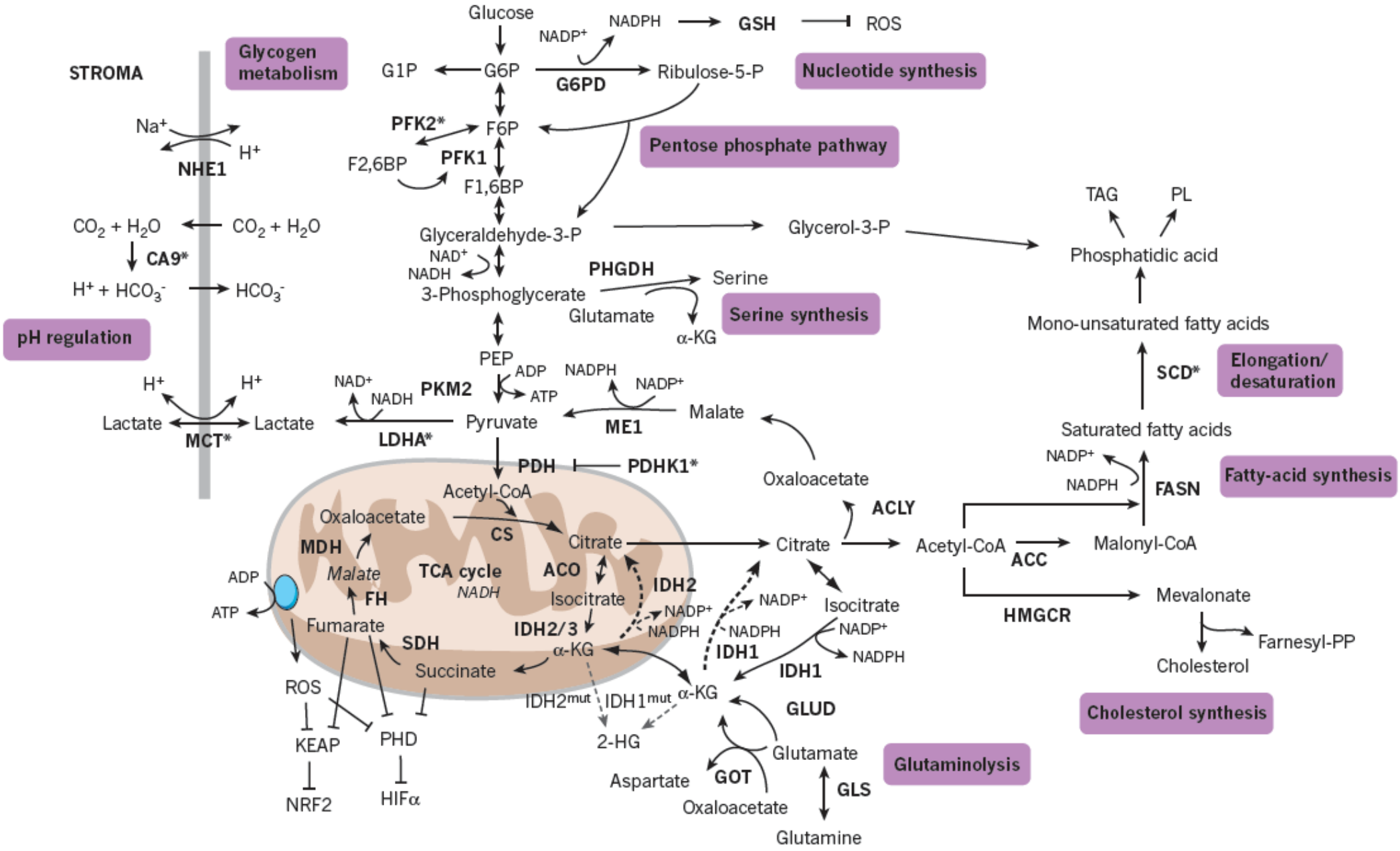
TUMOR METABOLISM



Schulze & Harris, Nature 2012

OUTLINE

- Role of cell metabolism

- Characteristics of tumor cell metabolism

- Factors promoting metabolic transformation in tumors

    1. *Challenges of tumor growth*
    2. *Hypoxia*
    3. *Nutrient Deprivation*
    4. *Abnormal gene expression*

- Role of Oncogenes and Tumor Suppressors in metabolic transformation

    Examples

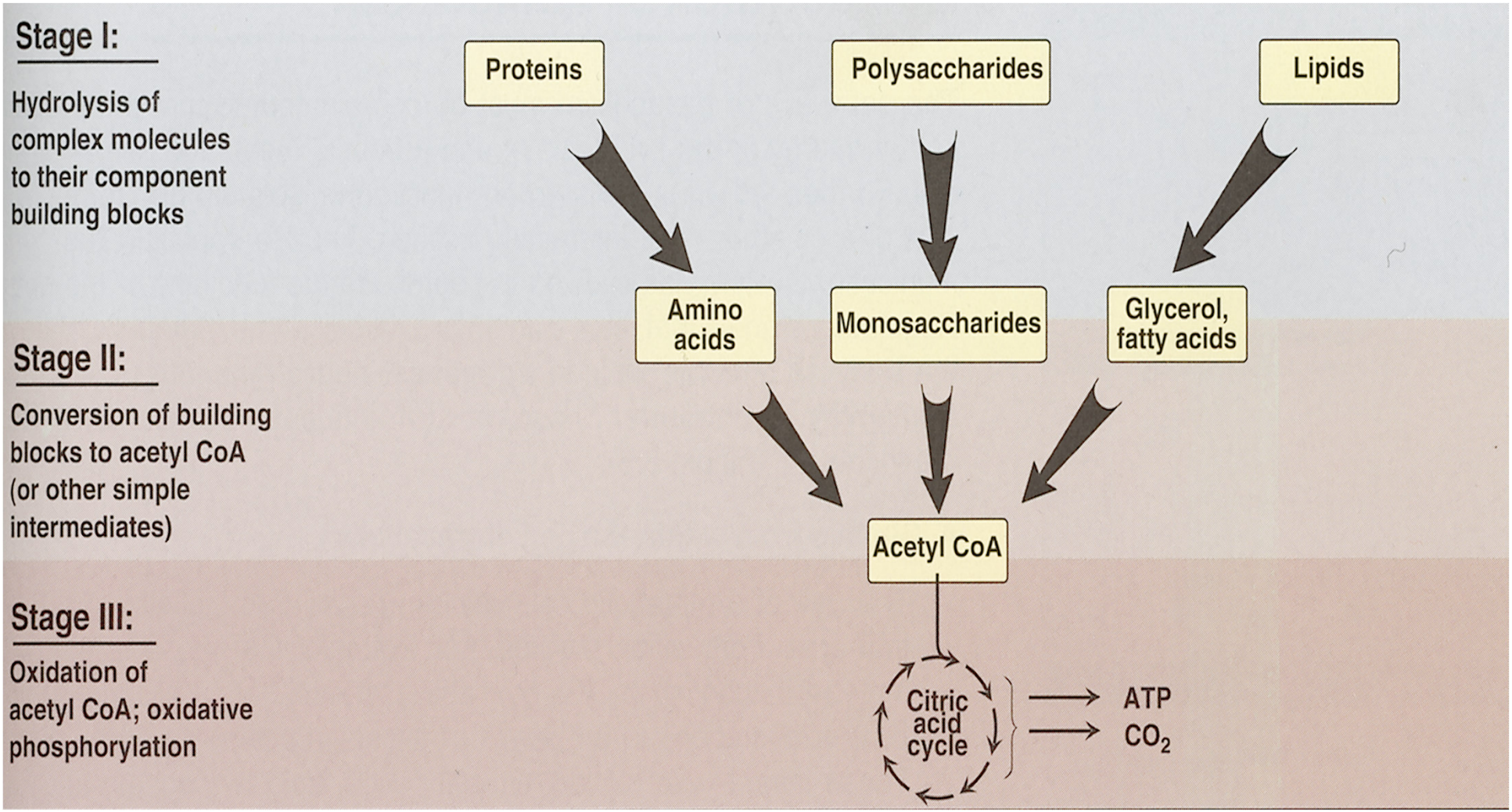
- Methods

- Perspectives and Future directions

    1. *Tumor Diagnostic*
    2. *Therapeutic intervention*

Cell Metabolism

" Intracellular chemical reactions that convert nutrients and endogenous molecules into the Energy and Matter (proteins, nucleic acids and lipids)"



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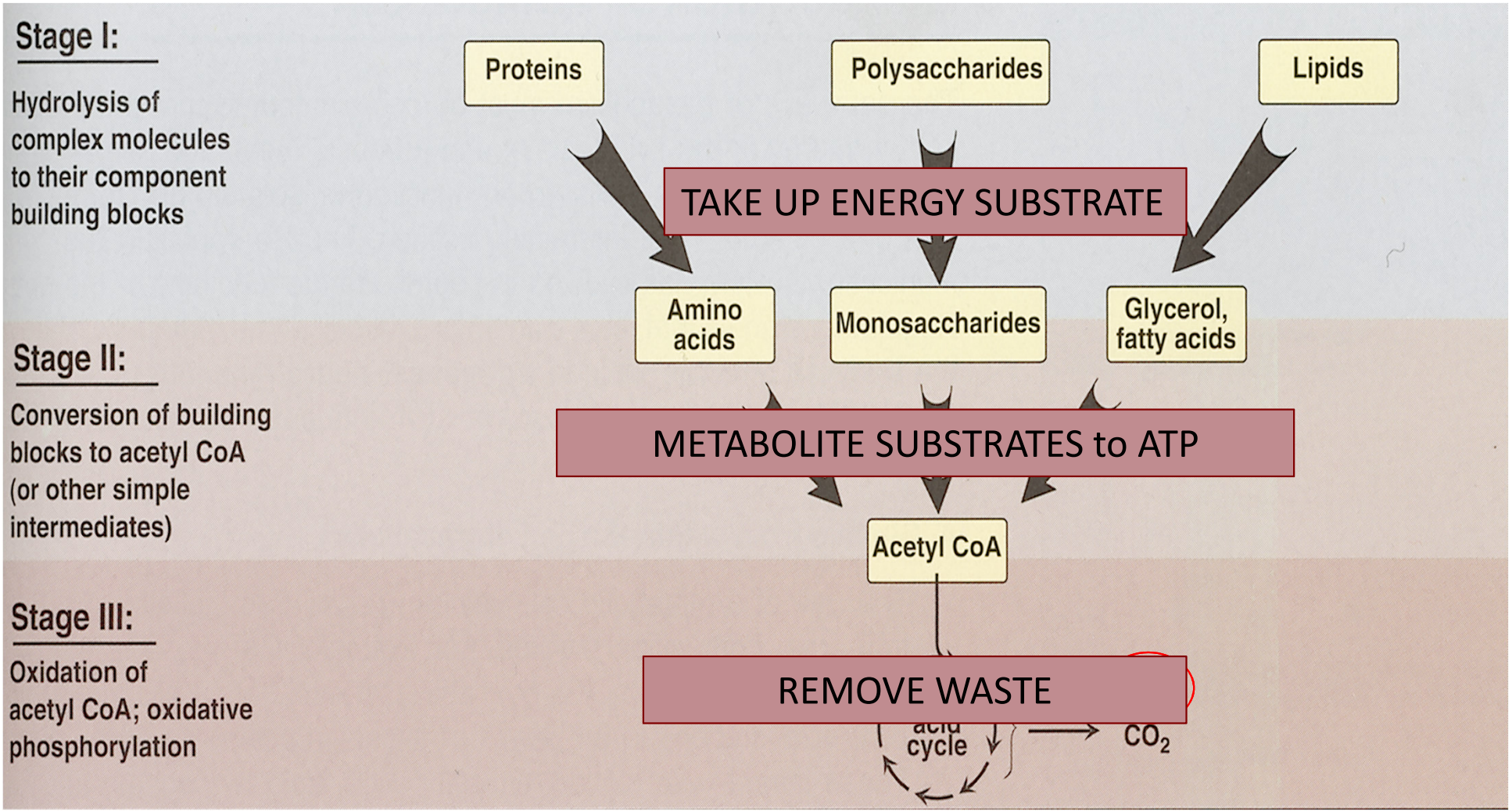
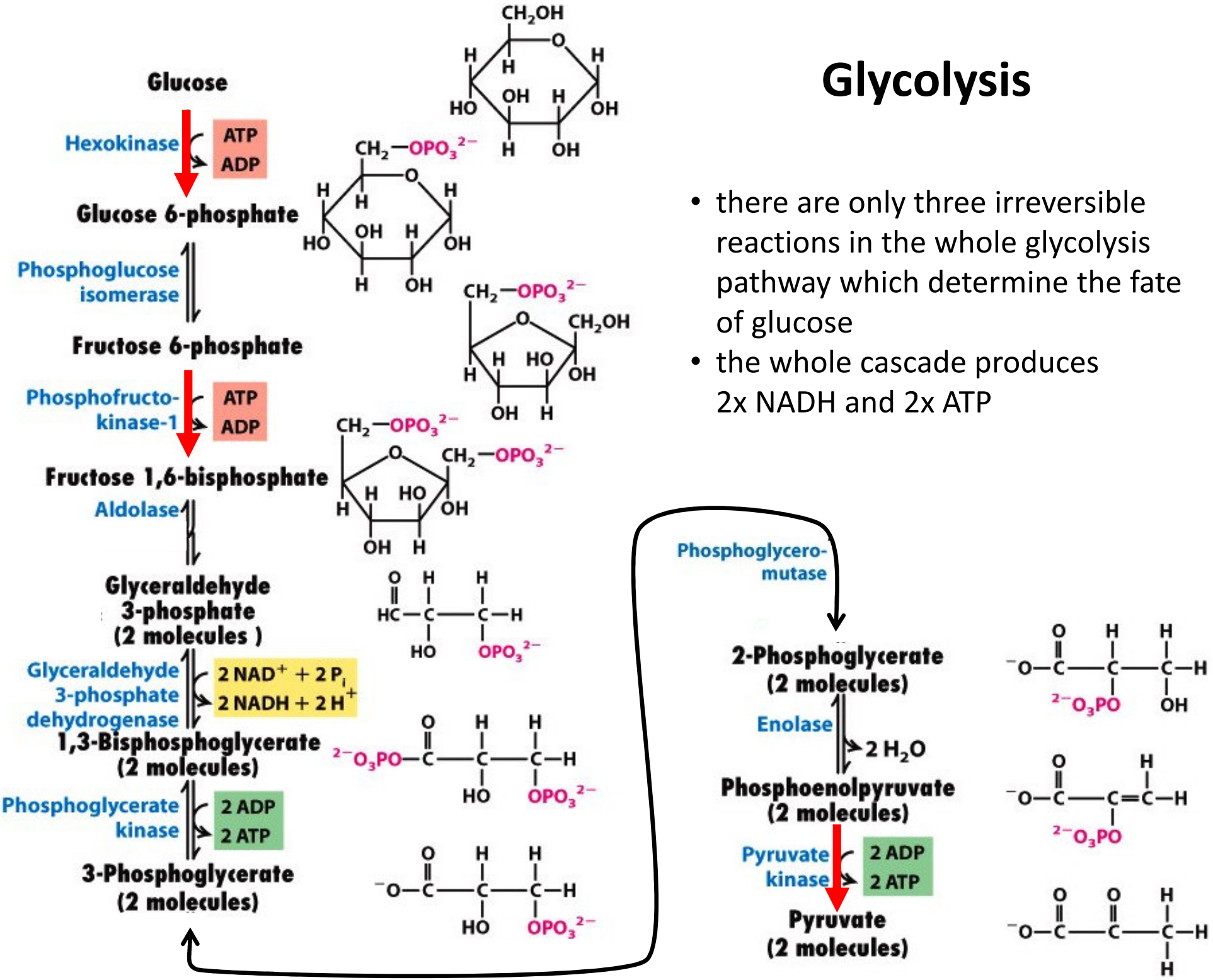
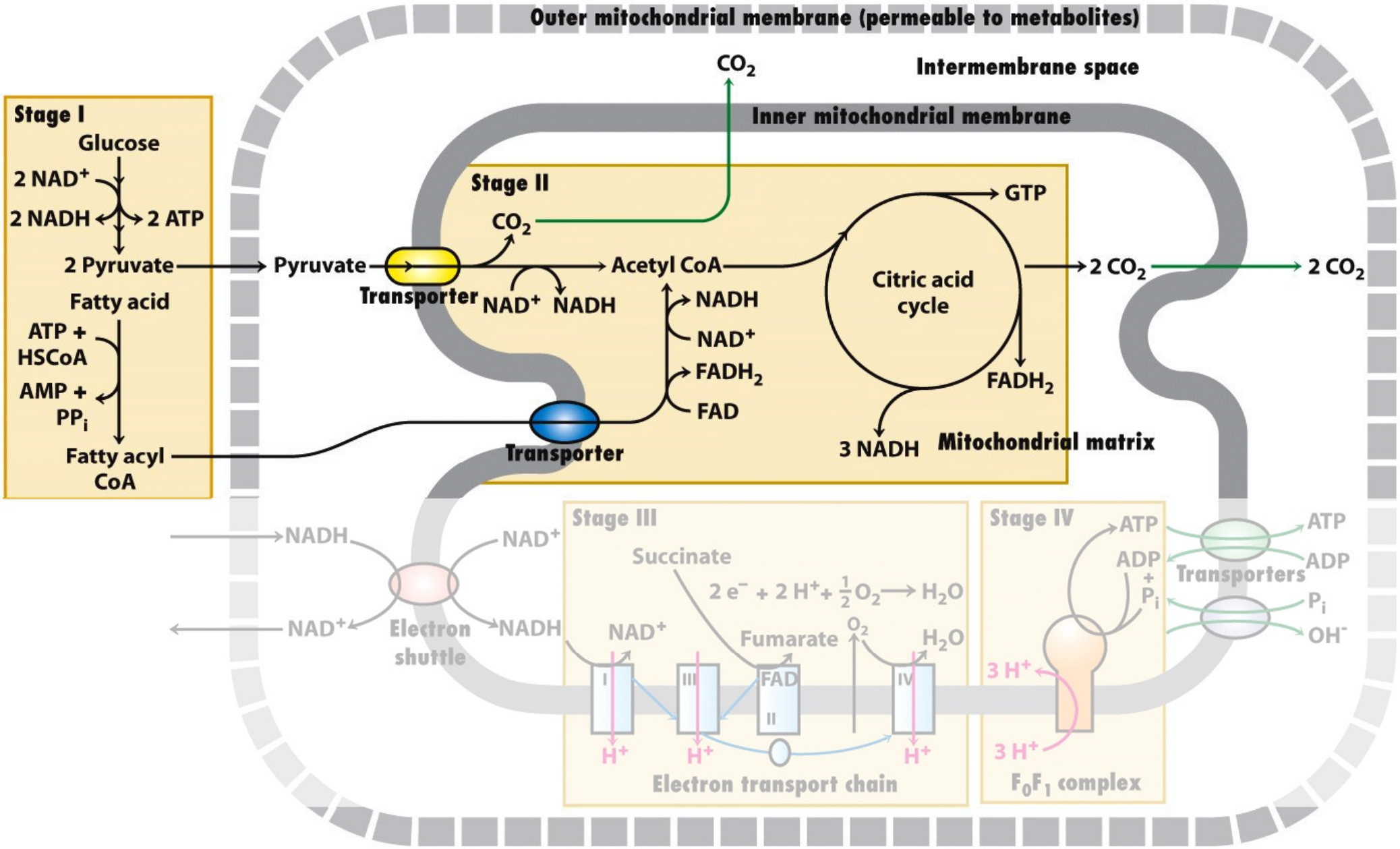


Glycolysis

- there are only three irreversible reactions in the whole glycolysis pathway which determine the fate of glucose
- the whole cascade produces 2x NADH and 2x ATP



Citric acid/tricarboxylic acid (TCA)/Krebs cycle



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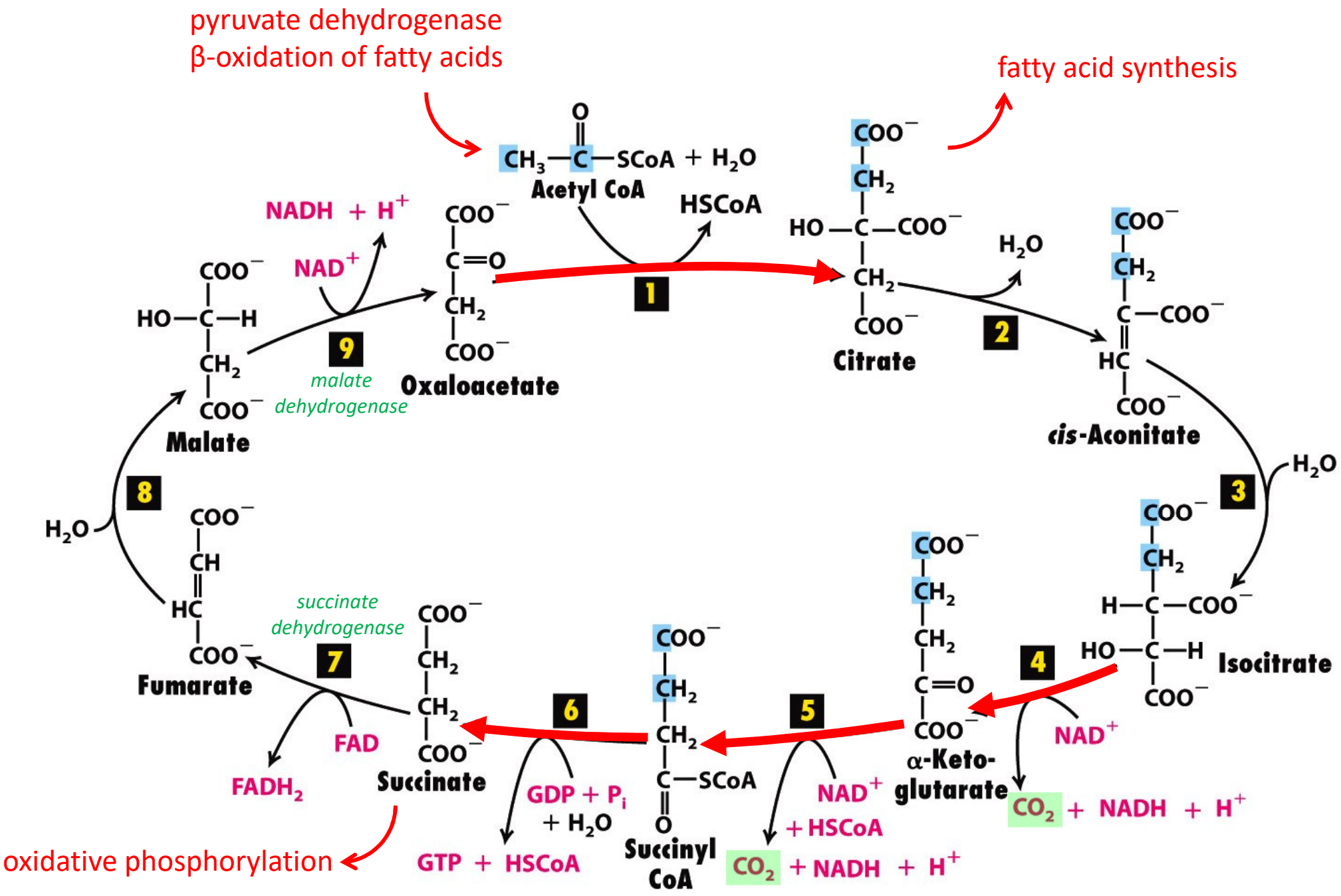
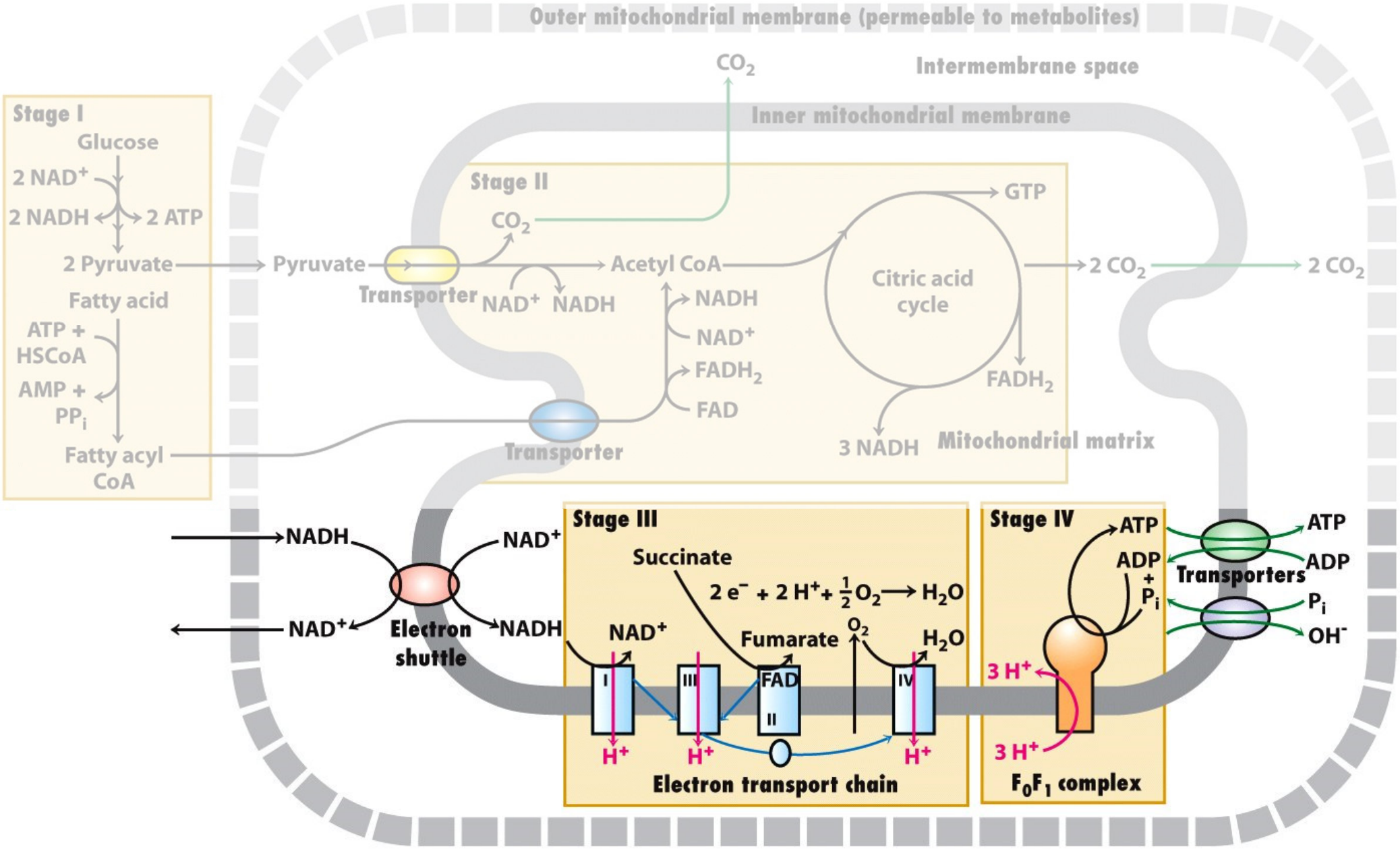
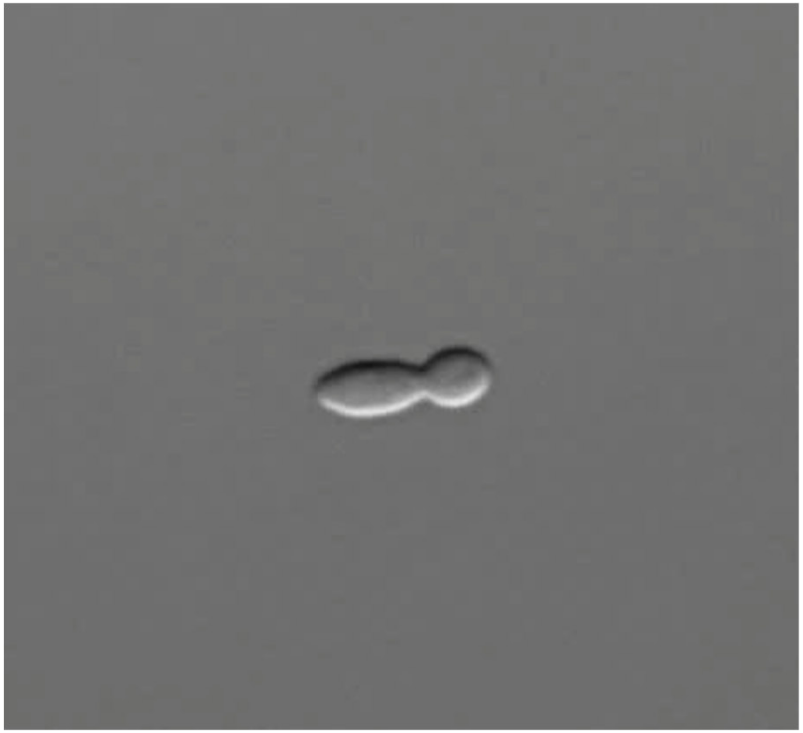


Oxidative phosphorylation



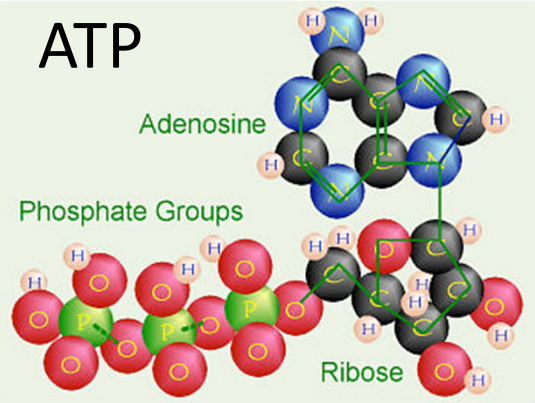
Regulation of nutrient consumption



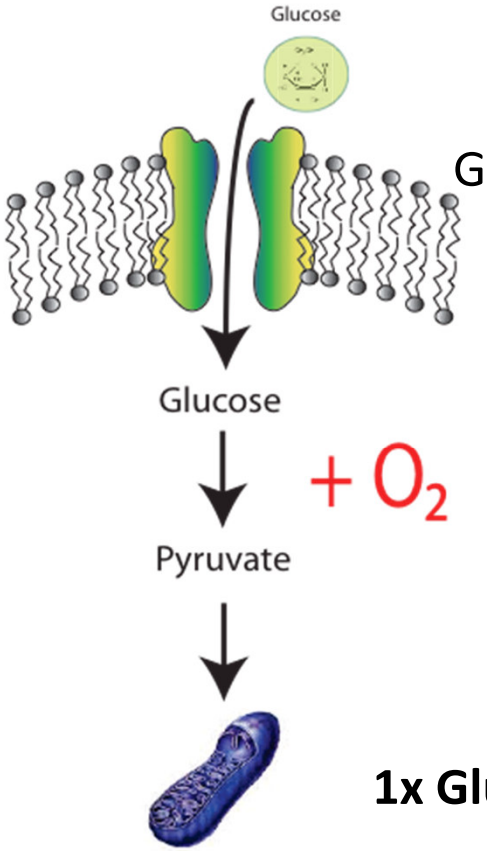
- yeast cells will constantly uptake glucose from their environment to support exponential growth until none is left
- much of the glucose is converted from the cell to ethanol even in the presence of oxygen
- yeast only shift their metabolism to oxidative phosphorylation when they deplete available glucose and must turn to ethanol to maintain survival

- metazoan cells, even when surrounded by nutrient-rich plasma and the extracellular fluid, do not import nutrients such as glucose in a constitutive manner
- without stimulation by growth factor signaling many cells even fail to consume nutrients in quantities sufficient to maintain cellular energy demands resulting in reduced cell size and finally cell death
- survival of growth-factor-deprived cells can be readily restored by combined expression of a plasma membrane glucose transporter (GLUT1) and the first enzyme of the glycolytic pathway hexokinase (HK)
- cancer cells accumulate oncogenic alterations that enhance PI-3 kinase, AKT, or upstream receptor tyrosine kinases result in constitutive glucose uptake and metabolism

Cell Metabolism



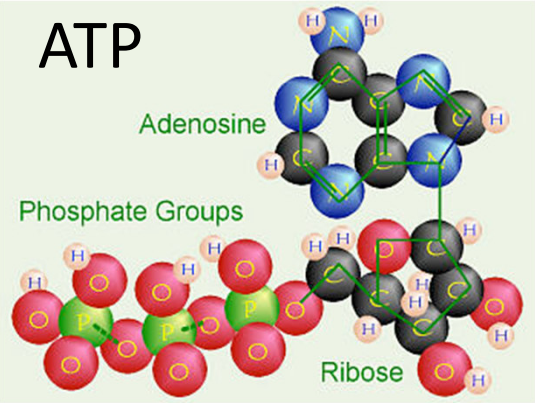
- drives all energy dependent processes in the cell
- mainly generated by **Glycolysis** and **Oxidative Phosphorylation**



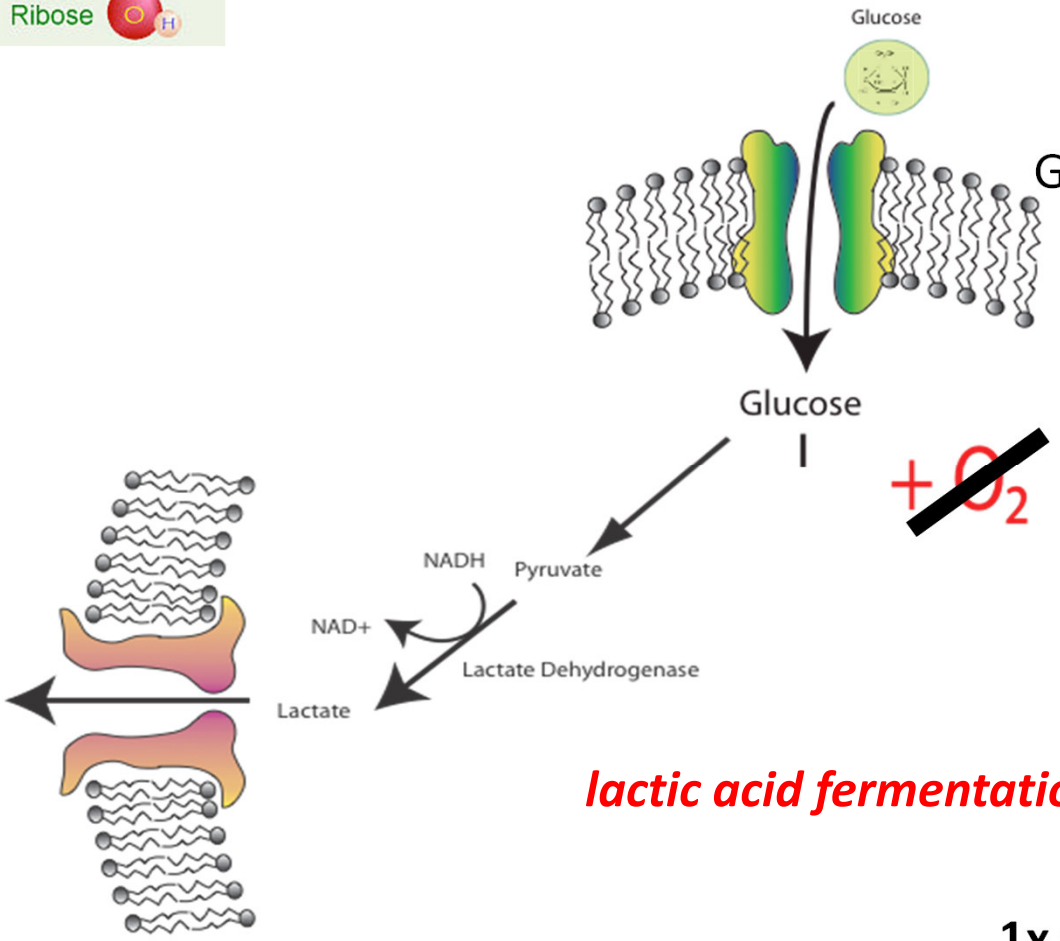
GLUT-transporters
(facilitated diffusion)

1x Glucose → **30 x ATP**

Cell Metabolism



- if O_2 is low, cells need to perform another reaction: **lactic acid fermentation** from pyruvate

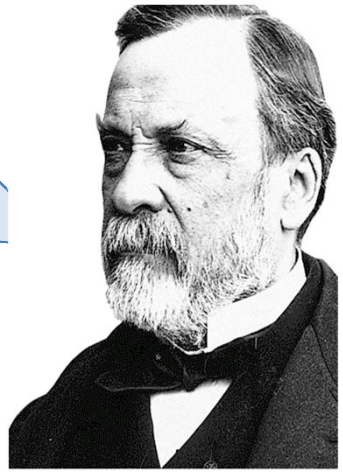


lactic acid fermentation

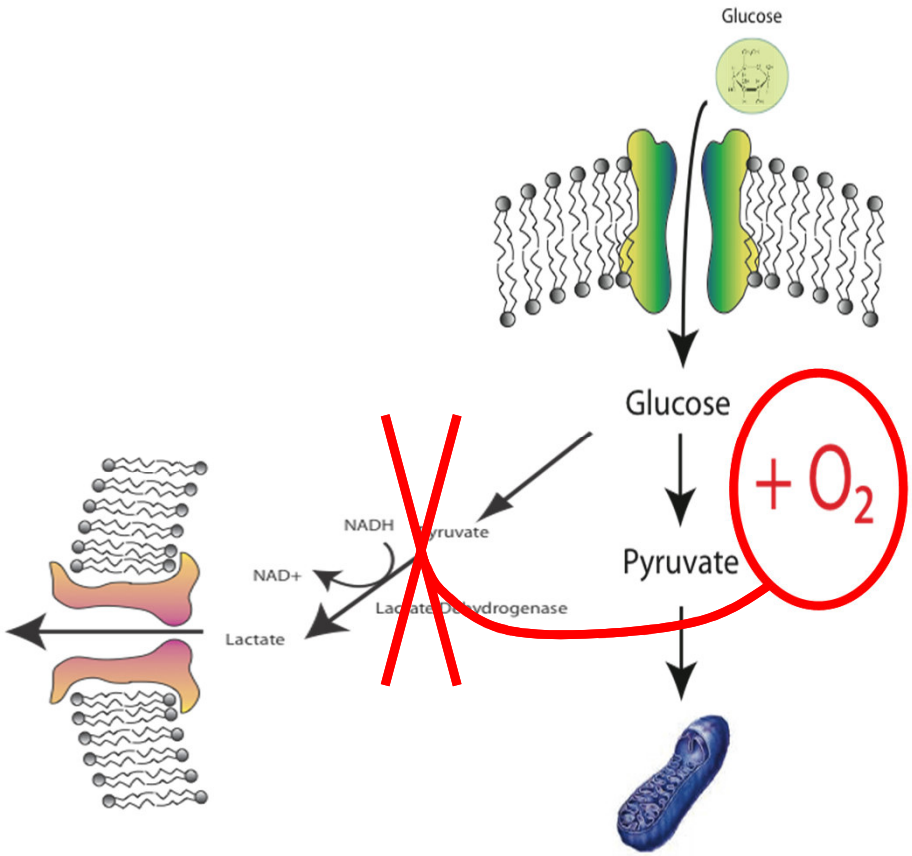
1x Glucose → 2 x ATP

Pasteur effect:

The presence of oxygen inhibits glucose metabolism via lactate



Louis Pasteur

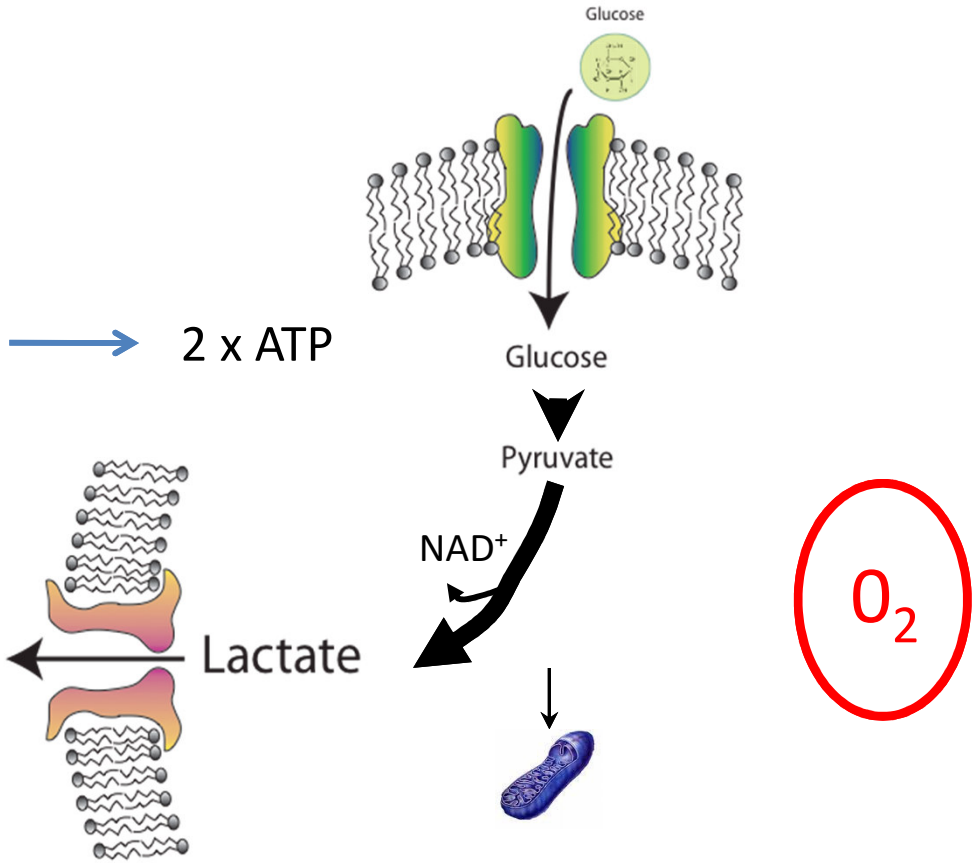


Warburg effect:

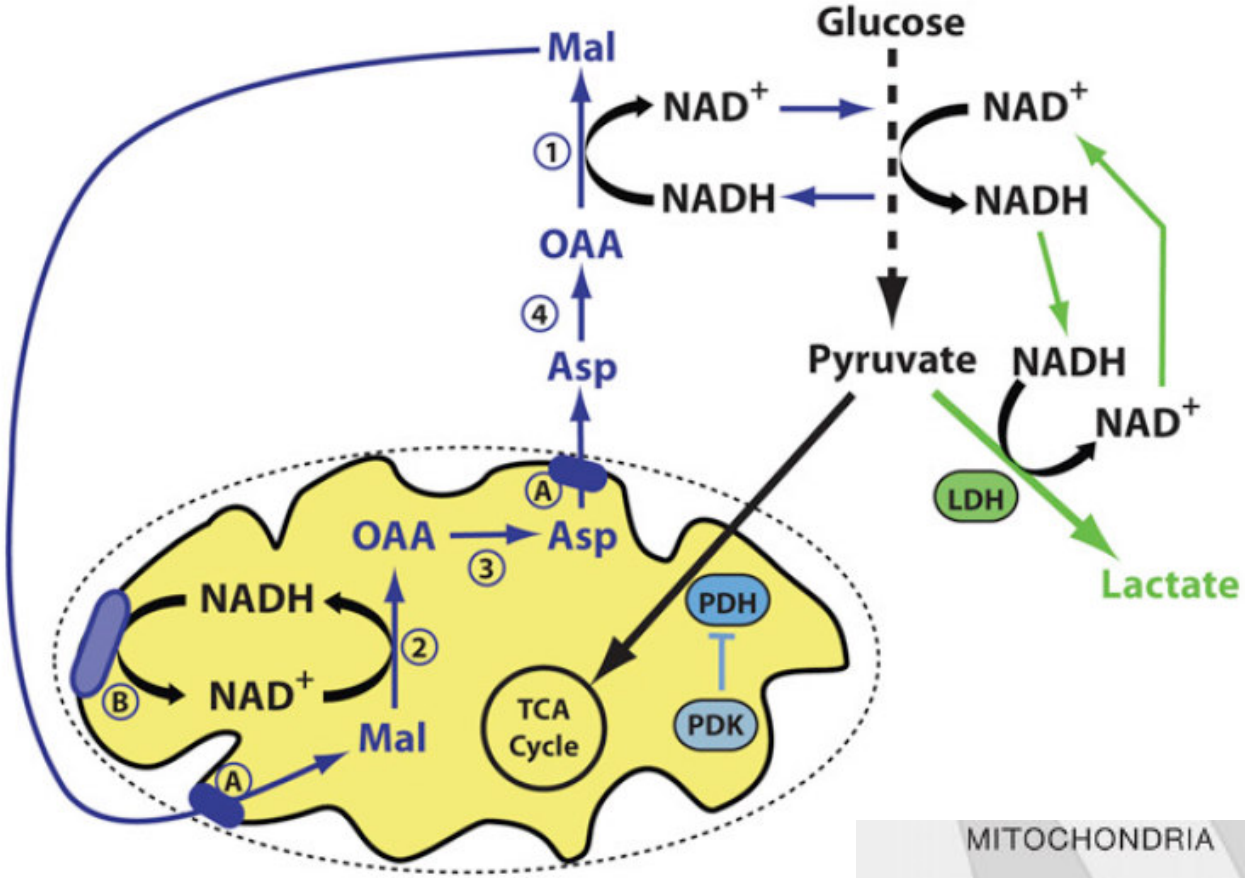
Presence of oxygen does not inhibit lactate fermentation in tumors

Warburg O (1956). "On the origin of cancer cells". *Science 123 (3191):*

1x Glucose → 2 x ATP

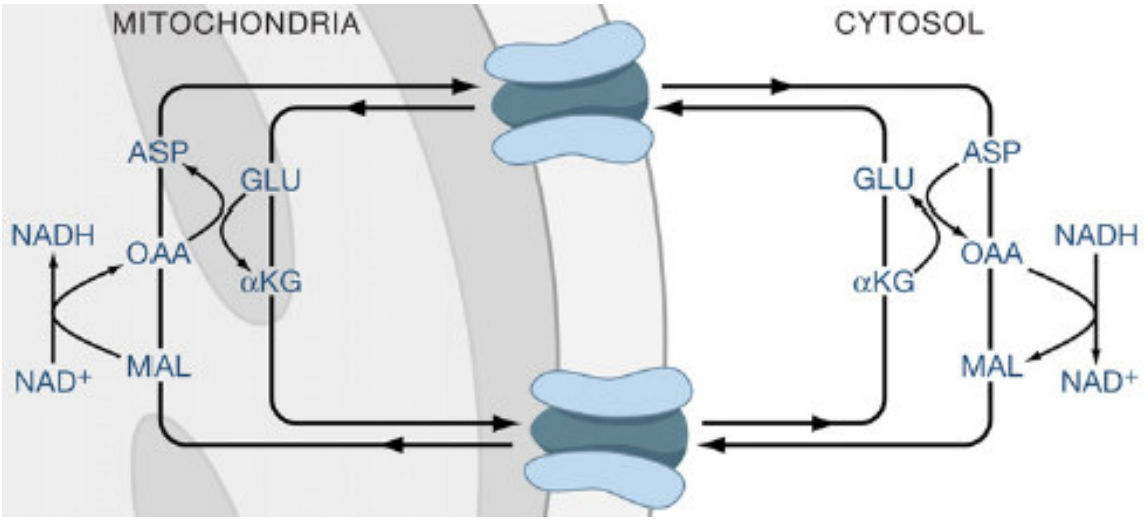


Balancing NAD+/NADH levels



in conditions of low glucose uptake and flux through glycolysis, the generated NADH is balanced by the mitochondrial malate-aspartate shuttle

in conditions of high glucose uptake and flux, the capacity of this shuttle is saturated and lactate dehydrogenase activity is required to regenerate NAD+



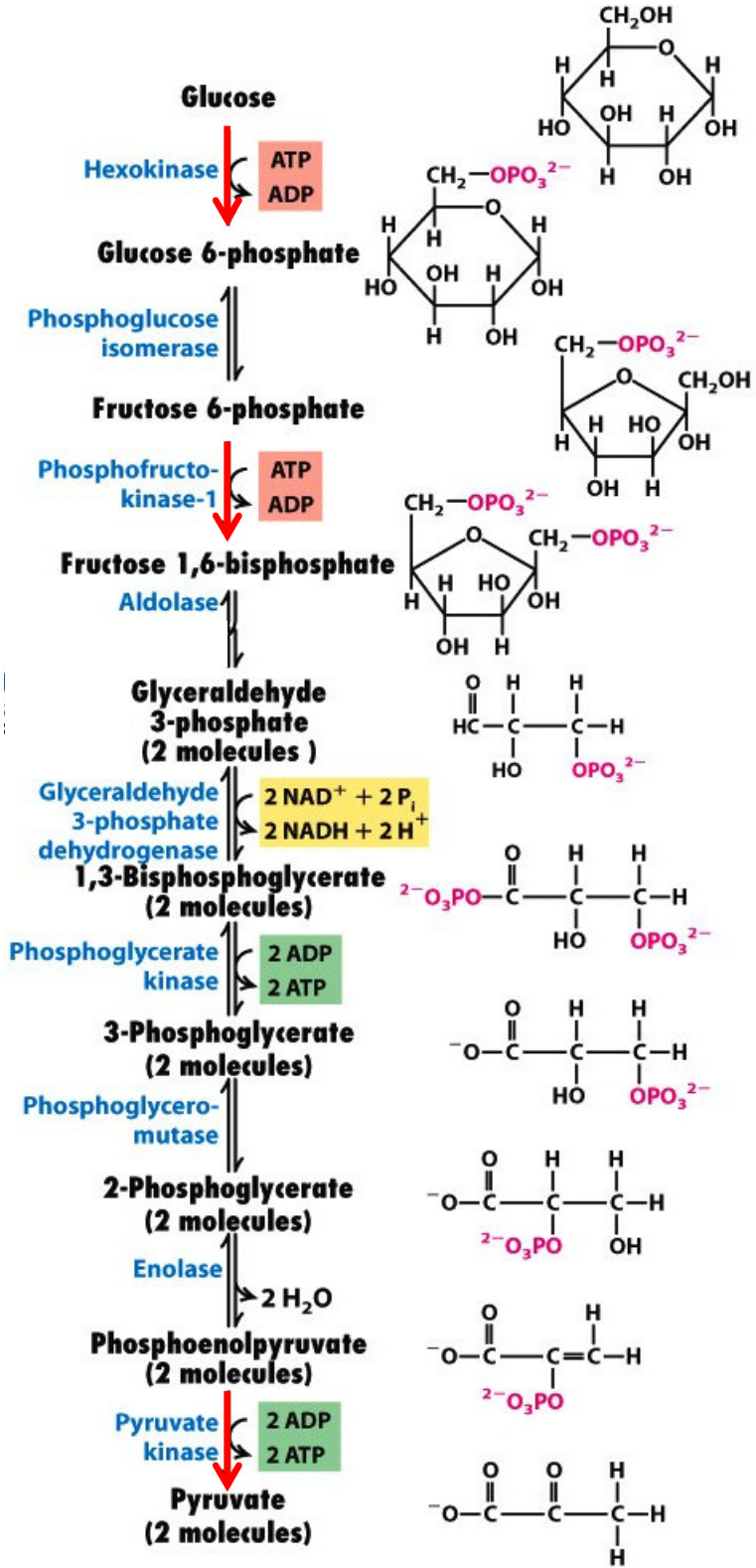
Why do cancer cells show the Warburg effect?

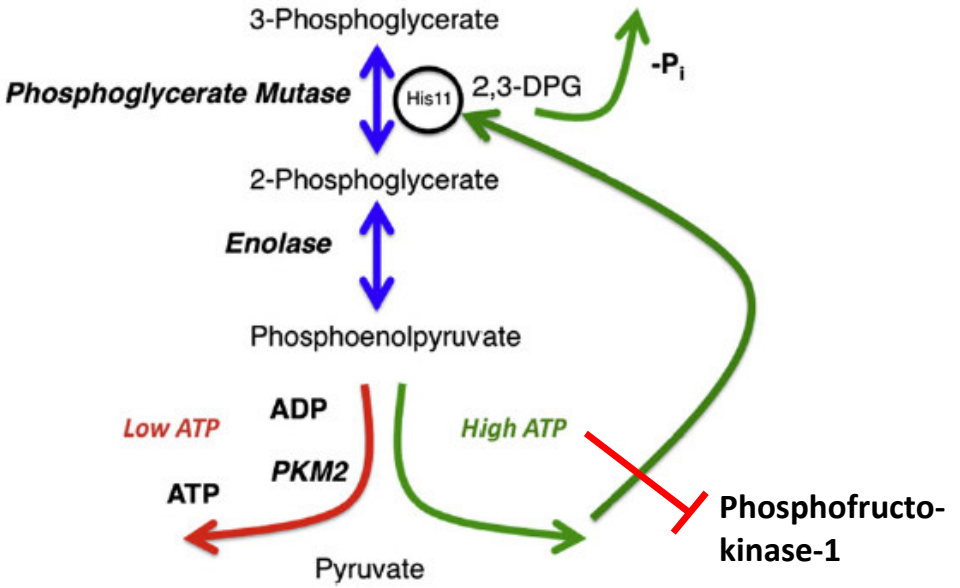
Warburg effect:
high rate of glycolysis followed by lactic acid fermentation in the cytosol (sometimes called aerobic glycolysis) leads to reduced ATP production per glucose (2 instead of 30 ATPs)

- ATP is rapidly metabolized and the ATP pool can turnover more than six times per minute; at such rates, a 10% decrease in ATP production would halve ATP levels in less than 1 min
- the concentration of ATP in cells is near 5mM, and this value is far above the Km for most kinases that use ATP as a substrate
- calculations suggest that the amount of ATP required for cell growth/division is far less than that required for basal cellular maintenance
- basal machinery consumes ATP for: maintenance of ion gradients via Na^+/K^+ and Ca^{2+} ATPases, steady-state protein and RNA synthesis, and intracellular trafficking
    - each peptide bond carries an energetic cost of about 3 kcal/mol (1 ATP + 2GTP per bond). However, half-lives of most proteins range from minutes to a few hours. Only about 10% of proteins have half-lives close to the time scales of cell cycling. Thus the majority of ATP consumed during protein synthesis maintains steady state levels of protein concentrations
    - Na^+/K^+ ATPase pumps: one molecule of ATP is consumed for every three Na^+ ions that are pumped out of cells to maintain approximately a 20 mM concentration in the cytosol. The rate of ion flux is around 100 nM/min per mg of protein; glucose uptake rates in cultured cells are around 100 nM/min per mg protein as well
- this means the use of ATP for basal homeostasis exceeds the amount used for the de novo synthesis of biomaterials

Glycolysis in cancer cells

- there are only three irreversible reactions in the whole glycolysis pathway which determine the fate of glucose
- the whole cascade produces 2x NADH and 2x ATP
- however, in cancer cells often an alternative pathway is used which even avoids ATP production (since high ATP levels inhibit phophofructokinase): here a phosphate group is transferred onto PGAM1 (phosphoglycerate mutase) which then creates 2,3DPG which spontaneously loses this phosphate group





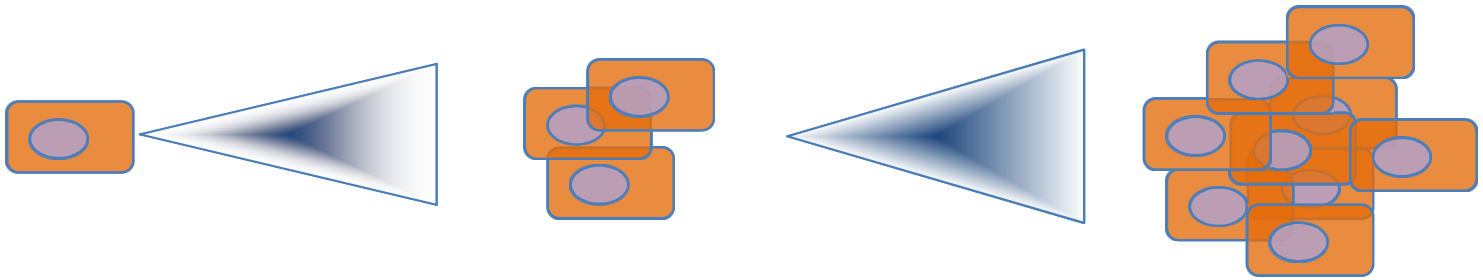
Why do cancer cells show the Warburg effect?

Warburg effect:
high rate of glycolysis followed by lactic acid fermentation in the cytosol (sometimes called aerobic glycolysis)

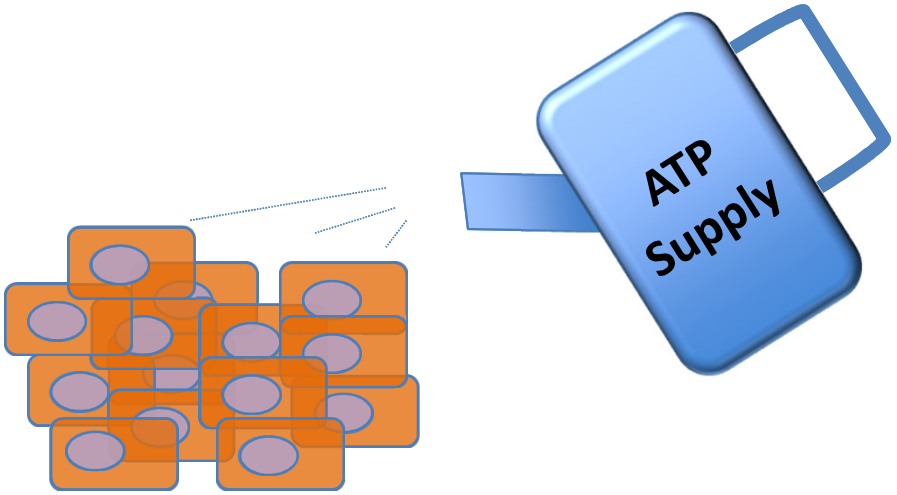
- glycolysis has the capacity to generate ATP at a faster rate than oxidative phosphorylation and so would be advantageous as long as glucose supplies are not limited
- glycolytic metabolism is an adaptation to hypoxic conditions during the early avascular phase of tumor development, as it allows for ATP production in the absence of oxygen <-> most tumors retain the capacity for oxidative phosphorylation and consume oxygen at rates similar to those observed in normal tissues
- aerobic glycolysis provides a biosynthetic advantage for tumor cells, since a high flux of substrate through glycolysis allows for effective shunting of carbon to key subsidiary biosynthetic pathways

Challenges of Tumour Metabolism

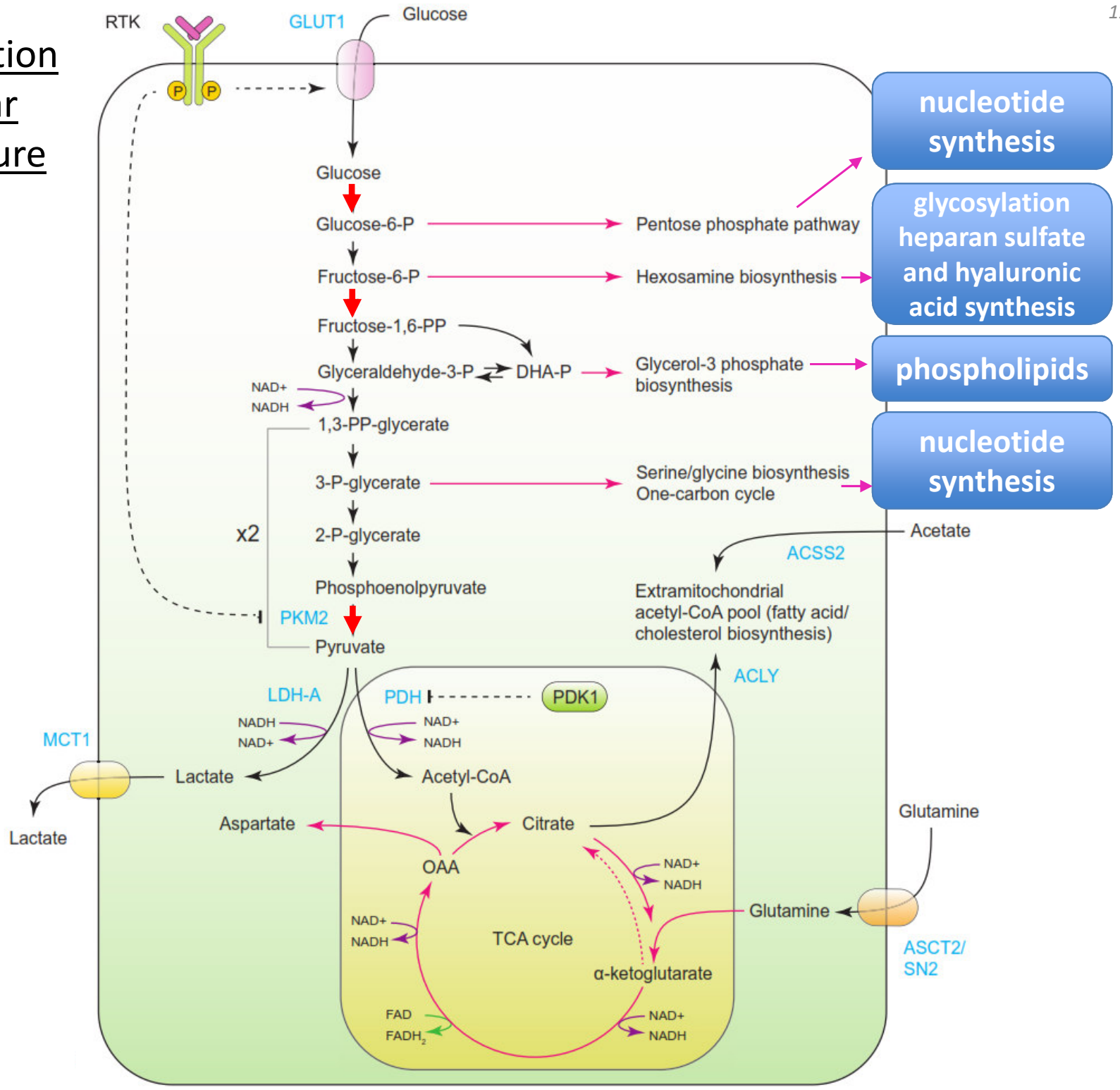
1. Adequate production of Macromolecular Precursors to ensure the growth



2. Sufficient Energy Supply generation to support tumour maintenance



Adequate production of macromolecular precursors to ensure growth



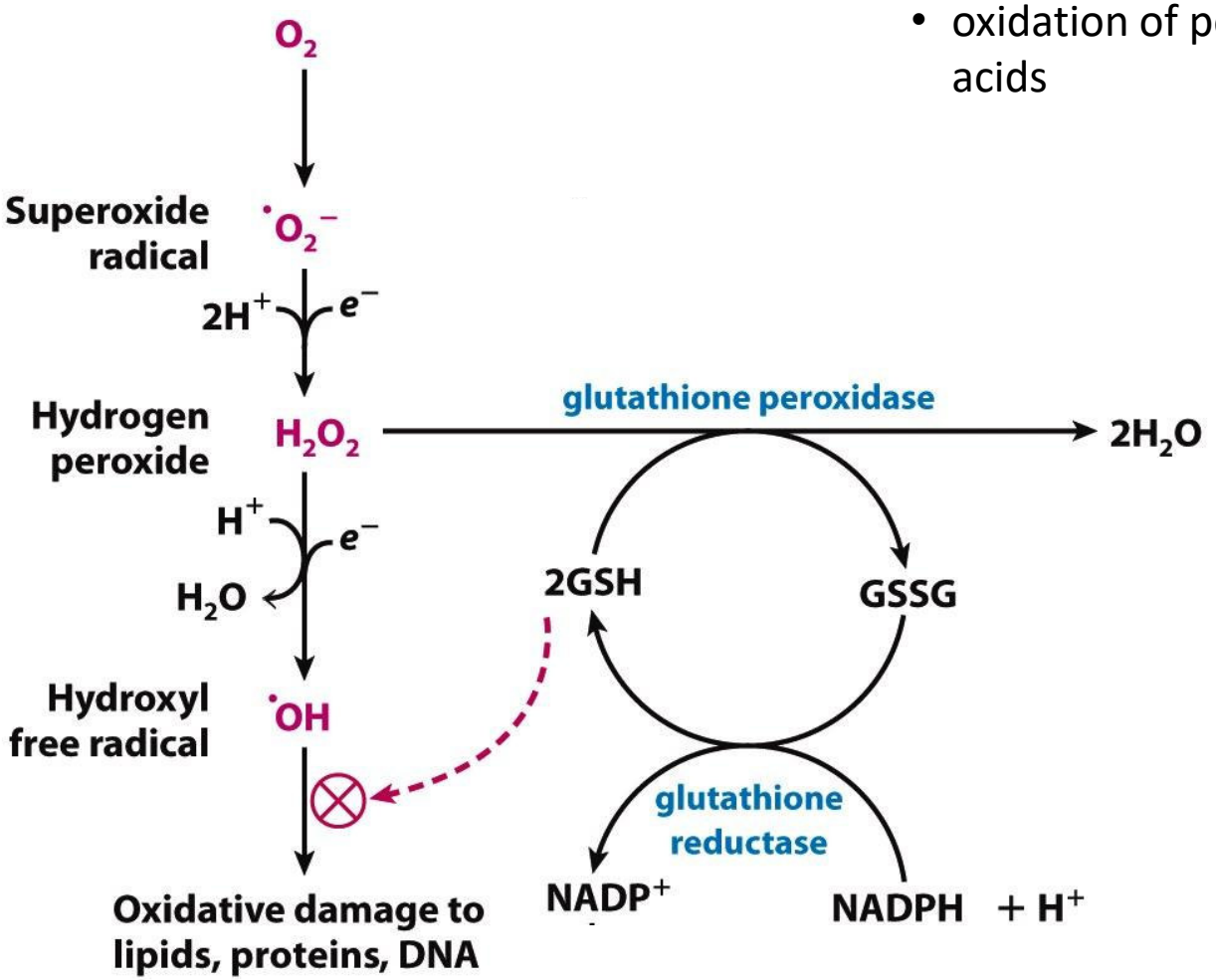
Tumor dependencies: Mechanisms of redox control

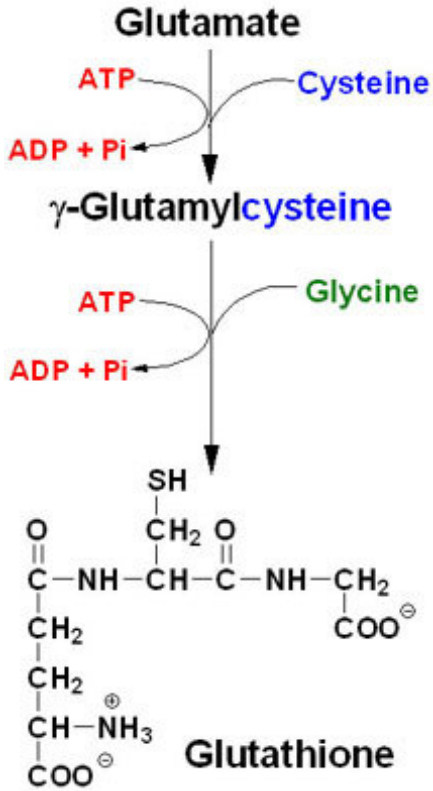
sources of ROS:

- mitochondrial electron transfer
- NADPH oxidases (Nox, Duox)
- Ero1-mediated disulfide bond formation in ER

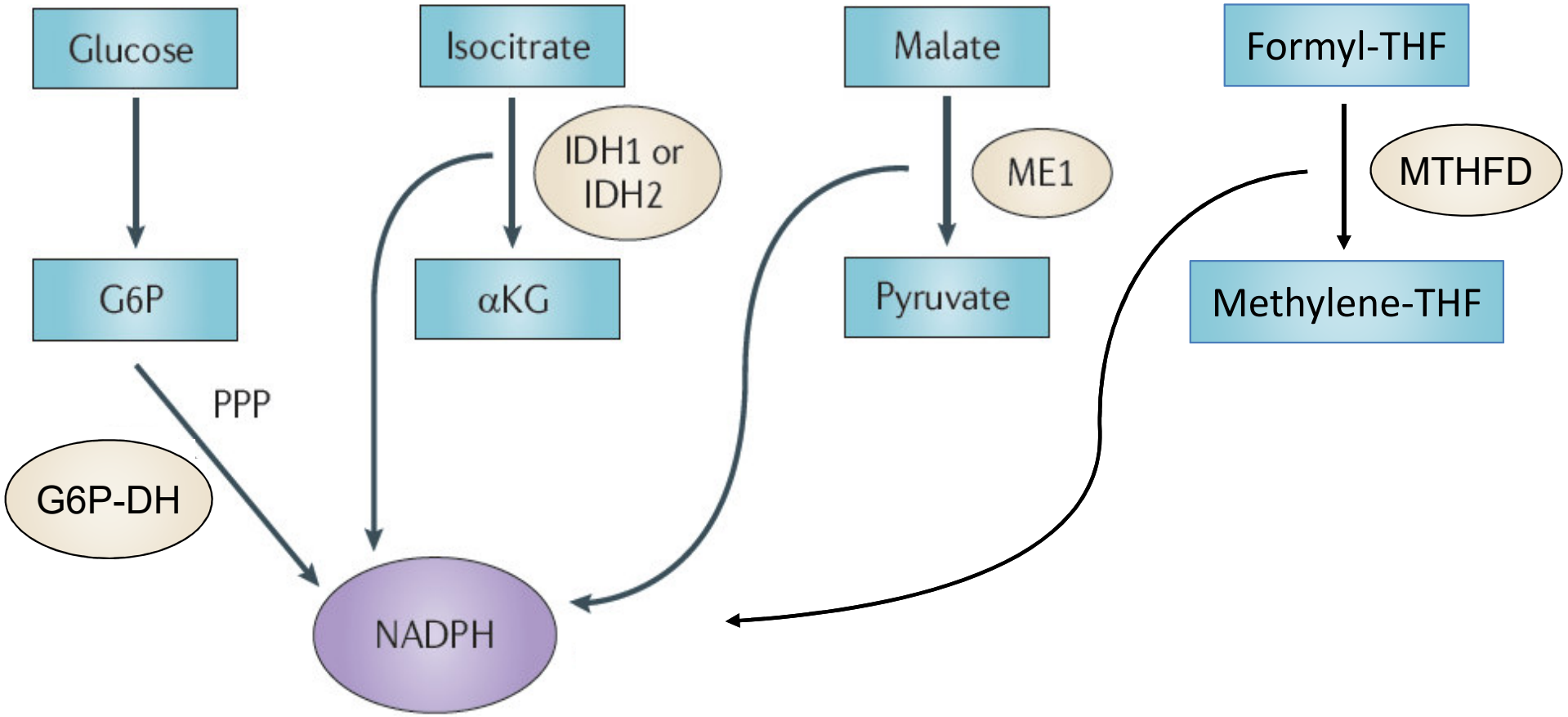
targets of ROS:

- cysteine oxidation (phosphatases)
- LRPH oxidation to carbonyls, MHY oxidation
- DNA/RNA damage
- oxidation of polyunsaturated fatty acids





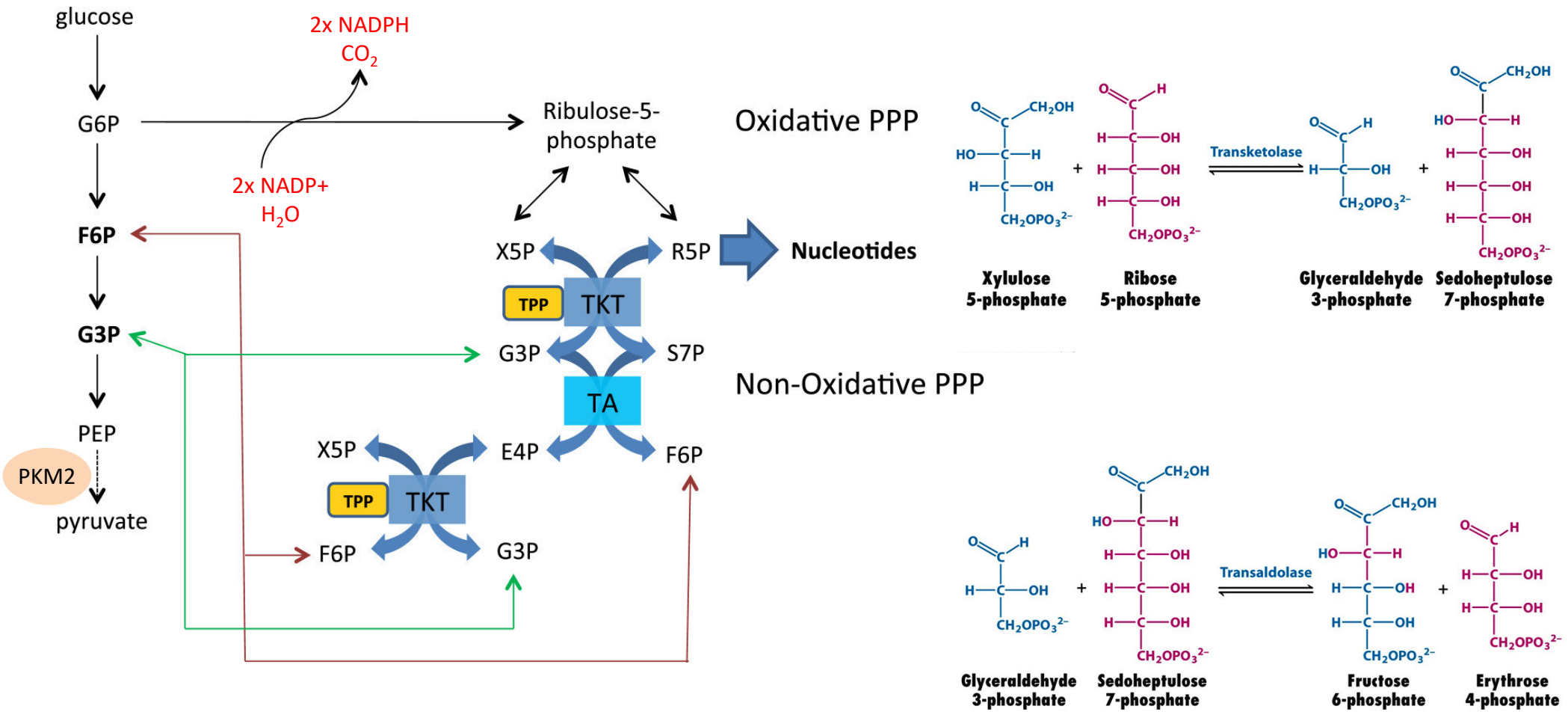
Sources of NADPH



<u>NADPH can only be generated by a few reactions:</u>

- in the **oxidative pentose phosphate pathway (PPP)** by G6P-DH
- in the **citrate cycle** by IDH (can occur in mitochondria or the cytoplasm)
- in the **malate-pyruvate shuttle** by malic enzyme (ME)
- in the **folate cycle** by methylene-tetrahydrofolate dehydrogenase (MTHFD)

Pentose-Phosphate pathway



- the **oxidative pentose phosphate pathway (PPP)** generates **NADPH** from glucose-6-phosphate (**G6P**) which is converted to ribose 5-phosphate (**R5P**) and xylulose 5-phosphate (**X5P**) for biosynthesis of nucleotides.
- alternatively, transketolase and –aldolase can shunt back F6P and G3P into the **non-oxidative PPP pathway** to produce R5P and X5P, but without **NADPH** production

Tumor dependencies: Nitrogen sources, amino acids

Essential amino acids
(cannot be synthetized and must be supplied in the diet):

phenylalanine (F)
valine (V)
threonine (T)
tryptophan (W)
methionine (M)
leucine (L)
isoleucine (I)
lysine (K)
histidine (H)

non-essential amino acids (NEAAs)

Limited synthesis:
arginine (R)
cysteine (C)
glycine (G)
glutamine (Q)
proline (P)
tyrosine (Y)

Dispensable:
alanine (A)
aspartic acid (D)
asparagine (N)
glutamic acid (E)
serine (S)

- asparagine synthetase (ASNS) is frequently upregulated in tumors and associated with poor prognosis
- **acute lymphoblastic leukemia cells lack ASNS expression leading to asparagine auxotrophy**

alternative nitrogen source:
ammonia NH_4^+
(produced by gut microbiota)

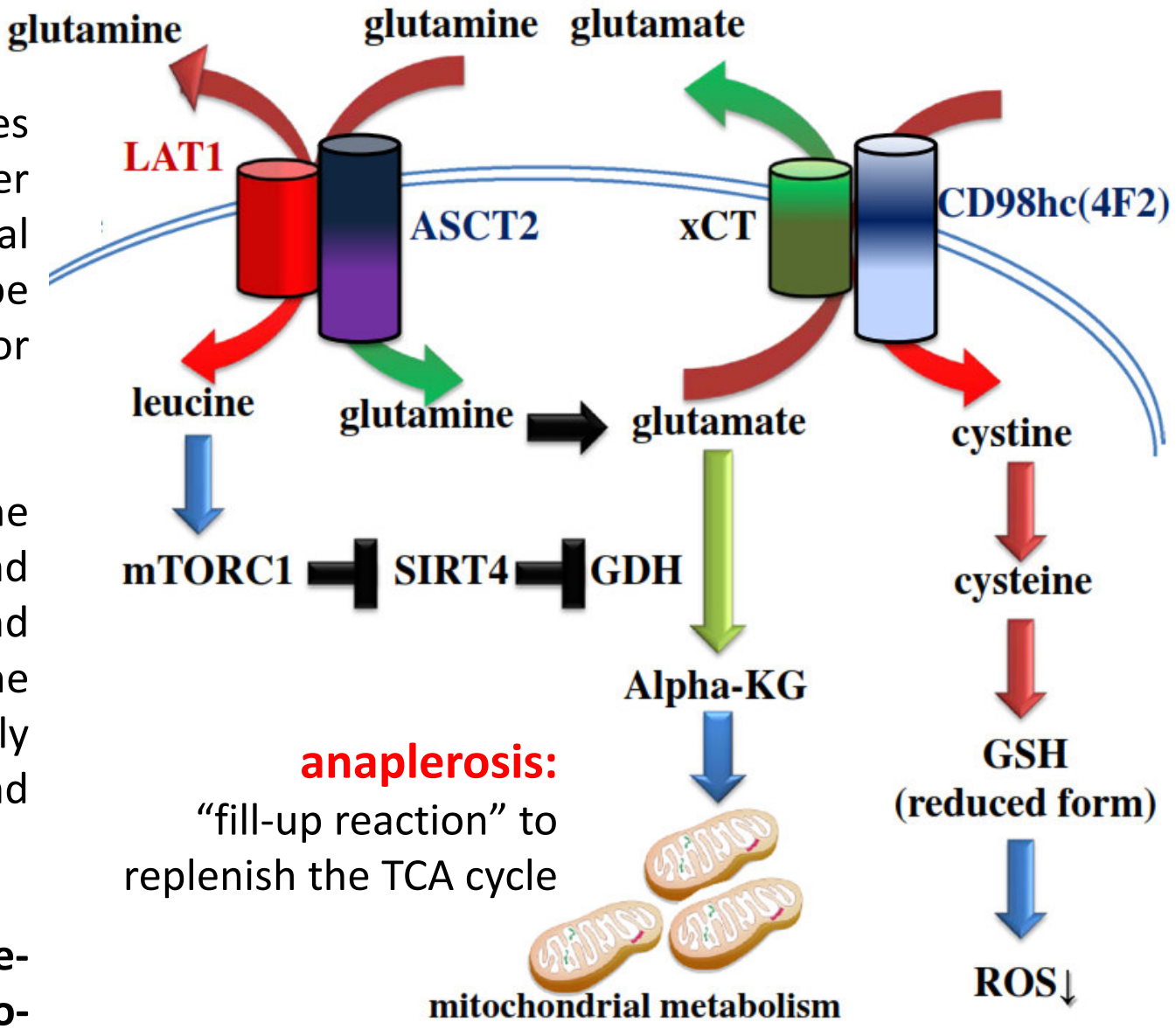
- arginine is produced by argininosuccinate lyase (ASL) and argininosuccinate synthase (ASS1) which are often missing/silenced in melanoma, renal cell carcinoma, and hepatocellular carcinoma, prostate cancer, and acute lymphoblastic leukemia
- this **auxotrophy for arginine** is being exploited for therapy

Central role of glutamine

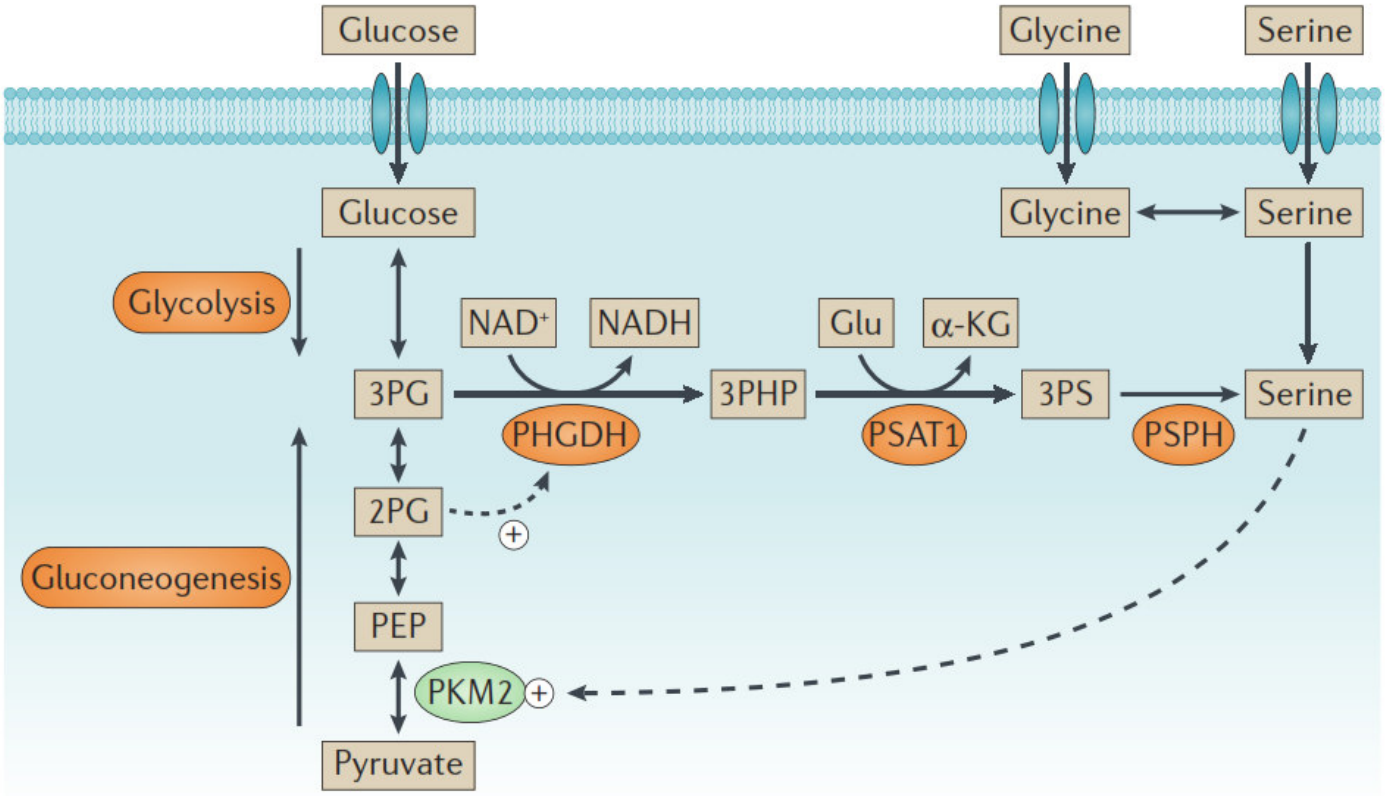
glutamine not only facilitates uptake of many other essential and non-essential amino acids but can also be used as energetic input for the mitochondrial TCA cycle

glutamine is used in the production of uracil and thymine (1x), cytosine and adenine (2x) and guanine (3x) as well as the assembly of both pyrimidine and purine rings (via aspartate)

=> **glutamine levels are rate-limiting for cell cycle pro-gression**

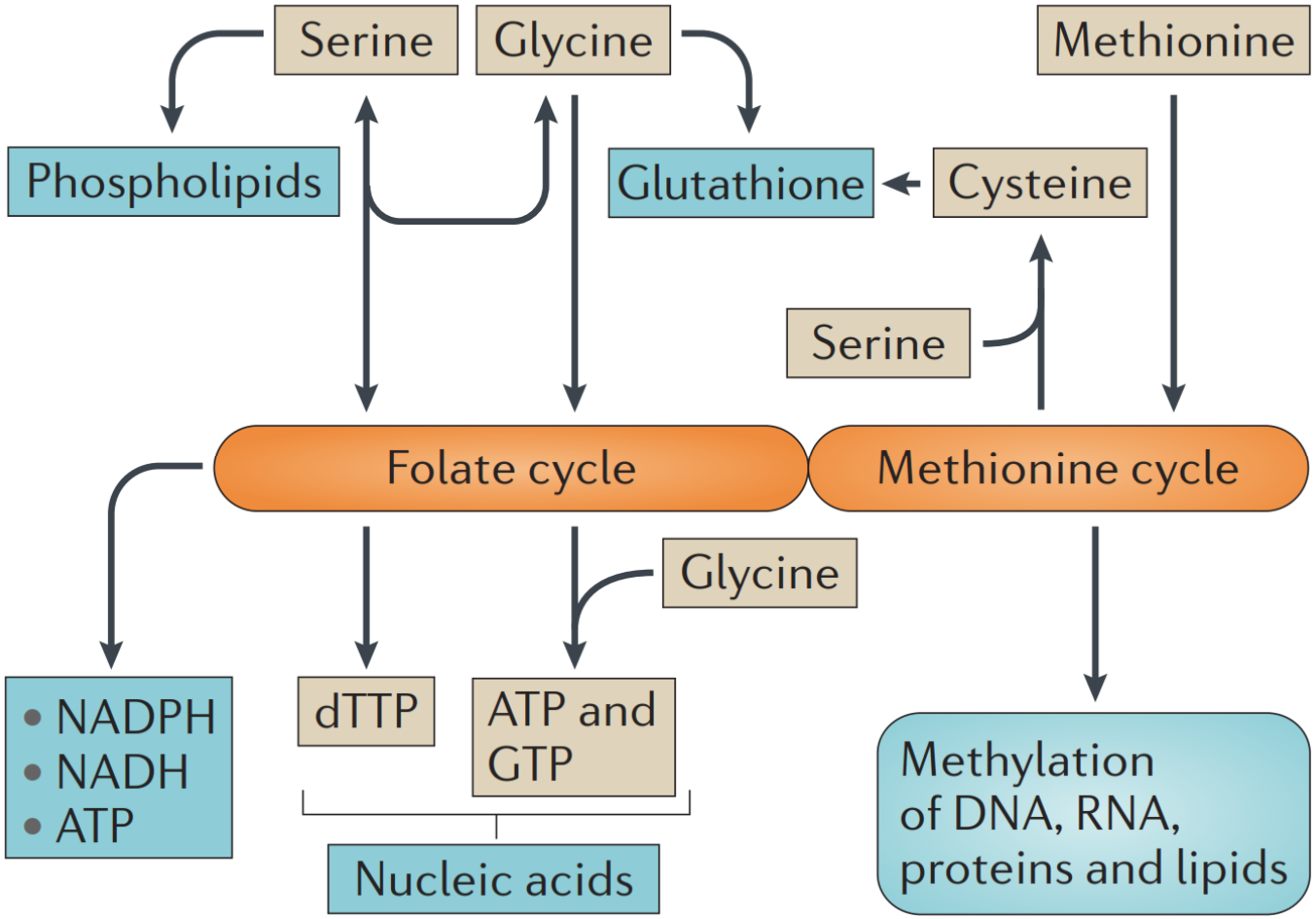


Serine production



- Phosphoglycerate dehydrogenase (PHGDH) catalyses the first and rate-limiting step of serine anabolism
- **PHGDH has been found amplified and upregulated in breast cancer and melanoma and its levels correlate with worse prognosis**
- block of PHGDH reduces cancer growth; inhibitors for PHGDH are entering clinical trials

Serine metabolism



- Serine has a unique metabolic role in the cell as a major substrate for the folate/one-carbon cycle
- One-carbon-THF (tetrahydrofolate) species are utilized as substrates for the biosynthesis of purines, thymidine, S-adenosylmethionine (SAM) and of up to 50% of all cellular NADPH
- **MTHFD2** (enzyme in the mitochondrial folate cycle) **is among the top three most frequently overexpressed metabolic enzymes in cancer**

Mechanisms of metabolic adaptation in cancer

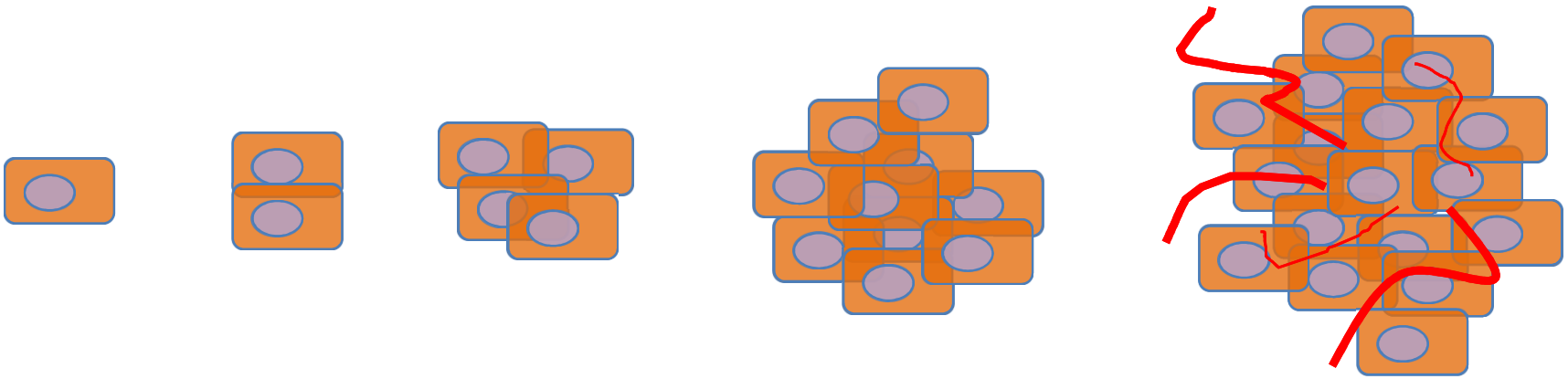
Increased glycolytic rate

High rates of self-catabolism

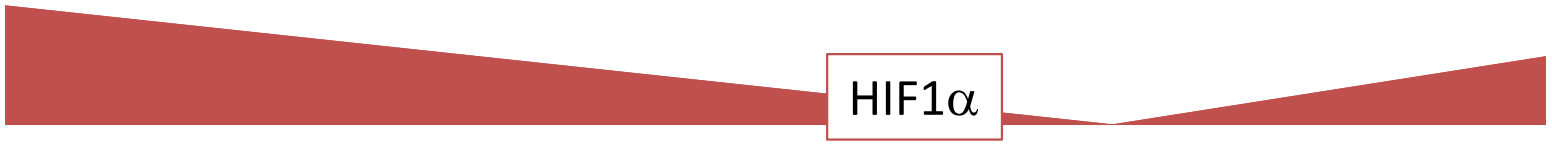
Alternative expression/splicing of metabolic enzymes

Alternative sources of energy

Metabolic Stress during tumour development



Tumour Growth



Nutrients,
Oxygen

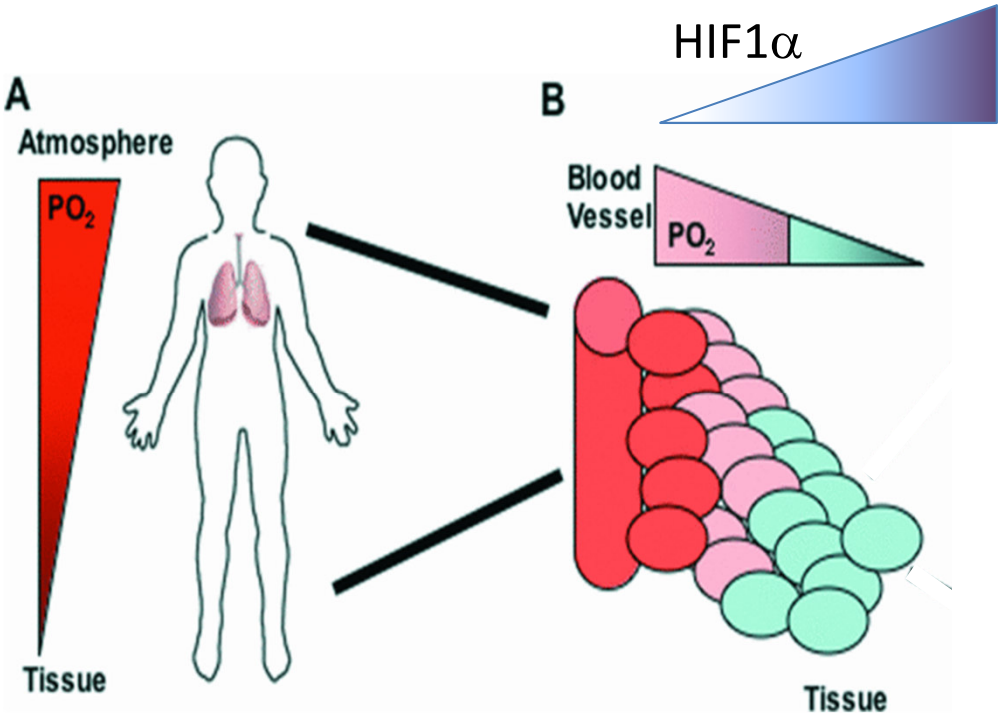
Metabolic Stress

Angiogenesis

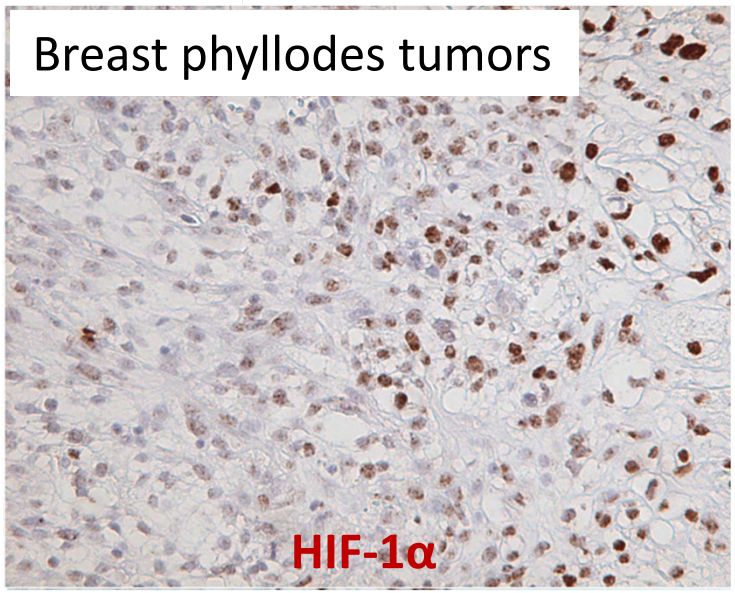
Metabolic Adaptation

Decreased concentration of Oxygen promotes induction of HIF1α

HIF1α

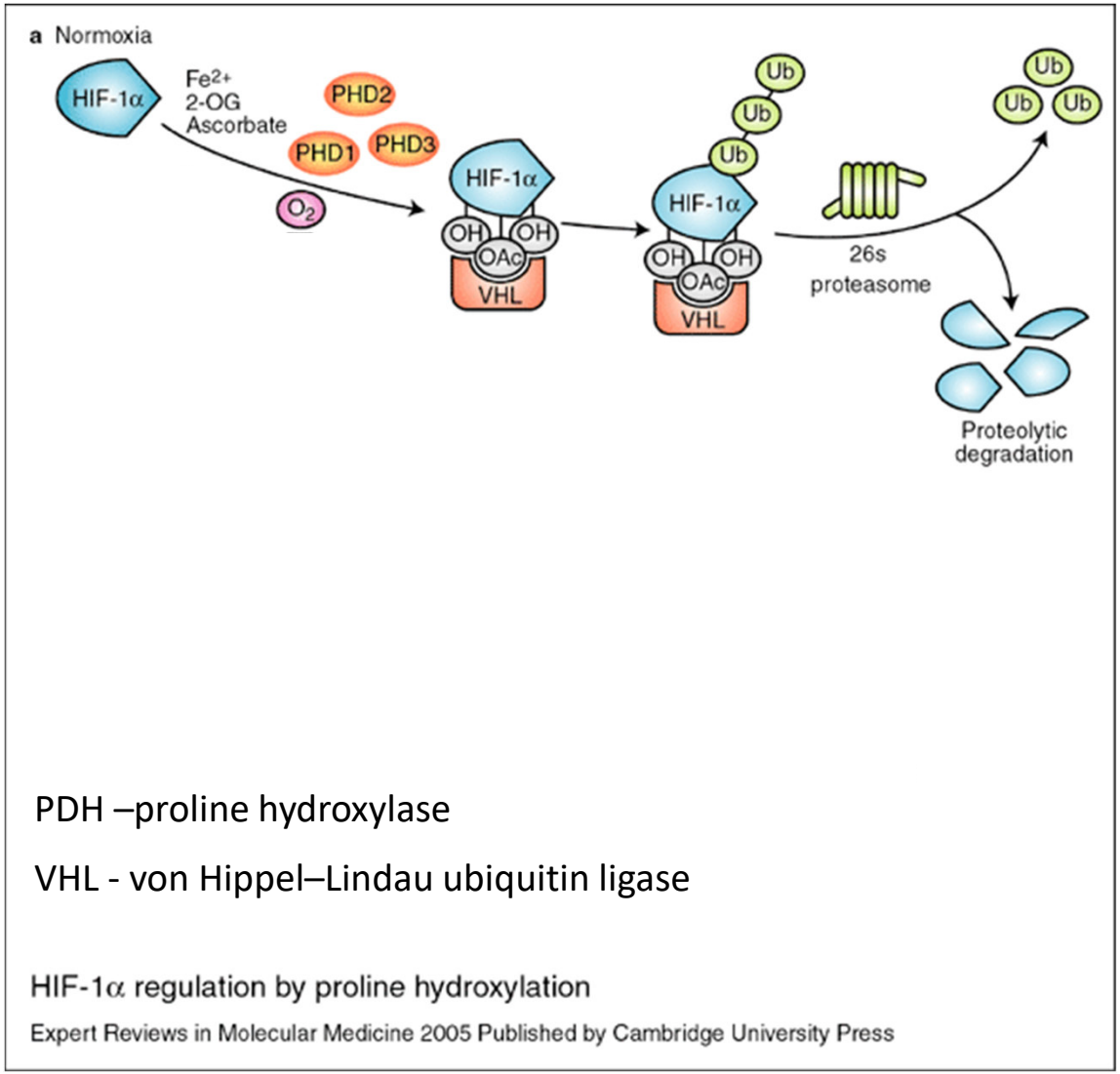


- Transcription Factor
- Exhibits rapid turnover in the presence of Oxygen
- Recognizes HRE element in the promoter region of target genes
- *Bona fide* HIF1α targets: promote proliferation, angiogenesis, survival



Kuijper et al. Breast Cancer Research 2005 7:R808

Turnover of HIF1α protein in the cell



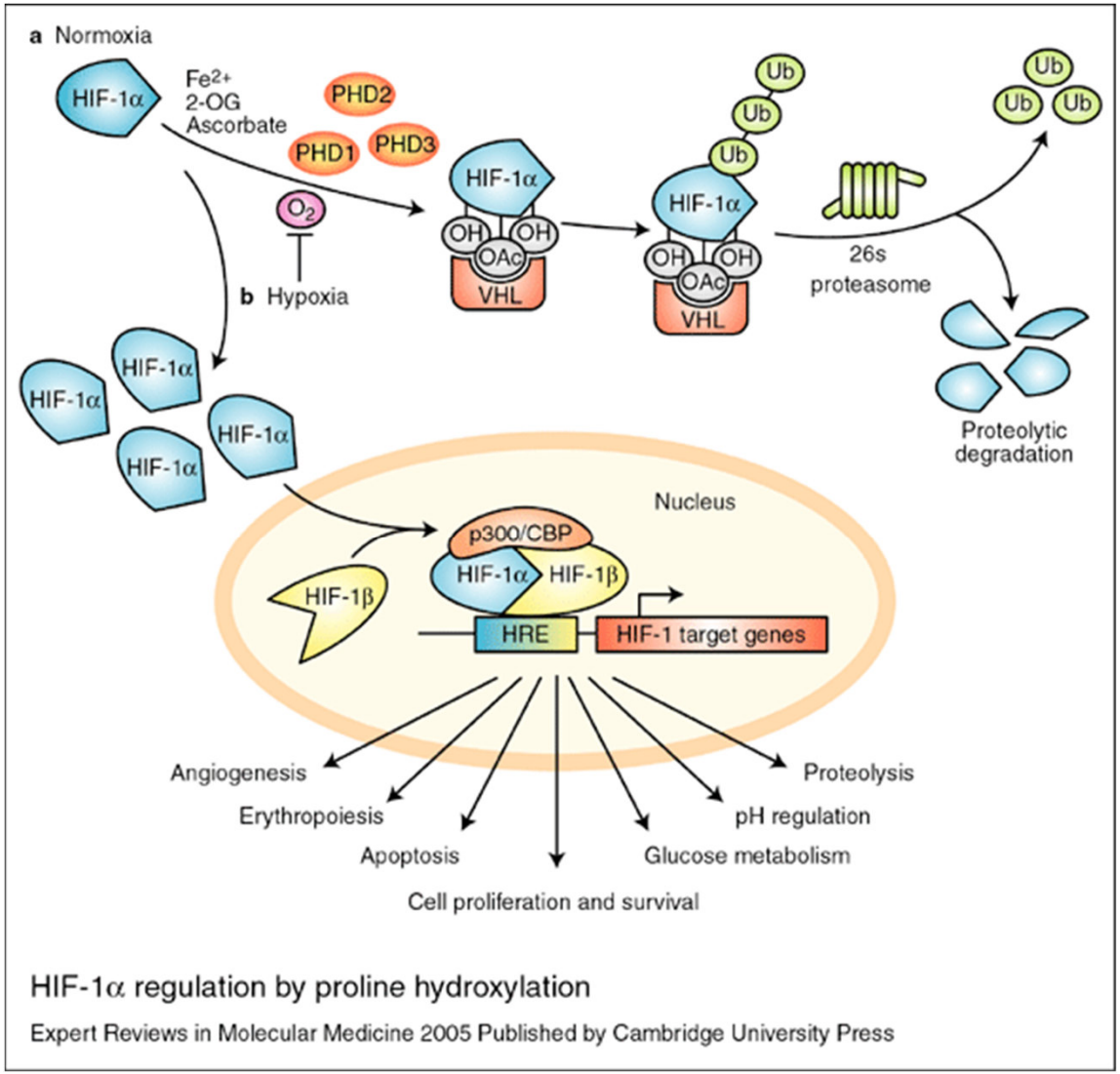
PDH –proline hydroxylase

VHL - von Hippel–Lindau ubiquitin ligase

HIF-1α regulation by proline hydroxylation

Expert Reviews in Molecular Medicine 2005 Published by Cambridge University Press

Turnover of HIF1α protein in the cell



HIF-1α regulation by proline hydroxylation

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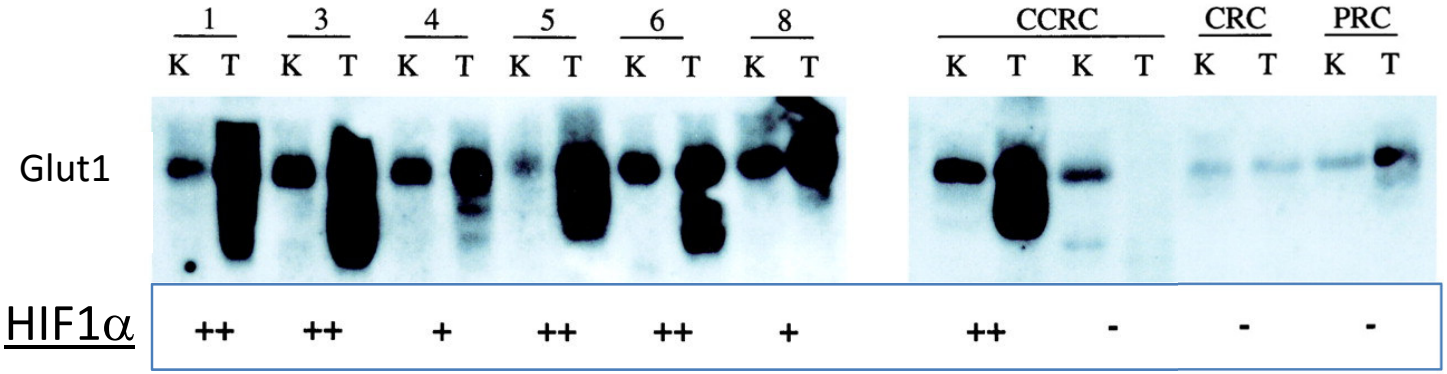
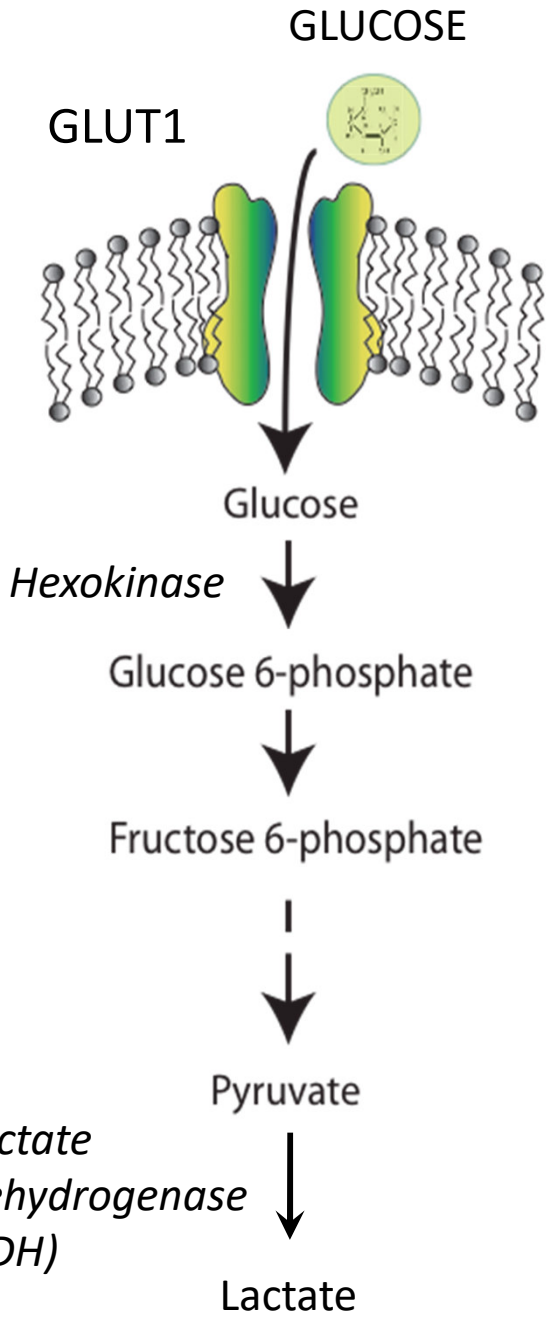
HIF1α regulates expression of genes involved in glycolysis

I. HIF1 α regulates glucose uptake by inducing GLUT1

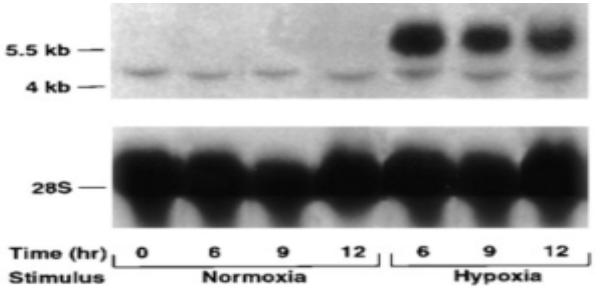


Wiesener M S et al. Cancer Res 2001;61:5215-5222

K- kidney (normal tissue)
T-Tumor



II. HIF1 α regulates glucose metabolic enzymes



Northern Blot
Hexokinase II

Lung adenocarcinoma

Riddle S R et al. Am J Physiol Lung Cell Mol Physiol 2000;278:L407-L416

Responses to nutrient deprivation

L-Lactate

NAD+ → NADH

Lactate dehydrogenase (LDH)

Pyruvate

Fatty Acids

TCA

Glutamate

Glutamine

Tumor Specific expression of Metabolic Enzymes

Case I – Hexokinase II

- There are four isoforms of mammalian hexokinases
- Hexokinase II has high affinity to glucose
- Hexokinase II is not expressed in normal brain, liver or pancreas
- Switch from Hexokinase I to Hexokinase II occurs during tumor progression

Tumor Specific expression of Metabolic Enzymes

Case II – PKM isoforms

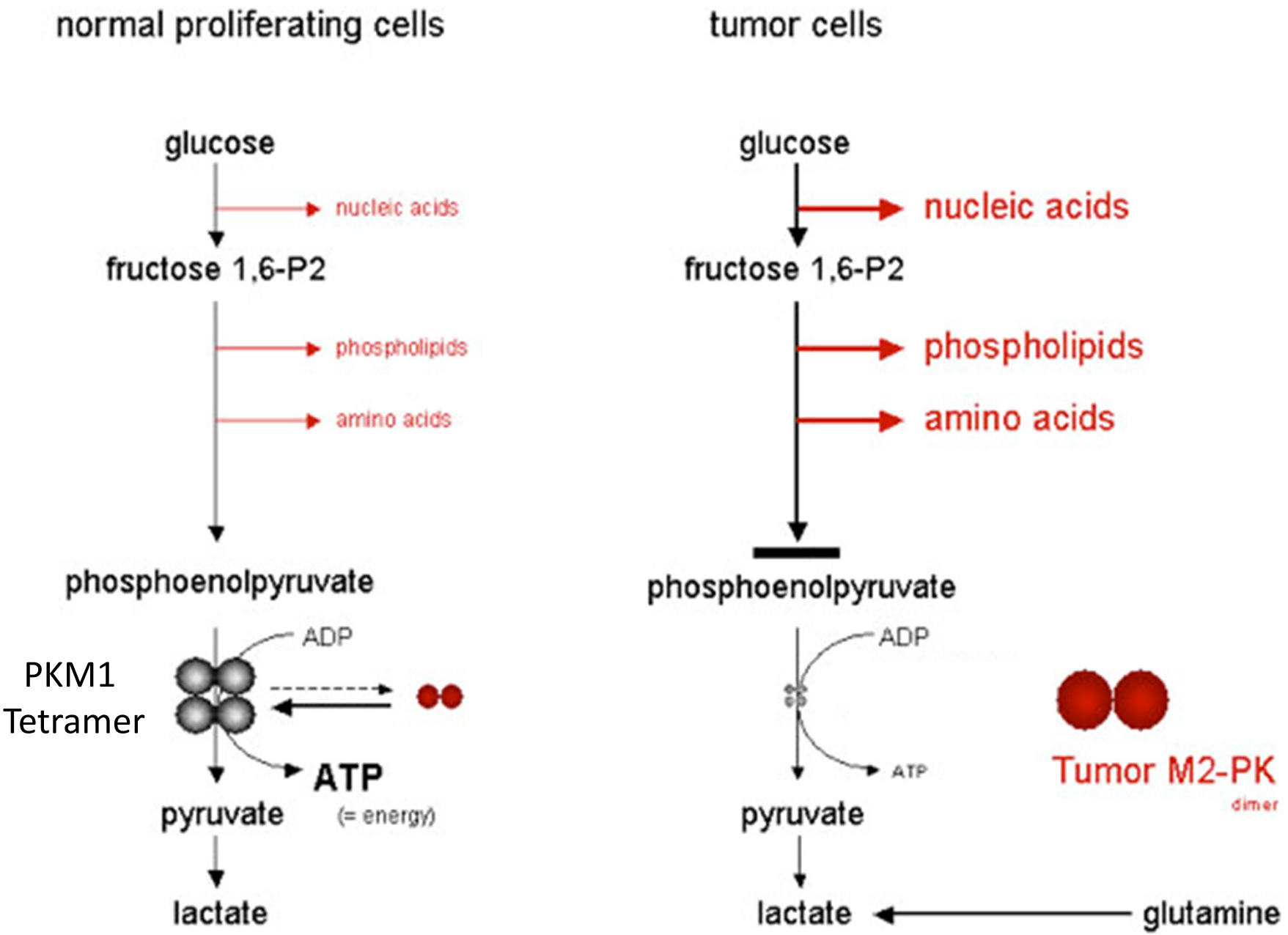
- The glycolytic enzyme pyruvate kinase (PKM) is alternatively spliced to two isoforms – PKM1 and PKM2

- Cells expressing PKM2 produce more lactate and consume less oxygen than cell expressing PKM1

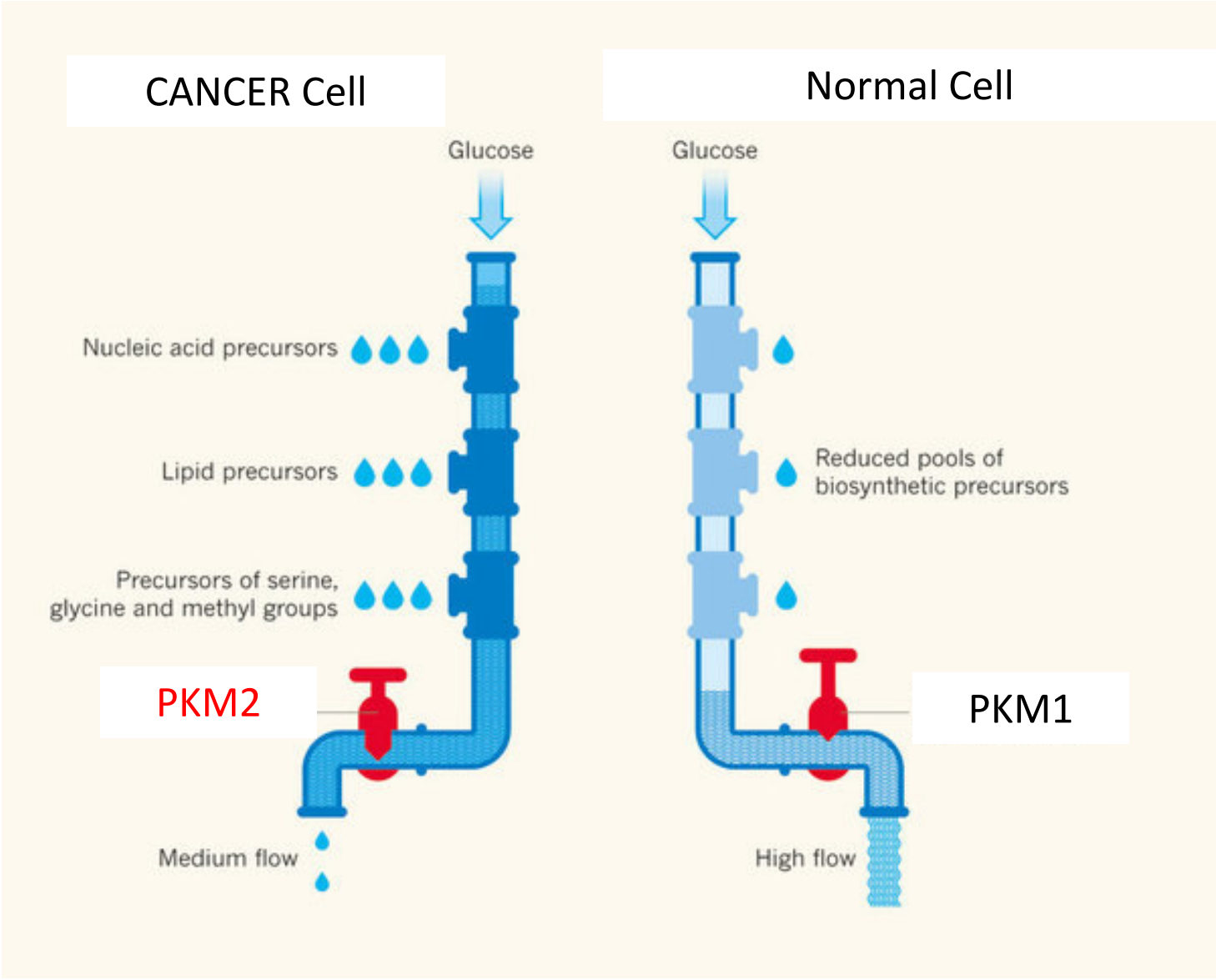
- All cancer cells studied to date exclusively express PKM2

- PKM2 is less active than PKM1

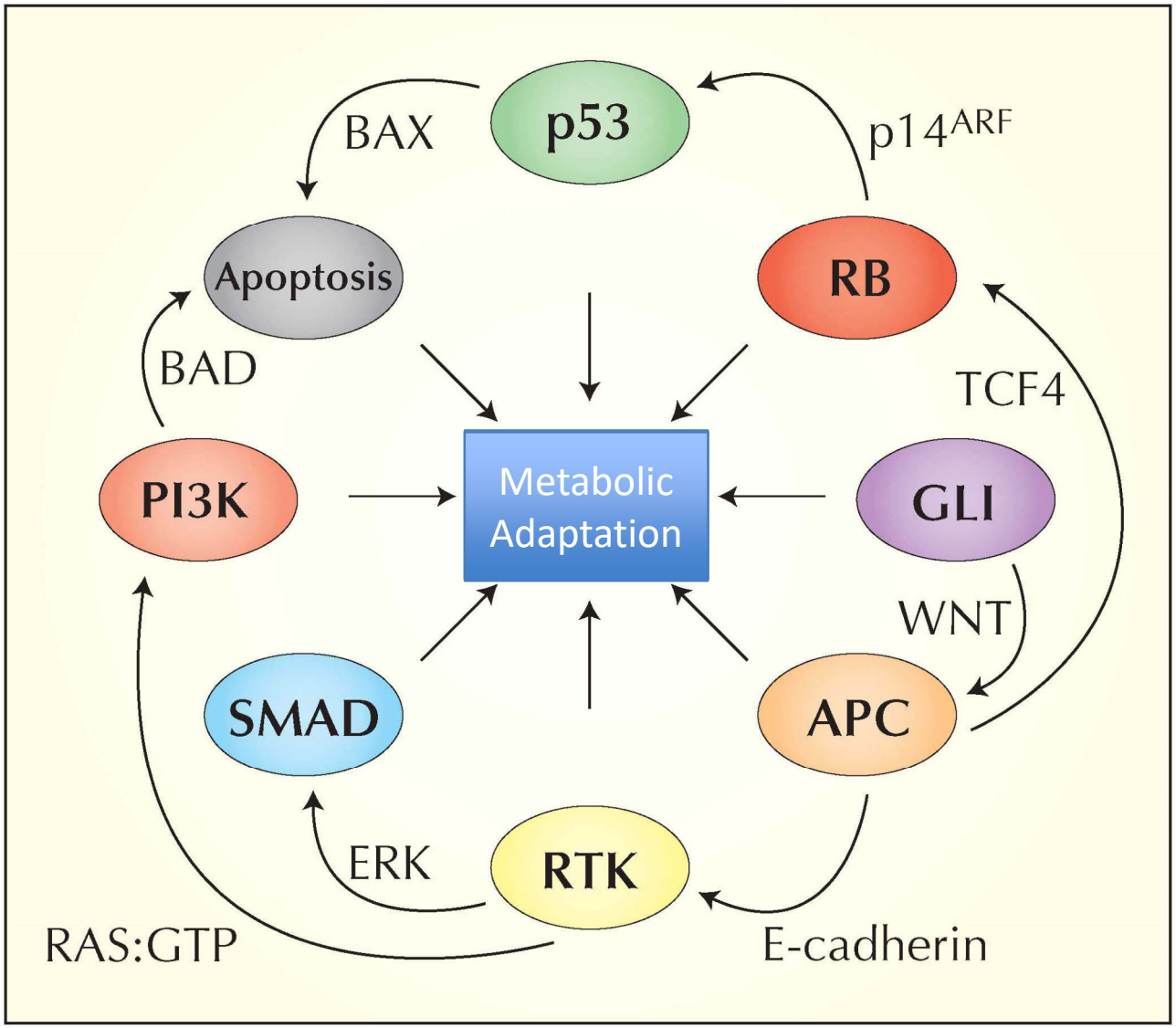
Tumor Specific expression of PKM2



Tumor Specific expression of PKM2

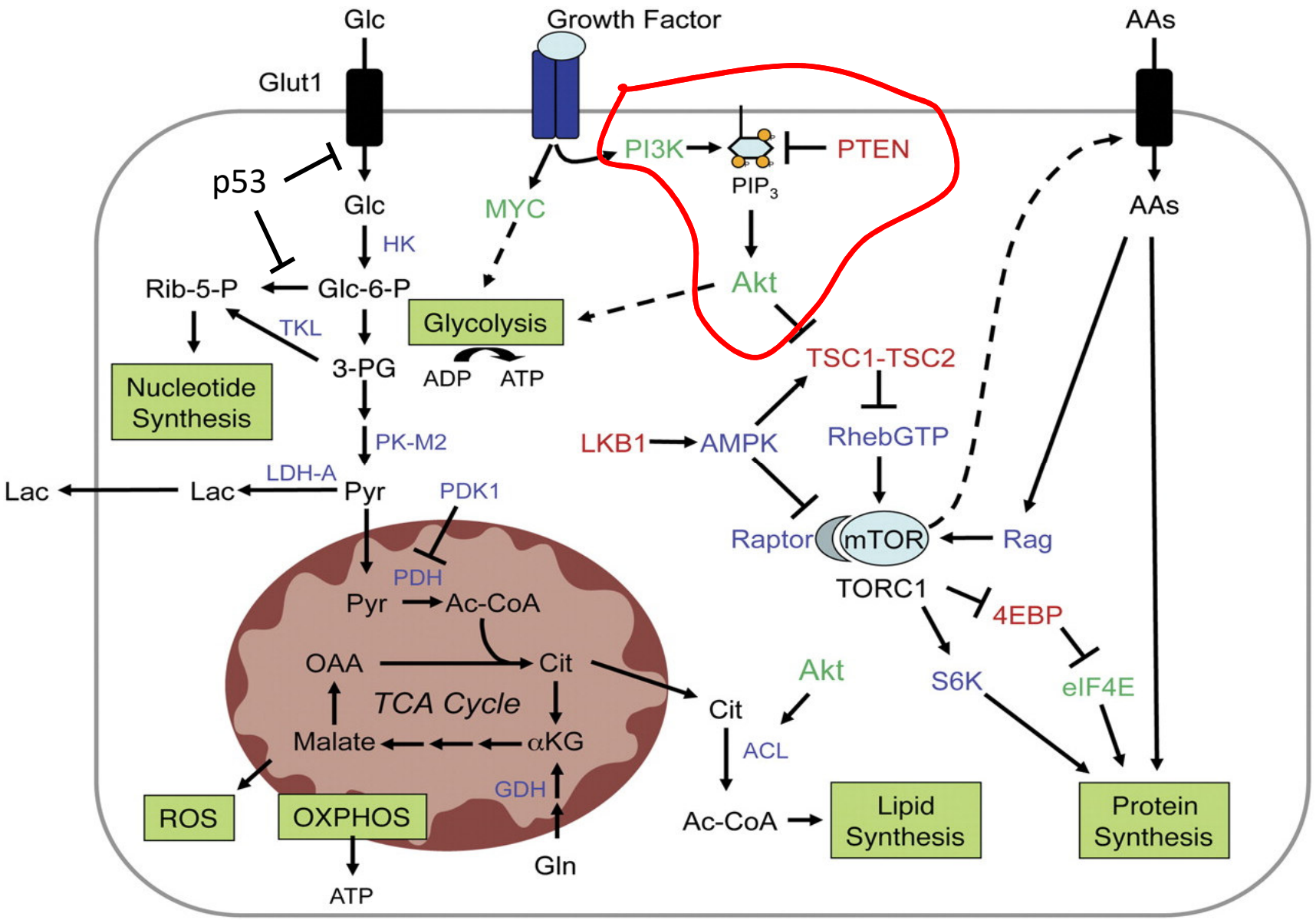


Links between Metabolism and Oncogenes/Tumor Suppressors



…….. Driving Forces Behind Tumor Metabolic Adaptation

Oncogenes and tumor suppressor in metabolic transformation



ONCOGENES

TUMOR SUPPRESSORS

Effectors

Increased activity of PI3K-AKT signaling

In tumors:

Constitutes one of the most common sets of mutations

Activating mutations in PI3K (a lipid kinase that regulated levels of phosphatidylinositol (PIP3))

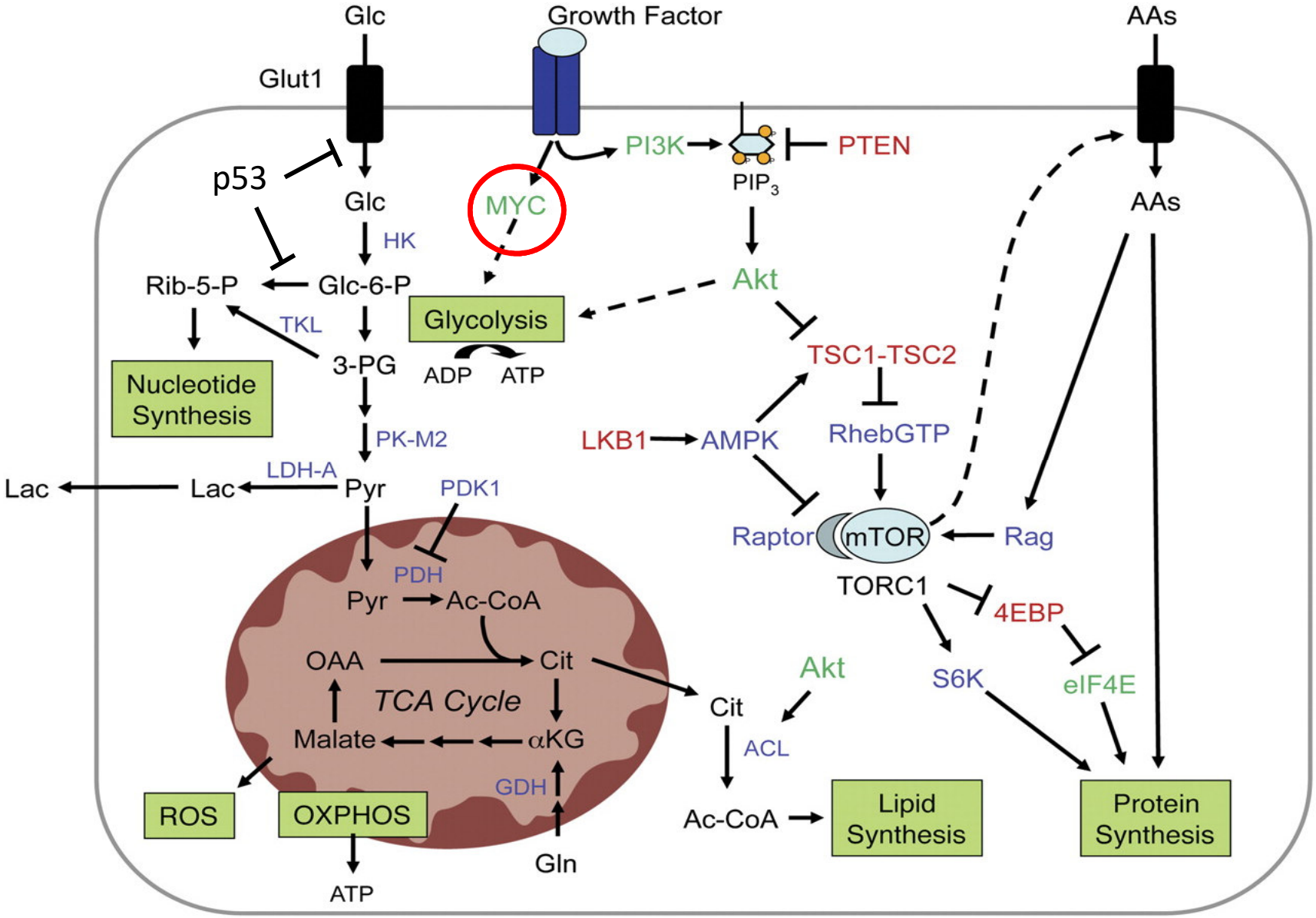
Activation of Growth Factor Receptors (mutations or amplification)

Loss of phosphatase PTEN

OUTCOME OF ACTIVATION

- Increase surface expression of nutrient transporters
- Akt-dependent stimulation of hexokinase and phosphofructokinase
- Enhanced transcription of genes involved in glycolysis and lipogenesis
- Enhanced protein translation through Akt-dependent mTOR activation

Oncogenes and tumor suppressor in metabolic transformation



ONCOGENES

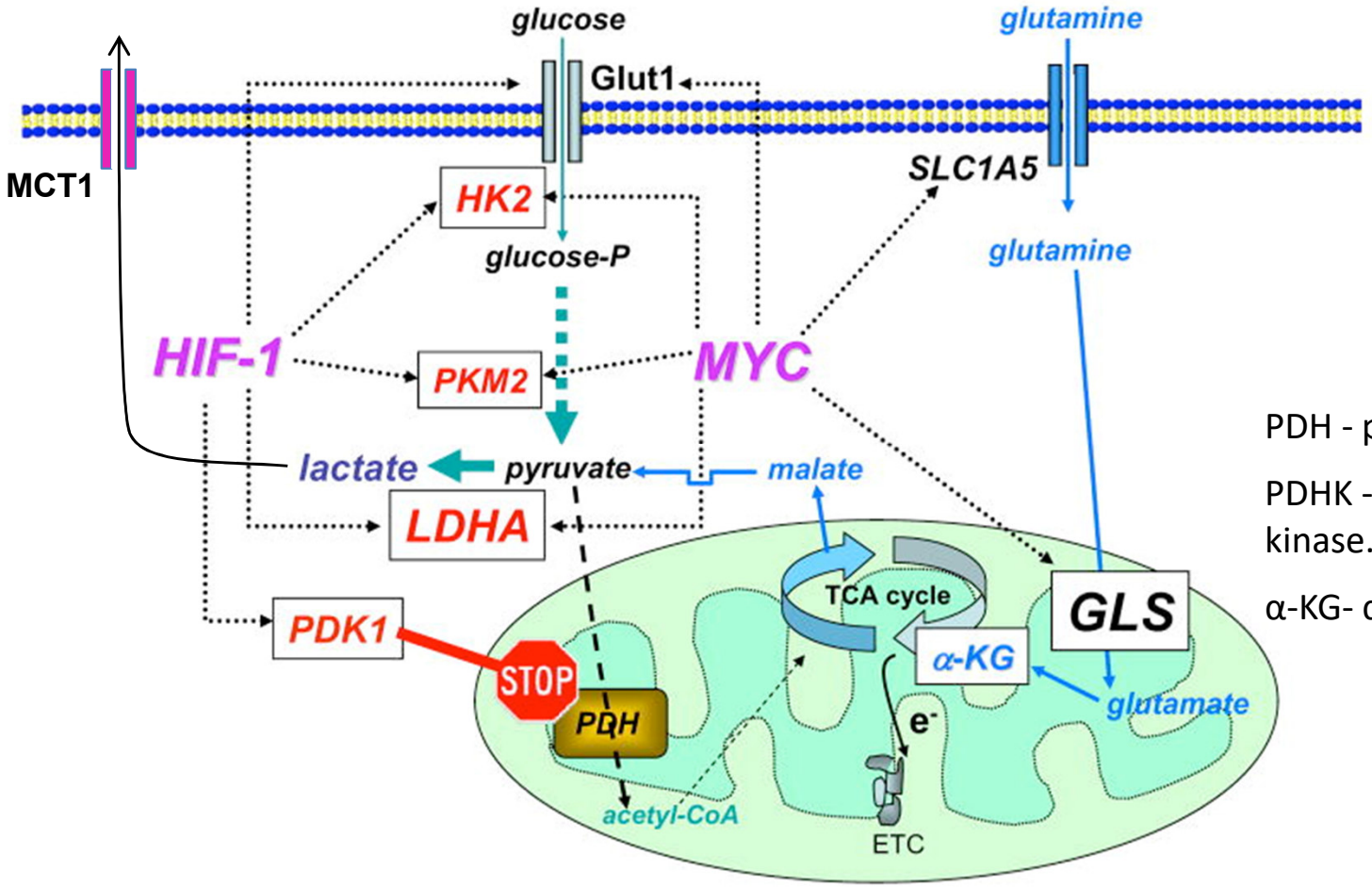
TUMOR SUPPRESSORS

Effectors

Myc regulates glucose and glutamine metabolism

• Regulates genes involved in glucose metabolism (glucose transporter Glut1, hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), pyruvate dehydrogenase kinase 1 (PDK1) and the lactate transporter MCT1

• Regulates gutamine metabolism through transporters (SLC1A5) and glutaminase (GLS).

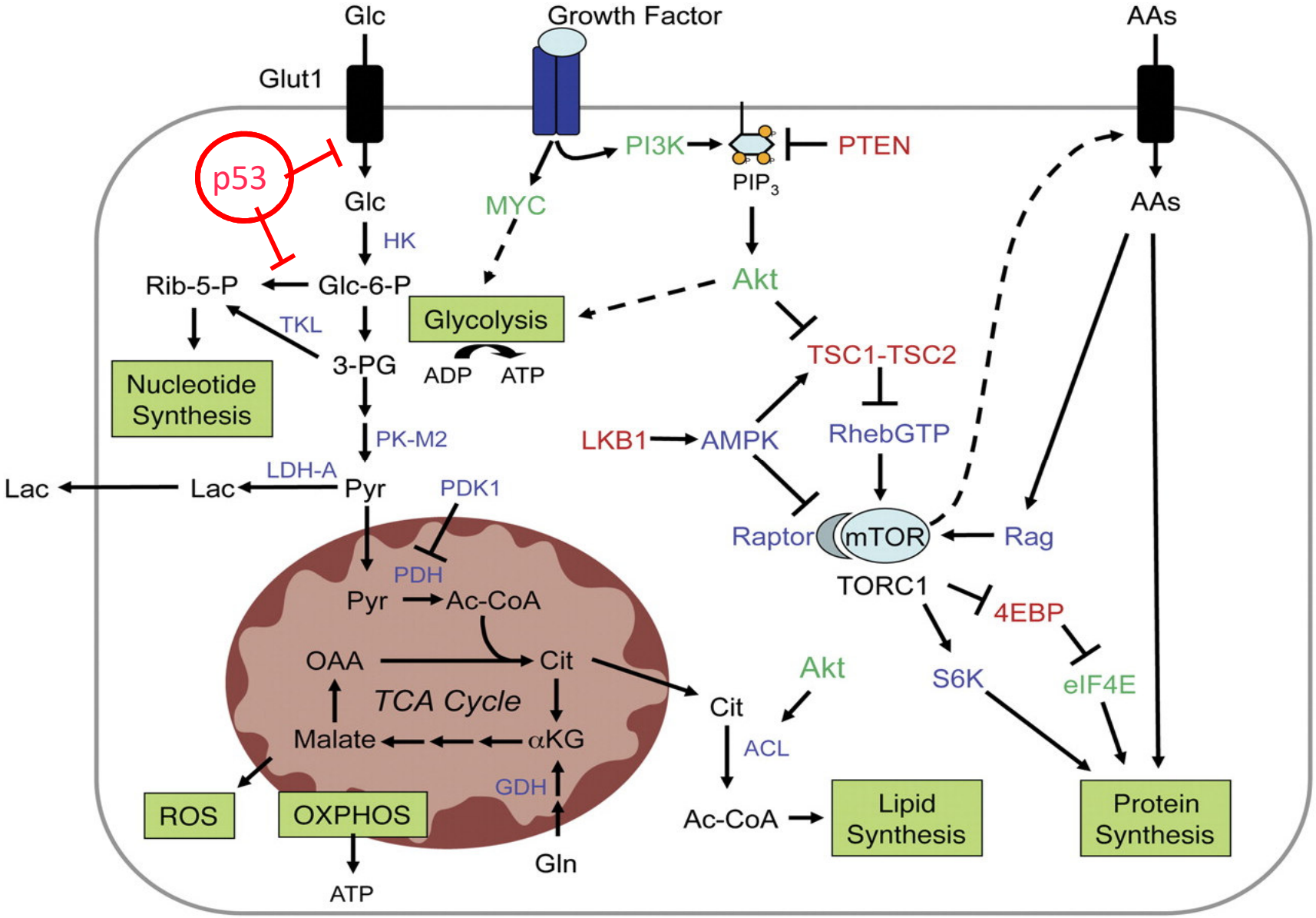


PDH - pyruvate dehydrogenase.

PDHK - pyruvate dehydrogenase kinase.

α-KG- α-ketoglutarate

Oncogenes and tumor suppressor in metabolic transformation



ONCOGENES

TUMOR SUPPRESSORS

Effectors

Tumor suppressor p53

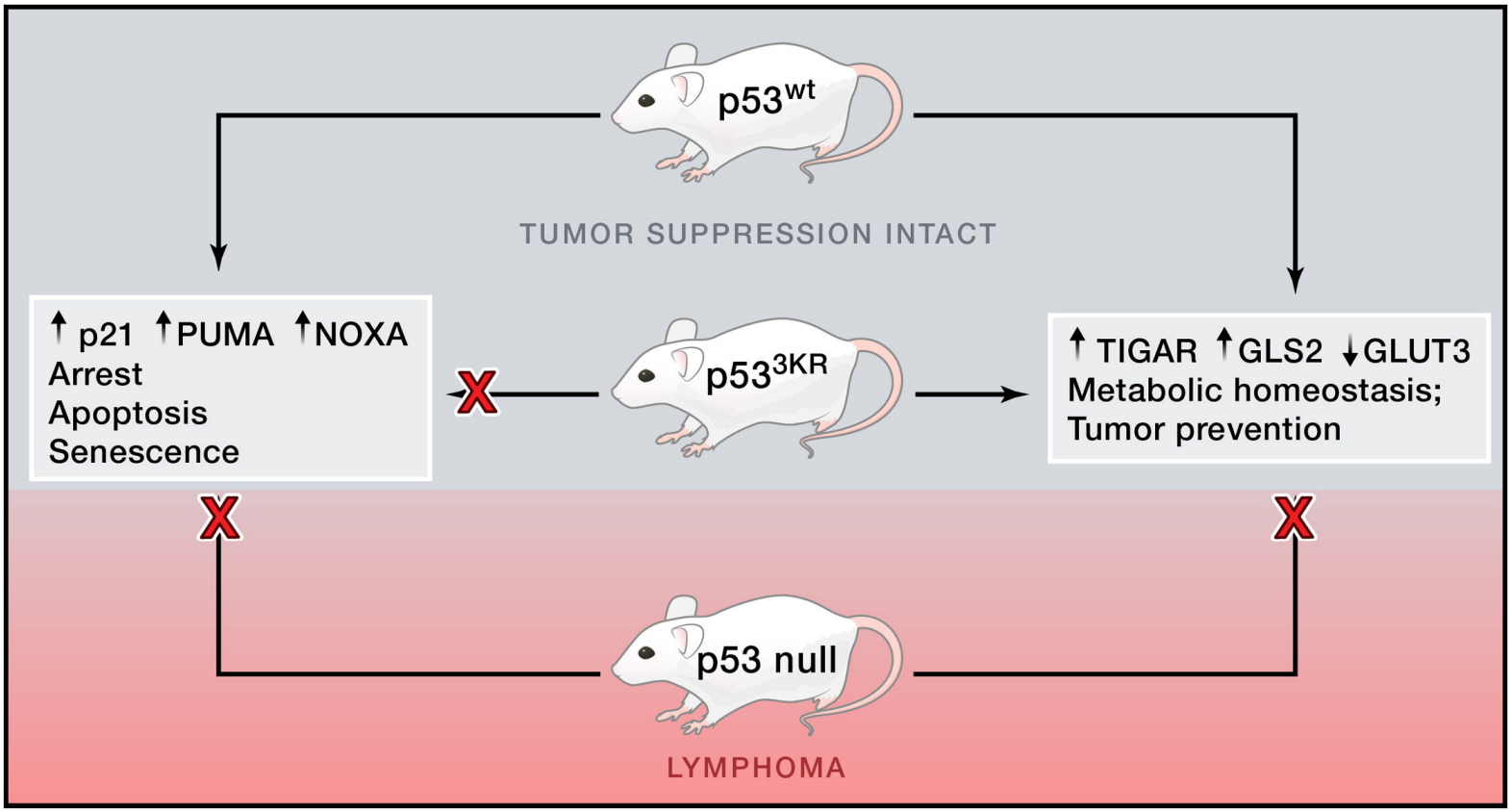
- Transcription Factor

- Activated in response to DNA damage, Hypoxia, Oxidative Stress, abnormal expression of Oncogenes

- Activation is achieved by post-translational modification and increase in p53 stability

- Activated p53 executes variety of cellular responses: growth arrest, apoptosis, senescence, metabolic transformation and …. more

P53: what is its mechanism of action ?



- wild-type p53 can induce many target genes, including p21, BAX, PUMA, and NOXA to drive cell-cycle arrest, apoptosis and senescence or regulate genes like TIGAR, GLS2 and GLUT3 that control metabolism and antioxidant defenses
- complete loss of p53 results in spontaneous development of lymphomas in mice
- mutating three lysines to arginine (K117R, K161R, K162R) to inhibit acetylation renders p53 incapable of inducing cell-cycle arrest, apoptosis, and senescence but retains its tumor suppressor activity
- while deleting the p53 downstream targets p21 + Puma + Noxa does not block p53's ability to suppress tumor formation.

Hock & Vousden, Cell 2012

p53 Targets and metabolic control

Up

SCO2 (the synthesis of cytrochrom c oxidase protein) that enables functional mitochondrion respiratory chain

TIGAR (TP53-induced glycolysis and apoptosis regulator) inhibits glycolysis by decreasing levels of fructose-2,6-biphosphate, a potent stimulator of glycolysis and inhibitor of gluconeogenesis

GLS2 (glutaminase) promotes glutaminolysis

Cpt1c (carnitine palmitoyltransferase) promotes β-oxidation of fatty acids

DRAM- damage regulated autophagy modulator, a lysosomal protein that induces macro-autophagy

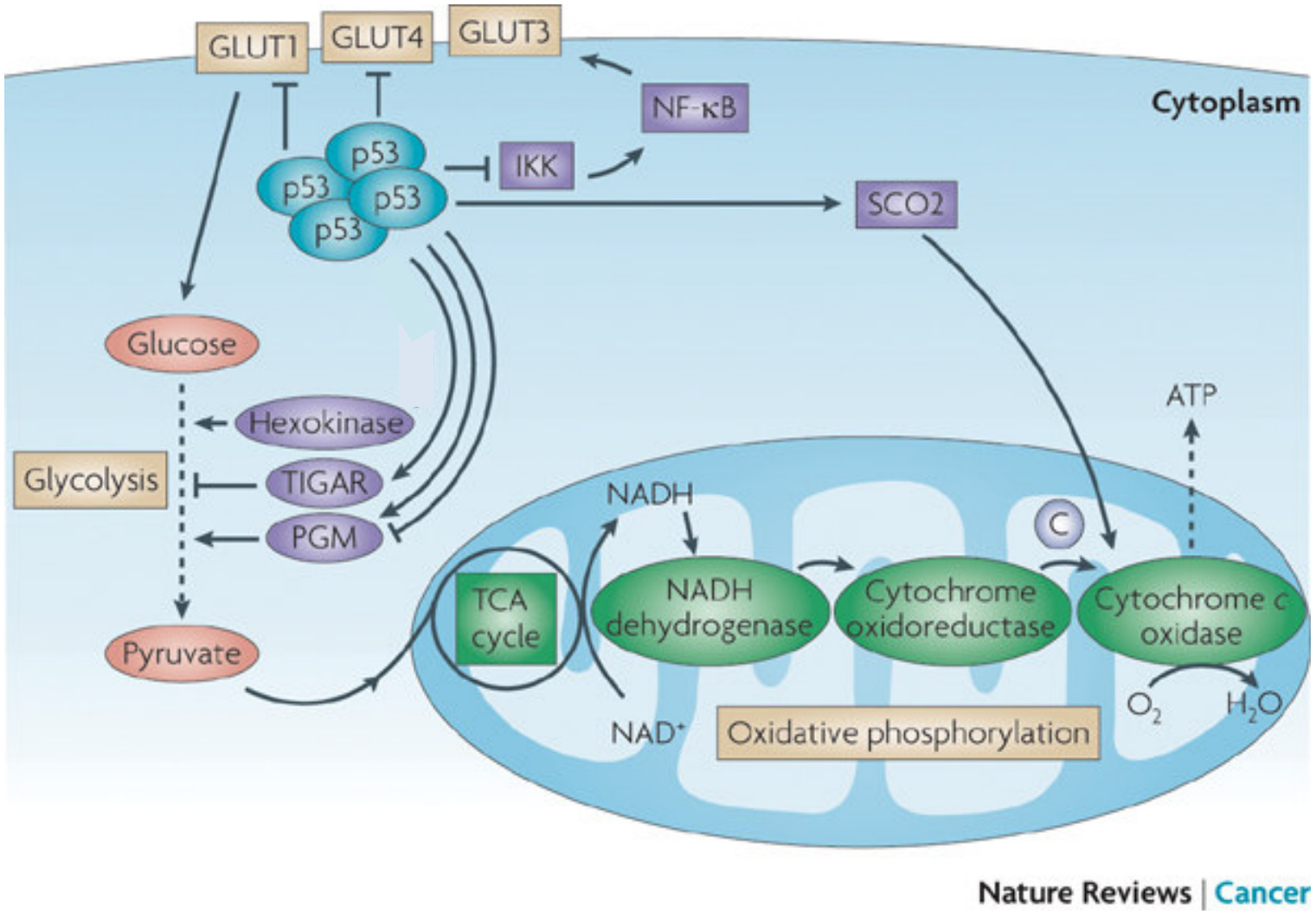
Down

Glut1 and Glut4- glucose transporters

PGM- phosphoglycerate mutase (catalyzes conversion of 3-phoshoglycerate to 2-phosphoglycerate)

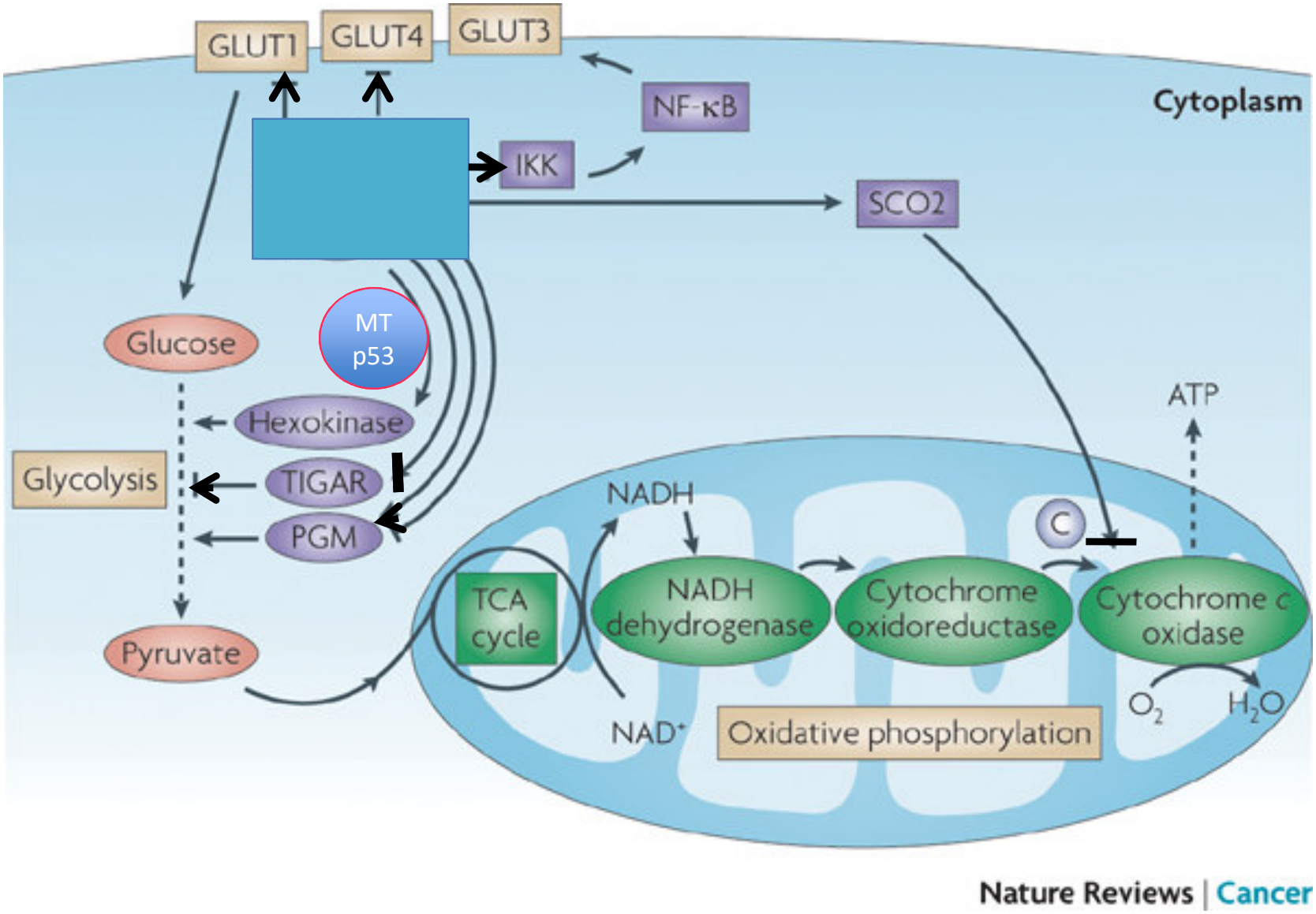
IKK (α and β)-phosphorylate inhibitors of NF-kappa-B, enabling NFkB-mediated upregulation of Glut3

p53 Targets and metabolic control



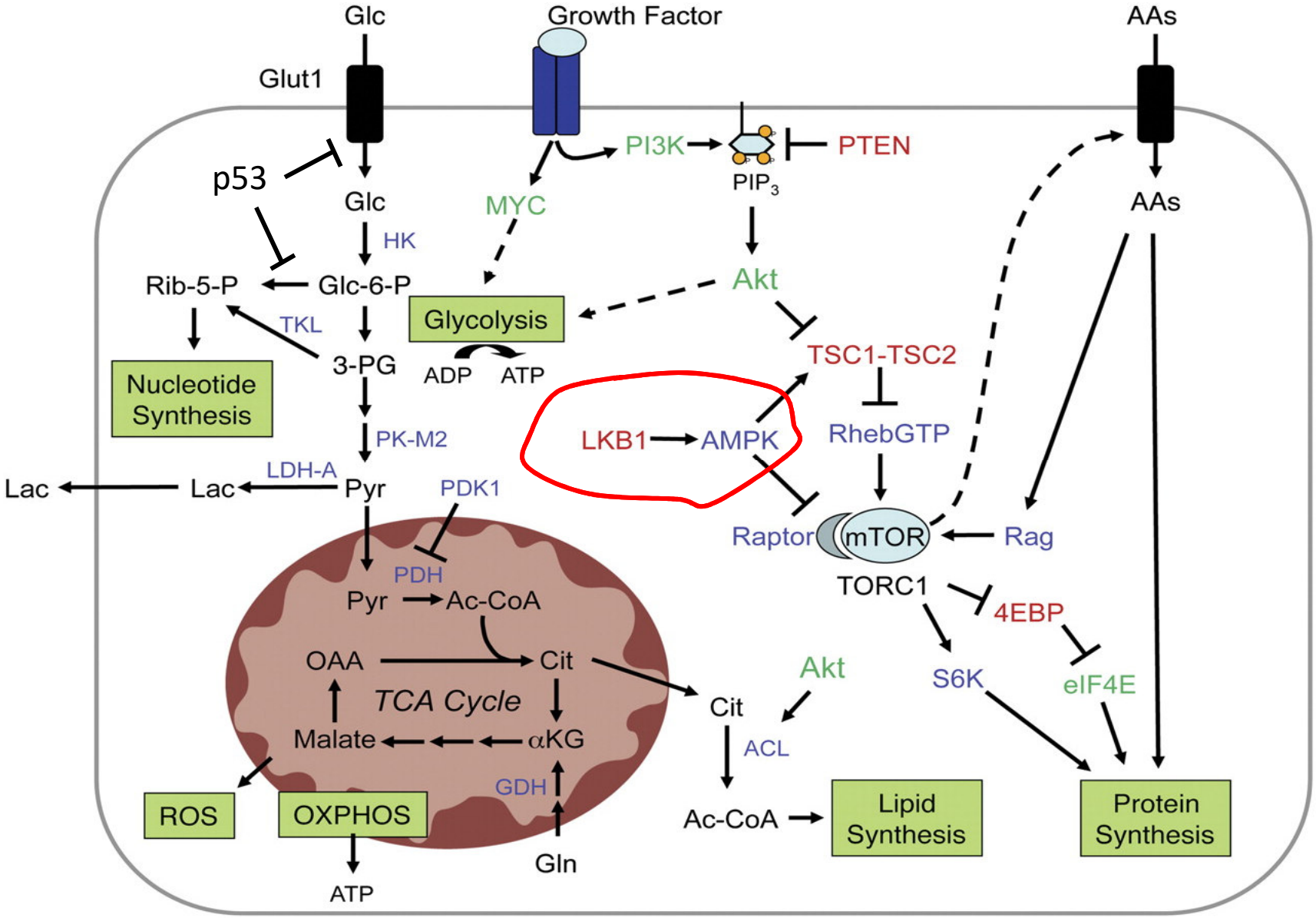
……Wt p53 activity favors the production of ATP by Oxidative Phosphorylation

Loss of p53 activity in tumors



……Loss of p53 activity favors glycolysis

Oncogenes and tumor suppressor in metabolic transformation



ONCOGENES

TUMOR SUPPRESSORS

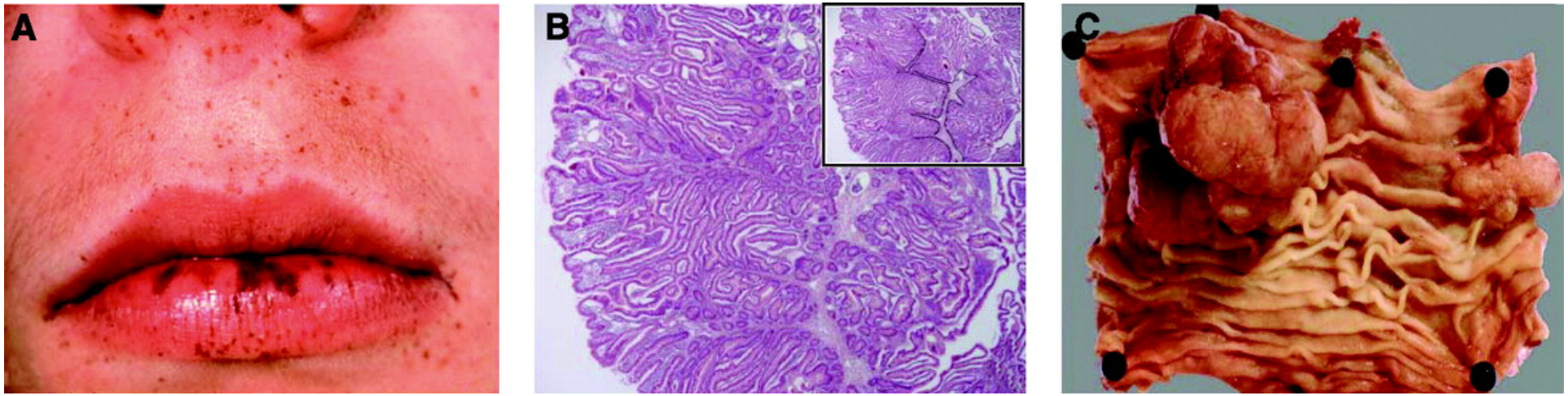
Effectors

Tumor suppressor function of LKB1

- Loss of S/T-kinase activity of LKB1 is associated with Peutz-Jeghers syndrome

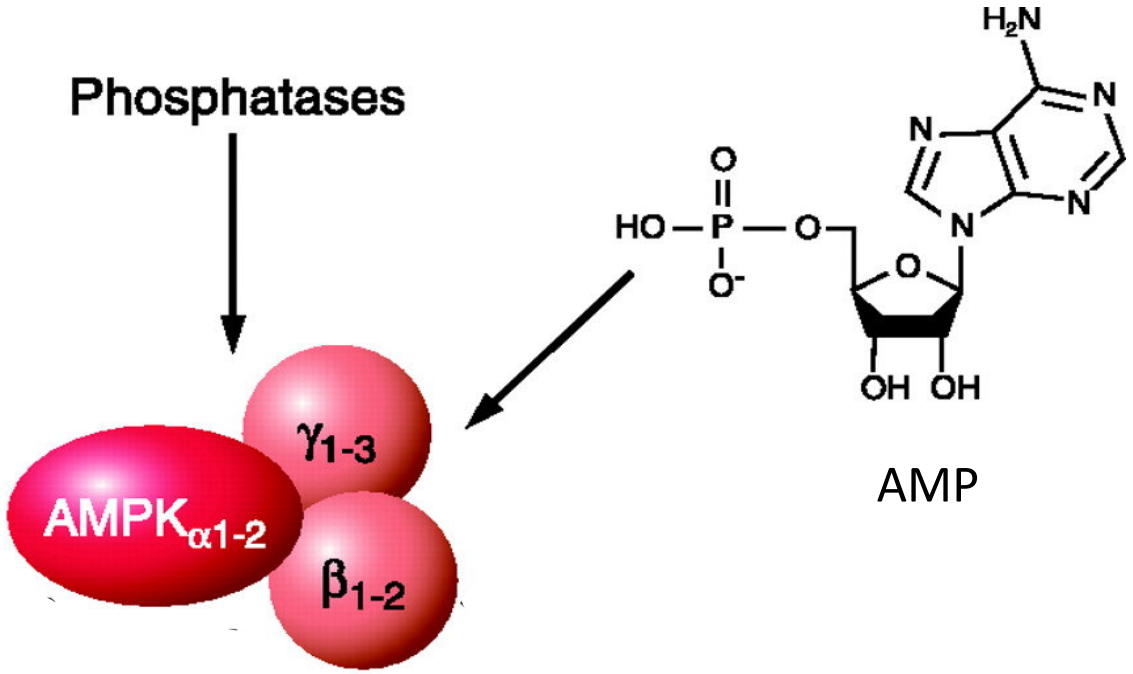
- Peutz-Jeghers syndrome – is characterized by benign overgrowth of differentiated tissues in the gastrointestinal tract

- Mutation in LKB1 are found in lung adenocarcinomas, squamous cell carcinomas, and cervical carcinomas



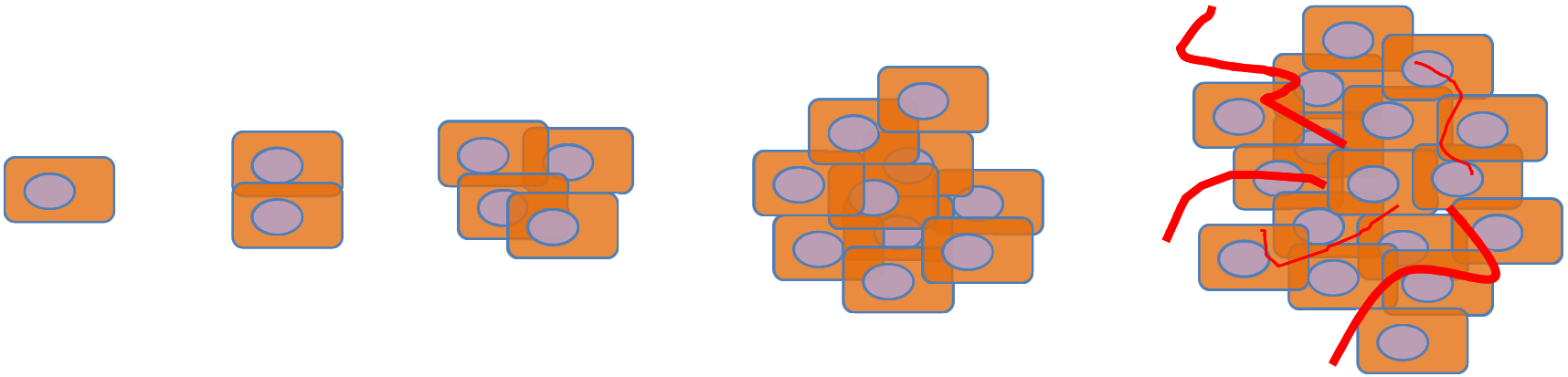
Jansen M et al. Physiol Rev 2009;89:777-798

LKB1 deficiency suppresses activity of AMPK



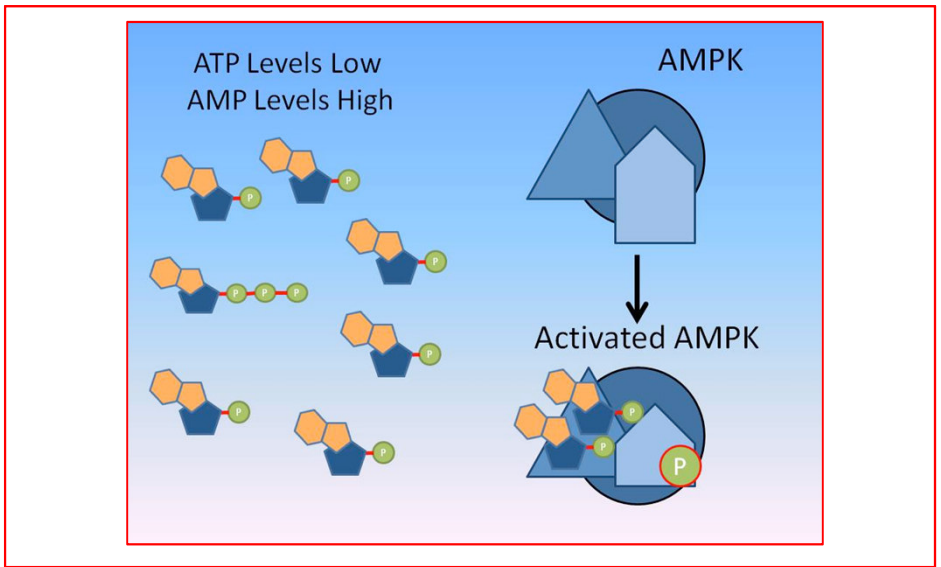
AMP

Metabolic Stress during tumour development

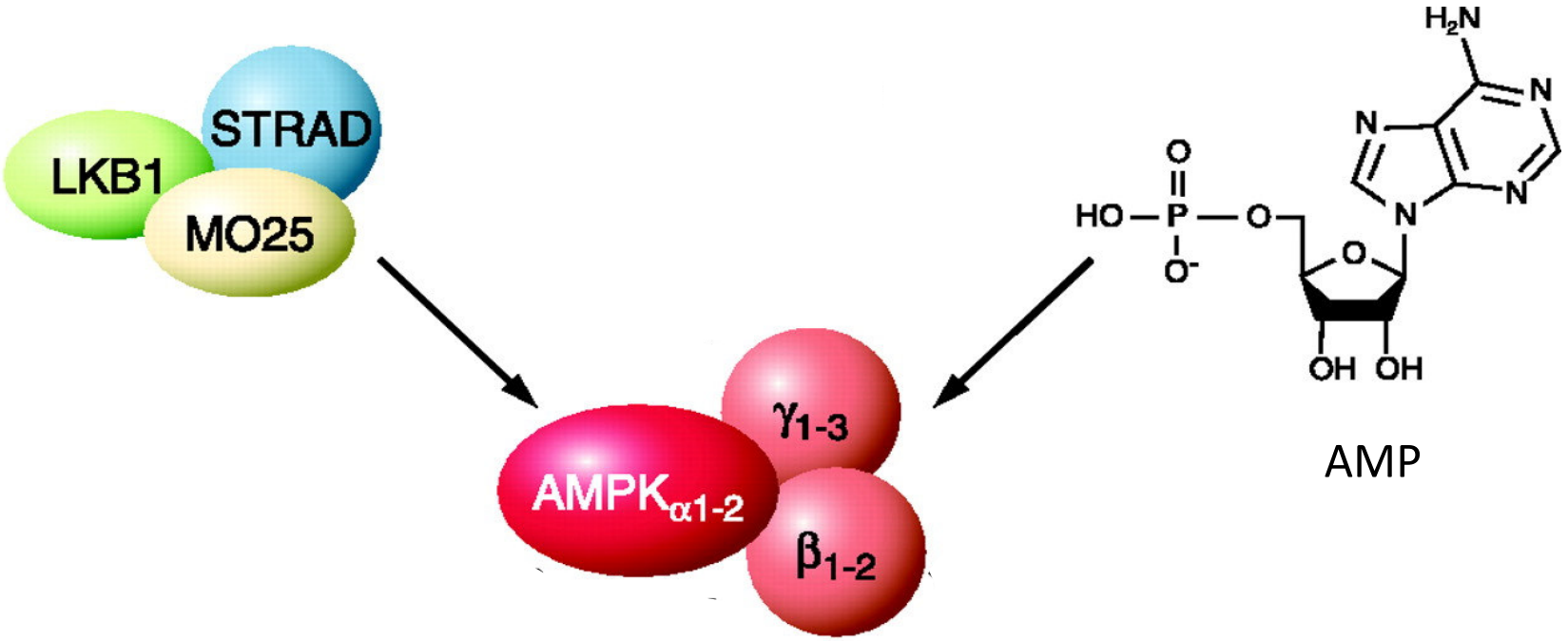


Tumour Growth

Nutrients,
Oxygen



LKB1 deficiency suppresses activity of AMPK

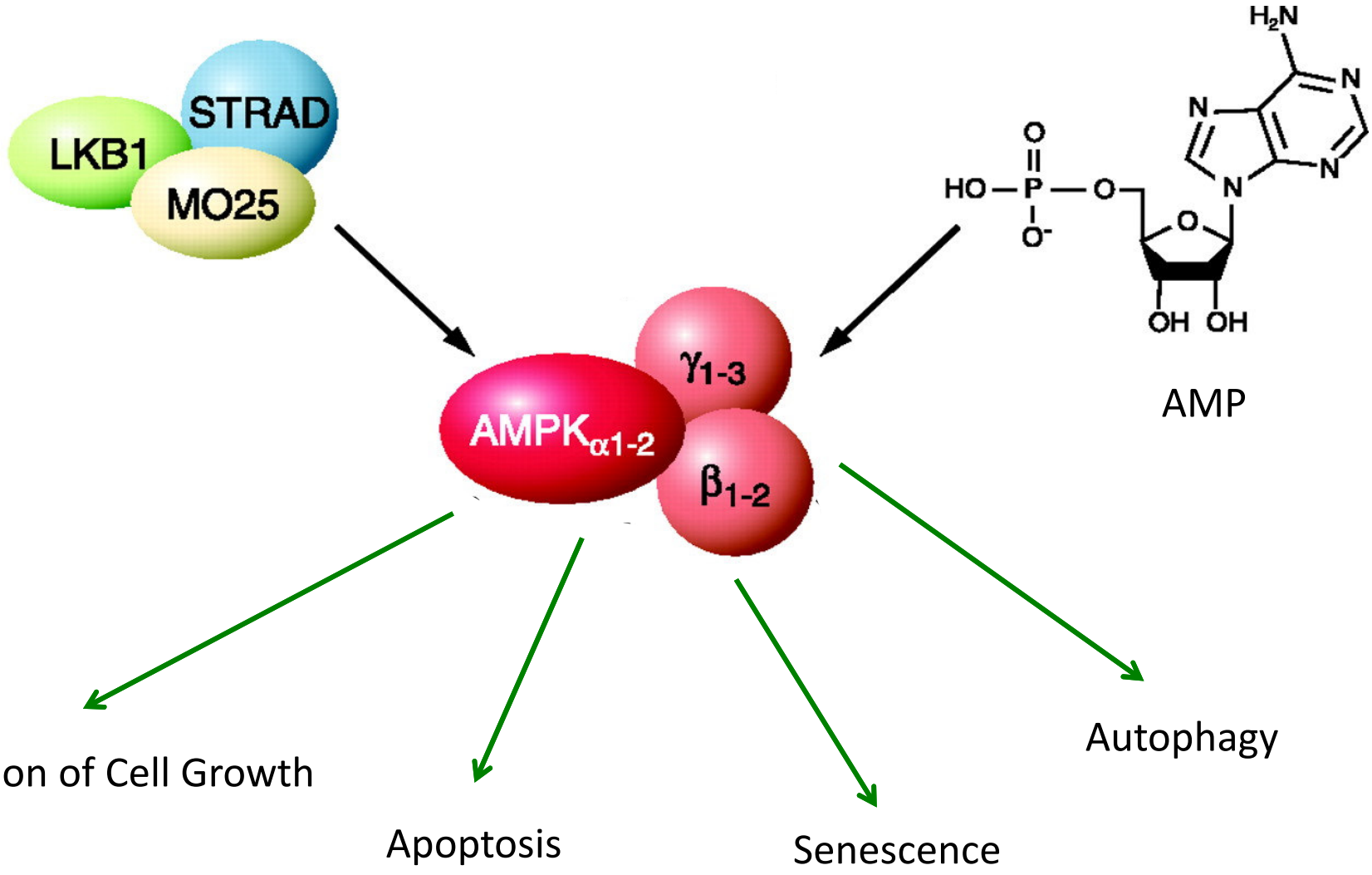


AMP

STRAD - Ste20 adaptor protein (function: stabilizes LKB1)

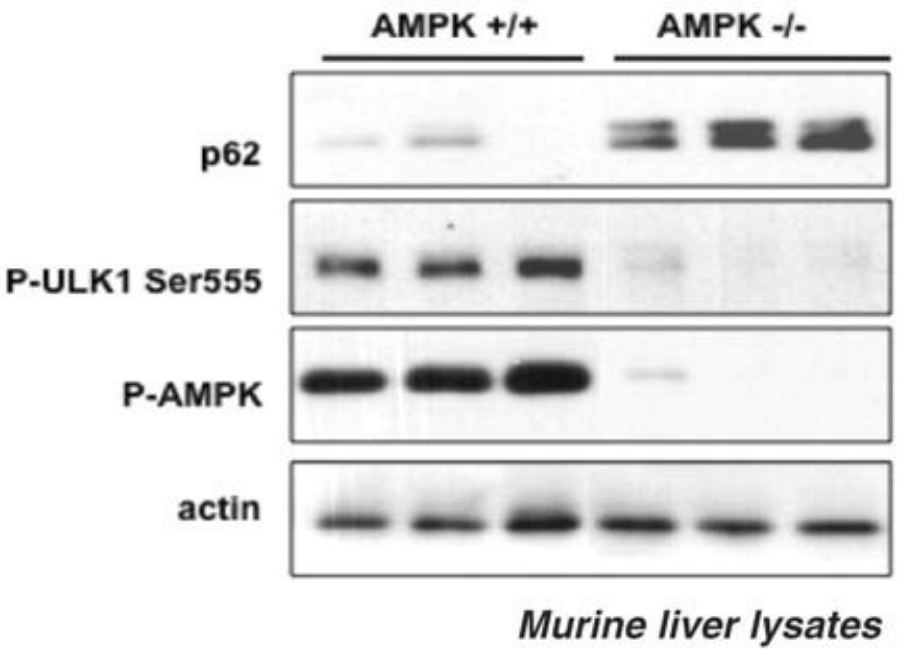
MO-25 - scaffolding protein (function: “keeps together”)

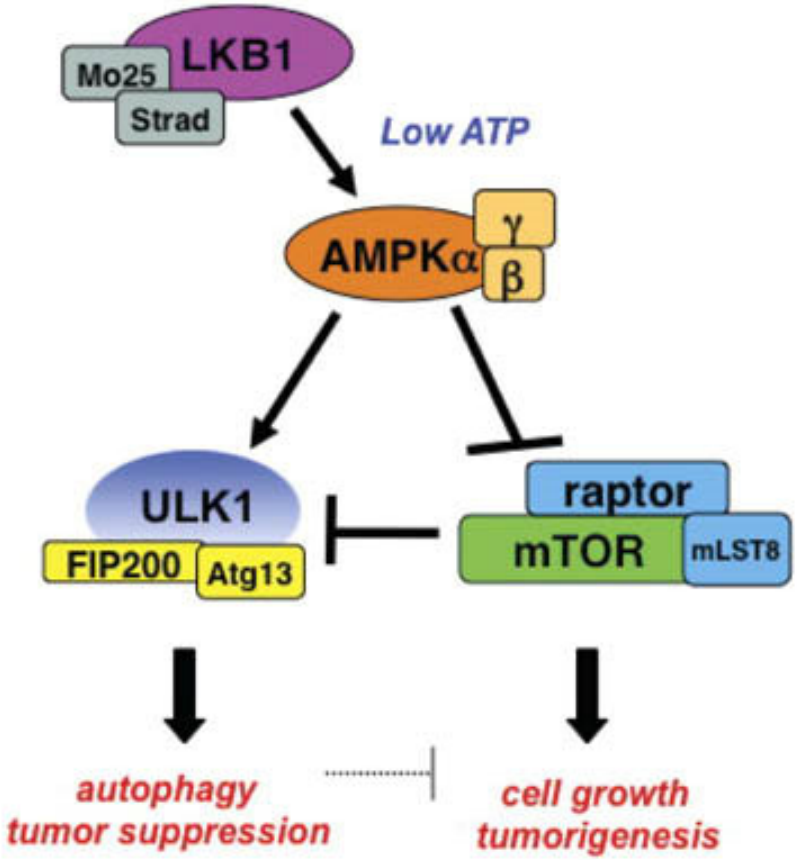
LKB1 deficiency suppresses activity of AMPK



CANCER RISK upon LKB1 loss

AMPK connects energy sensing to autophagy

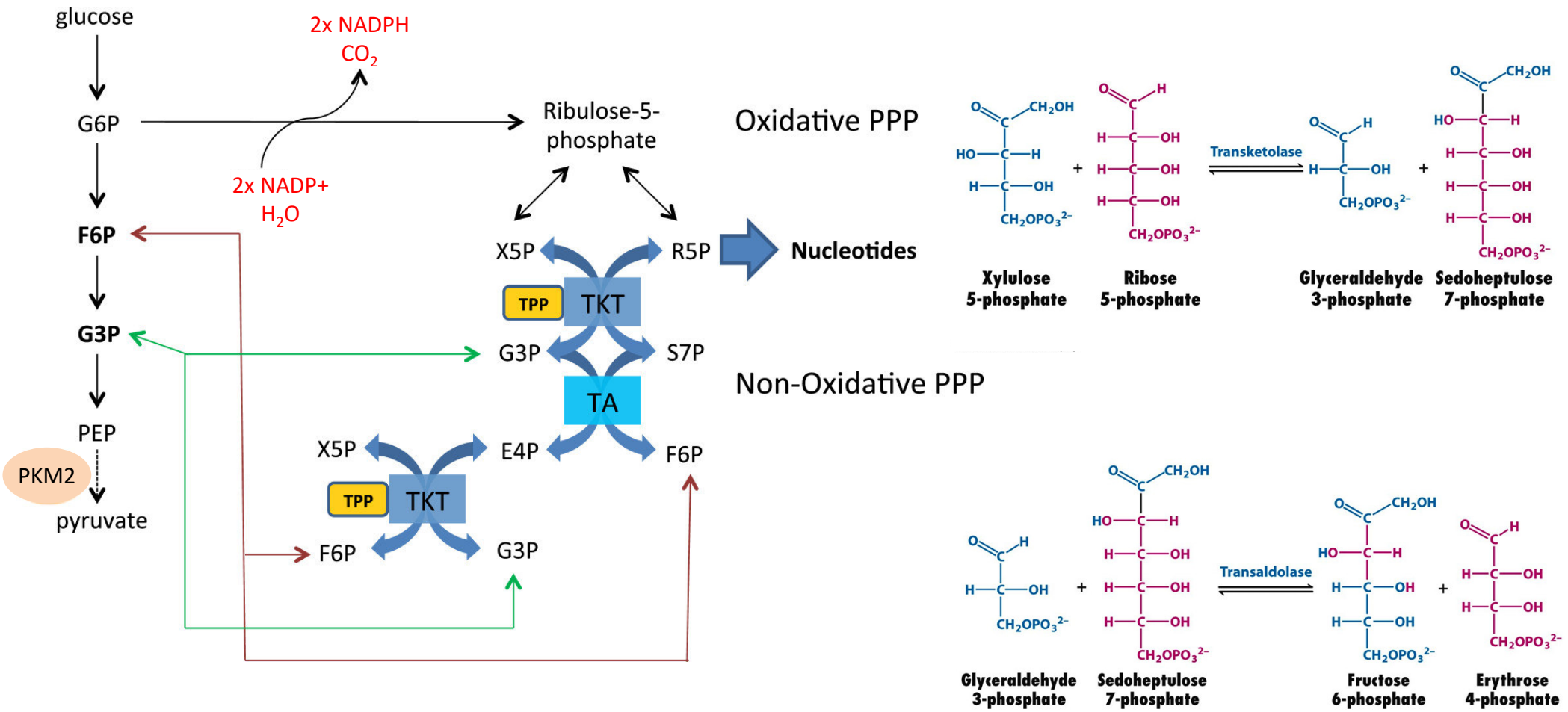




Egan D et al., Science (2011): 311 pp456-461

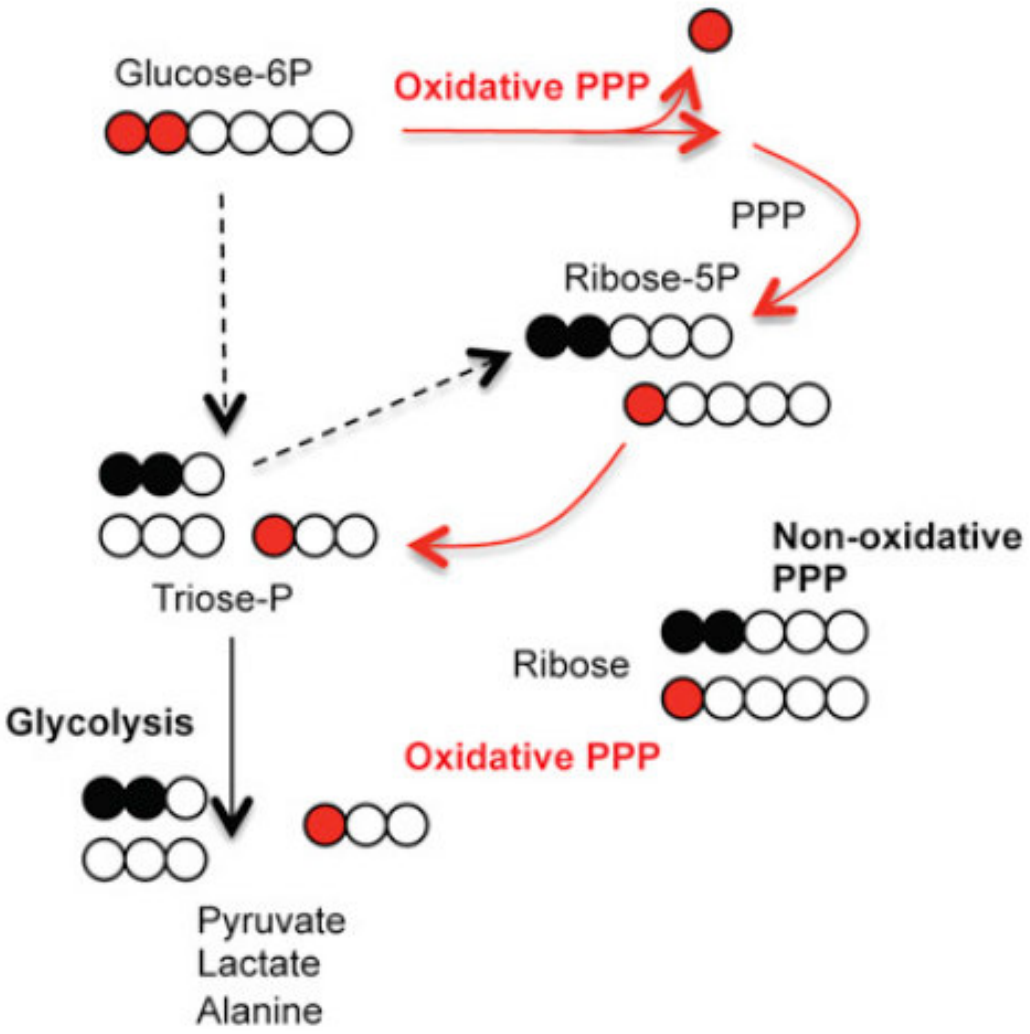
Methods to study tumor metabolism

Pentose-Phosphate pathway

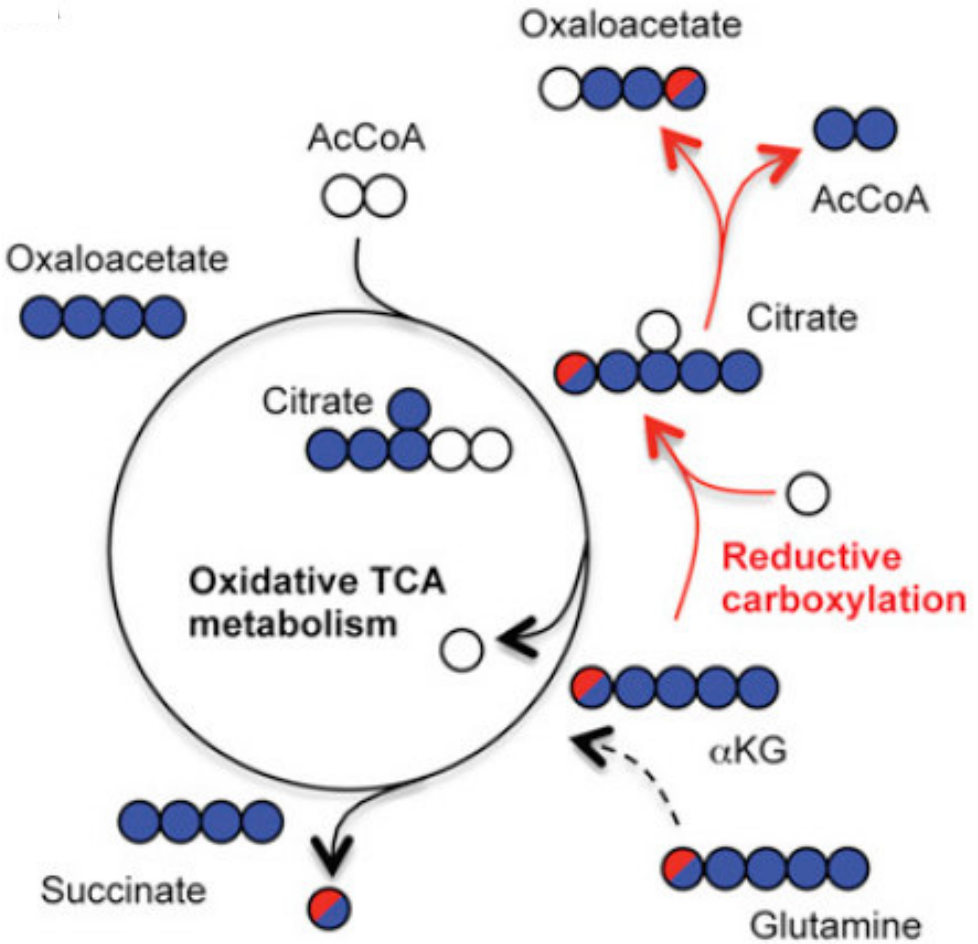


- normally, glucose-6-phosphate (**G6P**) entering the **oxidative pentose phosphate pathway (PPP)** is converted to ribose 5-phosphate (**R5P**) and xylulose 5-phosphate (**X5P**) for biosynthesis of nucleotides.
- alternatively, transketolase and –aldolase can shunted back F6P and G3P into the **non-oxidative PPP pathway** to produce R5P and X5P

Stable isotope tracers



glucose labeled on the first and second carbons ([1,2-$^{13}C_2$]glucose) is often used to determine relative flux through the **oxidative** and **non-oxidative PPP** by assessment of singly versus doubly labeled downstream intermediates

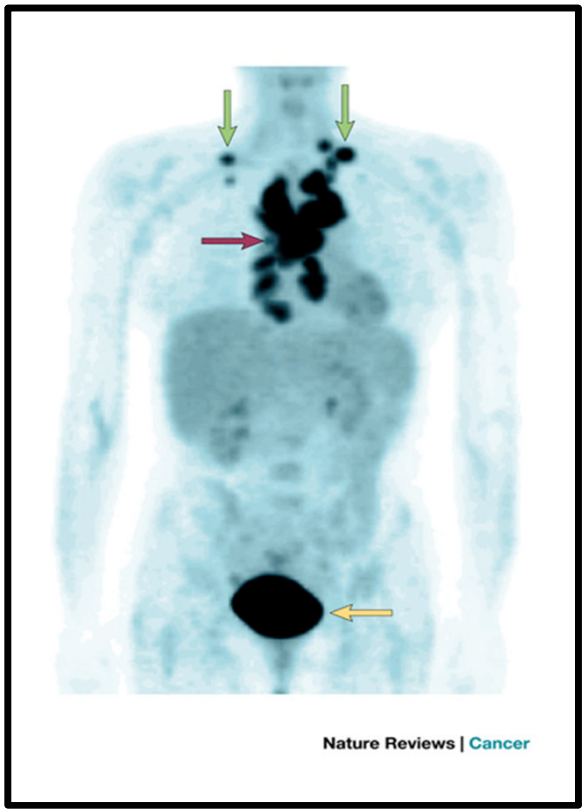


Uniformly labeled [U-$^{13}C_5$] glutamine (blue) or singly labeled [1-^{13}C] glutamine (red) provide independent means of distinguishing reductive carboxylation from oxidative TCA metabolism when measuring ^{13}C enrichment in citrate: Five carbon-labeled citrate suggests reductive carboxylation, while four-carbon-labeled citrate suggests oxidative TCA metabolism

Imaging Technique Positron-emission tomography

Deoxyglucose is taken by the cells but cannot be metabolized

Positron-emission tomography imaging with ^{18}fluorodeoxyglucose (FdG) of a patient with lymphoma.



The mediastinal nodes (purple arrow) and supraclavicular nodes (green arrows) show high uptake of FdG showing that tumours in these nodes have high levels of FdG uptake. The bladder (yellow arrow) also has high activity, because of excretion of the radionuclide.

AT CHUV: Prof. John Prior
john.prior@chuv.ch

Is it applicable for detection of cancer? Why?

Increased rate of glycolysis. FdG uptake and trapping occurs because of upregulation of glucose transporters (Glut1 and Glut3) and Hexokinases I and II

Isocitrate dehydrogenase
IDH

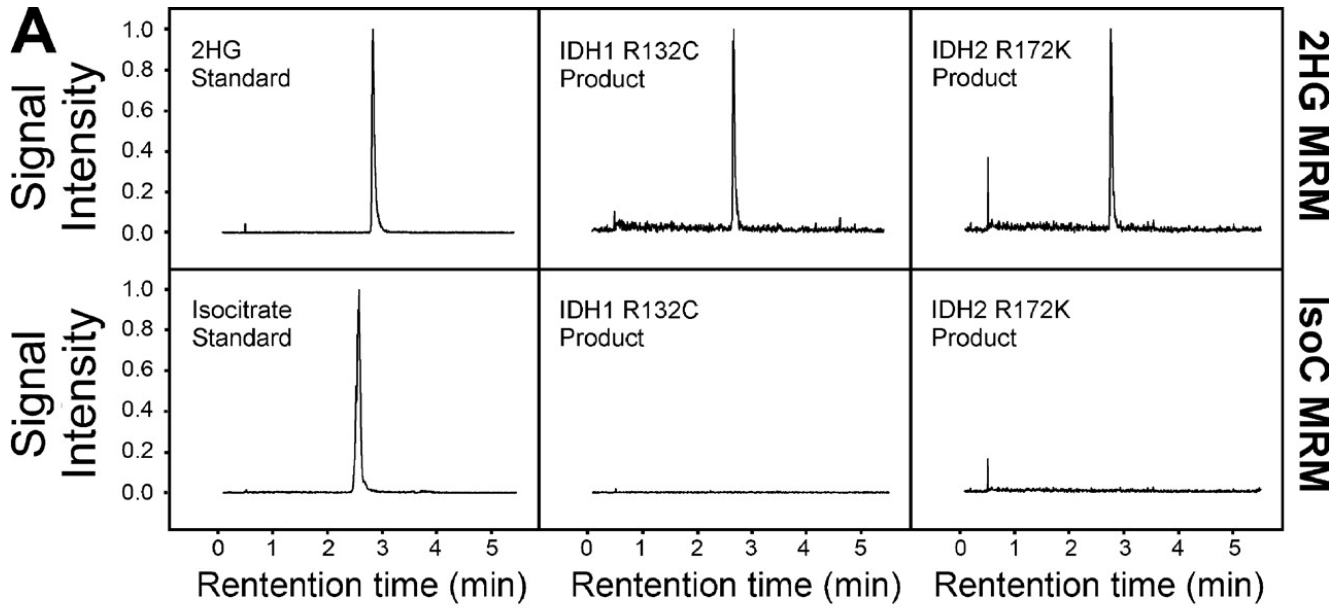
•There are 2 isoforms of mammalian IDH - IDH1 (cytoplasmic) or IDH2 (mitochondrial) -

•The large scale sequencing discovered IDH1 (Arg 132) or IDH 2 (Arg 140, Arg 172) are mutated in 90% of secondary GBMs and 18% of acute myeloid leukemias

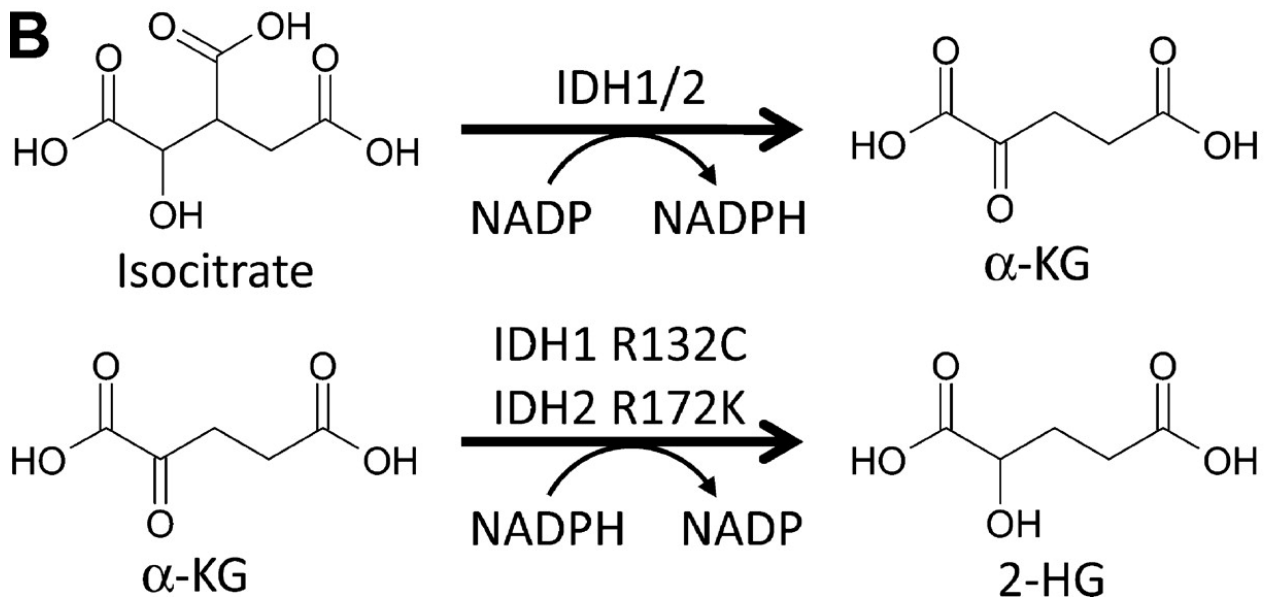
•IDH mutations observed in tumors are gain of function mutations

Reference: Dang L et al., Nature, 2009

Mutant IDH produces the onco-metabolite 2-HG



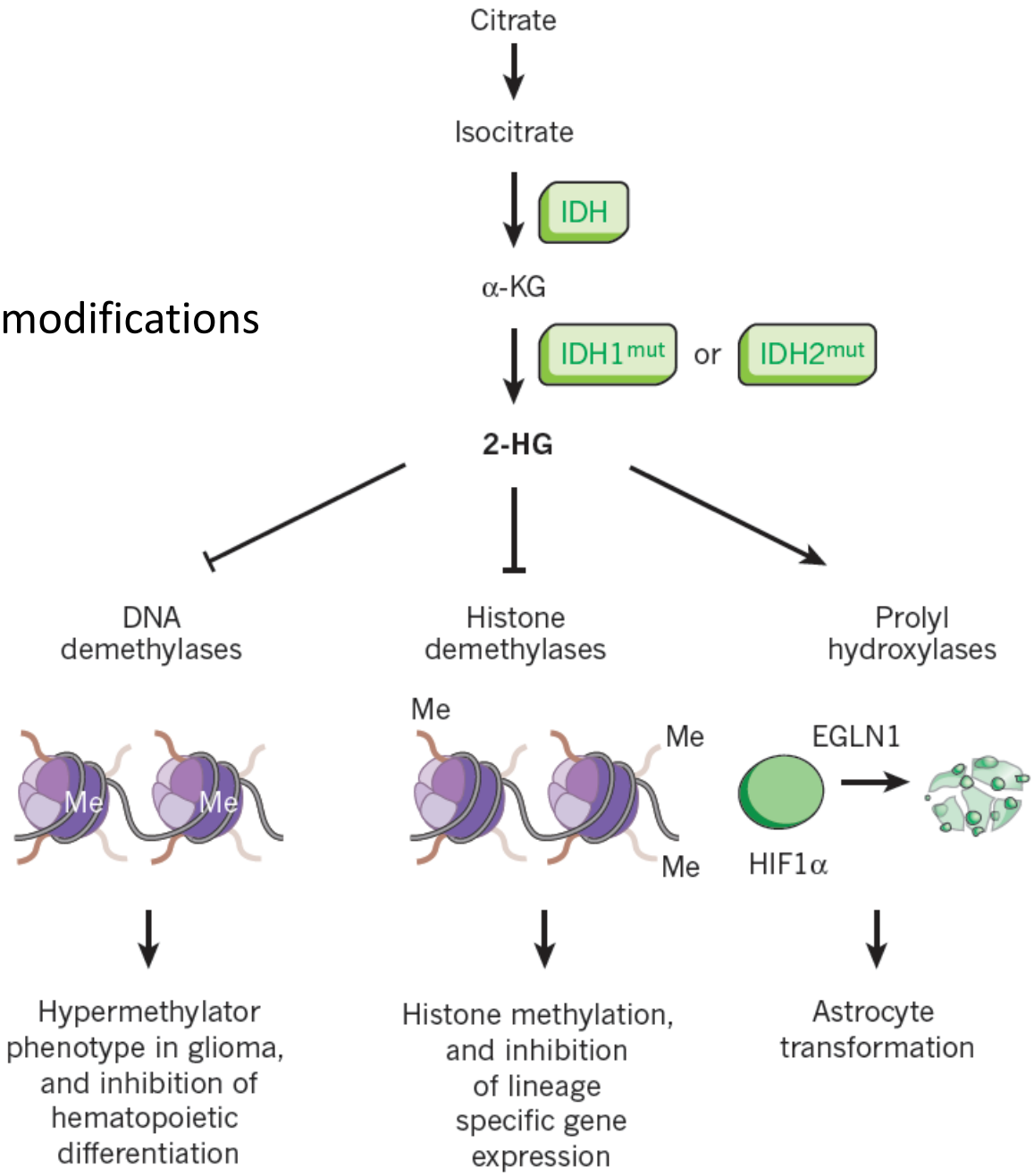
2-hydroxyglutarate (2-HG)



Gross S et al. J Exp Med 2010;207:339-344

2-hydroxyglutarate (2-HG)

- Potentially used as biomarker
- Promotes stability of HIF1α
- Impairs genome-wide epigenetic modifications



Post-translational protein modifications influenced by metabolic flux

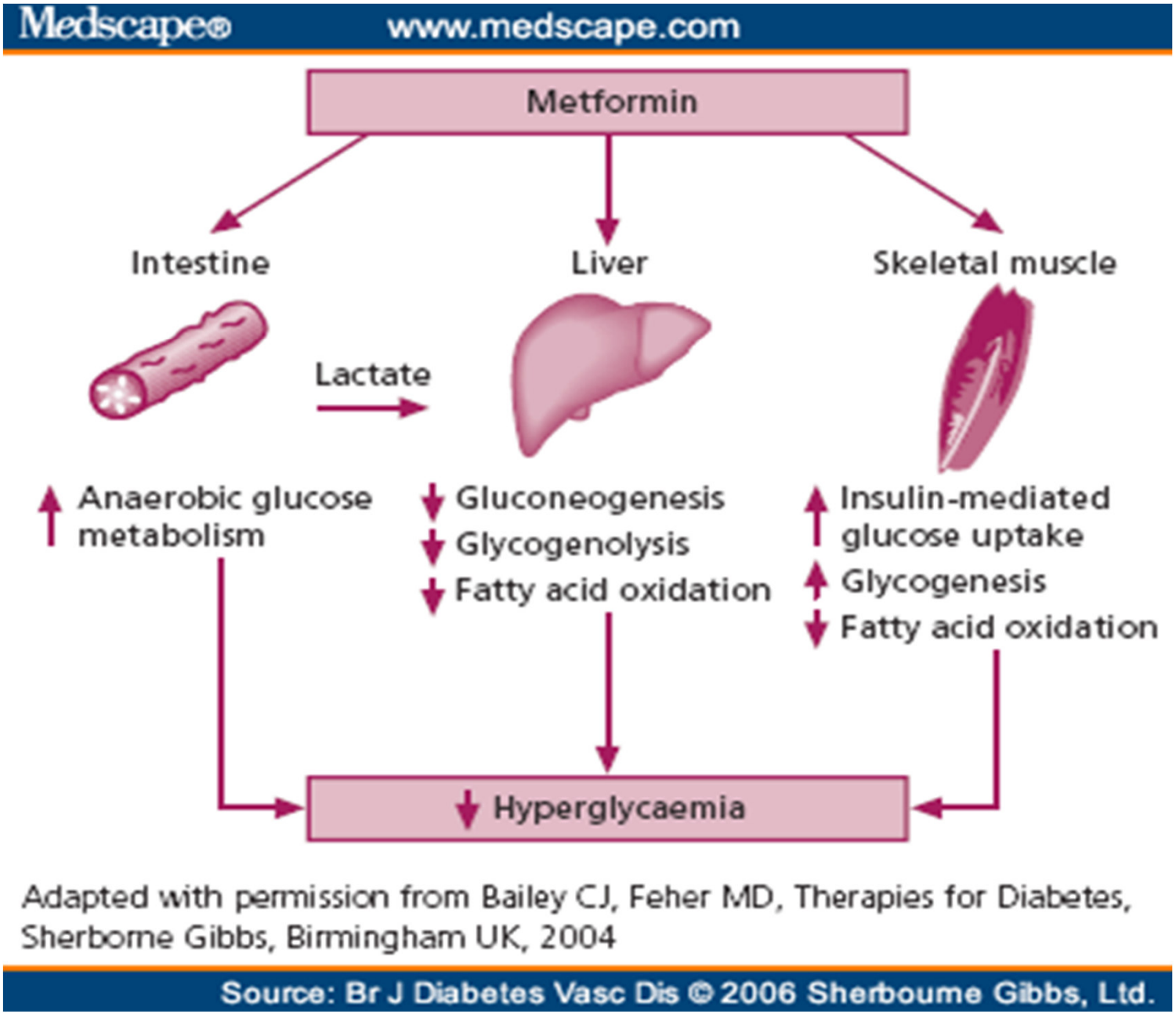
Examples of Mammalian Signal Transduction by Post-translational Modification

Modifications	Enzymes	Metabolic Substrate	Regulation
Glycosylation	OGT	GLCNAC	Metabolic Flux
Methylation	Methyltransferases	SAM	Metabolic Flux
Acetylation	Acetyltransferases	Acetyl-CoA	Metabolic Flux
ADP-ribosylation	PARPs	ADP-ribose, NAD^+	Metabolic Flux
Deacetylation	Sirtuins	NAD^+	Metaboic Flux
Cysteine Oxidation	-	superoxides	Metabolic Flux
Proly-hydroxylation	Prolyl-hydroxylase	α-ketoglutarate	Metabolic Flux

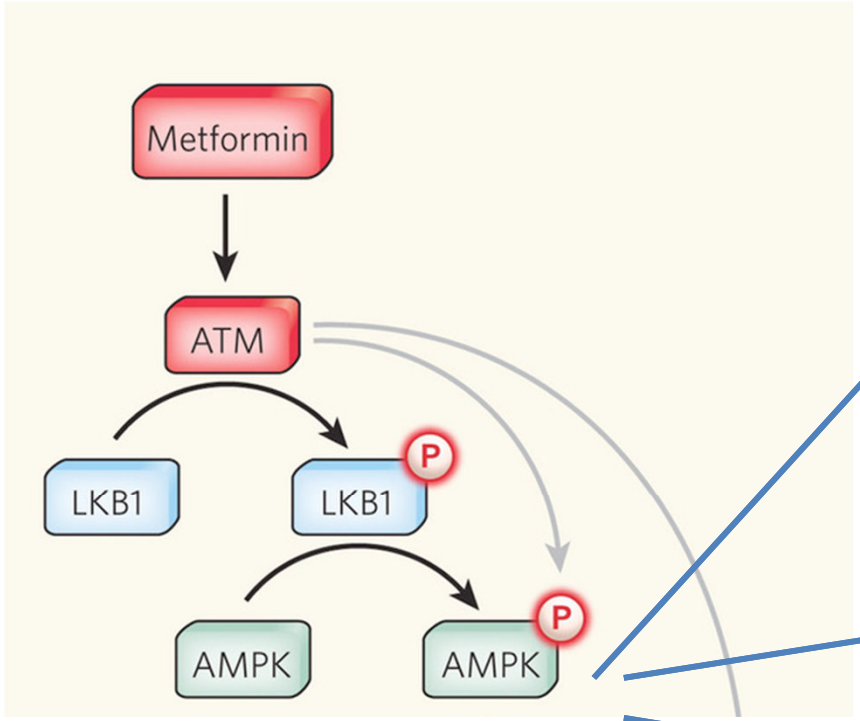
- in contrast to protein phosphorylation, other protein modifications are carried out by a relatively small number of enzymes (acetyltransferases, methyltransferases, GlcNAc transferases, ADP-ribosyltransferases, Prolyl-hydroxylases, etc.), which are much fewer than the total number of protein kinases
- importantly, the enzymes typically have Michaelis constants for their metabolic substrates that are close to the physiological concentrations and their levels are dynamically regulated by alterations in cellular metabolism

Metformin

Activator of AMPK



Metformin activates AMPK



Inhibition of Cell Growth

Senescence

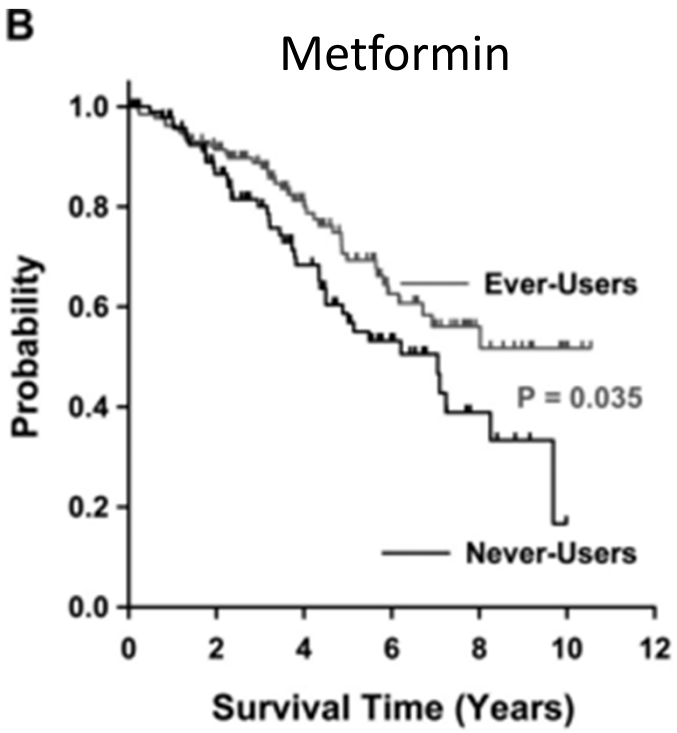
Autophagy

Apoptosis

Metformin

Reduced the risk of cancer in diabetic patients

E.g. prostate cancer patients.



Kaplan–Meier survival curves comparing ever-users and never-users

<u>Other Cancers:</u>

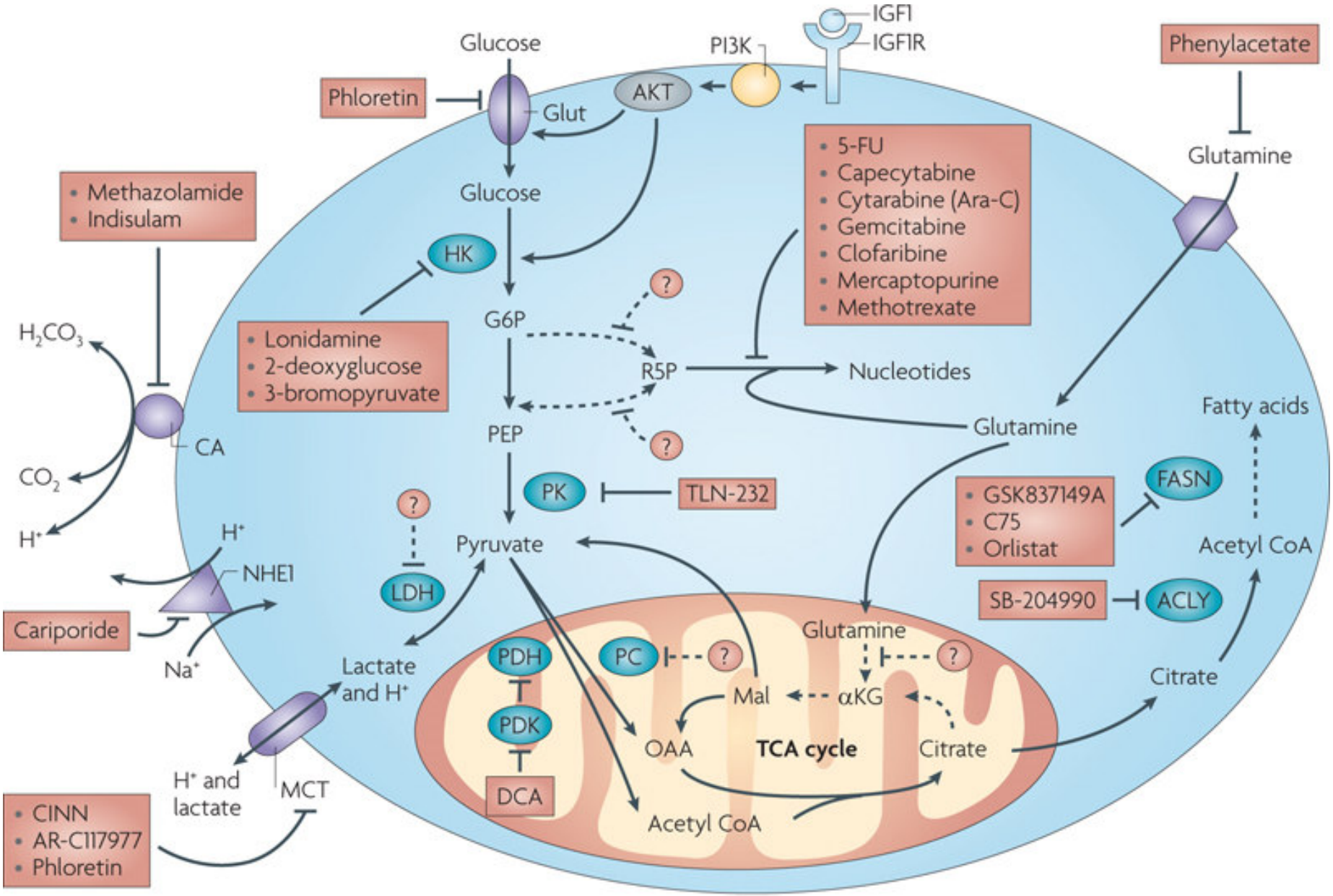
Lung

Breast

Colon

Pancreas

Pharmacological Inhibitors of tumor metabolism



Nature Reviews | Cancer

Diet and Tumor Metabolism

Ketogenic Diet:

Low-carbohydrates and High fat (medium-chain triglyceride)

Reference:

Seyfried, T. N., Kiebish, M., Mukherjee, P. & Marsh, J. Targeting energy metabolism in brain cancer with calorically restricted ketogenic diets. Epilepsia 49 (Suppl. 8), 114–116 (2008).

Zhou, W. et al. The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. Nutr. Metab. (Lond.) 4, 5 (2007).

Chu-Shore, C. J. & Thiele, E. A. Tumor growth in patients with tuberous sclerosis complex on the ketogenic diet. Brain Dev. 12 May 2009

Ser/Gly-free Diet:

Reference:

Maddocks OD, Berkers CR, et al. (2013) Serine starvation induces stress and p53-dependent metabolic remodelling in cancer cells.. Nature 493:542-6