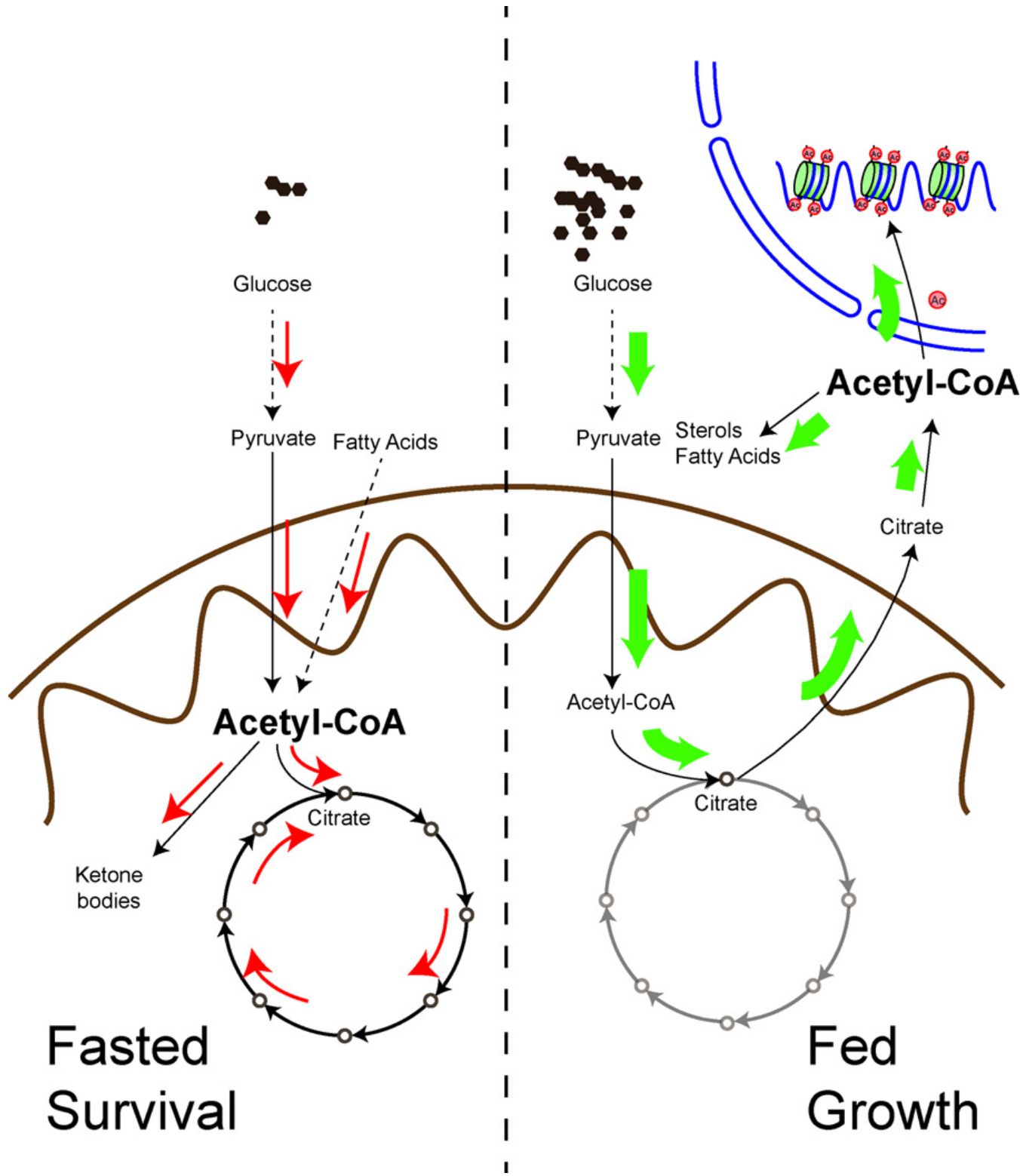


Carrer & Wellen, Curr Opin Biotechnol, 2015

Metabolites are sensed through acetyl-CoA-dependent acetylation

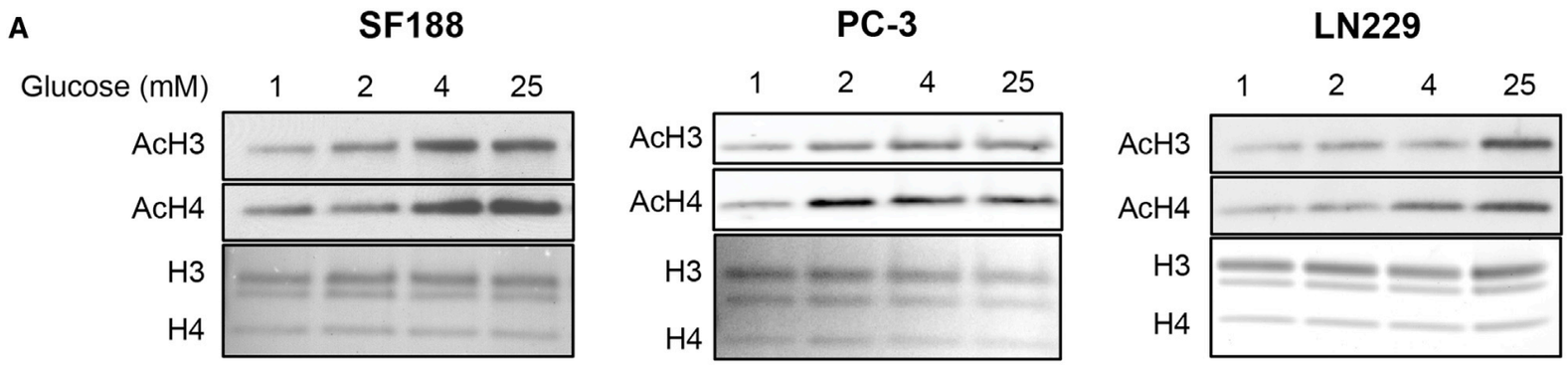


Nutrient abundance is sensed through acetyl-CoA-dependent histone acetylation



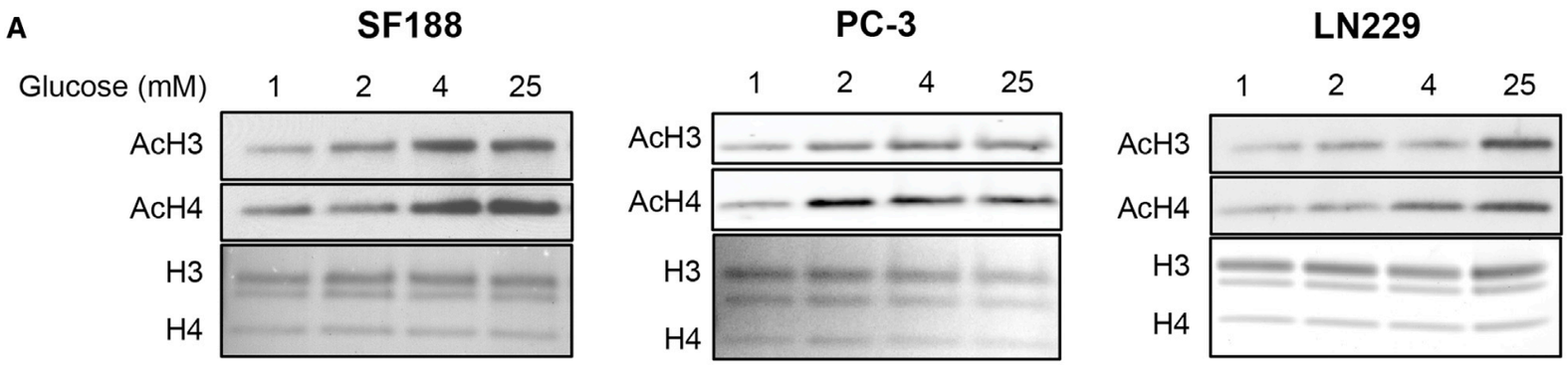
Akt-Dependent Metabolic Reprogramming Regulates Tumor Cell Histone Acetylation

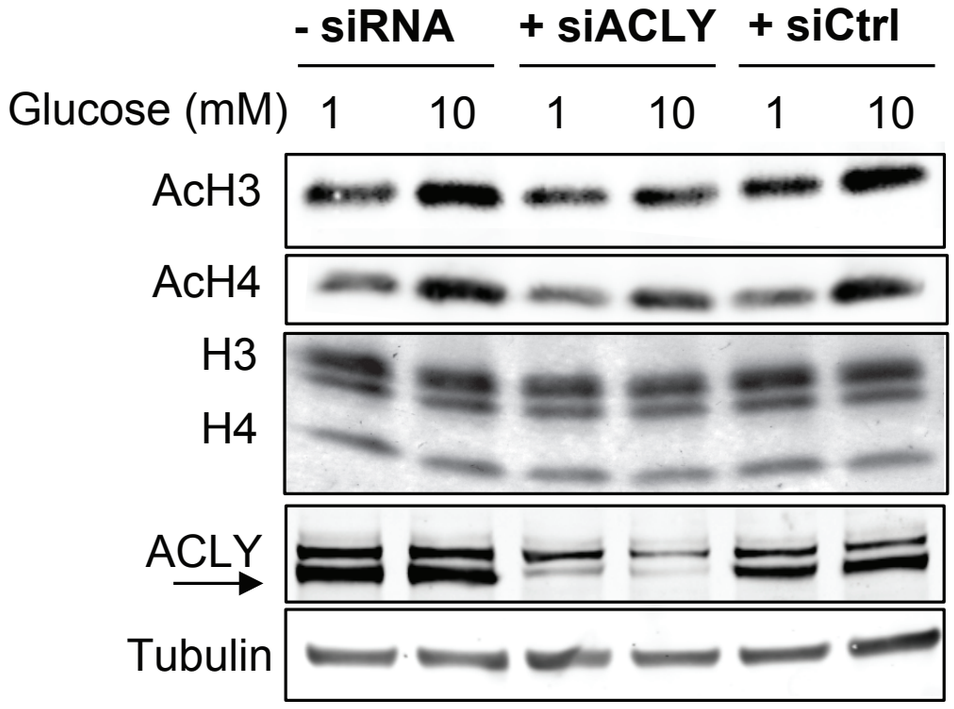
Joyce V. Lee,[1,2,11] Alessandro Carrer,[1,2,11] Supriya Shah,[1,2,11] Nathaniel W. Snyder,[3] Shuanzeng Wei,[4] Sriram Venneti,[5] Andrew J. Worth,[3] Zuo-Fei Yuan,[6] Hee-Woong Lim,[7] Shichong Liu,[6] Ellen Jackson,[1,2] Nicole M. Aiello,[2,8] Naomi B. Haas,[8] Timothy R. Rebbeck,[9] Alexander Judkins,[10] Kyoung-Jae Won,[7] Lewis A. Chodosh,[1,2] Benjamin A. Garcia,[6] Ben Z. Stanger,[2,8] Michael D. Feldman,[4] Ian A. Blair,[3] and Kathryn E. Wellen[1,2,*]



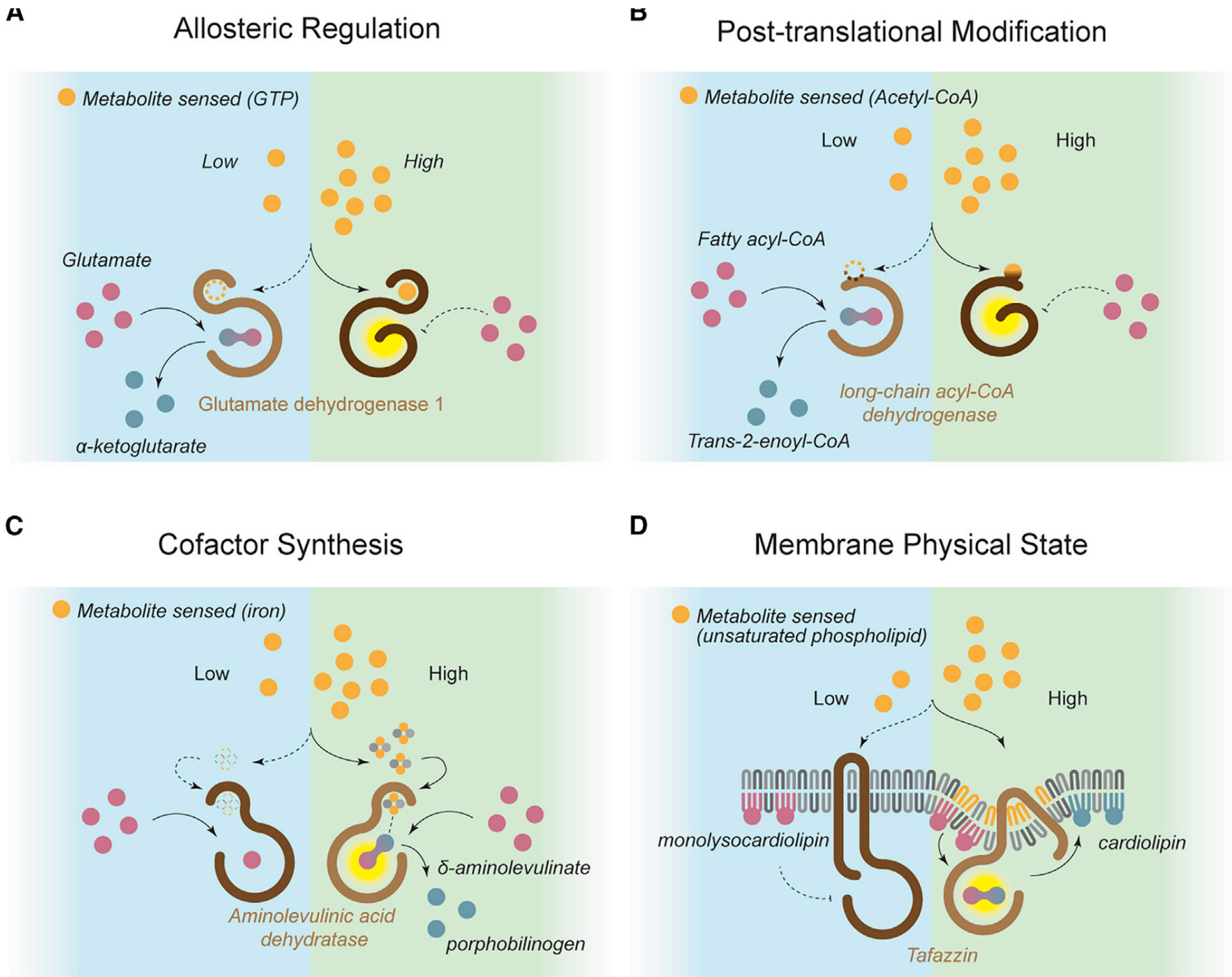
Akt-Dependent Metabolic Reprogramming Regulates Tumor Cell Histone Acetylation

Joyce V. Lee,[1,2,11] Alessandro Carrer,[1,2,11] Supriya Shah,[1,2,11] Nathaniel W. Snyder,[3] Shuanzeng Wei,[4] Sriram Venneti,[5] Andrew J. Worth,[3] Zuo-Fei Yuan,[6] Hee-Woong Lim,[7] Shichong Liu,[6] Ellen Jackson,[1,2] Nicole M. Aiello,[2,8] Naomi B. Haas,[8] Timothy R. Rebbeck,[9] Alexander Judkins,[10] Kyoung-Jae Won,[7] Lewis A. Chodosh,[1,2] Benjamin A. Garcia,[6] Ben Z. Stanger,[2,8] Michael D. Feldman,[4] Ian A. Blair,[3] and Kathryn E. Wellen[1,2,*]





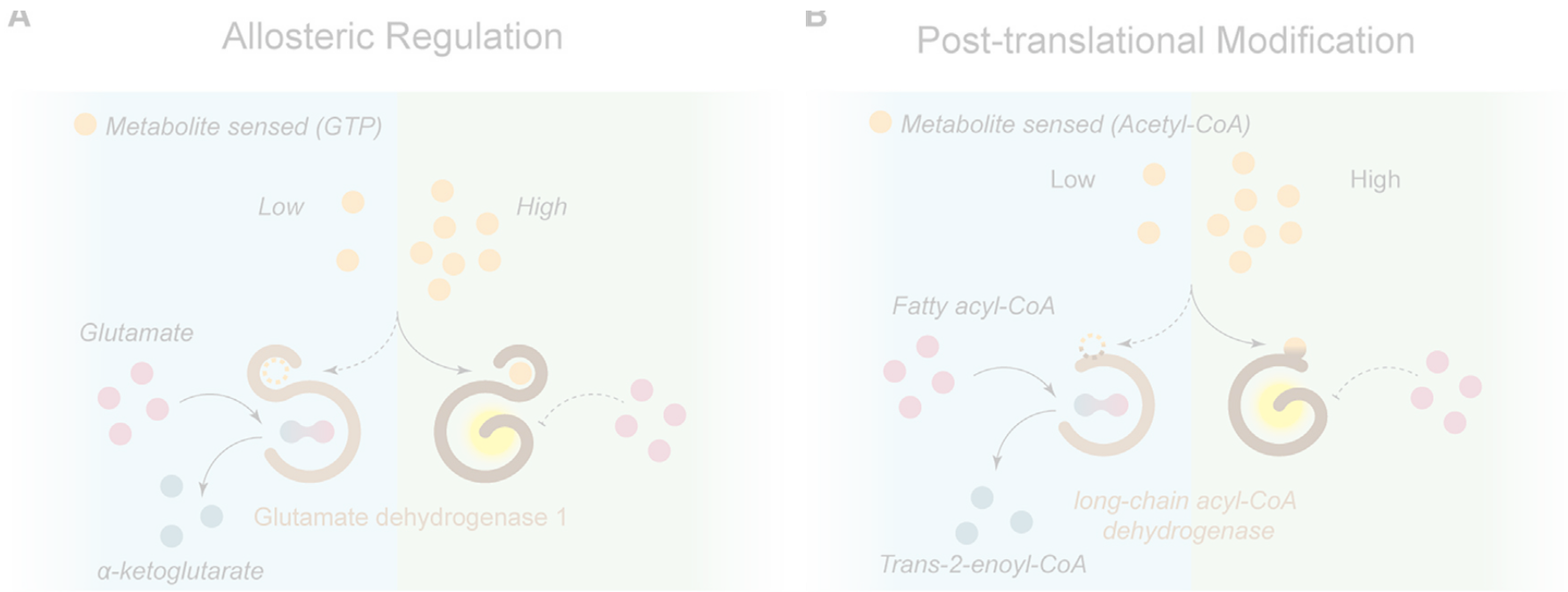
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

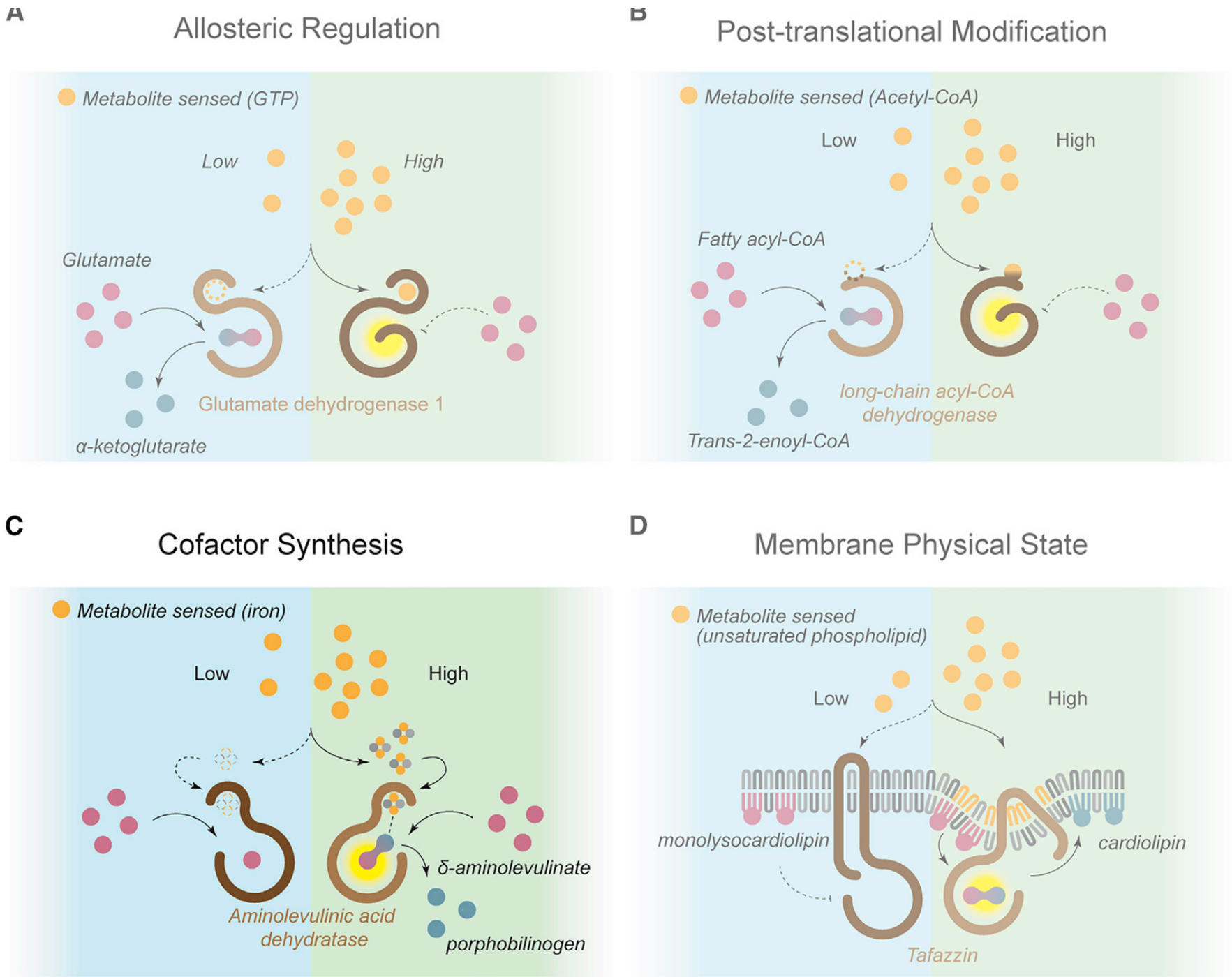
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

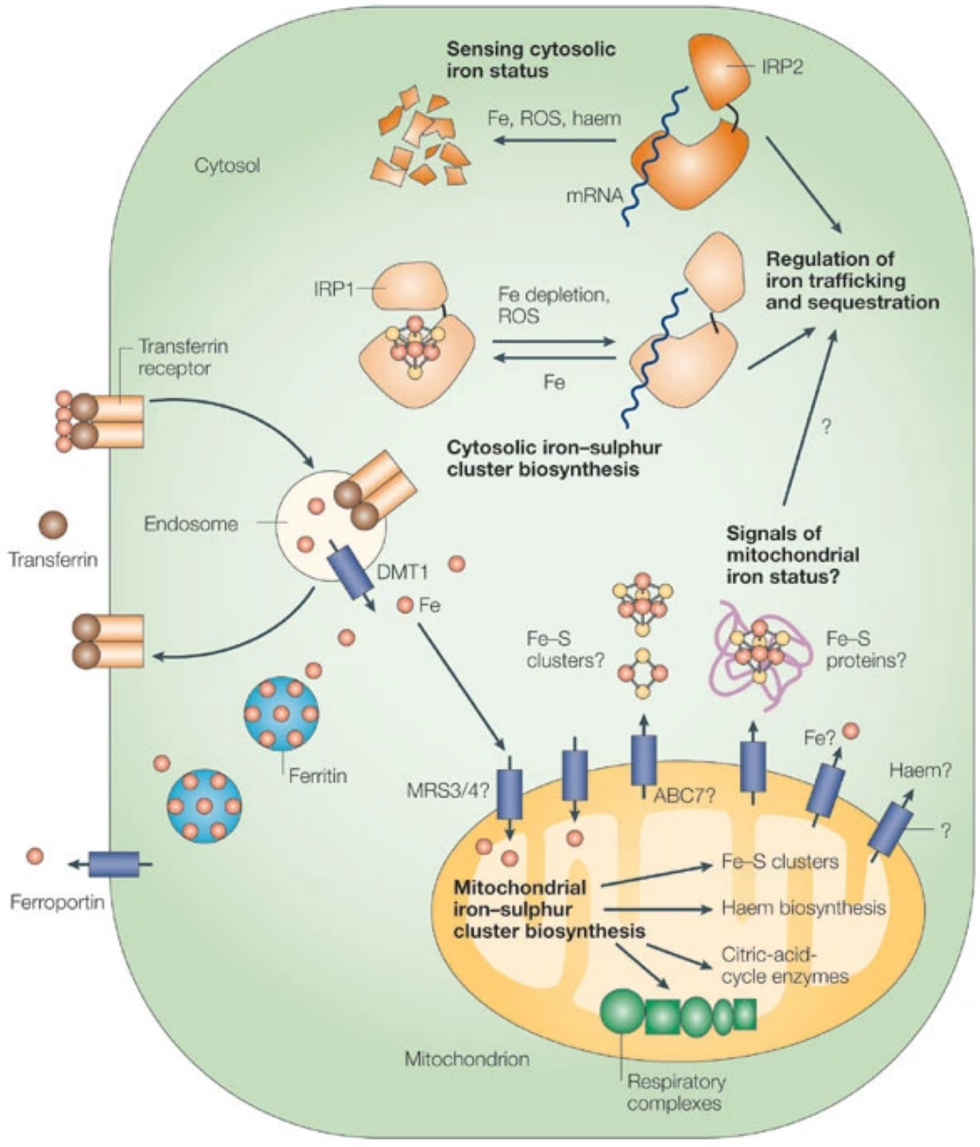
Metabolites are sensed through cofactor availability

Some metabolites are critical co-factors for enzymatic activity

Particularly useful when metabolite/co-factor synthesis and availability is highly compartmentalized

Mitochondria harbor the largest pool of intracellular iron, accounting for up to more than 50% of total cellular iron content.

Unbuffered free iron participates in Fenton reactions that produce reactive hydroxyl radicals. These toxic byproducts damage proteins, DNA, and lipid bilayers and trigger cell death through ferroptosis. Thus, iron levels must be sensed and kept within a narrow range.



Metabolites are sensed through cofactor availability

Some metabolites are critical co-factors for enzymatic activity

Particularly useful when metabolite/co-factor synthesis and availability is highly compartmentalized

Metabolites are sensed through cofactor availability

Some metabolites are critical co-factors for enzymatic activity

Particularly useful when metabolite/co-factor synthesis and availability is highly compartmentalized

TCA cycle intermediates are sensed through alpha-ketoglutarate (aKG)-dependent dioxygenases, a versatile group of iron-containing enzymes that includes key players in epigenetic regulation, oxygen sensing, lipid metabolism, and other critical processes.

These enzymes couple the decarboxylation of aKG with the oxidation of the substrate, and in many cases the predicted K_M of those enzymes to aKG overlaps with its physiological levels, suggesting that their activity may dynamically respond to intracellular aKG levels.

Metabolites are sensed through cofactor availability

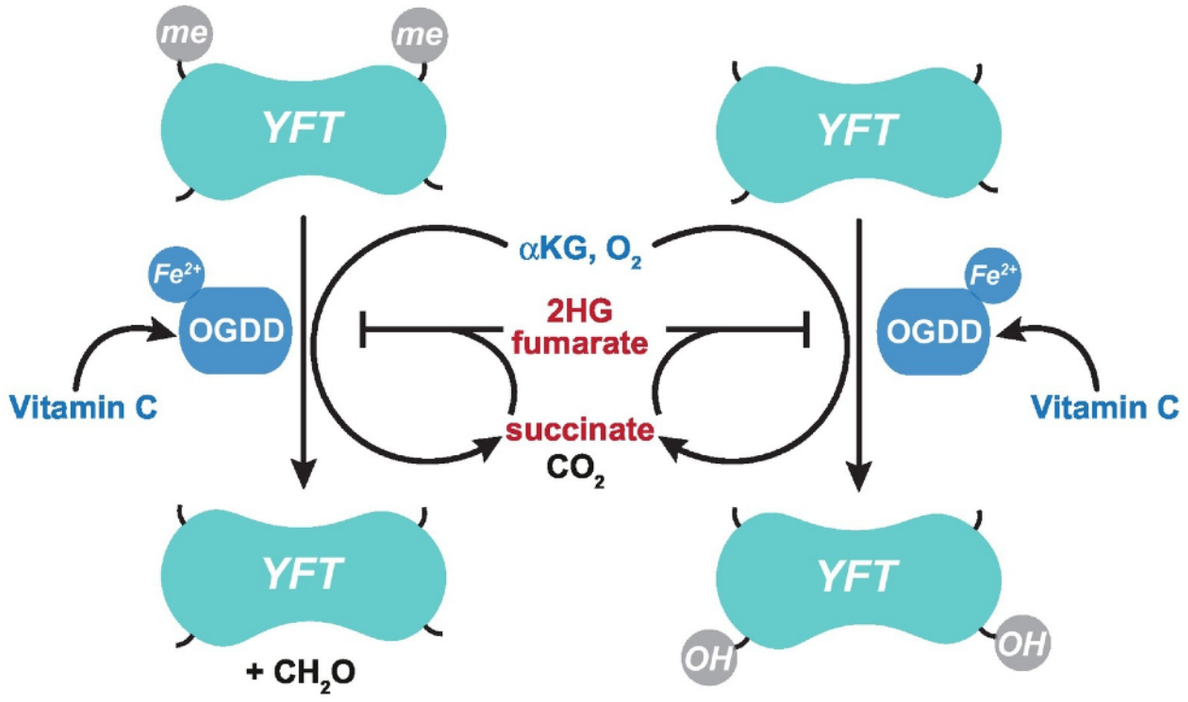
Some metabolites are critical co-factors for enzymatic activity

Particularly useful when metabolite/co-factor synthesis and availability is highly compartmentalized

TCA cycle intermediates are sensed through alpha-ketoglutarate (aKG)-dependent dioxygenases, a versatile group of iron-containing enzymes that includes key players in epigenetic regulation, oxygen sensing, lipid metabolism, and other critical processes.

These enzymes couple the decarboxylation of aKG with the oxidation of the substrate, and in many cases the predicted K_M of those enzymes to aKG overlaps with its physiological levels, suggesting that their activity may dynamically respond to intracellular aKG levels.

(B)



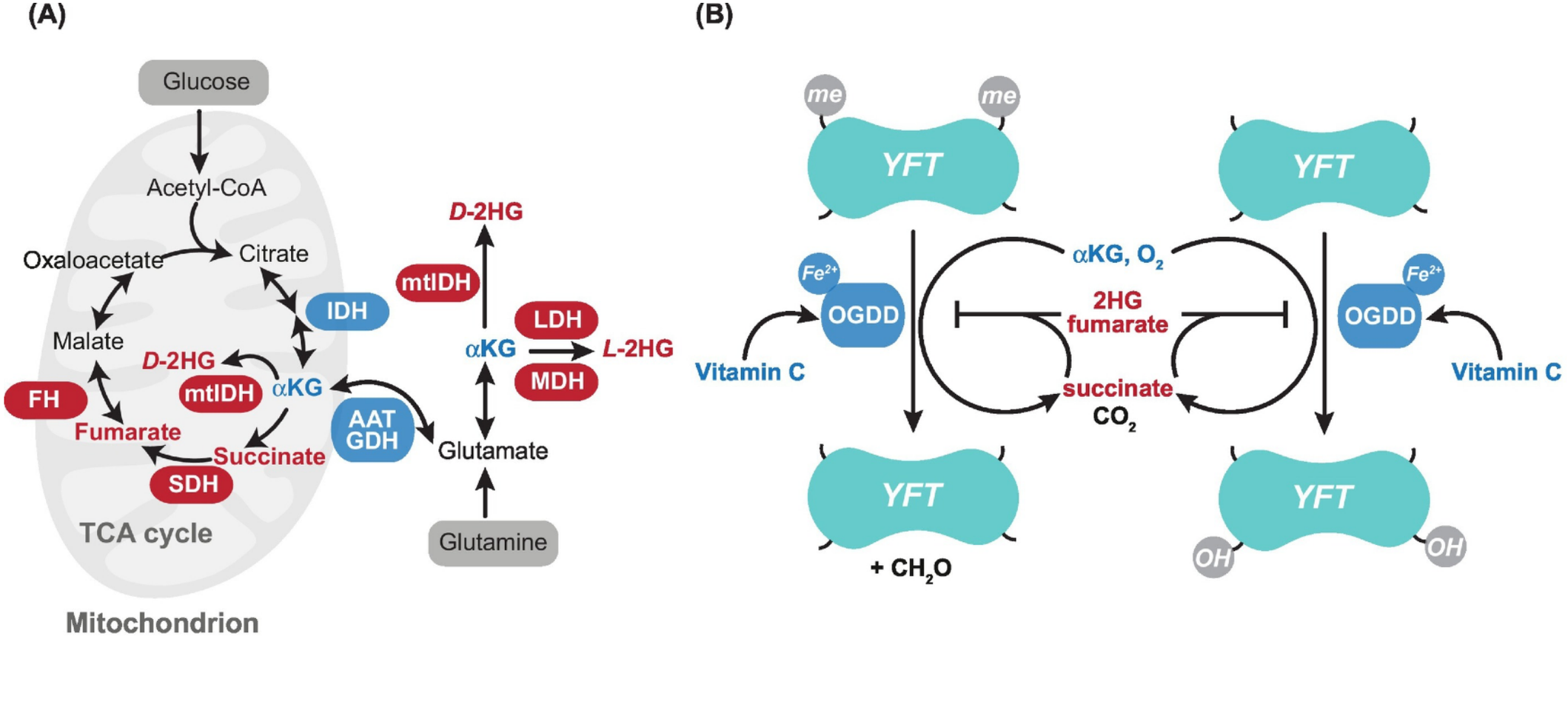
Metabolites are sensed through cofactor availability

Some metabolites are critical co-factors for enzymatic activity

Particularly useful when metabolite/co-factor synthesis and availability is highly compartmentalized

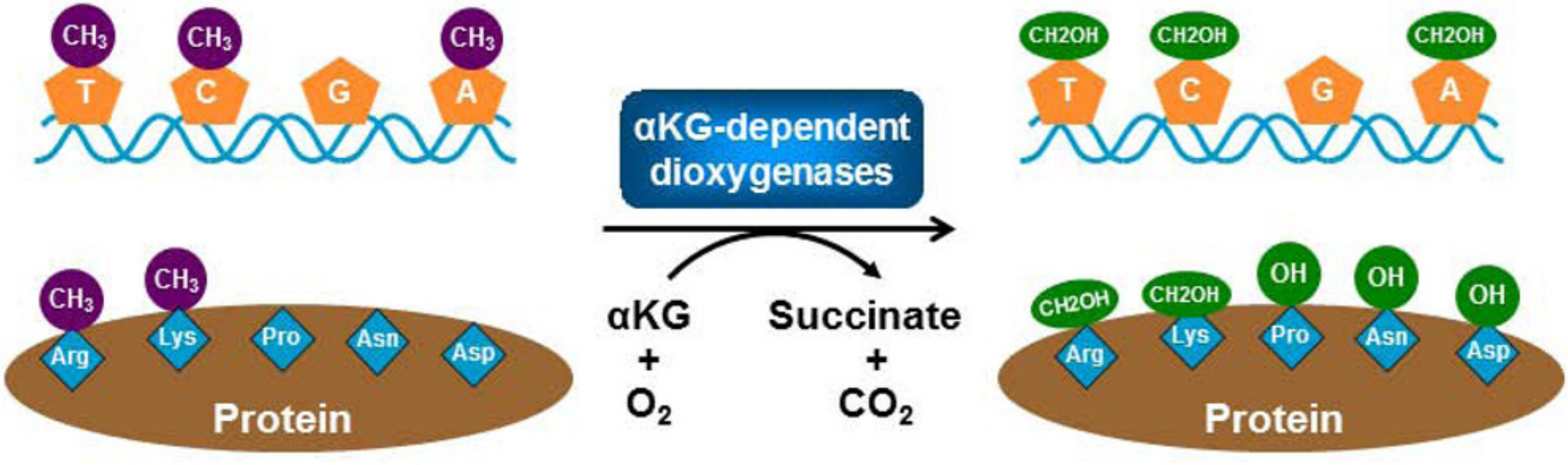
TCA cycle intermediates are sensed through alpha-ketoglutarate (aKG)-dependent dioxygenases, a versatile group of iron-containing enzymes that includes key players in epigenetic regulation, oxygen sensing, lipid metabolism, and other critical processes.

These enzymes couple the decarboxylation of aKG with the oxidation of the substrate, and in many cases the predicted K_M of those enzymes to aKG overlaps with its physiological levels, suggesting that their activity may dynamically respond to intracellular aKG levels.



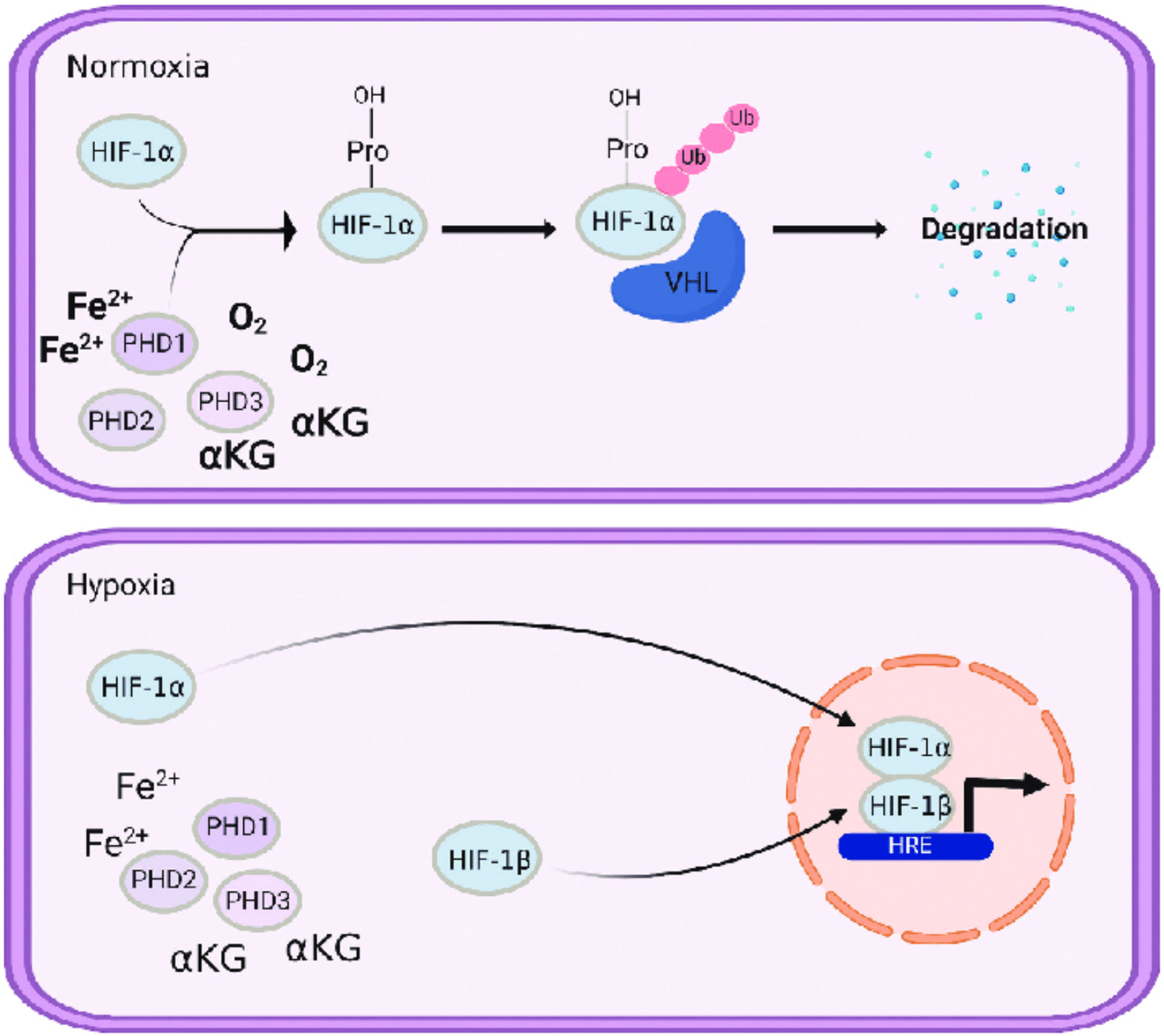
TCA cycle intermediates are sensed through alpha-ketoglutarate (aKG)-dependent dioxygenases, a versatile group of iron-containing enzymes that includes key players in epigenetic regulation, oxygen sensing, lipid metabolism, and other critical processes.

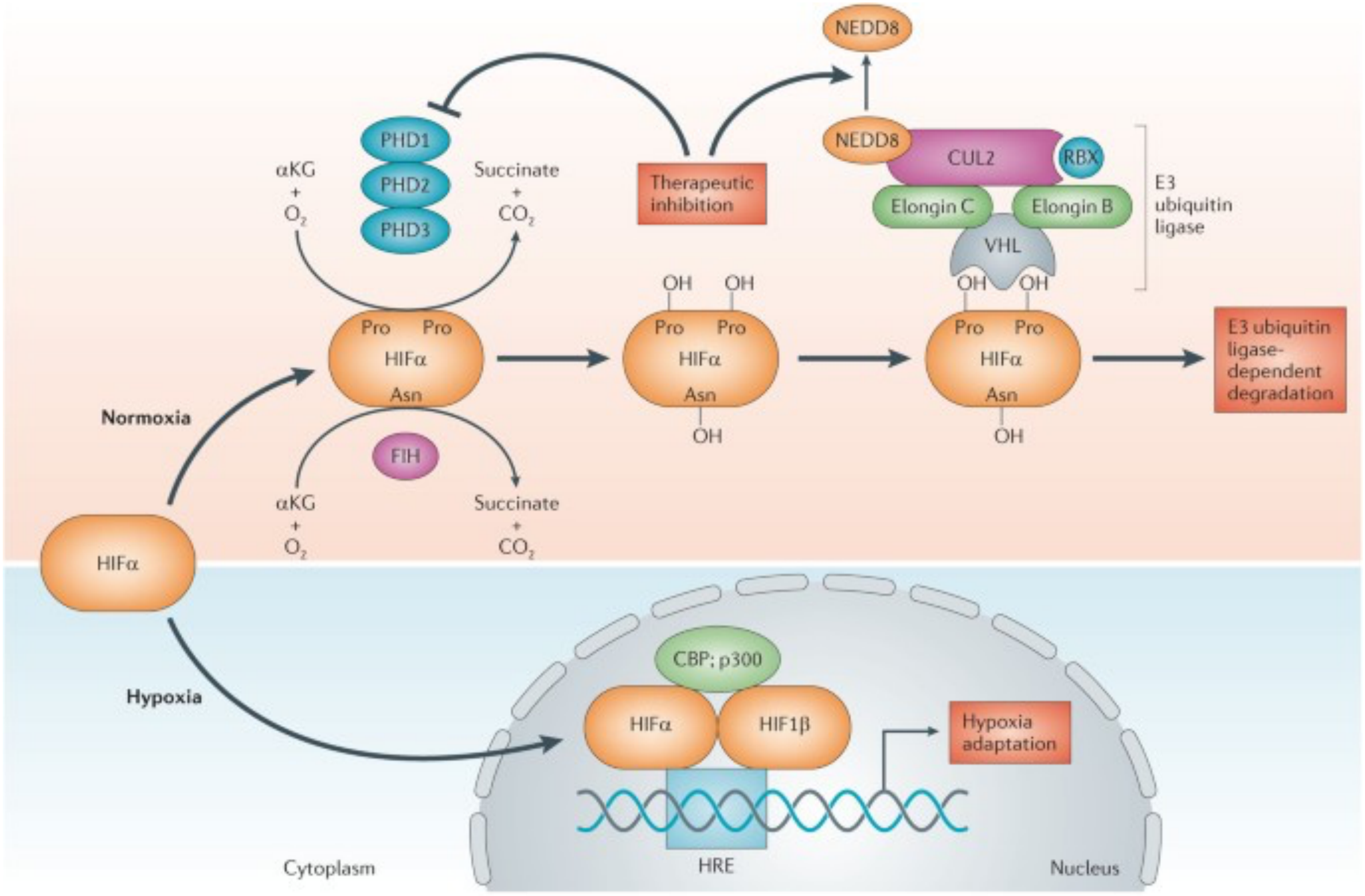
These enzymes couple the decarboxylation of aKG with the oxidation of the substrate, and in many cases the predicted K_M of those enzymes to aKG overlaps with its physiological levels, suggesting that their activity may dynamically respond to intracellular aKG levels.



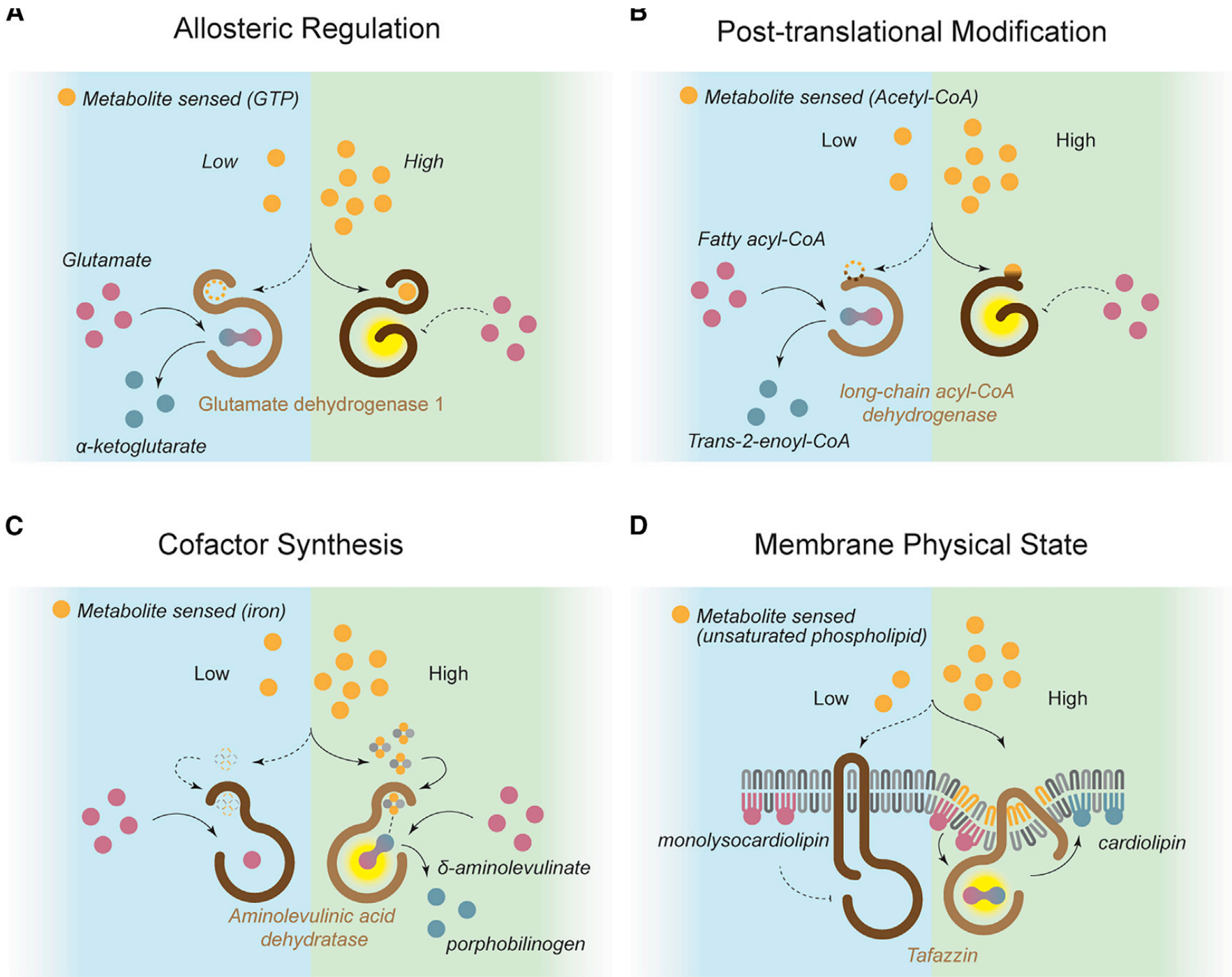
Metabolites are sensed through cofactor availability

aKG supports hypoxic response




NEDD8
NEDD8
CUL2
RBX
Elongin C
Elongin B
VHL
E3
ubiquitin
ligase
PHD1
PHD2
PHD3
αKG
+
O_2
Succinate
+
CO_2
Therapeutic
inhibition
OH OH
Pro Pro
HIFα
Asn
OH
E3 ubiquitin
ligase-
dependent
degradation
Normoxia
FIH
αKG
+
O_2
Succinate
+
CO_2
HIFα
Hypoxia
CBP; p300
HIFα
HIF1β
Hypoxia
adaptation
HRE
Cytoplasm
Nucleus

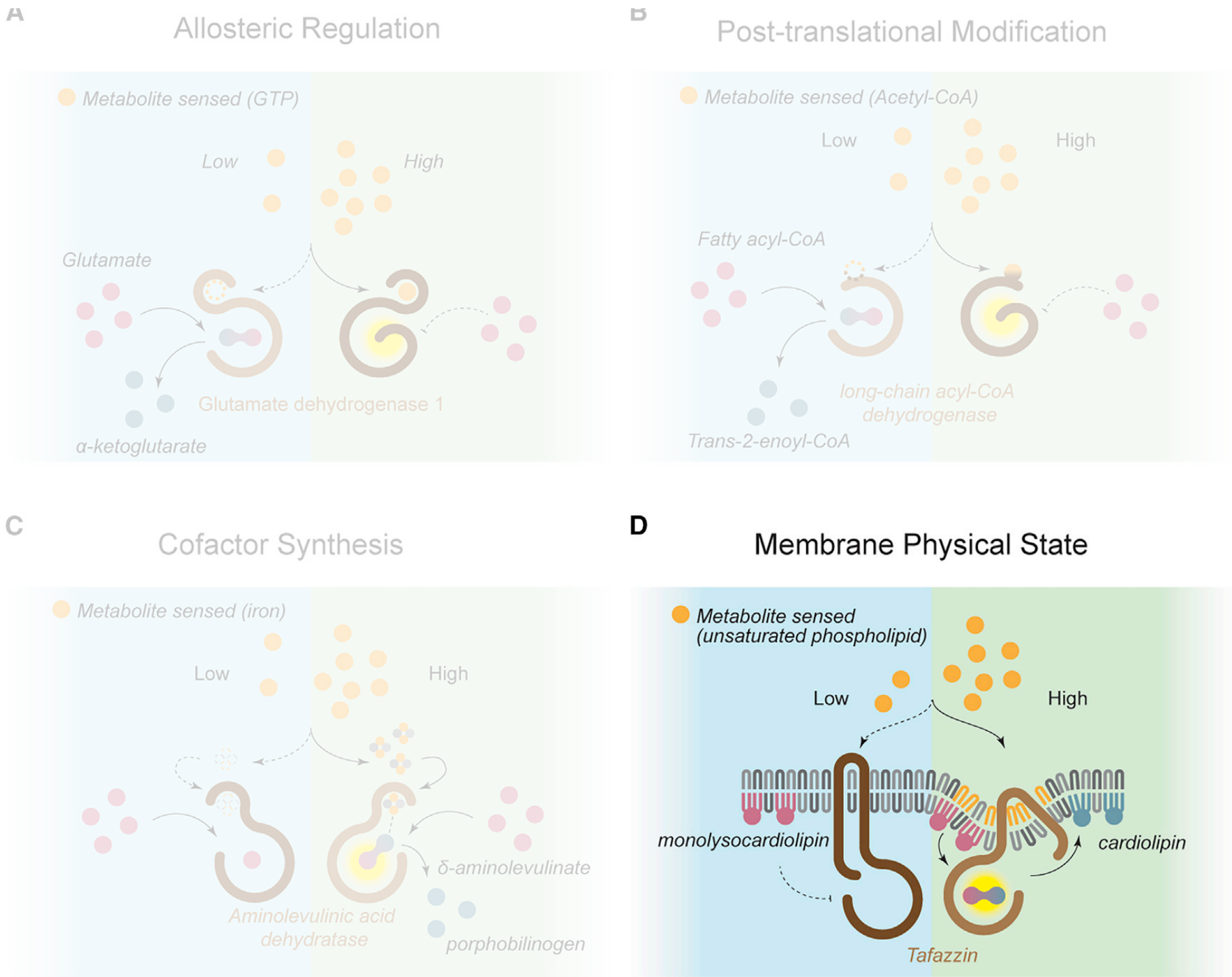
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

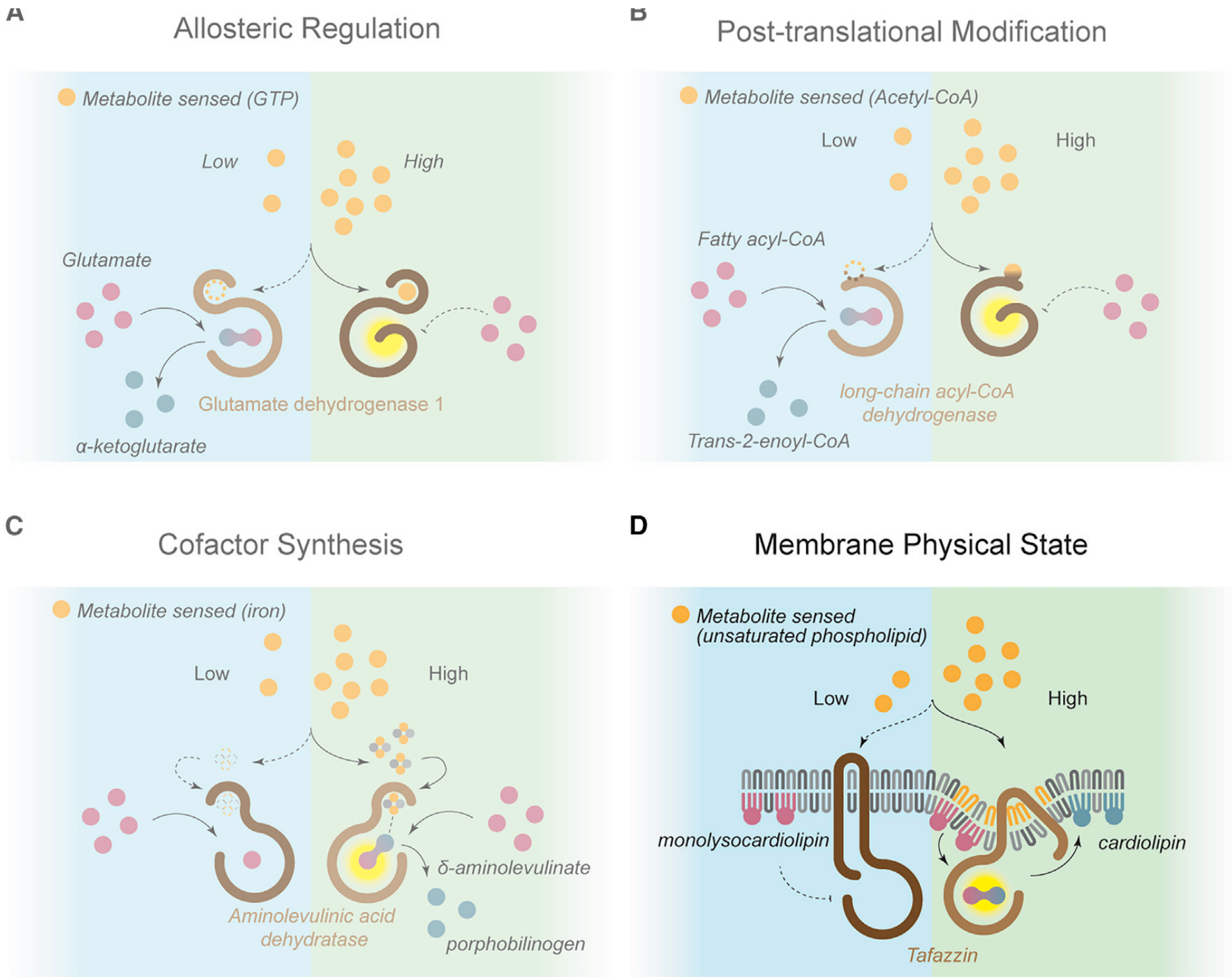
Metabolites are sensed by PROTEINS



Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

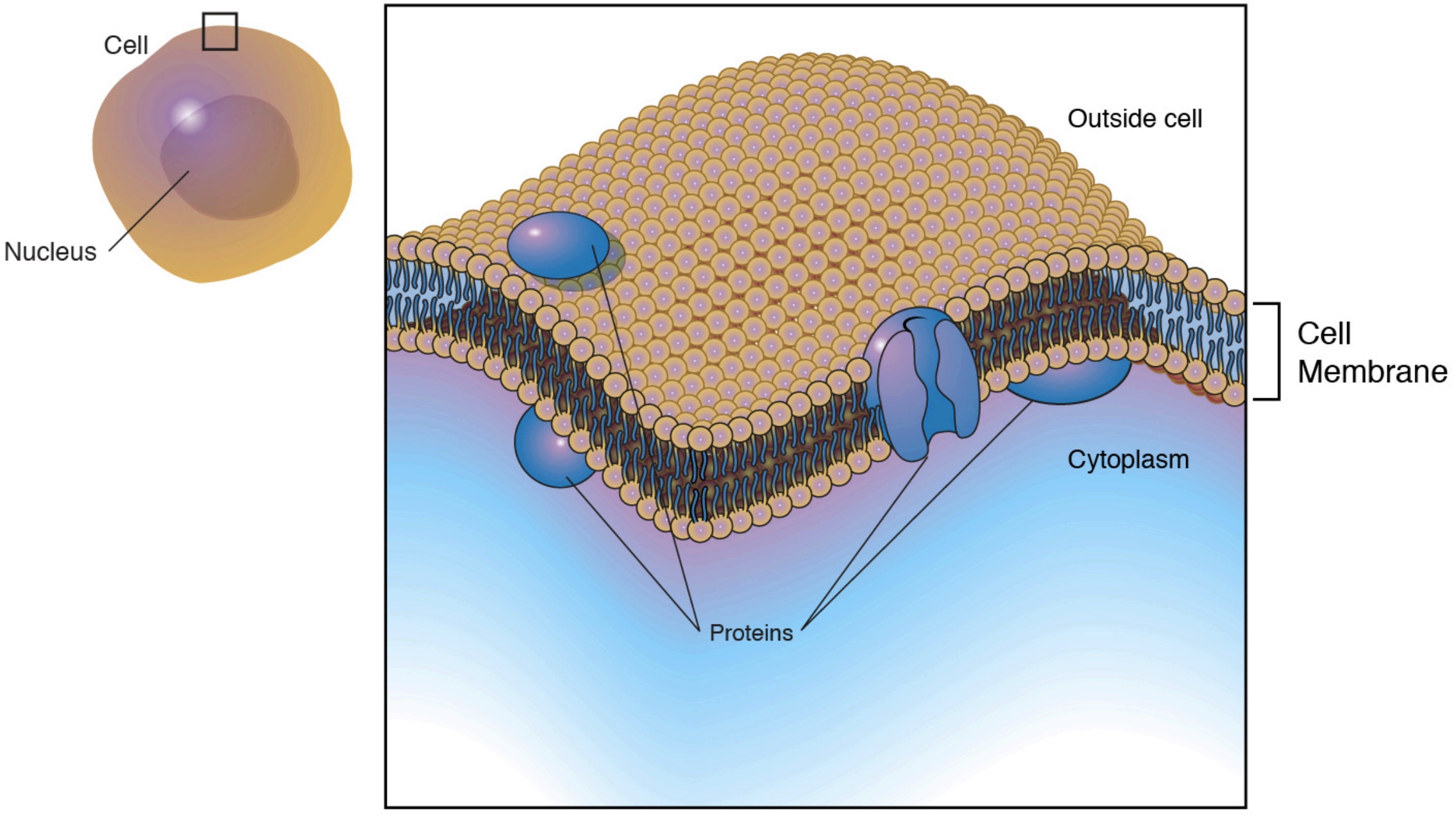
Metabolites are sensed by PROTEINS



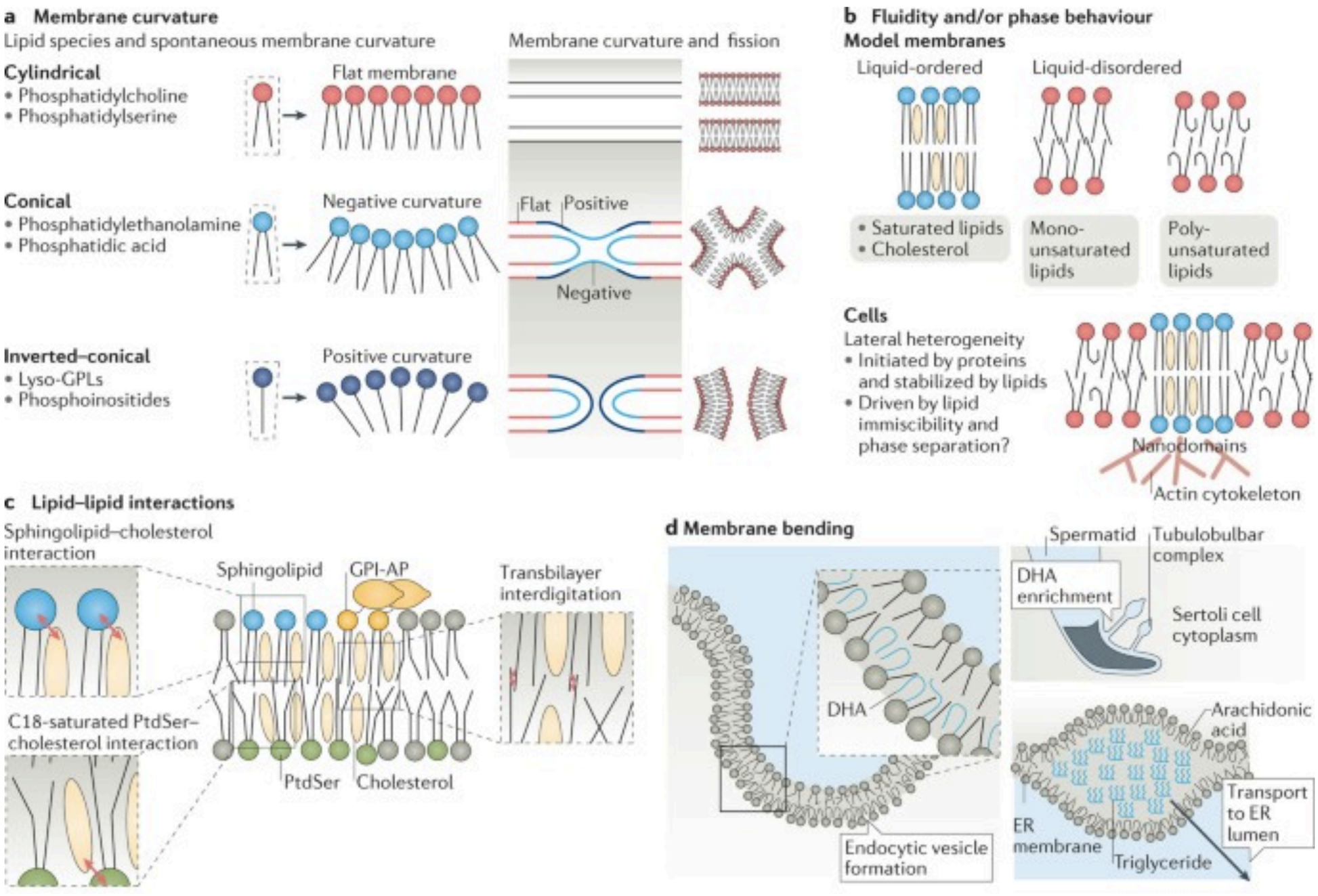
Several protein (or protein complex) sensors have been described, all functioning via 4 fundamental mechanisms

Liu & Birsoy, ***Mol Cell****, 2023*

Lipids are sensed through biophysical properties of cellular membranes



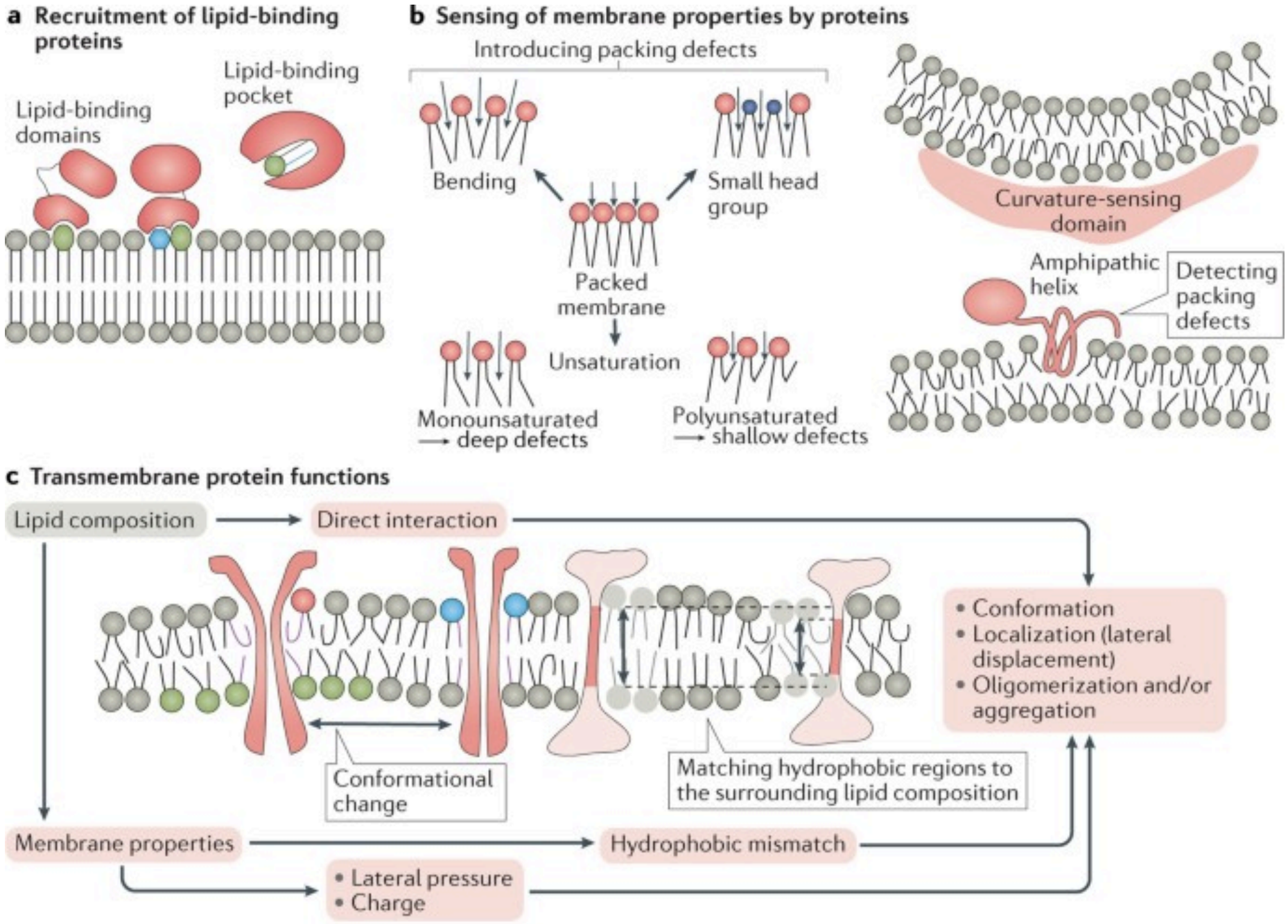
Lipids are sensed through biophysical properties of cellular membranes



Lipid composition of cellular membranes is highly heterogeneous and impacts biophysical properties

Harayama & Riezman, ***NRMCB****, 2019*

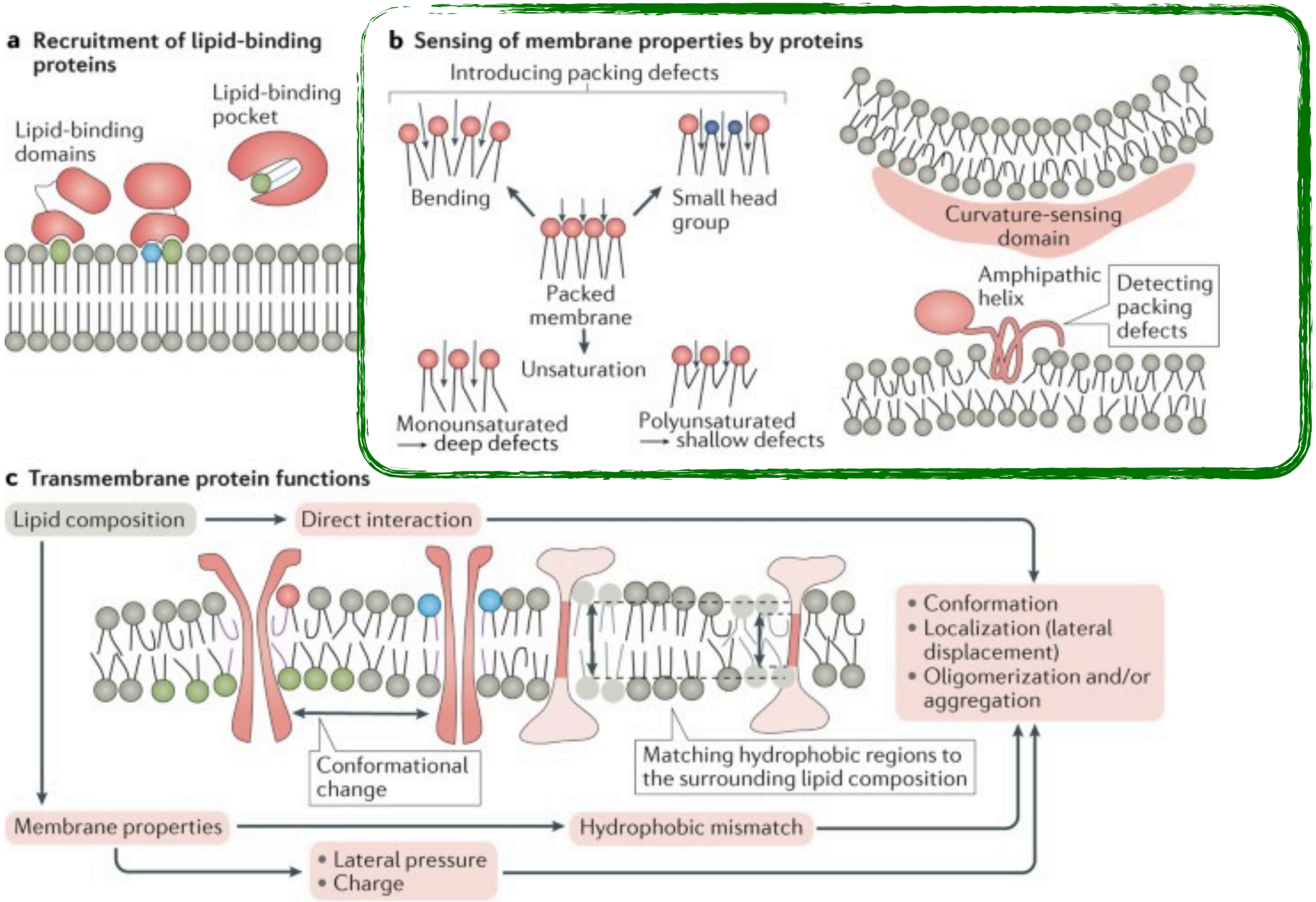
Lipids are sensed through biophysical properties of cellular membranes



Lipid composition of cellular membranes is highly heterogeneous and impacts biophysical properties

Harayama & Riezman, ***NRMCB****, 2019*

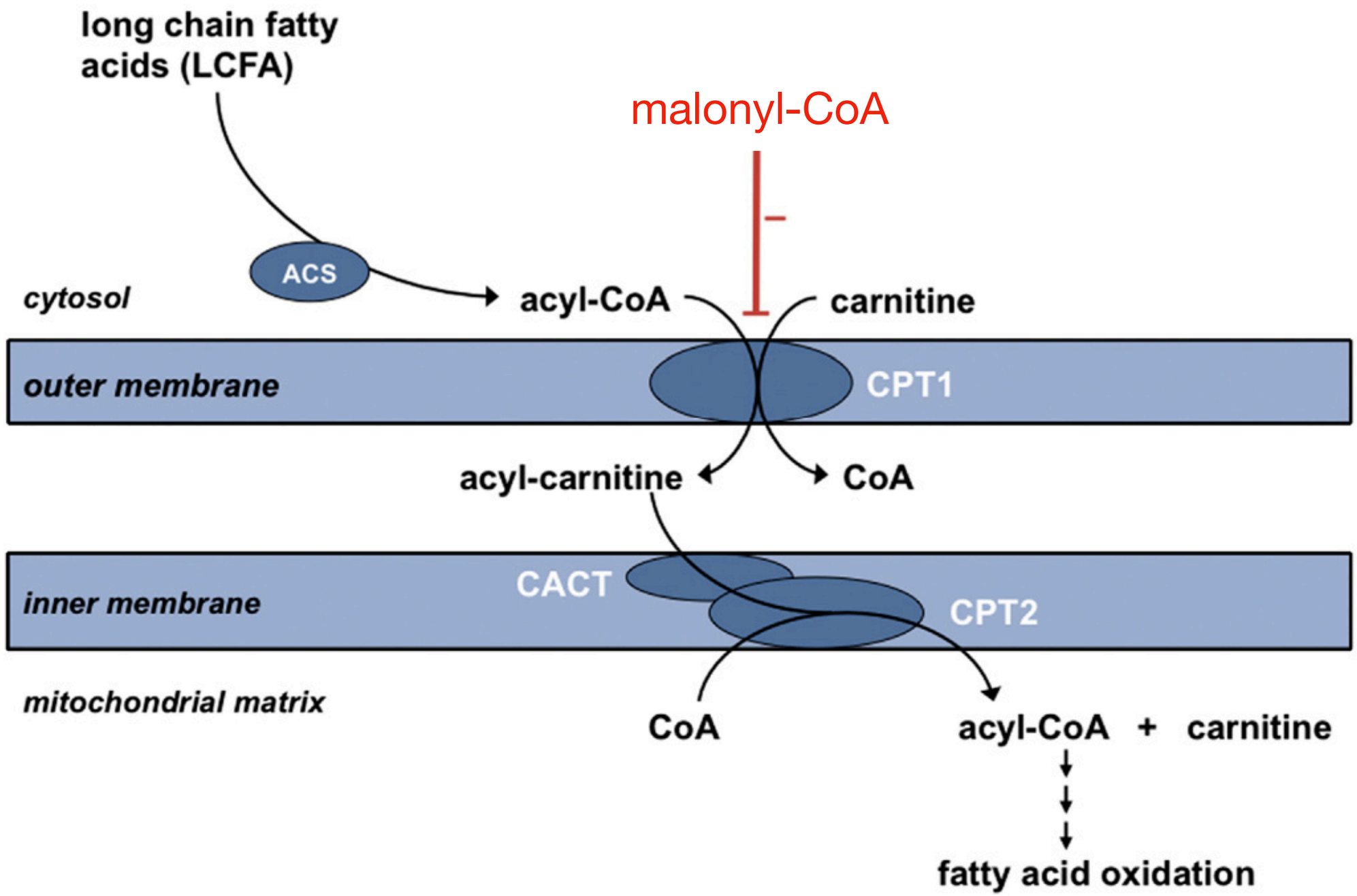
Lipids are sensed through biophysical properties of cellular membranes



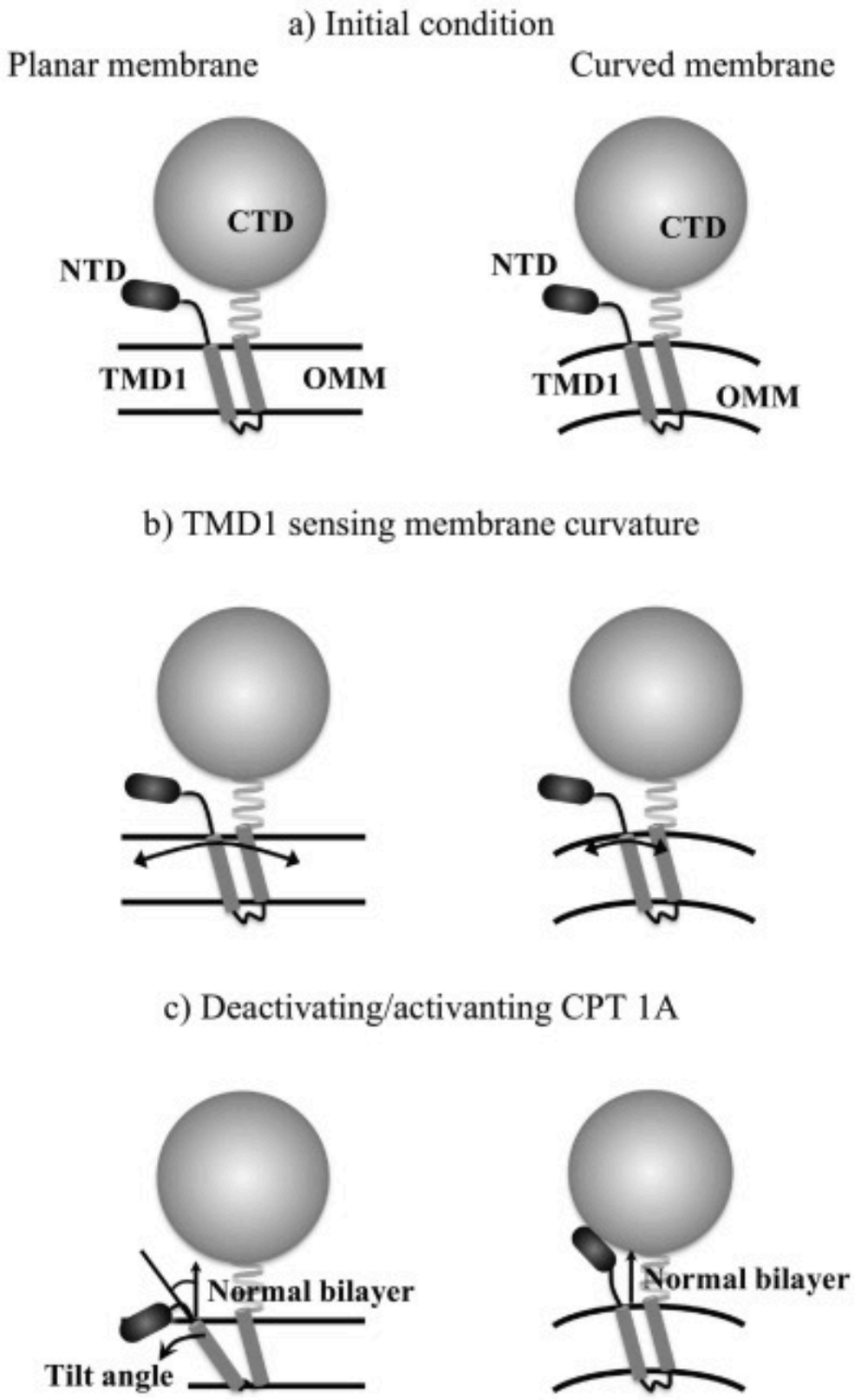
Lipid composition of cellular membranes is highly heterogeneous and impacts biophysical properties

Harayama & Riezman, ***NRMCB****, 2019*

Example: Carnitine Palmitoyltransferase 1A (CPT1A)



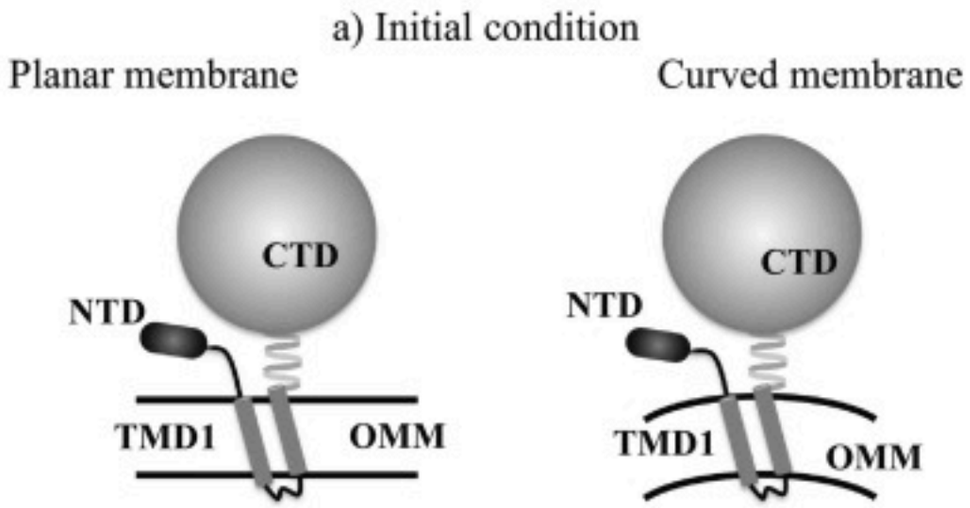
Example: Carnitine Palmitoyltransferase 1A (CPT1A)



CPT1A is a transmembrane protein at the outer mitochondrial membrane.

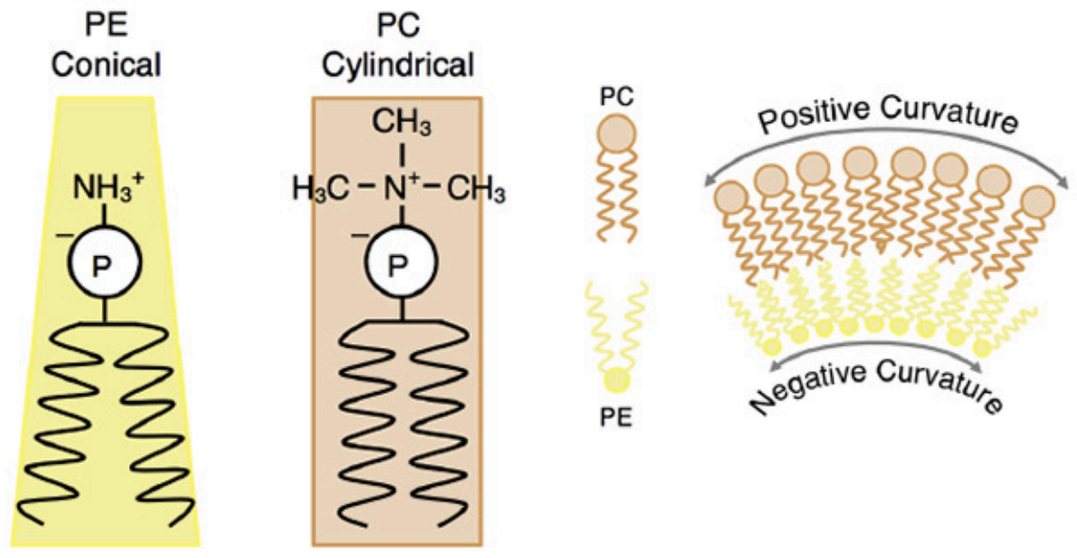
The N-terminal domain (NTD) of CPT1A is sensitive to the curvature of the membrane.

Example: Carnitine Palmitoyltransferase 1A (CPT1A)



CPT1A is a transmembrane protein at the outer mitochondrial membrane.

The N-terminal domain (NTD) of CPT1A is sensitive to the curvature of the membrane.

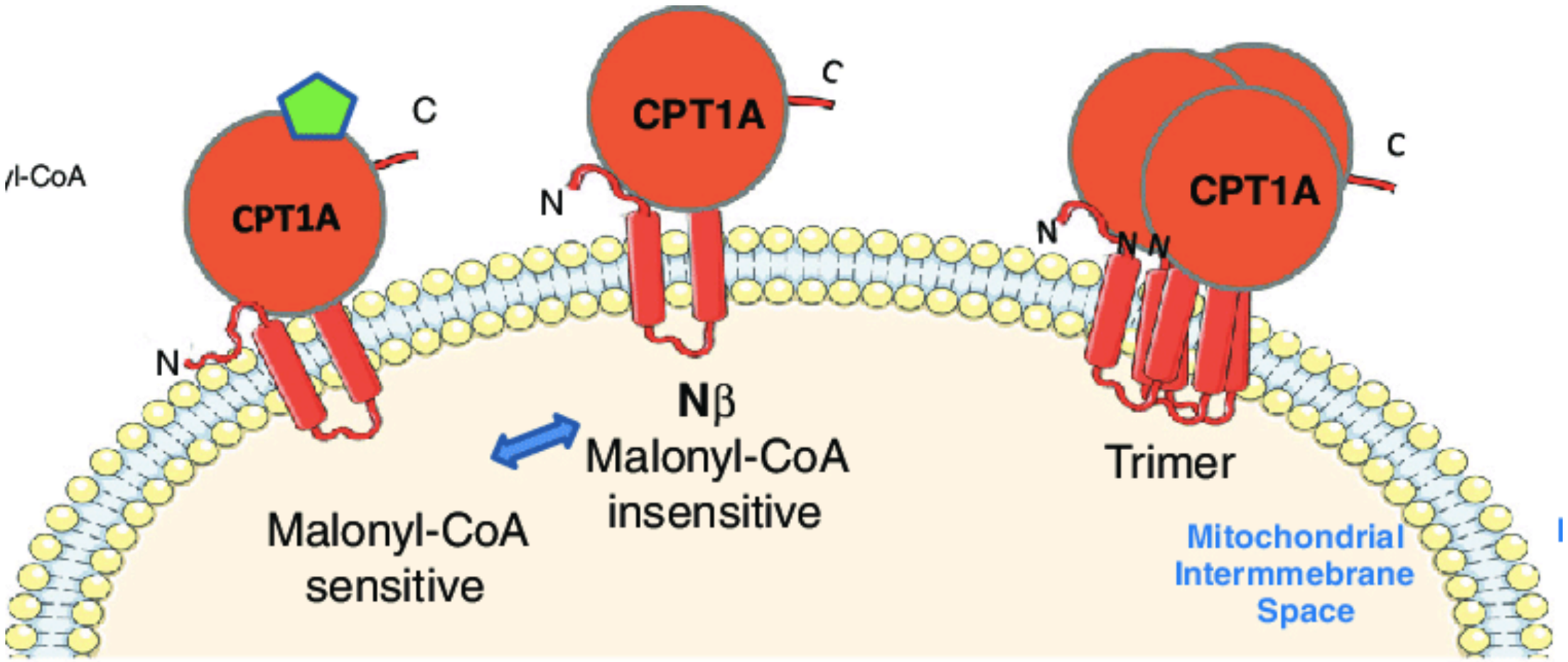


Membrane curvature is dictated by several factors including PL composition.

PE: phosphatidyl-ethanolamine

PC: phosphatidyl-choline

Example: Carnitine Palmitoyltransferase 1A (CPT1A)



The activity of CPT1A is regulated by PL abundance through biophysical interactions

Molecular mechanisms of nutrient sensing

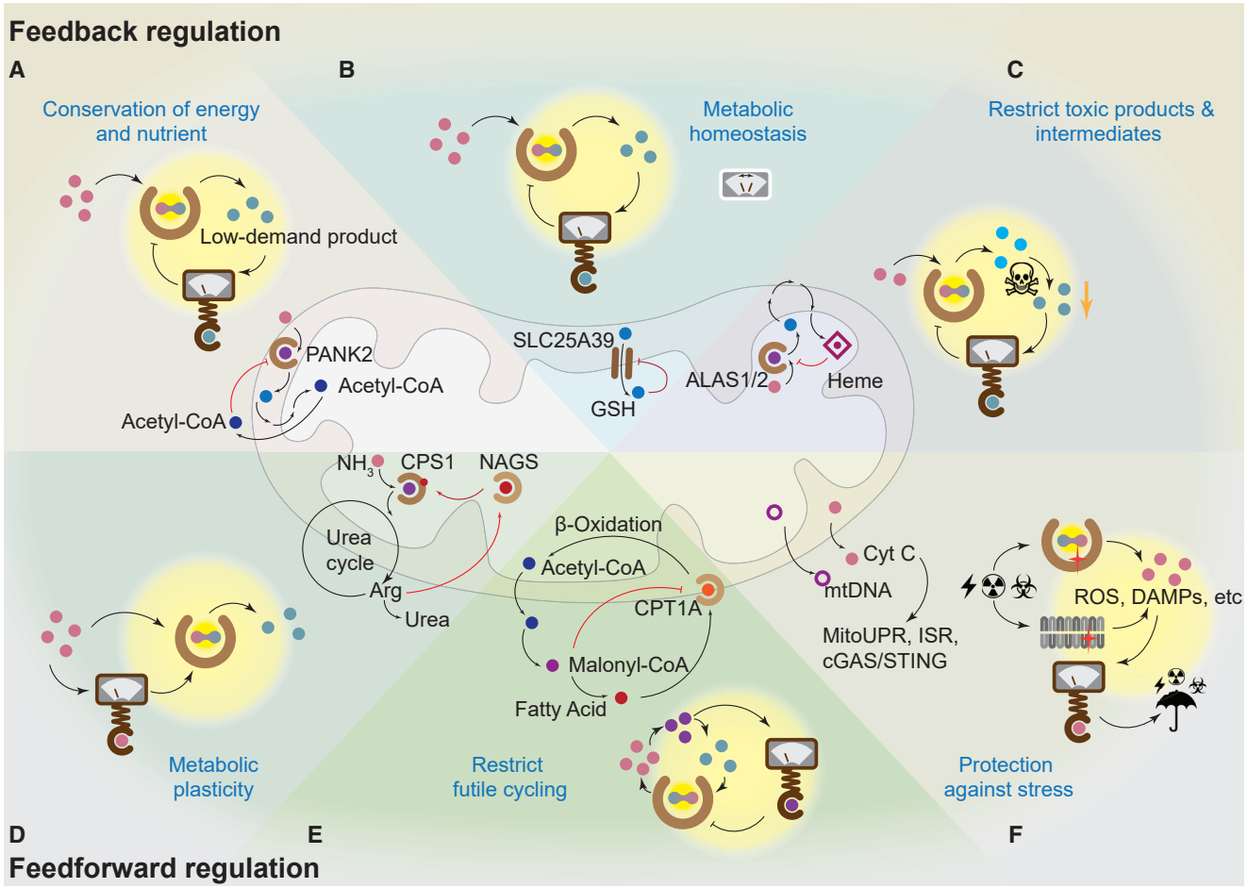
Molecular mechanisms of nutrient sensing

WHERE??

Membrane-enclosed organelles maintain distinct biochemical environments.

This creates a unique milieu for nutrient sensing.

Compartmentalization of nutrient sensing: MITOCHONDRIA



*Liu & Birsoy, **Mol Cell**, 2023*

(A) Feedback circuit that ensures metabolic conservation by limiting energy-consuming pathways. PANK2, a mitochondrial enzyme in the CoA synthesis pathway, is allosterically inhibited by CoA and acetyl-CoA.

(B) Feedback circuit dedicated to maintaining the mitochondrial levels of a metabolite. Glutathione has been observed to down-regulate its mitochondrial importer SLC25A39.

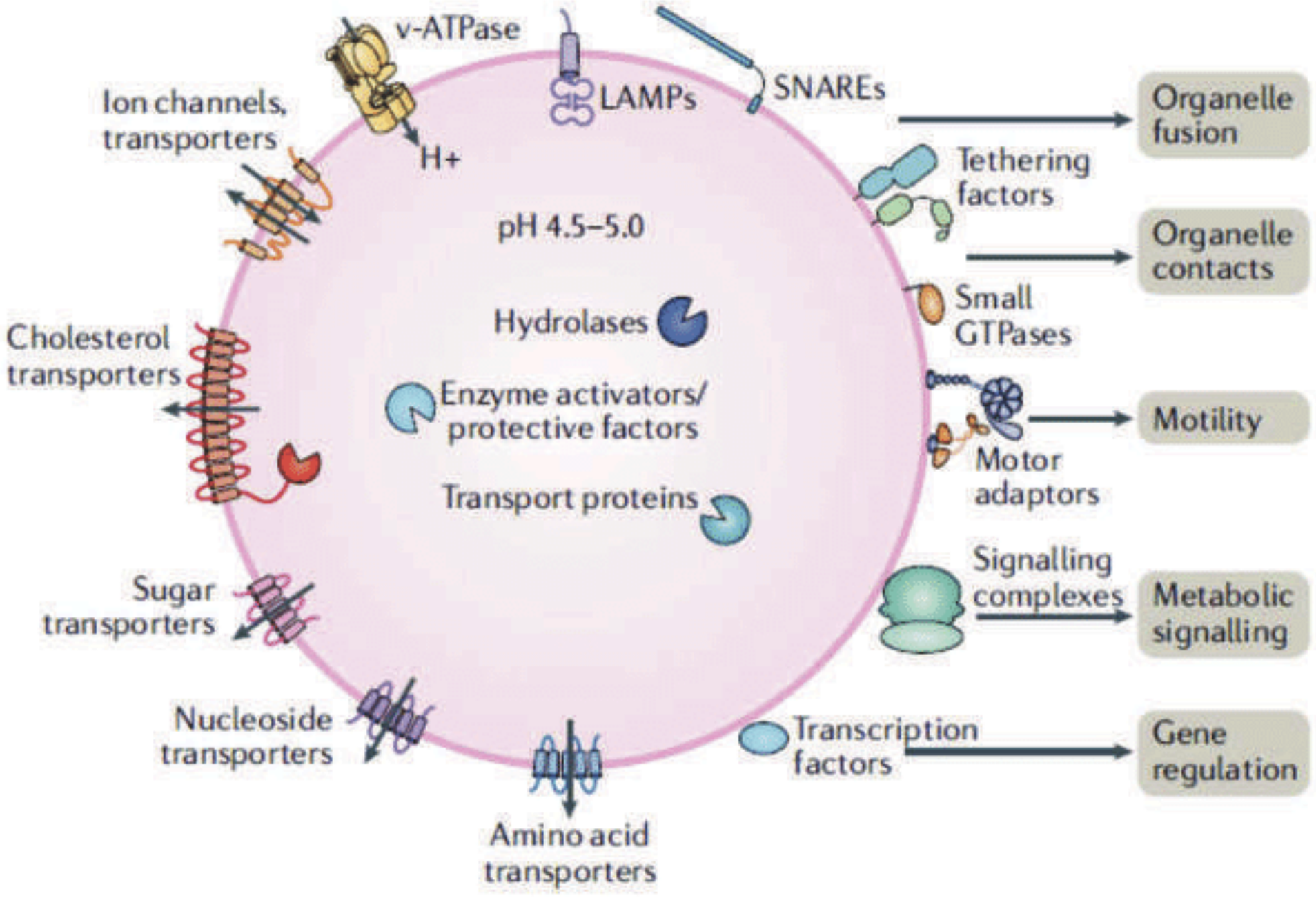
(C) Feedback circuit that restrains the production of toxic metabolites. Heme inhibits the import of the rate-limiting enzyme in its *de novo* synthesis, ALAS1/ALAS2, to avoid the accumulation of toxic intermediates.

(D) Feedforward circuit that enables metabolic plasticity. Arginine stimulates the synthesis of N-acetylglutamate, an allosteric activator of urea cycle enzyme CPS1, allowing robust activation of the urea cycle upon the influx of N.

(E) Feedforward circuit that prevents futile cycles. Fatty acid synthesis substrate malonyl-CoA inhibits the entrance of fatty acids into the reverse reaction, b-oxidation, by allosterically inhibiting CPS1.

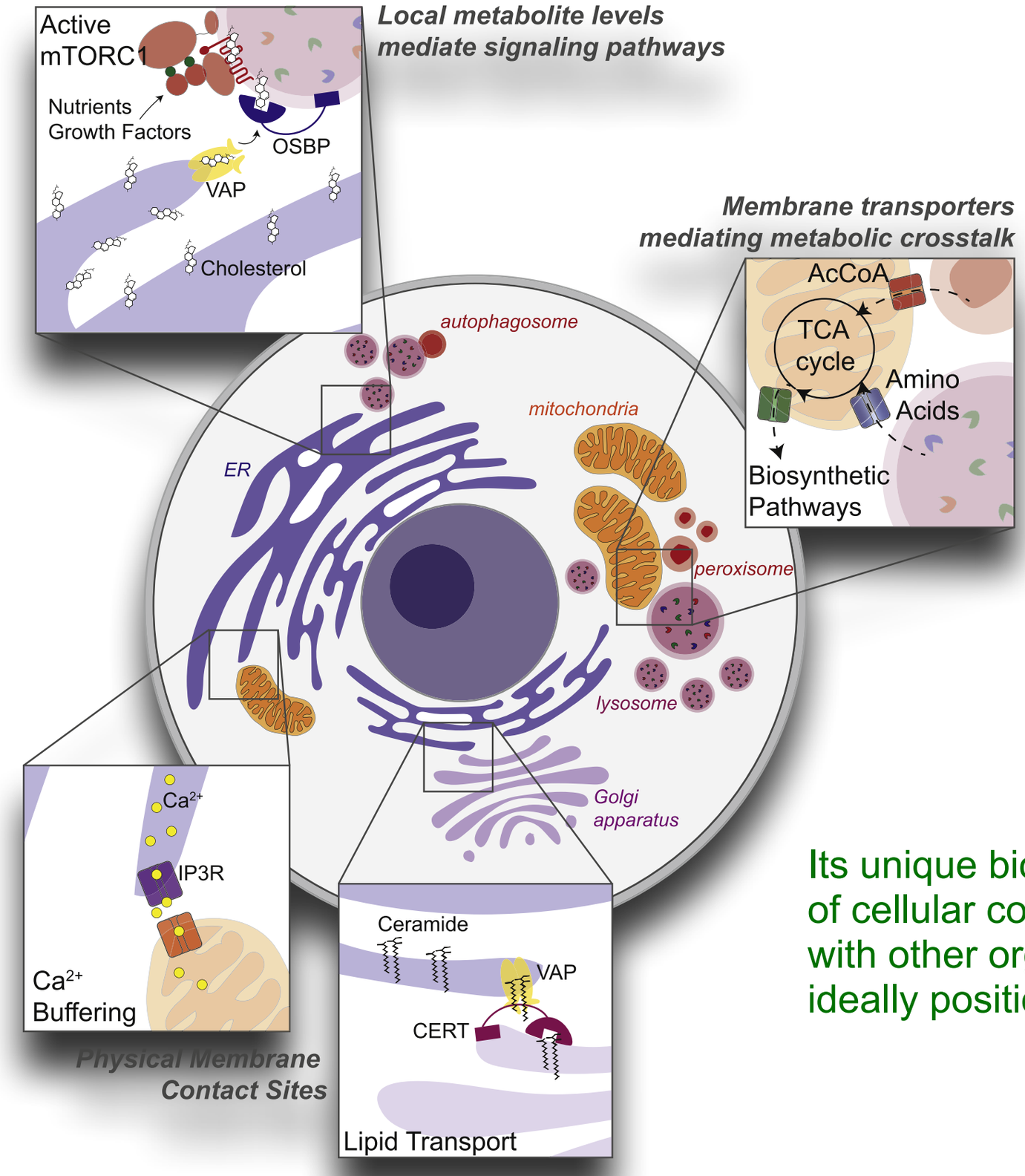
(F) Feedforward circuits that trigger adaptive responses to stress. The release of mitochondrial DNA or cytochrome *c* triggers stress response signaling via the cGAS-STING pathway or the integrated stress response (ISR).

Compartmentalization of nutrient sensing: LYSOSOMES



Ballabio & Bonifacino, ***NRMCB****, 2020*

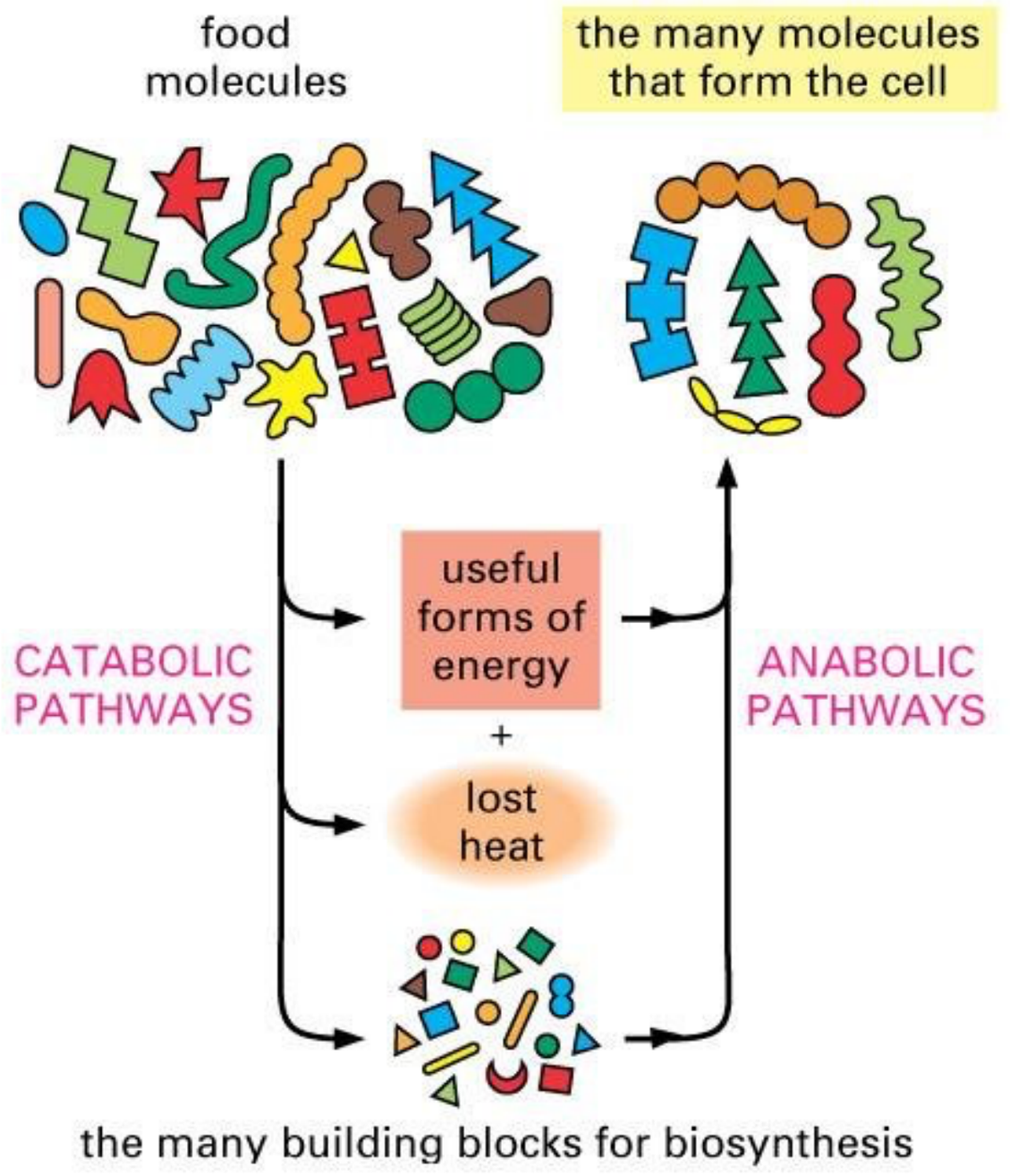
Compartmentalization of nutrient sensing: LYSOSOMES



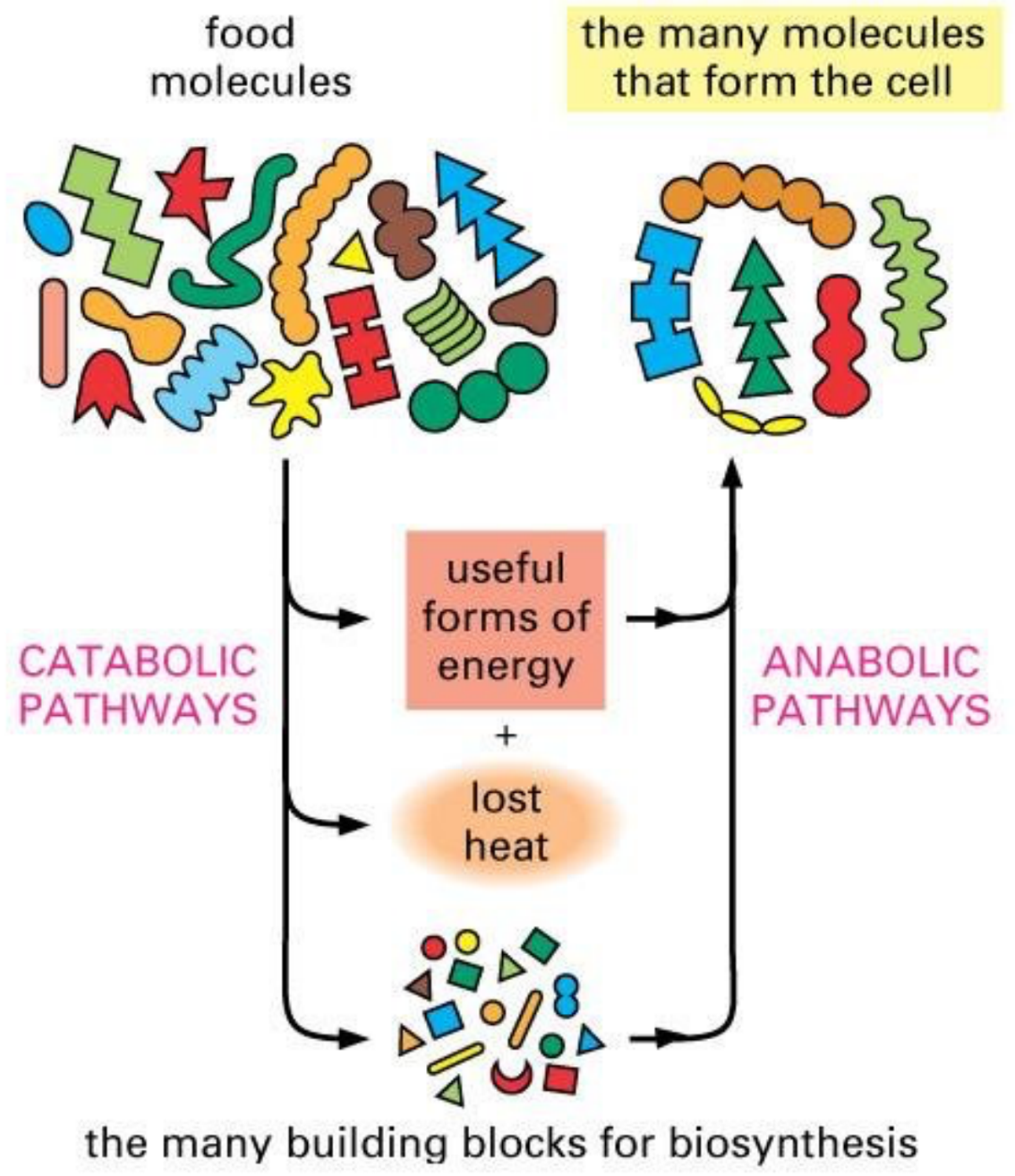
Its unique biochemical milieu, the scavenging of cellular components, the interconnections with other organelles, make the lysosome ideally positioned to sense metabolic inputs

Jain & Zoncu, ***Mol Metab****, 2021*

Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



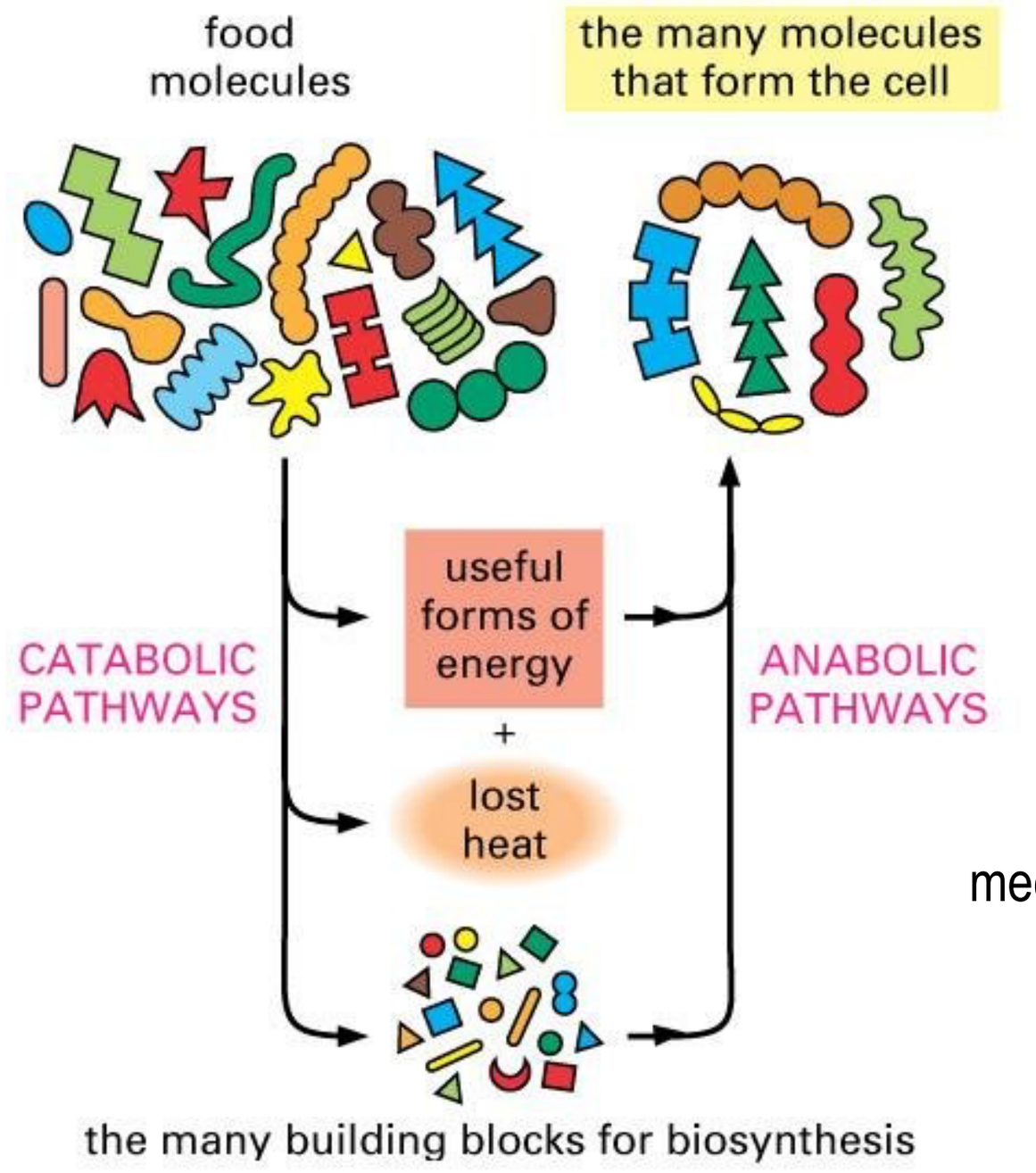
Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



AMPK

AMP-activated protein kinase

Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



AMPK

AMP-activated protein kinase

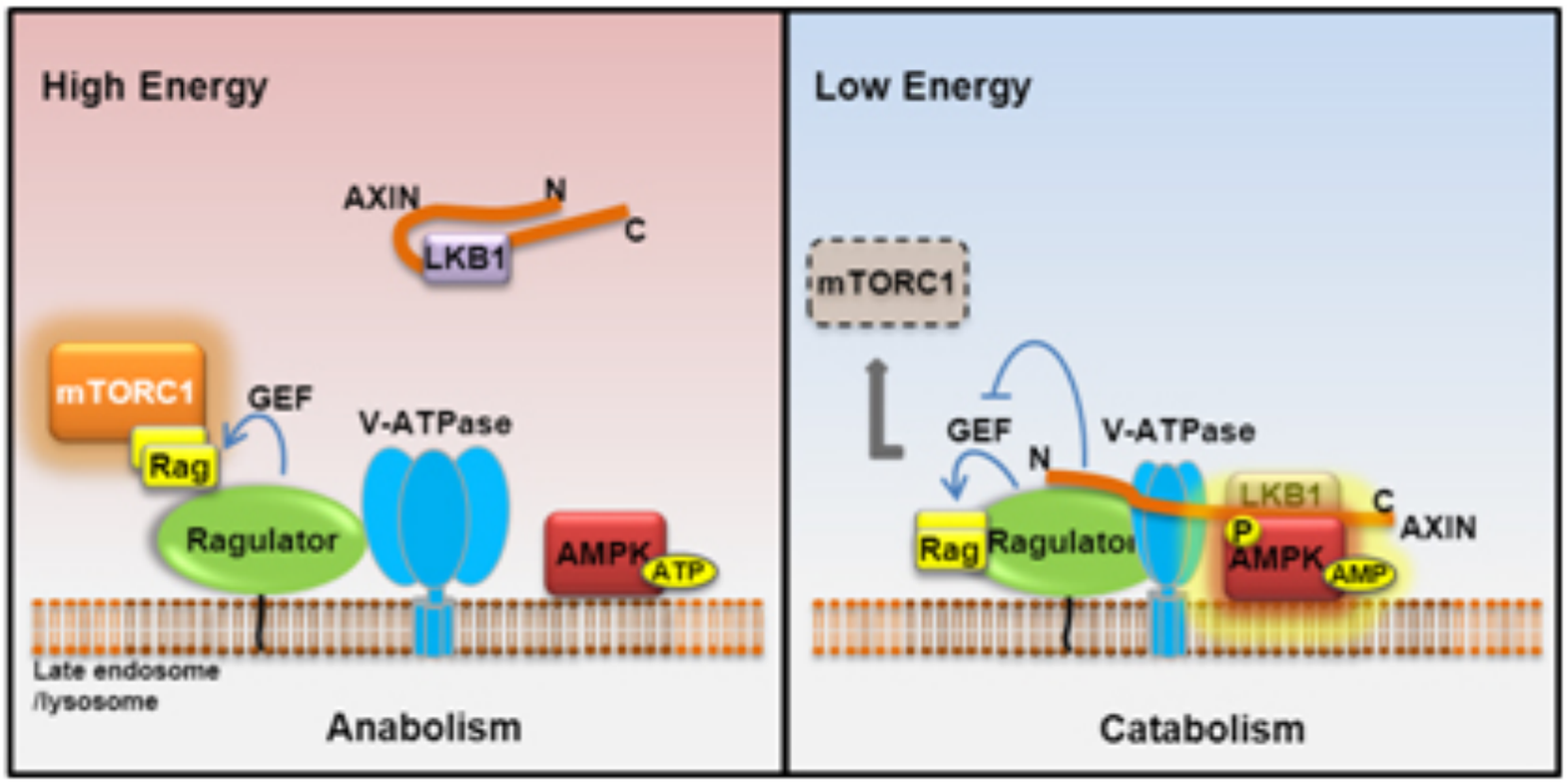
mTORC

mechanistic Target of Rapamycin

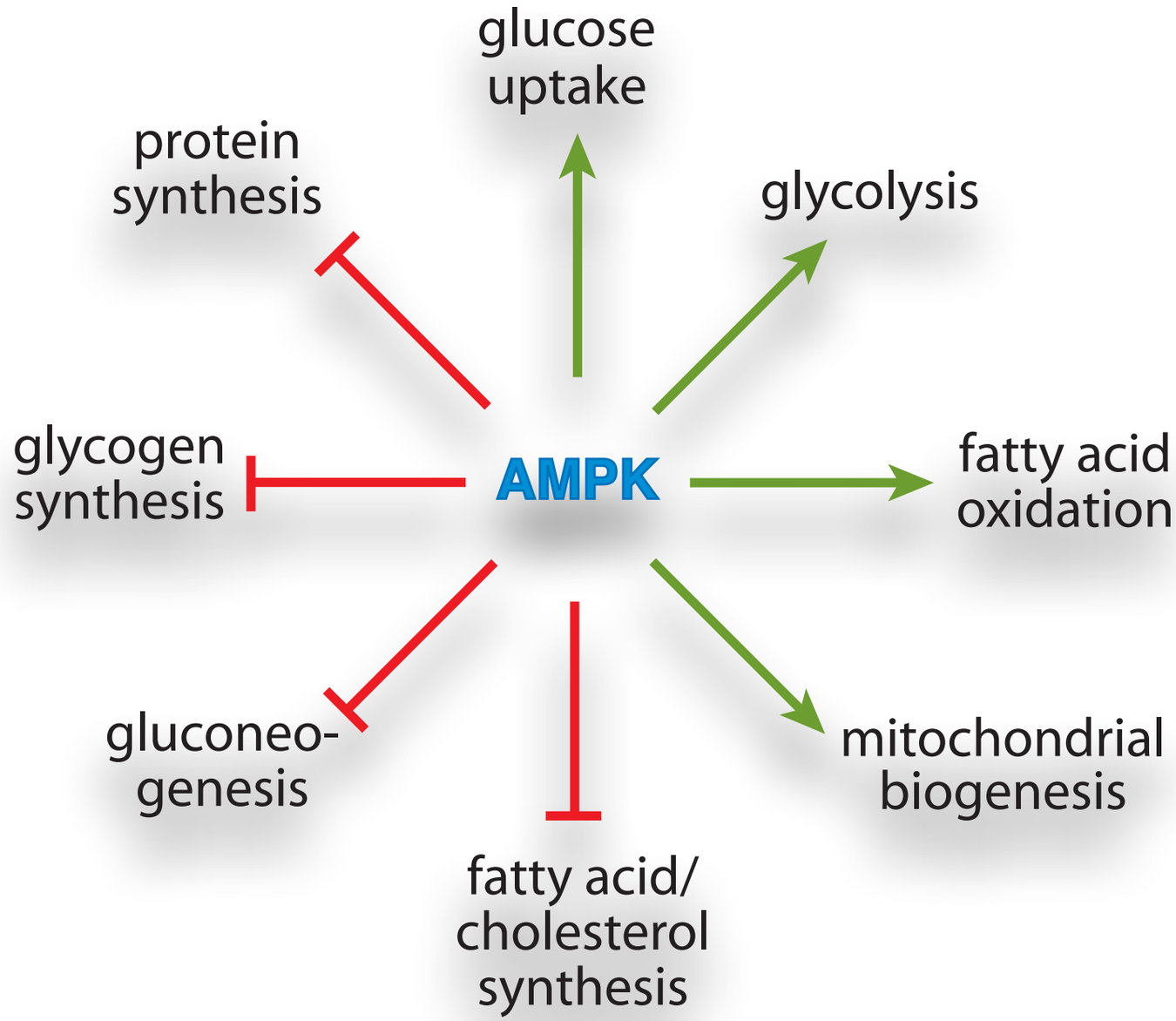
AMPK and mTORC are master regulator of catabolism and anabolism, respectively

Both activated at lysosomes, enabling co-regulation

AMPK and mTOR are both components of ancient conserved pathways that have evolved as a yin-yang-like antagonistic mechanism controlling catabolism and anabolism

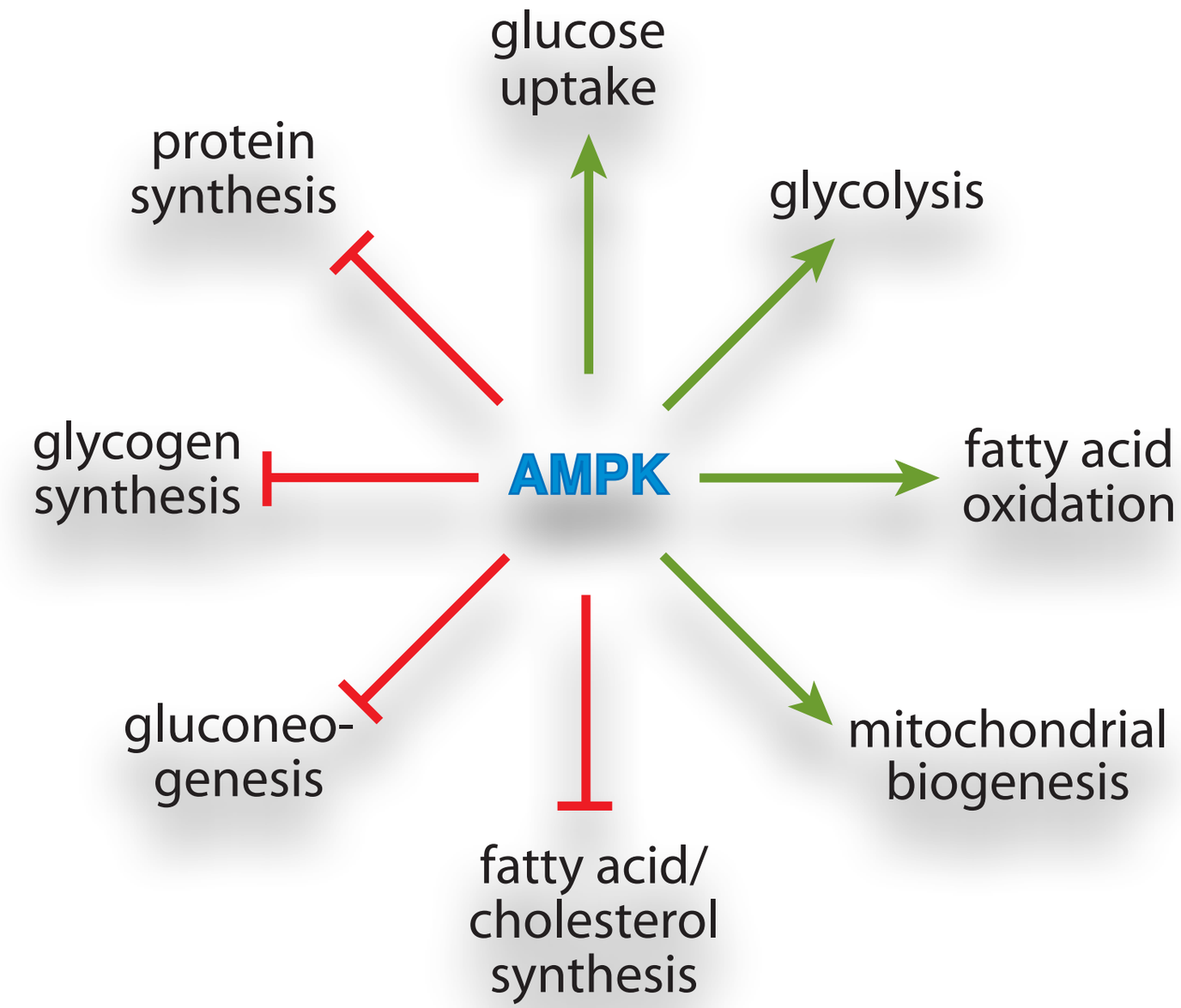


AMPK: Central regulator of glucose and lipid metabolism



1. **Promote glycolysis and FAO (catabolism)**
2. **Increase number of mitochondria**
3. **Blocks biosynthesis of macromolecules**

AMPK: Central regulator of glucose and lipid metabolism



1. **Promote glycolysis and FAO (catabolism)**
2. **Increase number of mitochondria**
3. **Blocks biosynthesis of macromolecules**

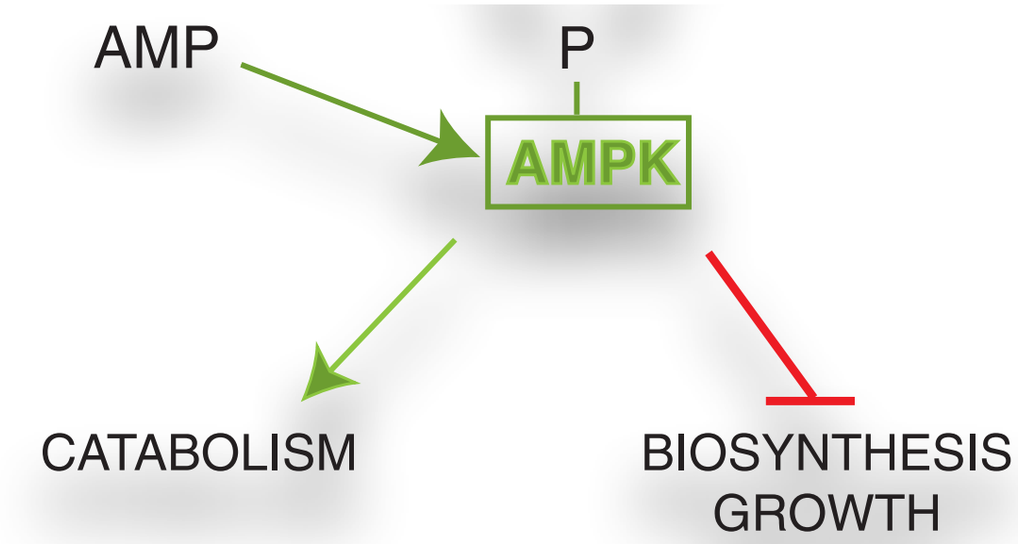
| Generate more ATP

Consume less ATP

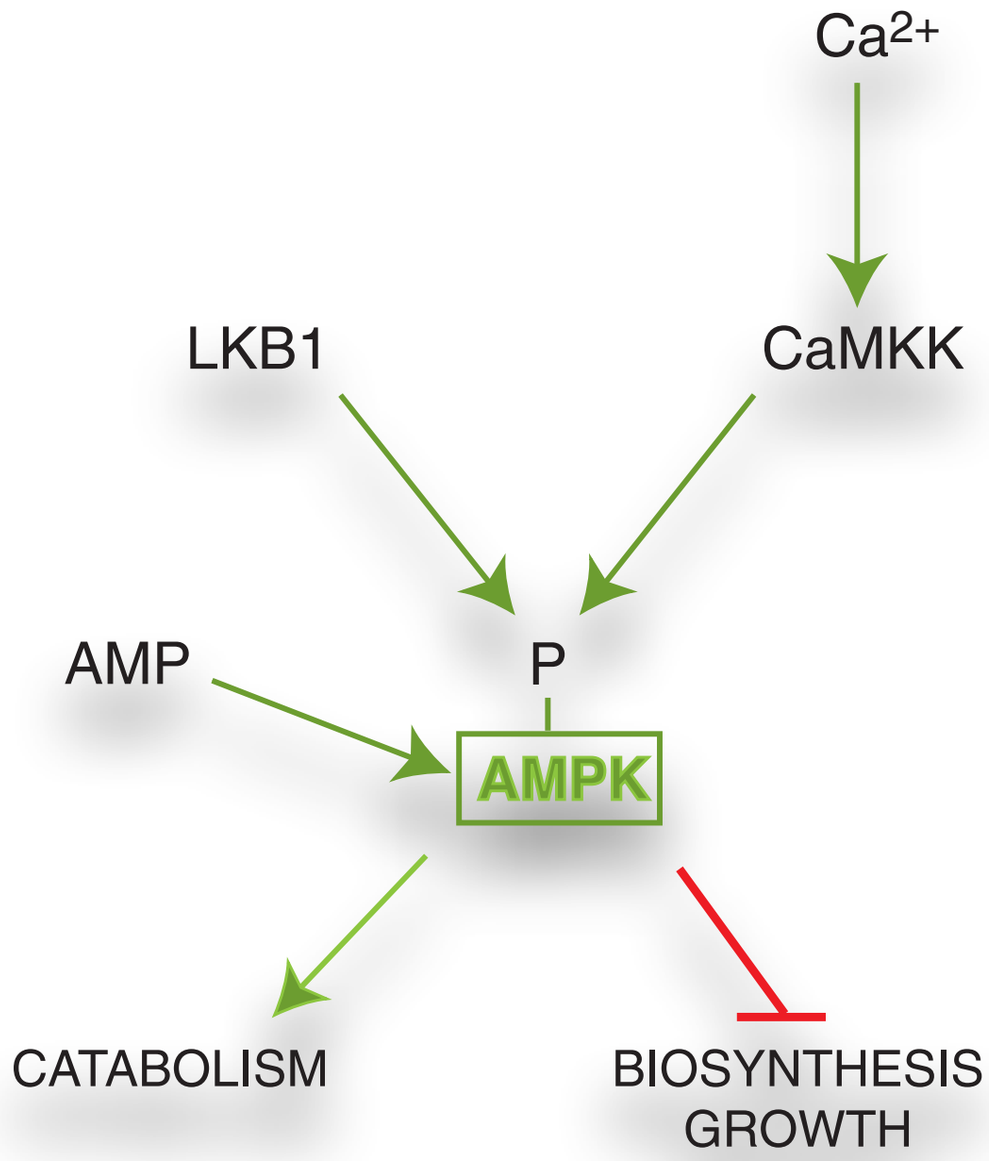
Cells constantly need to manage their energy consumption depending on the availability of nutrients and on their capacity to produce ATP.
When cellular ATP levels decrease, it is essential for cells to minimize energy consumption to avoid exhausting what is left of their resources. At the same time, emergency measures have to be taken to restore the cellular energy supply, such as increasing nutrient intake, activating alternative energy-producing pathways or turning over existing macromolecules into nutrients.

AMPK: Central regulator of glucose and lipid metabolism

The AMP–activated protein kinase (AMPK) is a highly conserved (all eukaryotic cells) metabolic checkpoint that acts as a sensor of ATP levels in the cell

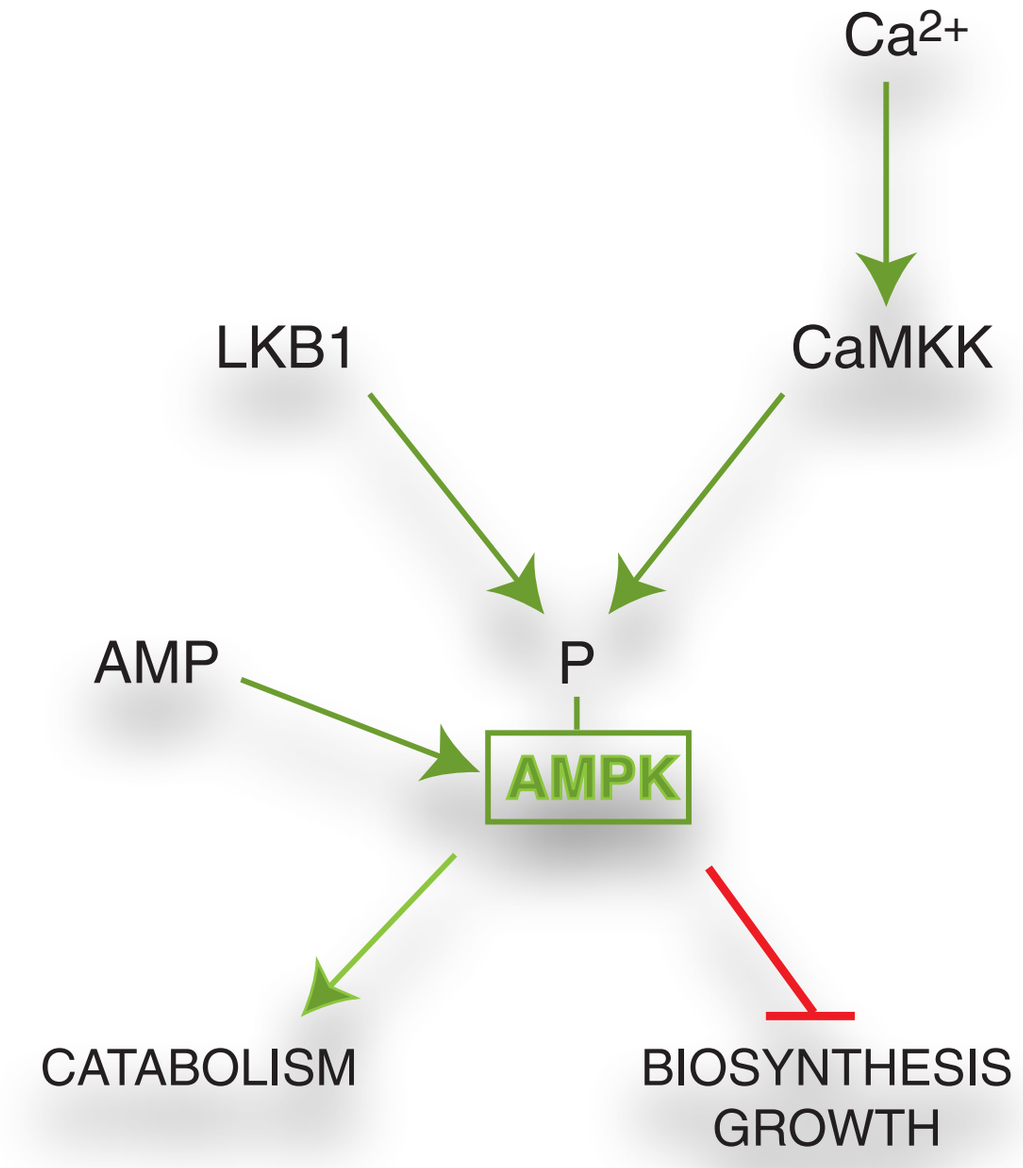


AMPK: Central regulator of glucose and lipid metabolism



The AMP–activated protein kinase (AMPK) is a highly conserved (all eukaryotic cells) metabolic checkpoint that acts as a sensor of ATP levels in the cell

AMPK: Central regulator of glucose and lipid metabolism

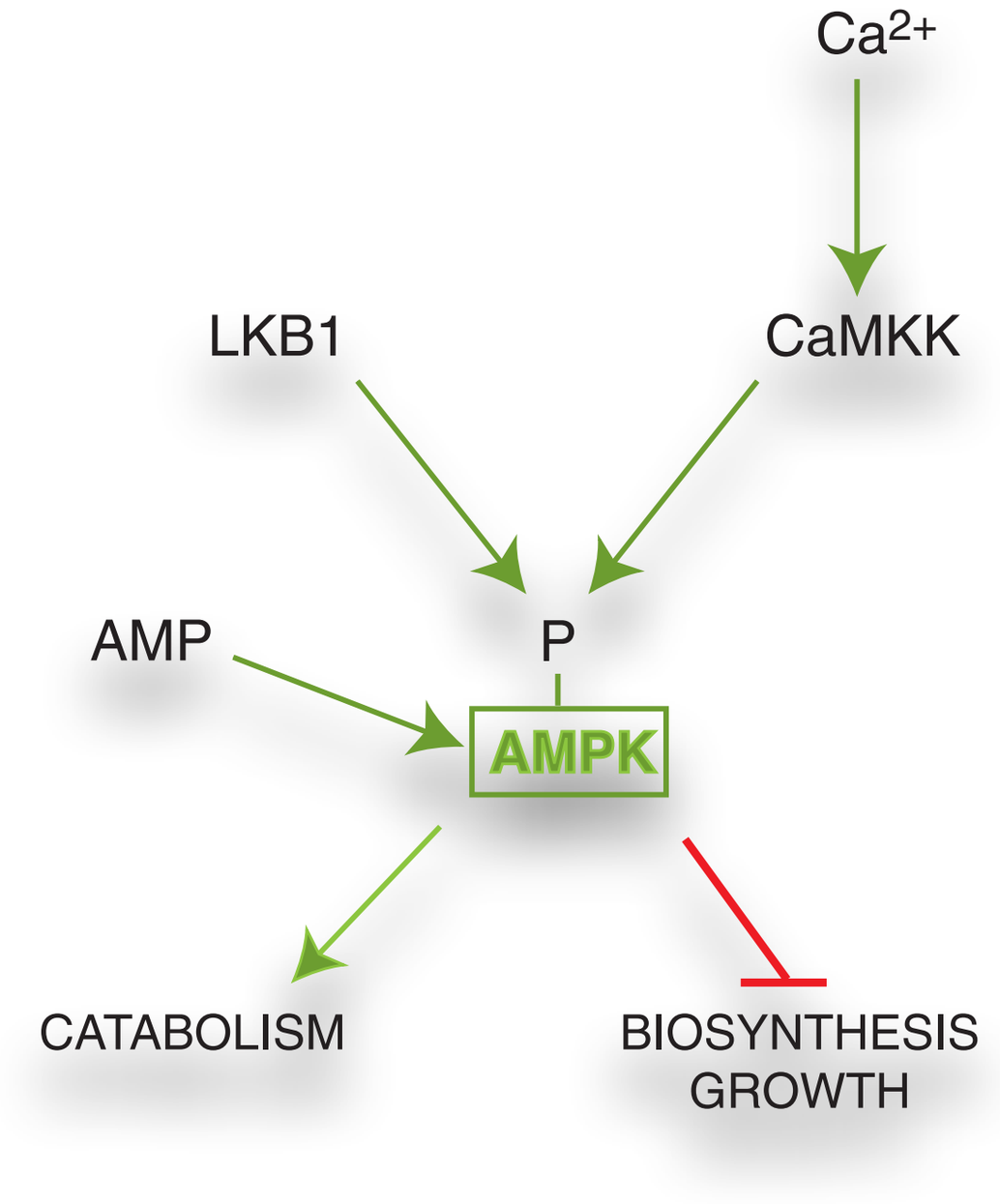


The AMP–activated protein kinase (AMPK) is a highly conserved (all eukaryotic cells) metabolic checkpoint that acts as a sensor of ATP levels in the cell

AMPK is regulated by 3 upstream kinases:

- Liver Kinase B1 (LKB1) - *ubiquitous*
- Calmodulin-dependent protein kinases α, β (CAMKKs) - *neurons*

AMPK: Central regulator of glucose and lipid metabolism



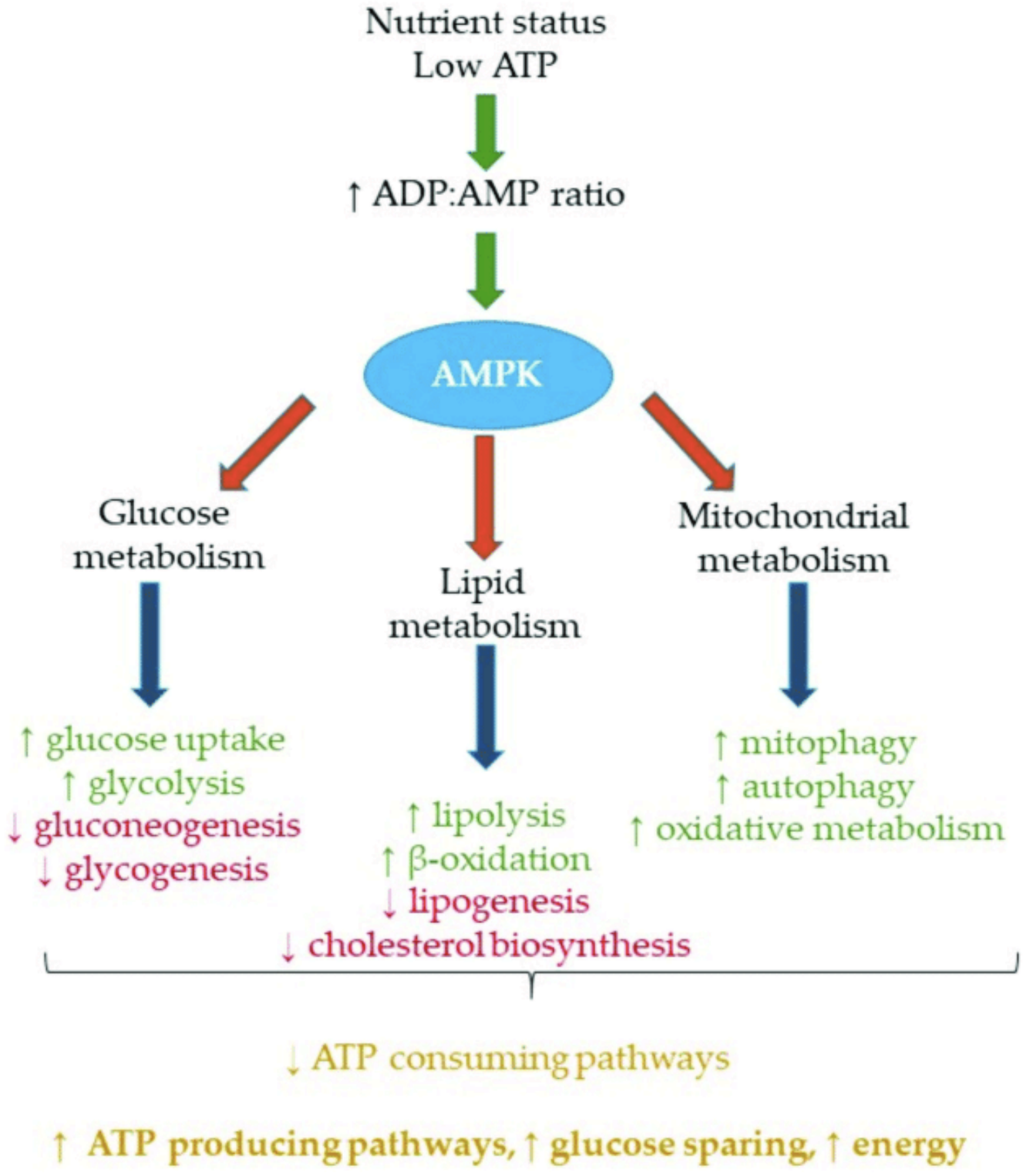
The AMP–activated protein kinase (AMPK) is a highly conserved (all eukaryotic cells) metabolic checkpoint that acts as a sensor of ATP levels in the cell

AMPK is regulated by 3 upstream kinases:

- Liver Kinase B1 (LKB1) - *ubiquitous*
- Calmodulin-dependent protein kinases α, β (CAMKKs) - *neurons*

AMPK was first discovered in 1973 as a mammalian protein kinase that is activated by changes in intracellular adenosine nucleotide levels (Carlson & Kim, ***J Biol Chem***)

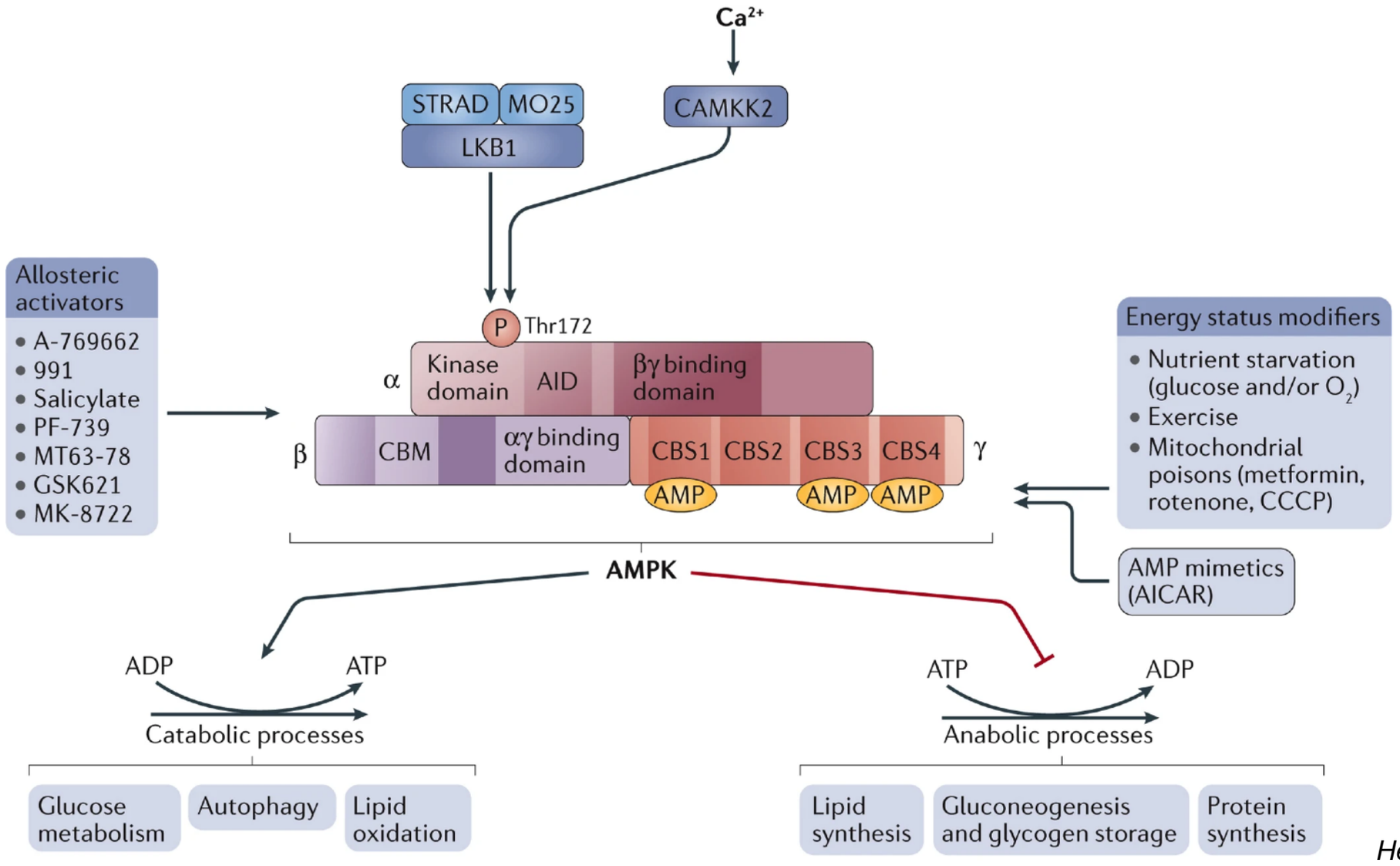
AMPK: energy sensor



AMPK: structure and function

AMPK is an obligate heterotrimeric kinase complex composed of a catalytic (α) subunit and two regulatory (β and γ) subunits.

The α subunit contains the kinase domain and a critical residue, Thr172, that is phosphorylated by upstream kinases. The β subunit contains a carbohydrate binding module that allows AMPK to associate with glycogen. The γ subunit enables AMPK to respond to changes in the ATP:AMP ratio as it contains four tandem cystathionine-β-synthase (CBS) domains that bind adenine nucleotides. Binding of AMP, and to a lesser extent ADP, to the γsubunit stimulates AMPK activity



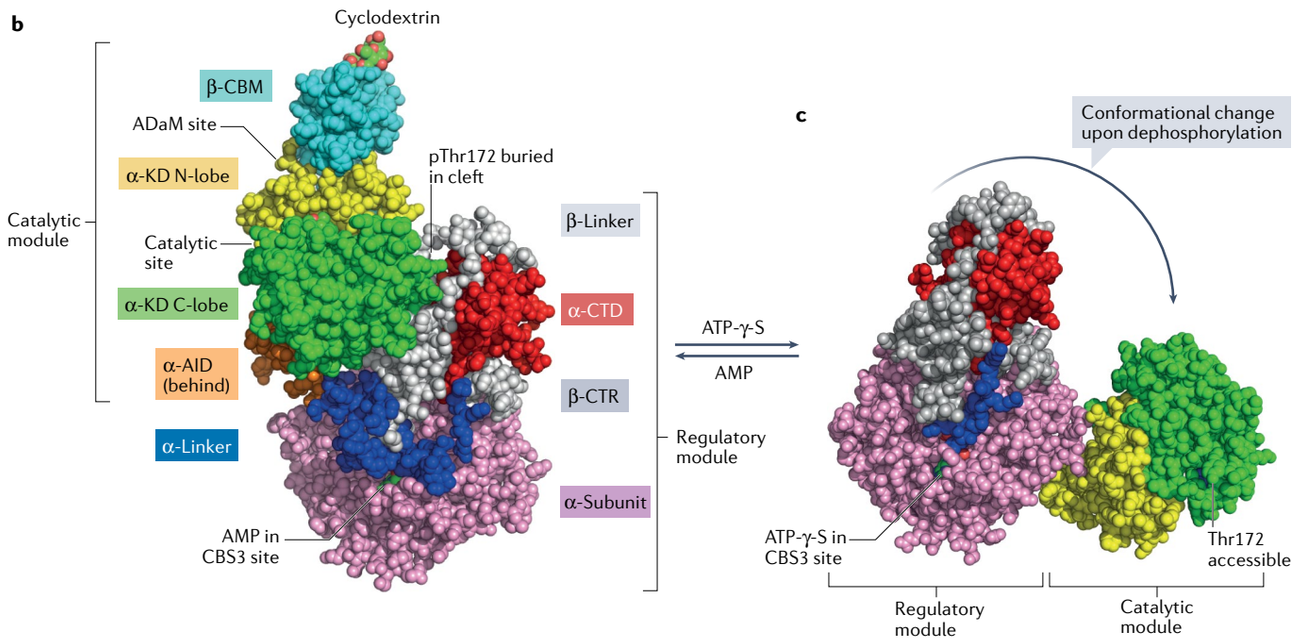
*Herzig & Shaw, **NRMCB**, 2018*

AMPK: structure and function

AMP binding to the γ subunit enhances AMPK activity through three distinct mechanisms:

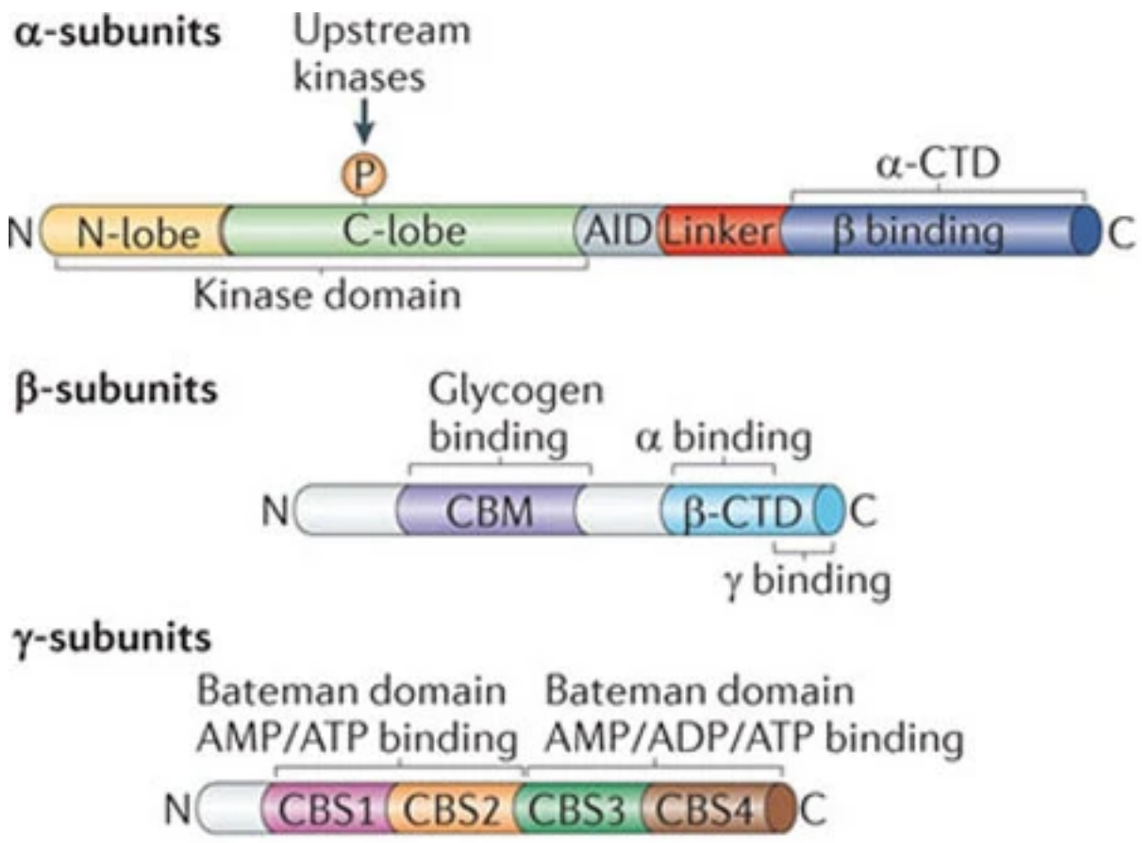
1. AMP has been proposed to stimulate phosphorylation of Thr172 by directly stimulating the activity of the upstream kinase or by an allosteric mechanism that would render AMPK a better substrate for the upstream kinase; however reports show no effect of AMP on the phosphorylation of Thr172 by the upstream kinase *in vitro*.
2. AMP inhibits the dephosphorylation of Thr172 by protecting it from phosphatases.
3. AMP causes allosteric activation of AMPK already phosphorylated on Thr172.

Several factors lead to AMPK activation, such as mitochondrial poisons and oxygen or glucose starvation, as well as exercise. Drugs that activate AMPK include the AMP mimetic AICAR and several small-molecule allosteric activators (listed on the left-hand side)



Steinberg & Hardie, ***NRMCB****, 2023*

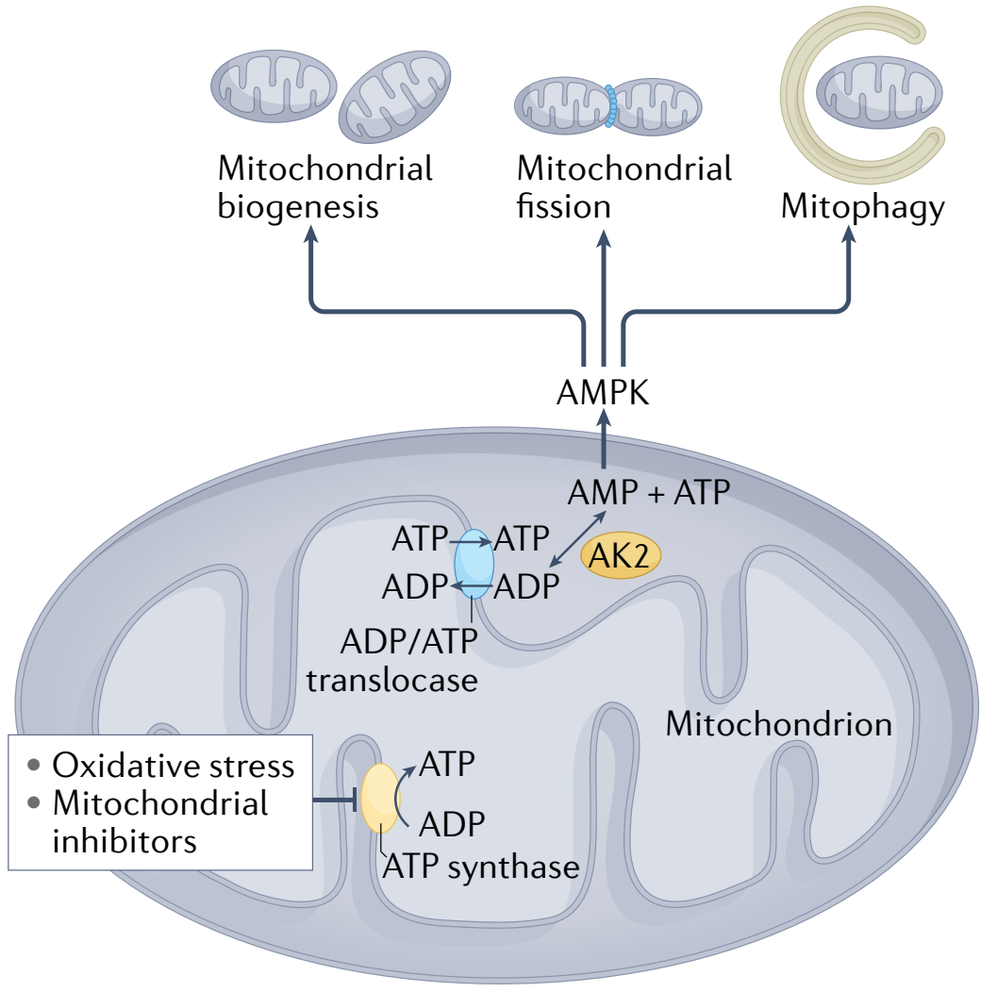
AMPK: structure and function



Nature Reviews | Molecular Cell Biology

How AMPK is activated?

Mitochondria are the major suppliers of ATP,
but are susceptible to damage by oxidative stress

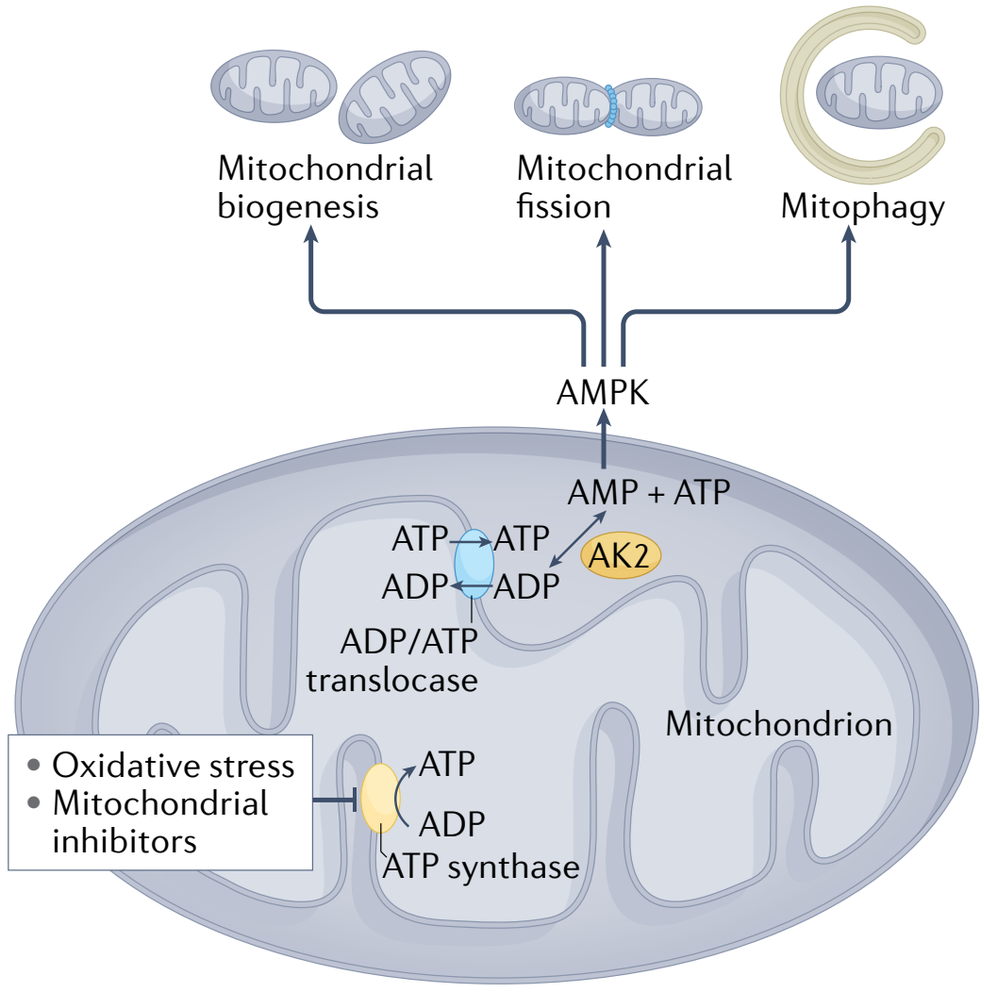


In fully energized, undamaged mitochondria the high ATP to ADP ratio drives the freely reversible adenylate kinase reaction (ATP + AMP ↔ 2ADP) towards ADP, thus keeping AMP at very low levels. However, impairments in mitochondrial function cause rising ADP to ATP ratios, driving the AK2 reaction in the opposite direction and causing an even larger increase in the AMP to ATP ratio. This activates AMPK by the canonical pathway

Steinberg & Hardie, ***NRMCB****, 2023*

How AMPK is activated?

Mitochondria are the major suppliers of ATP,
but are susceptible to damage by oxidative stress



(ATP + AMP ↔ 2ADP) is catalysed by the AK2 isoform of adenylate kinase, which is located in the mitochondrial intermembrane/ intra-cristae space.

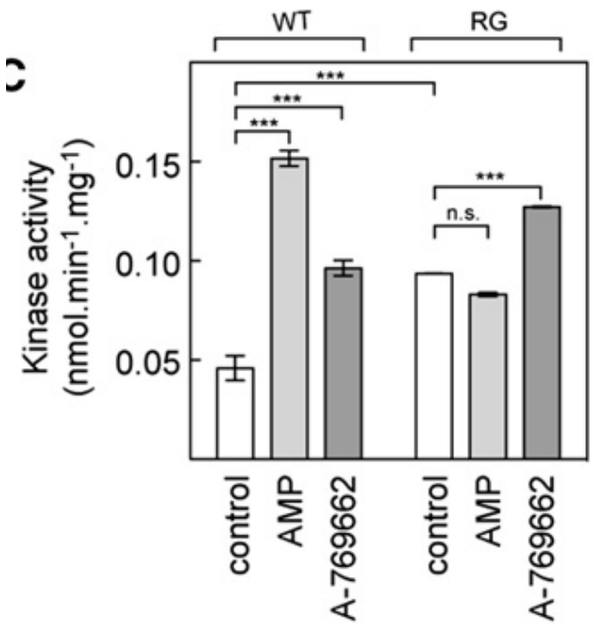
In fully energized, undamaged mitochondria the high ATP to ADP ratio drives the freely reversible adenylate kinase reaction (ATP + AMP ↔ 2ADP) towards ADP, thus keeping AMP at very low levels. However, impairments in mitochondrial function cause rising ADP to ATP ratios, driving the AK2 reaction in the opposite direction and causing an even larger increase in the AMP to ATP ratio. This activates AMPK by the canonical pathway

*Steinberg & Hardie, **NRMCB**, 2023*

Point mutations in the γ2 interfere with the binding of the regulatory nucleotides, AMP and ATP.

Here, they selected one of these mutations, R531G, that causes a severe loss of binding of AMP and ATP to CBS3, thus generating an AMP-insensitive complex.

They constructed isogenic HEK293 cells stably expressing either wild-type γ2 or γ2-R531G mutant and used them to test whether a variety of pharmacological agents and stresses that activate AMPK do so via increases in AMP

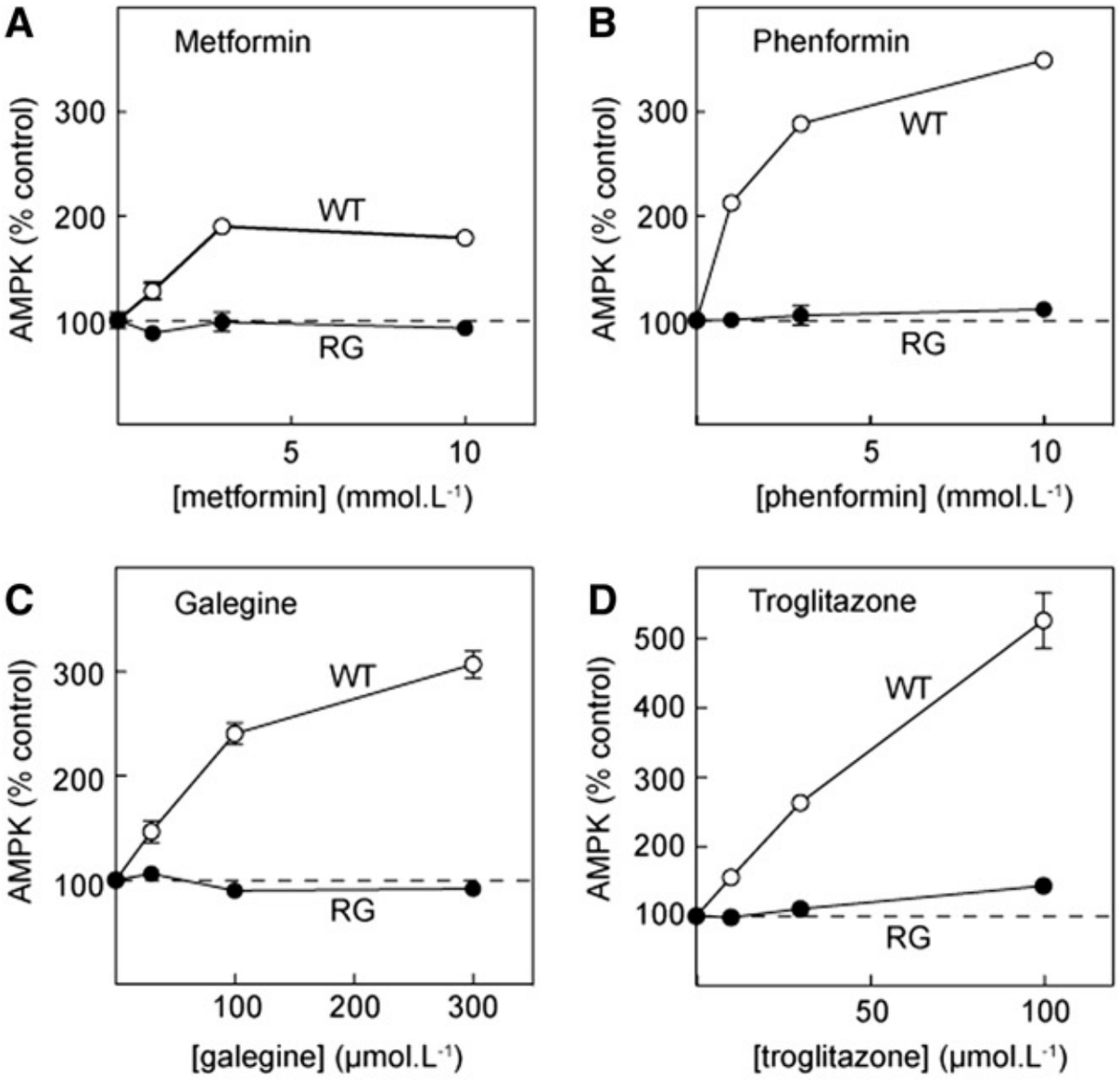


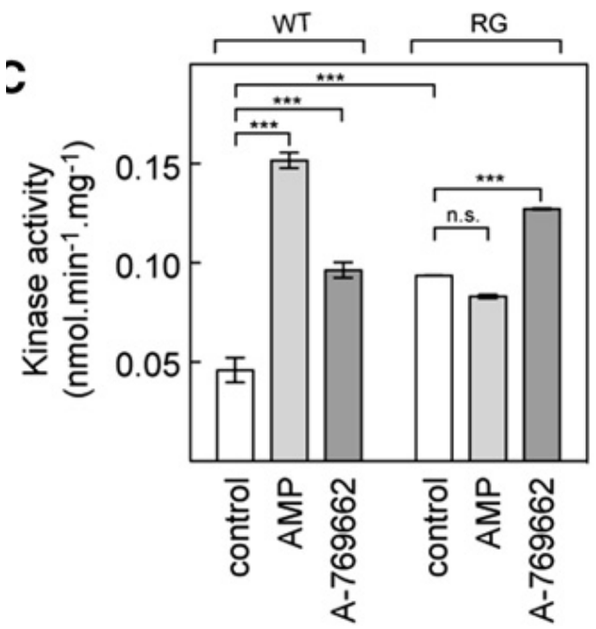
Hawley et al, ***Cell Metab****, 2010*

Point mutations in the γ2 interfere with the binding of the regulatory nucleotides, AMP and ATP.

Here, they selected one of these mutations, R531G, that causes a severe loss of binding of AMP and ATP to CBS3, thus generating an AMP-insensitive complex.

They constructed isogenic HEK293 cells stably expressing either wild-type γ2 or γ2-R531G mutant and used them to test whether a variety of pharmacological agents and stresses that activate AMPK do so via increases in AMP



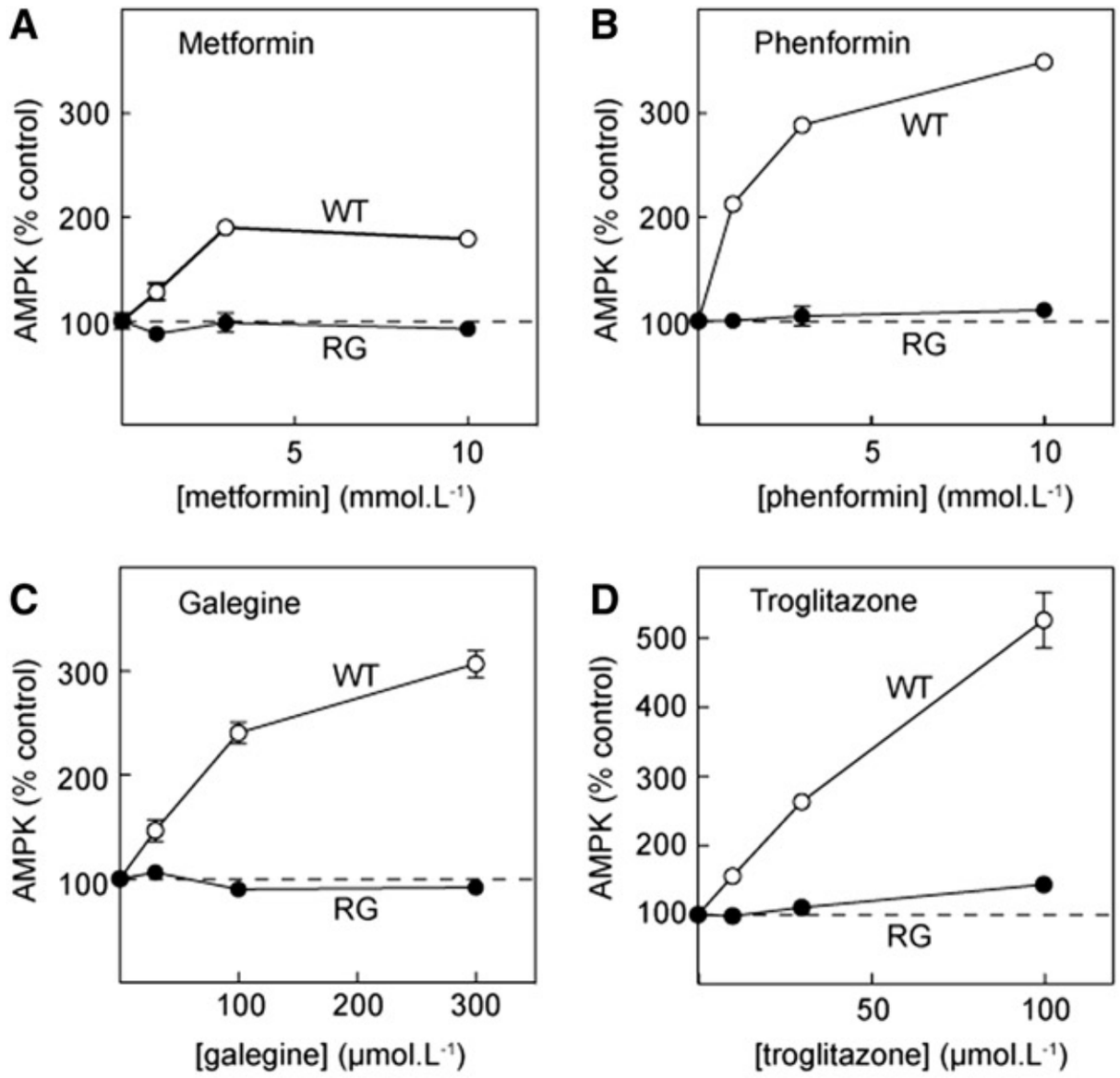


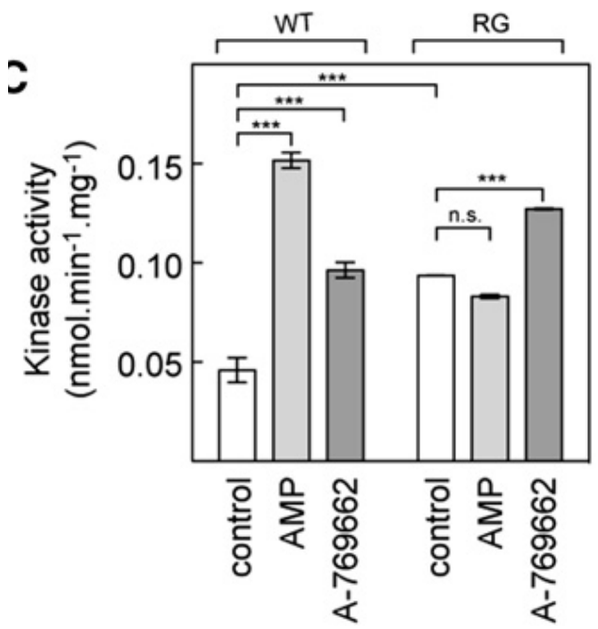
Hawley et al, ***Cell Metab****, 2010*

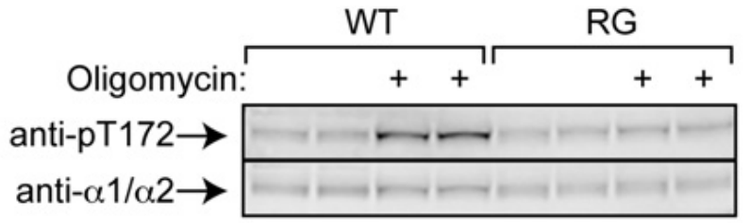
Point mutations in the γ2 interfere with the binding of the regulatory nucleotides, AMP and ATP.

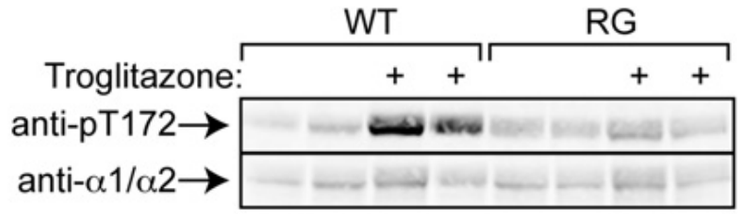
Here, they selected one of these mutations, R531G, that causes a severe loss of binding of AMP and ATP to CBS3, thus generating an AMP-insensitive complex.

They constructed isogenic HEK293 cells stably expressing either wild-type γ2 or γ2-R531G mutant and used them to test whether a variety of pharmacological agents and stresses that activate AMPK do so via increases in AMP



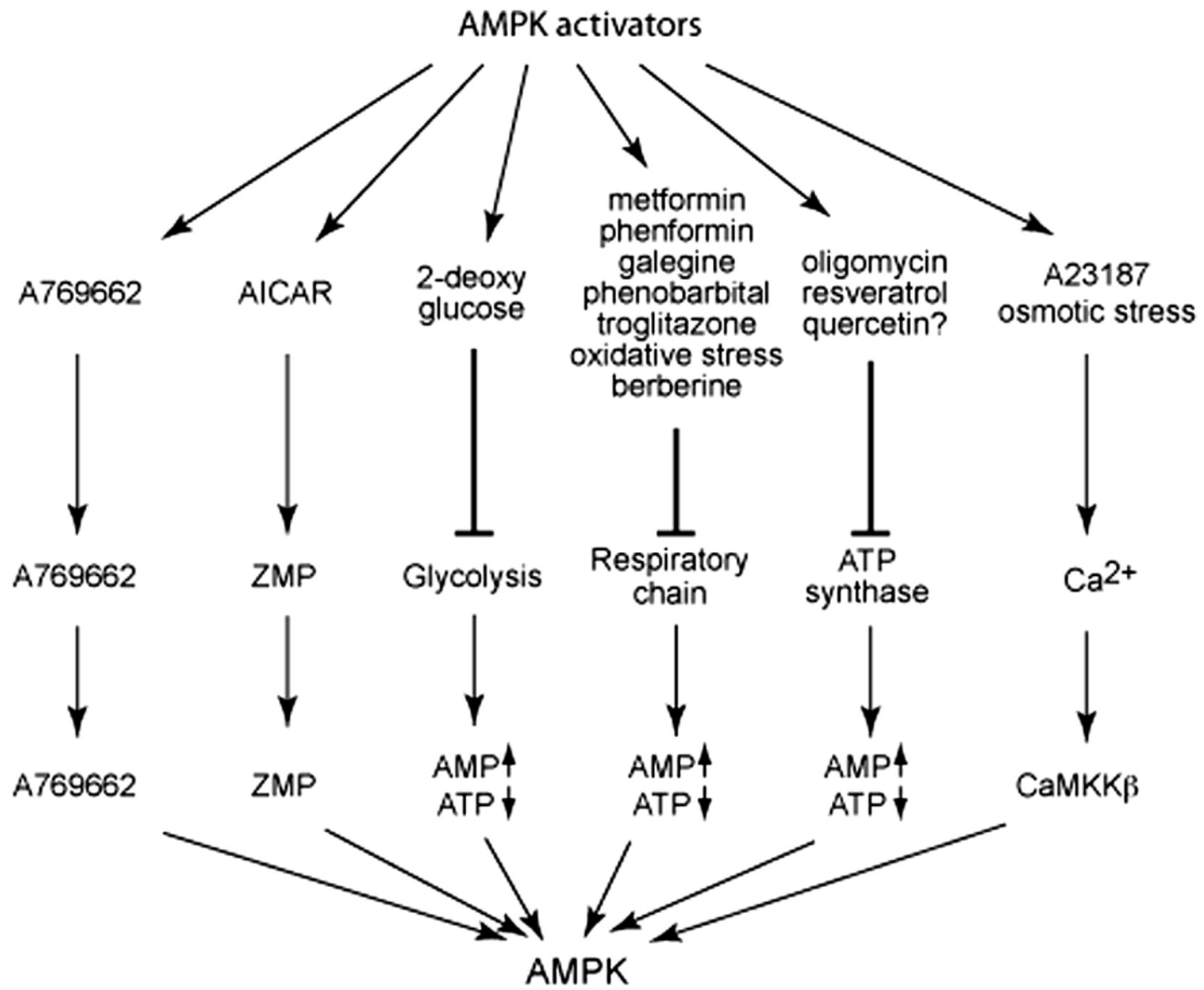






Hawley et al, ***Cell Metab****, 2010*

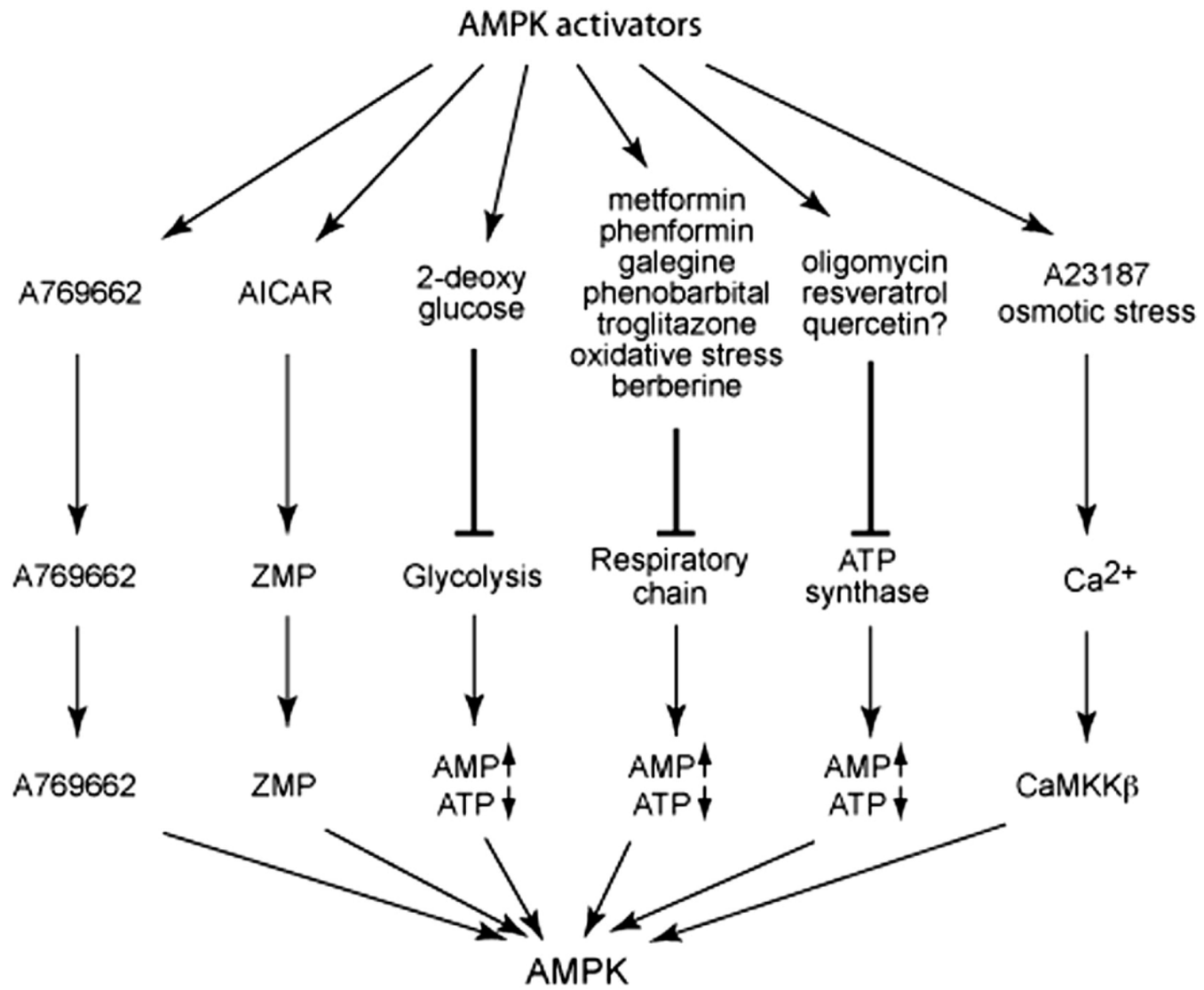
How AMPK is activated?



Six mechanisms for AMPK activation

Hawley et al, ***Cell Metab****, 2010*

How AMPK is activated?



Six mechanisms for AMPK activation

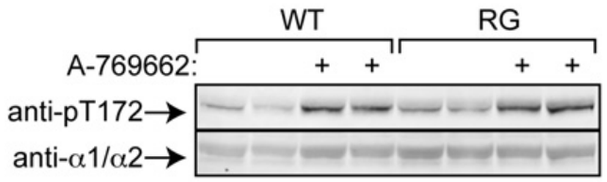
…. energy status sensing is part of the story….

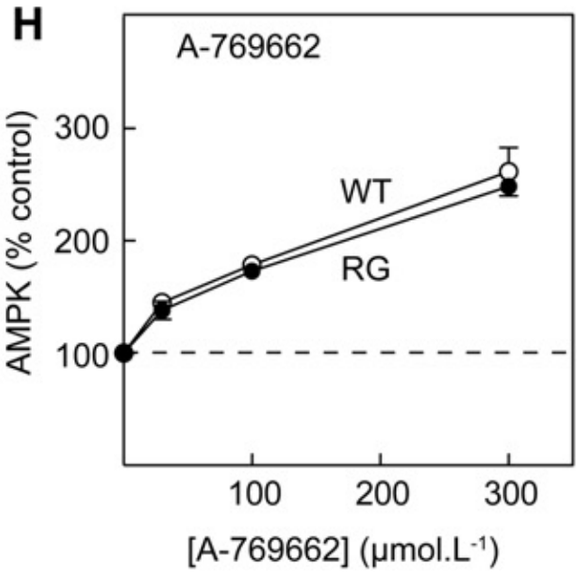
Hawley et al, ***Cell Metab****, 2010*

When AMPK is activated?

- Intense muscle contraction/activity
- Ischemia in cardiac muscle
- Oxidative stress in the liver
- Poor perfusion in tumors

A-769662 is an AMP-independent allosteric regulator of AMPK activation

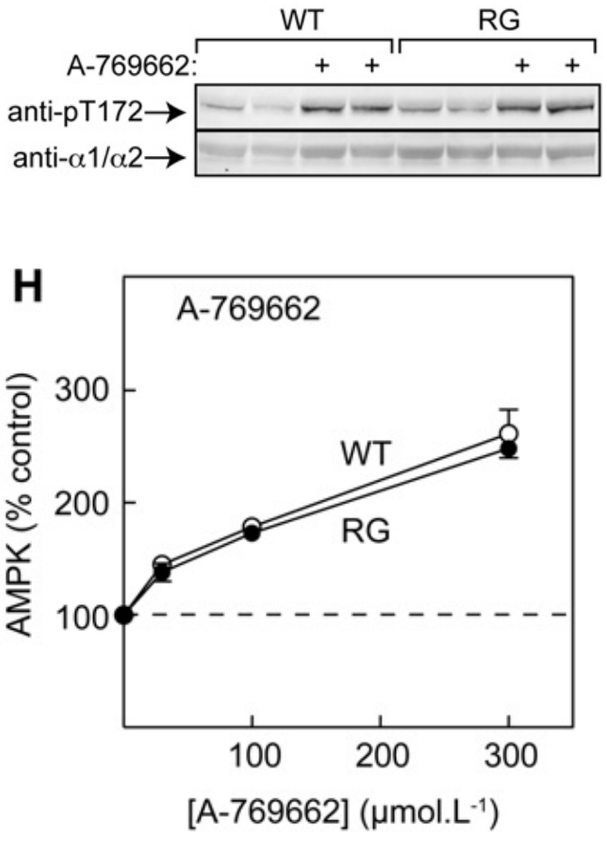




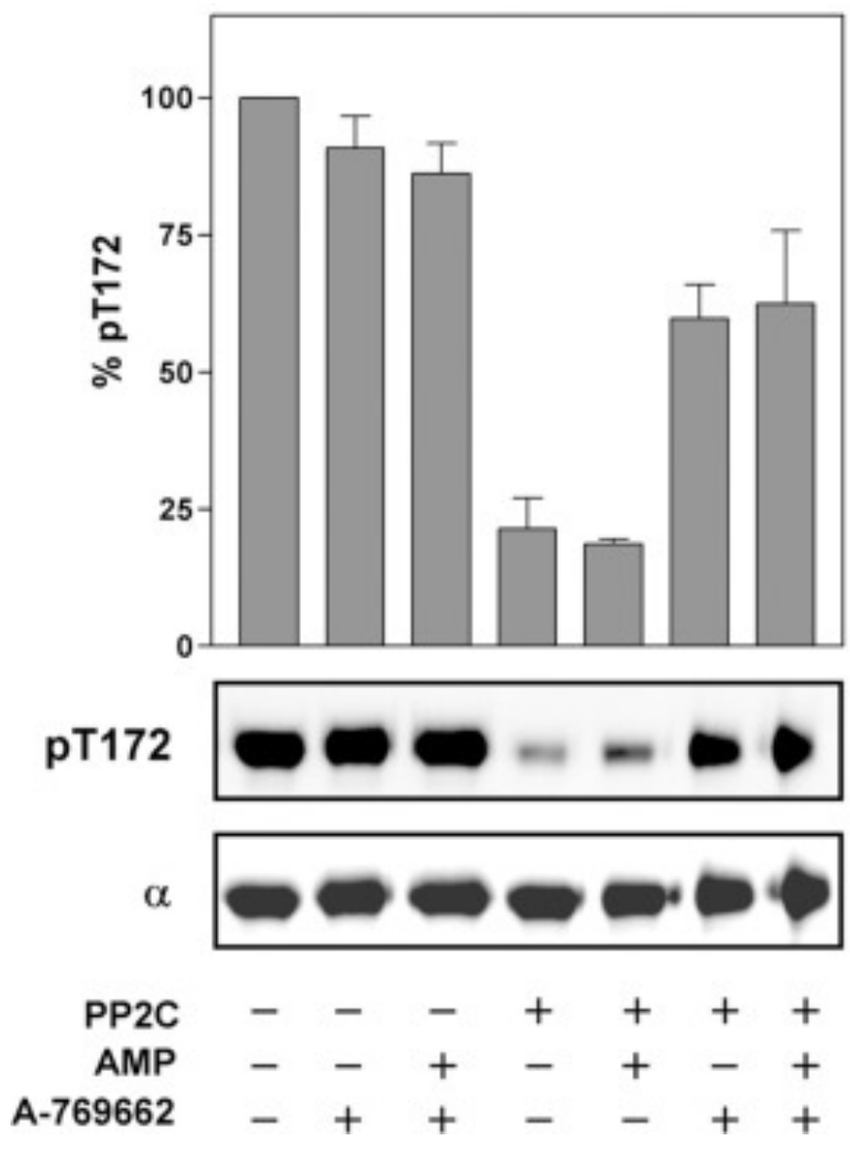
Hawley et al, ***Cell Metab****, 2010*

Sanders et al, ***JBC****, 2007*

A-769662 is an AMP-independent allosteric regulator of AMPK activation

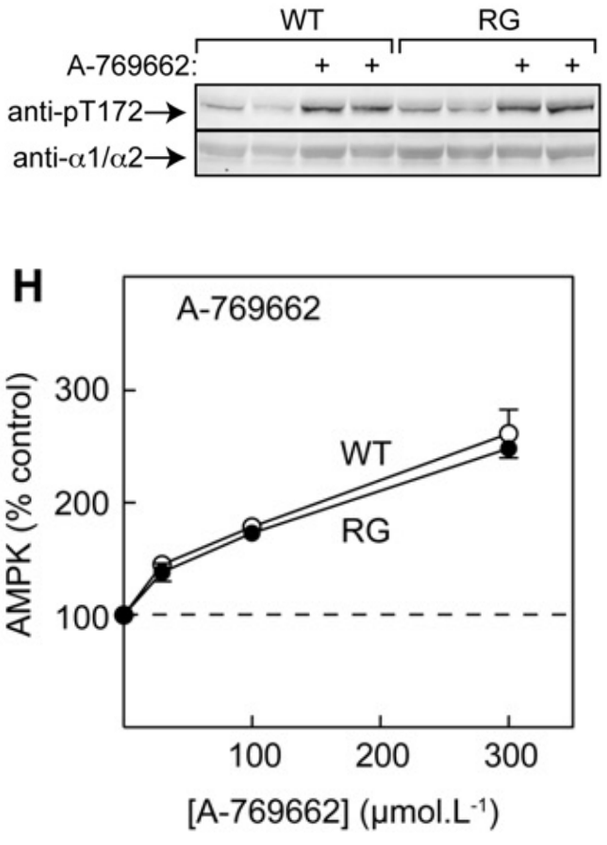


Hawley et al, ***Cell Metab****, 2010*

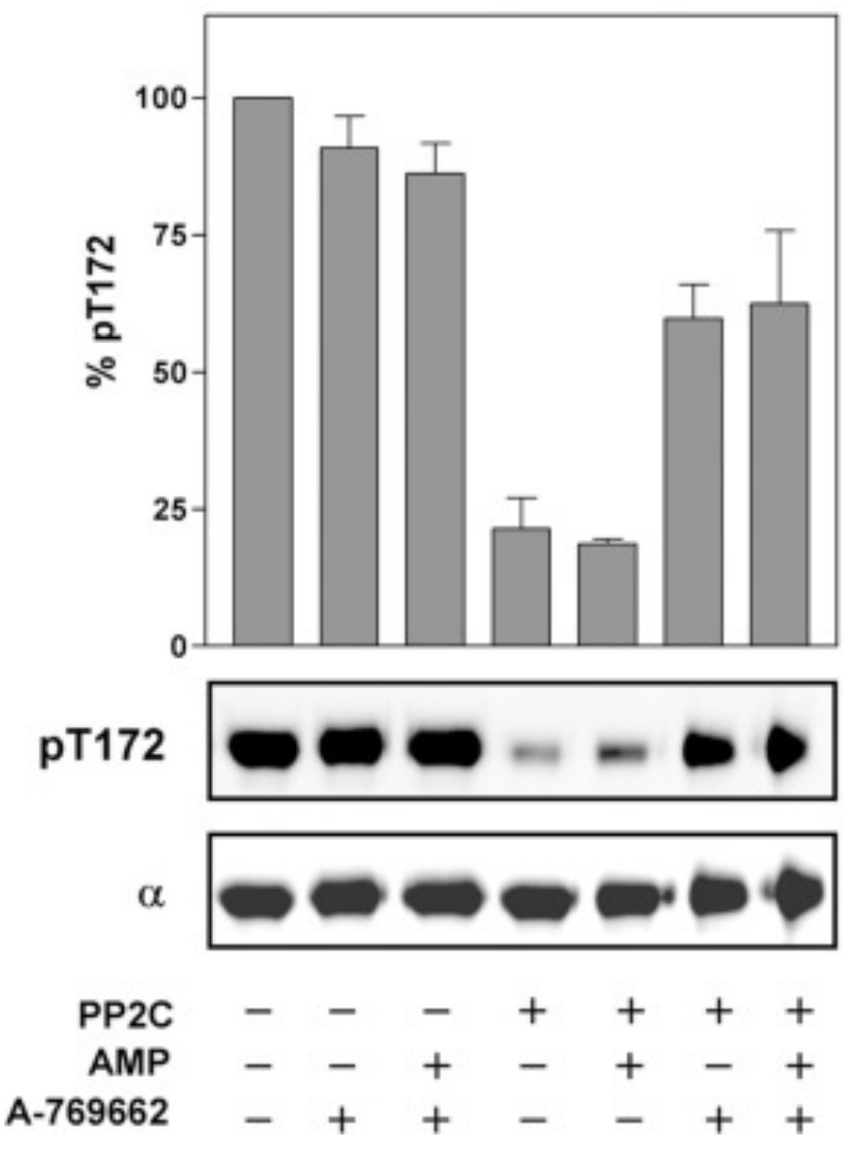


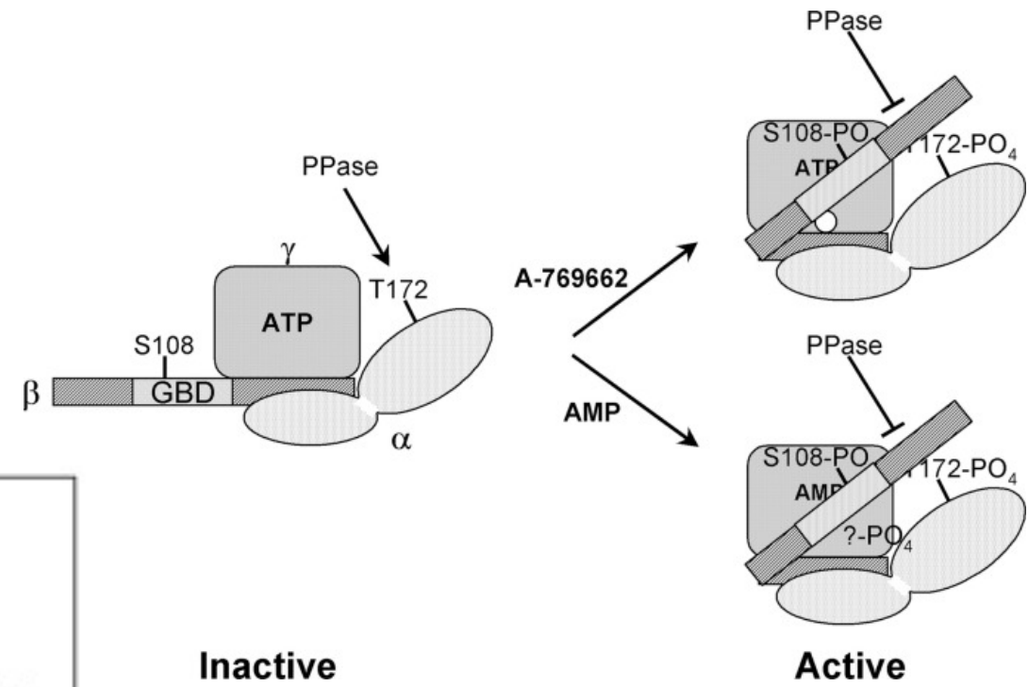
Sanders et al, ***JBC****, 2007*

A-769662 is an AMP-independent allosteric regulator of AMPK activation



*Hawley et al, **Cell Metab**, 2010*

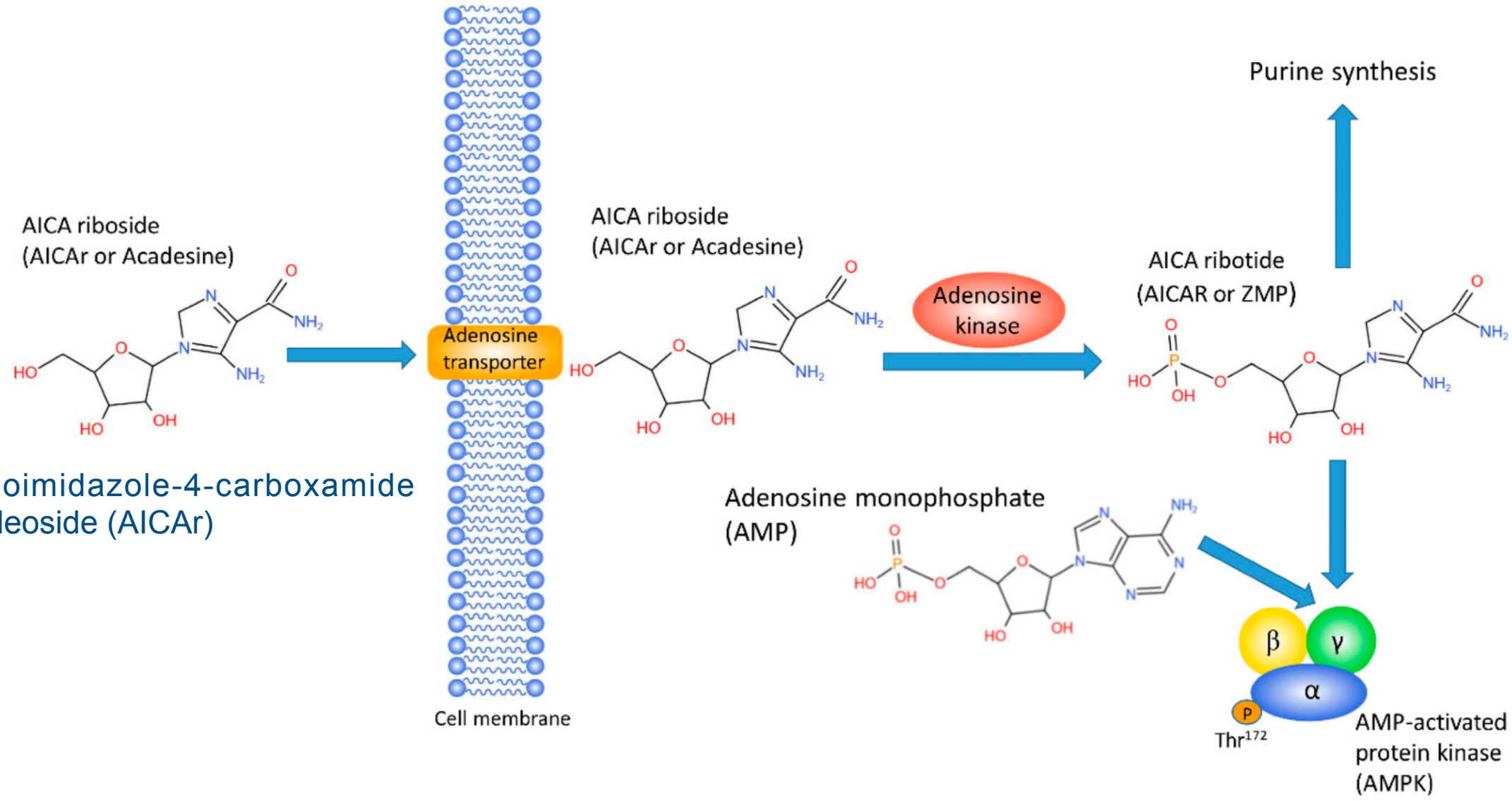




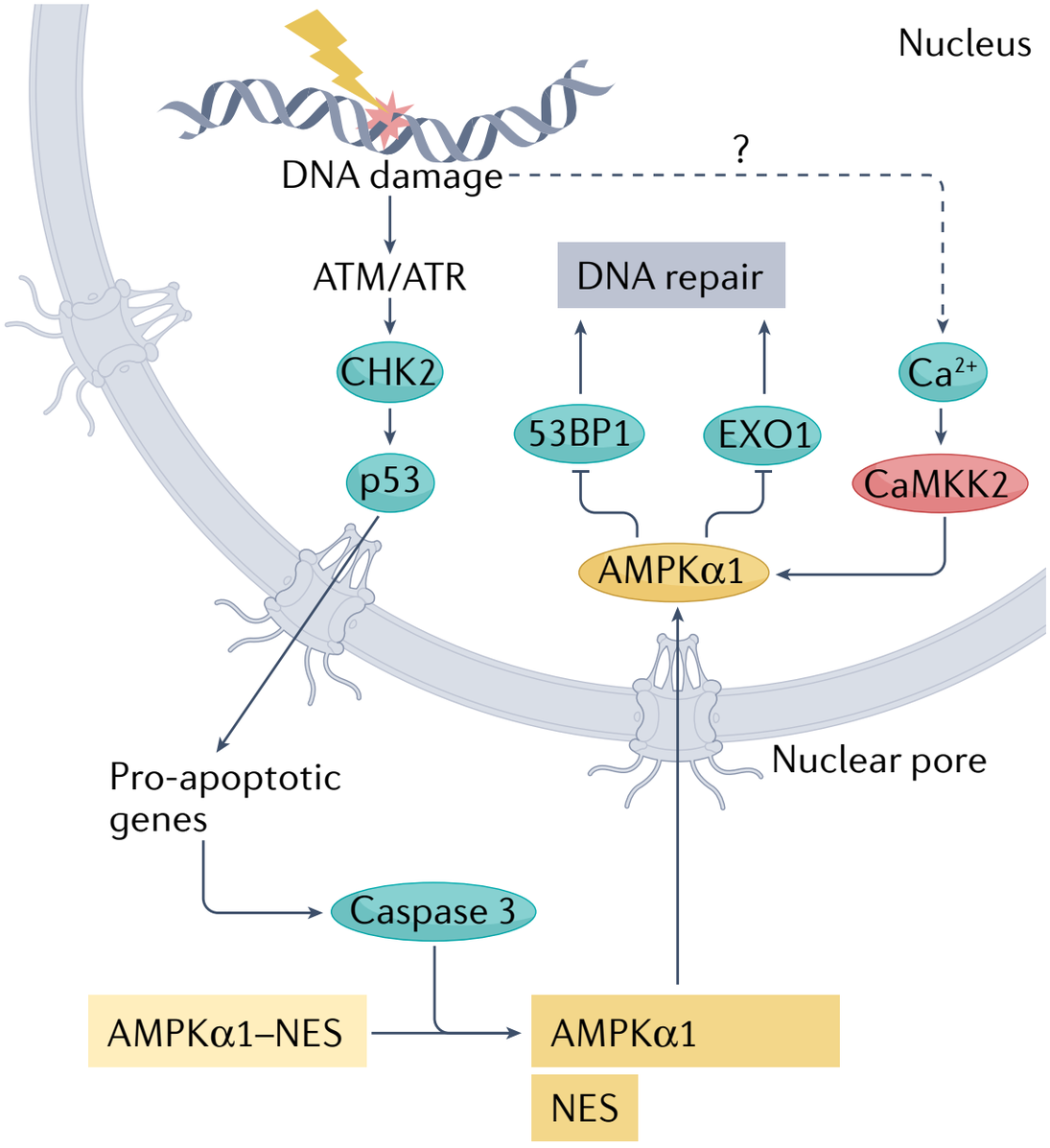
*Sanders et al, **JBC**, 2007*

AICAR is an AMP mimetic

5-Aminoimidazole-4-carboxamide ribonucleoside (AICAr)



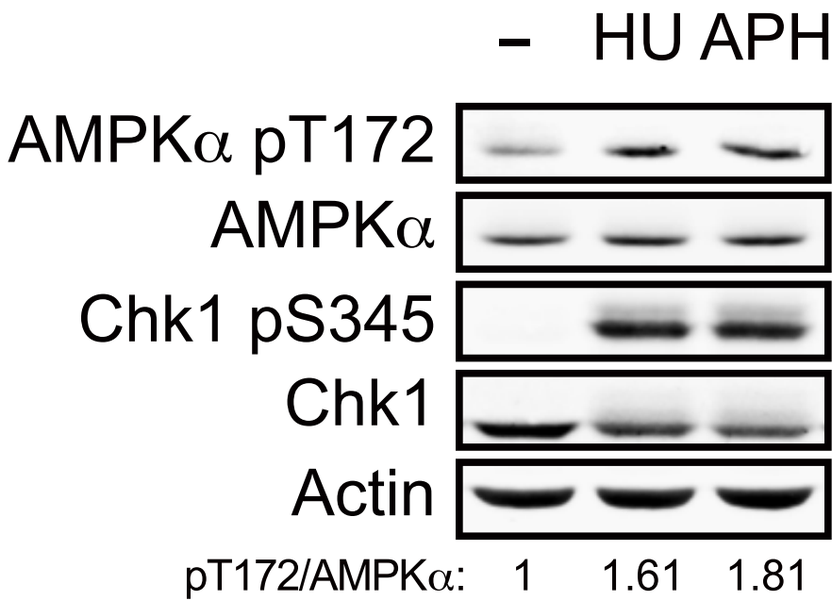
How AMPK is activated?

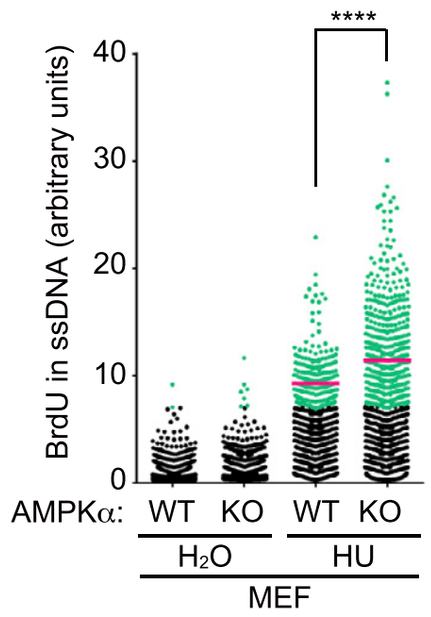


A number of NON-CANONICAL regulations of AMPK have emerged, activation by including DNA damage and damaged lysosomes.

*Steinberg & Hardie, **NRMCB**, 2023*

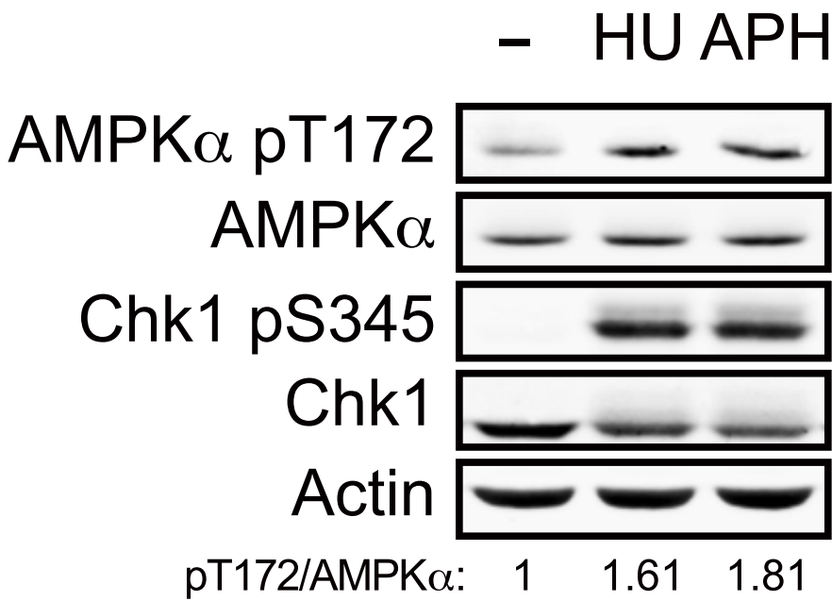
Ca^{2+}-Stimulated AMPK-Dependent Phosphorylation of Exo1 Protects Stressed Replication Forks from Aberrant Resection

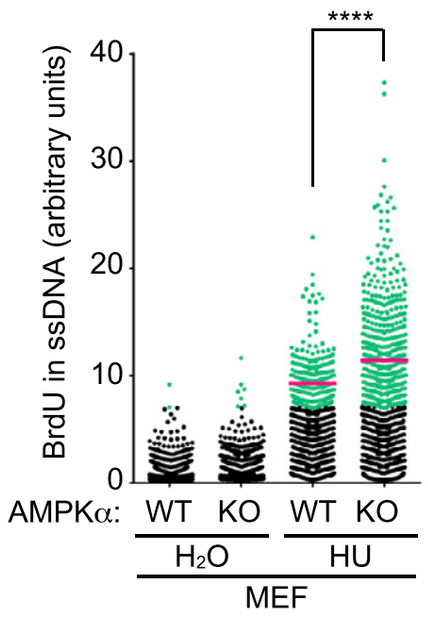




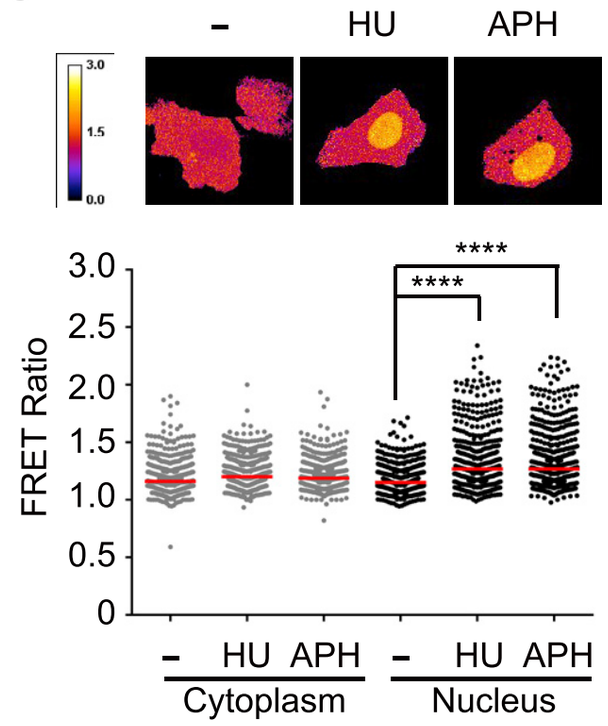
Li et al, ***Mol Cell****, 2019*

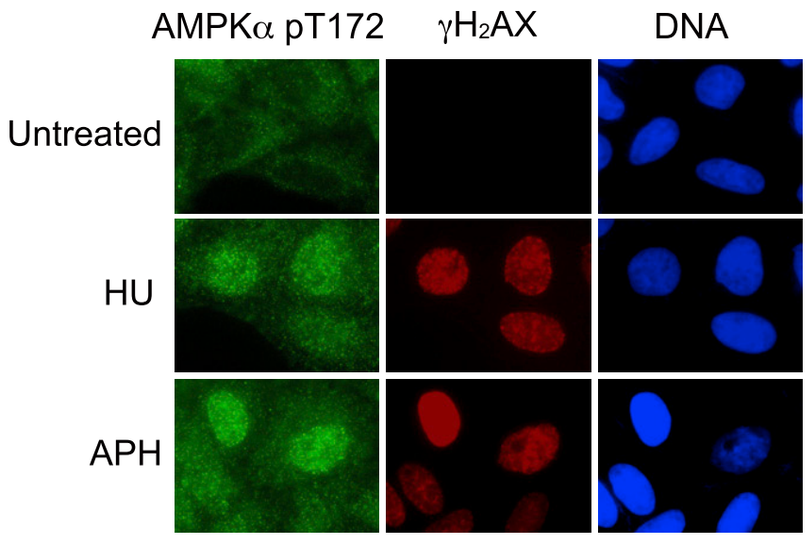
Ca^{2+}-Stimulated AMPK-Dependent Phosphorylation of Exo1 Protects Stressed Replication Forks from Aberrant Resection





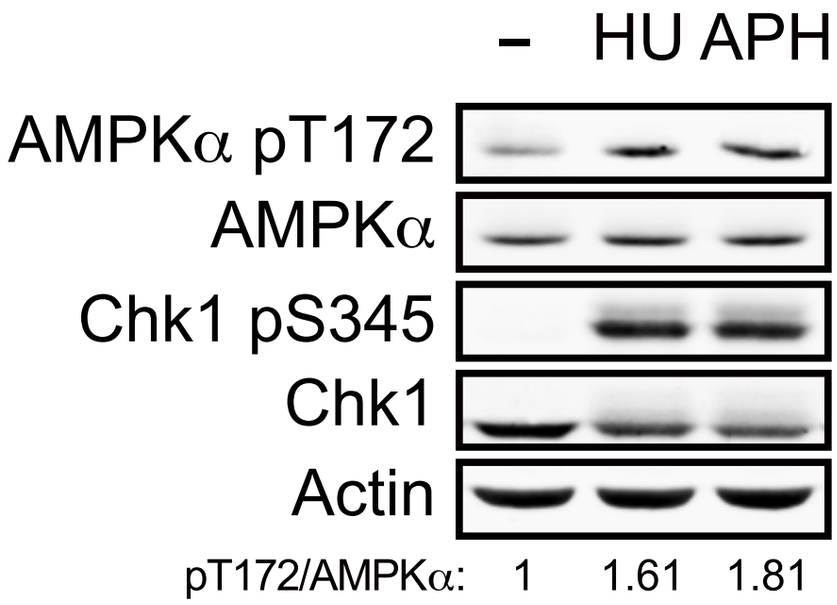
C

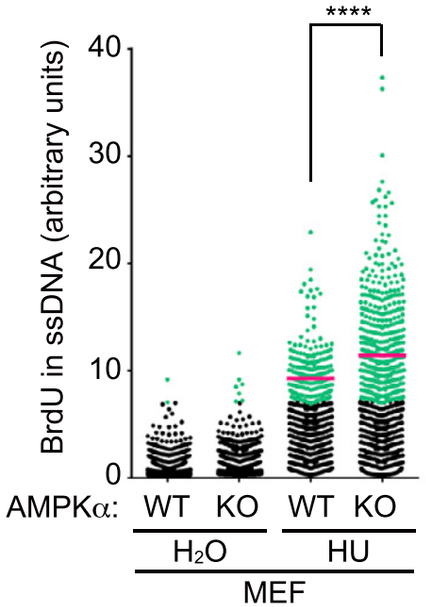




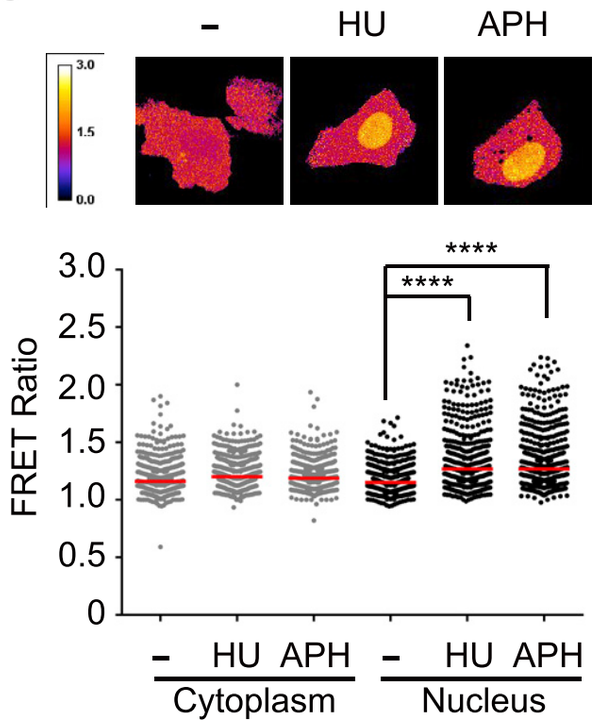
Li et al, ***Mol Cell****, 2019*

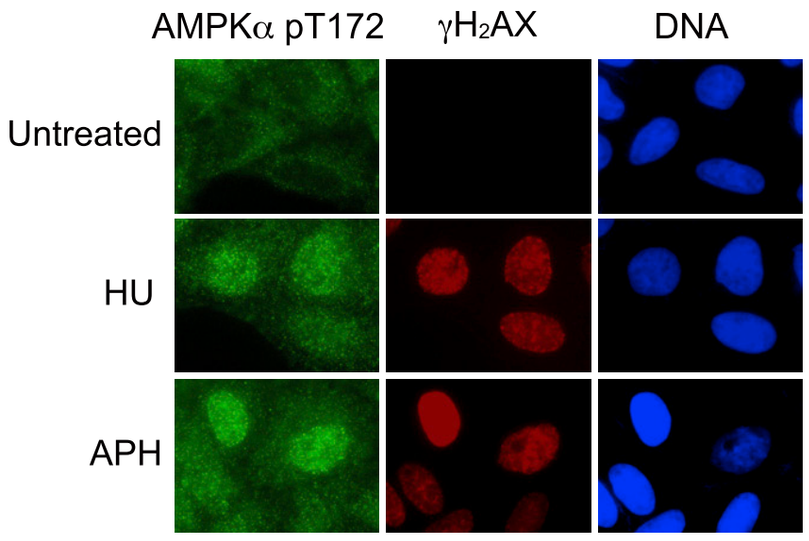
Ca^{2+}-Stimulated AMPK-Dependent Phosphorylation of Exo1 Protects Stressed Replication Forks from Aberrant Resection

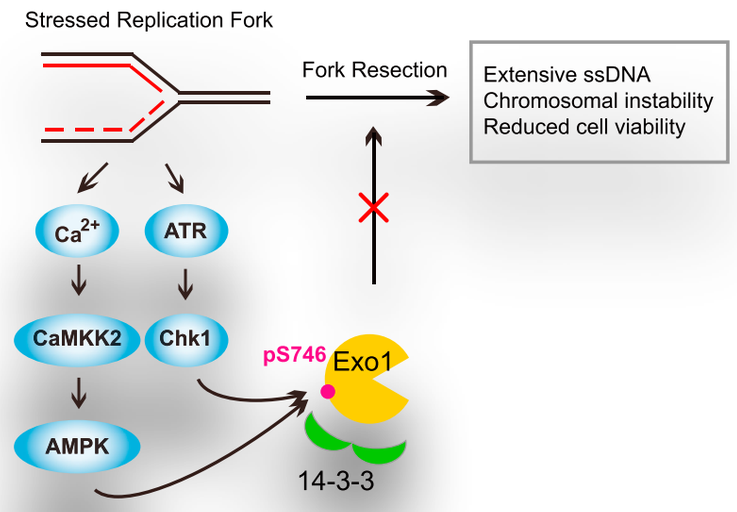




C

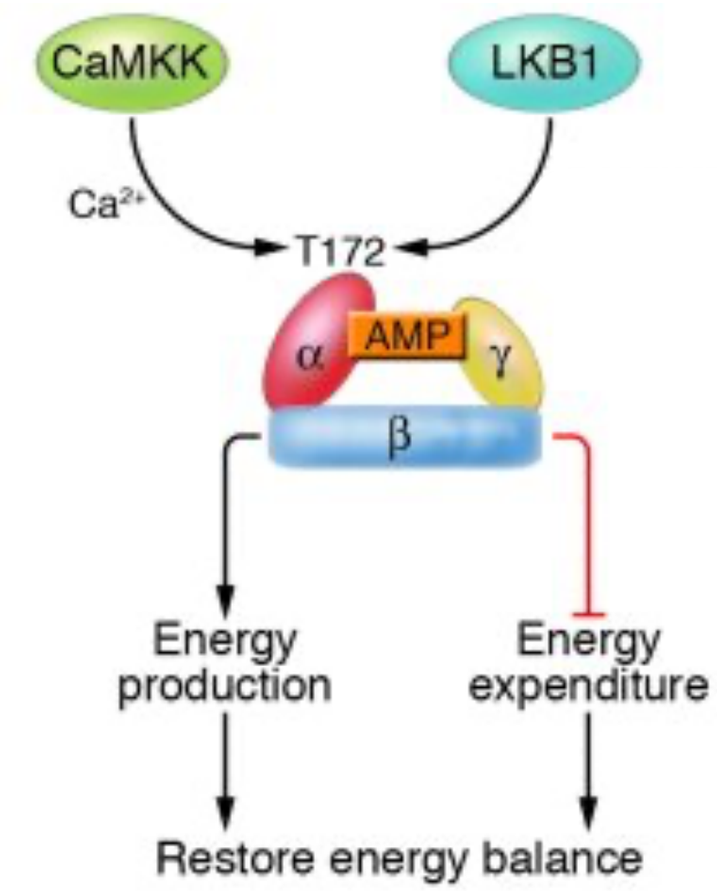






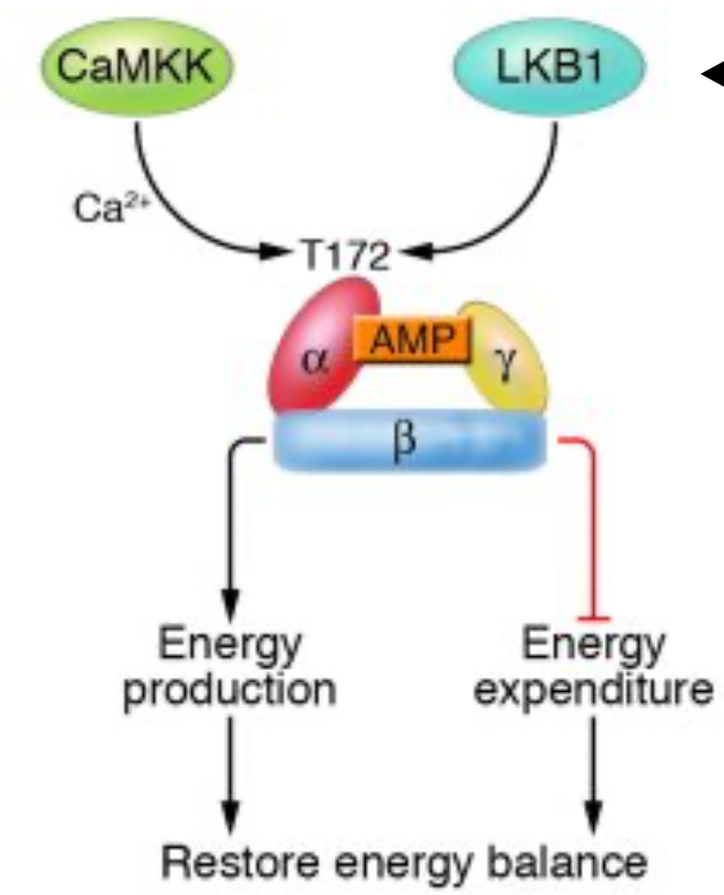
Li et al, ***Mol Cell****, 2019*

How AMPK is activated?



LKB1 (in complex with STRAD-MO25) is the major upstream kinase (and regulator) of AMPK in mammals. Yet, what factors elicit its activation remain elusive

How AMPK is activated?



ROS

PI3K / AKT

Post-Transl. Modif.

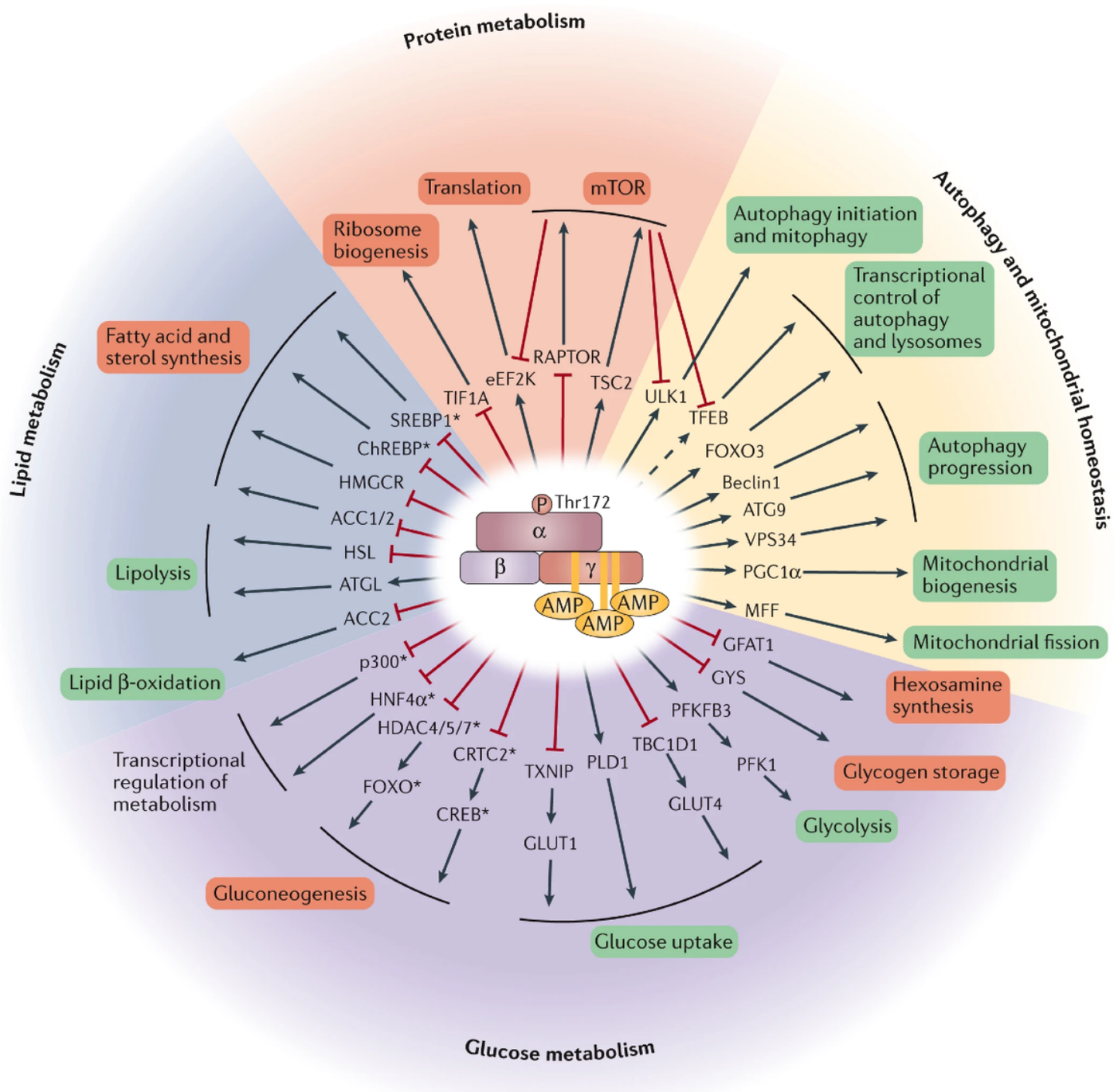
Ubiquitination

Calcium flux

LKB1 (in complex with STRAD-MO25) is the major upstream kinase (and regulator) of AMPK in mammals. Yet, what factors elicit its activation remain elusive

AMPK: activity and regulated pathways

Once activated, AMPK redirects metabolism towards increased catabolism and decreased anabolism through the phosphorylation of key proteins in multiple pathways, including mTOR complex 1 (mTORC1), glycolysis (PFK1) and fatty acid synthesis (ACC1/2)

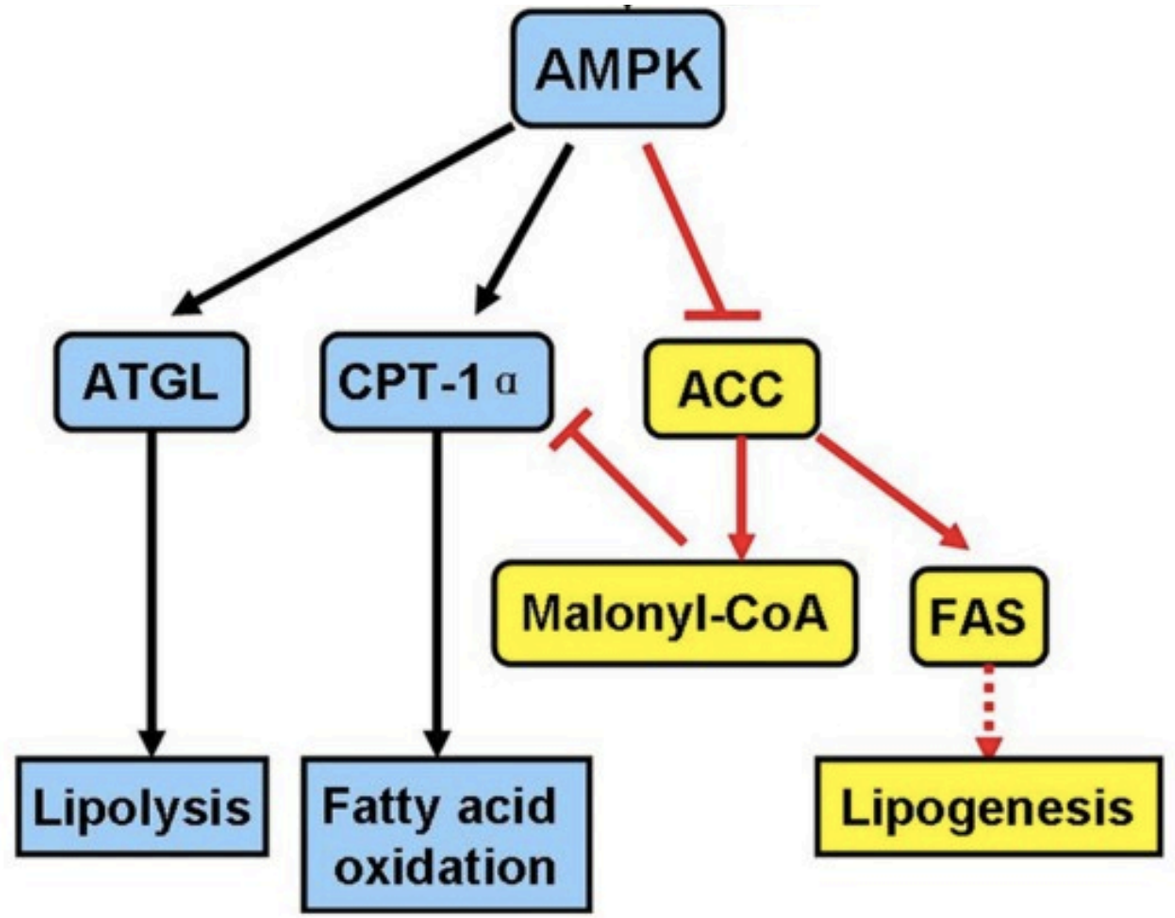


*Herzig & Shaw, **NRMCB**, 2018*

AMPK: inhibition of FAS and stimulation of FAO

AMPK inhibits multiple biosynthetic pathways under conditions of energy shortage.

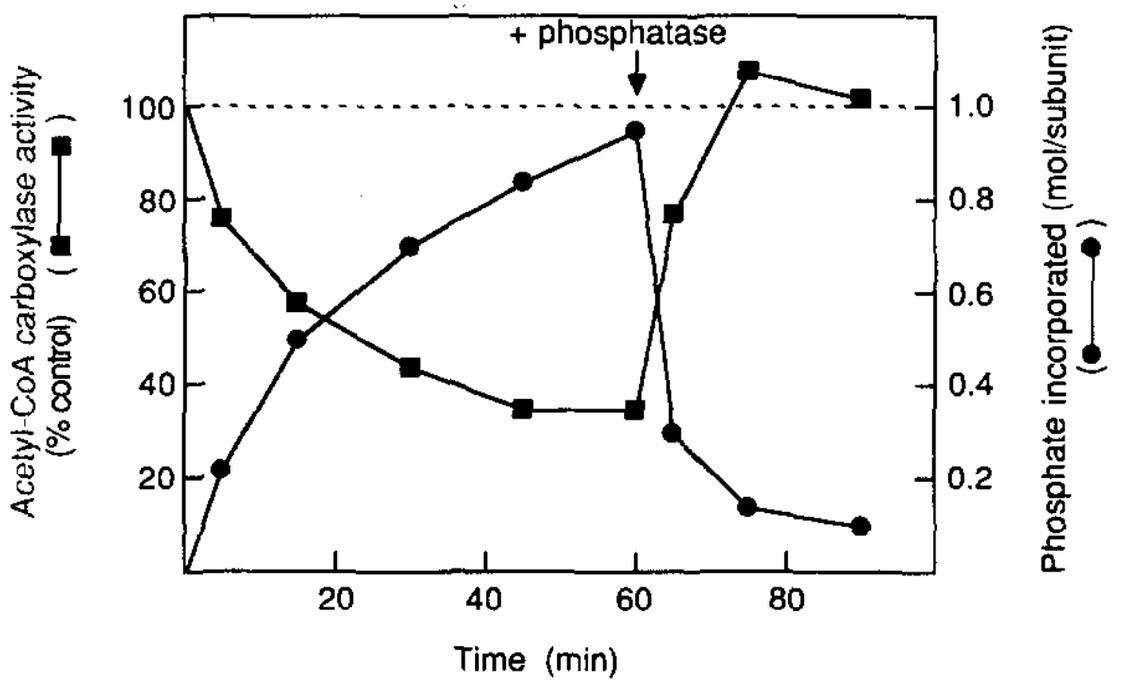
The first pathway to be identified was the inhibition of lipid and sterol synthesis by AMPK through inhibitory phosphorylation of the Acetyl-CoA Carboxylases (ACC1 and ACC2), which catalyze the first step in *de novo* lipid synthesis, and inhibitory phosphorylation of HMGCoA Reductase (HMGCR), which catalyzes the rate-limiting step in cholesterol synthesis

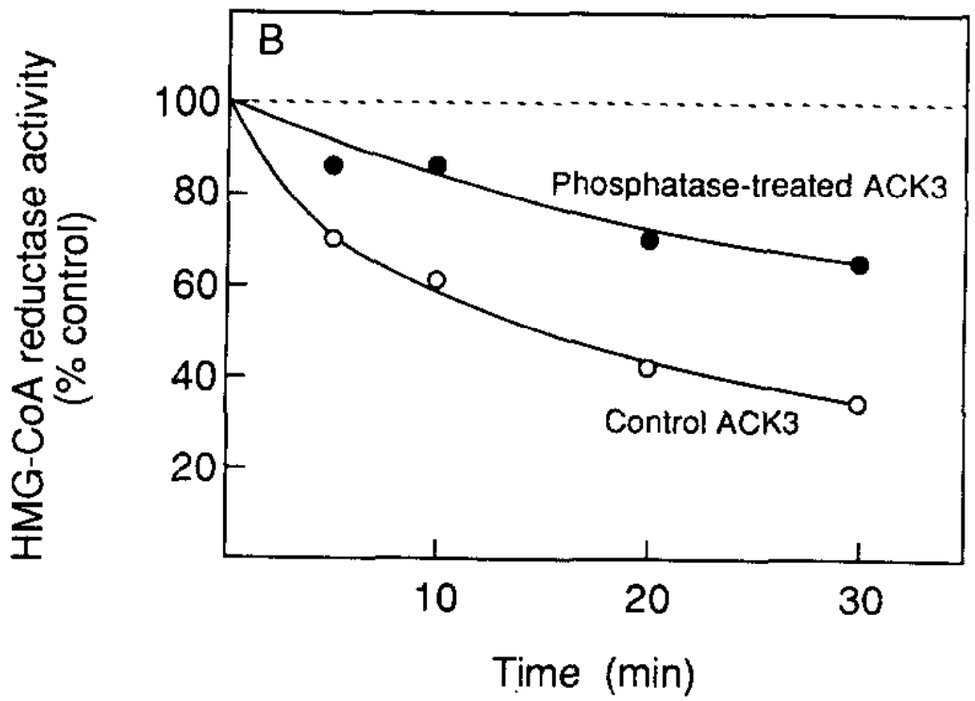


AMPK: inhibition of FAS and stimulation of FAO

A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis

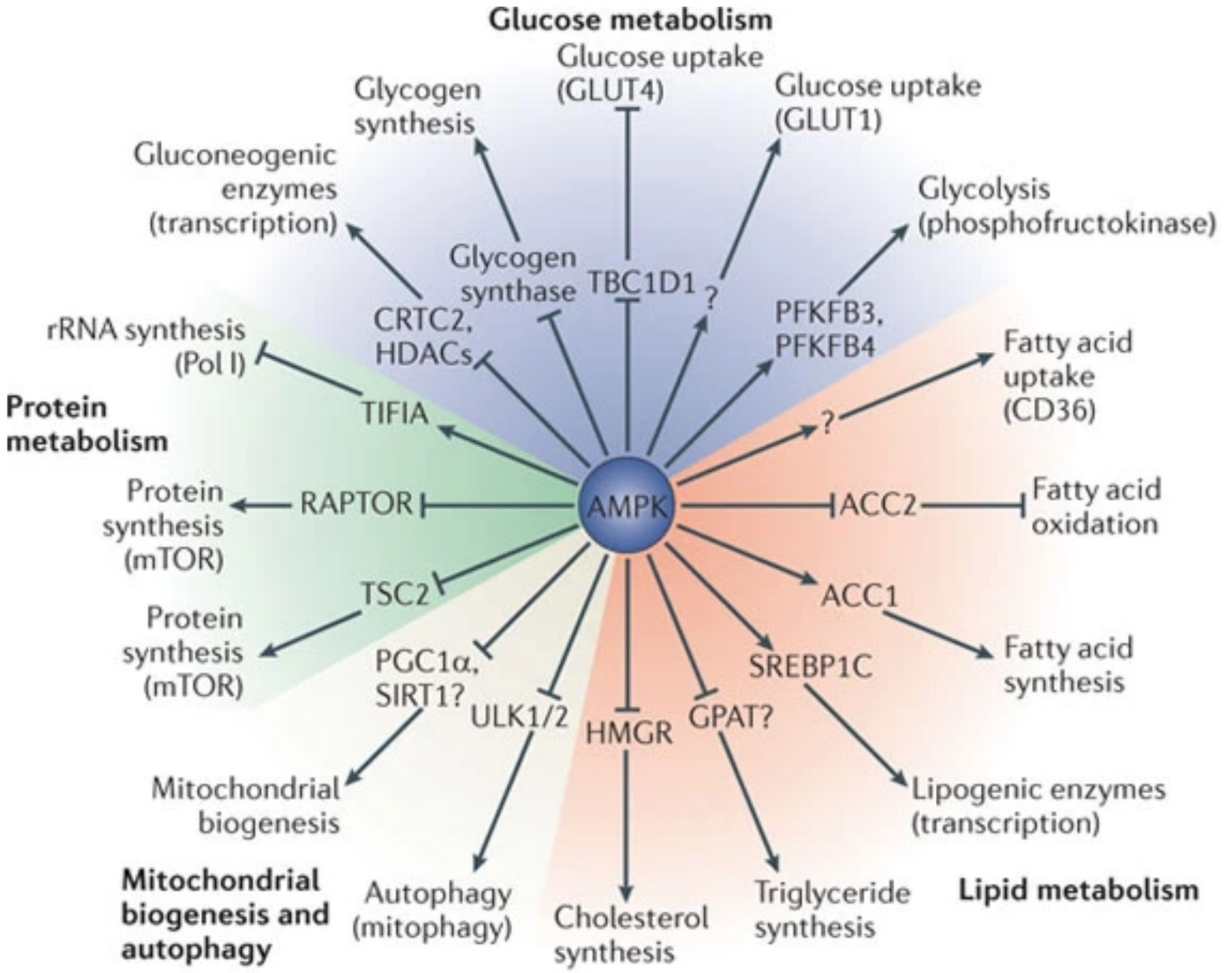
David Carling, Victor A. Zammit+ and D. Grahame Hardie





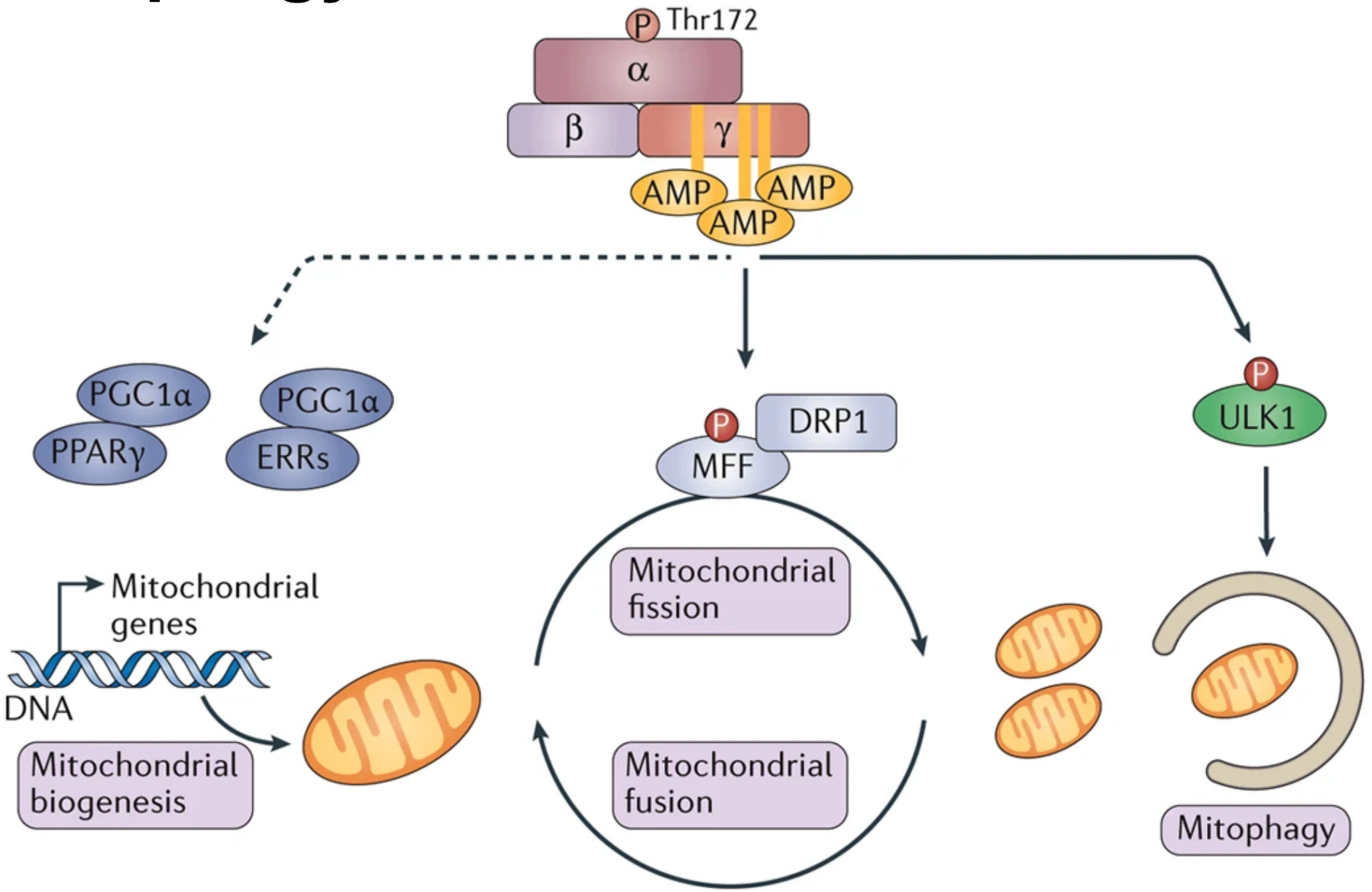
Carling, Zammit & Hardie, ***FEBS Lett****, 1987*

AMPK: activity and regulated pathways



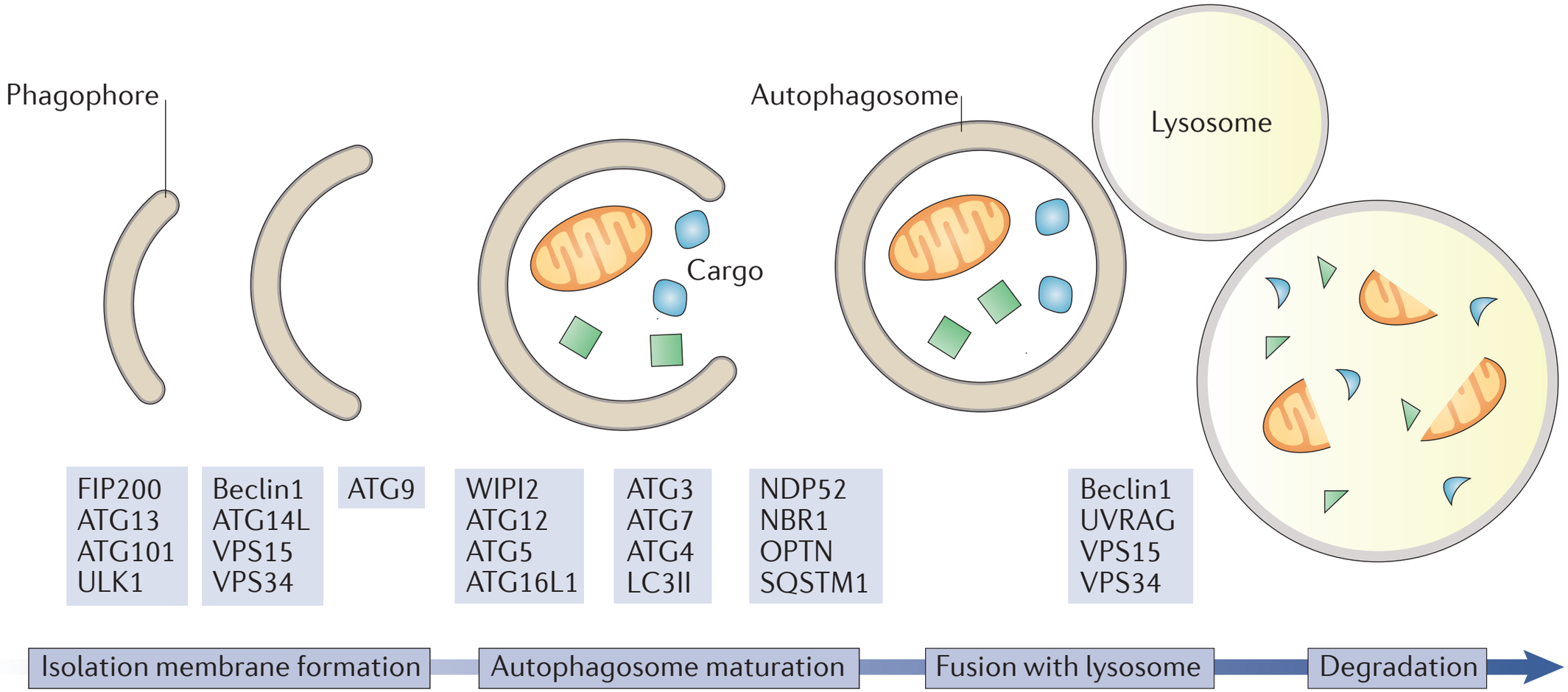
Nature Reviews | Molecular Cell Biology

AMPK: regulation of mitochondrial homeostasis and autophagy



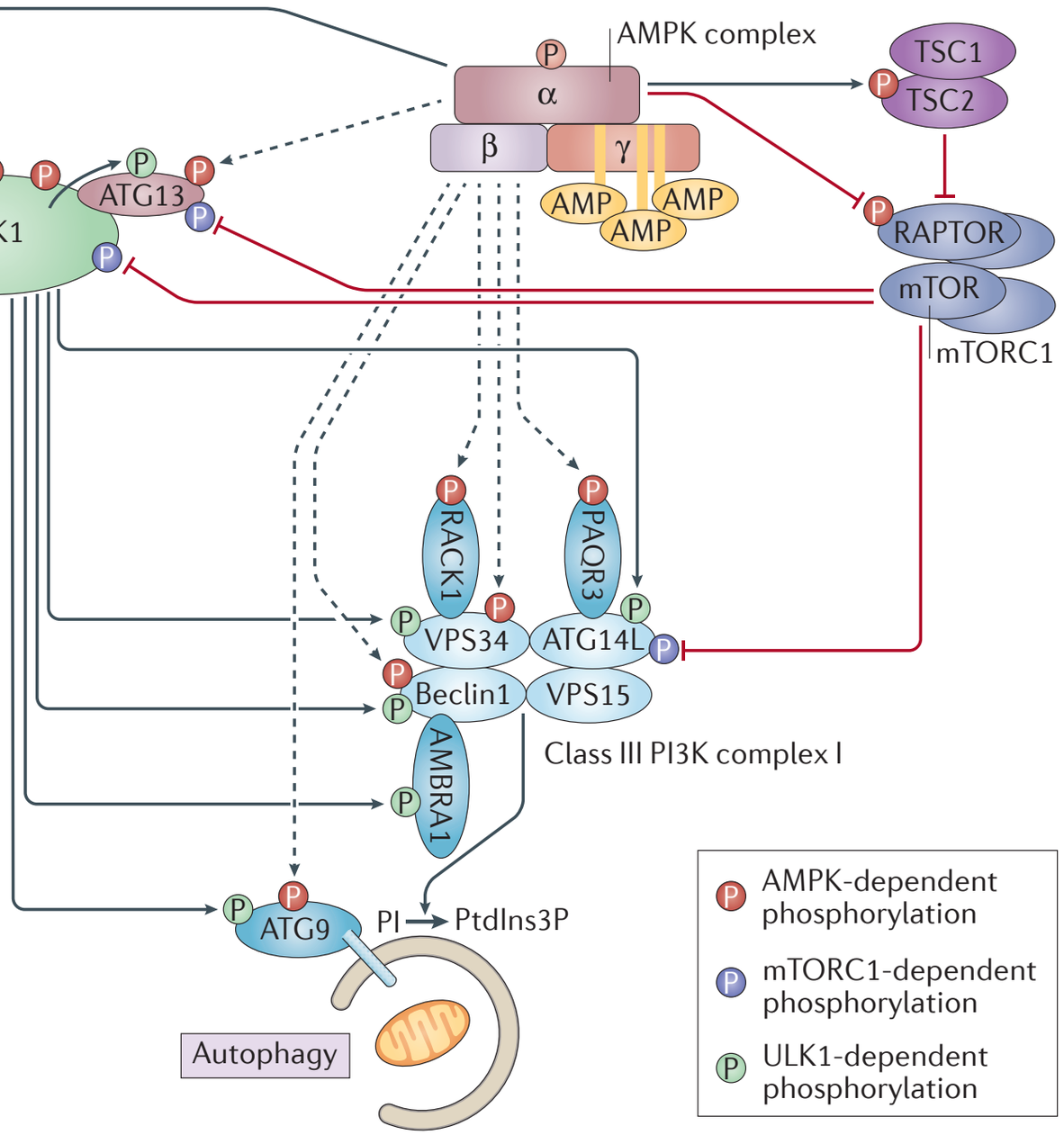
Nature Reviews | Molecular Cell Biology

AMPK: induction of autophagy



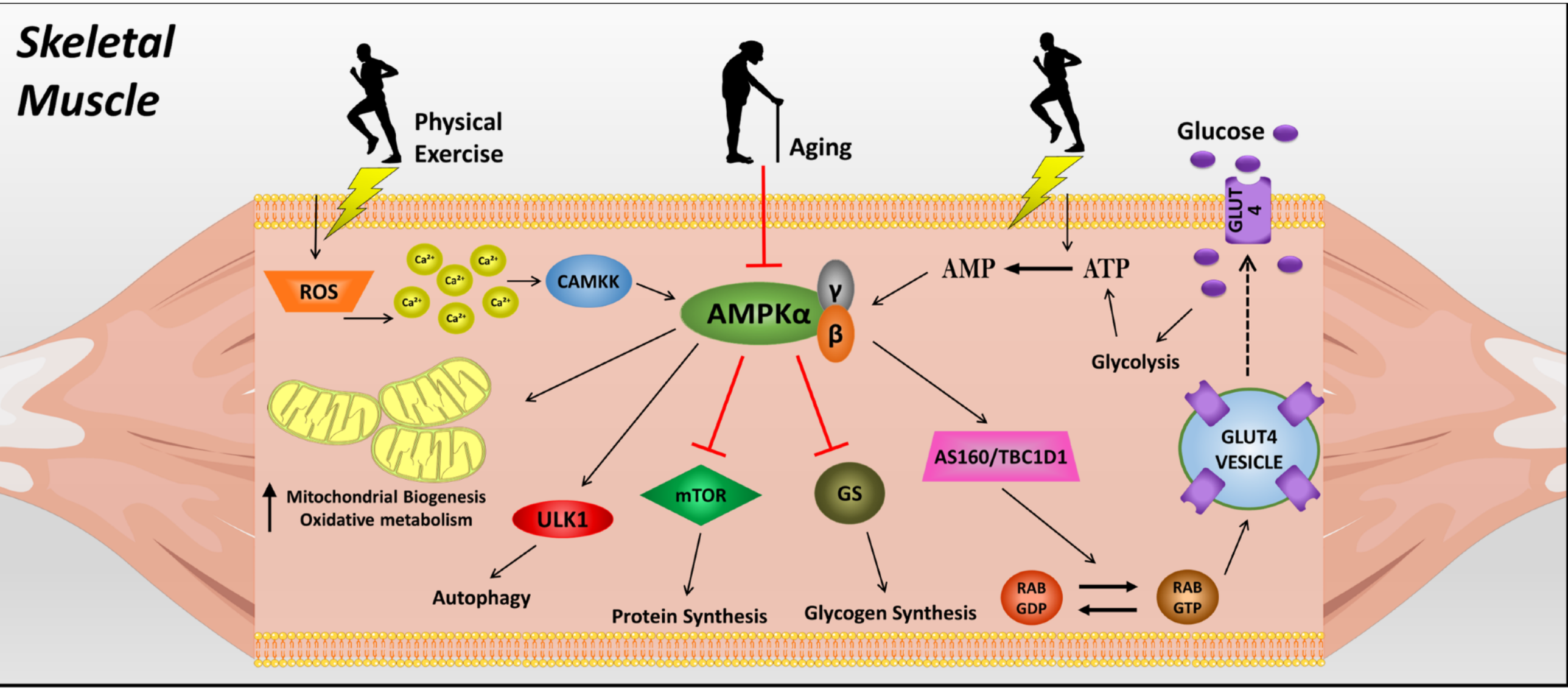
Autophagy is a process by which cells digest their own components using a specialized machinery of adaptors and effectors. It begins with the generation of the autophagosome and recognition of the cargo, followed by the maturation of the autophagosome and fusion with lysosomes. The term itself means ‘self-eating’ and was first coined by Belgian scientist and Nobel Prize laureate Christian de Duve. Autophagy serves two main functions: **it enables the degradation of cellular structures** that are too large for other surveillance pathways, such as the ubiquitin–proteasome system, and **it allows cells to survive starvation by recycling building blocks** such as amino acids to sustain essential cell functions. **Autophagy can be either a bulk recycling of cytosolic components or a targeted removal of macromolecules and even organelles.** In particular, removal of mitochondria by autophagy, a process called mitophagy, has been shown to require the canonical autophagy machinery as well as specific markers at the surface of damaged mitochondria that signal their removal. Genes essential for autophagy (ATGs) have been discovered by screening for genes that are required for autophagosome formation in the yeast Saccharomyces cerevisiae during nitrogen starvation239,240. Yoshinori Ohsumi was awarded the Nobel Prize in physiology or medicine in 2016 for the discovery of the ATG genes. Since the 1990s, the molecular events controlling autophagy execution have been characterized, and the role of ATG genes in controlling various steps of the autophagy pathway has been demonstrated. **The first ATG gene to be cloned, ATG1, encodes a protein kinase required for the initiation of autophagy. Its mammalian homologue, ULK1, plays a similar role.**

AMPK: induction of autophagy



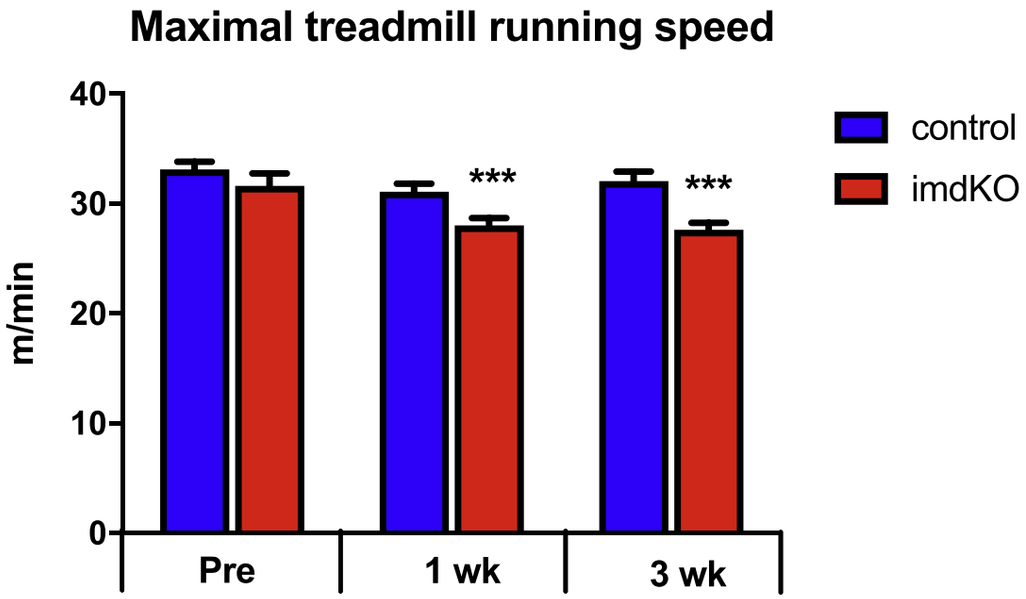
*Herzig & Shaw, **NRMCB**, 2018*

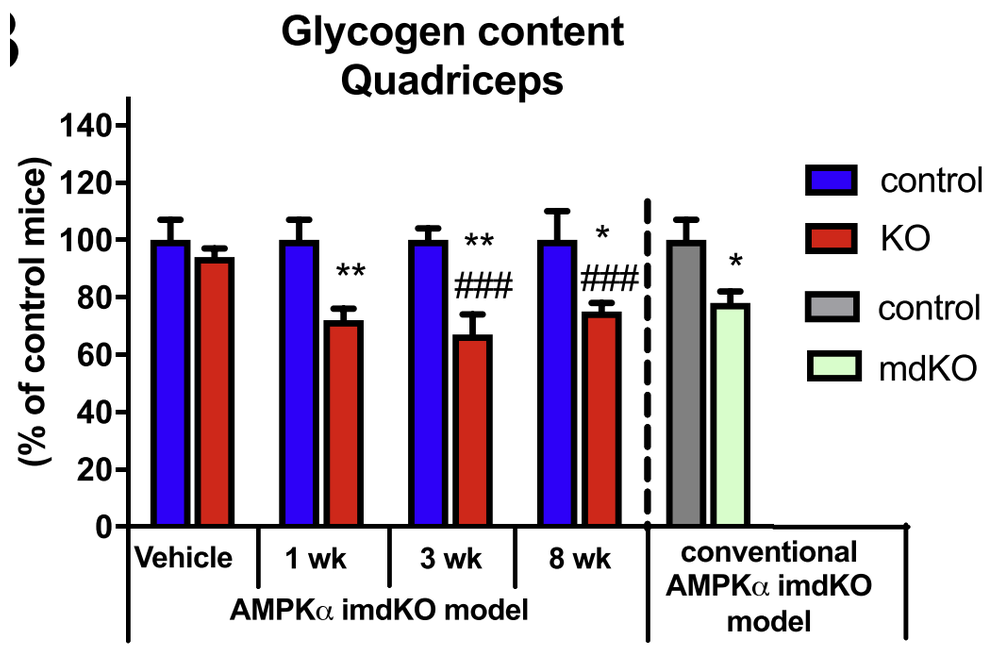
Physiological consequences of AMPK activation

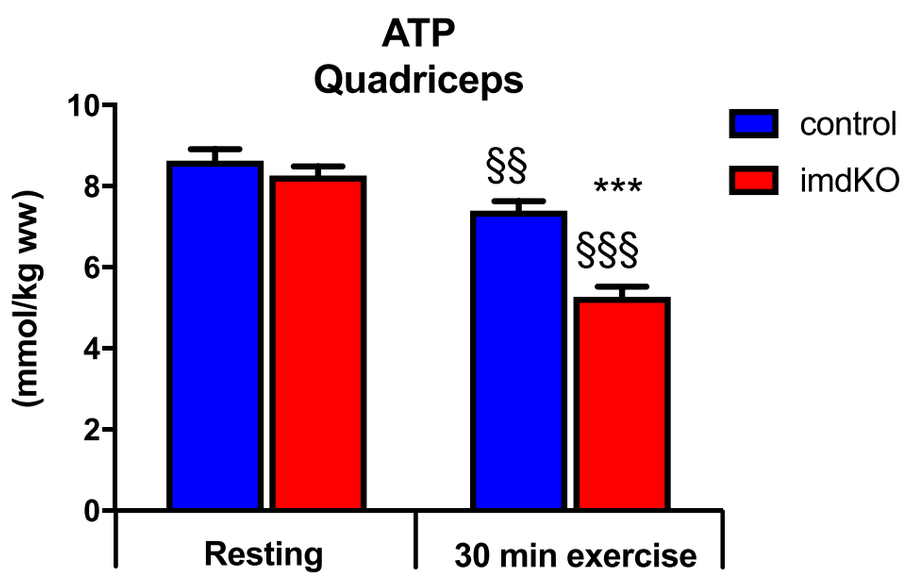


AMPK preserves energy expenditure and optimizes ATP generation: important to sustain exercise in skeletal muscle cells

Inducible deletion of skeletal muscle AMPKα reveals that AMPK is required for nucleotide balance but dispensable for muscle glucose uptake and fat oxidation during exercise



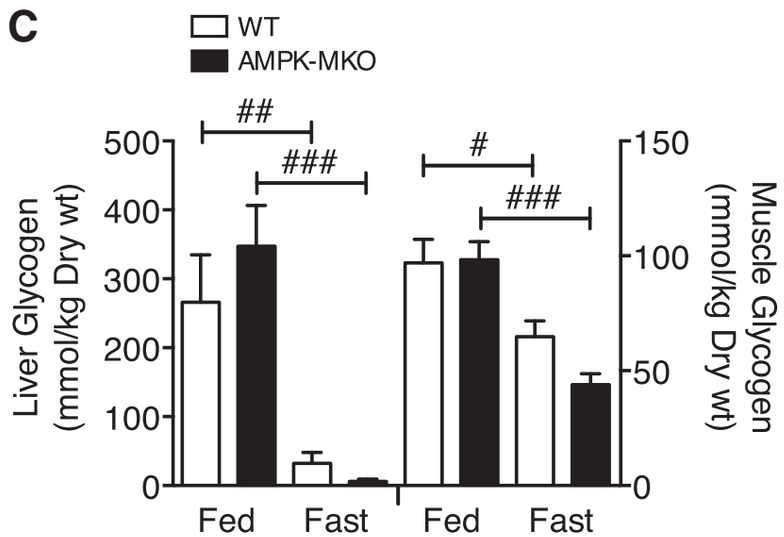


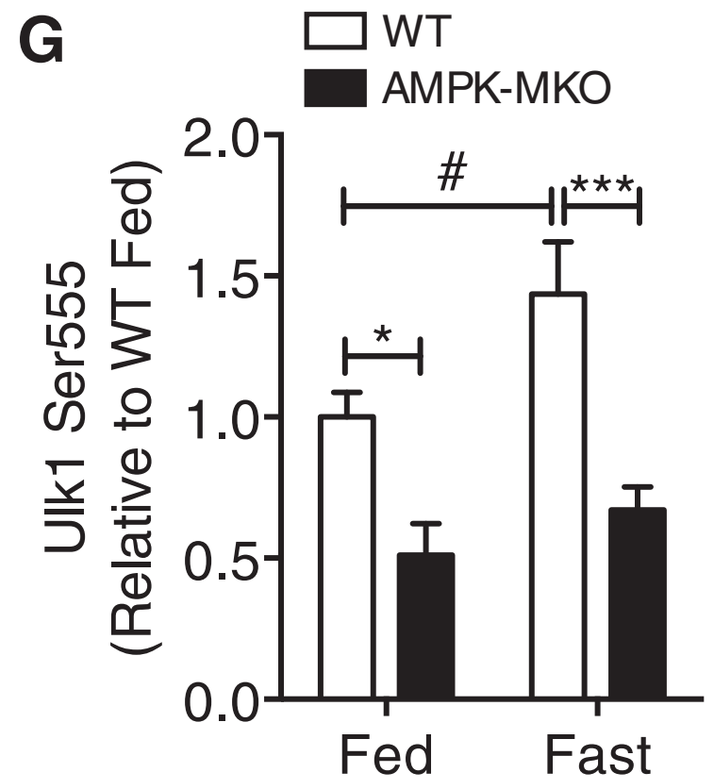


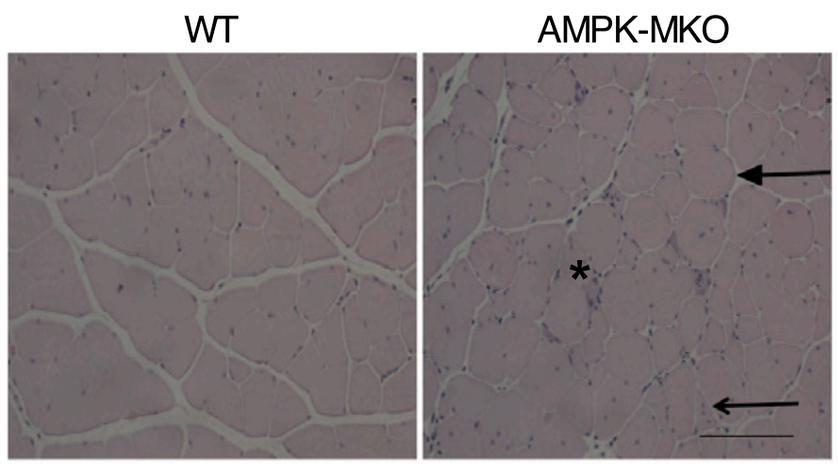
*Hingst et al, **Mol Metab**, 2020*

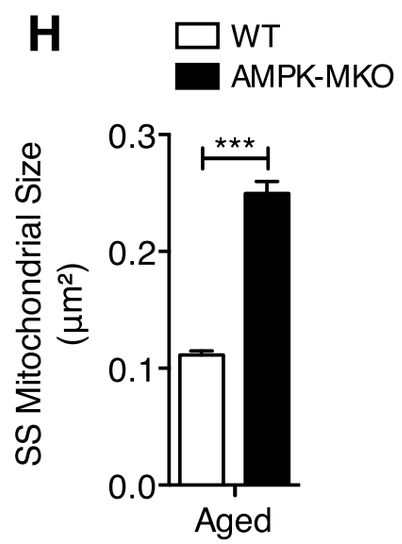
AMPK Activation of Muscle Autophagy Prevents Fasting-Induced Hypoglycemia and Myopathy during Aging

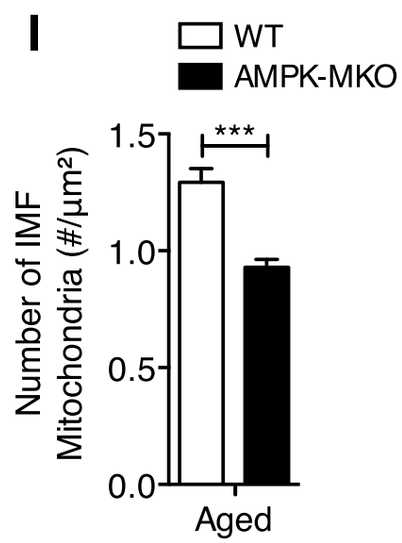
Adam L. Bujak,[1] Justin D. Crane,[1,2] James S. Lally,[1] Rebecca J. Ford,[1] Sally J. Kang,[1] Irena A. Rebalka,[1] Alex E. Green,[1] Bruce E. Kemp,[5] Thomas J. Hawke,[3] Jonathan D. Schertzer,[2,4] and Gregory R. Steinberg[1,4,*]





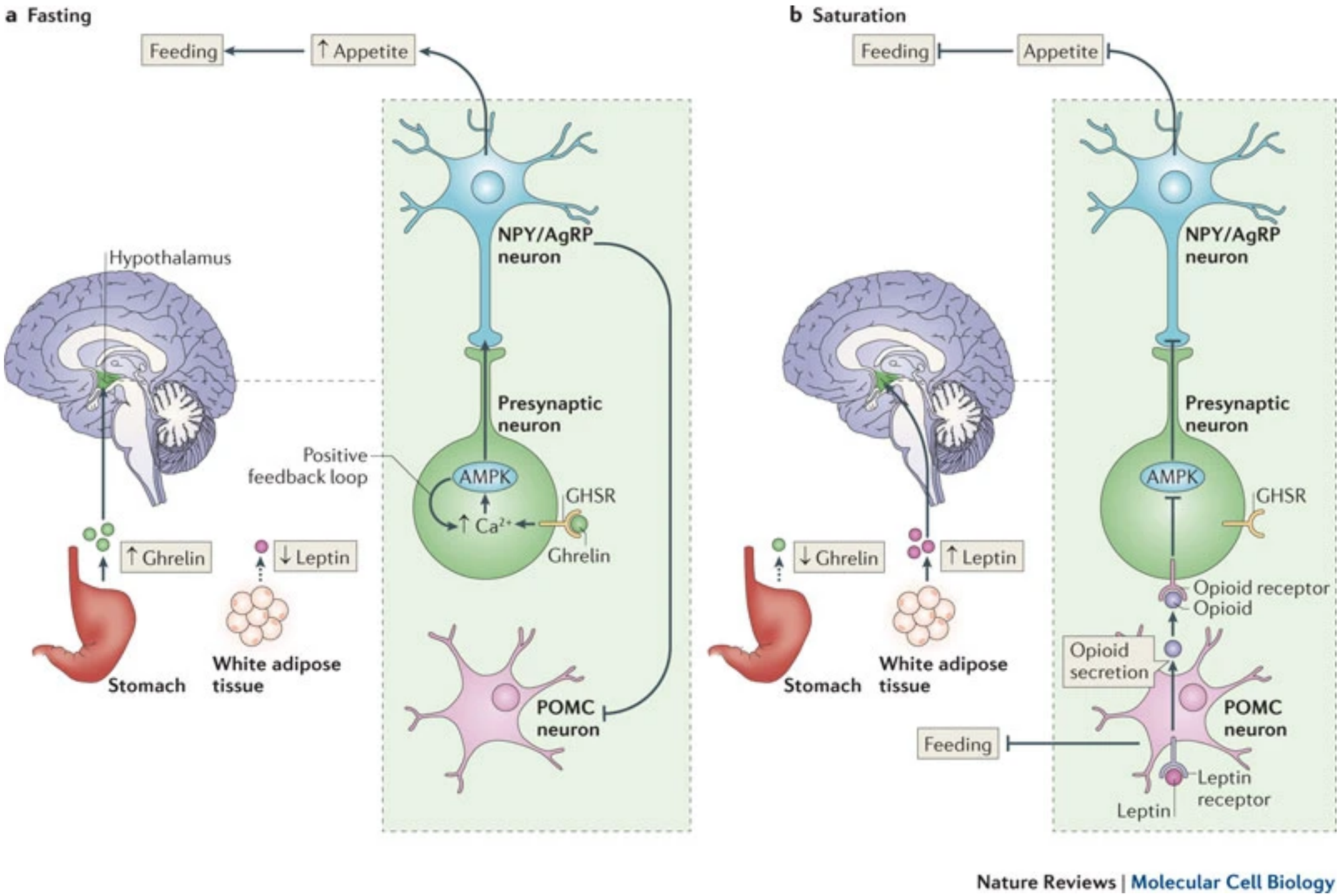






Bujak et al, ***Cell Metab****, 2015*

AMPK: regulation of appetite

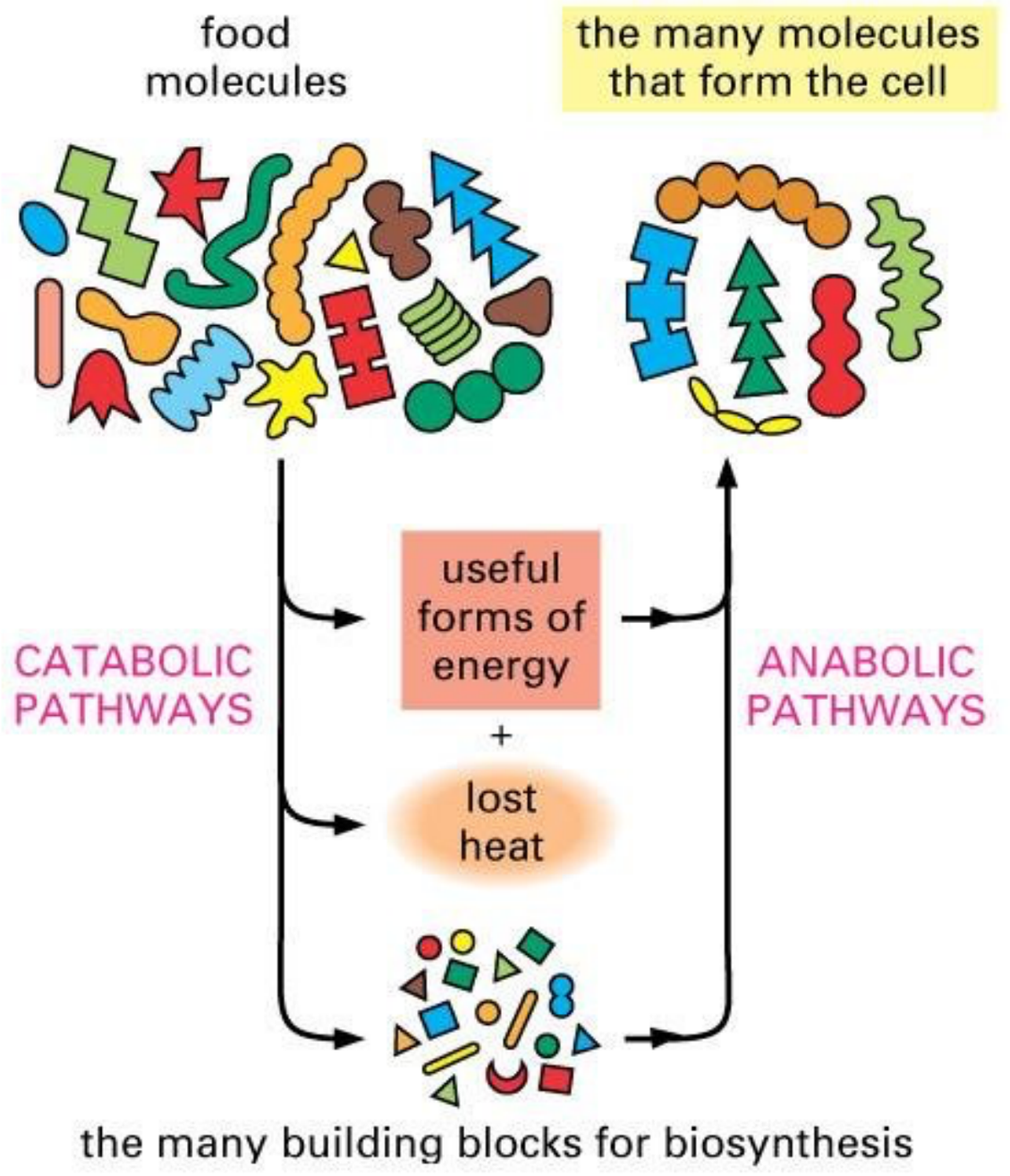


NPY/AgRP: neuropeptide Y and agouti-related protein-expressing neurons

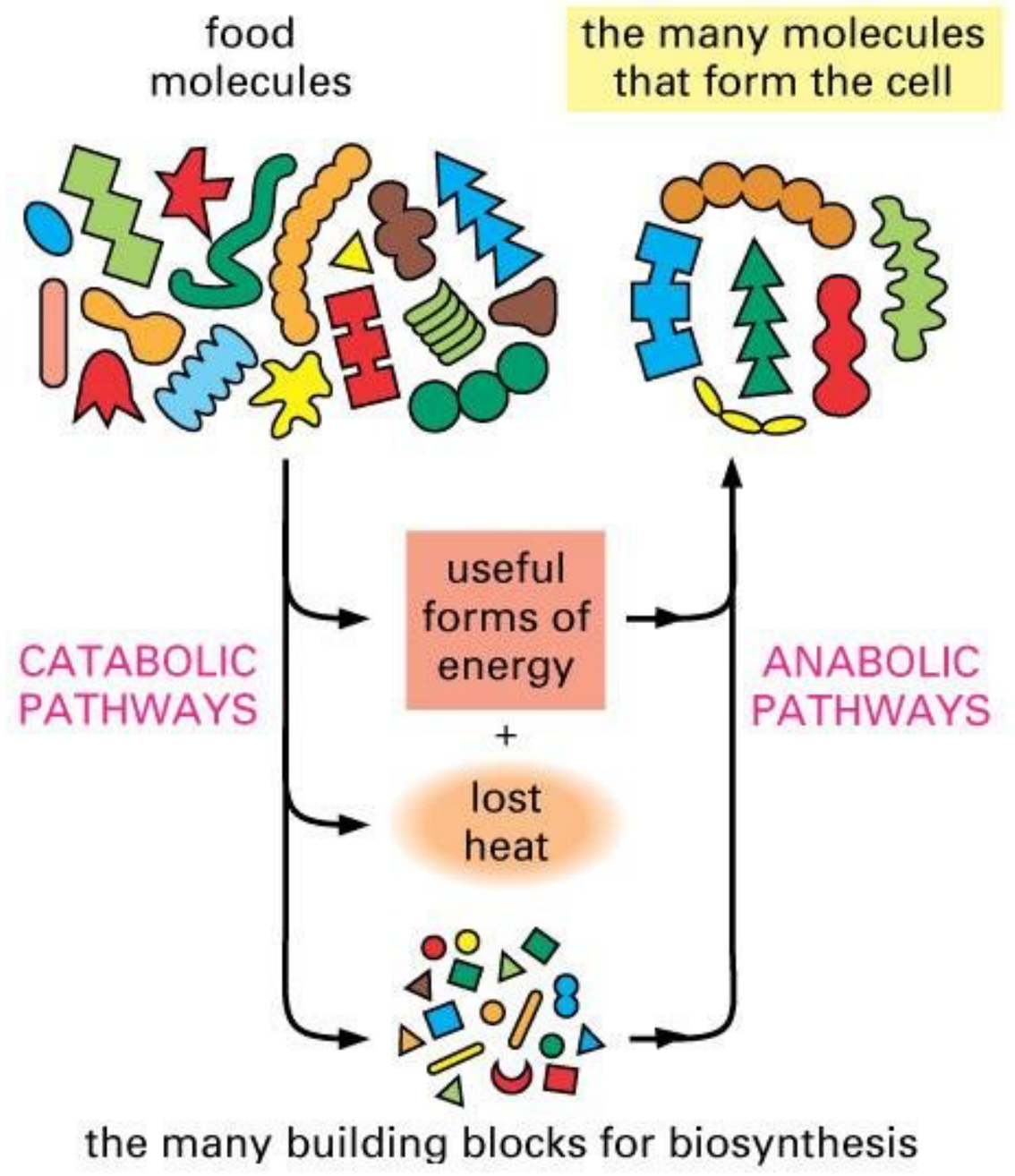
POMC: pro-opiomelanocortin-expressing neurons

Herzig & Shaw, ***NRMCB****, 2018*

Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



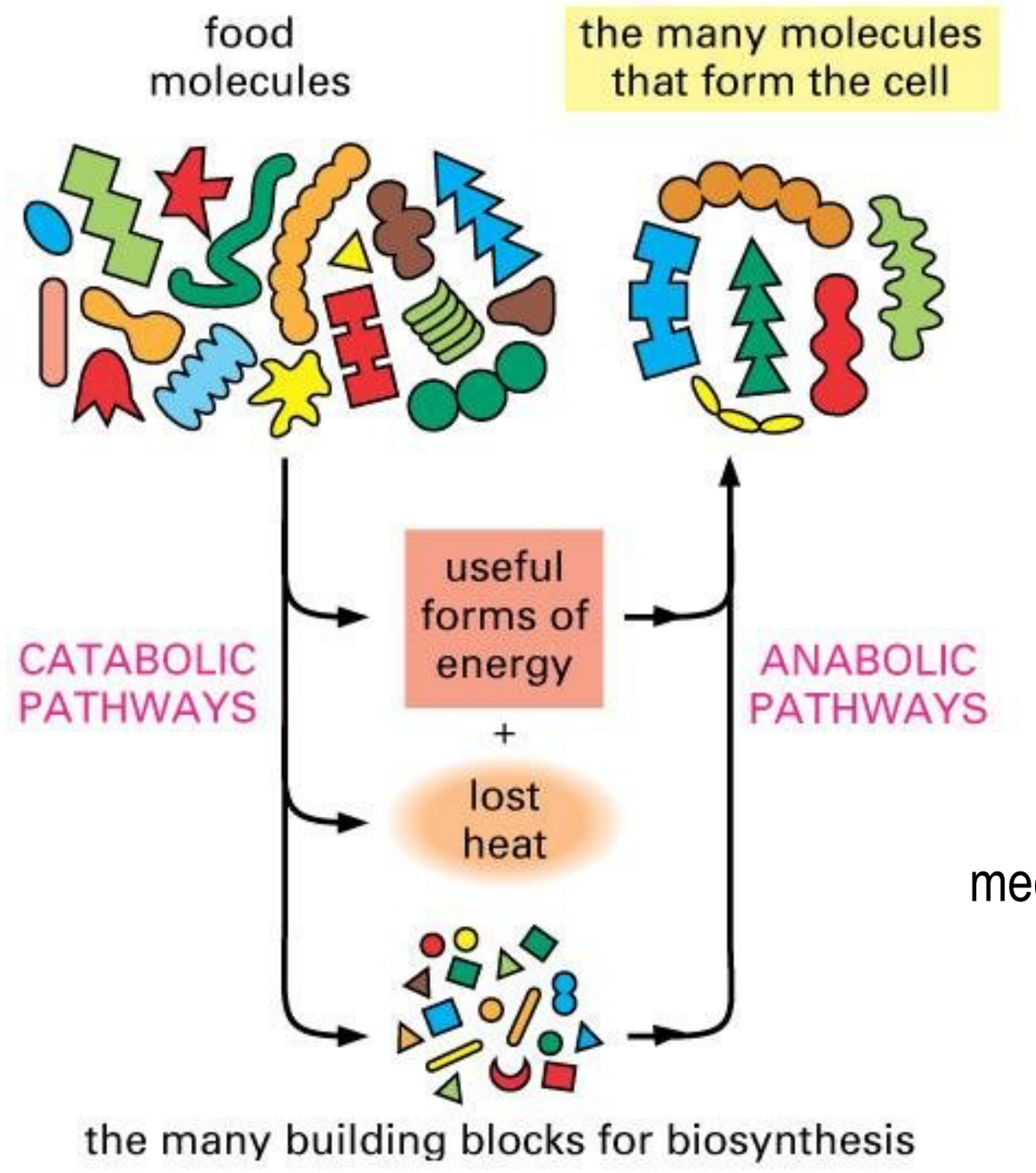
Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



AMPK

AMP-activated protein kinase

Catabolism and Anabolism are juxtaposed and regulated by nutrient sensing



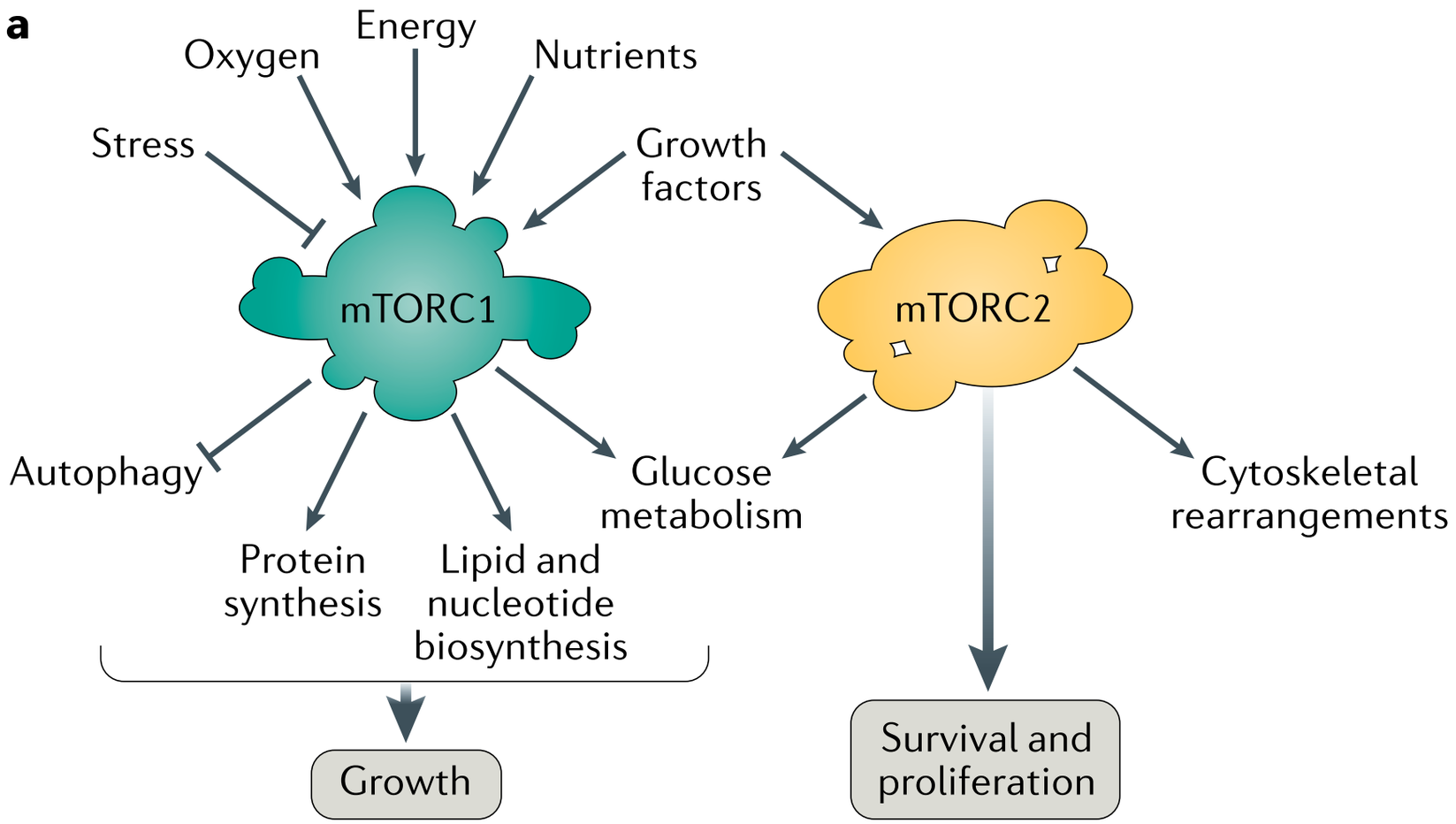
AMPK

AMP-activated protein kinase

mTORC

mechanistic Target of Rapamycin

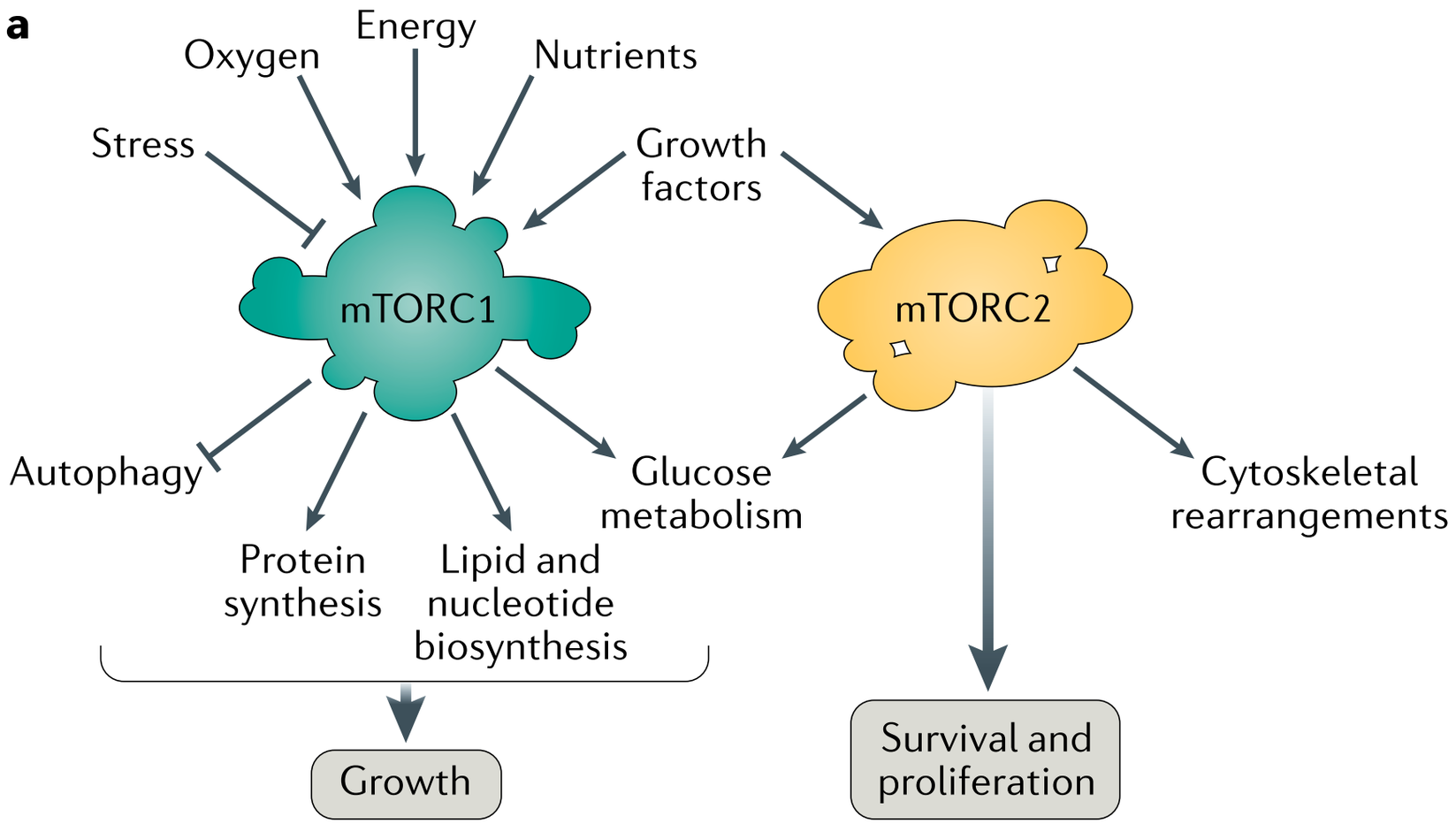
Two protein complexes coordinate nutrient/ signaling sensing and anabolism/growth



mTORC: mechanistic (previously: mammalian) Target of Rapamycin Complex

*Liu & Sabatini, **NRMCB**, 2020*

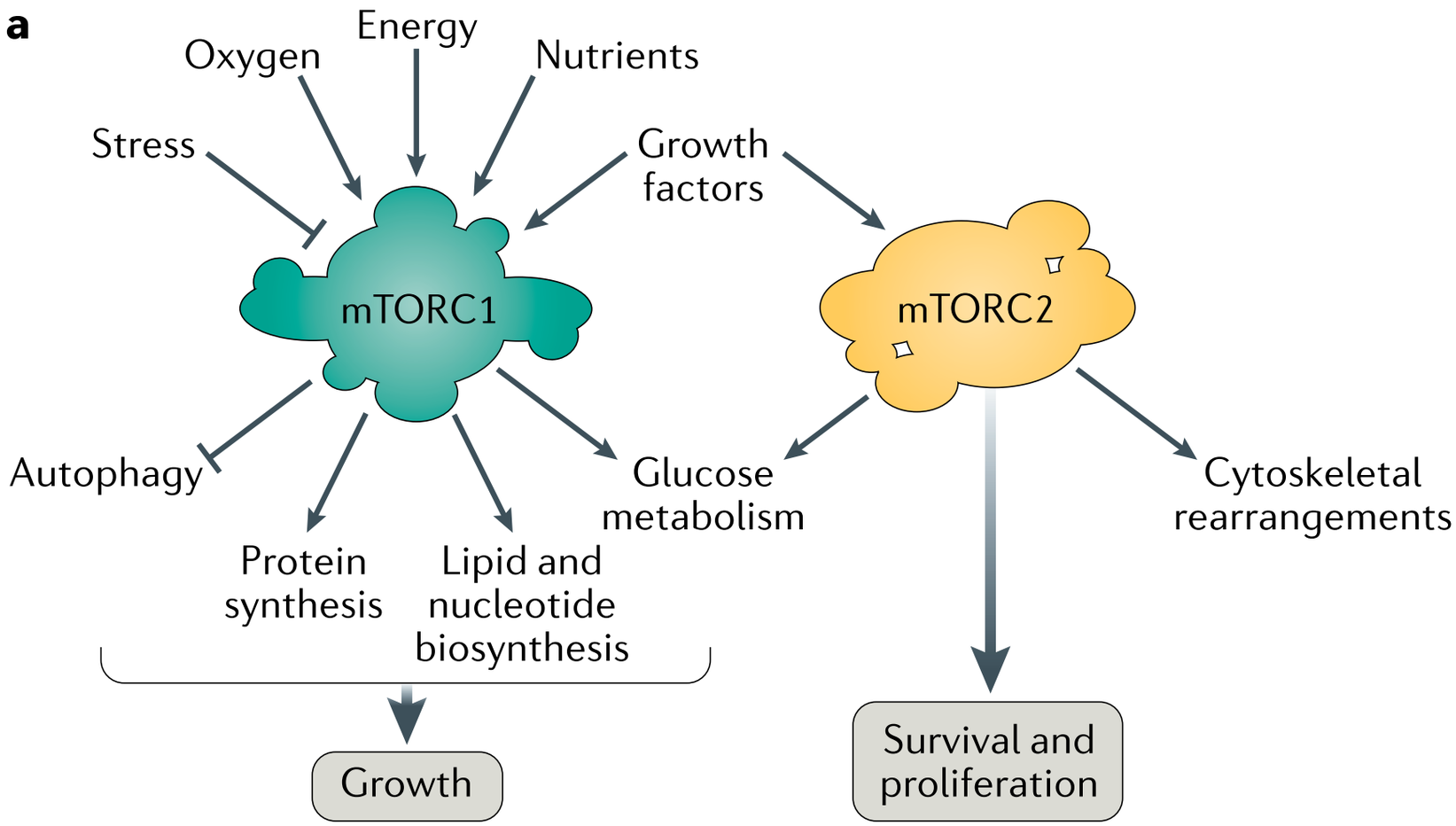
Two protein complexes coordinate nutrient/ signaling sensing and anabolism/growth



mTORC: mechanistic (previously: mammalian) Target of Rapamycin Complex

*Liu & Sabatini, **NRMCB**, 2020*

Two protein complexes coordinate nutrient/ signaling sensing and anabolism/growth

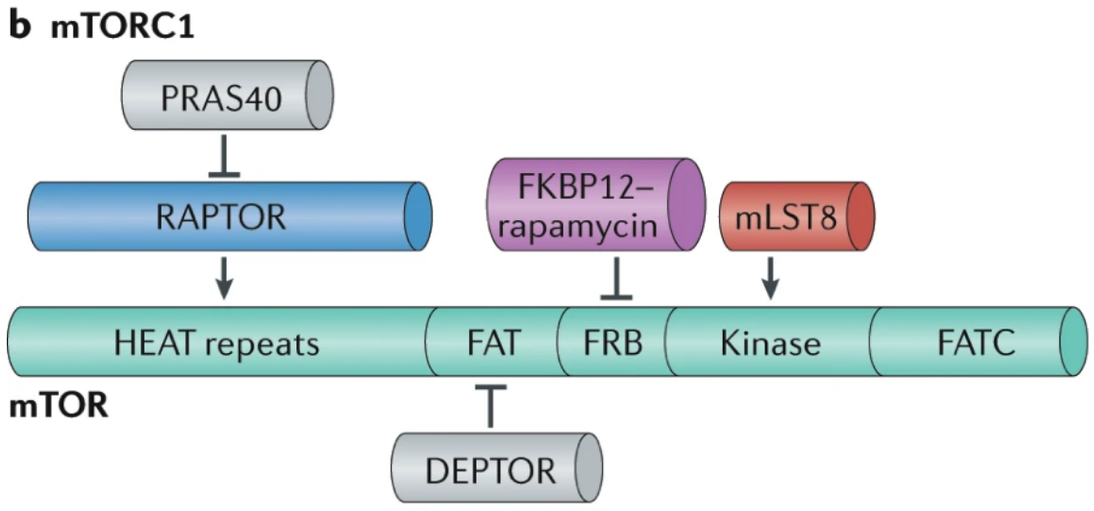


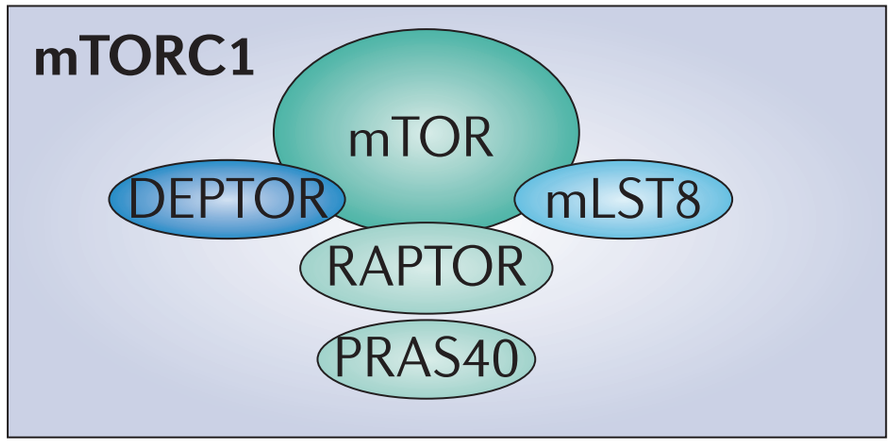
mTORC: mechanistic (previously: mammalian) Target of Rapamycin Complex

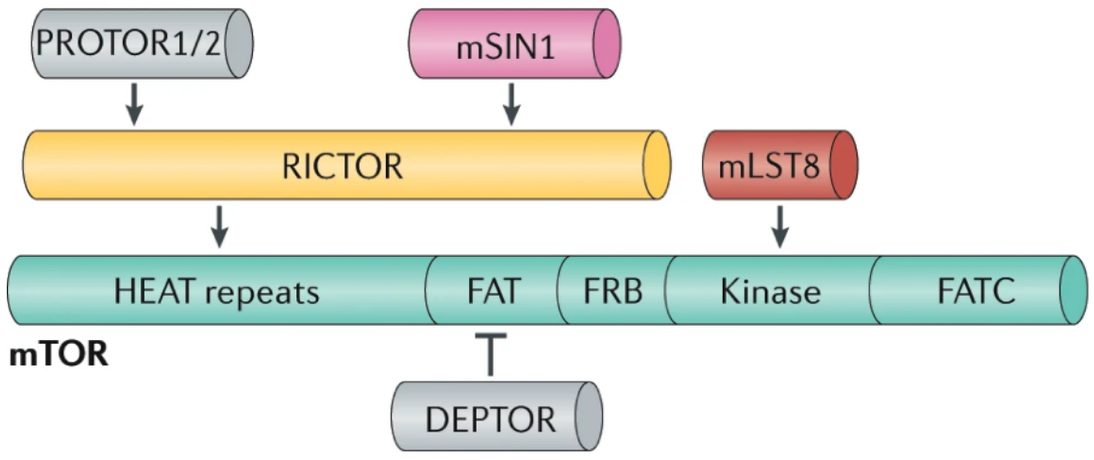
Macrolide with potent anti fungal activity isolated in 1964 from bacteria found in the *Rapa Nui* island. This compound was later found to have immunosuppressive, antitumour and neuroprotective properties, generating significant clinical excitement

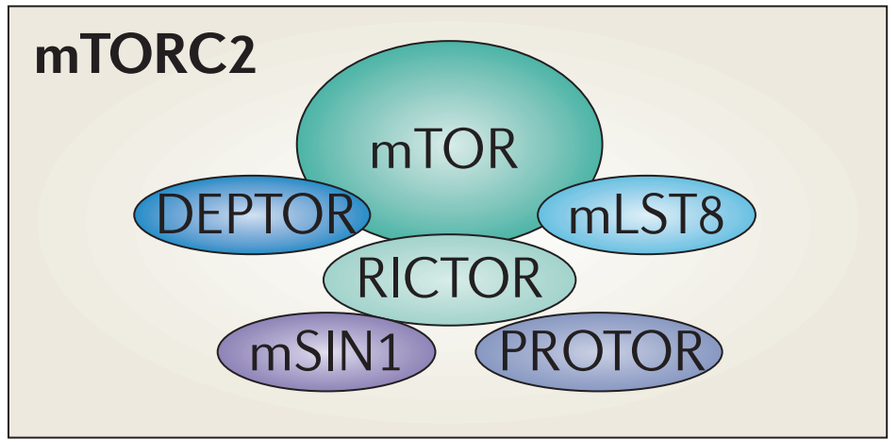
Liu & Sabatini, ***NRMCB****, 2020*

mTORC complexes: structure



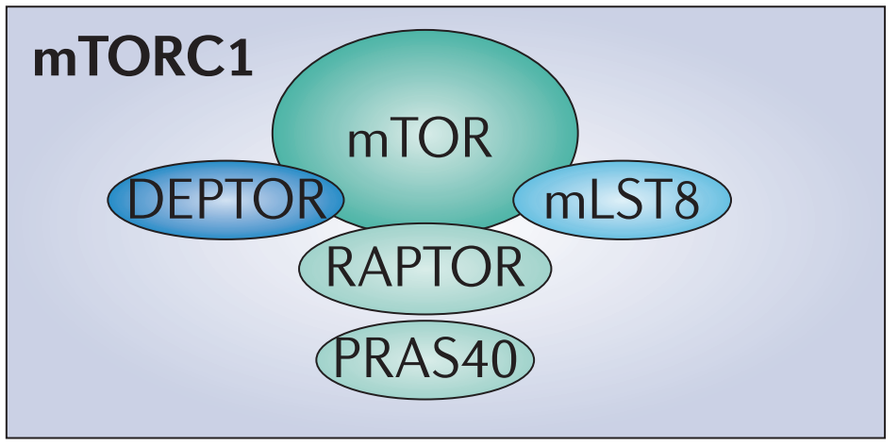


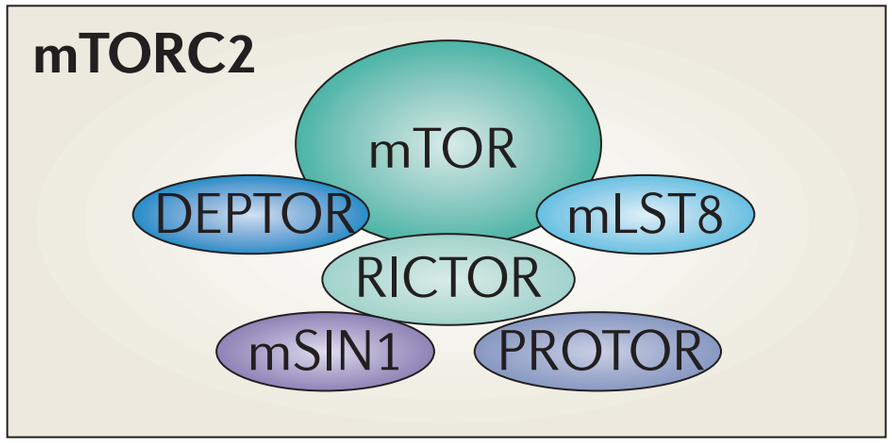




mTOR is a is a 289kDa serine/threonine protein kinase in the PI3K-related protein kinases (PIKK) family. In mammals, it constitutes the catalytic subunit of two distinct complexes known as mTOR complex 1 (mTORC1) and mTORC2. These complexes are distinguished by their accessory proteins and their differential sensitivity to rapamycin, as well as by their unique substrates and functions

mTORC complexes: structure

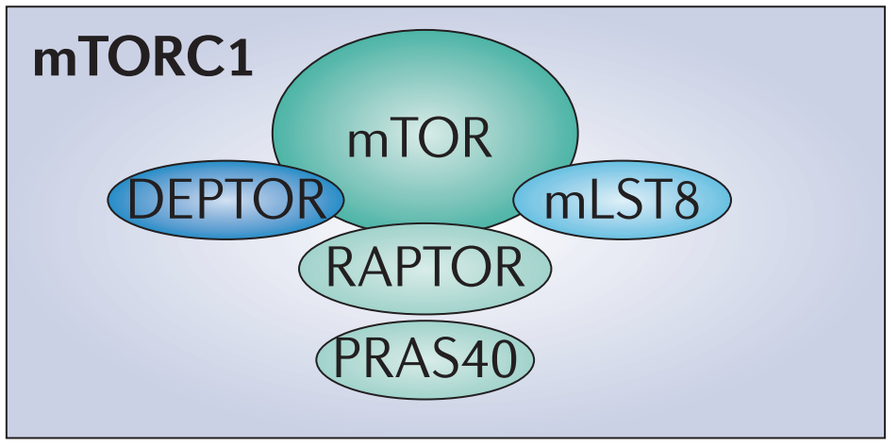


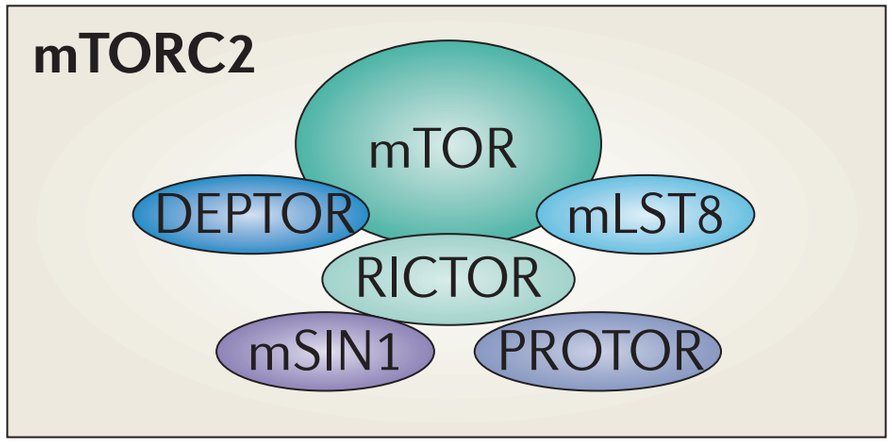


The overall organization of both mTORC1 and mTORC2 is that of a dimer: each complex includes two copies of mTOR and of their respective accessory subunits (differ in part).

Three core components: mTOR, mammalian lethal with SEC13 protein 8 (mLST8, also known as GβL - stabilizing role) and a unique defining subunit, the scaffold protein regulatory-associated protein of mTOR (RAPTOR/ RICTOR - localization and substrate specificity)

mTORC complexes: structure



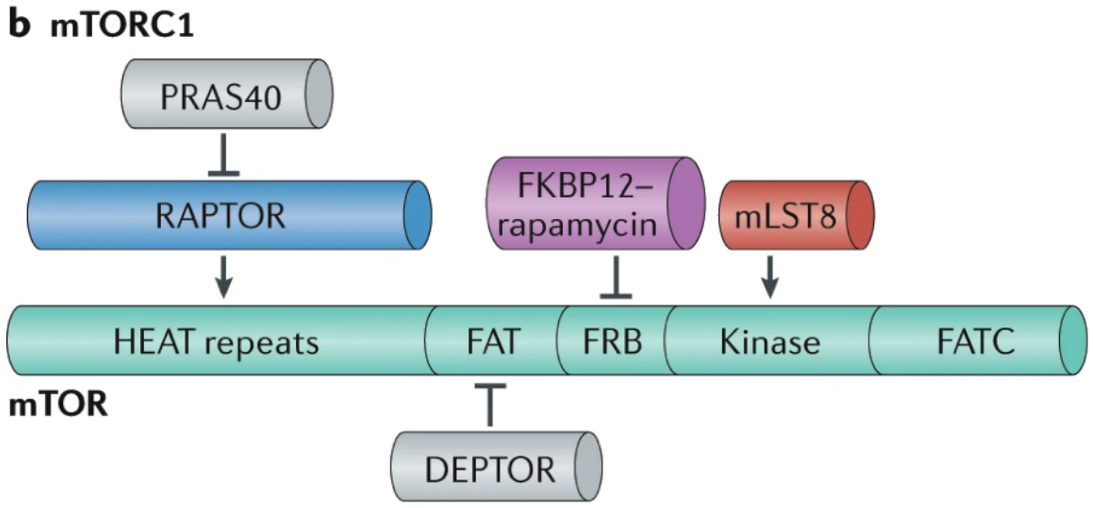


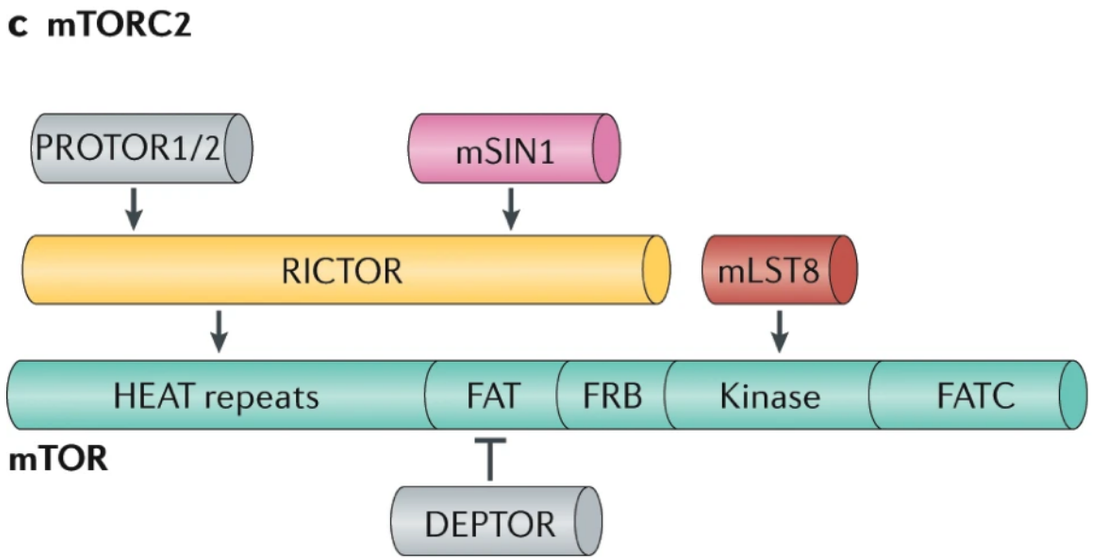
In isolation, this complex is relatively inactive; a recent structure suggests that key residues in the kinase domain of mTOR may only shift into a catalytic position after the complex binds its essential activator, the small GTPase Rheb (*Yang et al, **Nature**, 2017*)

mTORC2 retains the ability to phosphorylate its substrates upon acute rapamycin treatment

mSIN1 has a phospholipid-binding pleckstrin homology domain, which may help mTORC2 assemble on the plasma membrane

mTORC: structure





The mTOR N-terminus comprises an array of helical HEAT repeats that form two α-solenoids packed against each other, known as the ‘horn’ and the ‘bridge’ domains (*binding of regulators*).

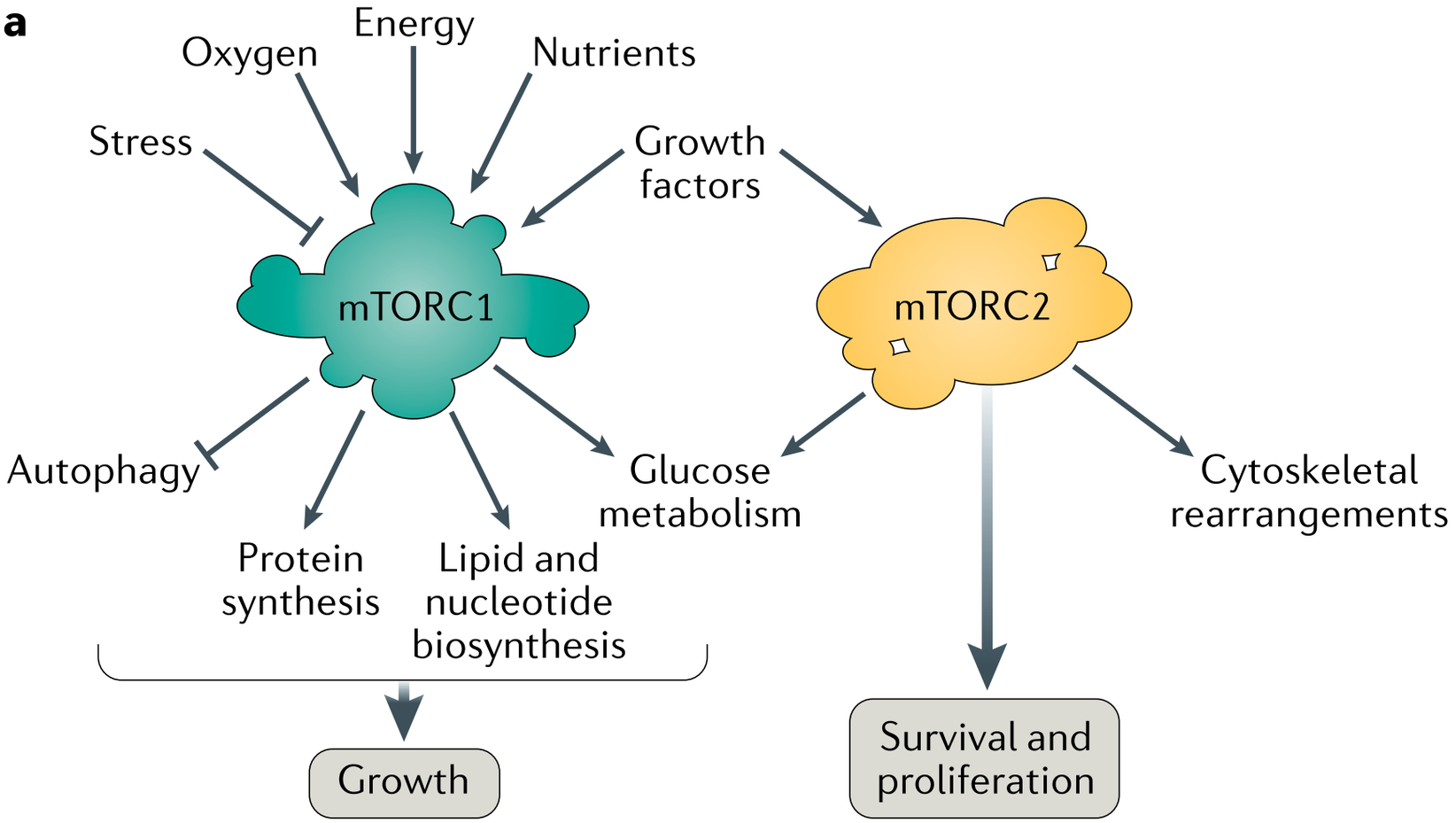
The HEAT domain enables its recruitment at the lysosomal surface.

As in other PIKK family kinases, the FAT domains serve as organizing centres of the complex, as they clamp onto and anchor the kinase domains, horn, and bridge.

The active site of mTOR contains a substrate-binding groove that consists of the activation loop, portions of the mLST8 binding site, and the FATC domain. The FRB domain and mLST8 narrow the active site cleft to prevent non-target proteins from binding

Goul, Peruzzo & Zoncu, ***NRMCB****, 2023*

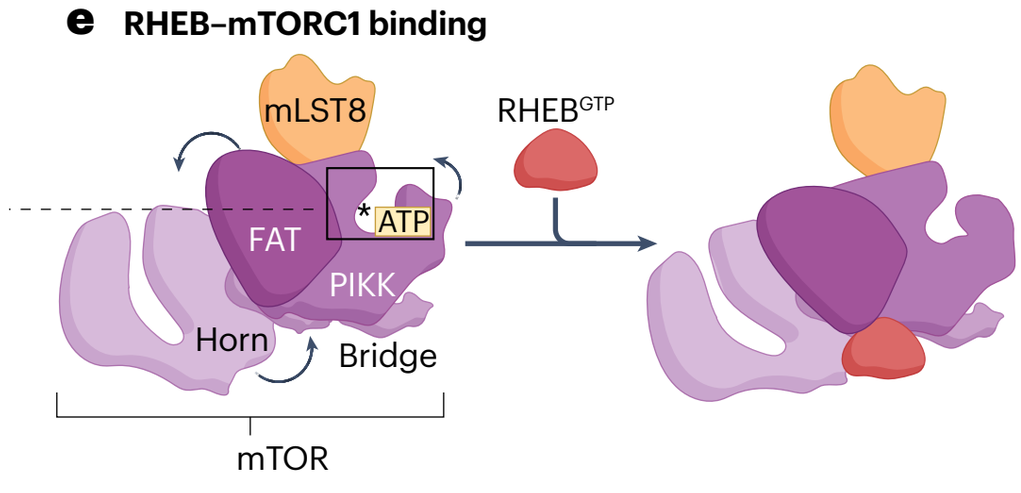
mTORC complexes have different activating cues and effectors



mTORC1 is sensible to both nutrients and growth factors

mTORC2 is sensible solely to growth factors

How is mTORC1 activated?



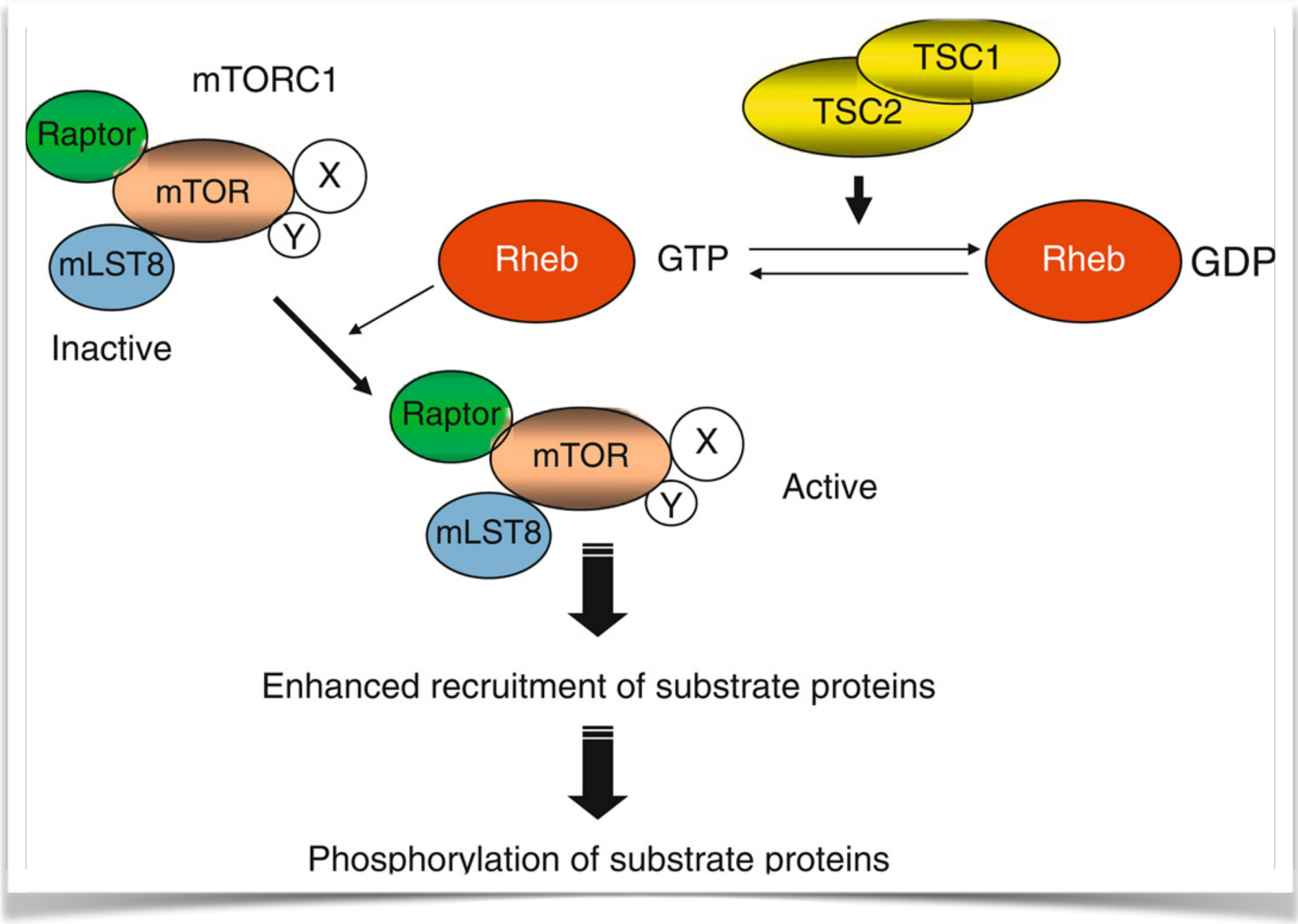
Regulators of mTORC1 converge on the lysosome-associated **RHEB** (Ras homologue enriched in brain) guanosine triphosphatases (**GTPases**) that **modulate its kinase activity**. RHEB is active in the GTP-bound state, stimulating mTORC1 through physical interactions that allosterically reorient the kinase active site, thereby favoring substrate phosphorylation.

The recruitment of mTORC1 to lysosomes, which enables its interaction with RHEB, is mediated by the heterodimeric **Rag GTPases**, and occurs in the presence of glucose, amino acids and other nutrients.

The requirement for both RHEB and Rag GTPases ensures that growth signaling occurs according to a 'co-incidence detection' principle, that is, only when the required intracellular building blocks and extracellular growth-promoting instructions are simultaneously present.

*Goul, Peruzzo & Zoncu, **NRMCB**, 2023*

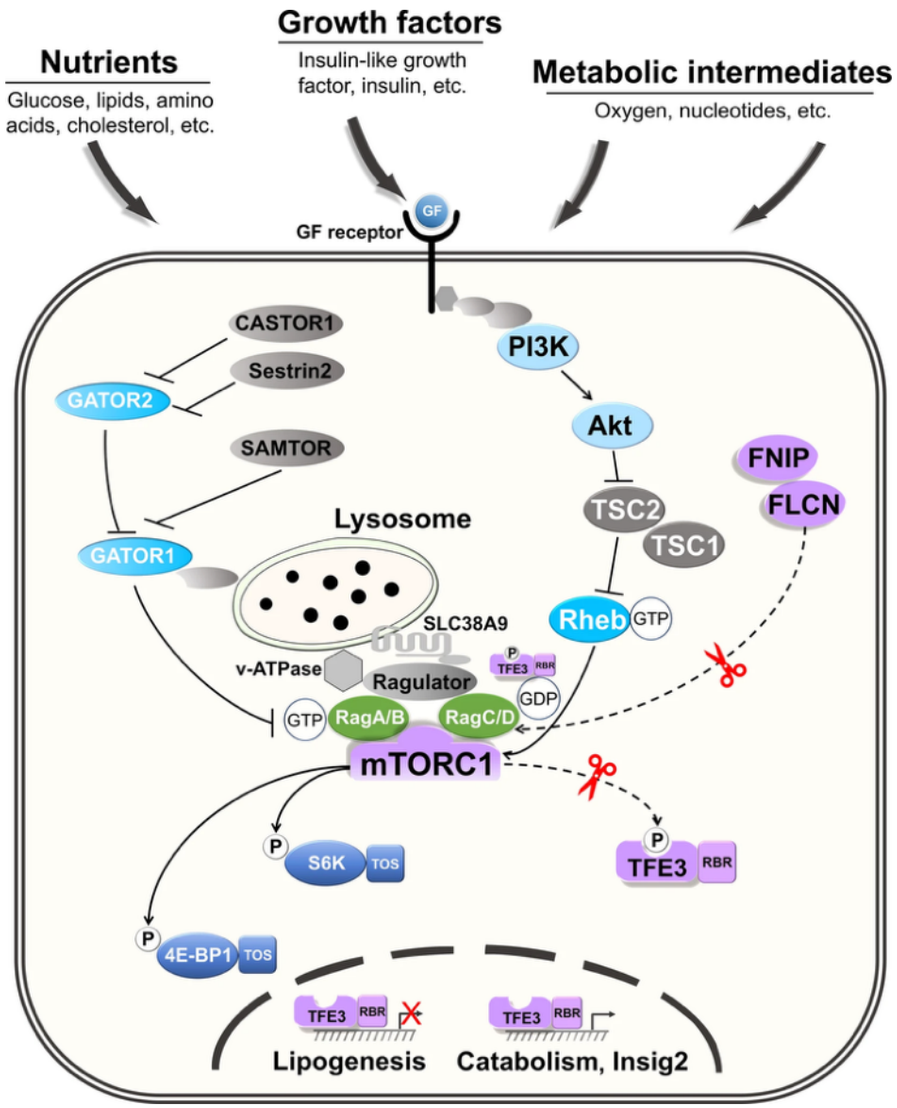
How is mTORC1 activated?



RHEB in its GTP-bound state interacts with mTORC1 and activates it. This involves enhanced recruitment of substrate proteins resulting in their phosphorylation. RHEB-GTP is converted to RHEB-GDP by the action of Tuberous Sclerosis Complex TSC1/TSC2 GAP (GTPase Activating Protein)

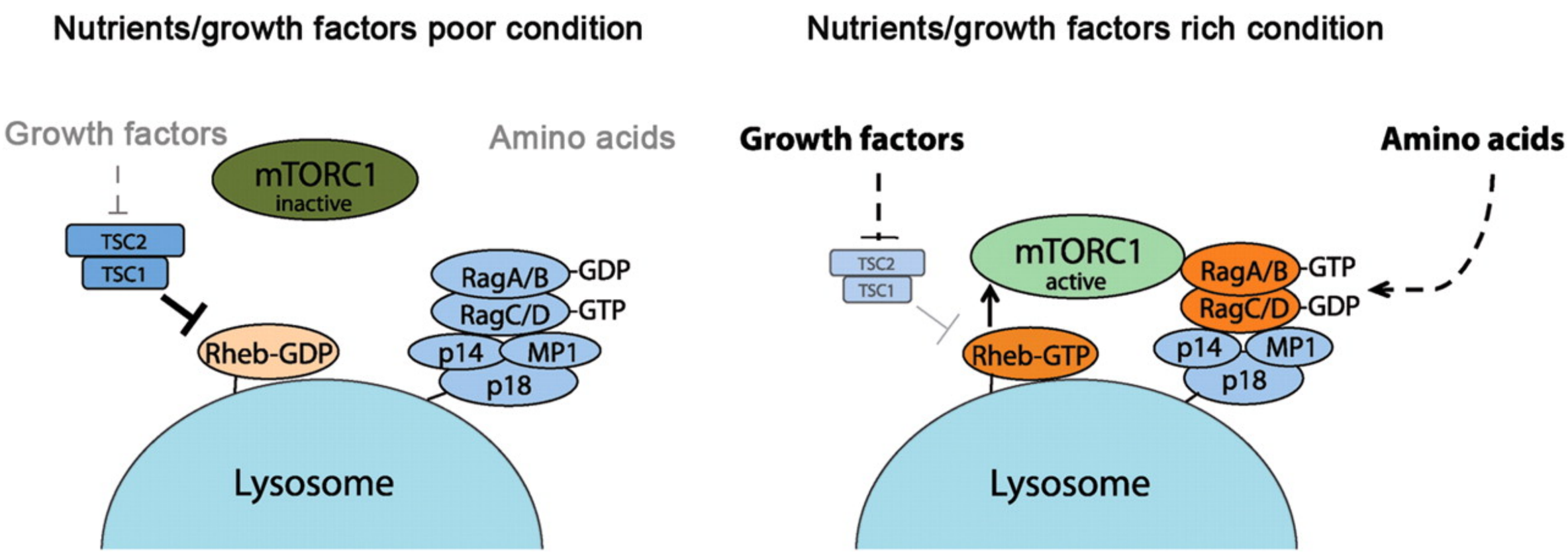
How is mTORC1 activated?

In response to nutrients, mTORC1 translocates from the cytoplasm to the lysosomal surface, where it is activated by growth factors via PI3K– AKT signaling.



AKT inhibits the TSC1–TSC2 complex, which is a GTPase- activating protein (GAP) for the small GTPase RHEB. GTP-bound RHEB directly binds and activates mTORC1 at the lysosome

mTORC1 activation

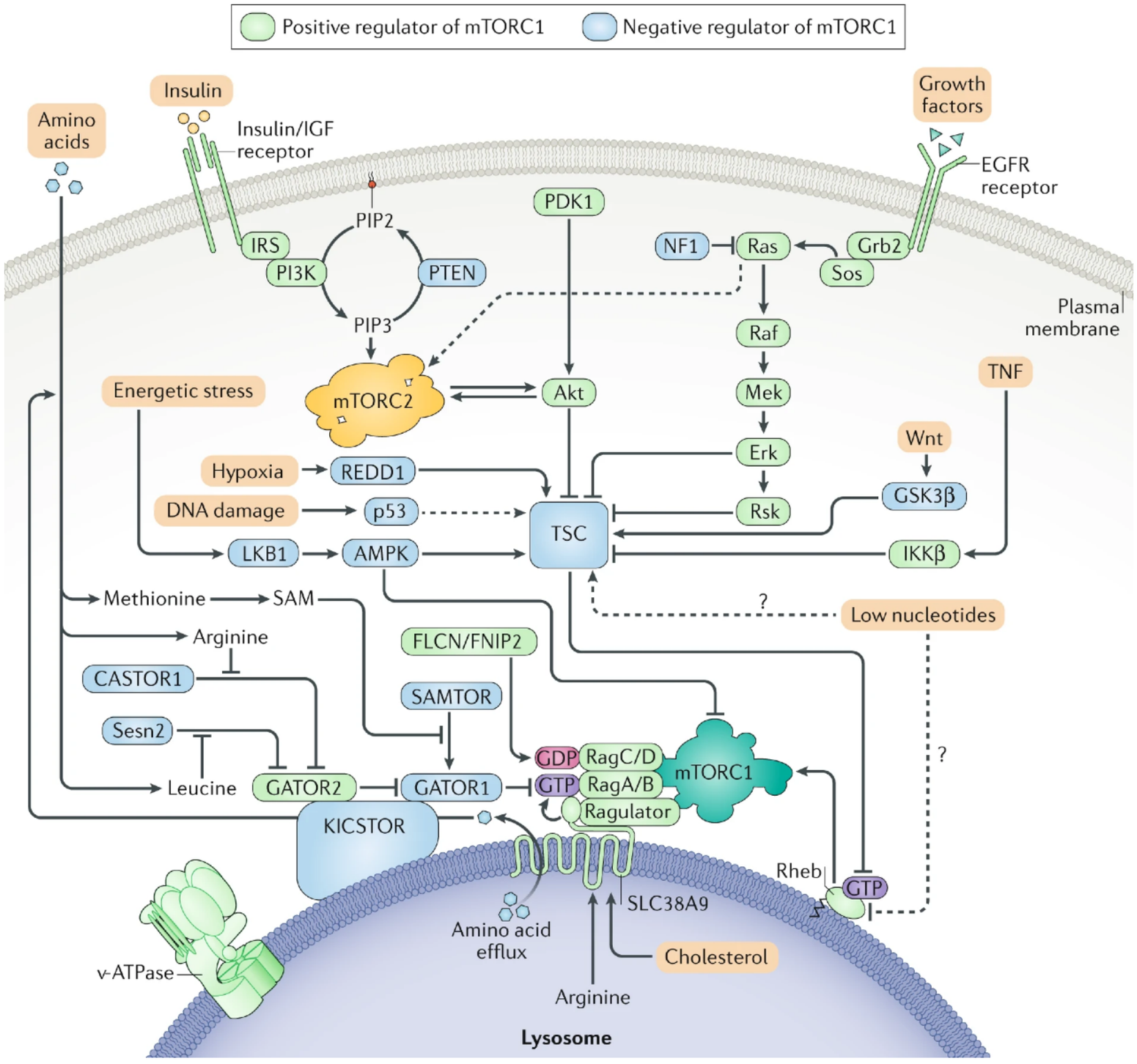


Nutrients are sensed by RAGULATOR proteins to recruit mTORC1 at the lysosome

Growth factors trigger AKT signaling to promote RHEB-GTP state and activate mTOR kinase

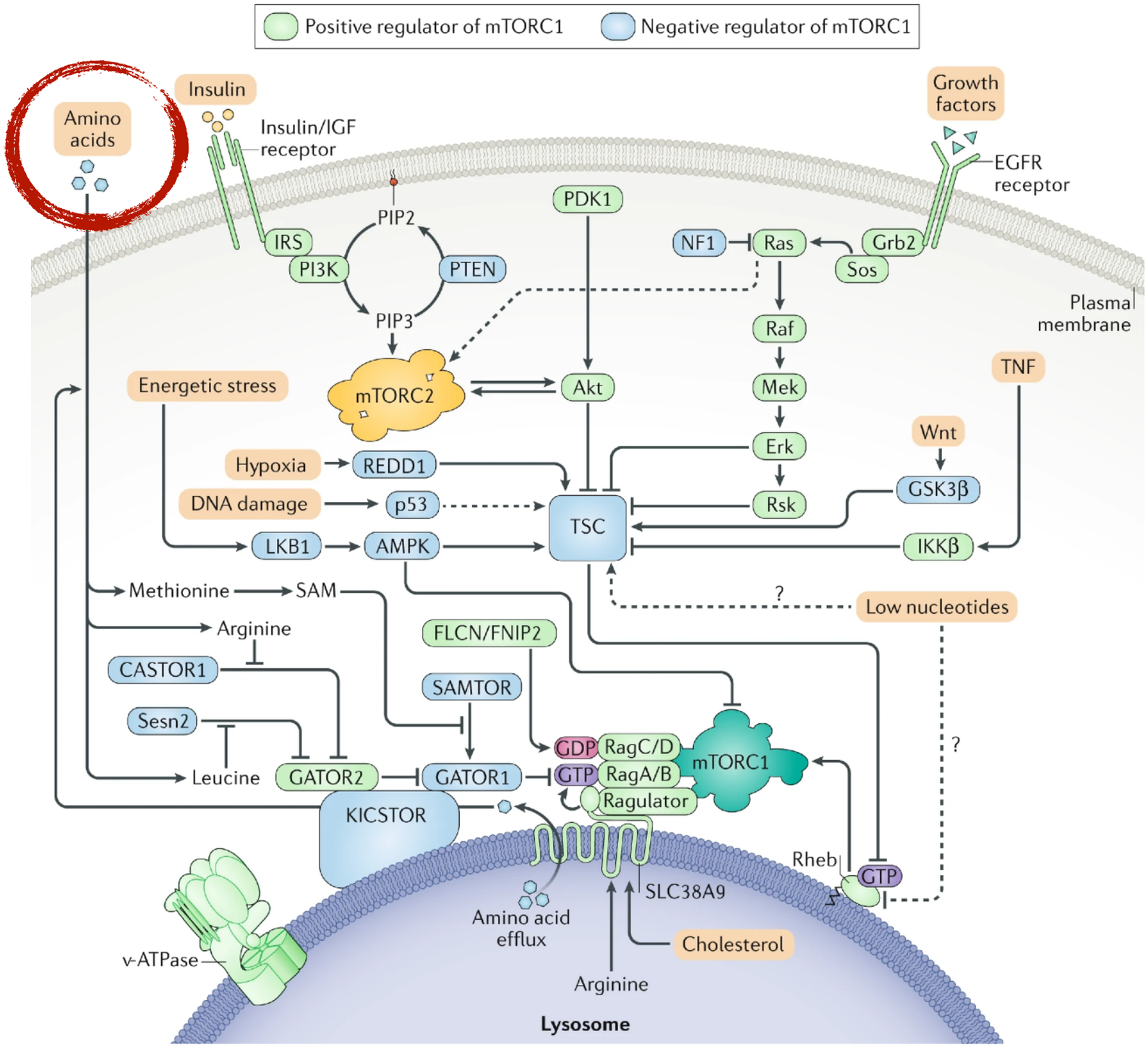
The requirement for both RHEB and Rag GTPases ensures that growth signaling occurs according to a 'co-incidence detection' principle, that is, only when the required intracellular building blocks and extracellular growth-promoting instructions are simultaneously present.

What is sensed by RAG-mTORC1?



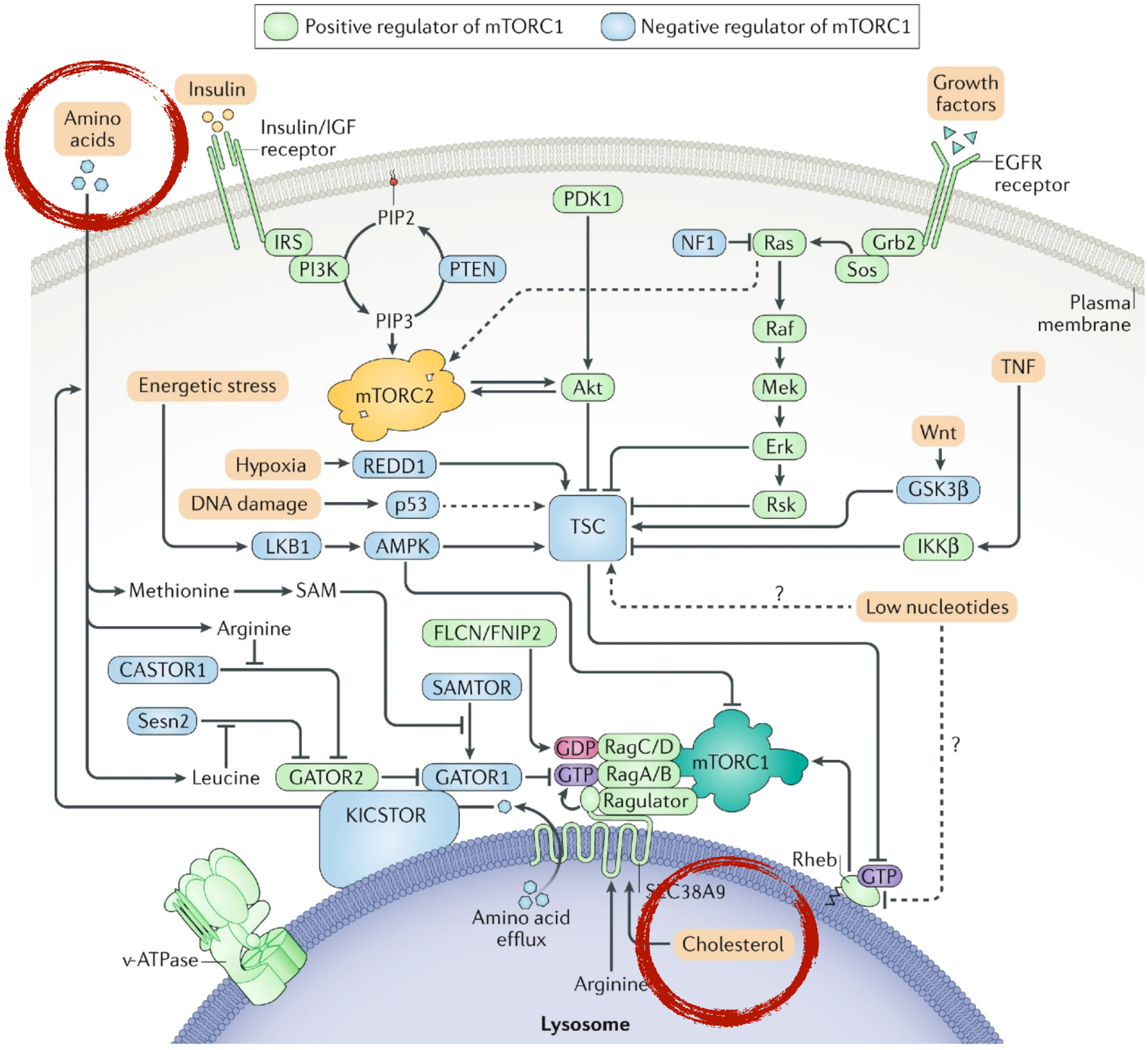
…. lots of things!

What is sensed by RAG-mTORC1?



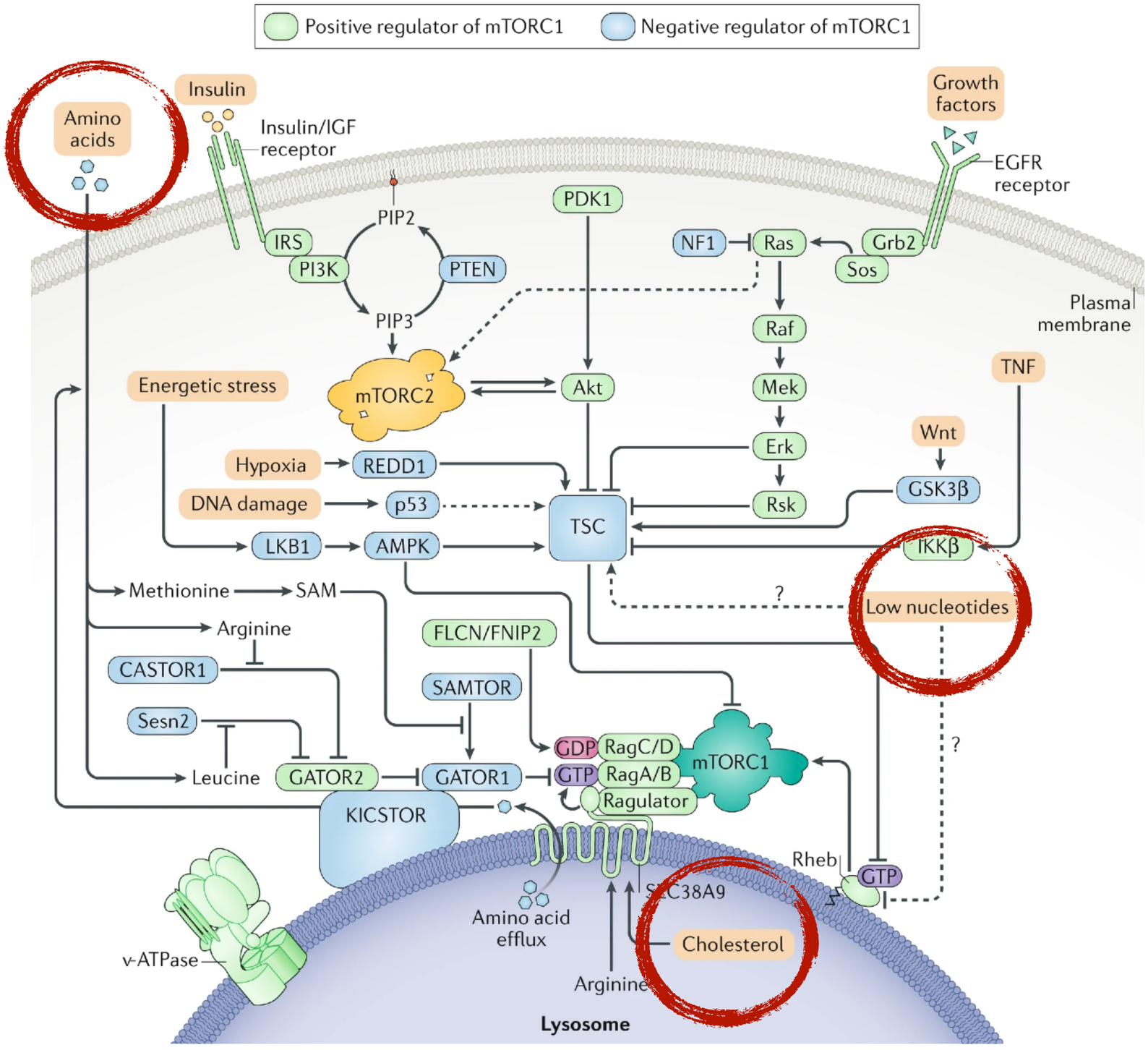
…. lots of things!

What is sensed by RAG-mTORC1?

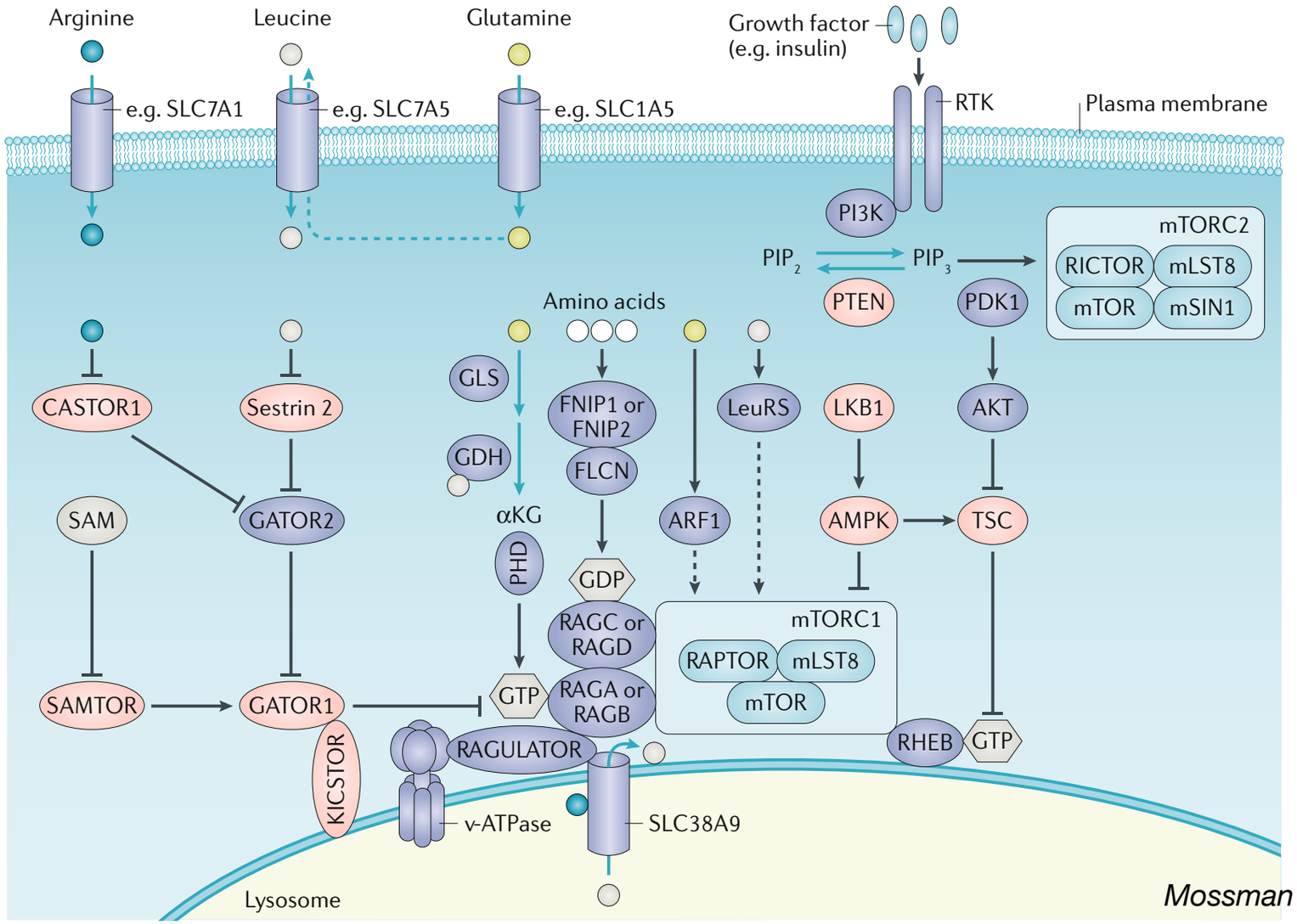


…. lots of things!

What is sensed by RAG-mTORC1?



…. lots of things!



Mossman et al, ***Mat Rev Cancer****, 2018*

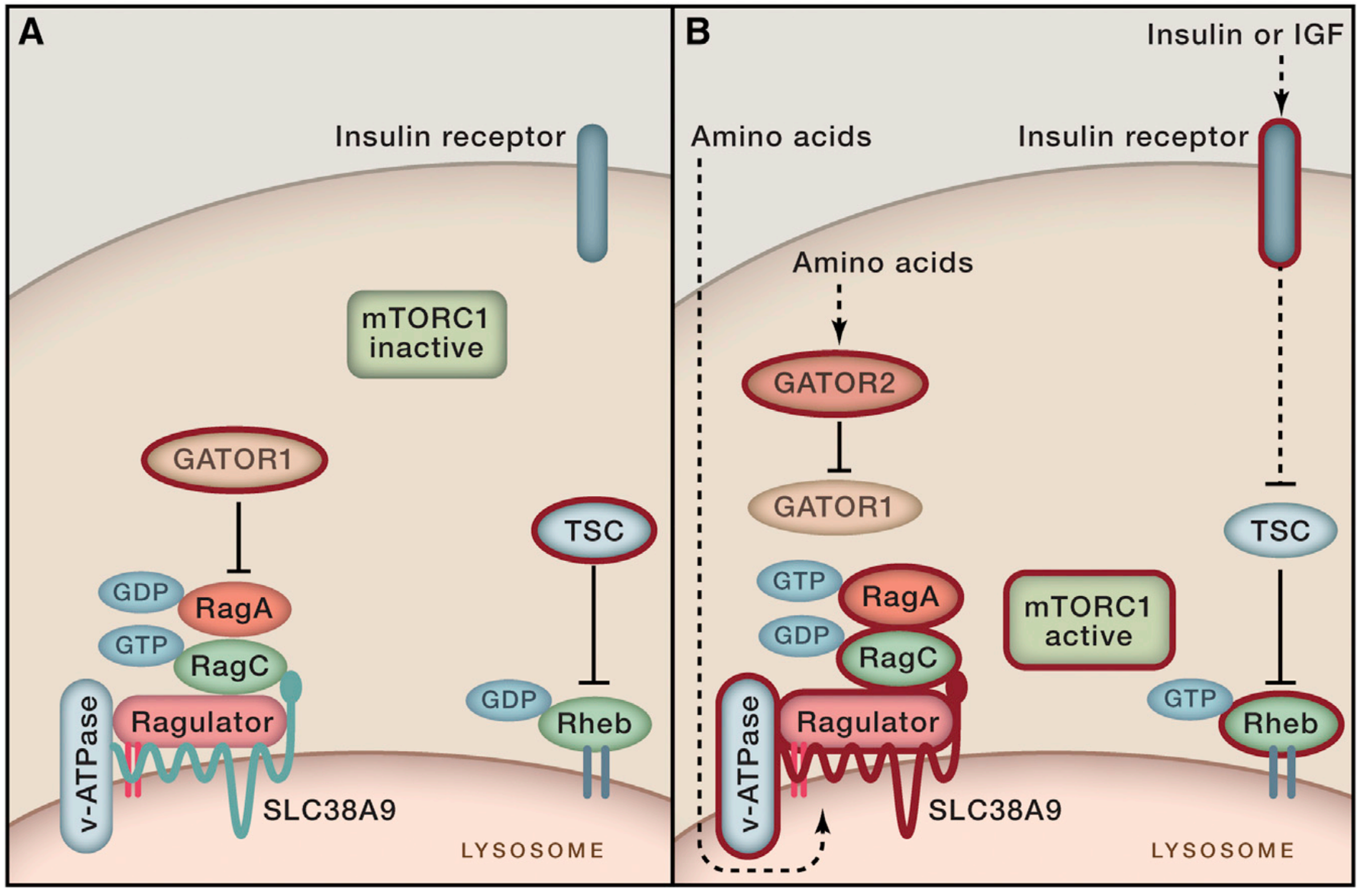
Nutrients, in particular amino acids, promote lysosomal localization of mTORC1 via the RAS-related GTP-binding proteins (RAGs), thereby enabling mTORC1 to encounter RHEB.

RAGs are small GTPases that form obligate heterodimers. RAGA or RAGB associates with RAGC or RAGD.

In the active state, GTP-bound RAGA or RAGB and GDP-bound RAGC or RAGD bind RAPTOR and thereby recruit mTORC1 to the lysosomal surface.

The nucleotide binding status of the RAGs is tightly regulated by amino acids obtained from intracellular synthesis, protein turnover or extracellular sources via specific transporters.

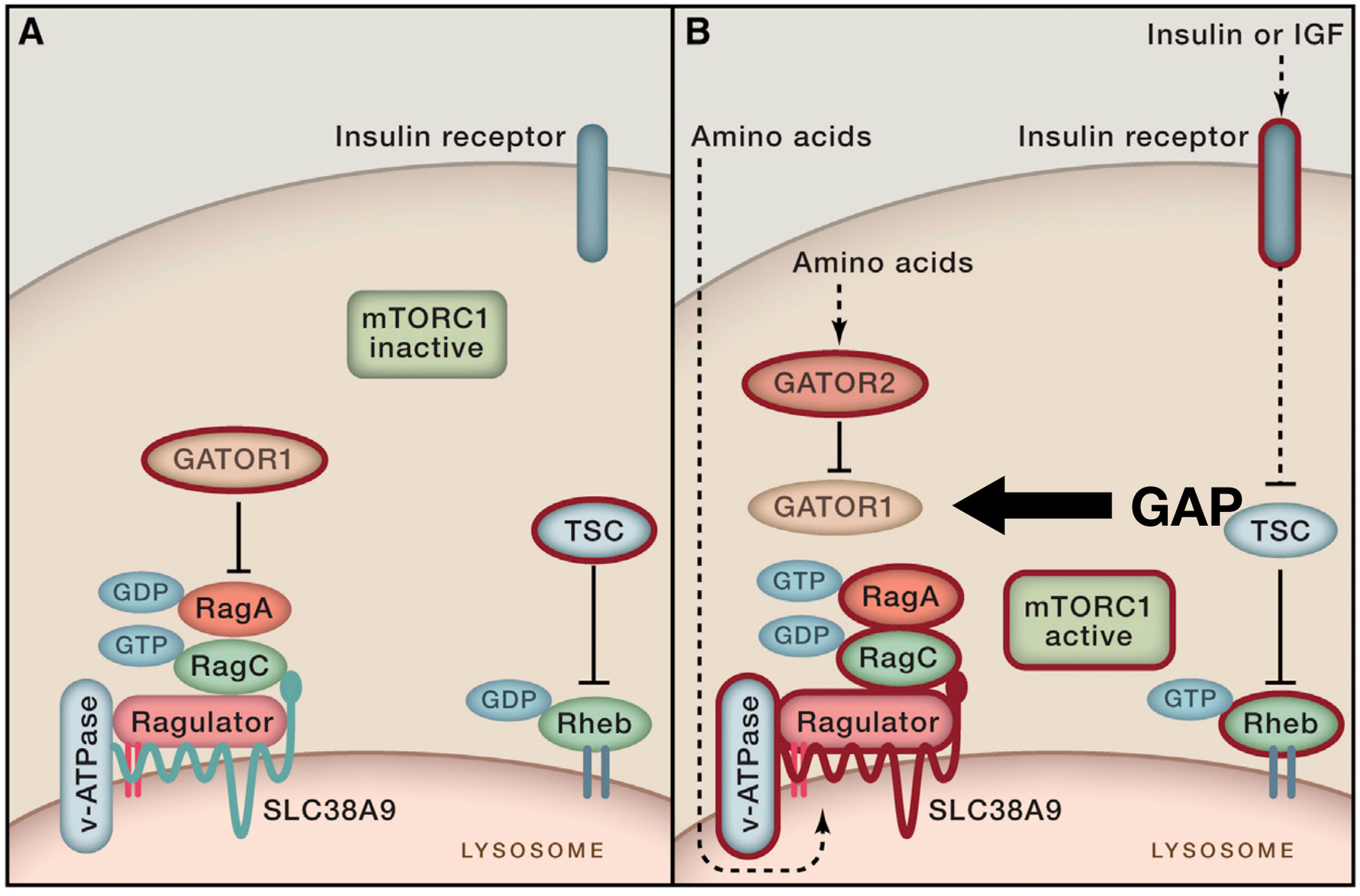
The lysosome is an ideal compartment to sense anabolic demands and activate mTORC1



(A) In the absence of amino acids and growth factors, mTORC1 is inactive. This is controlled by two separate signaling pathways. First, GATOR1 is an active GAP toward RagA, causing it to become GDP bound. In this state, mTORC1 does not localize to the lysosomal surface.

(B) In the presence of amino acids and growth factors, mTORC1 is active. Amino acids within the lysosome signal through SLC38A9 to activate the amino acid sensing branch. Ragulator is active, causing RagA to be GTP bound. This binding state is reinforced by the fact that GATOR1 is inactive in the presence of amino acids, as GATOR2 inhibits it. The Rag heterodimer in this nucleotide conformation state recruits mTORC1 to the lysosomal surface.

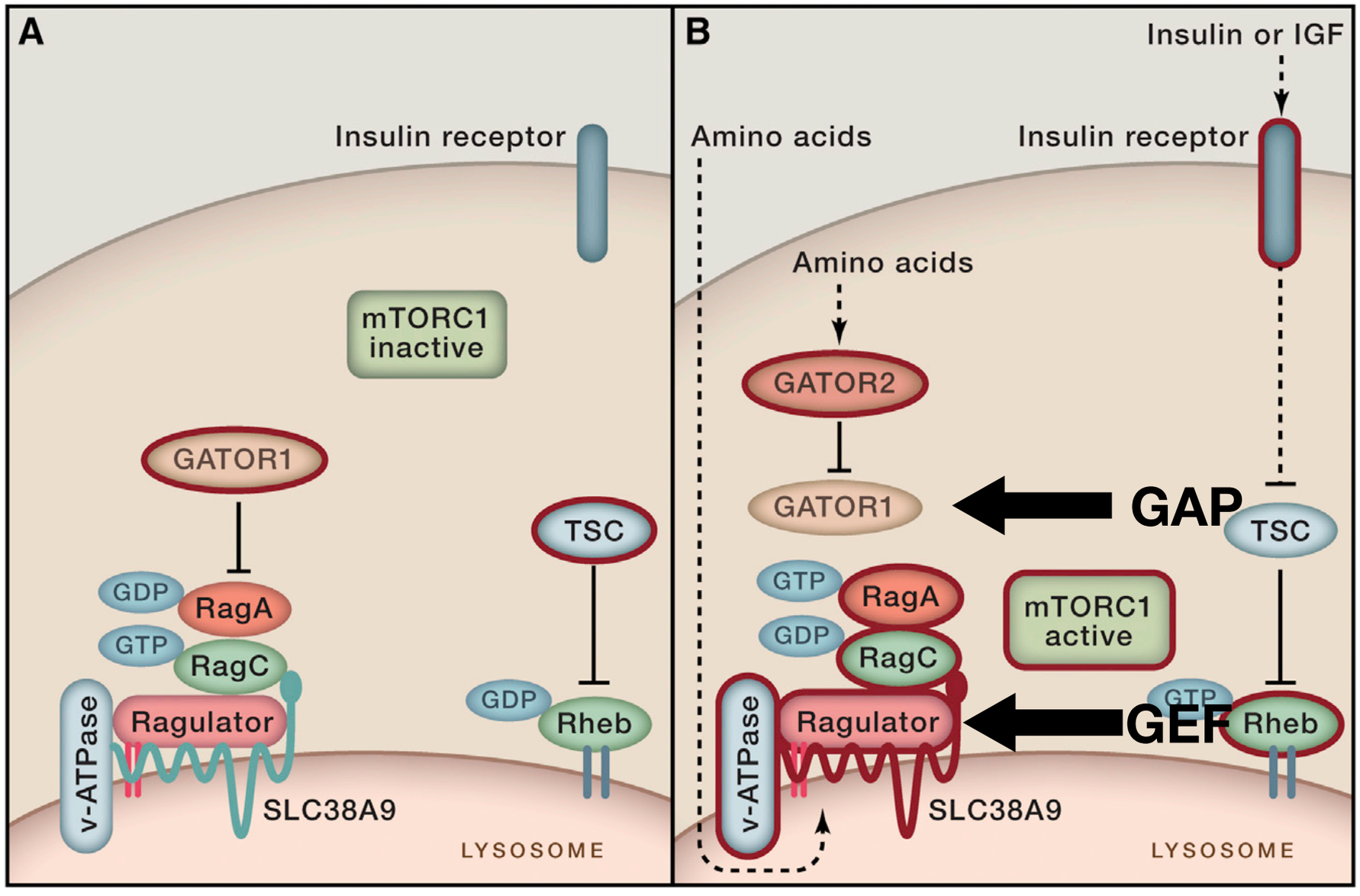
The lysosome is an ideal compartment to sense anabolic demands and activate mTORC1



(A) In the absence of amino acids and growth factors, mTORC1 is inactive. This is controlled by two separate signaling pathways. First, GATOR1 is an active GAP toward RagA, causing it to become GDP bound. In this state, mTORC1 does not localize to the lysosomal surface.

(B) In the presence of amino acids and growth factors, mTORC1 is active. Amino acids within the lysosome signal through SLC38A9 to activate the amino acid sensing branch. Ragulator is active, causing RagA to be GTP bound. This binding state is reinforced by the fact that GATOR1 is inactive in the presence of amino acids, as GATOR2 inhibits it. The Rag heterodimer in this nucleotide conformation state recruits mTORC1 to the lysosomal surface.

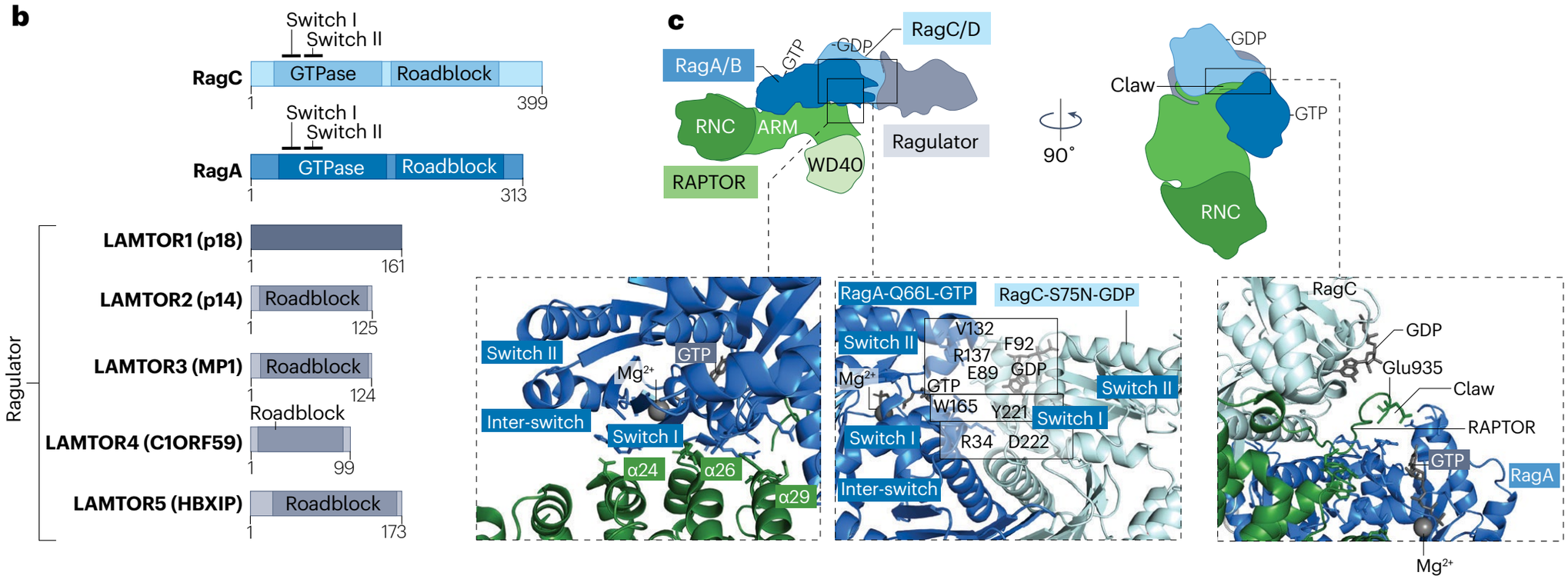
The lysosome is an ideal compartment to sense anabolic demands and activate mTORC1



(A) In the absence of amino acids and growth factors, mTORC1 is inactive. This is controlled by two separate signaling pathways. First, GATOR1 is an active GAP toward RagA, causing it to become GDP bound. In this state, mTORC1 does not localize to the lysosomal surface.

(B) In the presence of amino acids and growth factors, mTORC1 is active. Amino acids within the lysosome signal through SLC38A9 to activate the amino acid sensing branch. Ragulator is active, causing RagA to be GTP bound. This binding state is reinforced by the fact that GATOR1 is inactive in the presence of amino acids, as GATOR2 inhibits it. The Rag heterodimer in this nucleotide conformation state recruits mTORC1 to the lysosomal surface.

Sensing of amino acids by mTORC1 is mediated by RAGs-Ragulator complex

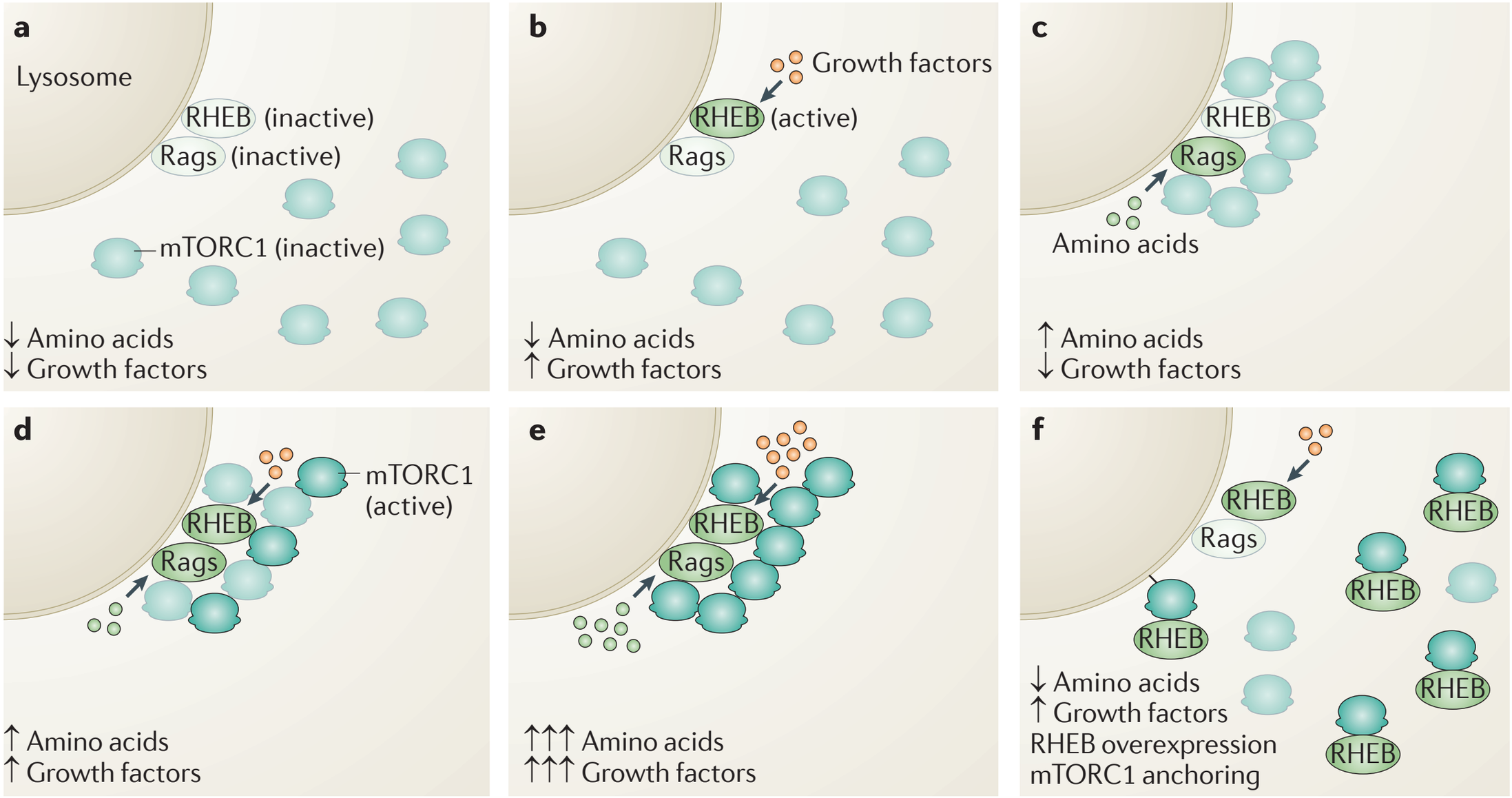


The Rag GTPases are anchored to the lysosome by the pentameric Ragulator complex, which is composed of late endosomal/lysosomal adaptor and MAPK and MTOR activator 1 (LAMTOR1; also known as p18), LAMTOR2 (p14), LAMTOR3, LAMTOR4 and LAMTOR5

SLC38A9 is specifically required for mTORC1 activation by Arg present within the lysosome lumen. Arg is not a substrate of SLC38A9 but, rather, allosterically promotes the interaction of SLC38A9 with Ragulator– Rag GTPases, thereby contributing to switching or stabilizing RagA/B to the active (mTORC1-binding) state. Moreover, through SLC38A9, Arg stimulates the efflux of Leu and other non-polar essential amino acids from the lysosome lumen.

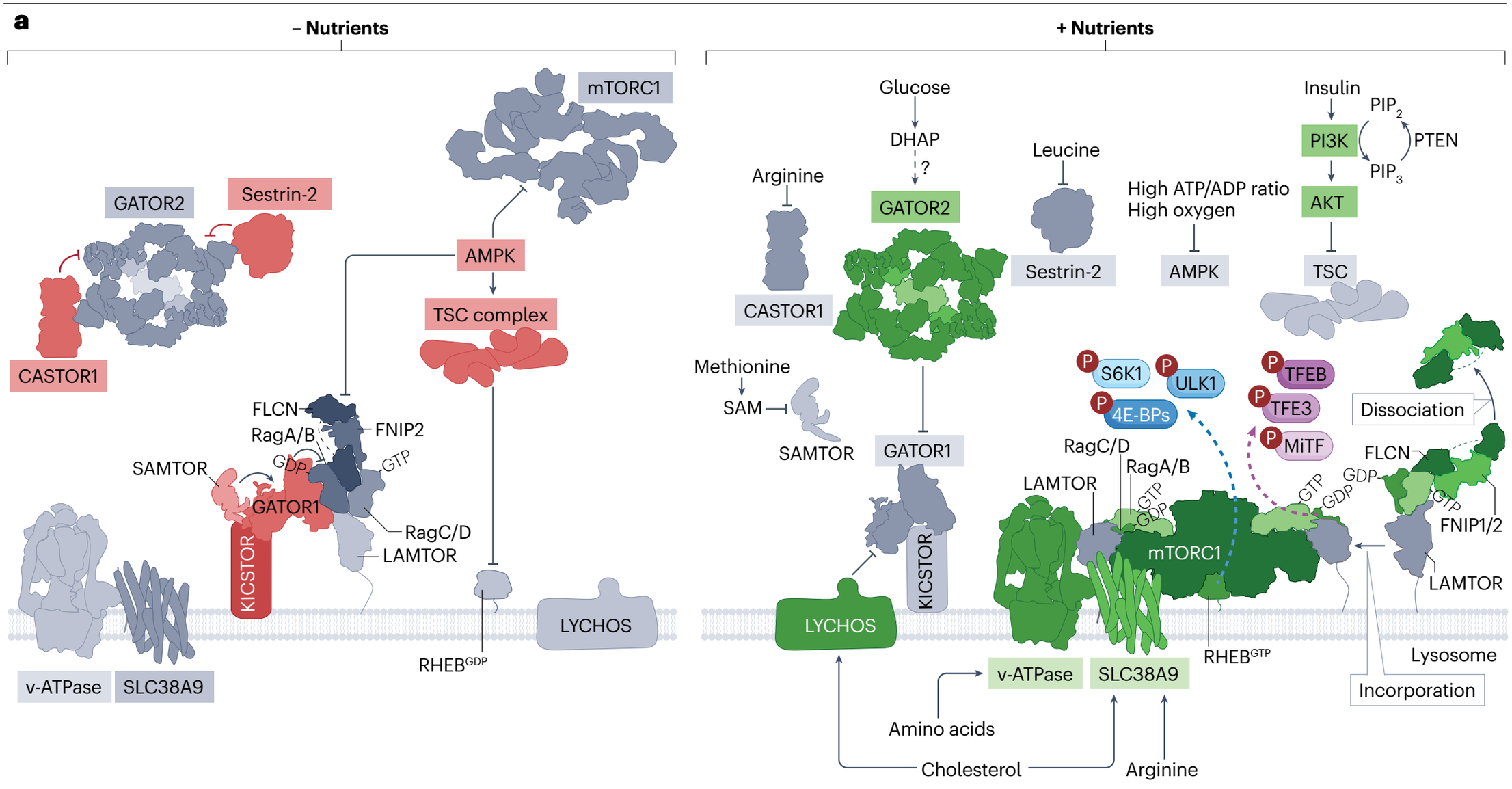
Goul, Peruzzo & Zoncu, ***NRMCB****, 2023*

mTORC1 activation



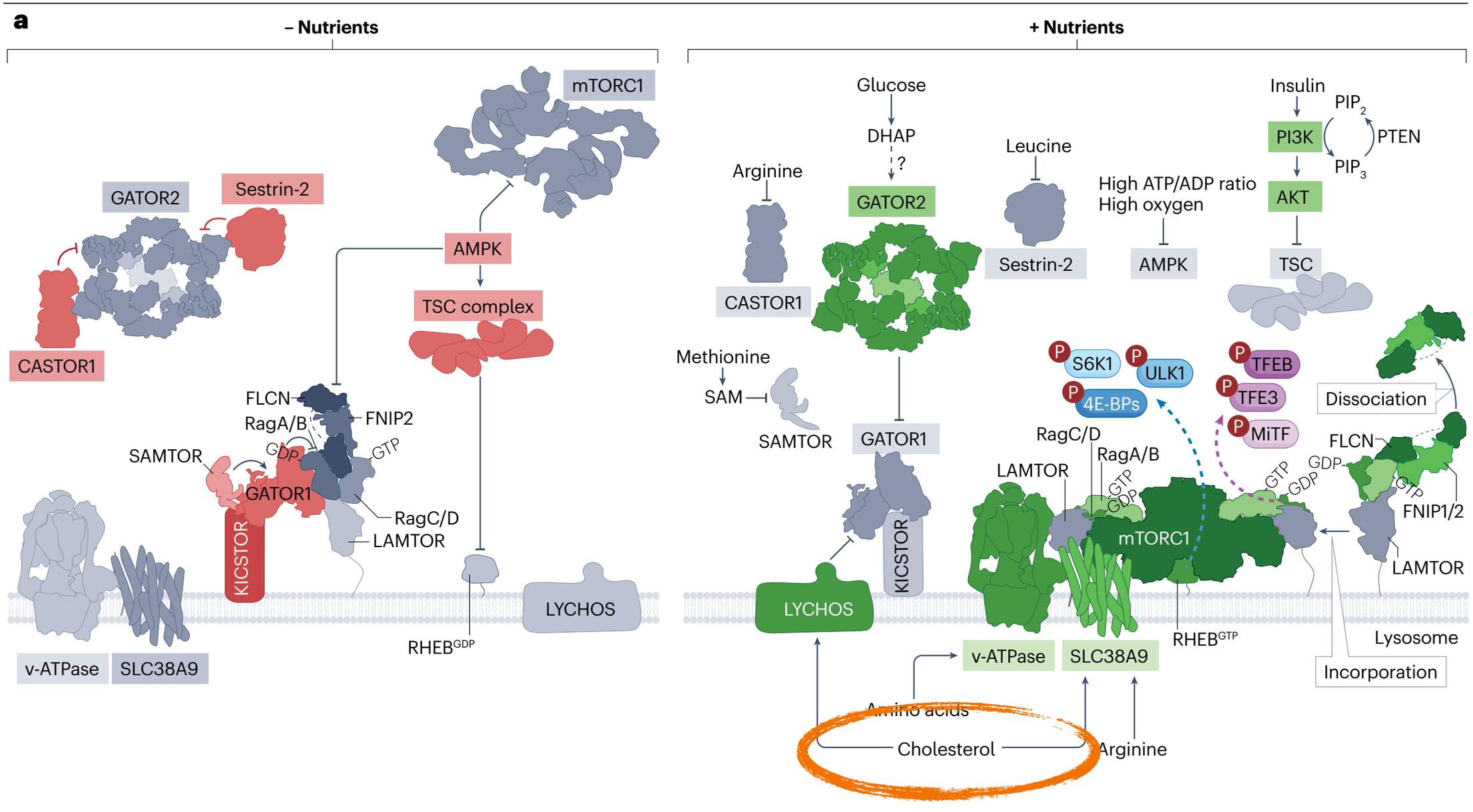
Zoncu et al, ***NRMCB****, 2011*

What is sensed by RAG-mTORC1?



Goul, Peruzzo & Zoncu, ***NRMCB****, 2023*

What is sensed by RAG-mTORC1?

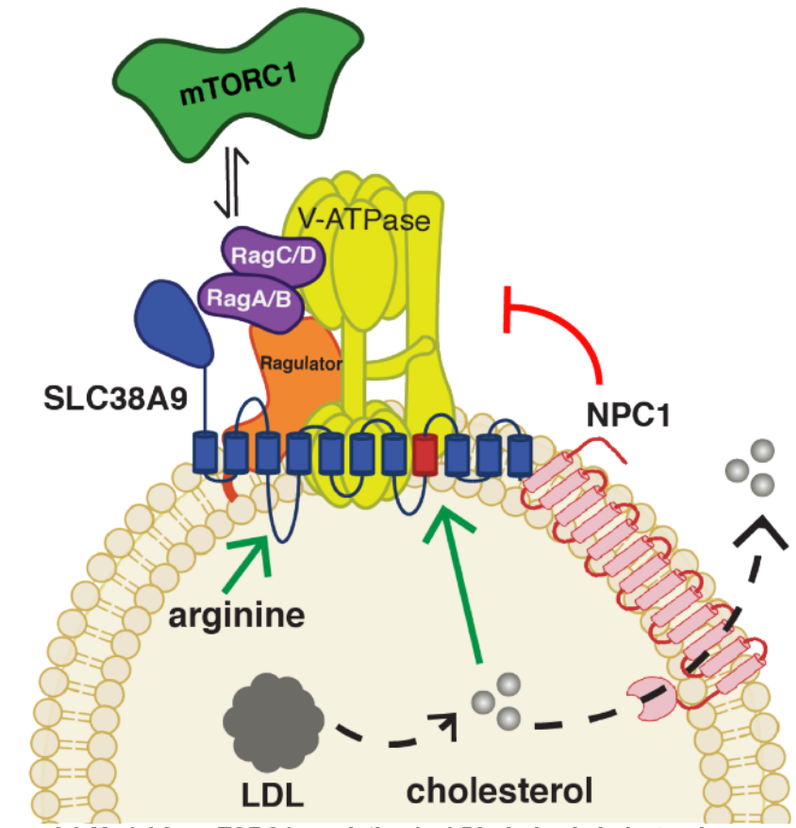


*Goul, Peruzzo & Zoncu, **NRMCB**, 2023*

CHOLESTEROL SENSING

Lysosomal cholesterol activates mTORC1 via an SLC38A9–Niemann-Pick C1 signaling complex

Castellano et al, ***Science****, 2017*

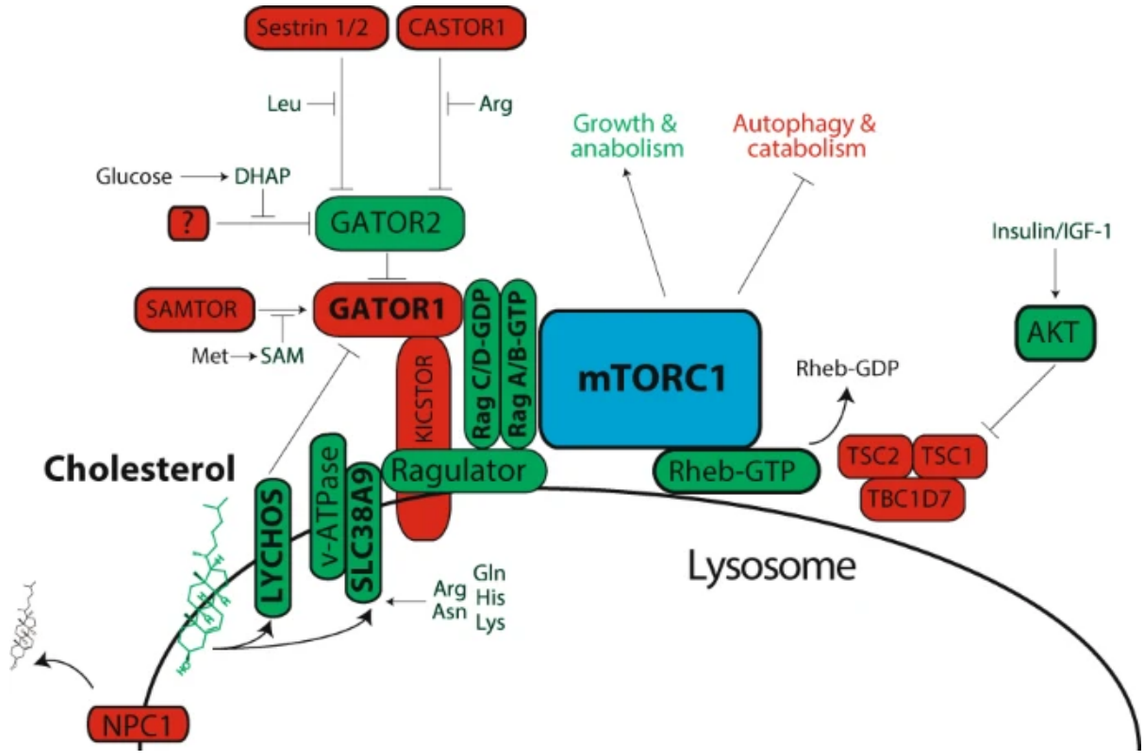


ıure 2.9 Model for mTORC1 regulation by LDL-derived cholesterol

SIGNAL TRANSDUCTION

Lysosomal GPCR-like protein LYCHOS signals cholesterol sufficiency to mTORC1

Shin et al, ***Science****, 2022*



The nucleotide-binding state of the Rags is controlled by protein complexes including the Ragulator, a GEF for RagA and RagB; and GATOR1, a GAP for RagA and RagB.

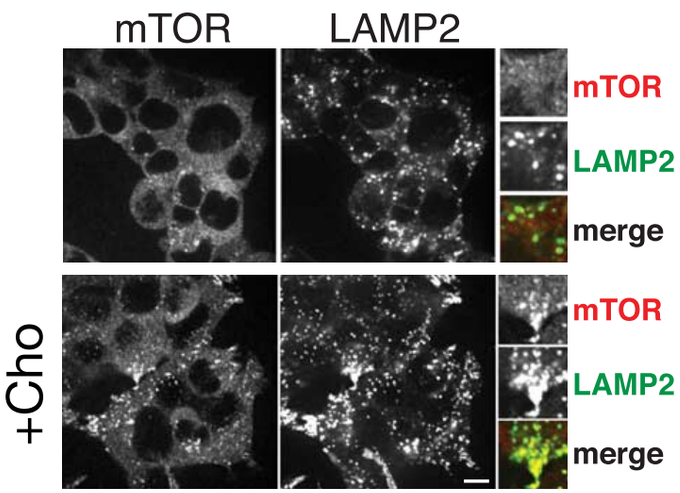
Cholesterol binds to SLC38A9 and regulates the Ragulator-Rag GTPase complex.

Cholesterol is also sensed by the GPCR LYCHOS; when cholesterol is high, LYCHOS activates mTORC1 activity by sequestering GATOR1

CHOLESTEROL SENSING

Lysosomal cholesterol activates mTORC1 via an SLC38A9–Niemann-Pick C1 signaling complex

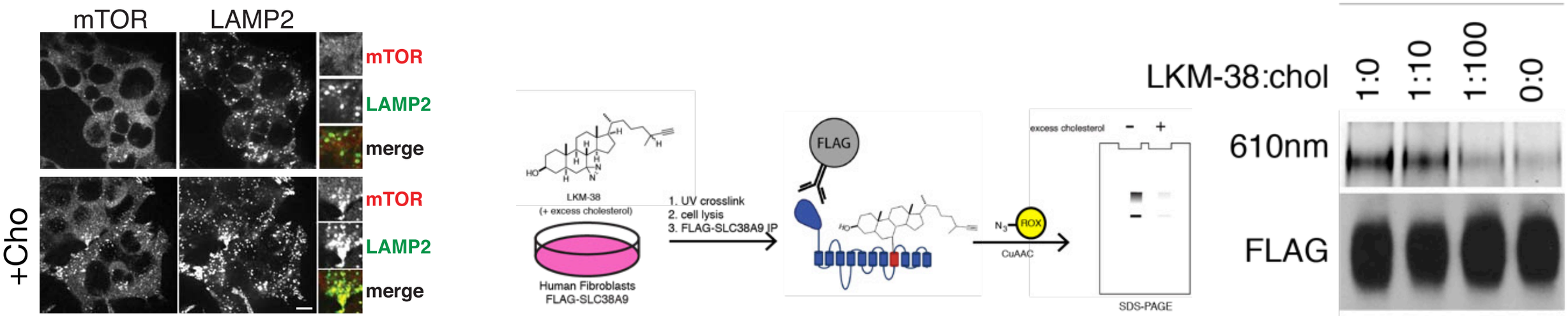
Castellano et al, ***Science****, 2017*



CHOLESTEROL SENSING

Lysosomal cholesterol activates mTORC1 via an SLC38A9–Niemann-Pick C1 signaling complex

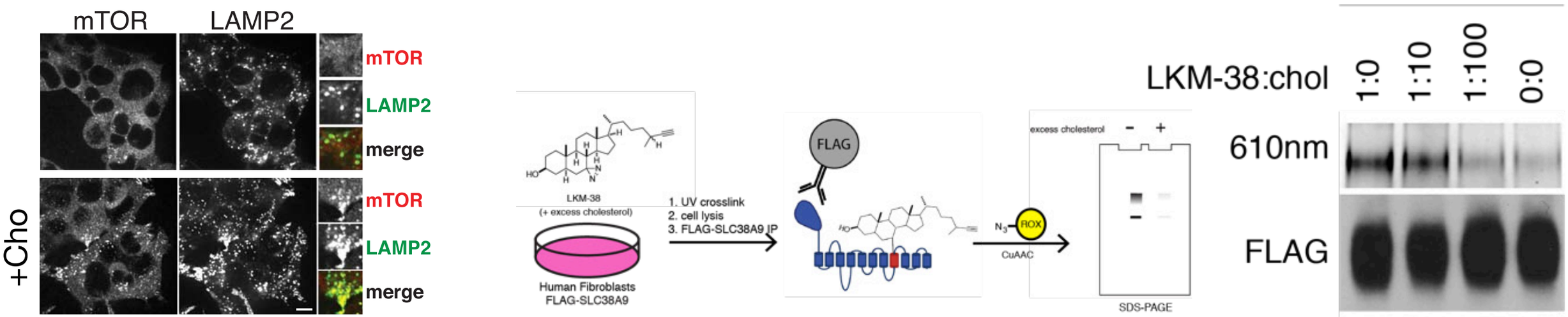
Castellano et al, ***Science****, 2017*

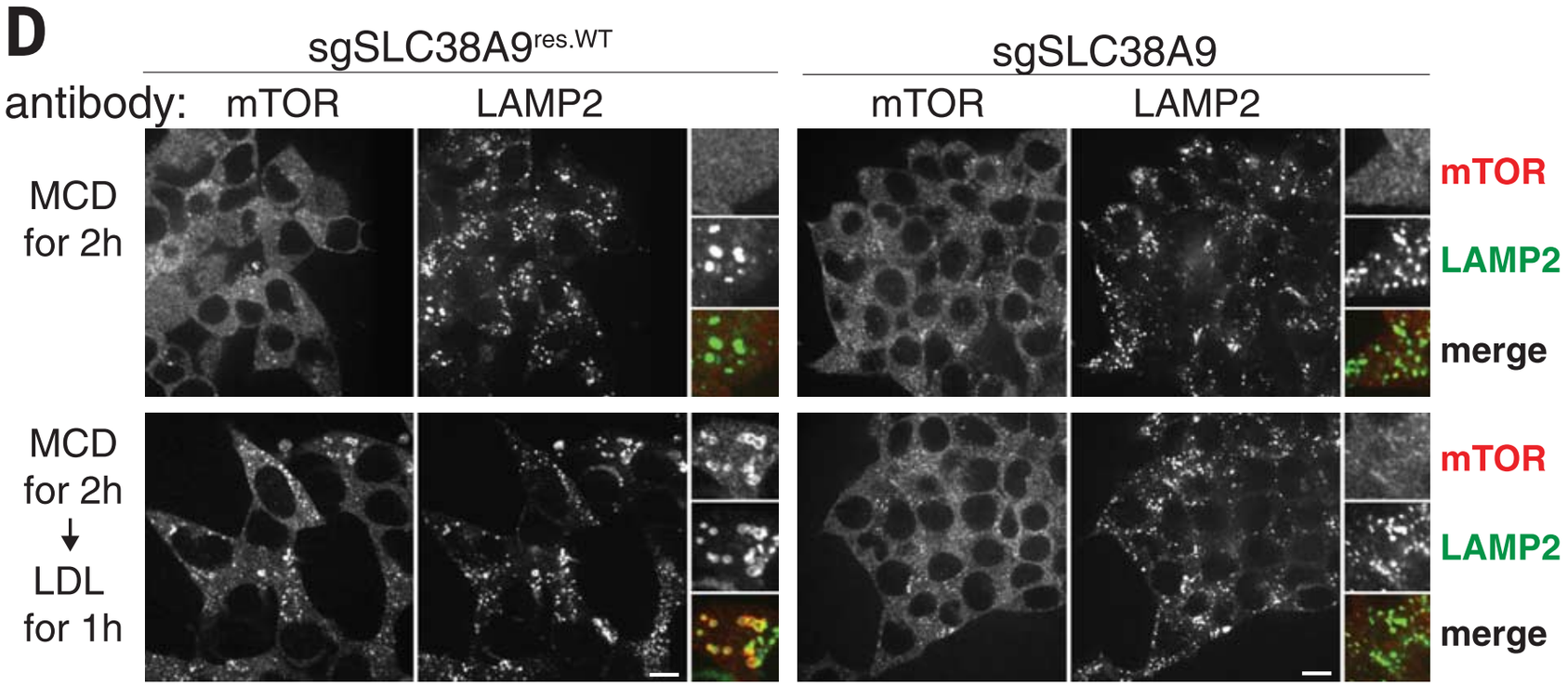


CHOLESTEROL SENSING

Lysosomal cholesterol activates mTORC1 via an SLC38A9–Niemann-Pick C1 signaling complex

Castellano et al, ***Science****, 2017*





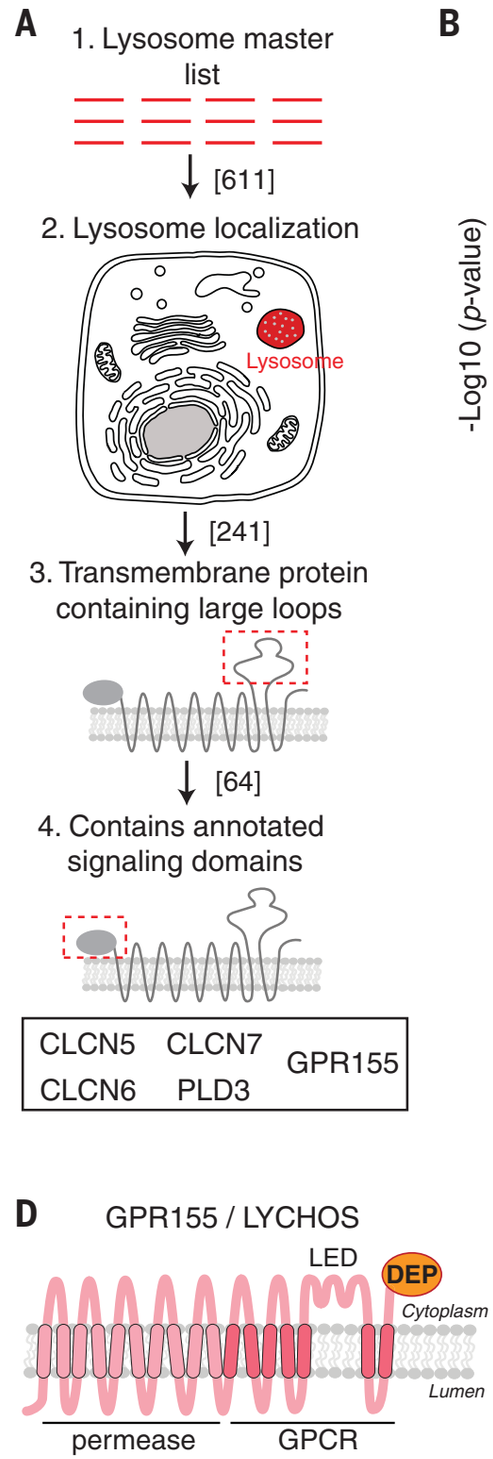
SIGNAL TRANSDUCTION

Lysosomal GPCR-like protein LYCHOS signals cholesterol sufficiency to mTORC1

Shin et al, ***Science****, 2022*

One important player is the lysosomal transmembrane protein, SLC38A9, which participates in cholesterol- dependent activation of mTORC1 through conserved sterol-interacting motifs within its transmembrane domains. However, SLC38A9 primarily relays arginine abundance to mTORC1, whereas a dedicated sensor for cholesterol remains to be identified.

More generally, it is likely that the lysosome has as yet undiscovered nutrient sensors that could regulate cellular metabolism through mTORC1-dependent or independent pathways.



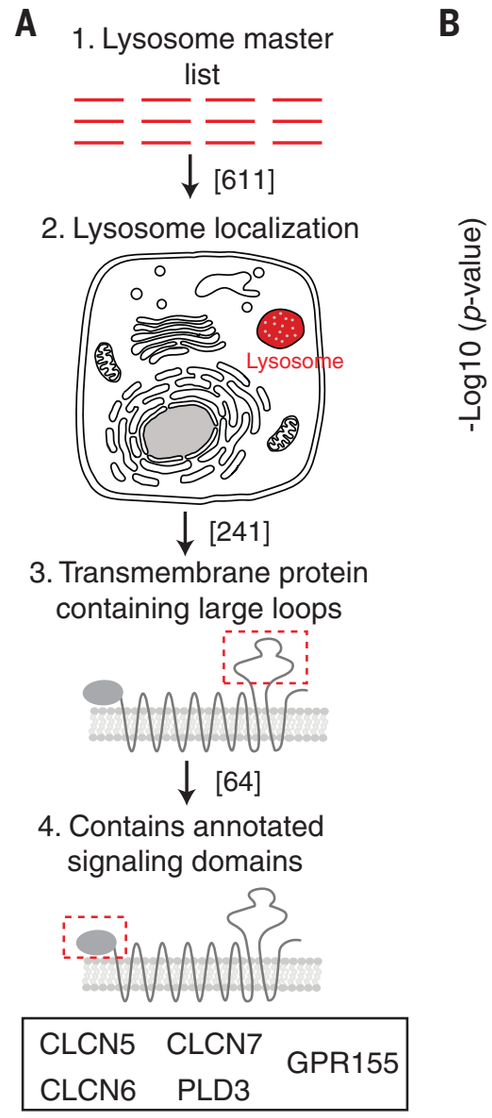
SIGNAL TRANSDUCTION

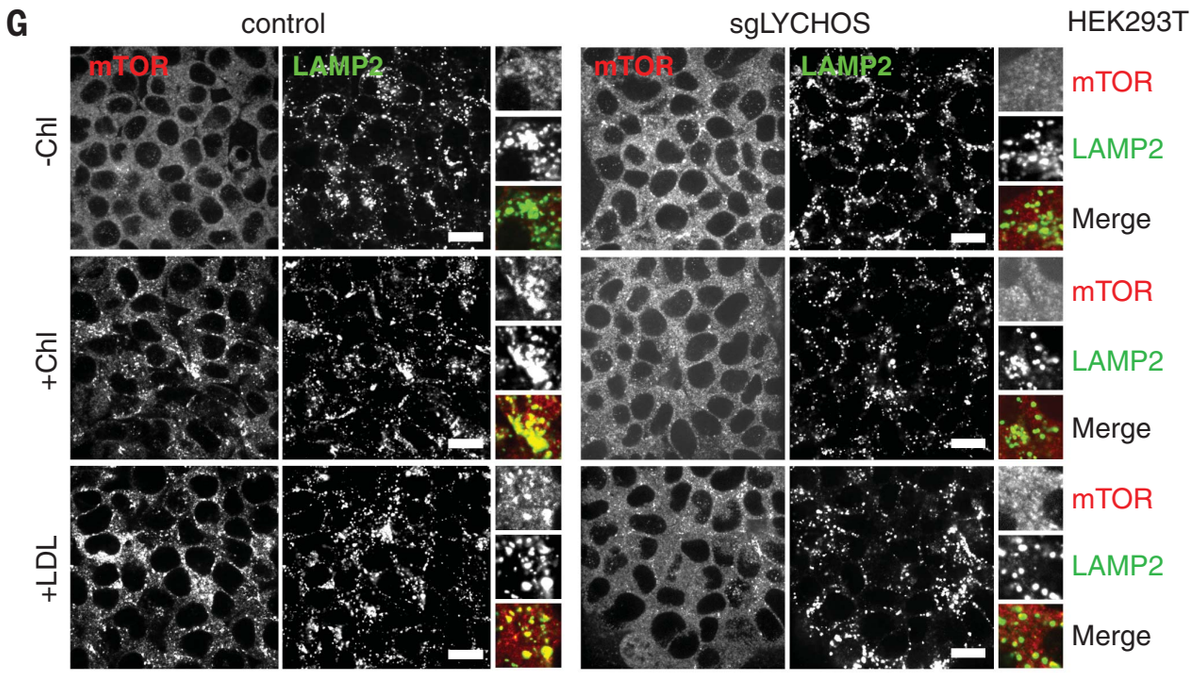
Lysosomal GPCR-like protein LYCHOS signals cholesterol sufficiency to mTORC1

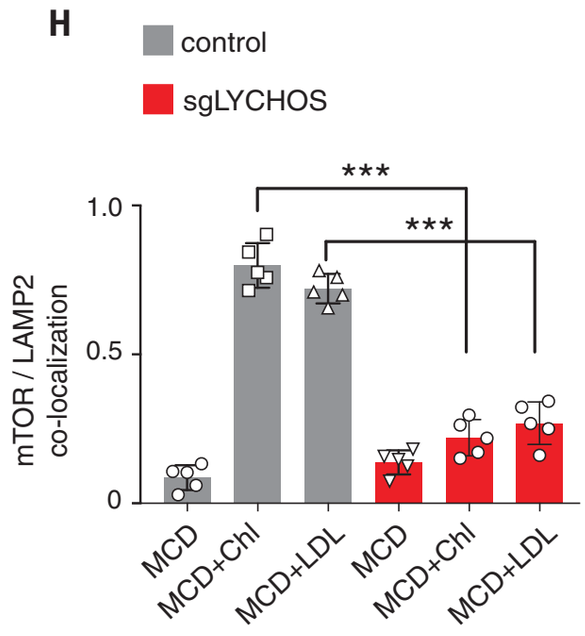
Shin et al, ***Science****, 2022*

One important player is the lysosomal transmembrane protein, SLC38A9, which participates in cholesterol- dependent activation of mTORC1 through conserved sterol-interacting motifs within its transmembrane domains. However, SLC38A9 primarily relays arginine abundance to mTORC1, whereas a dedicated sensor for cholesterol remains to be identified.

More generally, it is likely that the lysosome has as yet undiscovered nutrient sensors that could regulate cellular metabolism through mTORC1-dependent or independent pathways.



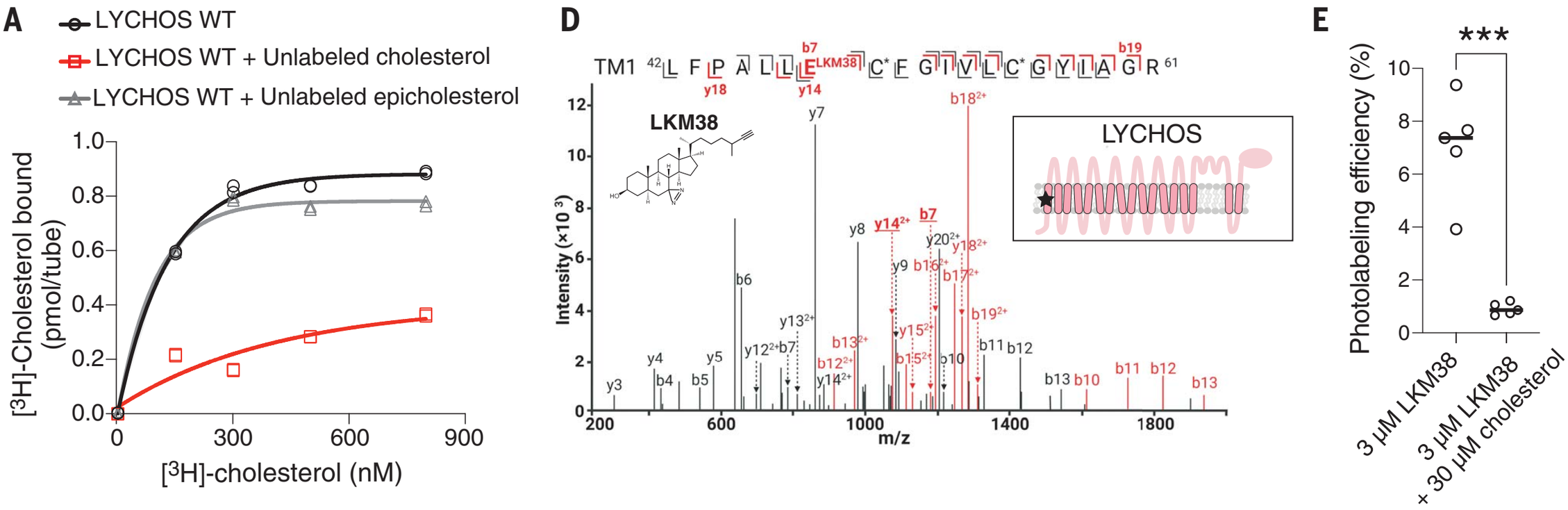


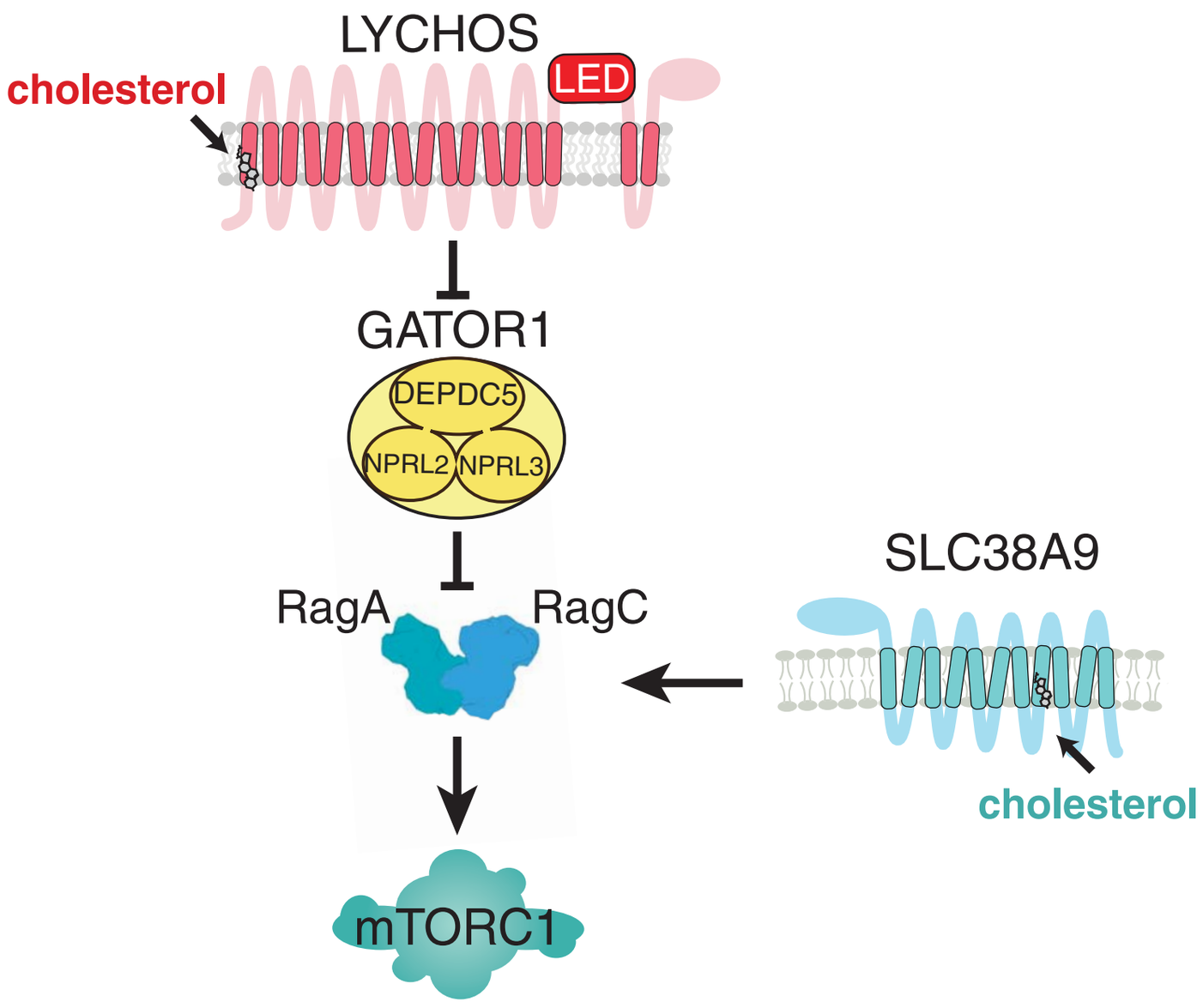


SIGNAL TRANSDUCTION

Lysosomal GPCR-like protein LYCHOS signals cholesterol sufficiency to mTORC1

Shin et al, ***Science****, 2022*



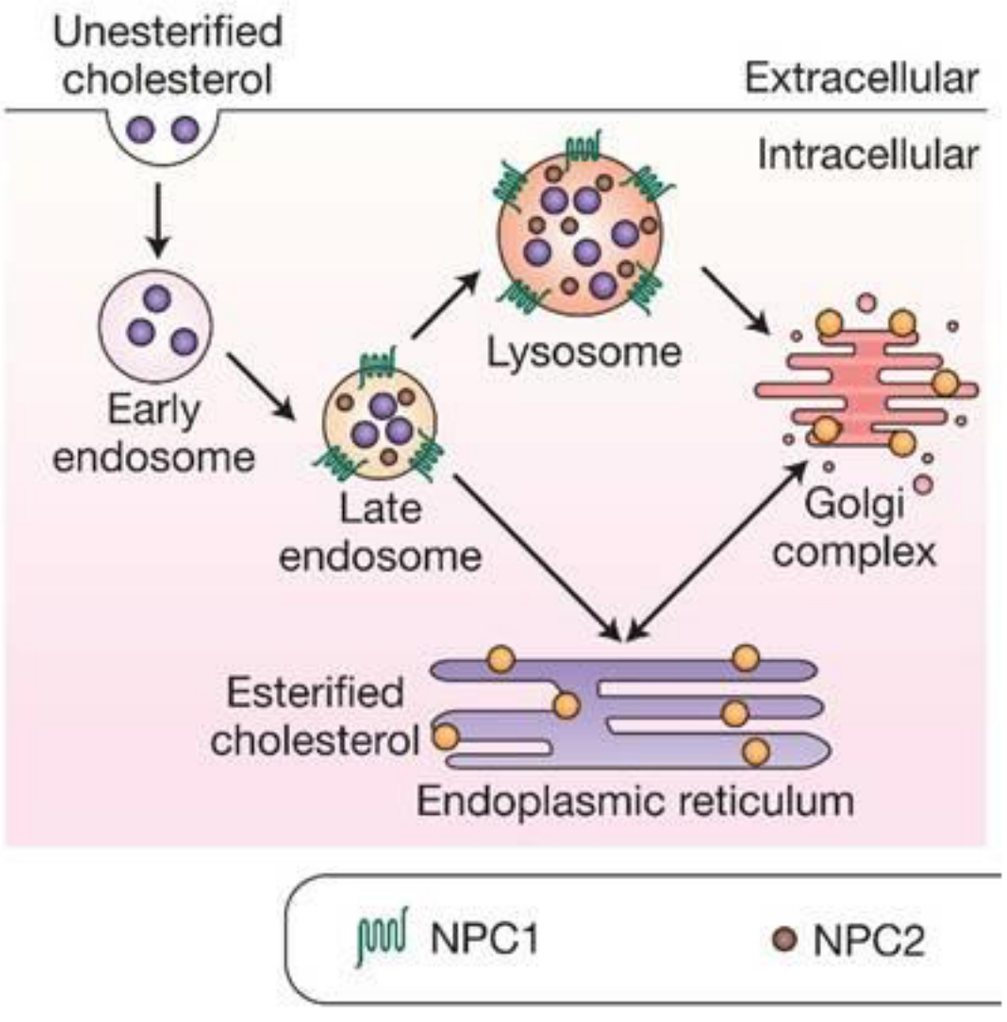


Shin et al, ***Science****, 2022*

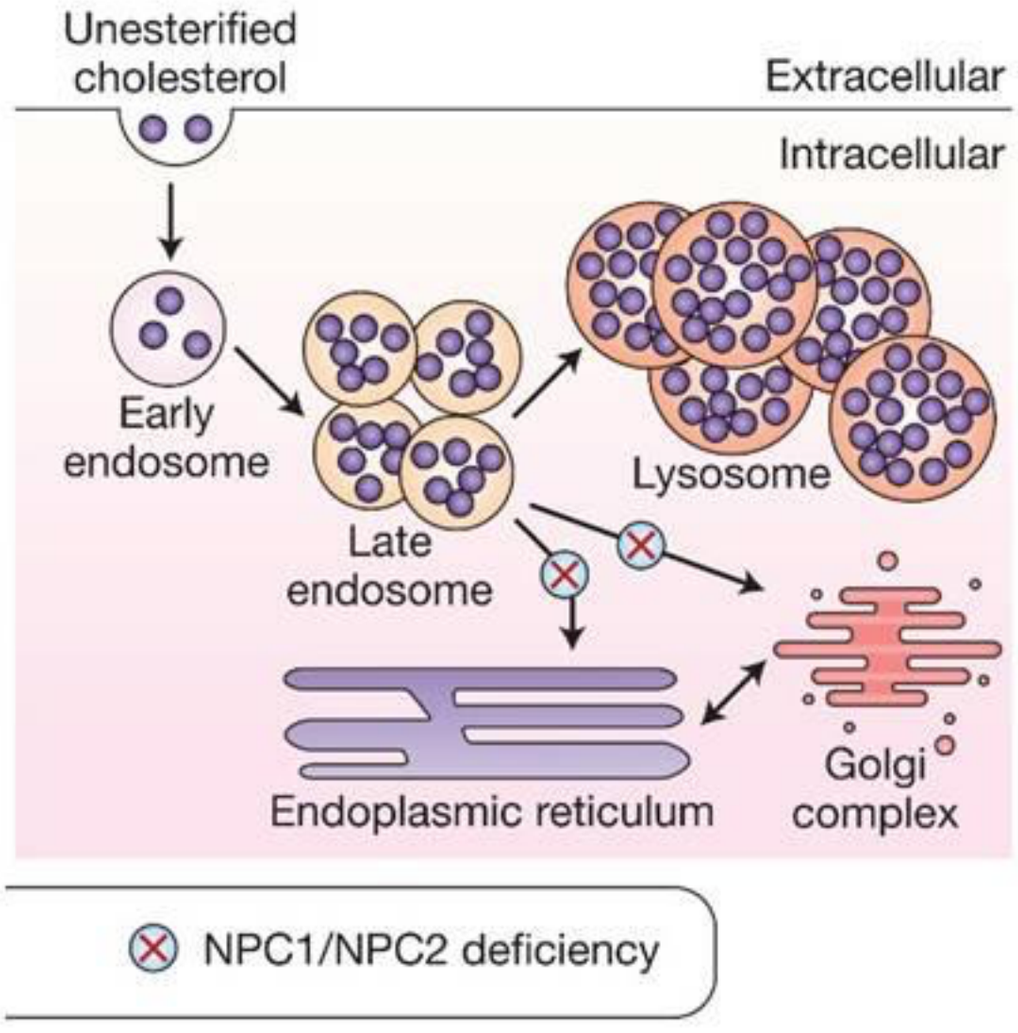
Castellano et al, ***Science****, 2017*

NPC disease –
dysfunction of a cholesterol transport protein NPC1/NPC2

Normal conditions



NPC disease



SYMPTOMS

* PROGRESSIVE NEUROLOGIC
  ~ ALMOST ALL AFFECTED INDIVIDUALS

EARLY INFANTILE ONSET

~ ↓ MUSCLE TONE

~ DELAY in DEVELOPMENTAL MOTOR MILESTONES

→ DEVELOPMENTAL REGRESSION

INFANTILE & CHILDHOOD ONSET

~ CLUMSINESS
~ LEARNING DIFFICULTIES
~ UNSTEADY GAIT
~ DIFFICULTY SWALLOWING
~ SLURRED SPEECH
~ SEIZURES or CATAPLEXY

TEENAGE & ADULT ONSET

~ PSYCHIATRIC SYMPTOMS
~ PROGRESSIVE COGNITIVE IMPAIRMENT

JAUNDICE

~ COMMON in NEWBORNS
~ RARE in OL

Vanier, ***J Rare Dis****, 2010*

SYMPTOMS

* PROGRESSIVE NEUROLOGIC
  - ALMOST ALL AFFECTED INDIVIDUALS

EARLY INFANTILE ONSET

- ↓ MUSCLE TONE
- DELAY in DEVELOPMENTAL MOTOR MILESTONES

→ DEVELOPMENTAL REGRESSION

INFANTILE & CHILDHOOD ONSET

- CLUMSINESS
- LEARNING DIFFICULTIES
- UNSTEADY GAIT
- DIFFICULTY SWALLOWING
- SLURRED SPEECH
- SEIZURES or CATAPLEXY

TEENAGE & ADULT ONSET

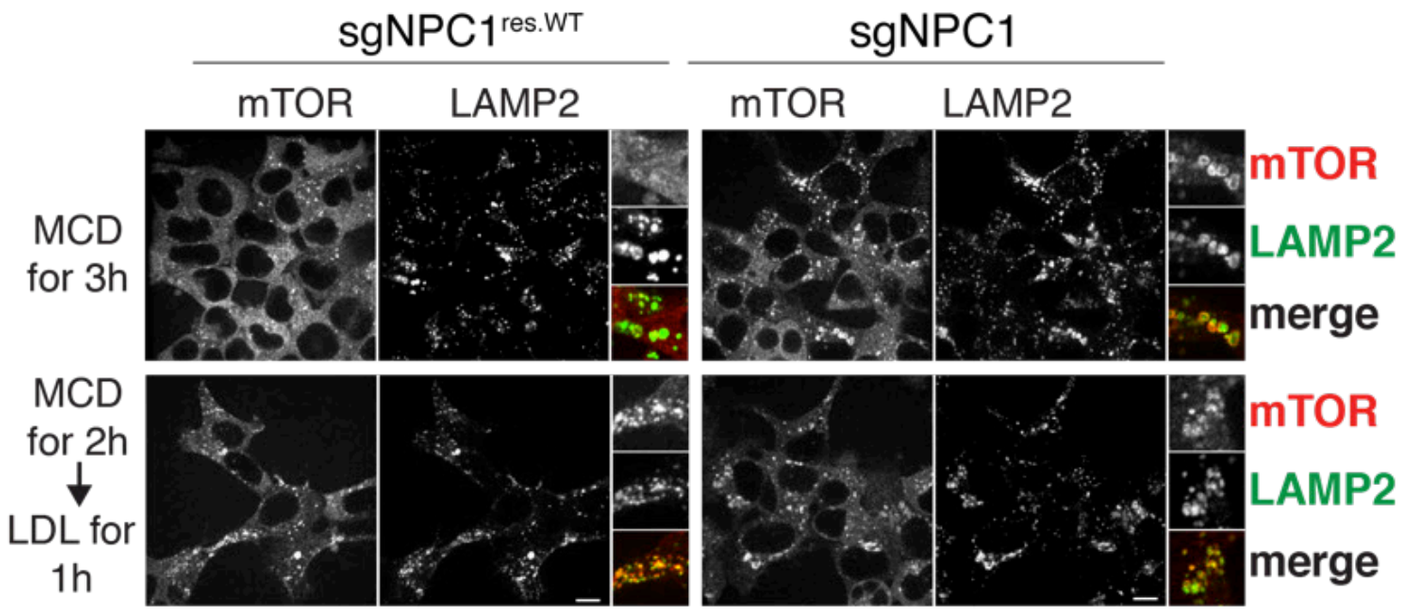
- PSYCHIATRIC SYMPTOMS
- PROGRESSIVE COGNITIVE IMPAIRMENT

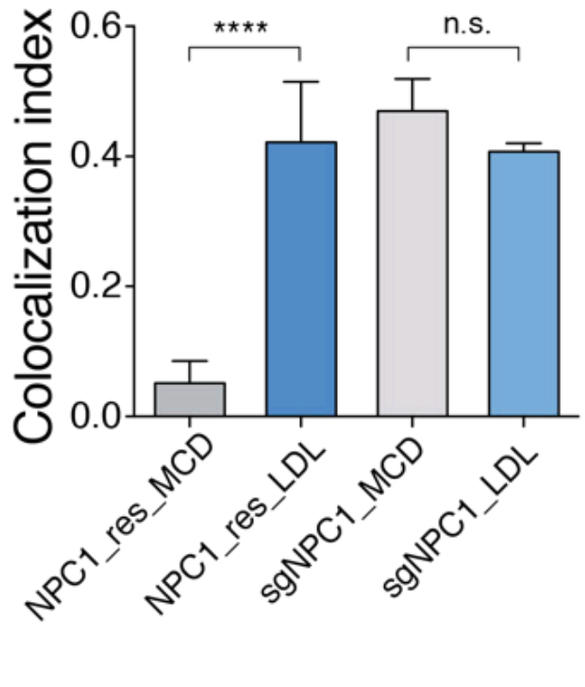
JAUNDICE

- COMMON in NEWBORNS
- RARE in OL

Vanier, ***J Rare Dis****, 2010*

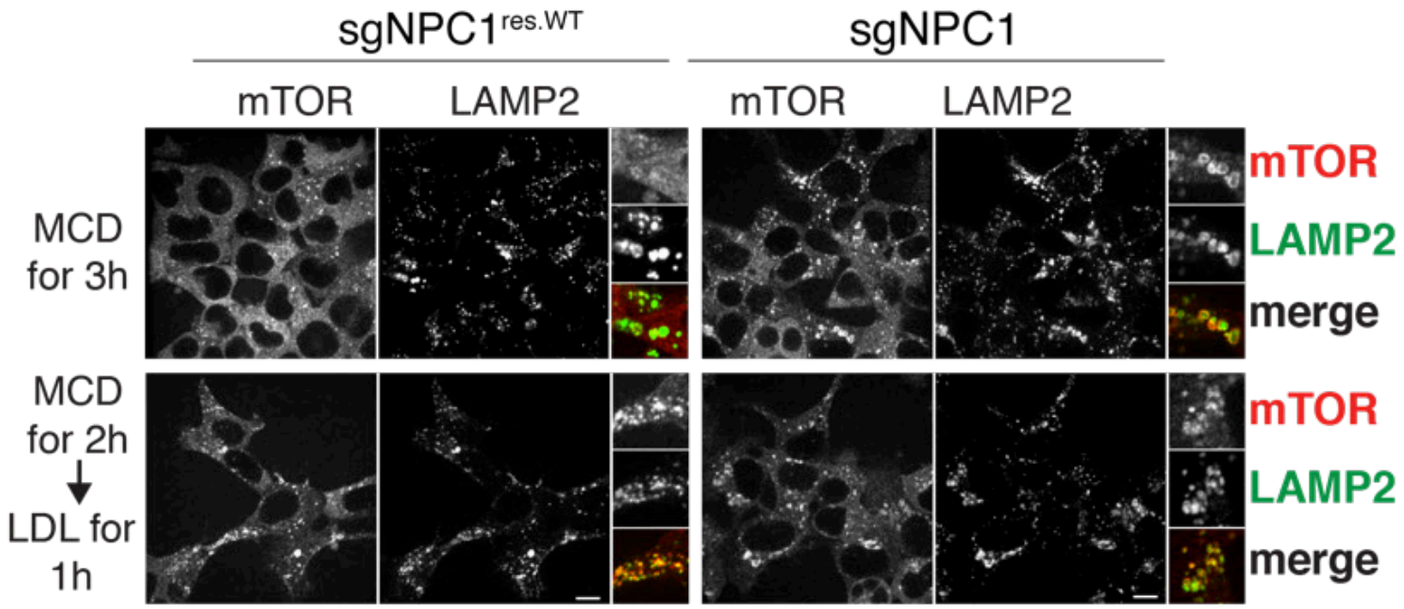
mTORC1 is hyper activated in Niemann-Pick Type C

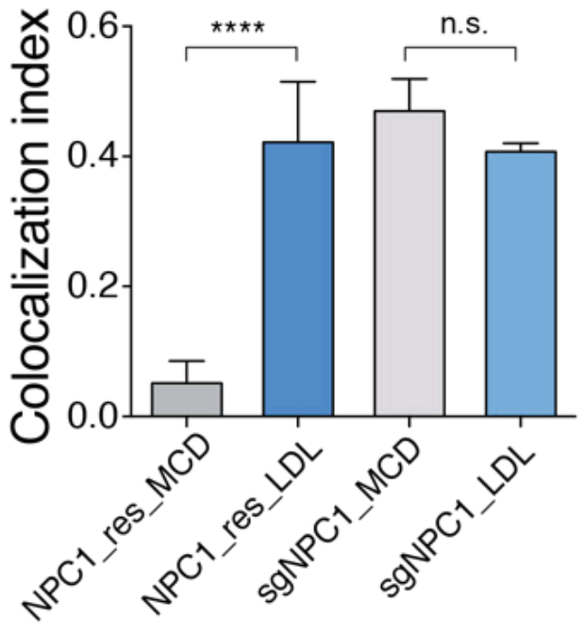


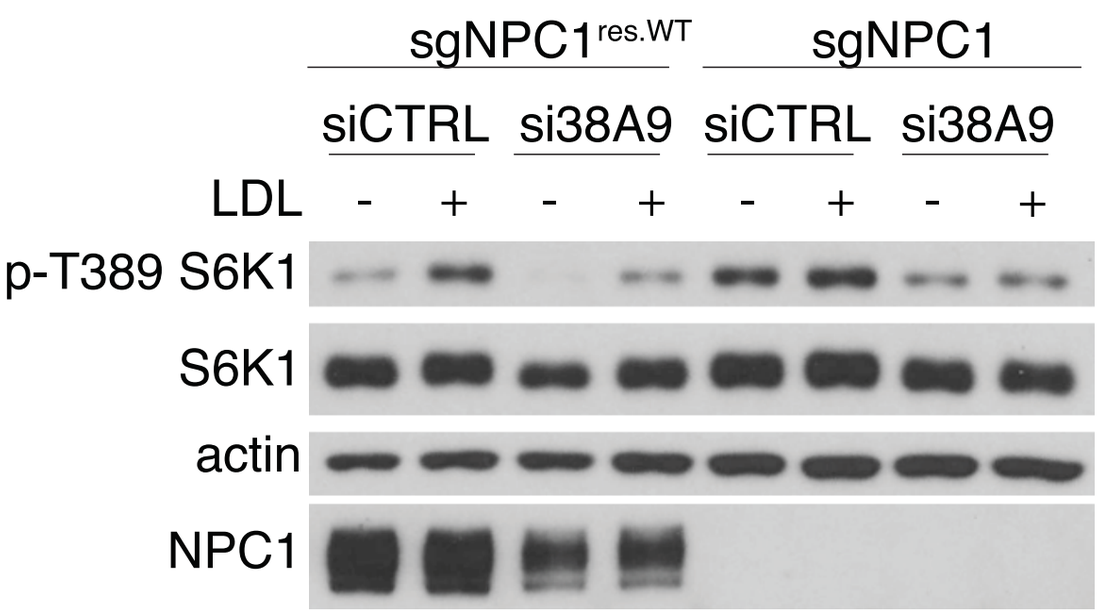


Davies et al, ***Dev Cell****, 2022*
Castellano et al, ***Science****, 2017*

mTORC1 is hyper activated in Niemann-Pick Type C

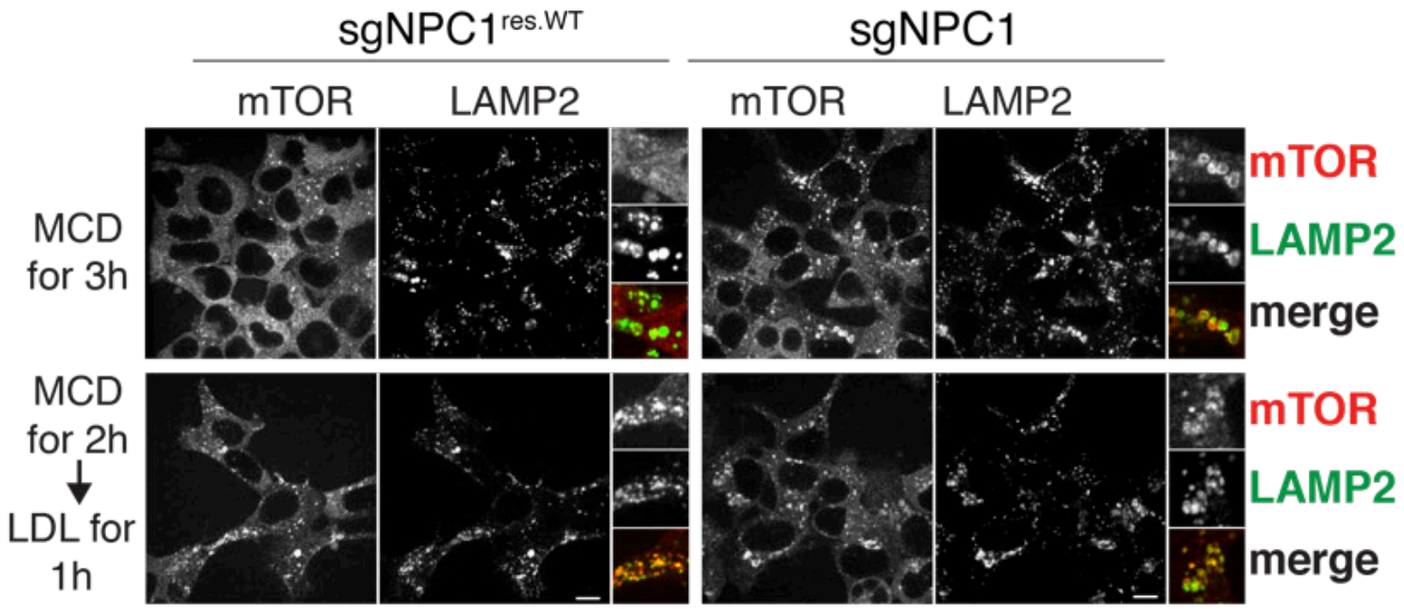


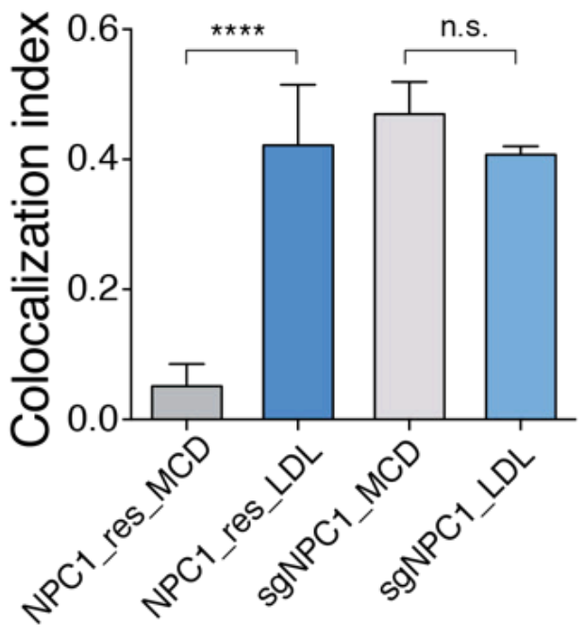


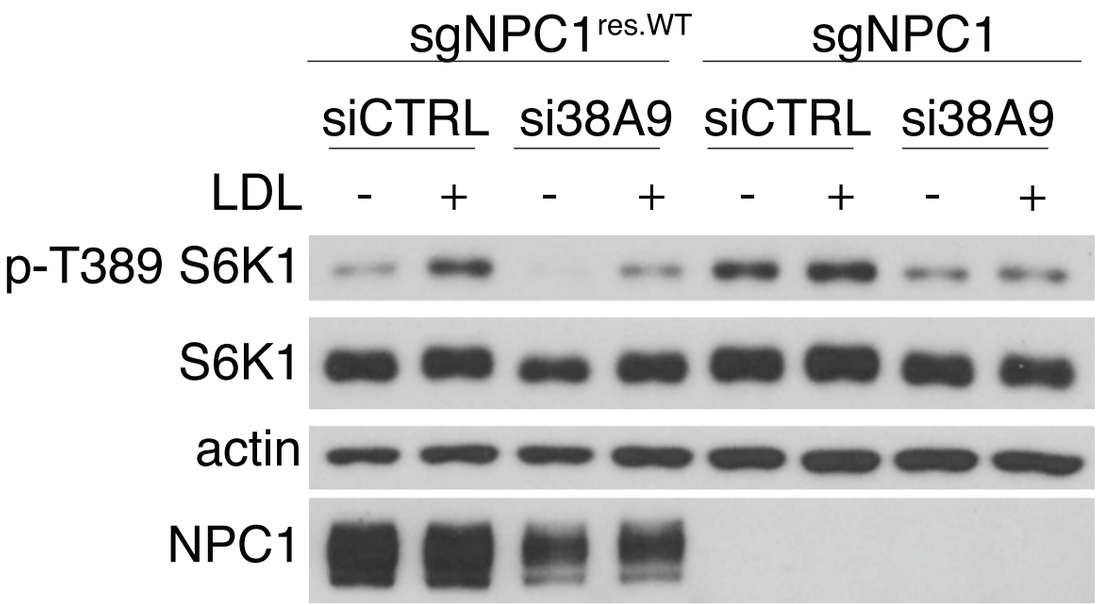


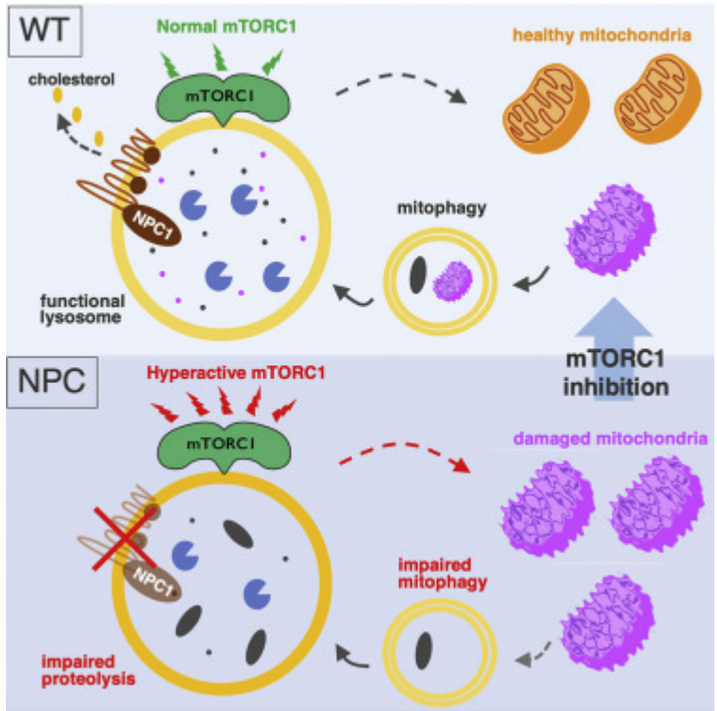
Davies et al, ***Dev Cell****, 2022*
Castellano et al, ***Science****, 2017*

mTORC1 is hyper activated in Niemann-Pick Type C



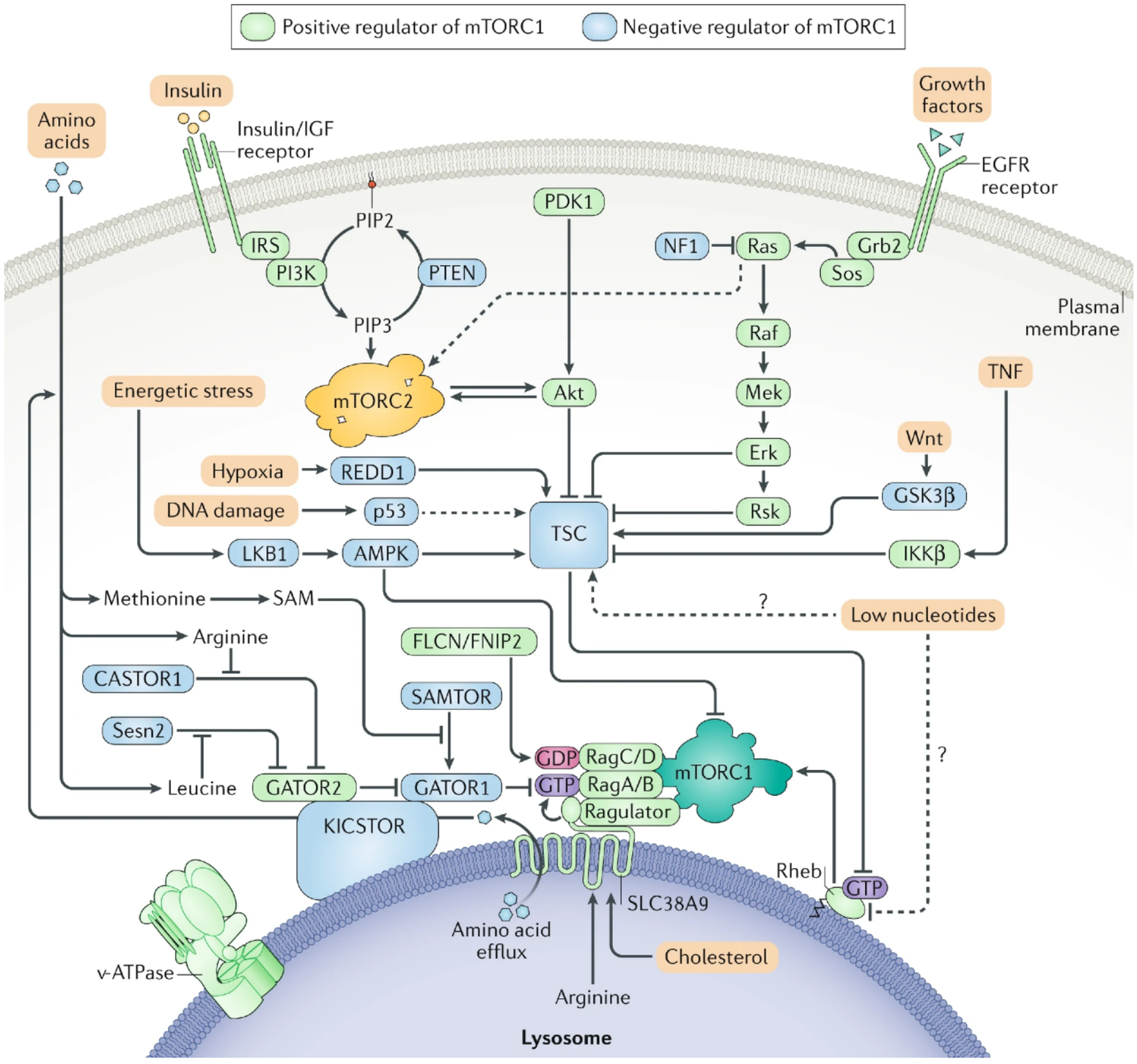






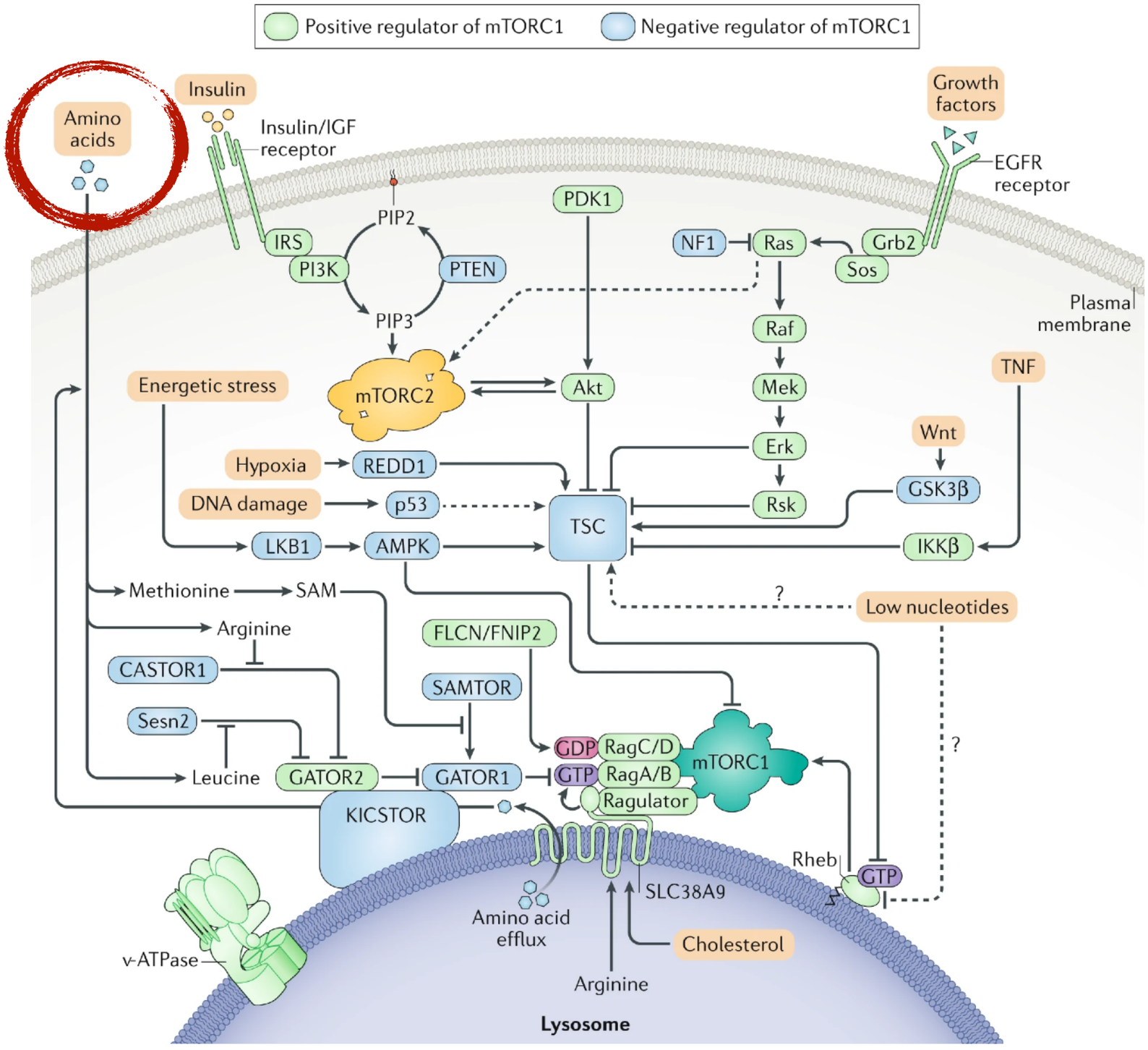
Davies et al, ***Dev Cell****, 2022*
Castellano et al, ***Science****, 2017*

What is sensed by RAG-mTORC1?



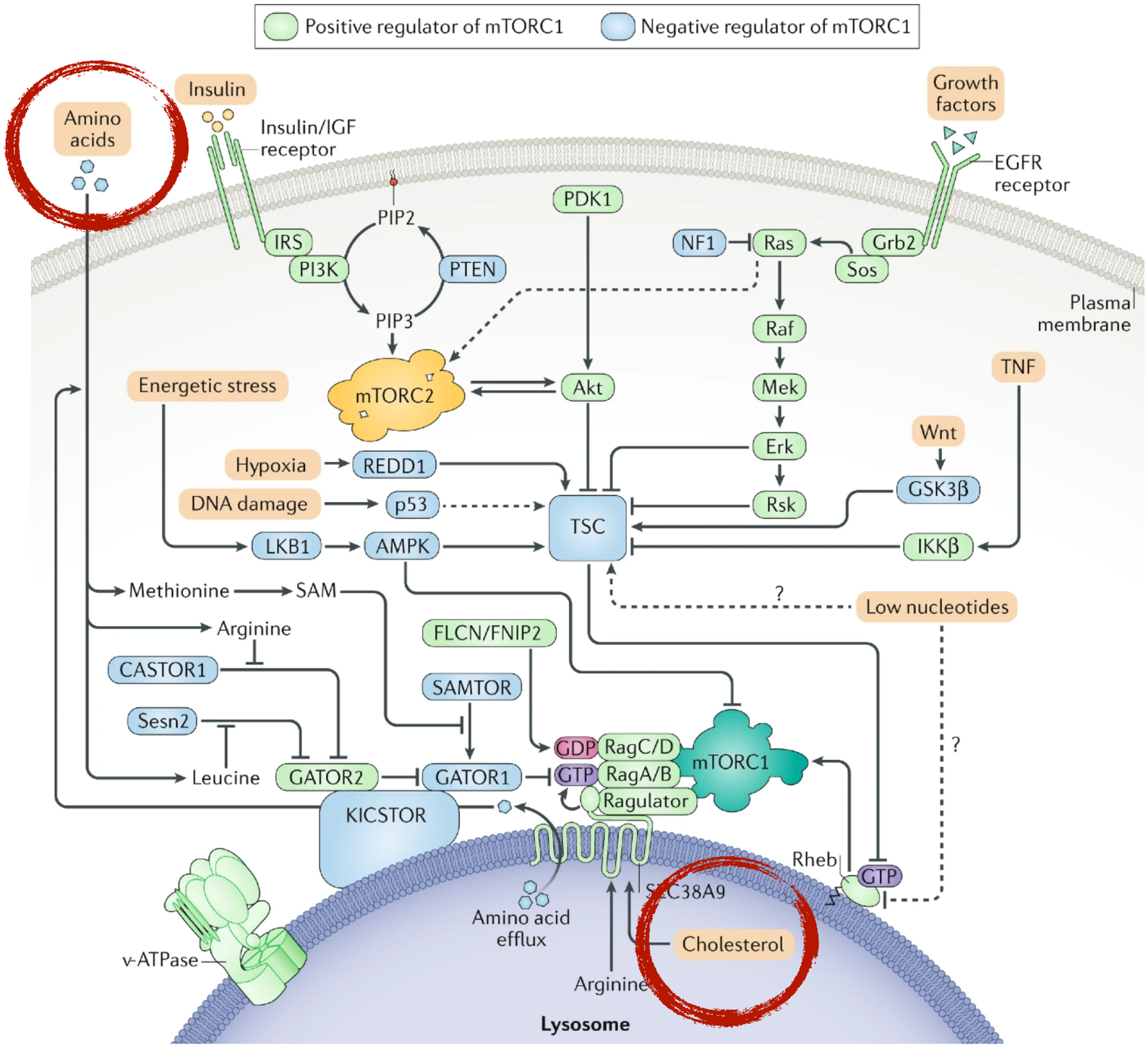
…. lots of things!

What is sensed by RAG-mTORC1?



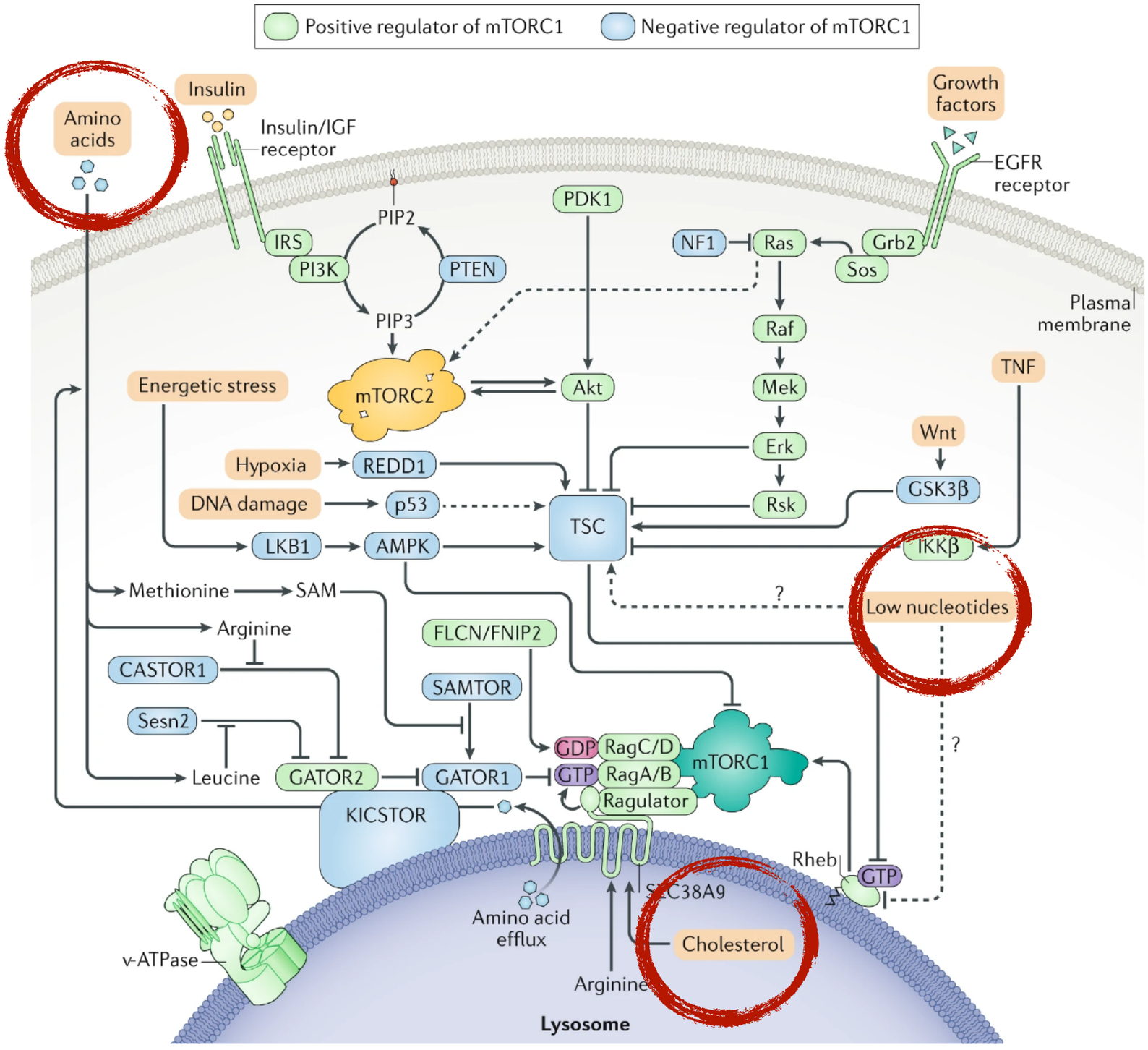
…. lots of things!

What is sensed by RAG-mTORC1?



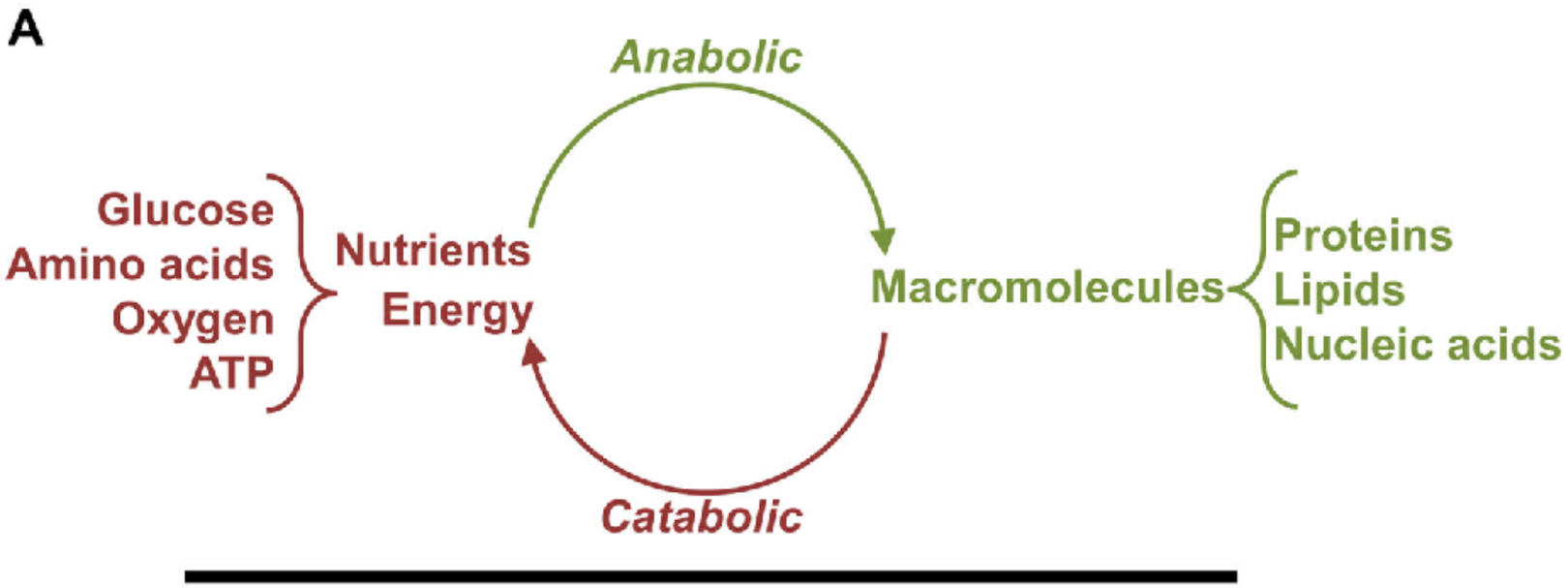
…. lots of things!

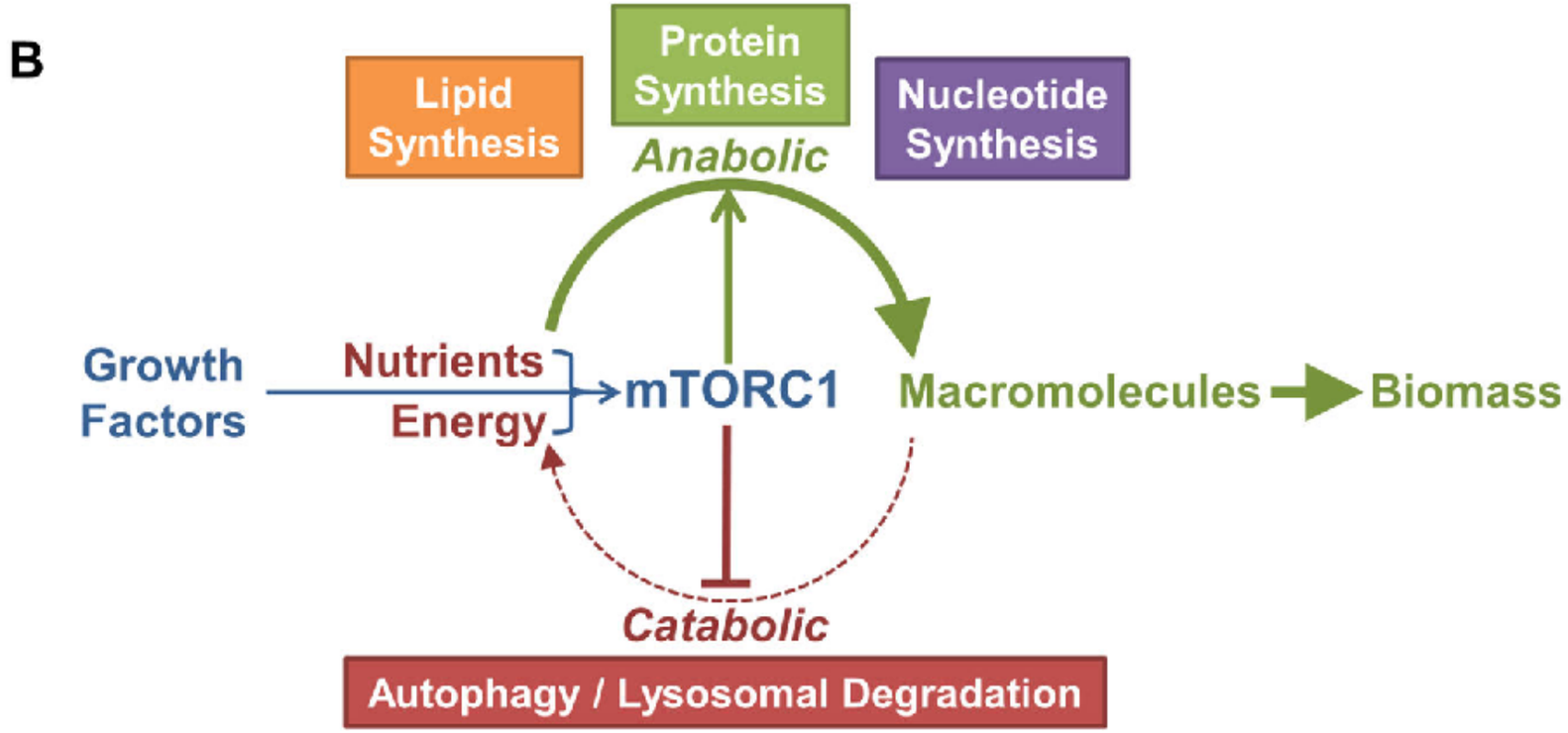
What is sensed by RAG-mTORC1?



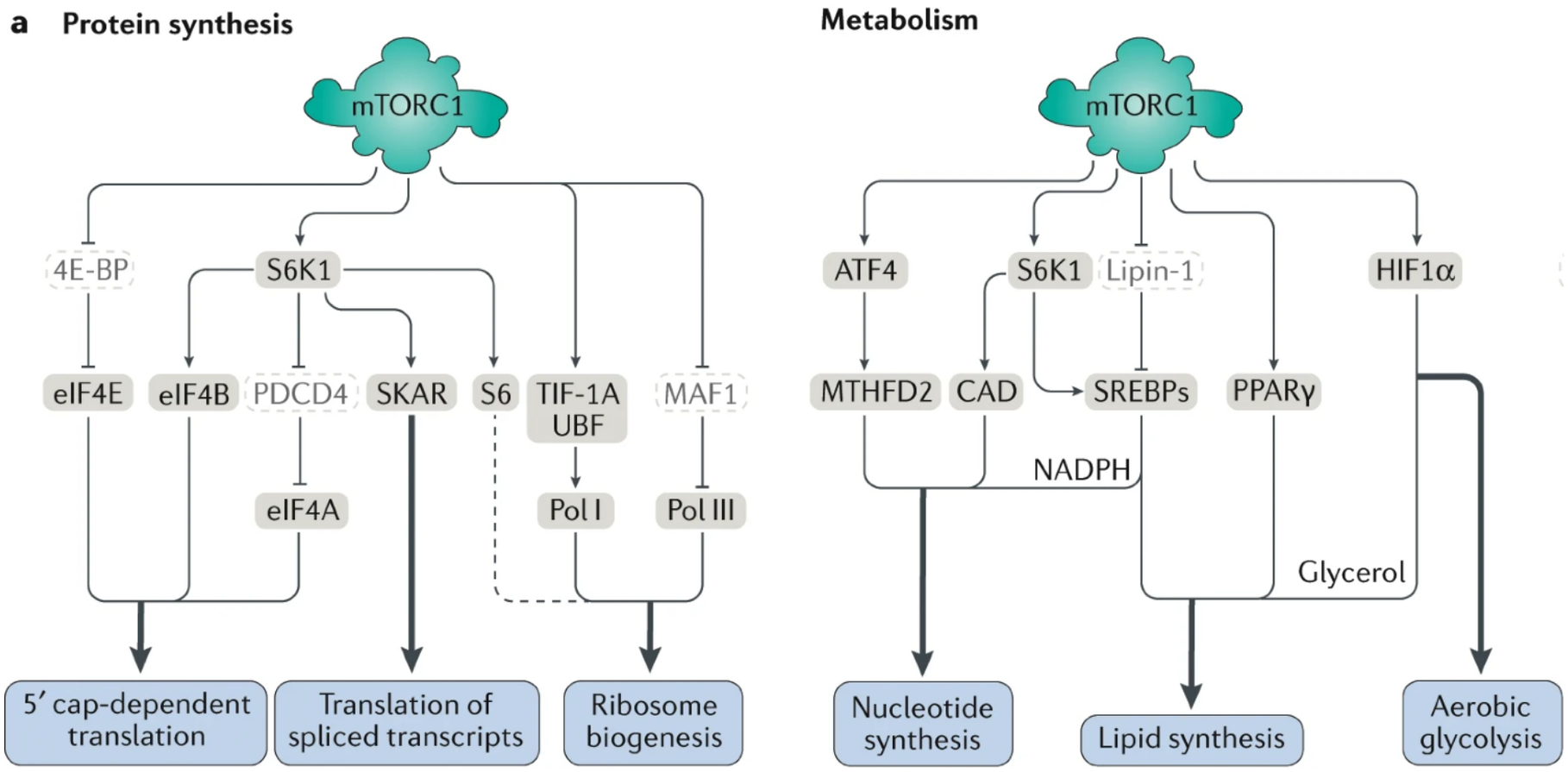
…. lots of things!

mTORC1: Central regulator anabolism



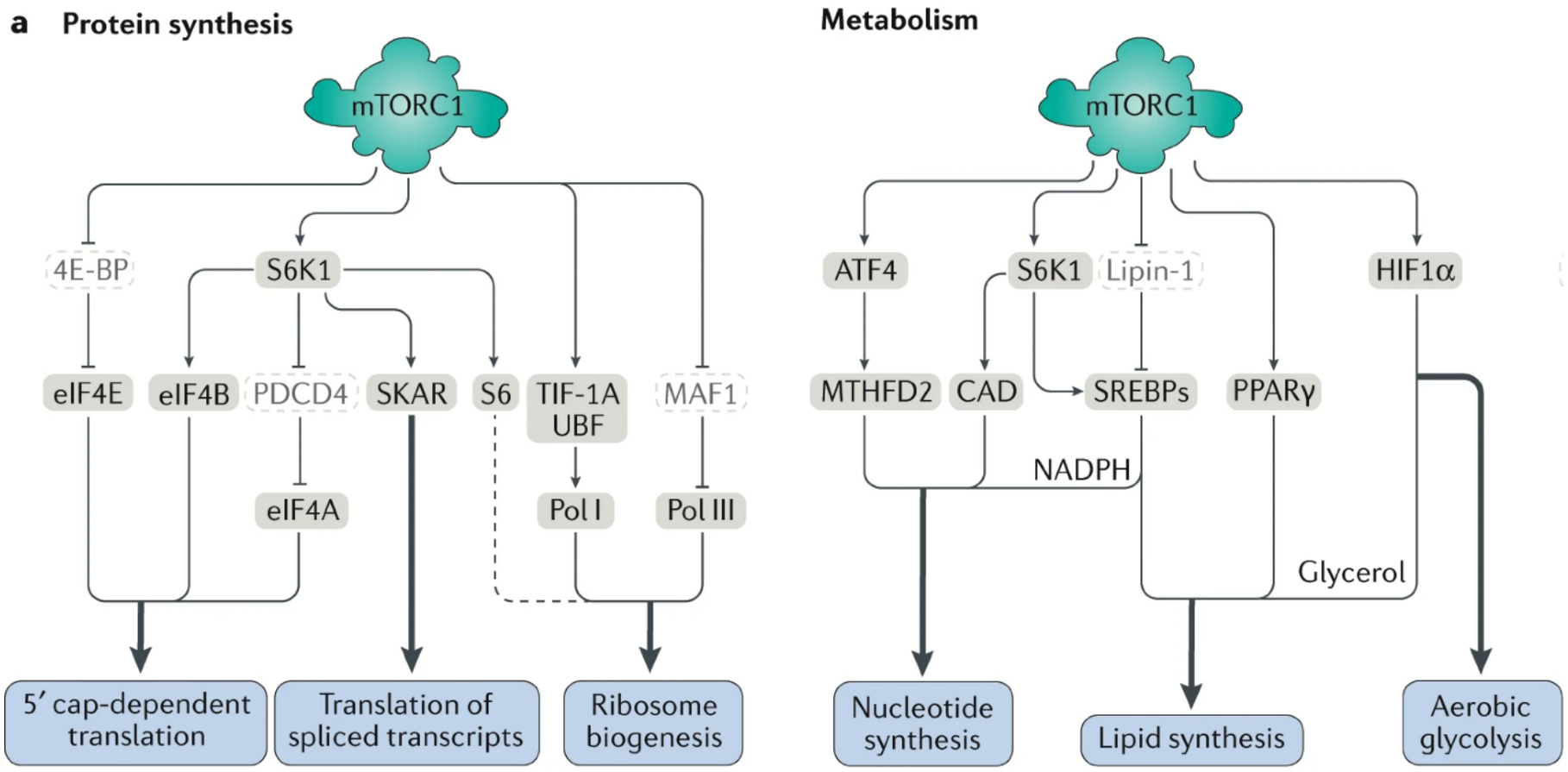


mTORC1: Central regulator anabolism



Liu & Sabatini, ***NRMCB****, 2020*

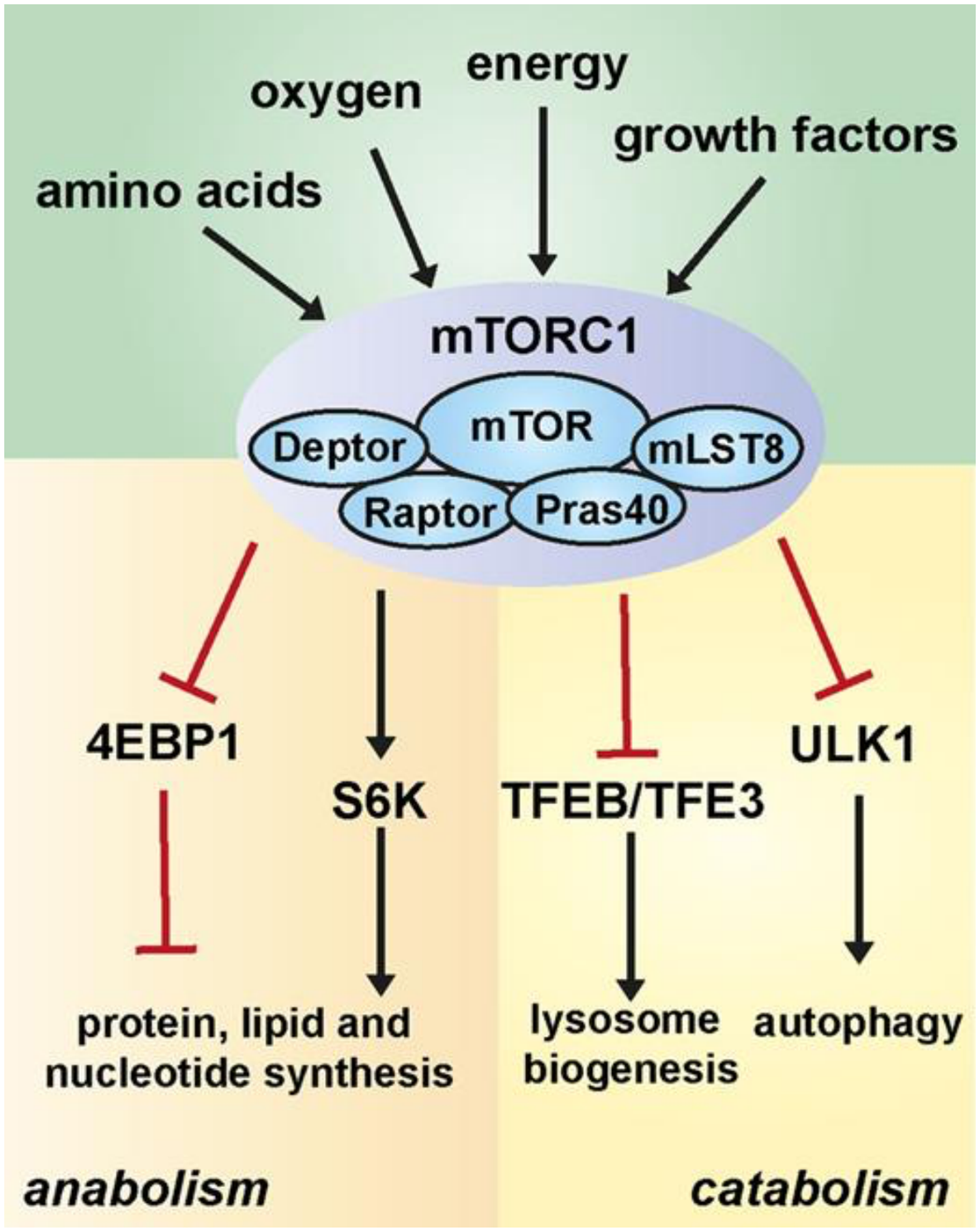
mTORC1: Central regulator anabolism



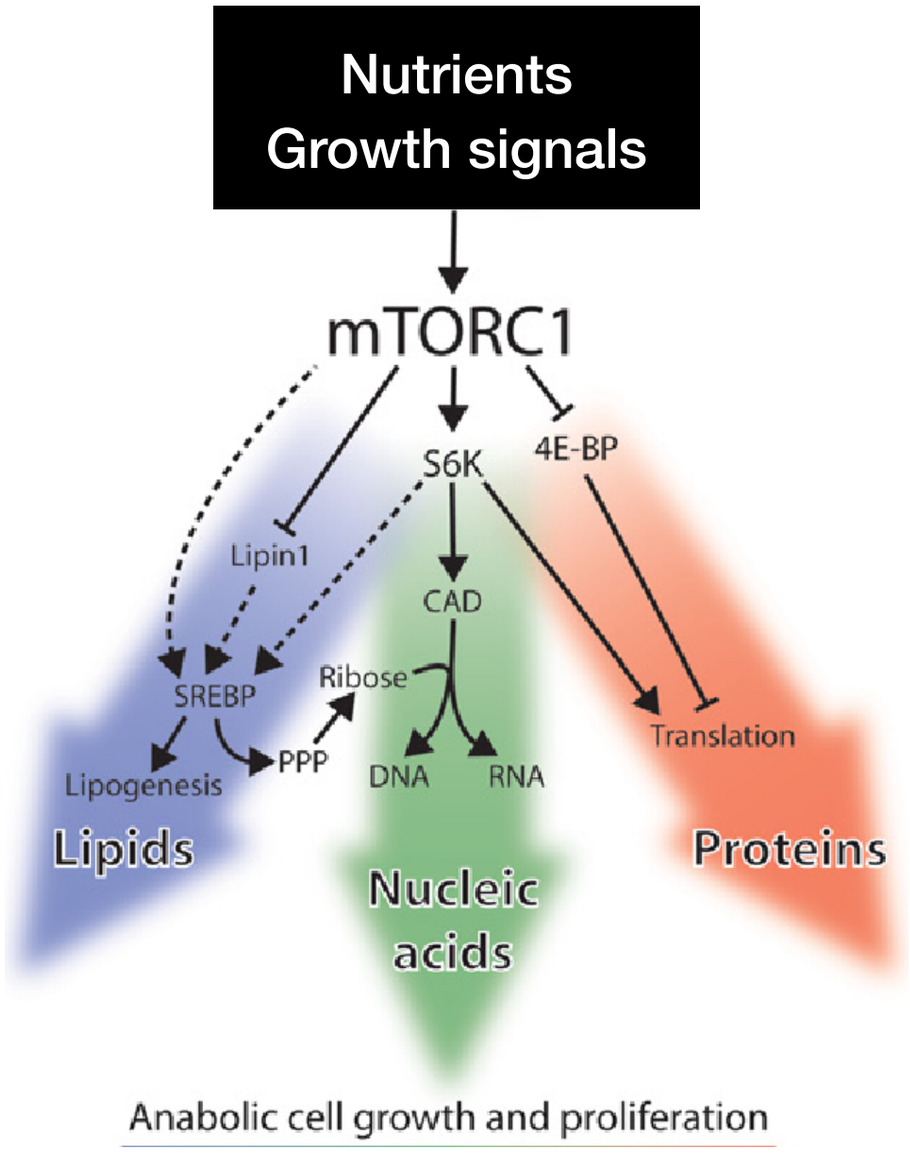
Activation of protein synthesis. Protein synthesis is the most energy-intensive and resource-intensive process in growing cells. It is therefore tightly regulated by mTORC1, which promotes protein synthesis by phosphorylating the eukaryotic initiation factor 4E binding proteins (4EBPs) and p70 S6 kinase 1 (S6K1).

*Liu & Sabatini, **NRMCB**, 2020*

mTORC1 canonical targets (and mediators) are 4EBP1 and S6K



mTORC1 canonical targets (and mediators) are 4EBP1 and S6K



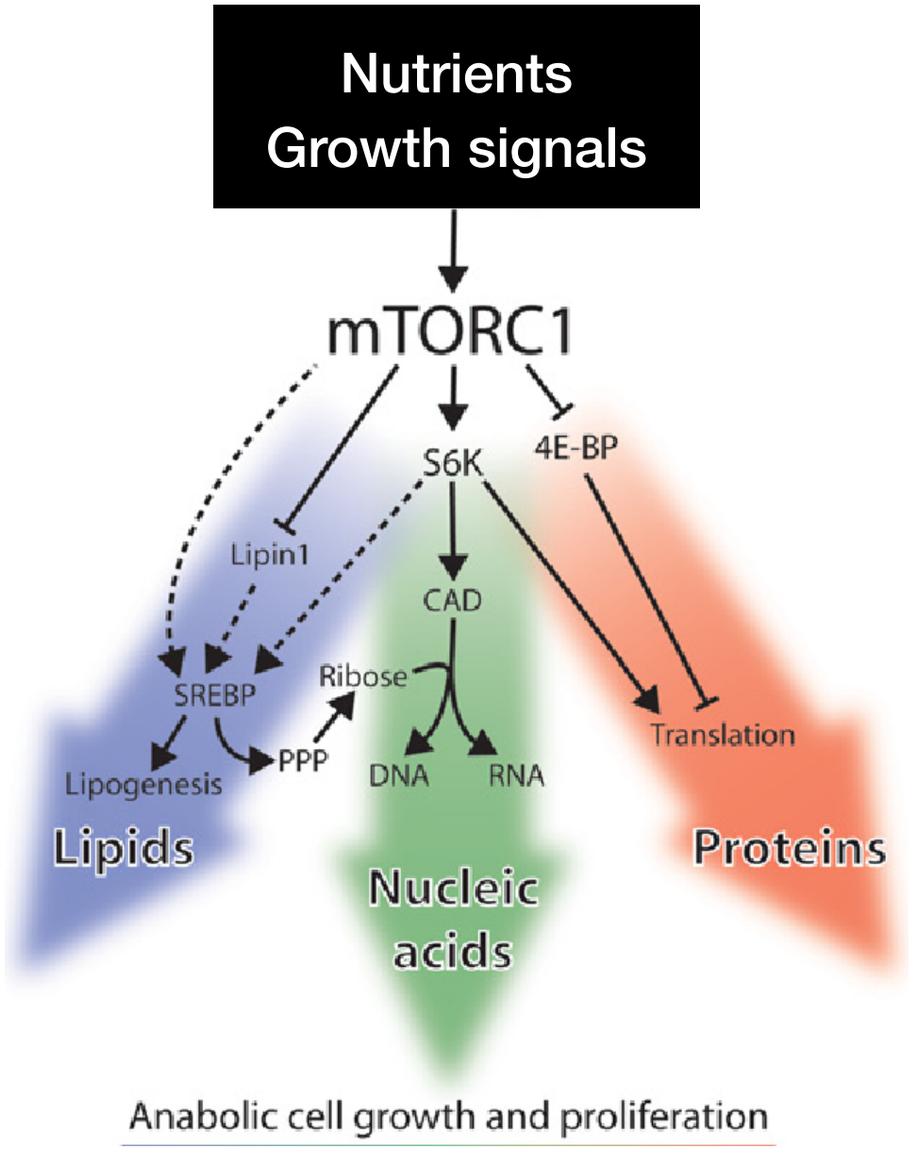
In its unphosphorylated state, 4EBP1 suppresses translation by binding and sequestering eukaryotic translation initiation factor 4E (eIF4E), an essential component of the eIF4F cap-binding complex.

S6K1 phosphorylates its namesake target, ribosomal protein S6, a component of the 40S subunit. The function of S6 phosphorylation remains ambiguous.

mTORC1 canonical targets (and mediators) are 4EBP1 and S6K

Effects on metabolism are multifold, and still emerging. Generally speaking, mTORC1 enhances several processes. These include:

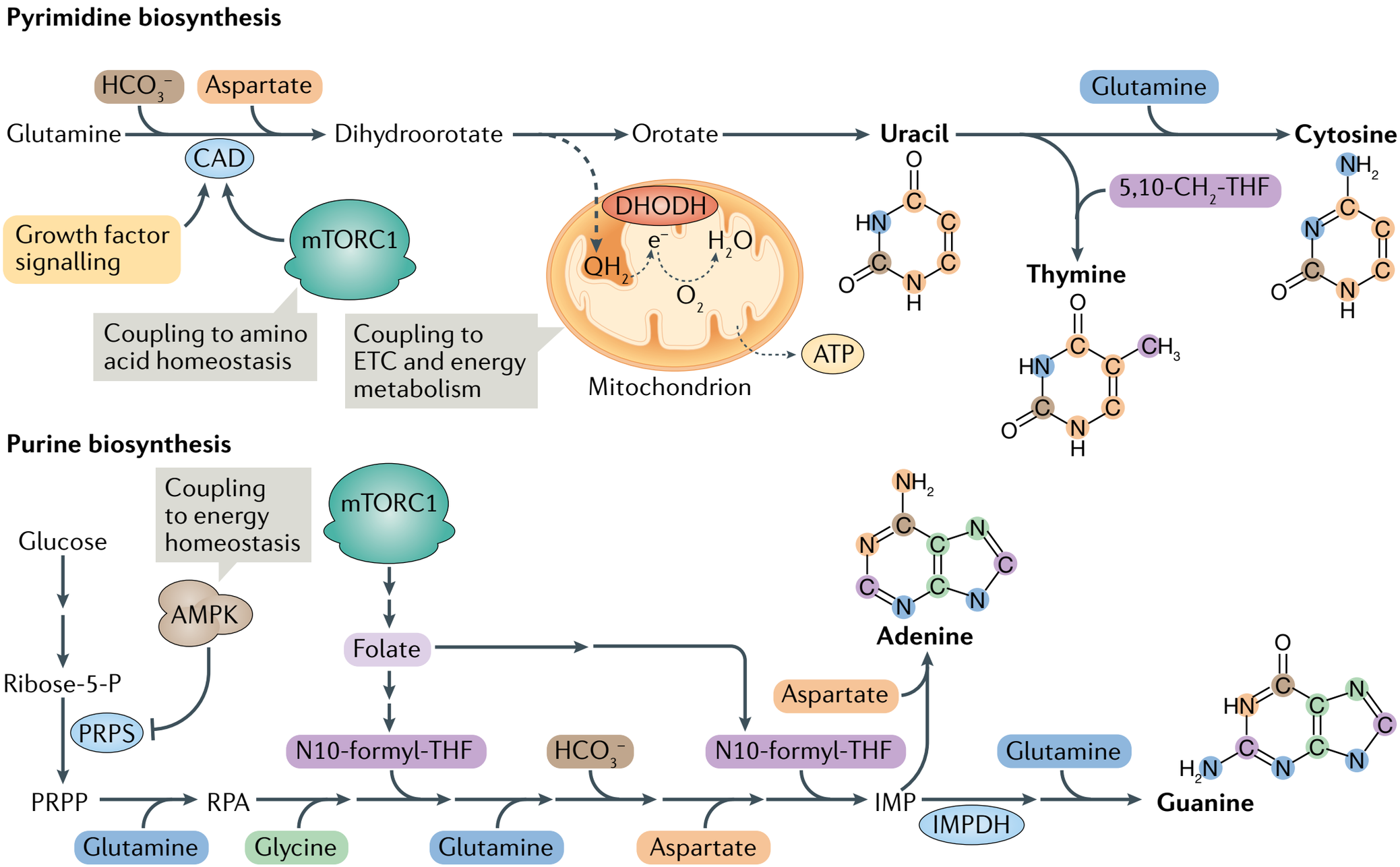
- Nucleotide synthesis
- Lipid synthesis
- Cholesterol biosynthesis
- Glycogen synthesis
- PPP
- Ser/Gly biosynthesis
- *Glycolysis?*
- *Mitochondria biogenesis?*
- *Mitochondria QC?*



In its unphosphorylated state, 4EBP1 suppresses translation by binding and sequestering eukaryotic translation initiation factor 4E (eIF4E), an essential component of the eIF4F cap-binding complex.

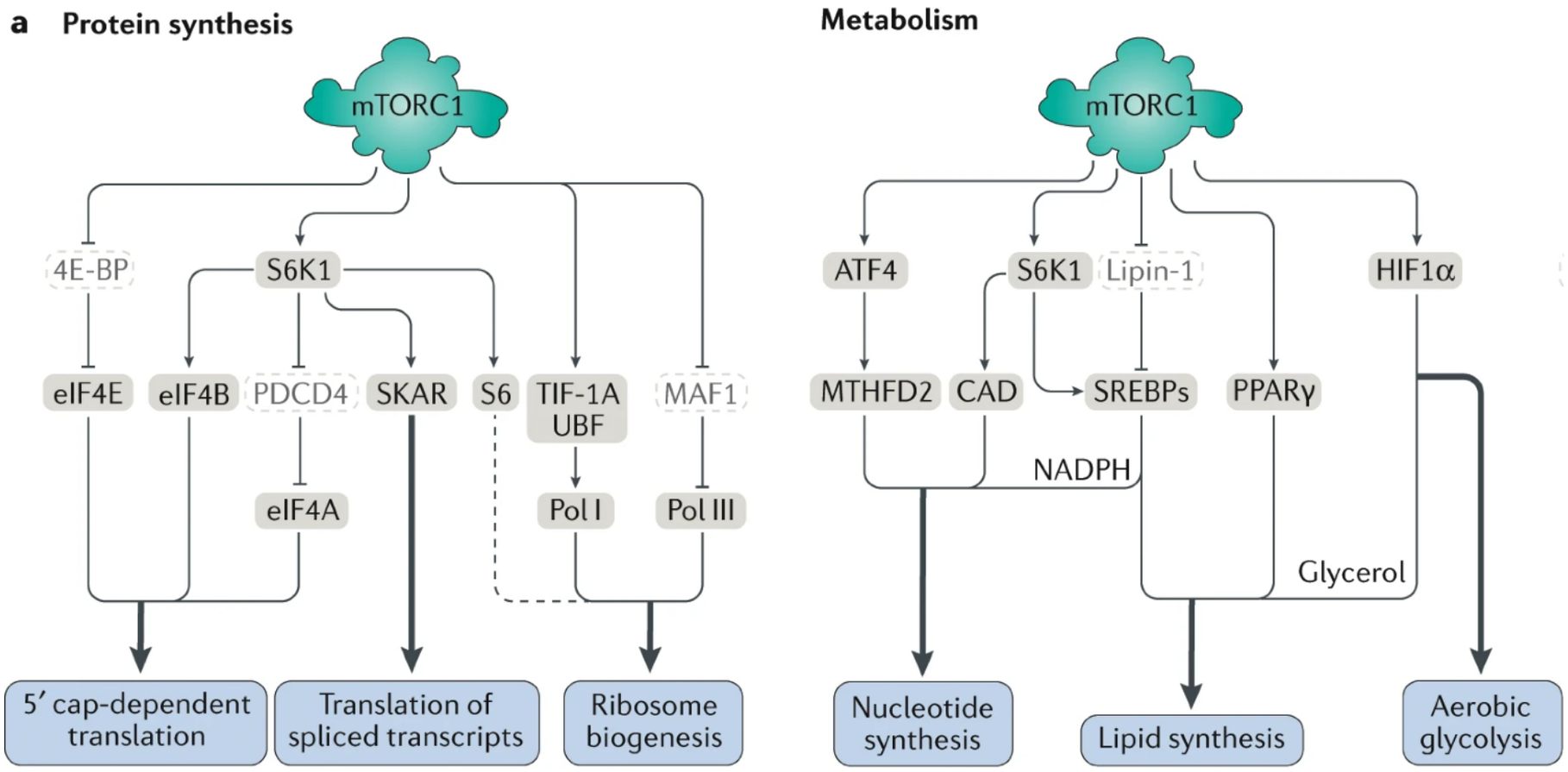
S6K1 phosphorylates its namesake target, ribosomal protein S6, a component of the 40S subunit. The function of S6 phosphorylation remains ambiguous.

mTORC1 stimulates nucleotide biosynthesis



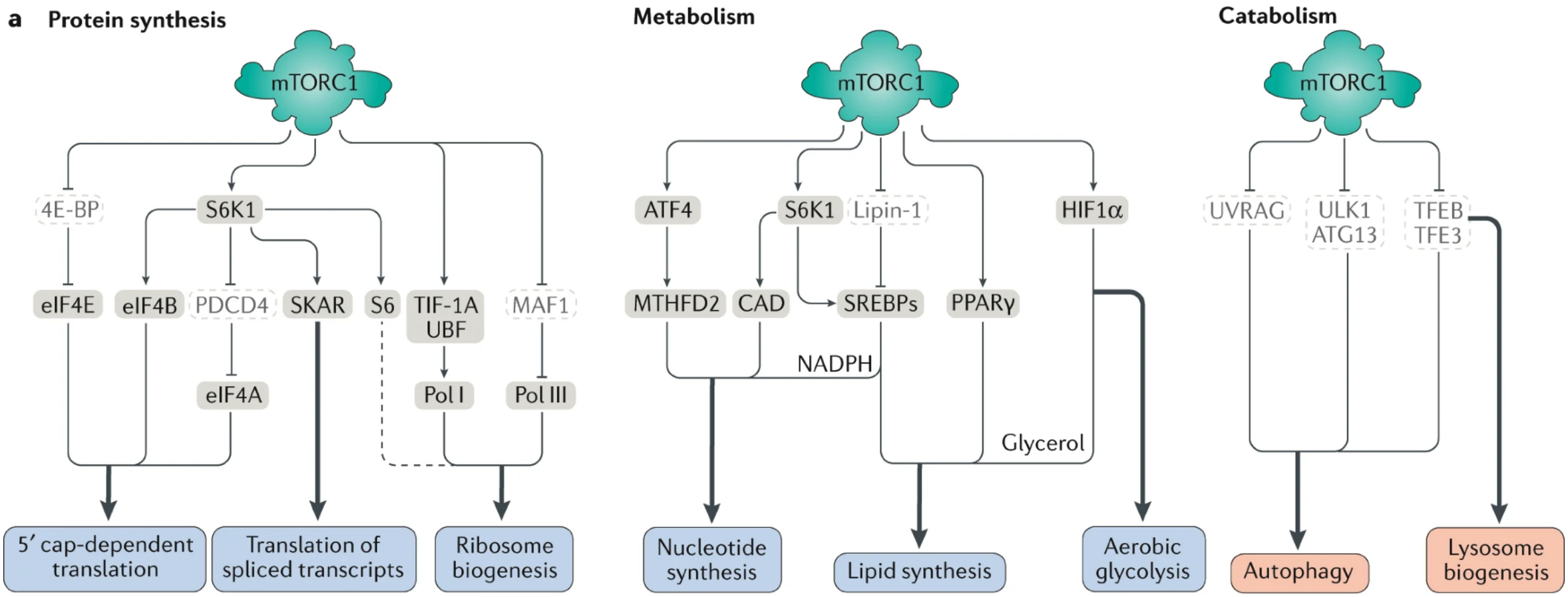
*Zhu & Thompson, **NRMCB**, 2019*

mTORC1: Central regulator anabolism



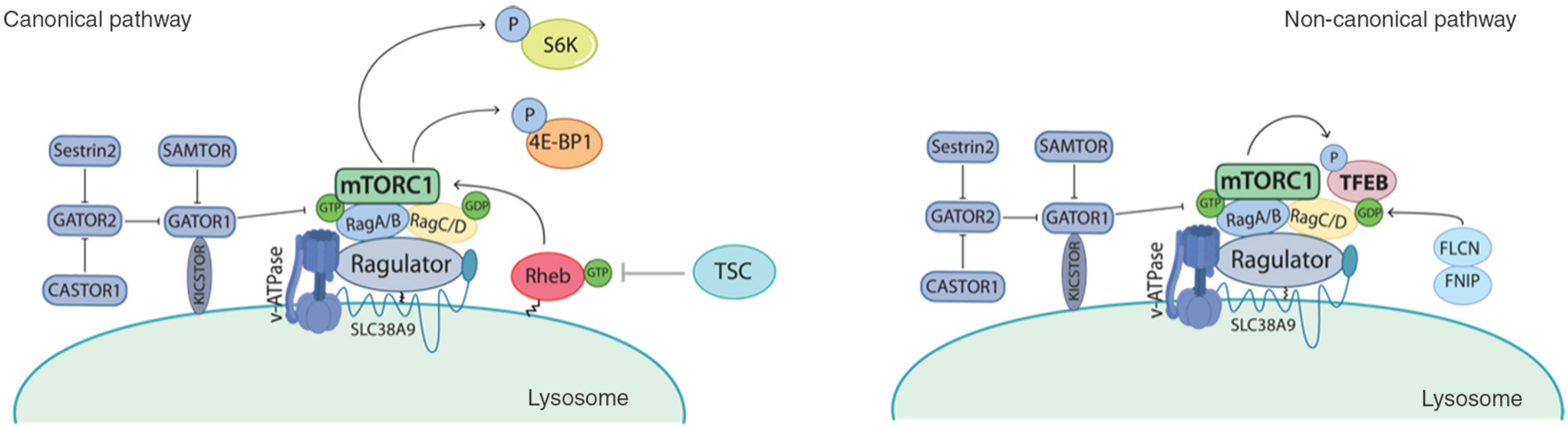
Liu & Sabatini, ***NRMCB****, 2020*

mTORC1: Central regulator anabolism



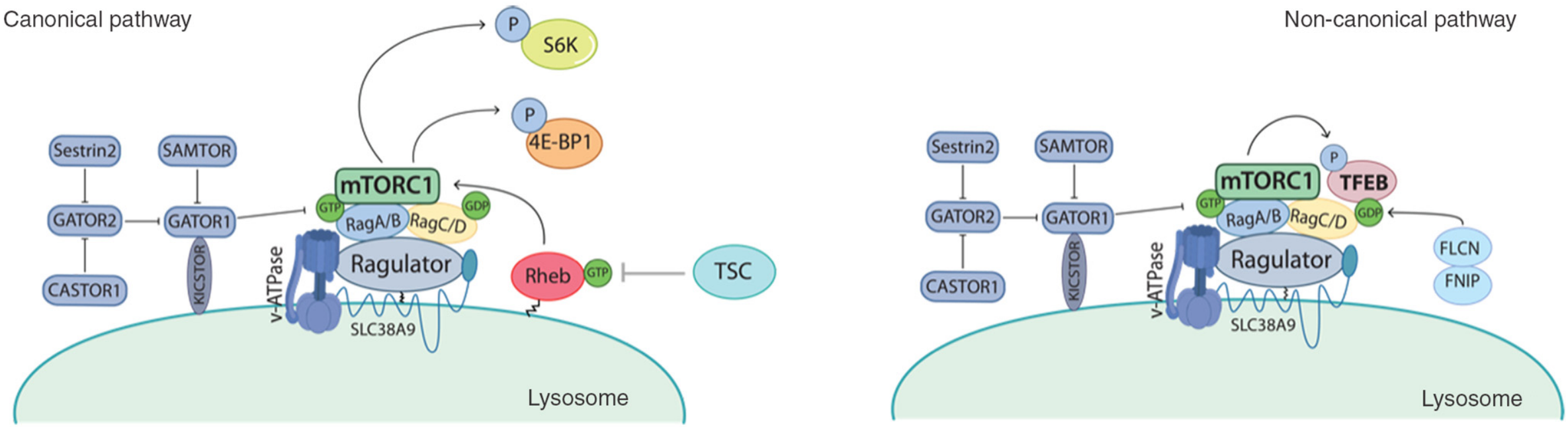
*Liu & Sabatini, **NRMCB**, 2020*

mTORC1 blocks autophagy through “non-canonical” mechanisms



Napolitano, Di Malta & Ballabio, ***Trends Cell Biol****, 2022*

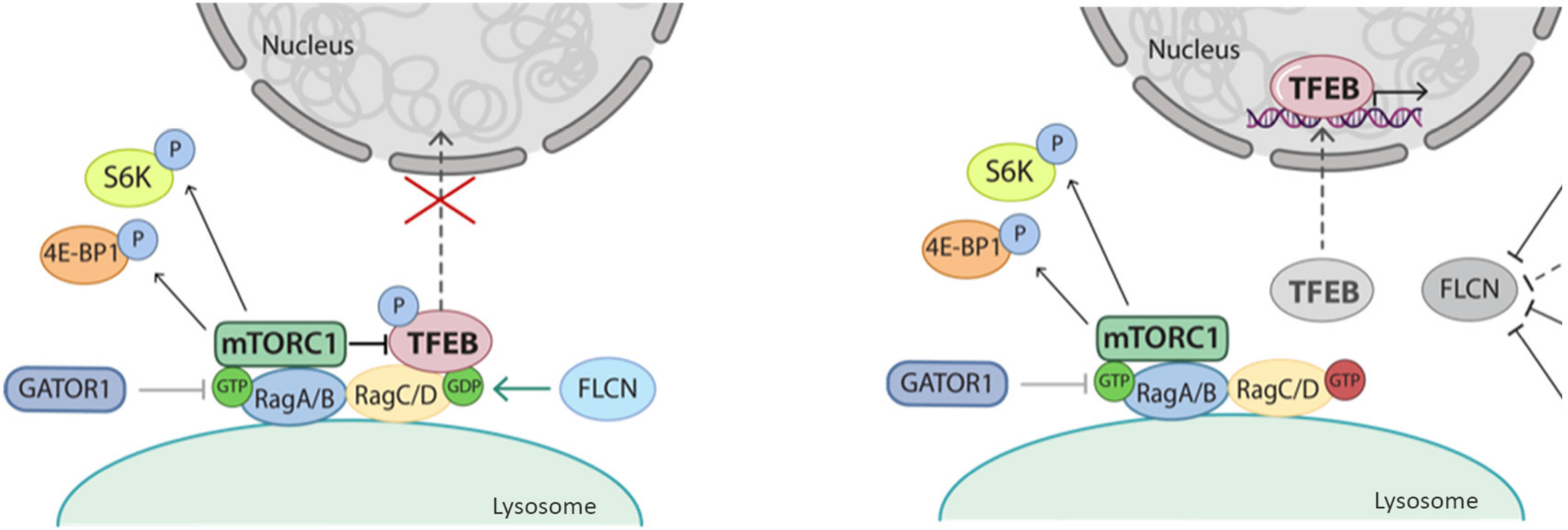
mTORC1 blocks autophagy through “non-canonical” mechanisms



mTORC1 can phosphorylate MiT-TFE transcription family members interacting with Follicular (FLCN) as GEF

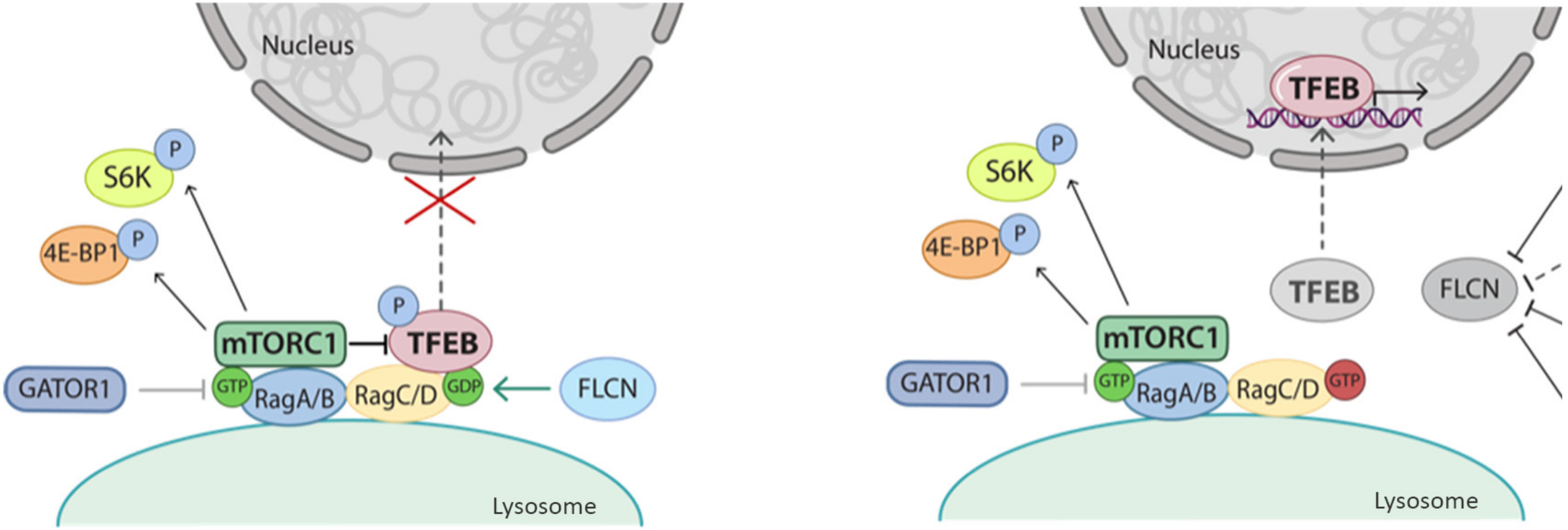
Napolitano, Di Malta & Ballabio, ***Trends Cell Biol****, 2022*

mTORC1 blocks autophagy through “non-canonical” mechanisms



Napolitano, Di Malta & Ballabio, ***Trends Cell Biol****, 2022*

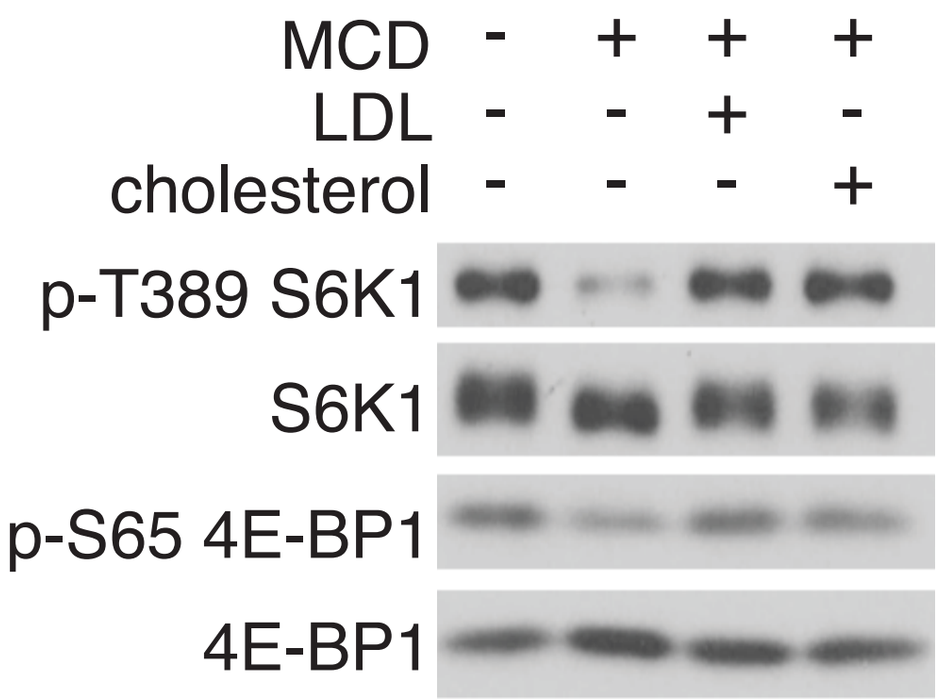
mTORC1 blocks autophagy through “non-canonical” mechanisms

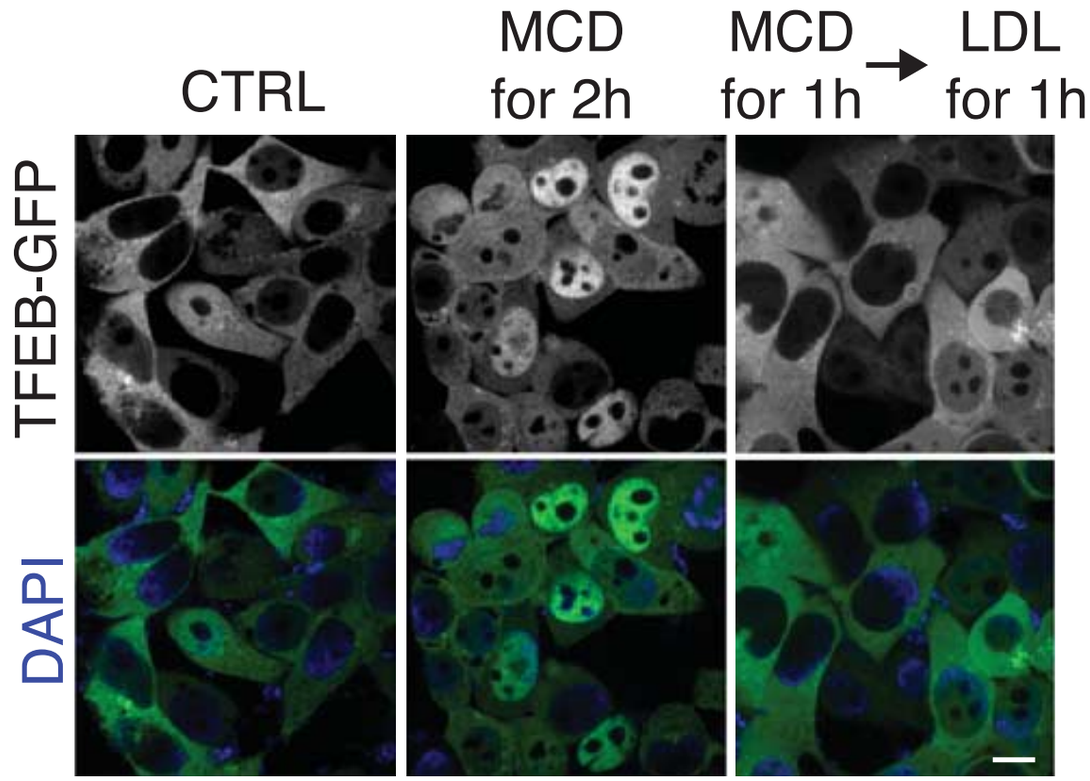


When TFEB is phosphorylated, it is retained in the cytoplasm (inactive)

Napolitano, Di Malta & Ballabio, ***Trends Cell Biol****, 2022*

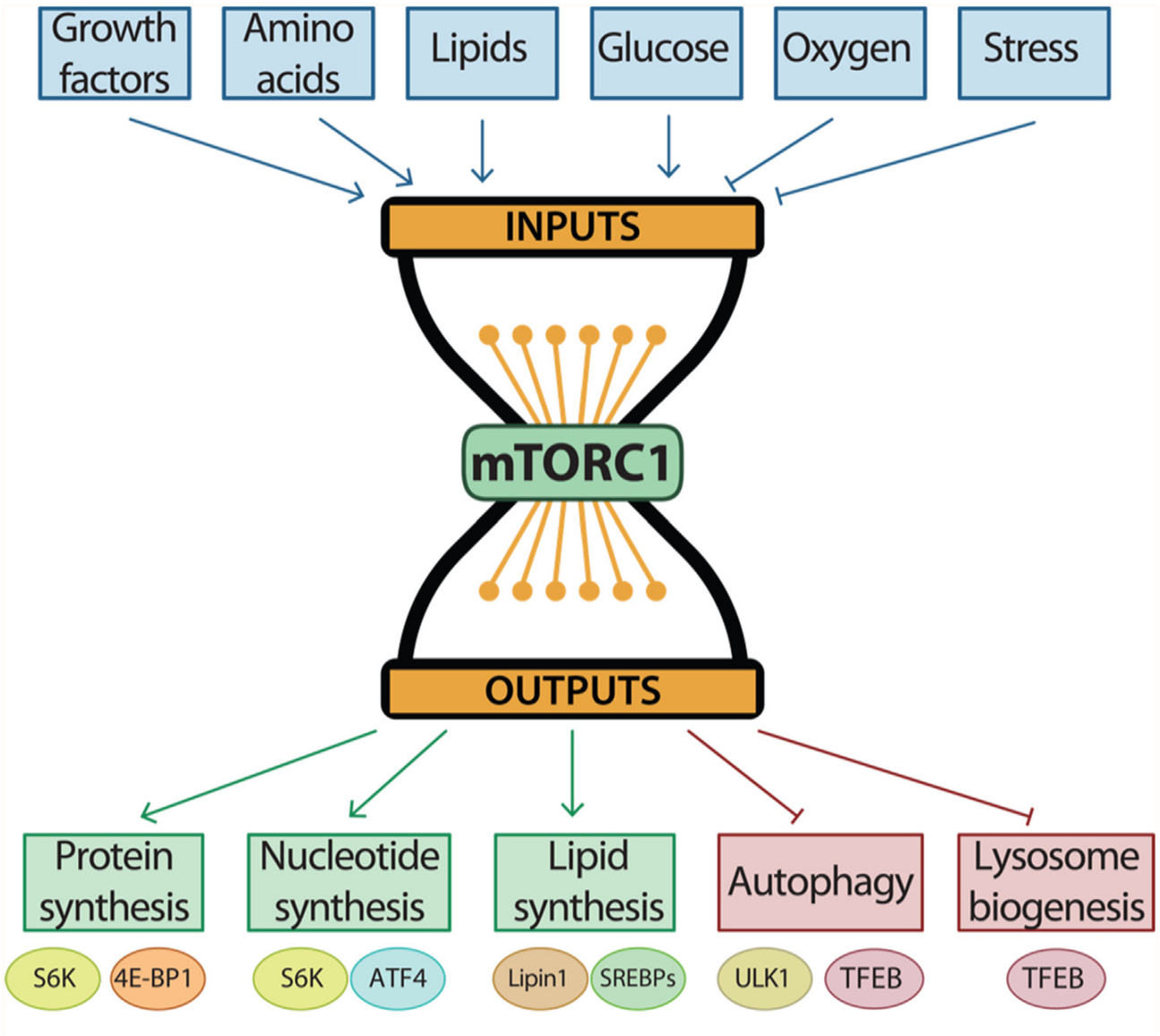
mTORC1 activation excludes TFEB from the nucleus





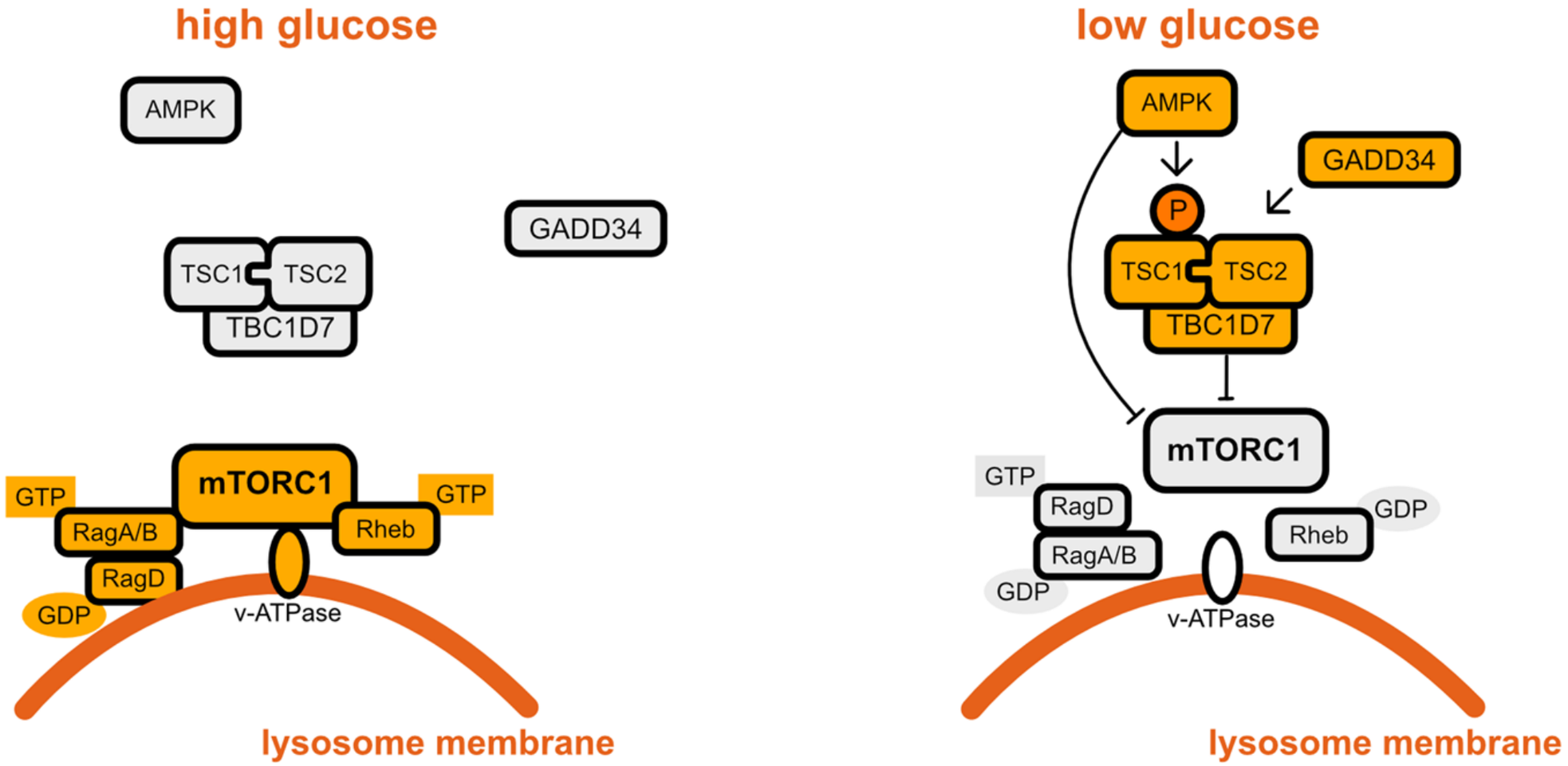
Castellano et al, ***Science****, 2017*

mTORC1: Signal integrator and central regulator anabolism

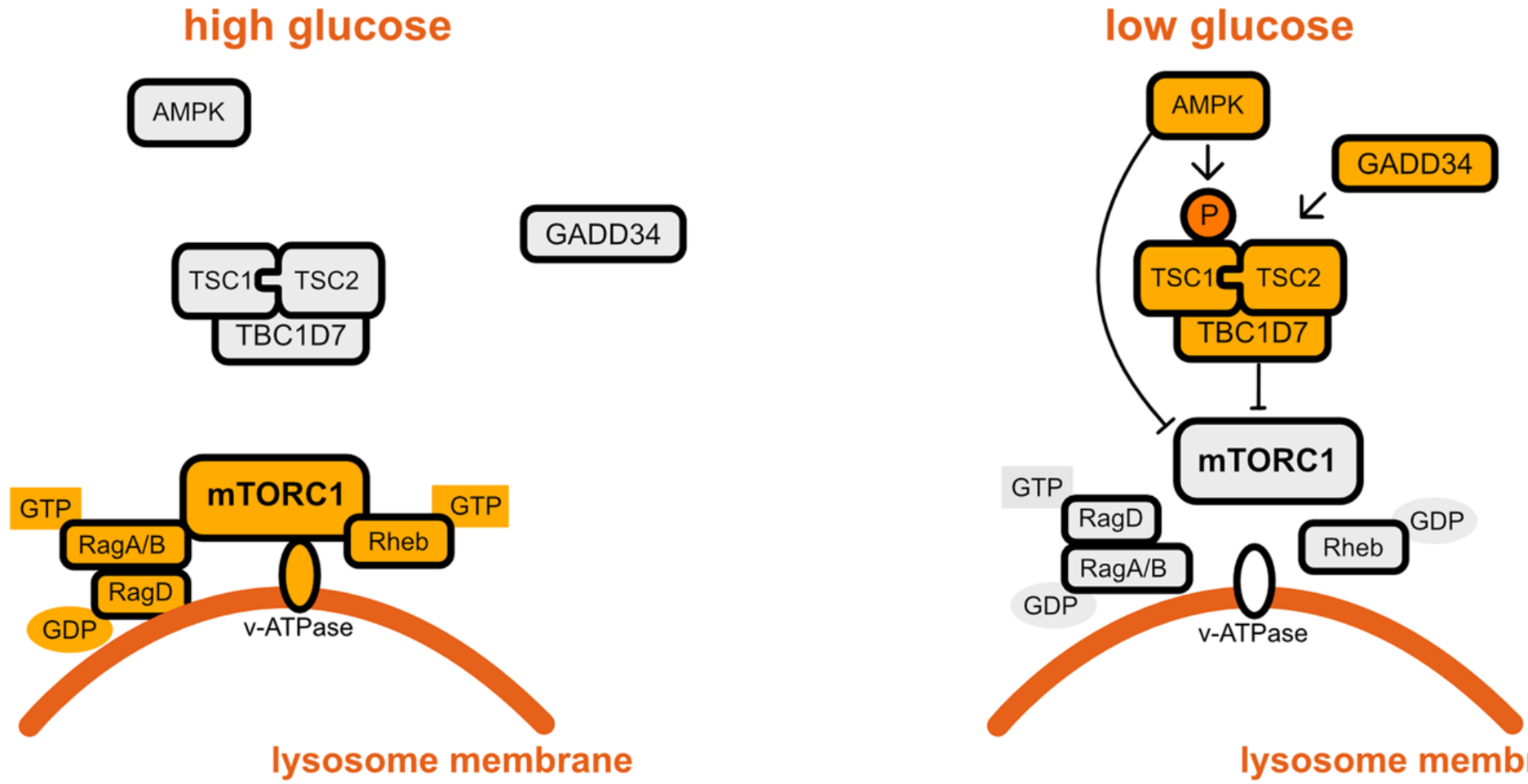


Napolitano, Di Malta & Ballabio, ***Trends Cell Biol****, 2022*

AMPK1 suppresses mTORC1



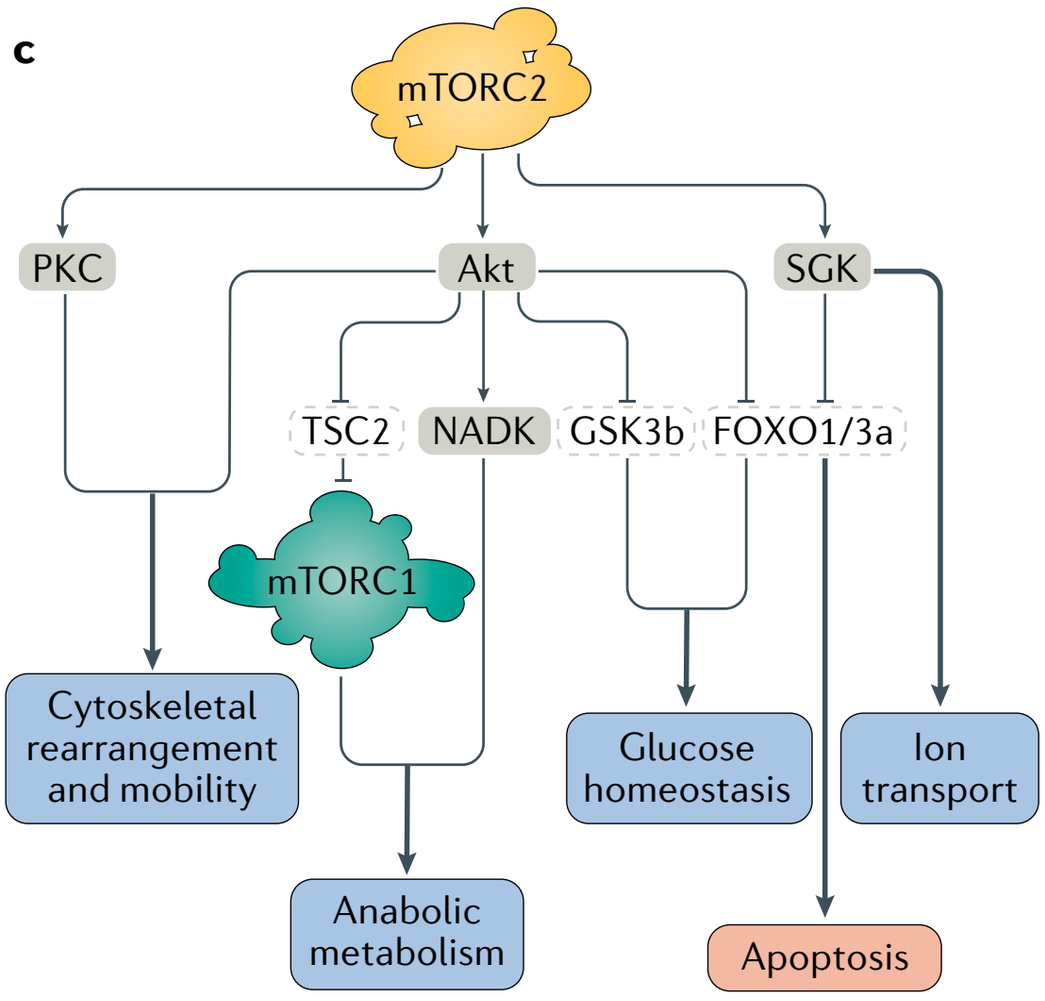
AMPK1 suppresses mTORC1



AMPK1 activates the TSC complex (inhibiting mTORC1)

AMPK - mTORC crosstalk ensure proper balance between anabolism and catabolism

mTORC2: Signal mediator for growth and survival



*Liu & Sabatini, **NRMCB**, 2020*

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Sensors

⬆AMPK

⬇mTORC1

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Sensors

⬆AMPK

⬆Sirtuins

⬇mTORC1

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Sensors

⬆AMPK

⬆Sirtuins

⬇mTORC1

Effectors

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Sensors

⬆AMPK

⬆Sirtuins

⬇mTORC1

Effectors

Energy expenditure

Energy status

Calorie restriction

(Glucose limitation, serum starvation, exercise)

Sensors

⬆AMPK

⬆Sirtuins

⬇mTORC1

Effectors

Energy optimization

(Remodeling of mitochondria, autophagy)

Energy expenditure

Sirtuins:

Seven family members (mammals): SIRT1 - SIRT7

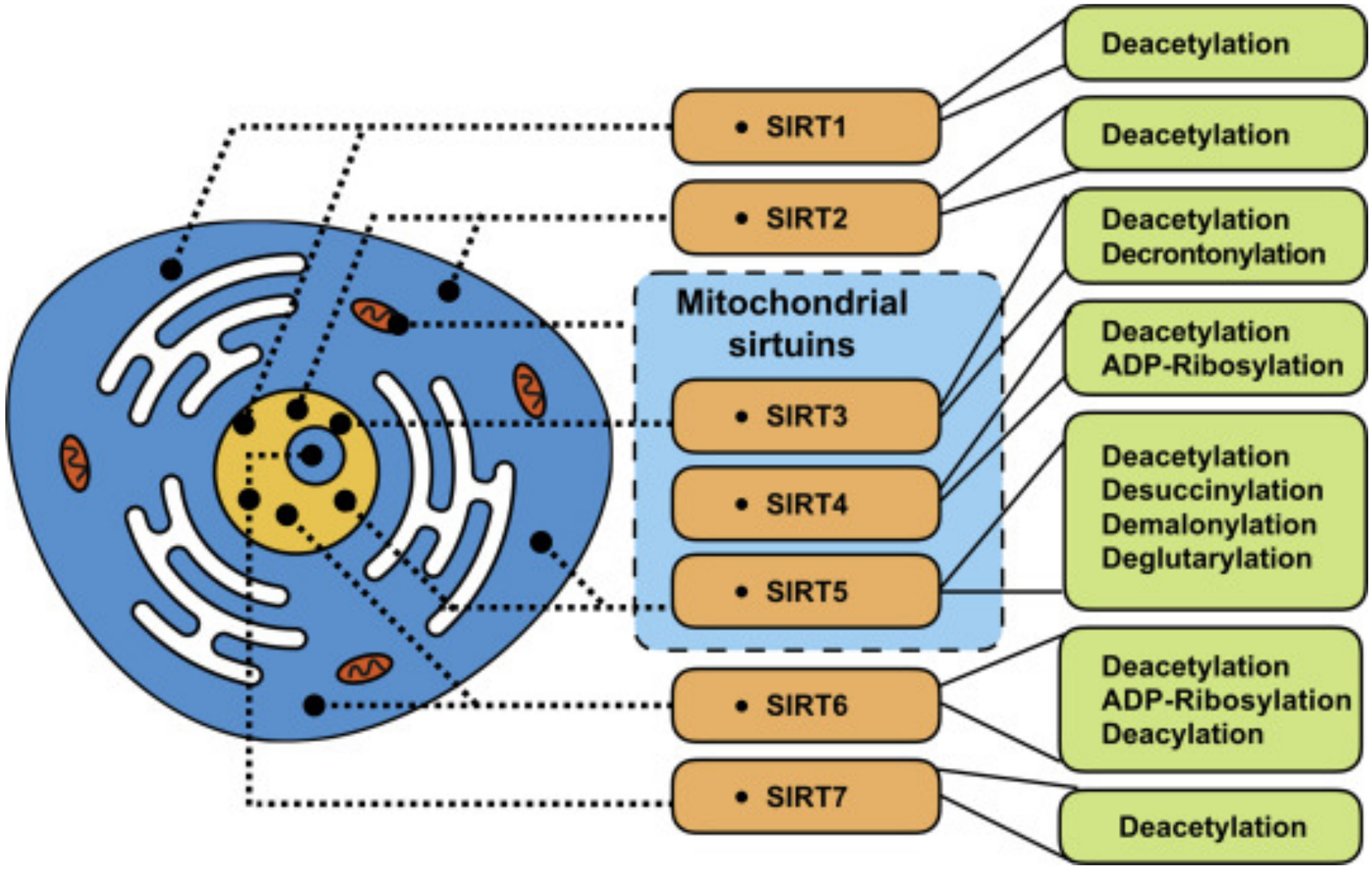
Protein deacetylases (also involved in ADP-ribosylation)

Localized at different compartments

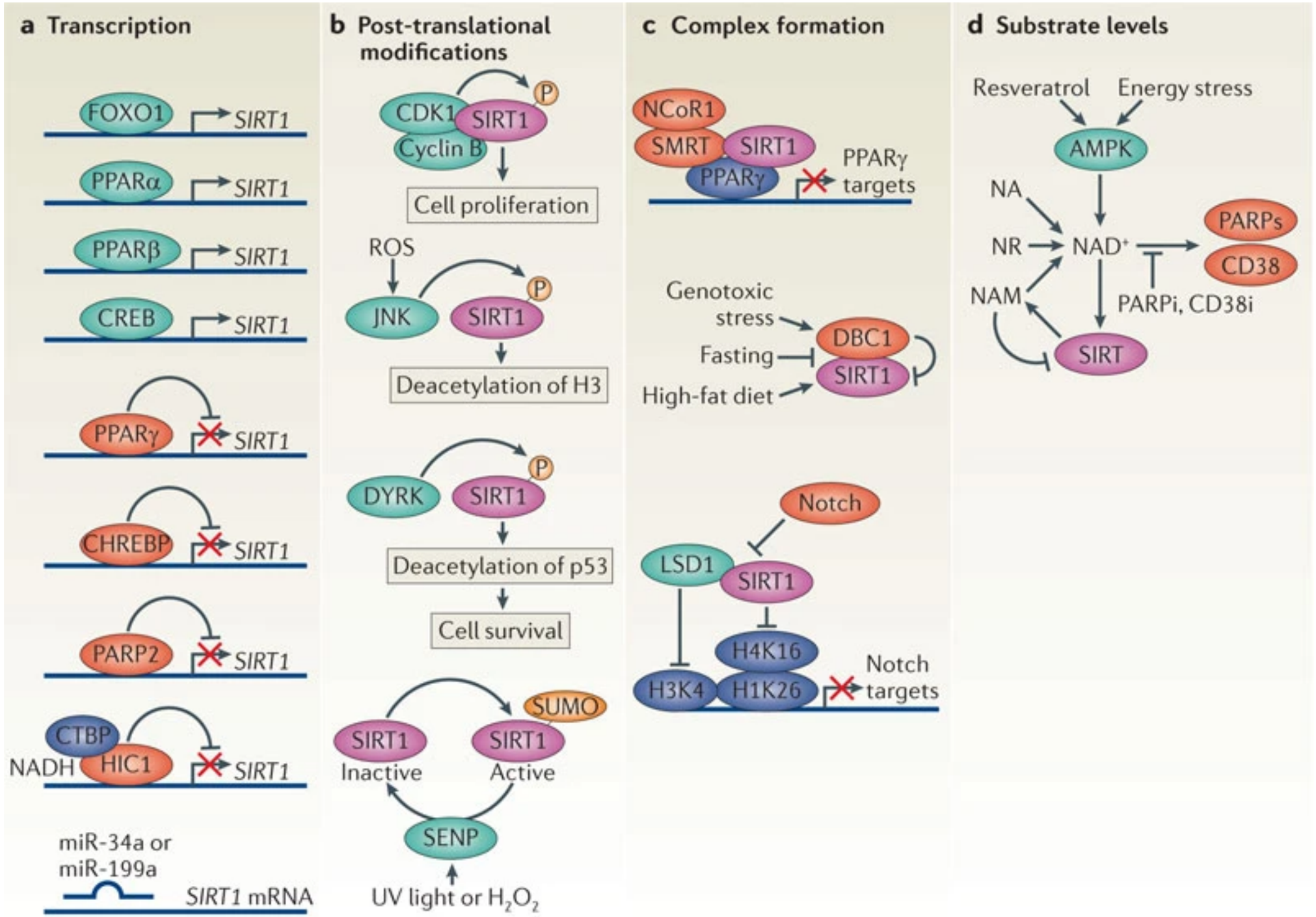
Depend on NAD+ (activated by calorie restriction)

Involved in metabolism and aging

Sirtuins:

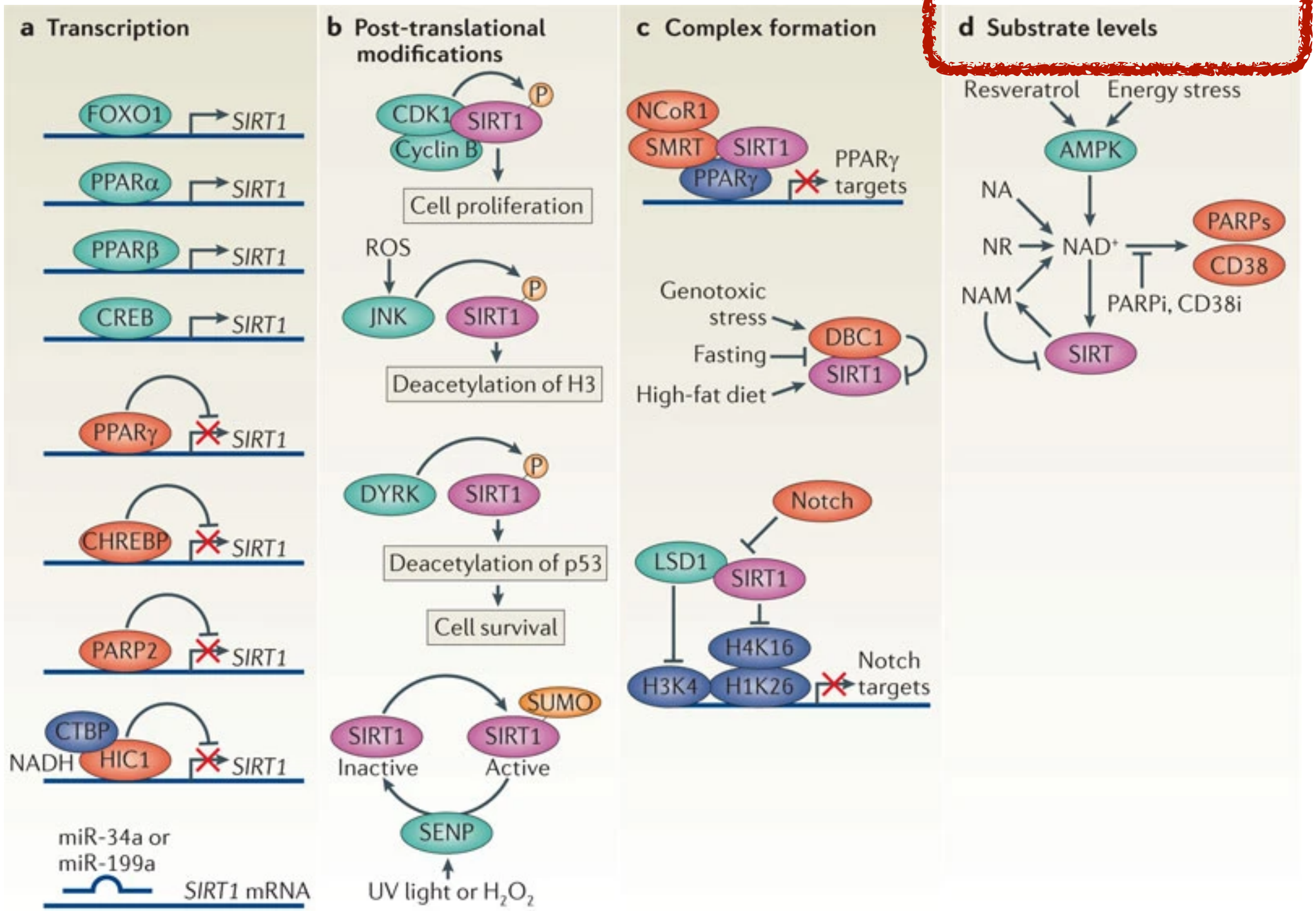

• SIRT1
• SIRT2
Mitochondrial sirtuins
• SIRT3
• SIRT4
• SIRT5
• SIRT6
• SIRT7
Deacetylation
Deacetylation
Deacetylation
Decrontonylation
Deacetylation
ADP-Ribosylation
Deacetylation
Desuccinylation
Demalonylation
Deglutarylation
Deacetylation
ADP-Ribosylation
Deacylation
Deacetylation

Sirtuins activity is regulated



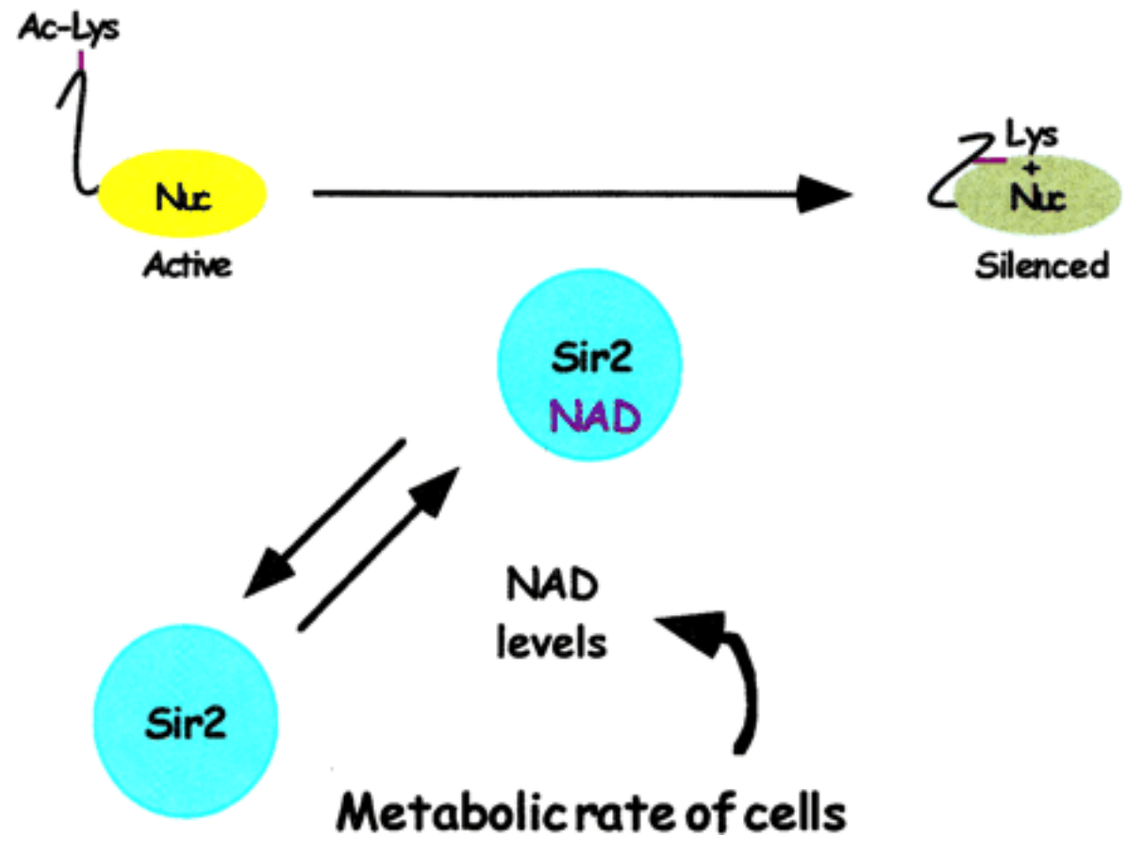
Houtkooper et al, ***NRCMB****, 2012*

Sirtuins activity is regulated



Houtkooper et al, ***NRCMB****, 2012*

Sirtuins depend on NAD+ availability



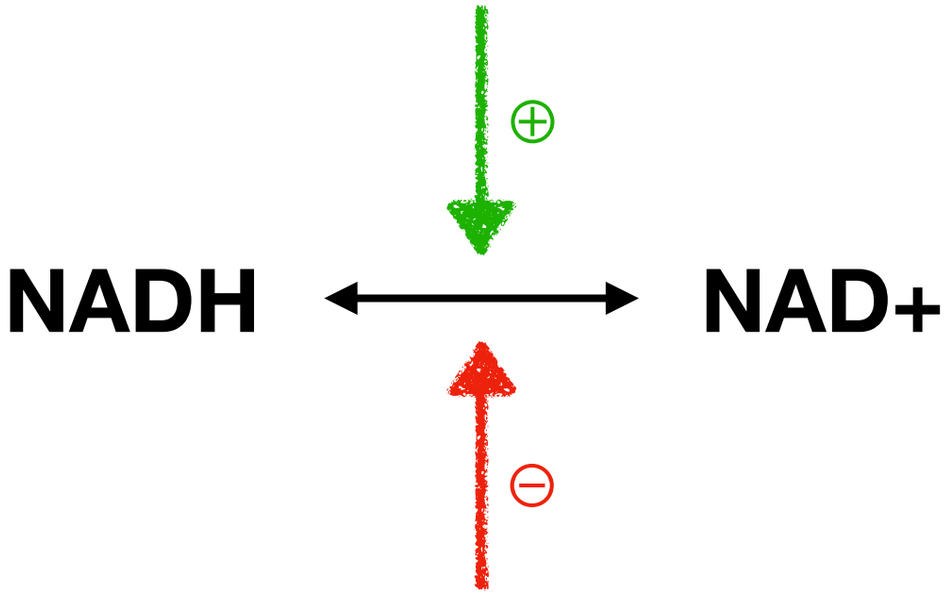
The NAD+-dependence of deacetylase activity supports the hypothesis that Sirtuins could act as metabolic sensors, capable of modulating gene expression according to the metabolic state of the cell

Guarente, ***Genes & Dev****, 2000*

NAD+ levels decrease under conditions that stimulate its conversion to its reduced form, NADH

ETC / OXPHOS
Fatty acid synthesis
Pyruvate metabolism
Lipid desaturation

NAD+-producing reactions

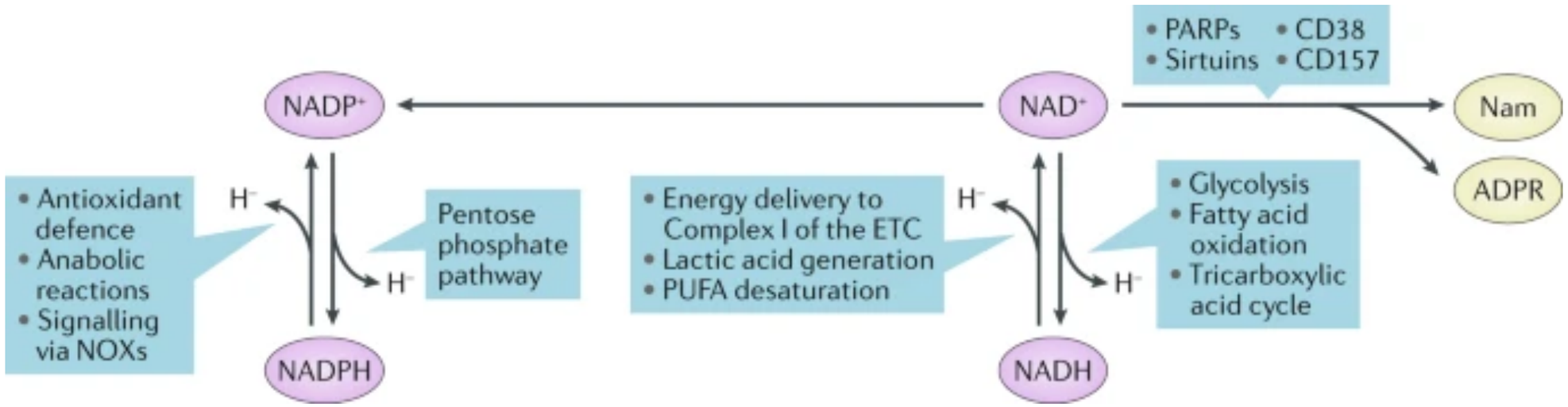


De novo synthesis
Salvage pathways

NAD+-consuming reactions

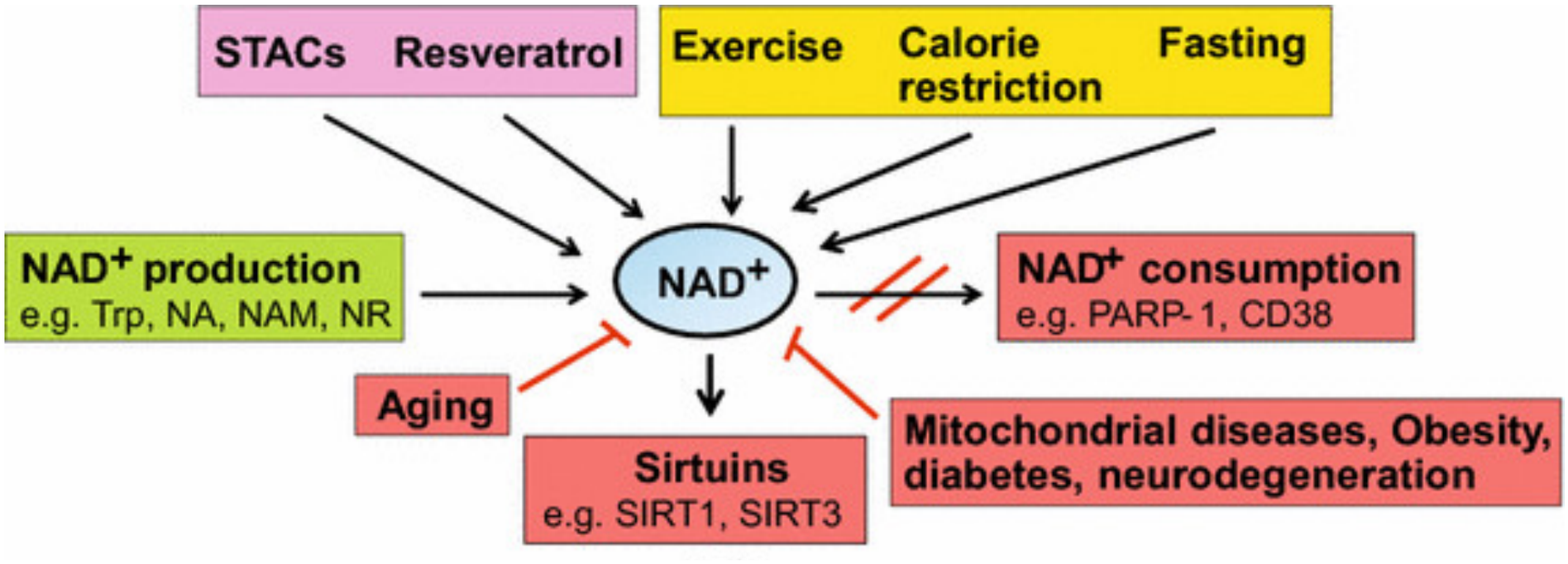
Glycolysis
TCA cycle
Fatty Acid ox
NAD phosphorylation (NADP+)
Specific PTMs

NAD+ homeostasis is sensitive to metabolic status of the cell

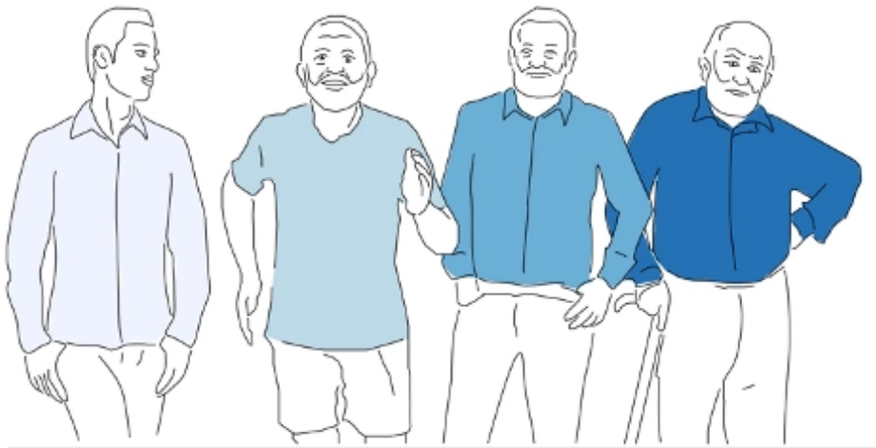


Ralto et al, ***Nat Rev Nephrol****, 2019*

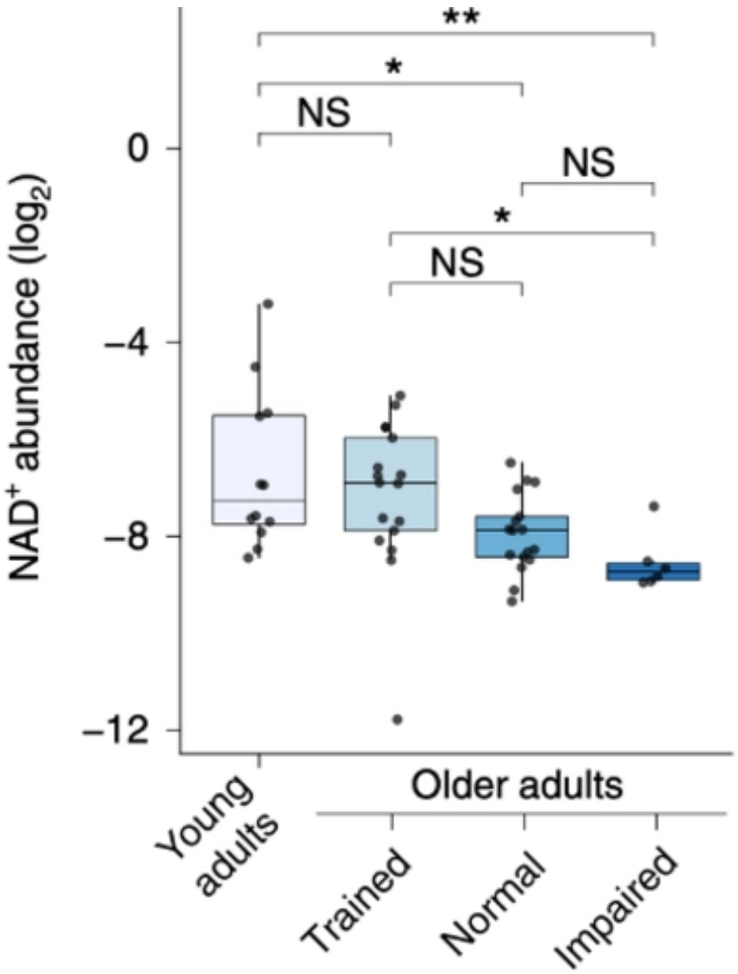
NAD+ levels rise in muscle, liver and white adipose tissue during fasting, caloric restriction and exercise



While high-fat diet in mice / obesity reduces the NAD+/NADH ratio

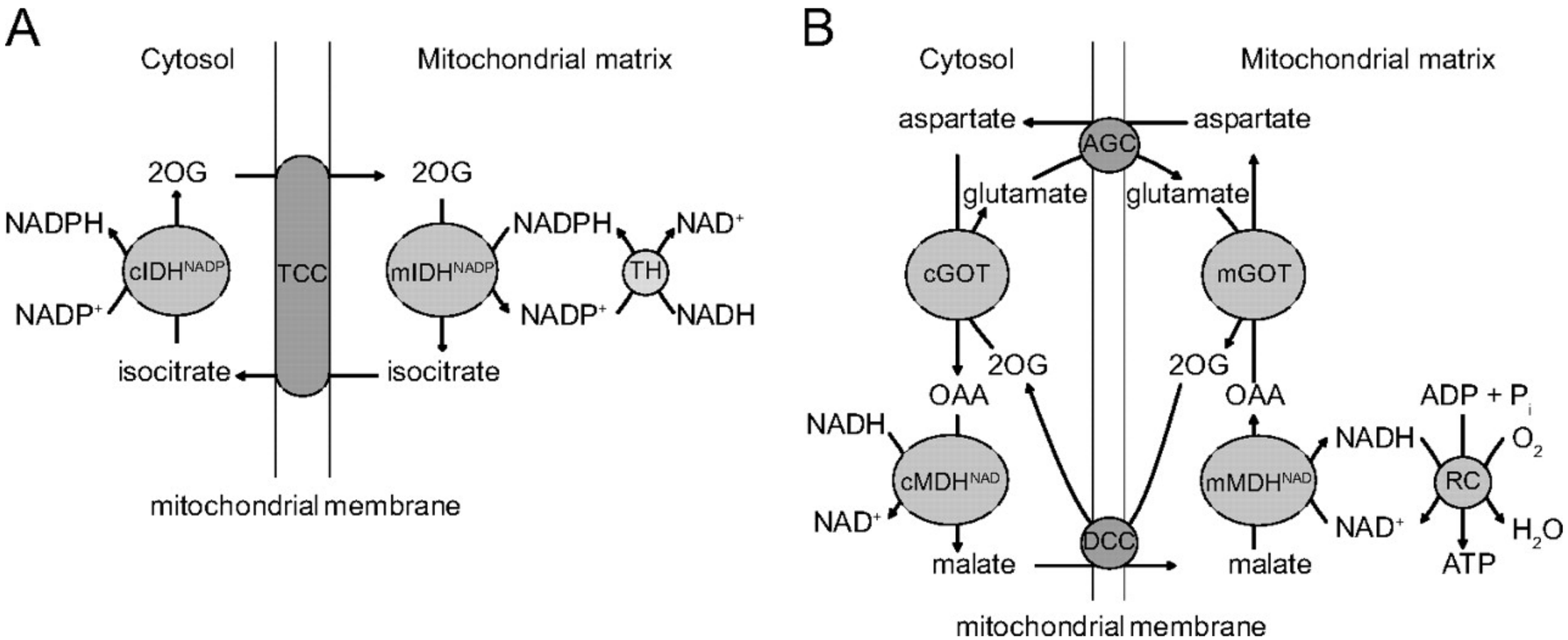


	Young	Older adults		
		Trained	Normal	Impaired
Participants	12	17	17	6
Age range (yr)	20–30	65–80	65–80	65–80
Male (%)	58	65	53	50
BMI (kg m^{-2})	22.7	23.9	25.8	27
Steps per day	10,153	13,671	10,197	6,608
Time high activity (%)	2.5	5.2	2.2	1.0

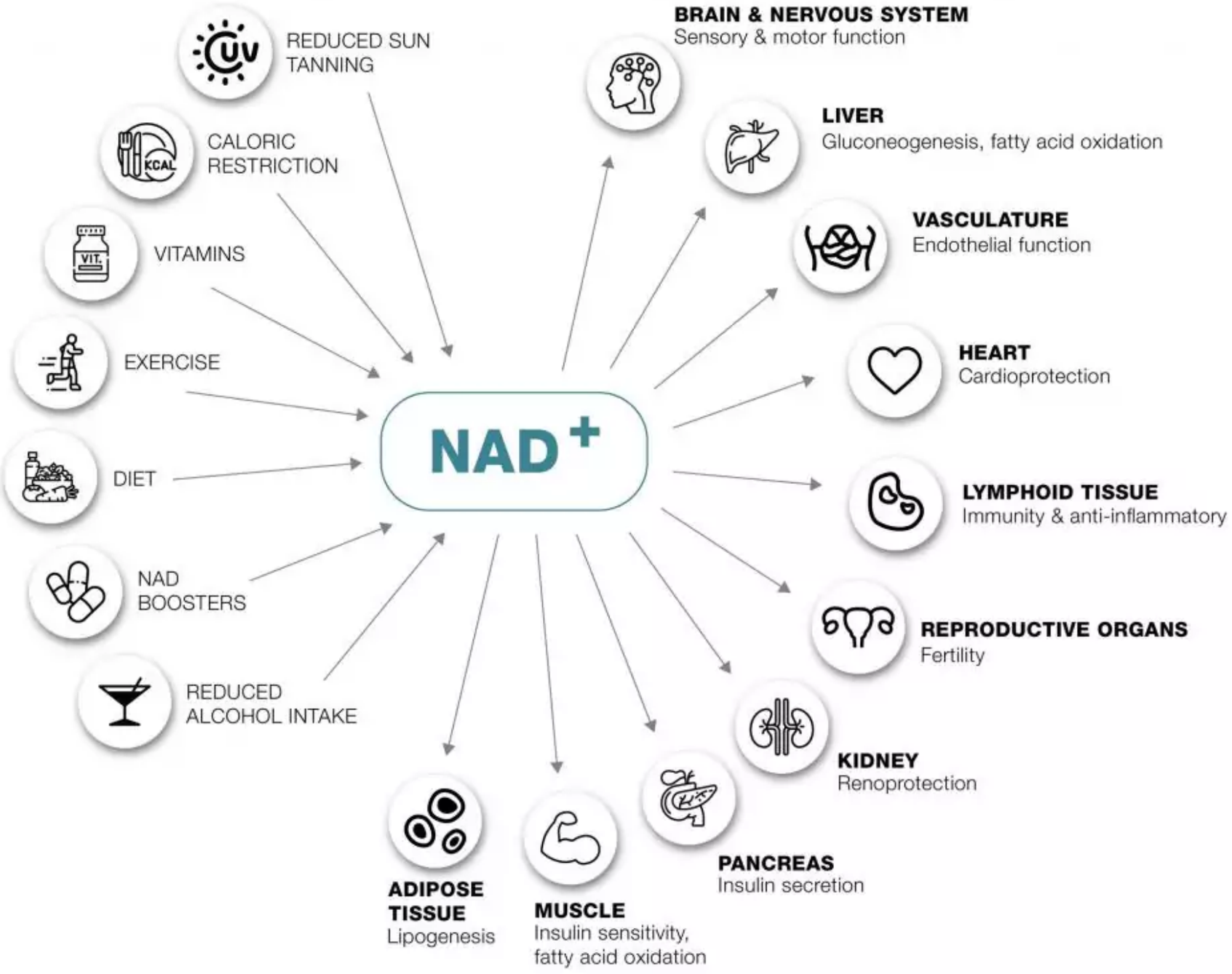


Jannsen et al, ***Nat Aging****, 2022*

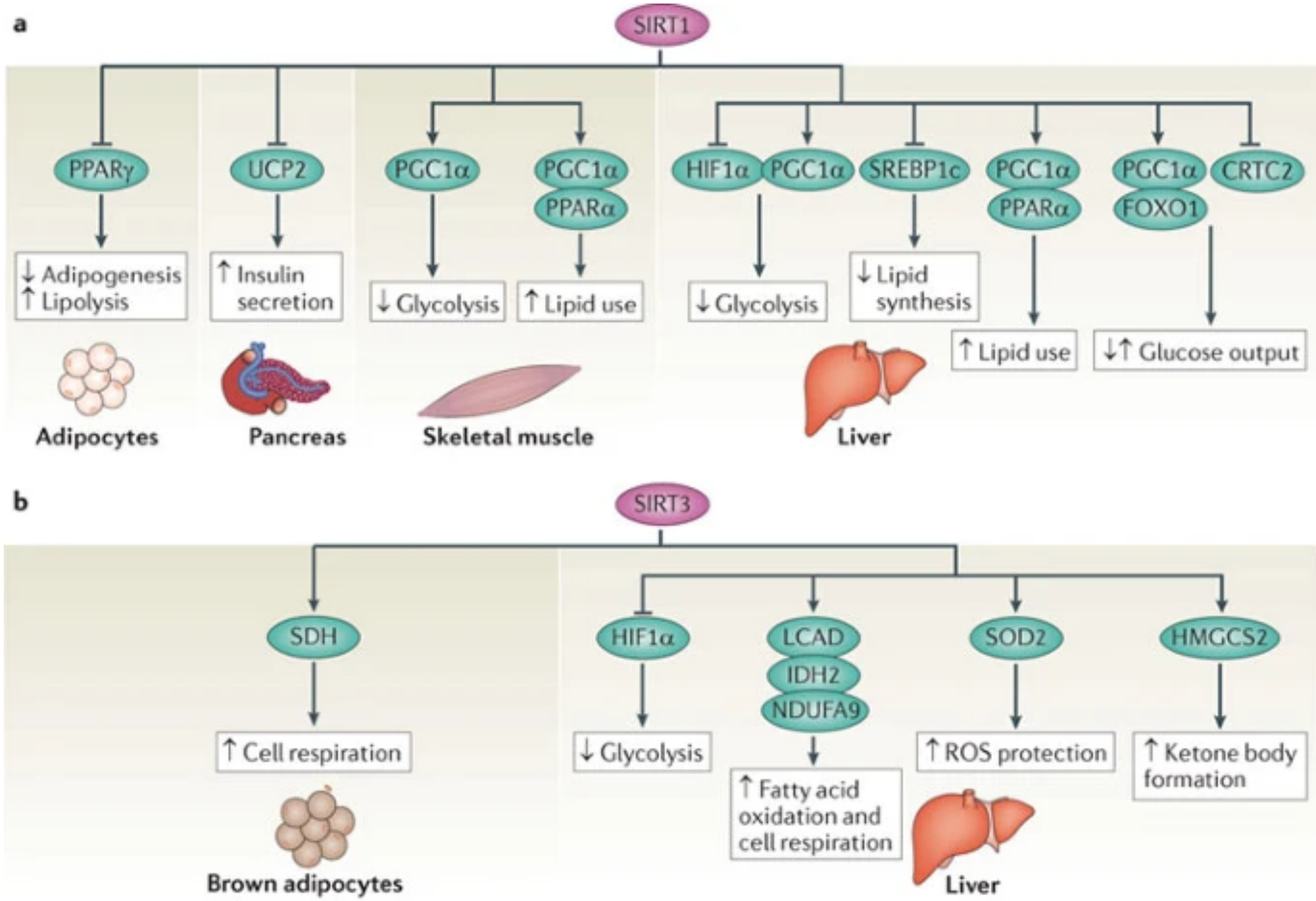
NAD+ is compartmentalized



NAD+ levels fluctuate and impact tissue-specific functions

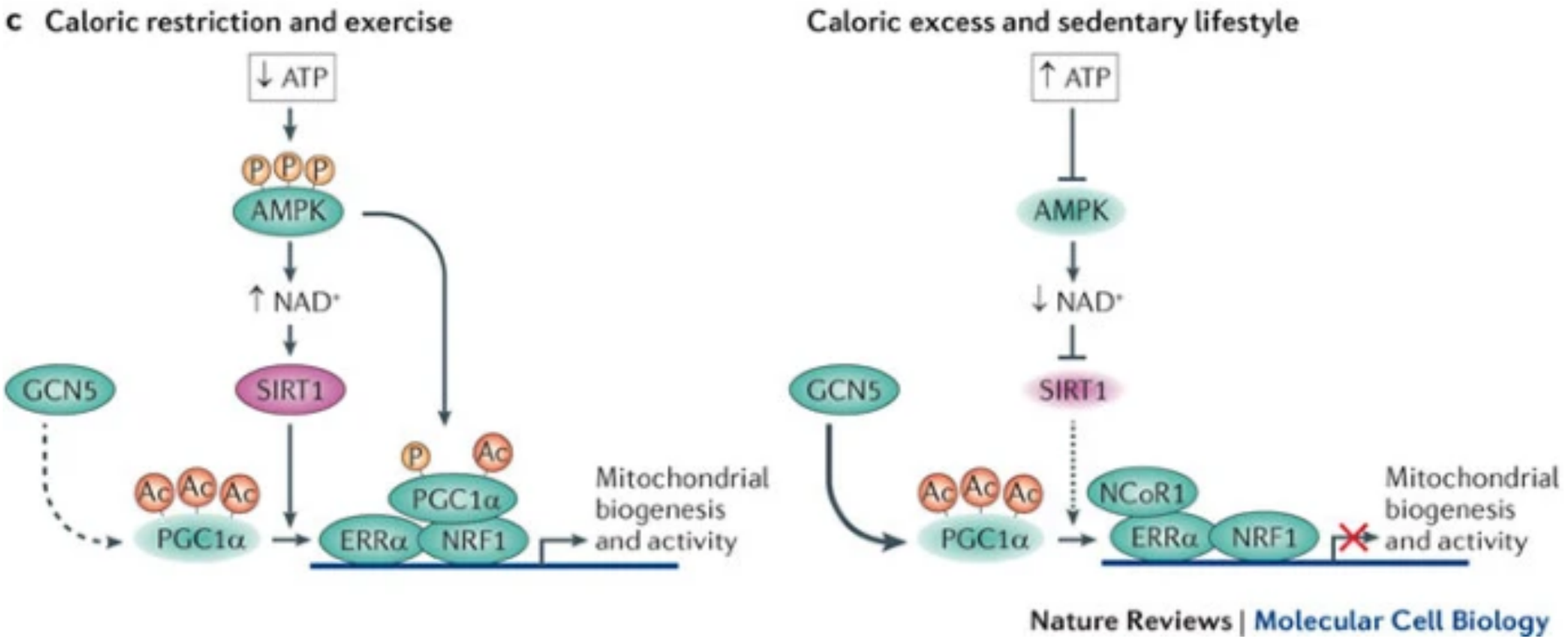


Sirtuins regulate metabolism



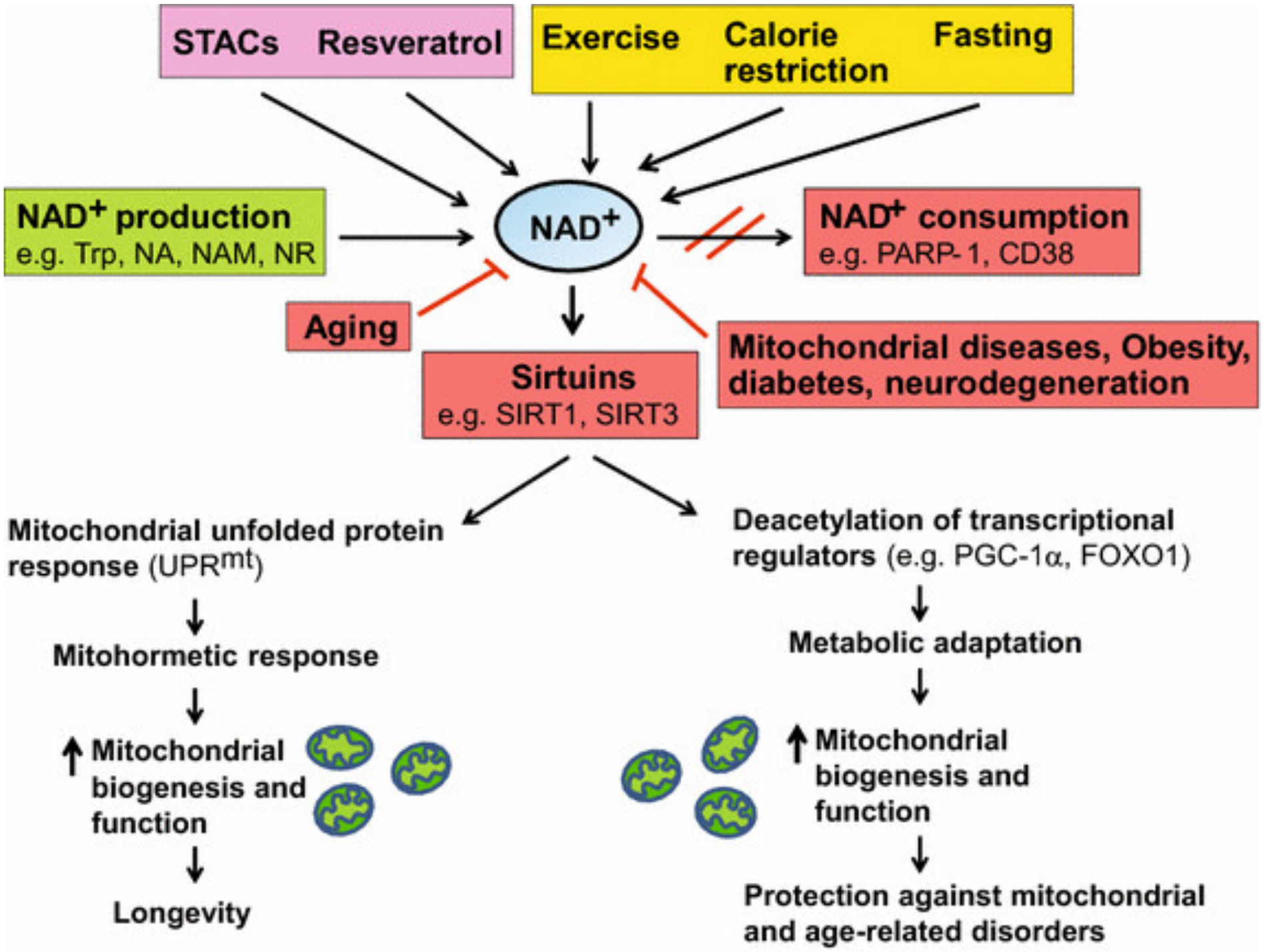
Houtkooper et al, ***NRCMB****, 2012*

Sirtuins regulate metabolism



Houtkooper et al, ***NRCMB****, 2012*

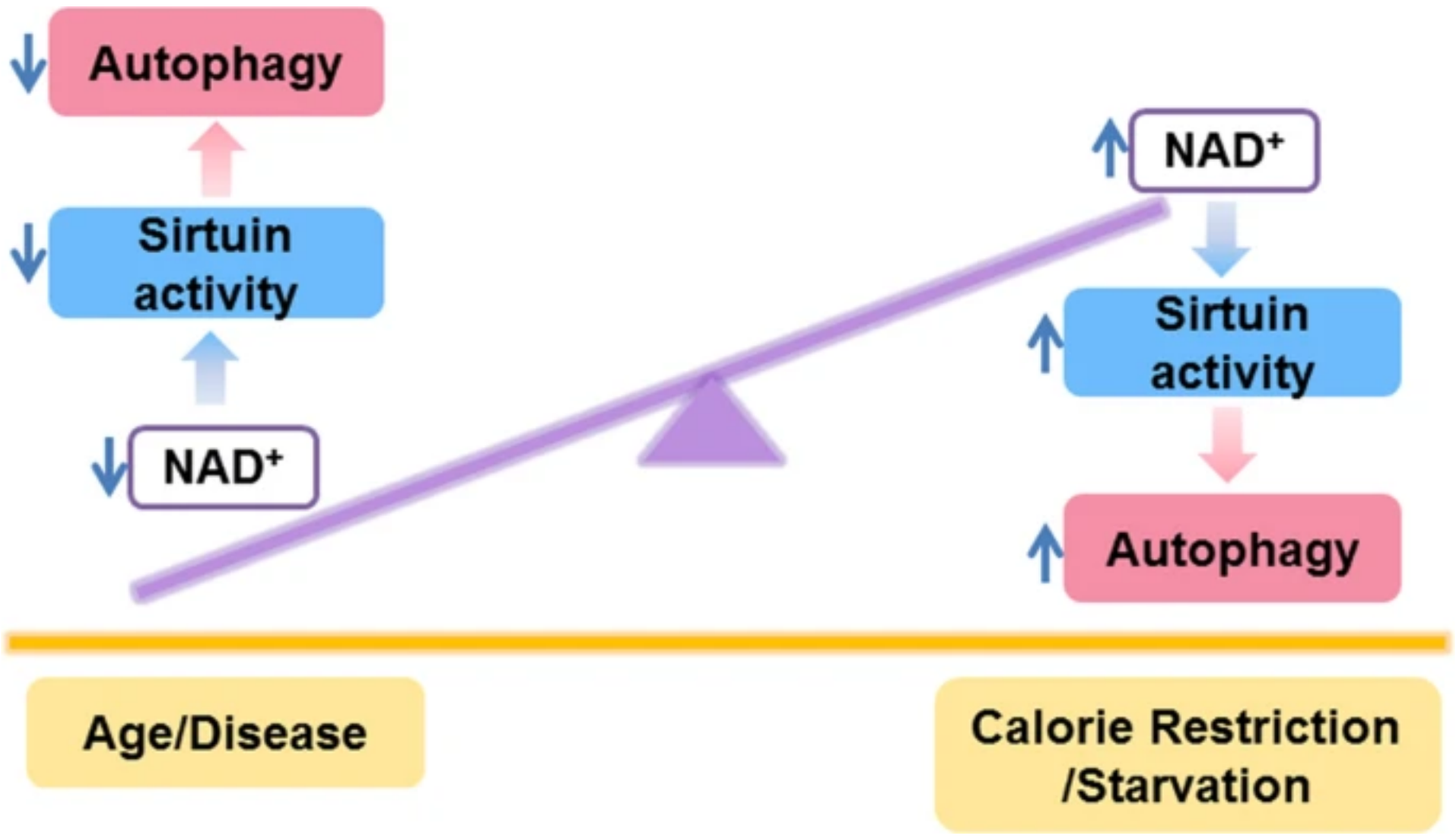
Sirtuins regulate mitochondria fitness



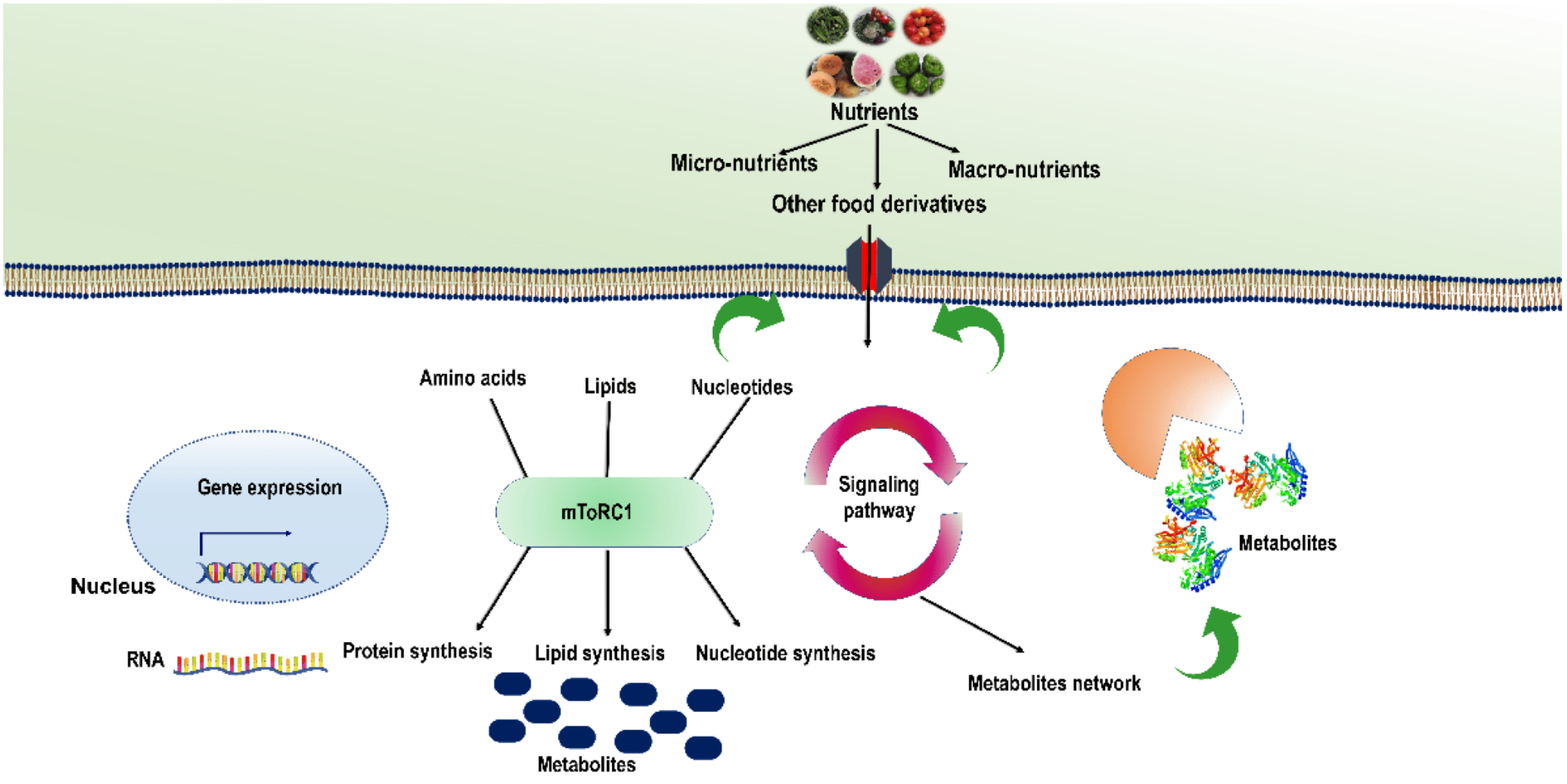
Sirtuins and aging

	Lifespan increase		Beneficial health effects	
	Dietary restriction	Mutations/ drugs	Dietary restriction	Mutations/ drugs
 Yeast	3 fold	10 fold	Extended reproductive period	Extended reproductive period, decreased DNA damage/mutations
 Worms	2-3 fold	10 fold	Resistance to misexpressed toxic proteins	Extended motility Resistance to mis-expressed toxic proteins and germ-line cancer
 Flies	2 fold	60-70%	None reported	Resistance to bacterial infection, extended ability to fly
 Mice	30-50%	30-50% (~100% in combination with DR)	Protection against cancer, diabetes, atherosclerosis,cardio-myopathy, autoimmune, kidney and respiratory diseases, reduced neurogeneration	Reduced tumor incidence, protection against age-dependent cognitive decline, cardio-myopathy, fatty liver and renal lesions. Extended insulin sensitivity
 Monkeys	Trend noted	Not tested	Prevention of obesity, protection against diabetes, cancer and cardiovascular disease	Not tested
 Humans	Not determined	Not determined (GHR deficient subjects reach old age)	Prevention of obesity, diabetes, hypertension Reduced risk factors for cancer and cardiovascular disease	Possible reduction in cancer and diabetes

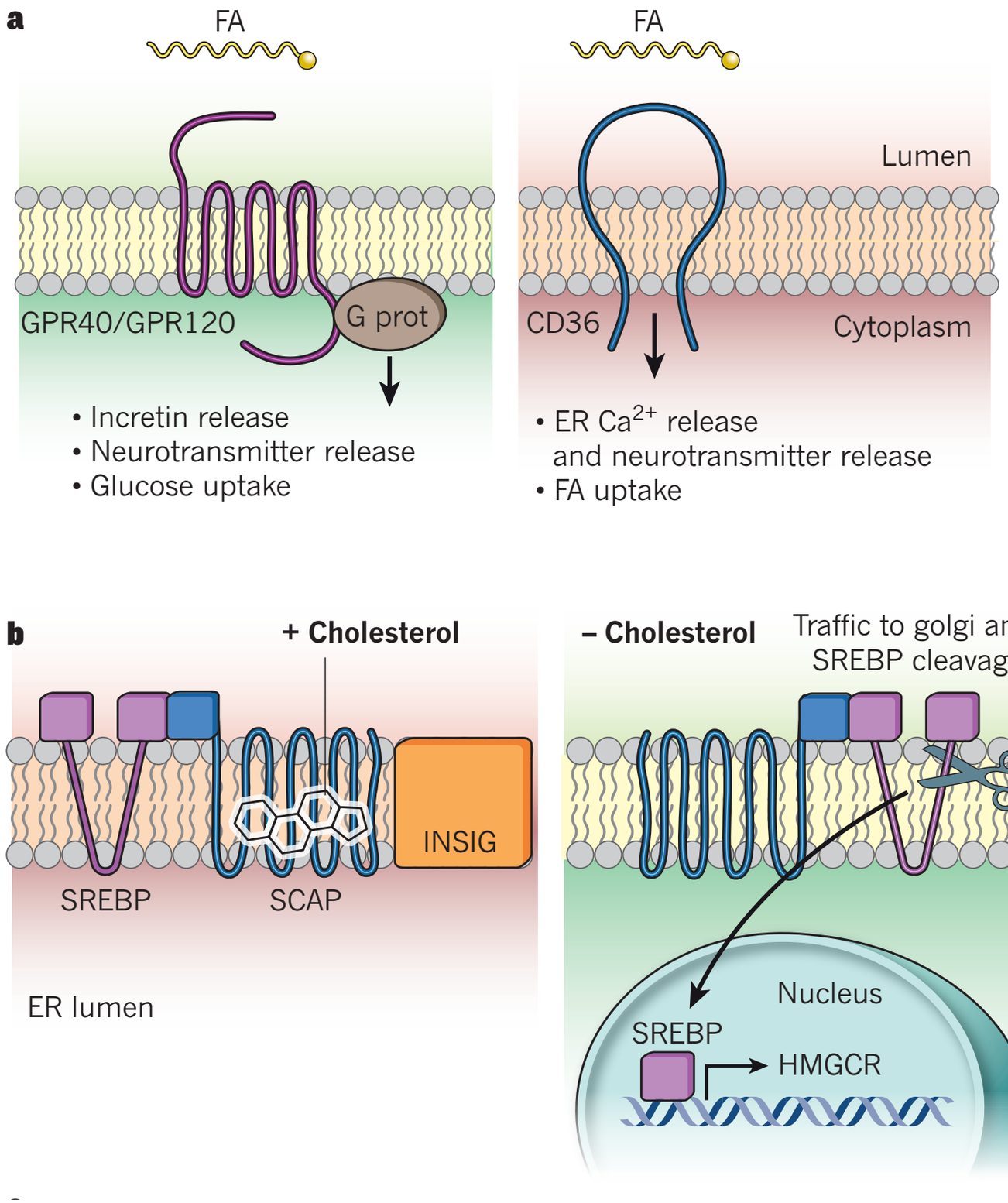
Sirtuins and aging



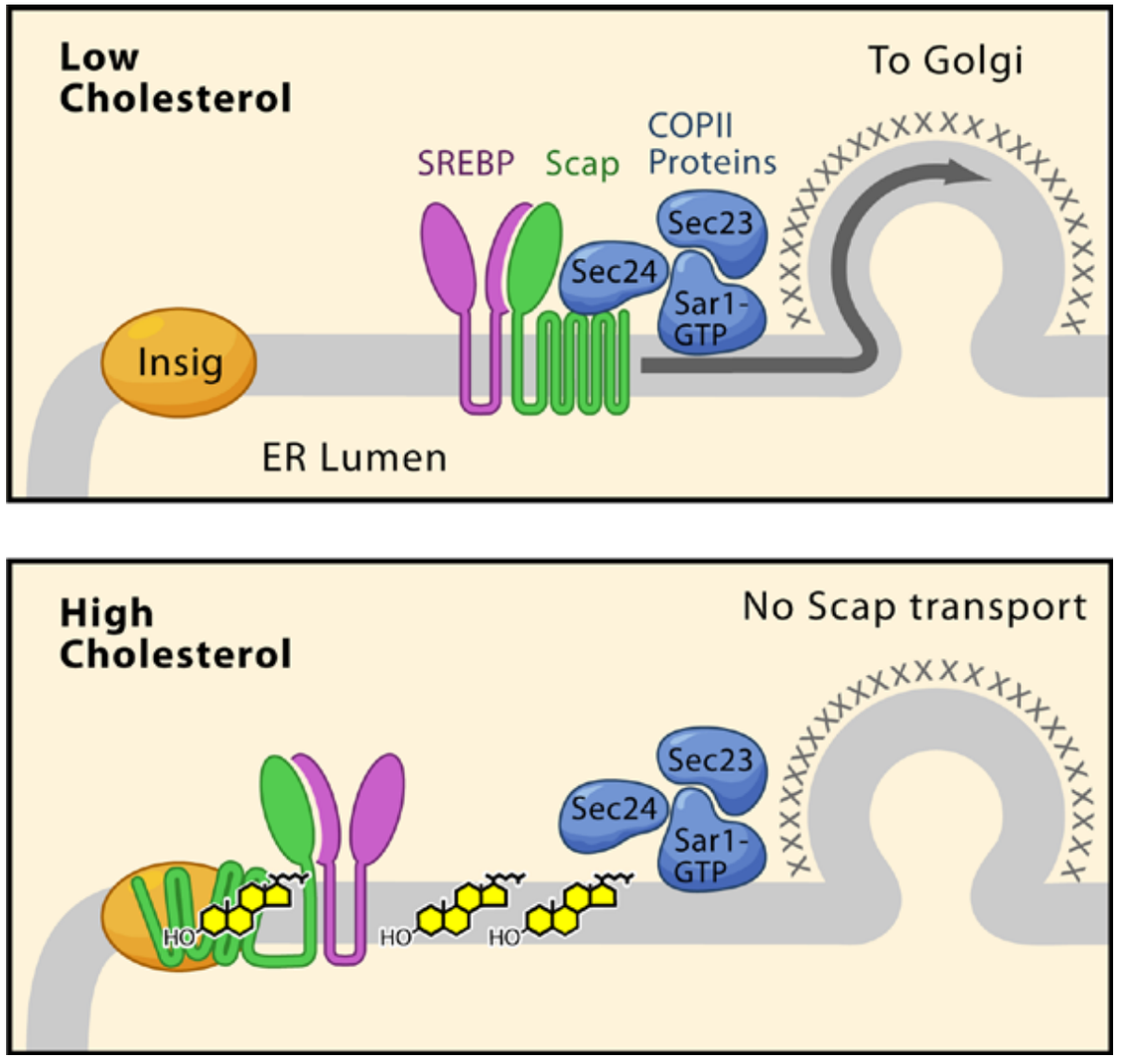
Ablation of sirtuins decreases lifespan (healthspan??) in yeast and worms, while their OE prolongs it


Nutrients
Micro-nutrients
Macro-nutrients
Other food derivatives
Amino acids
Lipids
Nucleotides
Gene expression
Nucleus
RNA
mToRC1
Protein synthesis
Lipid synthesis
Nucleotide synthesis
Metabolites
Signaling pathway
Metabolites network
Metabolites

Lipid sensing



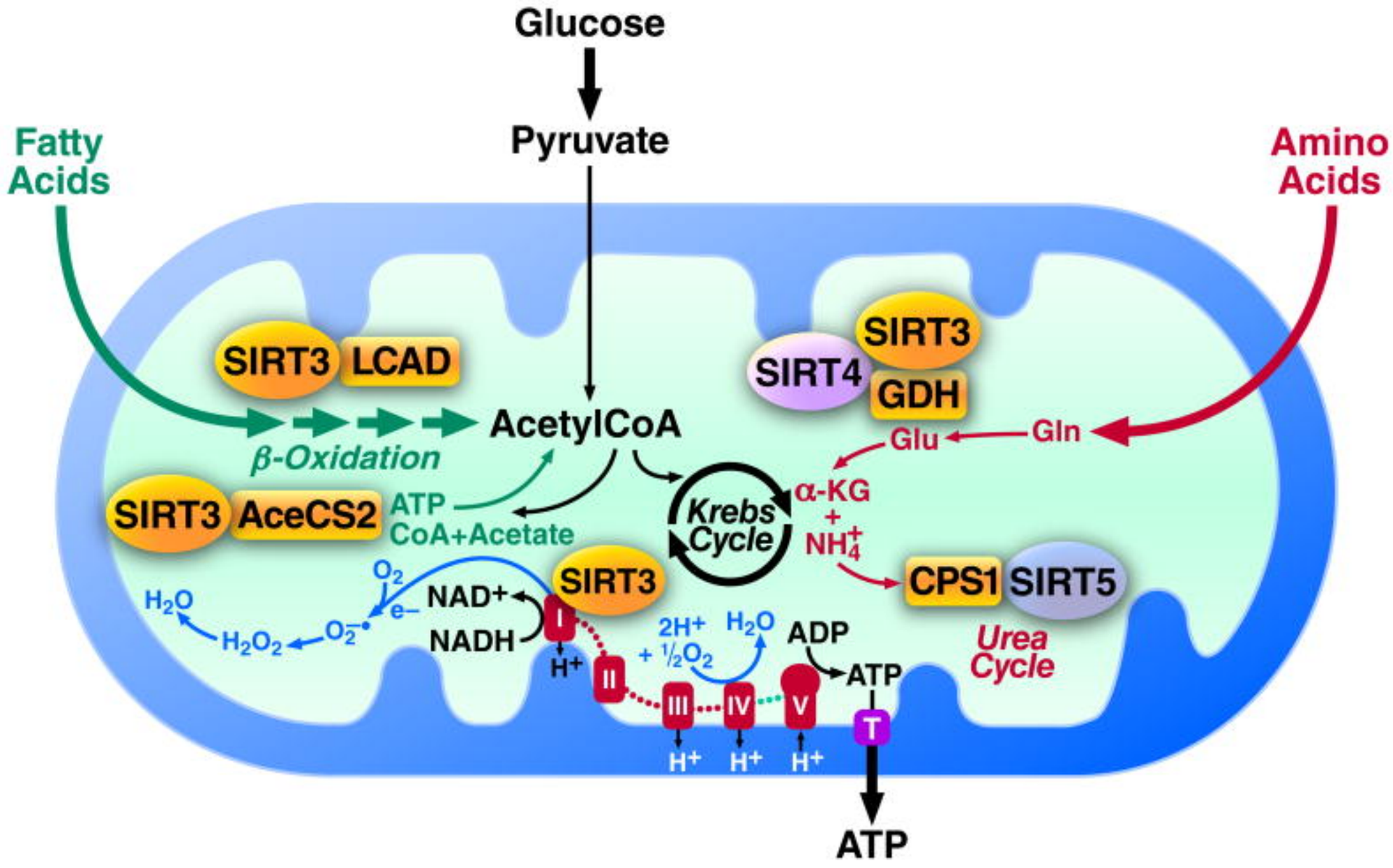
Cholesterol sensing is mediated by SCAP and SREBP



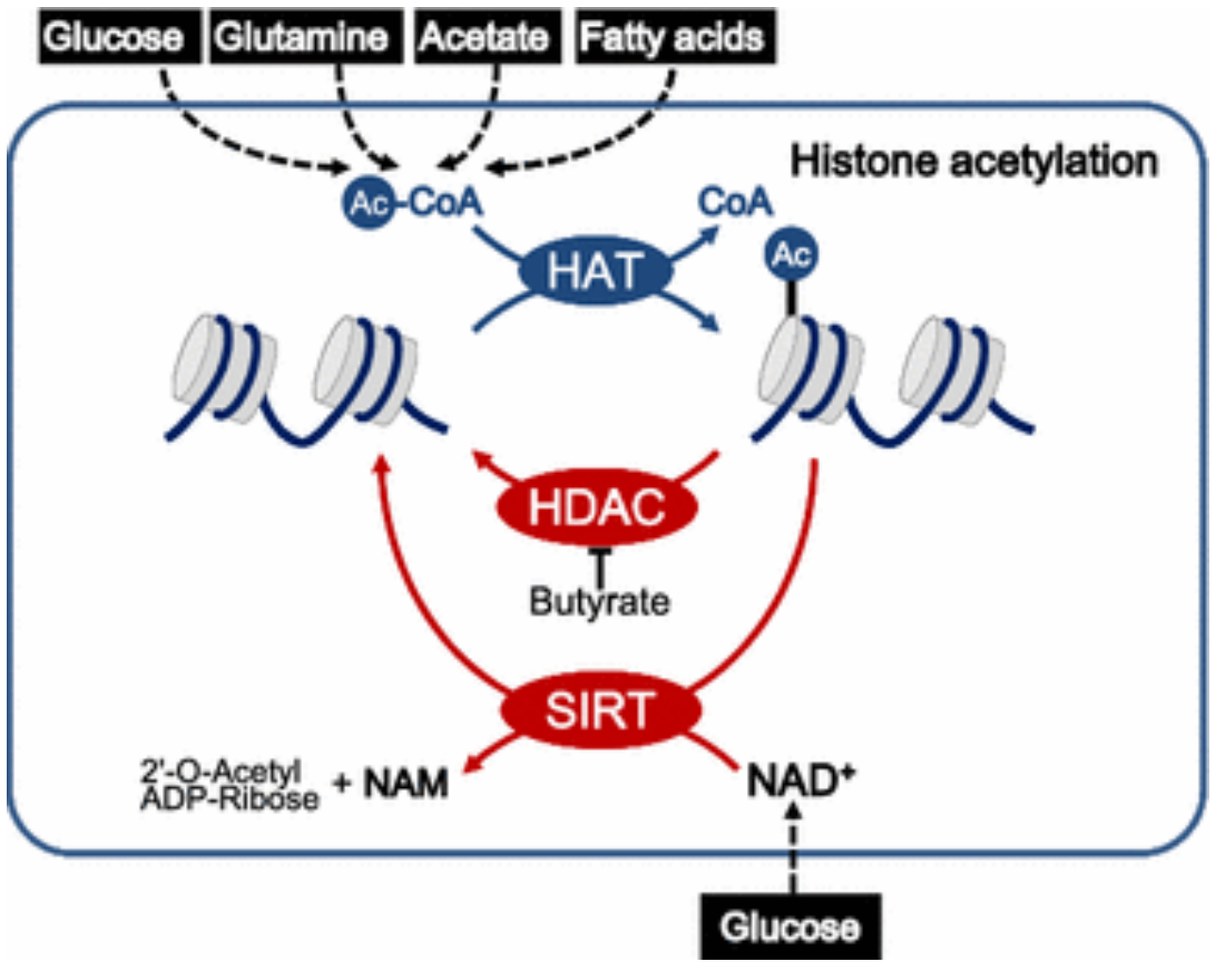
When animal cells are deprived of sterols, Scap escorts SREBPs from the ER to Golgi by binding to Sec24, a component of the Sar1/Sec23/Sec24 complex of the COPII protein coat. Once in the Golgi, the SREBPs are proteolytically processed to generate their nuclear forms that activate genes for cholesterol synthesis and uptake.

Cholesterol negatively regulates ER-to-Golgi transport by binding to Scap, thereby changing its conformation and triggering the binding of Scap to Insig, an ER anchor protein. Insig prevents the binding of Scap to COPII proteins, thereby halting transport of SREBPs to the Golgi.

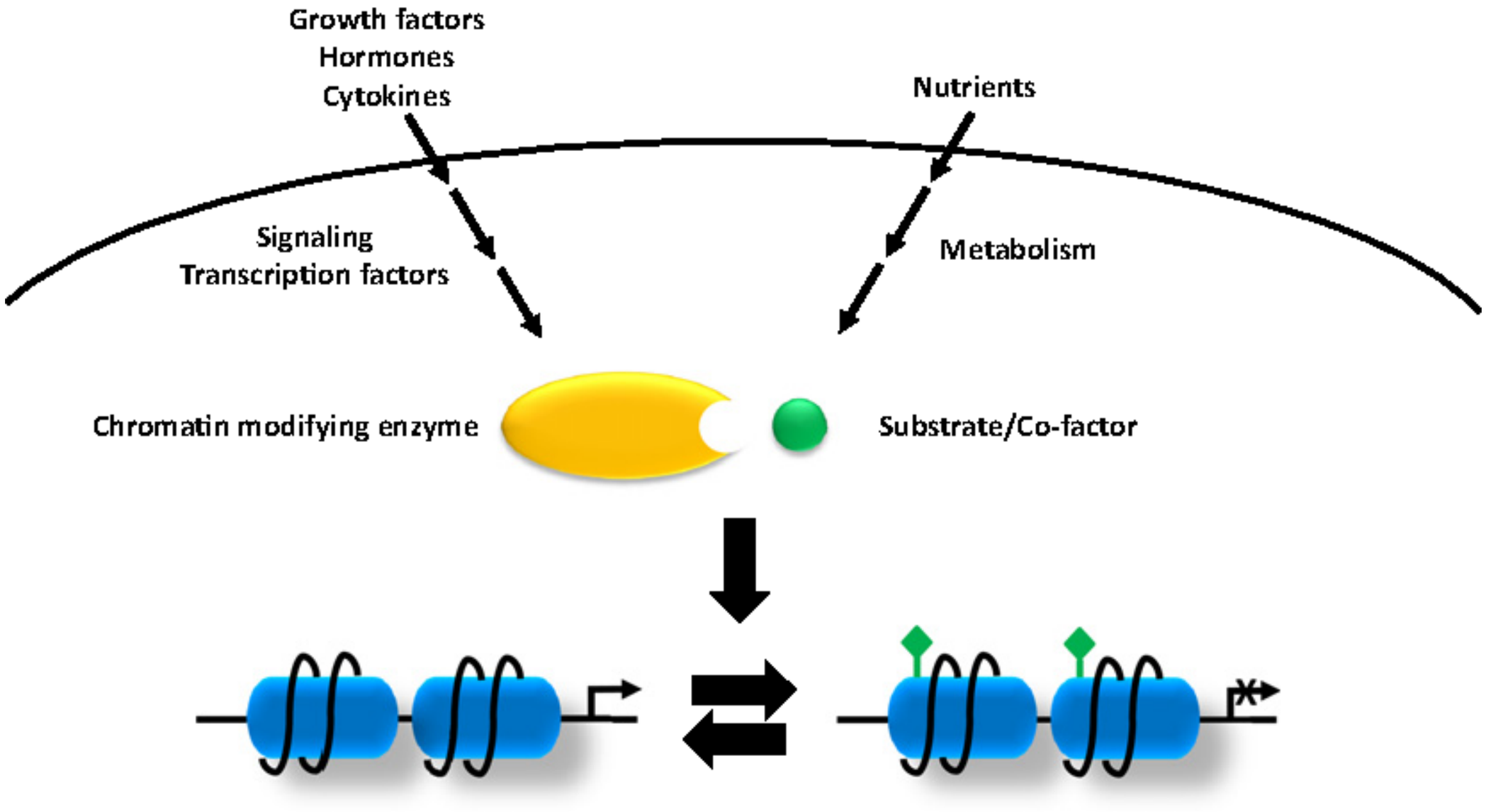
Nutrient availability impacts acetyl-CoA levels



…which can signal to the nucleus

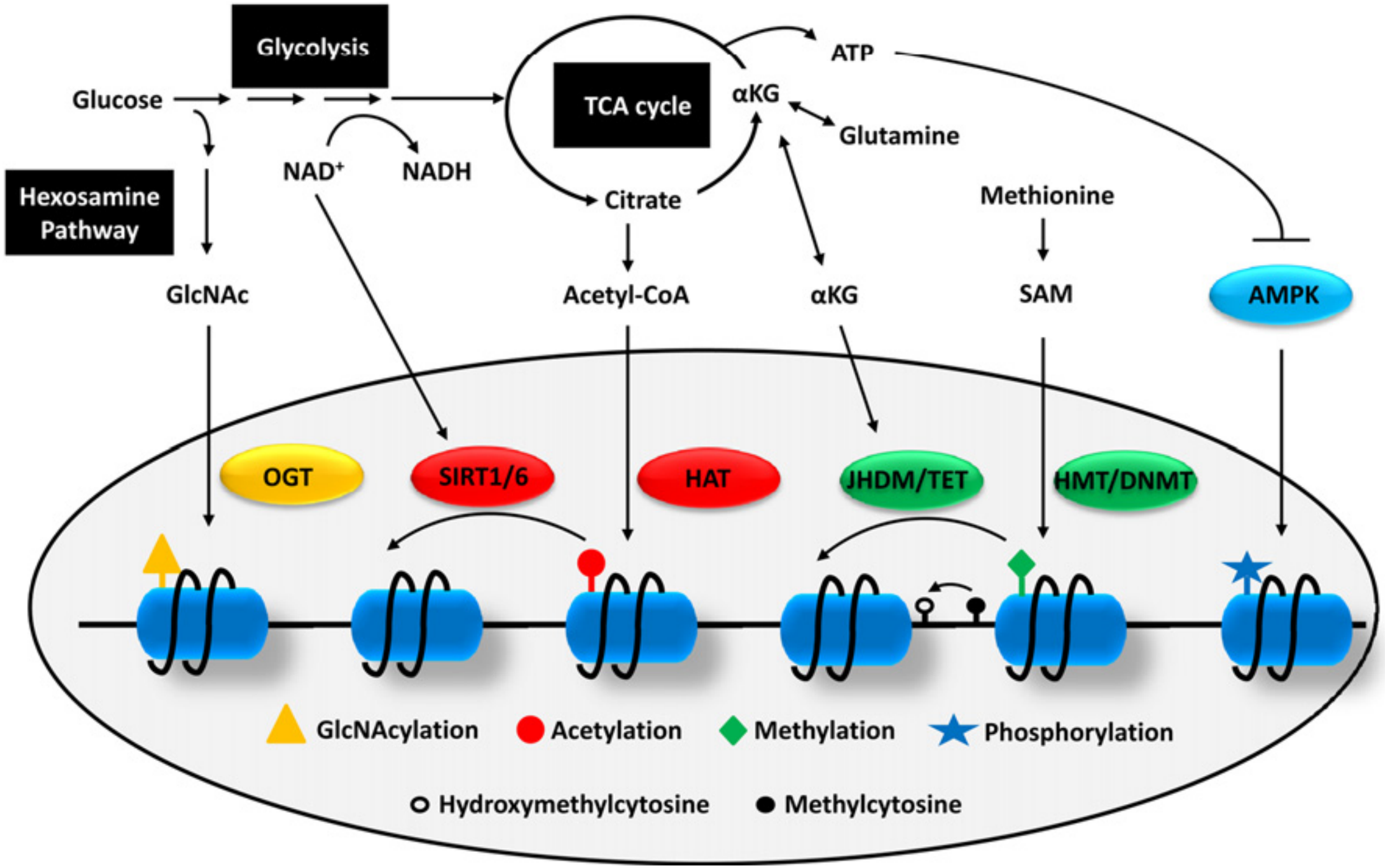


Metabolites integrate nutrient availability in the nucleus

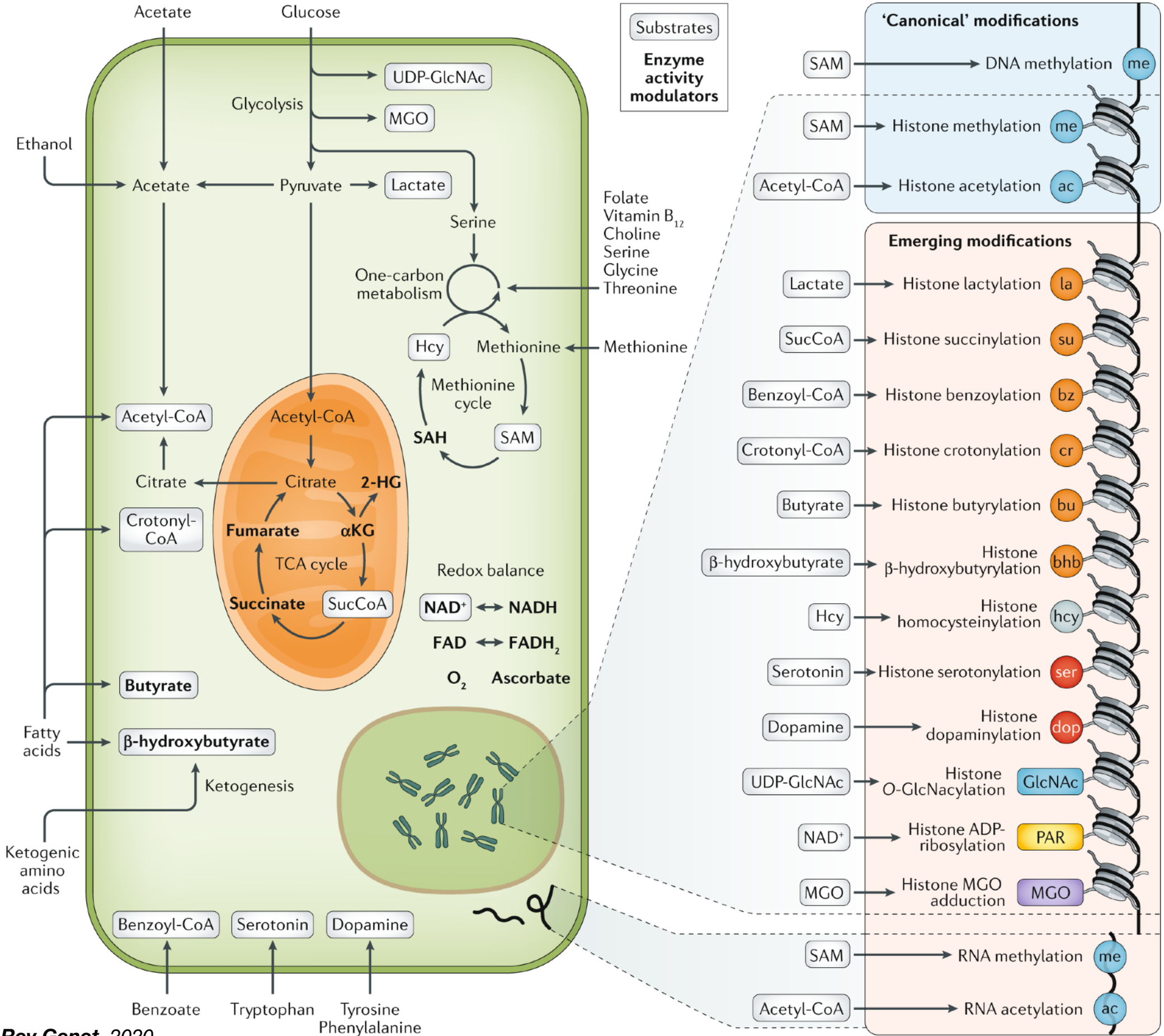


Lu & Thompson, ***Cell Metab****, 2012*

Metabolites integrate nutrient availability in the nucleus



Lu & Thompson, ***Cell Metab****, 2012*



Dai et al, ***Nat Rev Genet****, 2020*

When is nutrient sensing important?

Physiology:

When a cell changes microenvironment - *adaptation*

To regulate changes in cell state - *differentiation*

To regulate growth - *development*

To integrate dietary inputs - *fed/fast state*

To integrate circadian oscillations - *day/night cycles*

Pathology:

Cancer

Metabolic syndrome / obesity

Maladaptive responses (dysplasia, hypertrophy, …)

Neurological disorders