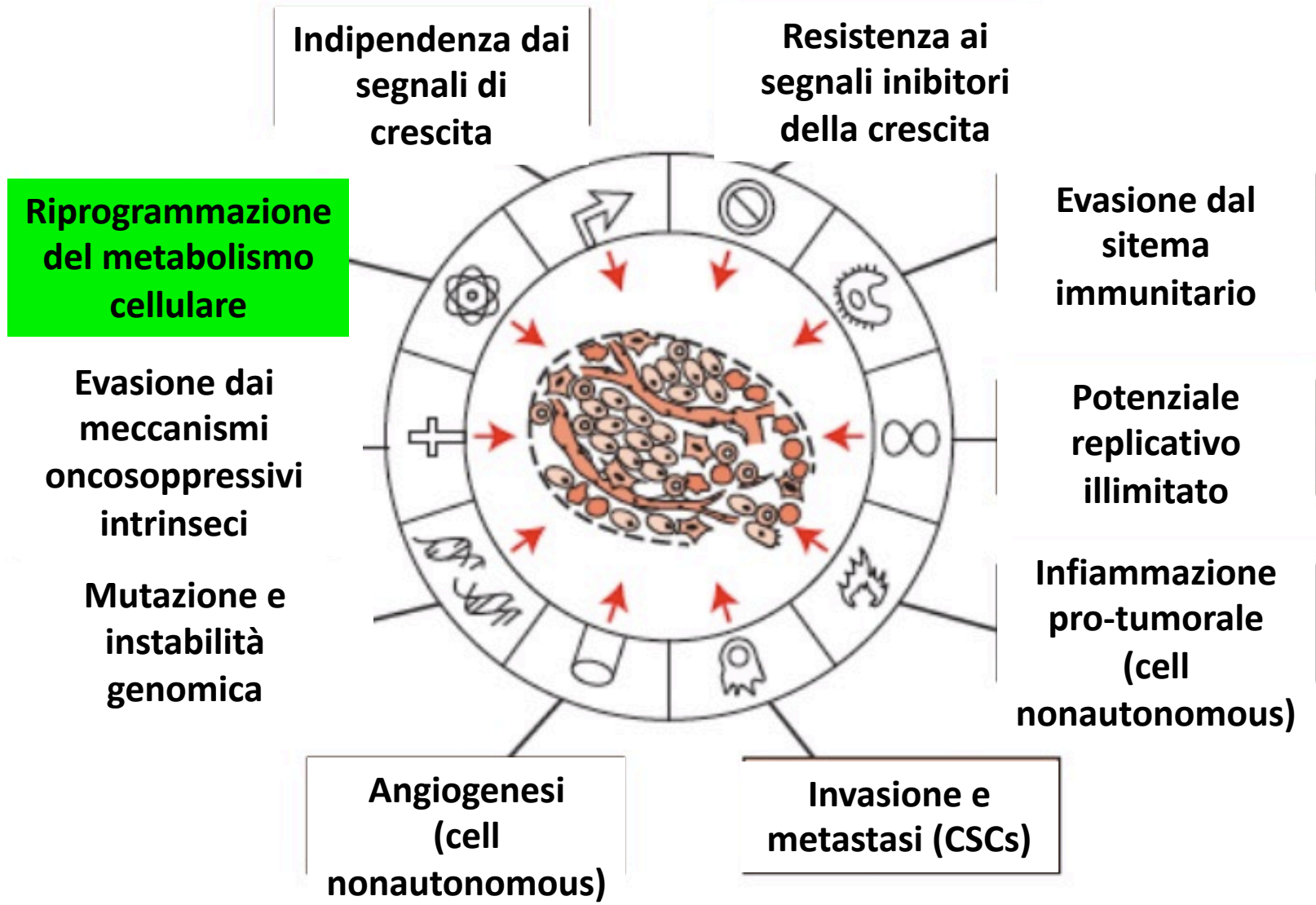


Corso di Biologia Cellulare del Cancro

AA 2020-2021

**LA RIPROGRAMMAZIONE DEL METABOLISMO
NEL CANCRO**

La riprogrammazione del metabolismo è un hallmark del cancro

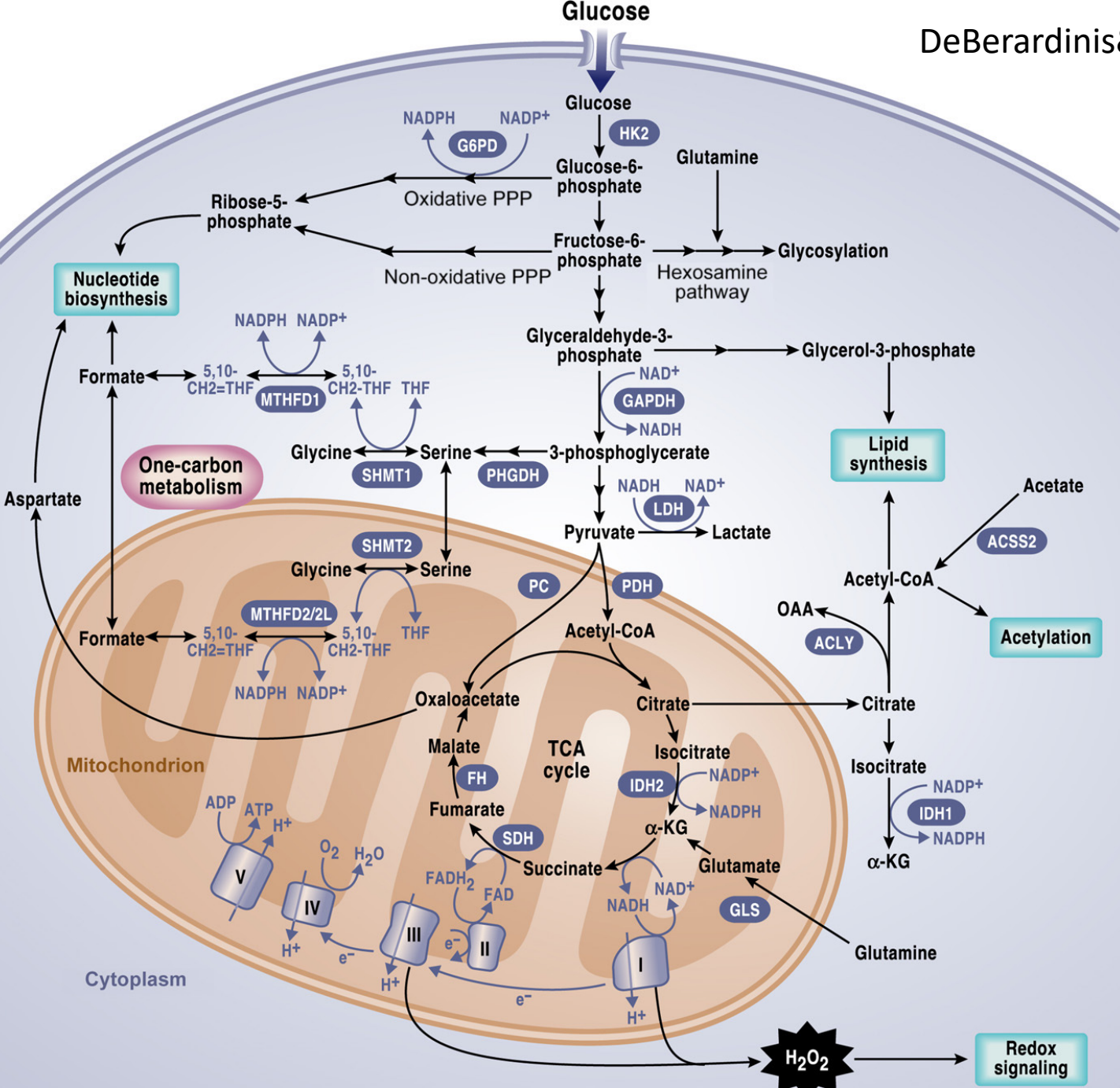


Il metabolismo cellulare

**L'insieme delle reazioni chimiche intracellulari
che convertono i nutrienti in energia (ATP)
e biomolecole (proteine, acidi nucleici e lipidi)**

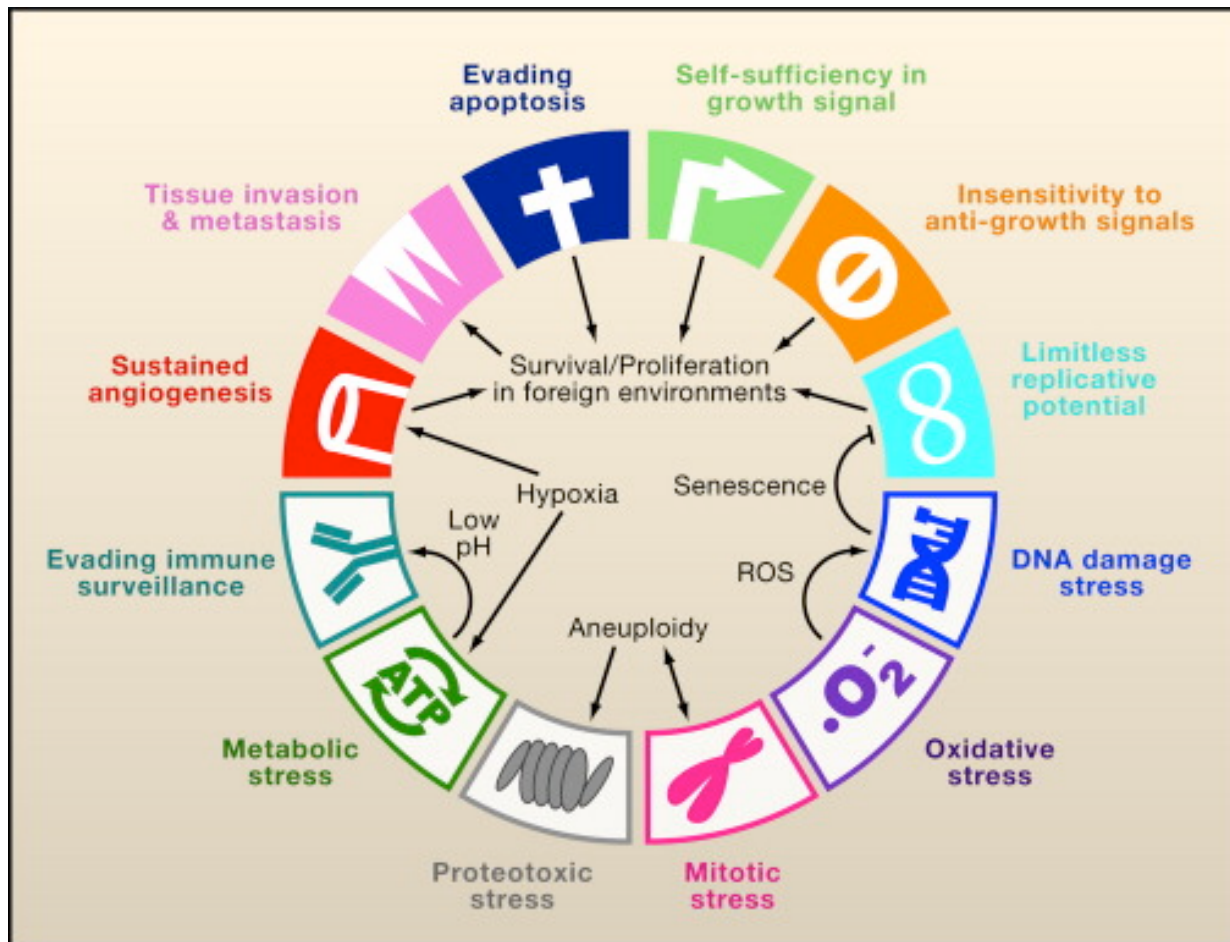
Attività bioenergetiche = generazione di ATP dai nutrienti

Attività anaboliche = generazione di biomassa dai nutrienti



H₂O₂ → **Redox signaling**

La riprogrammazione del metabolismo è un hallmark del cancro



Le cellule tumorali necessitano di riprogrammare il metabolismo per far fronte alle richieste energetiche e biosintetiche associate al loro elevato tasso di proliferazione e per adattarsi al microambiente.

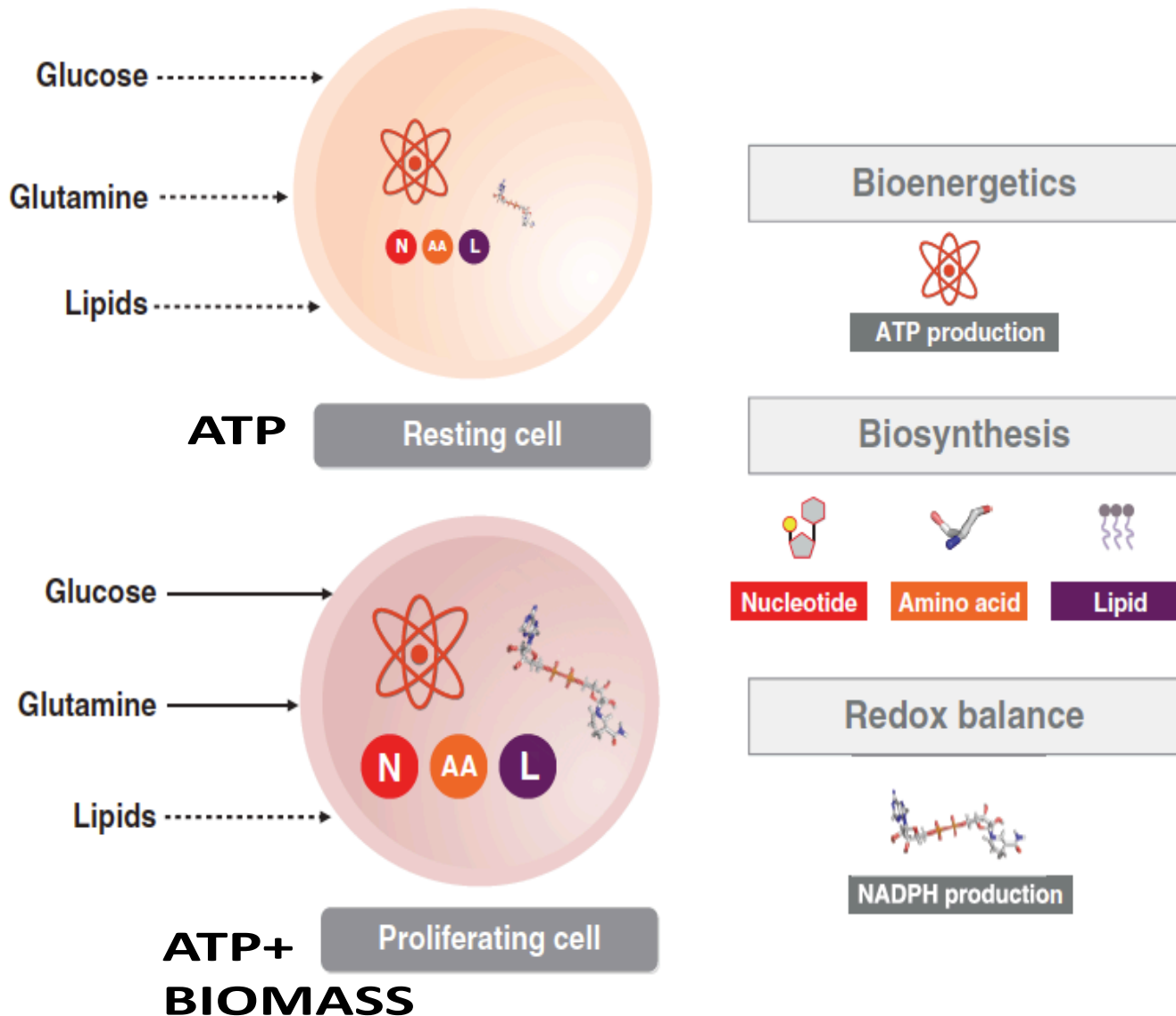
Domande:

- 1. In cosa consiste la riprogrammazione del metabolismo nel cancro?**
- 2. Quali vantaggi selettivi conferisce alle cellule tumorali?**
- 3. Come possono essere sfruttate queste conoscenze ai fini terapeutici?**

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Attività metaboliche di cellule quiescenti e proliferanti

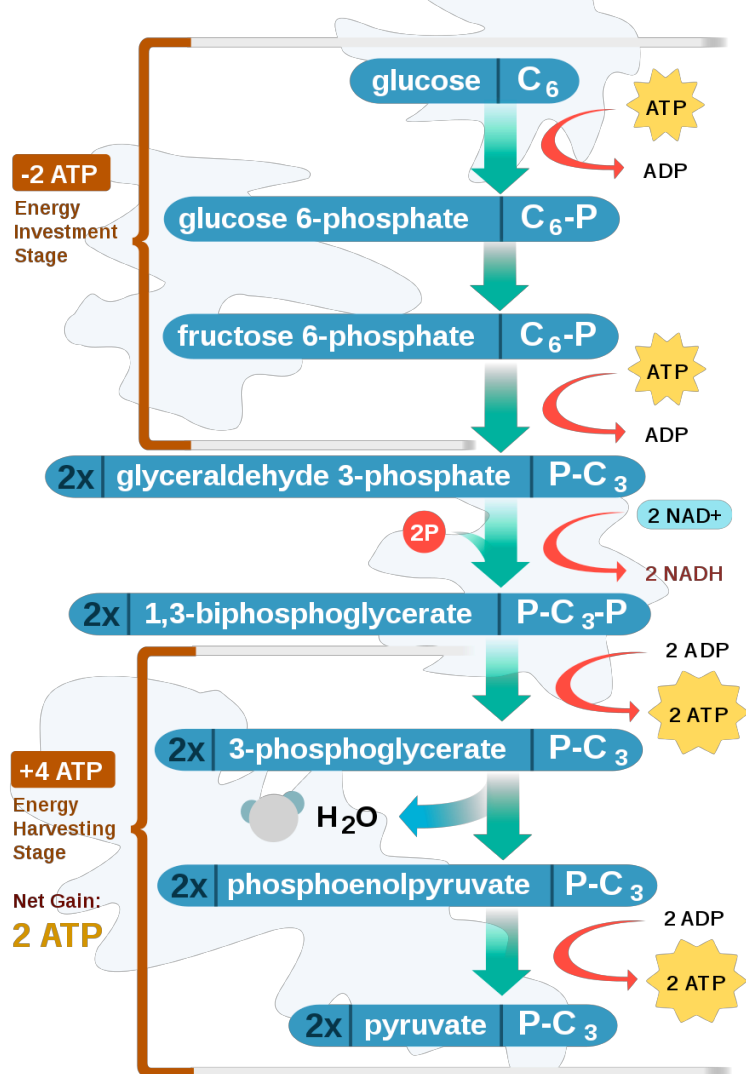


Domande:

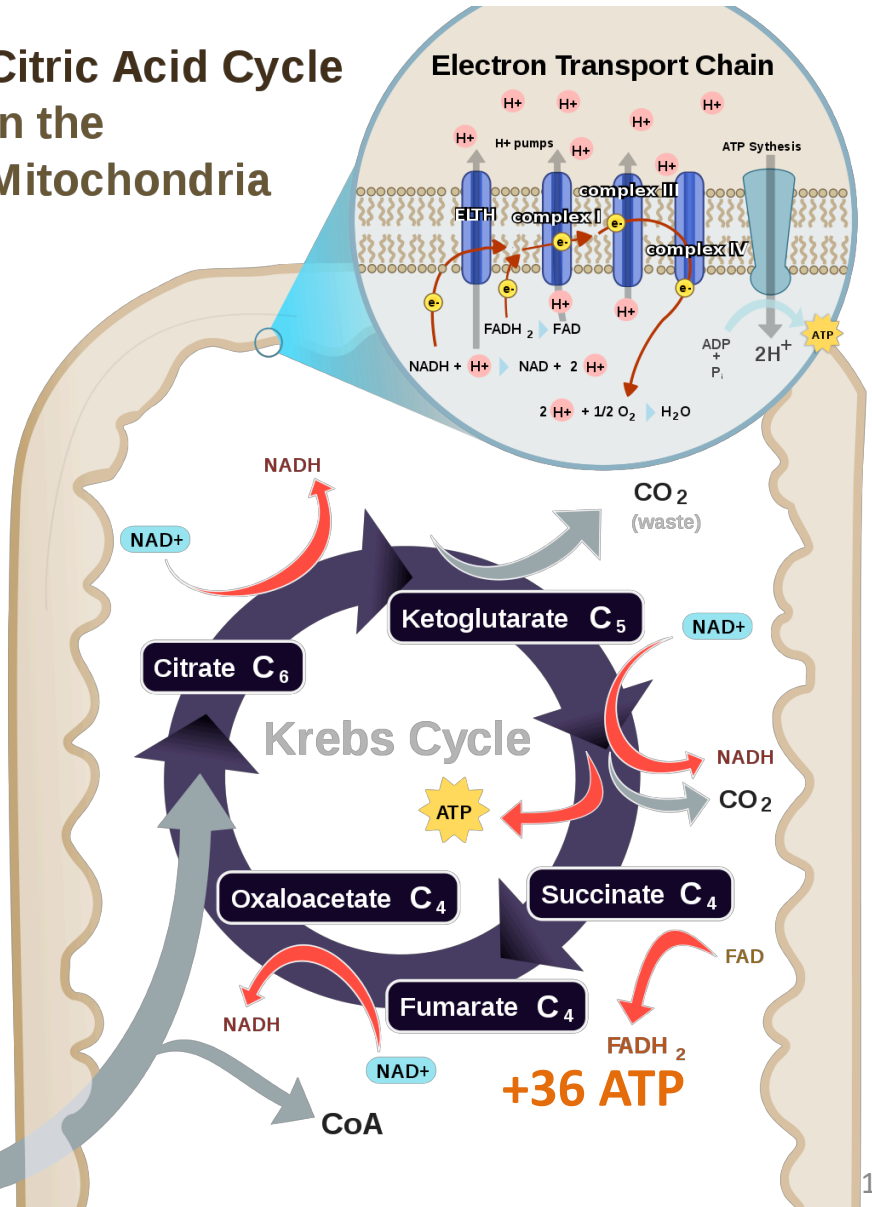
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2. Quali vantaggi selettivi conferisce alle cellule tumorali?
3. Come possono essere sfruttate queste conoscenze ai fini terapeutici?
 - a. Alterazioni dei **processi bioenergetici** (glicolisi, glutaminolisi, beta-ossidazione)
 - b. Aumento della **biosintesi** (nucleotidi, proteine, lipidi)
 - c. Alterazione del **bilancio redox**
 - d. Mutazioni di geni metabolici e produzione di **oncometaboliti**

Generazione di ATP dal glucosio: glicolisi, TCA e OxPhos

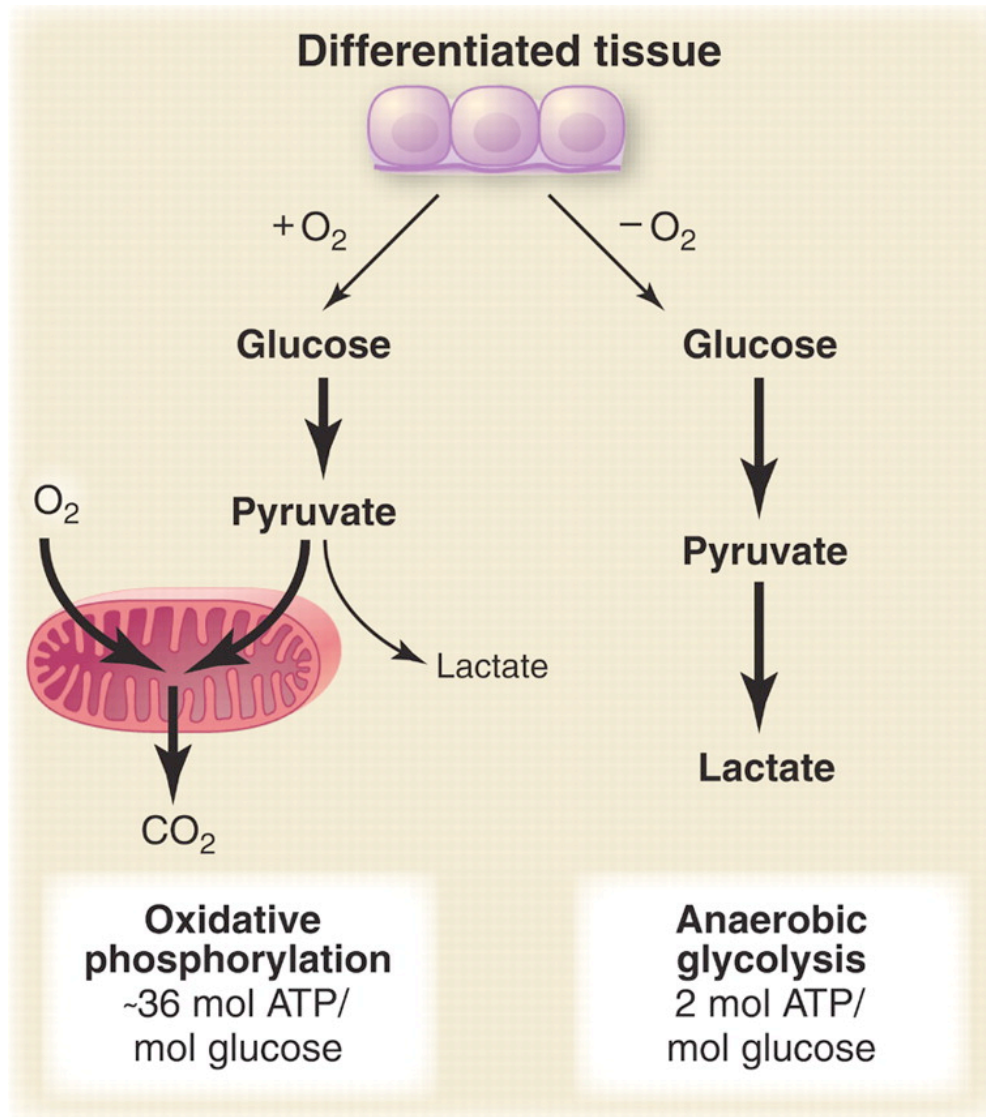
Glycolysis in the Cytoplasm



Citric Acid Cycle in the Mitochondria



L'effetto Warburg e la glicolisi aerobica



L'effetto Warburg

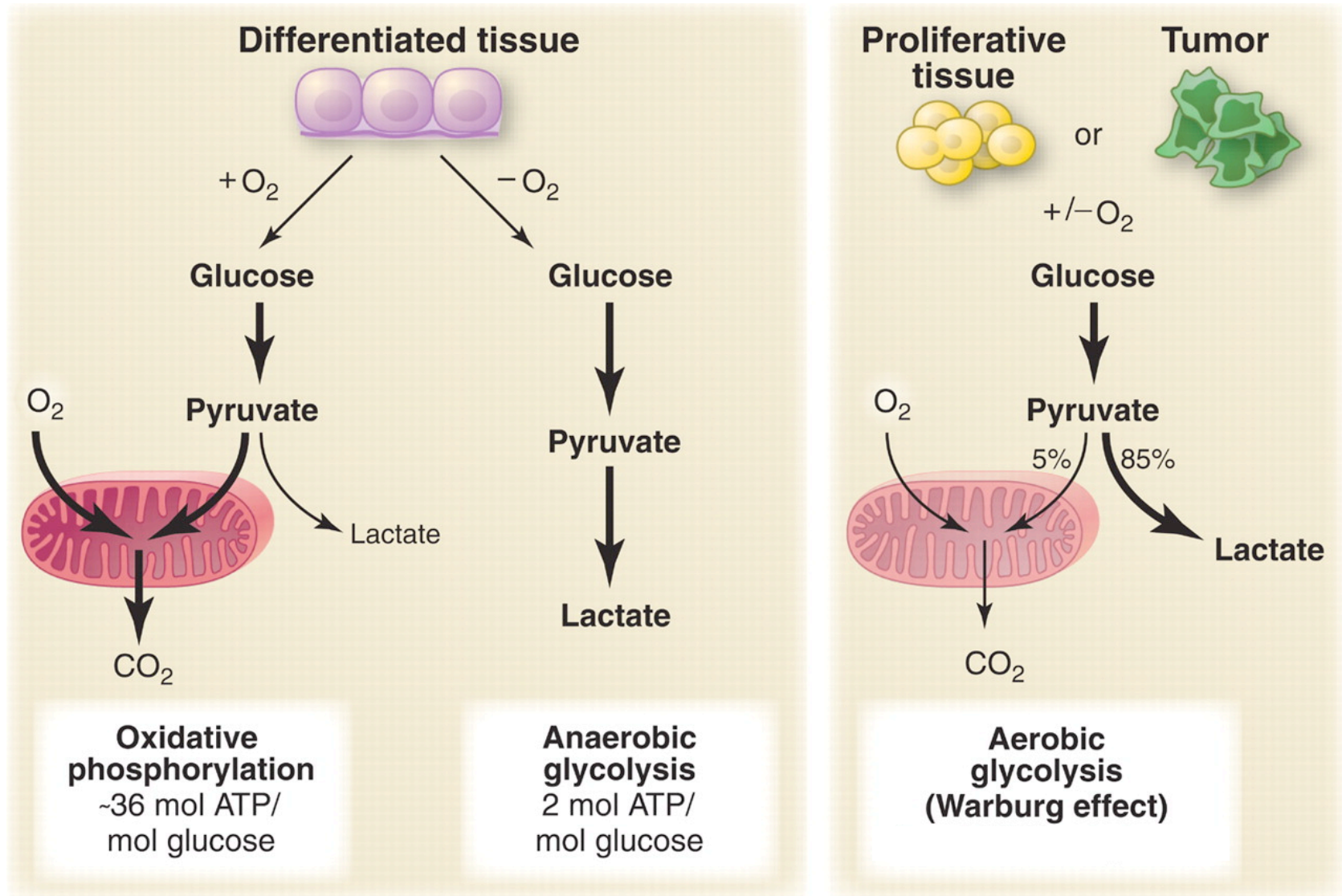
Warburg osservò che **cellule tumorali consumano grandi quantità di glucosio** e producono lattato (fermentazione del piruvato) anche in condizioni normossiche



Otto Heinrich Warburg

Nobel prize 1931

L'effetto Warburg e la glicolisi aerobica



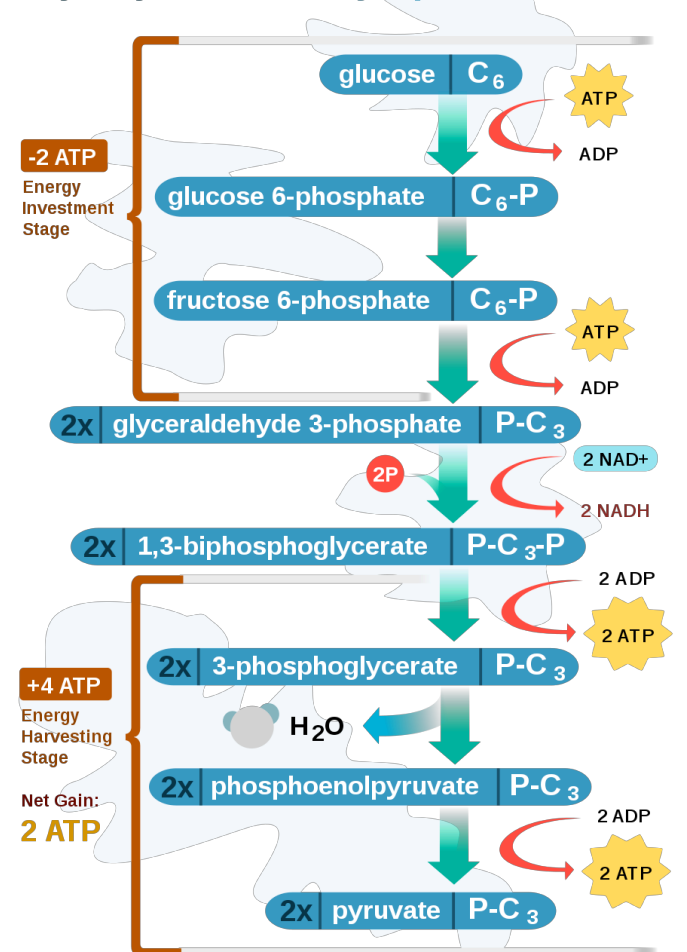
Organismi unicellulari hanno un metabolismo basato sulla fermentazione

By definition, **cancer** involves the inappropriate proliferation of cells, and the metabolic phenotype of cancer cells must represent a solution that regulates metabolic pathways to achieve a balance between **ATP production** and **biomass production**.

A major source of cellular energy and new cell mass is **glucose**. Glucose is metabolized via **glycolysis** to pyruvate, which can be oxidatively metabolized to CO₂ in the tricarboxylic acid (TCA) cycle to generate large amounts of ATP through the process of oxidative phosphorylation. Pyruvate also can be reductively metabolized to organic acids or alcohols (e.g., lactate, acetate, or ethanol), a process known as fermentation.

Glucose fermentation does not require oxygen, but it is far less efficient than the TCA cycle coupled to oxidative phosphorylation in generating ATP. **Despite decreased efficiency in ATP production, many fast-growing unicellular organisms rely primarily on glucose fermentation during proliferation regardless of oxygen availability.**

Glycolysis in the Cytoplasm



Thus, cancer cells revert to a metabolic phenotype that is characteristic of rapidly dividing cells, which suggests that aerobic glycolysis **must provide advantages** during proliferation



La glicolisi è meno efficiente del TCA/Oxphos in termini energetici, ma può generare ATP velocemente in presenza di abbondante glucosio

Glycolysis is inefficient in terms of ATP production, as it generates only two ATP molecules per molecule of glucose, whereas complete oxidation of one glucose molecule by oxidative phosphorylation can generate up to 36 ATP molecules. Despite its low efficiency in ATP yield per molecule of glucose, aerobic glycolysis can generate more ATP than oxidative phosphorylation by producing ATP at a **faster rate**. Cancer cells thus maintain ATP levels by simply “burning” more glucose.

Le cellule tumorali utilizzano i mitocondri e l'OxPhos per produrre ATP, tuttavia la maggiorparte del glucosio è utilizzata nella glicolisi aerobia

The great majority of tumor cells have the capacity to produce energy through glucose oxidation (that is, the process by which glucose-derived carbons enter the TCA cycle and are oxidized to CO₂, producing ATP through oxidative phosphorylation).

Furthermore, limiting glycolytic ATP production by inhibiting the activity of pyruvate kinase fails to prevent tumorigenesis, suggesting that the major role of glycolysis is not to supply ATP.

Moreover, mitochondrial metabolism is necessary for cancer cell proliferation and tumorigenesis. Thus, despite their high glycolytic rates, most cancer cells generate the majority of ATP through mitochondrial function.

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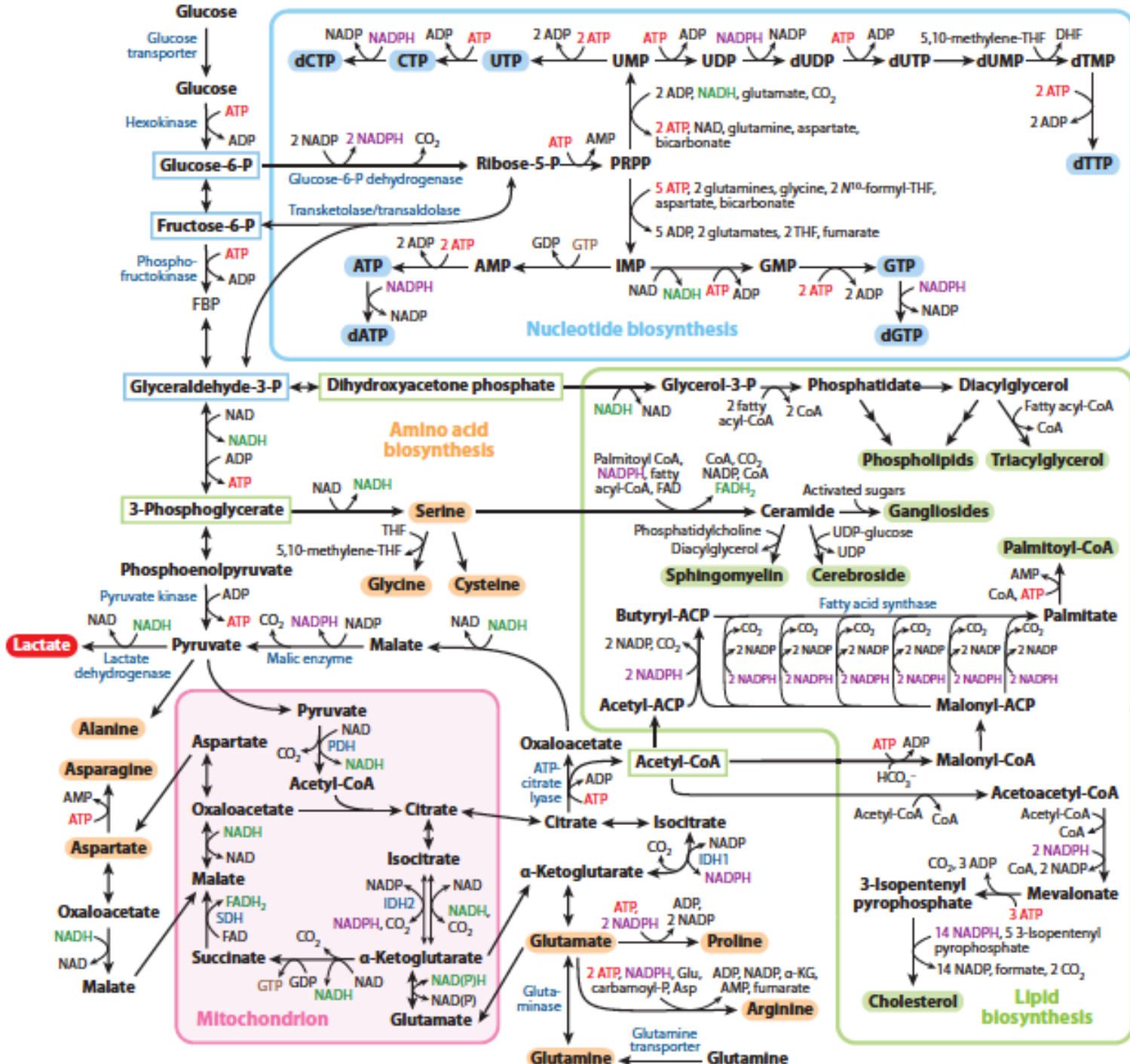
Supporta crescita e proliferazione aberrante

= meccanismi importanti fin dalle prime fasi in cui il tumore sopporta limitazioni di nutrienti

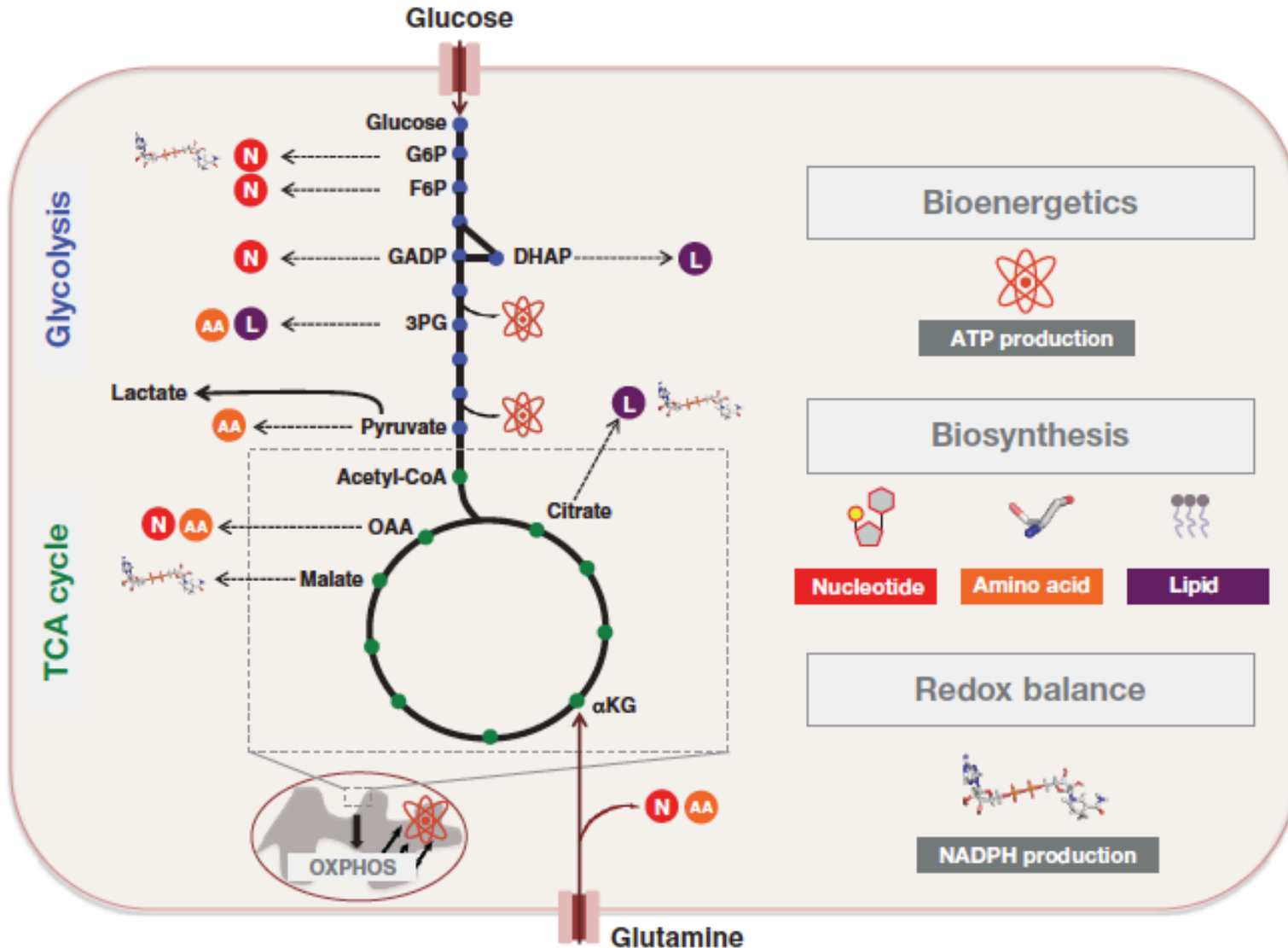
Supporta la sopravvivenza tumorale in condizioni di stress.

= meccanismi che possono assumere importanza durante evoluzione tumorale/cascata metastatica

Il programma bioenergetico delle cellule tumorali in 3 punti



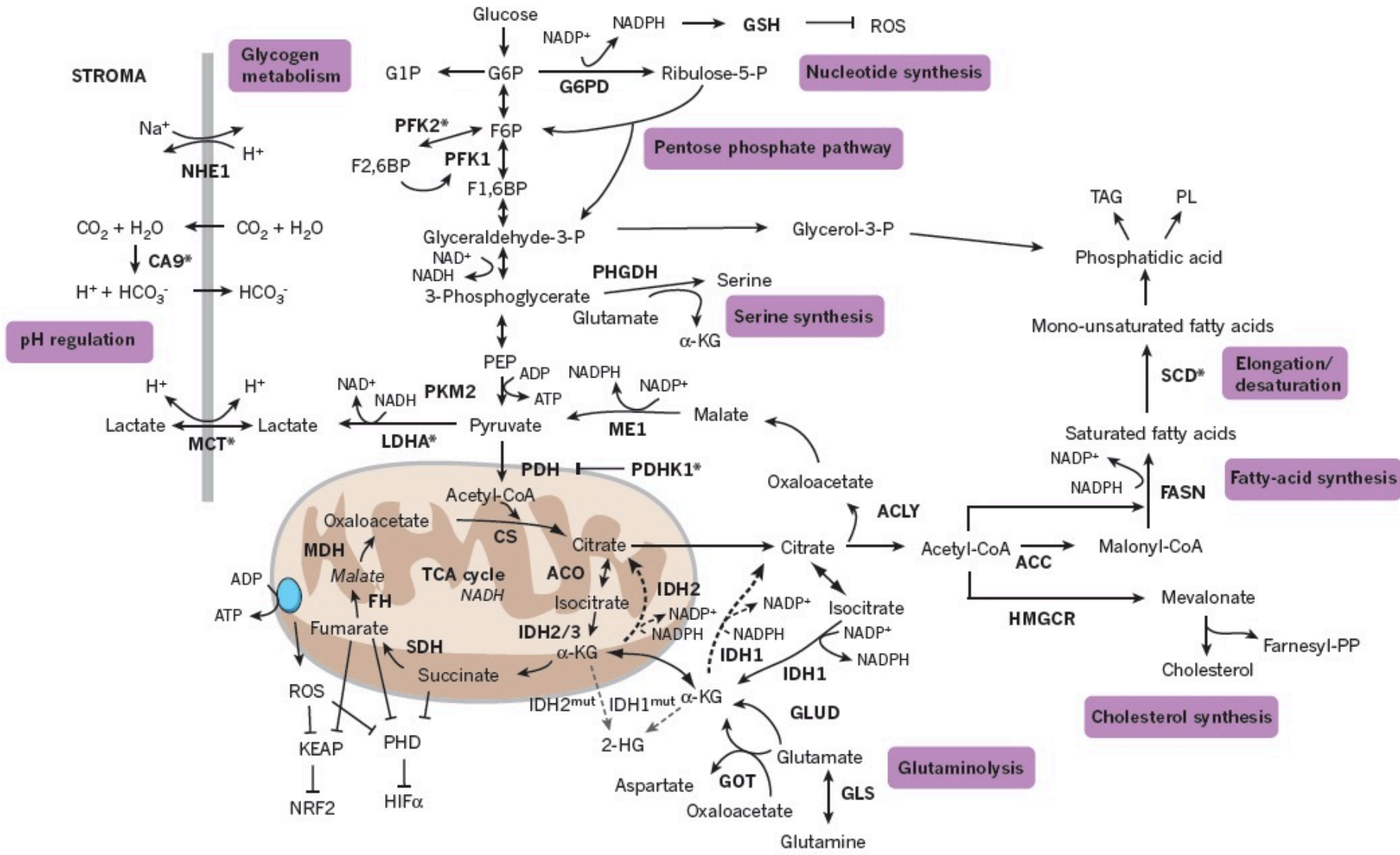
Vantaggi della glicolisi aerobia



Permette l'assimilazione del glucosio in precursori biosintetici per la generazione di biomassa

Vantaggi della glicolisi aerobia

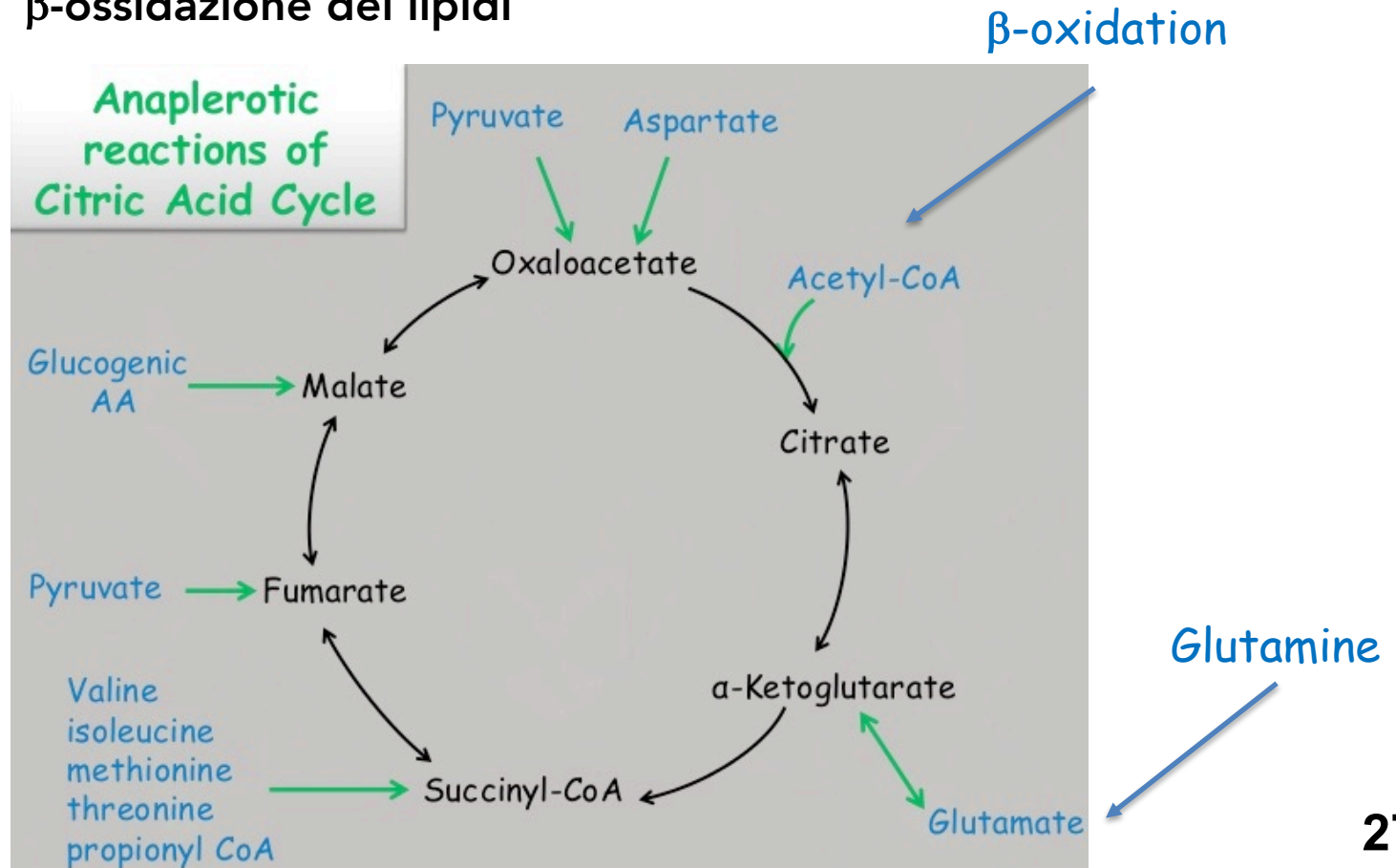
- ✓ Permette l'assimilazione del glucosio in precursori biosintetici per la generazione di biomassa e la generazione di NADPH
- ✓ Permette la generazione di ATP in condizioni ipossiche
- ✓ Risulta nella secrezione di lattato nel microambiente.
Conseguenze:
 - abbassamento del pH e protonazione di farmaci basici = chemioresistenza
 - immunosoppressione



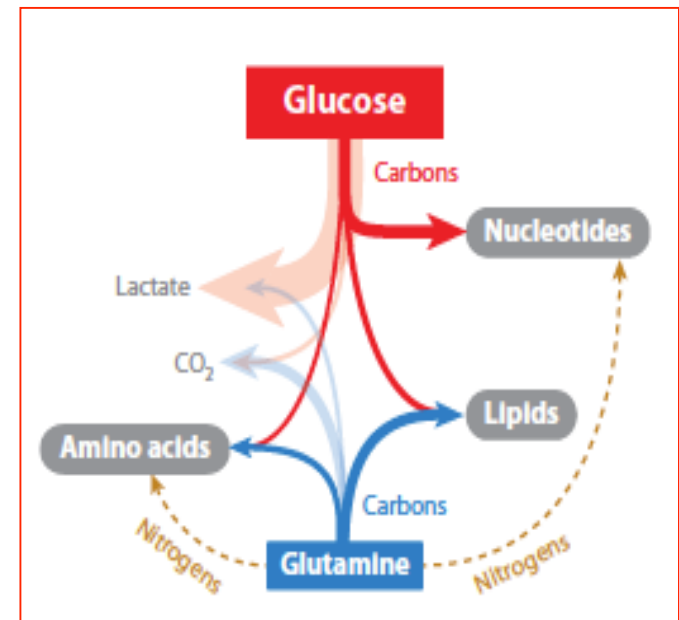
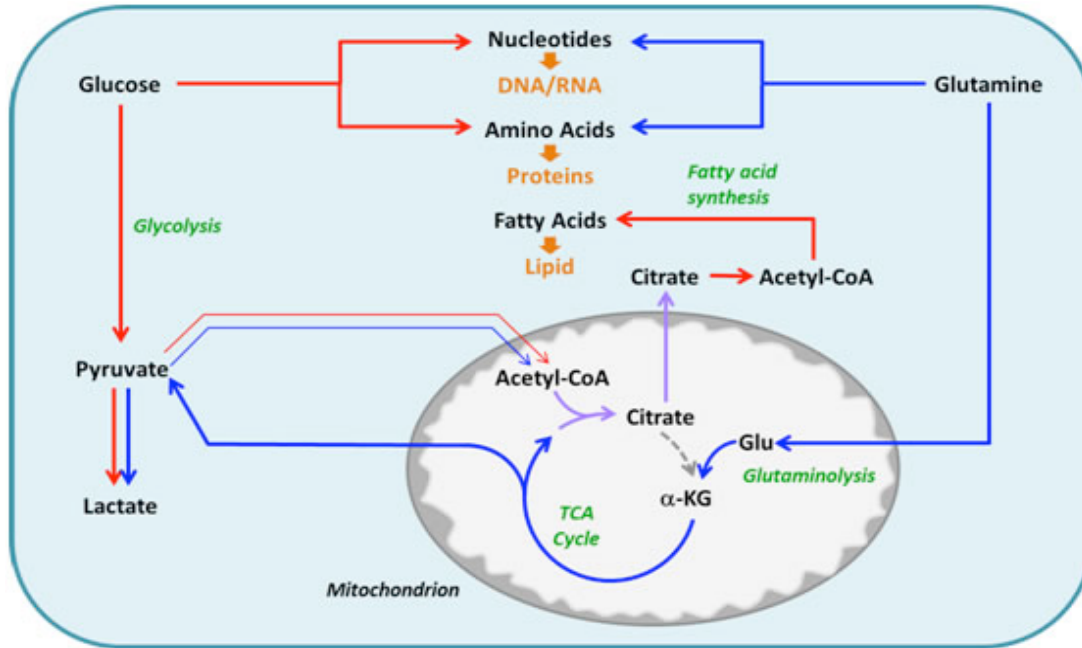
Vie anaplerotiche

Reazioni che generano intermedi del TCA

1. GLUTAMINOLISI (produce α -ketoglutarato)
2. Carbosilazione del piruvato
3. Ossidazione di AA
4. β -ossidazione dei lipidi



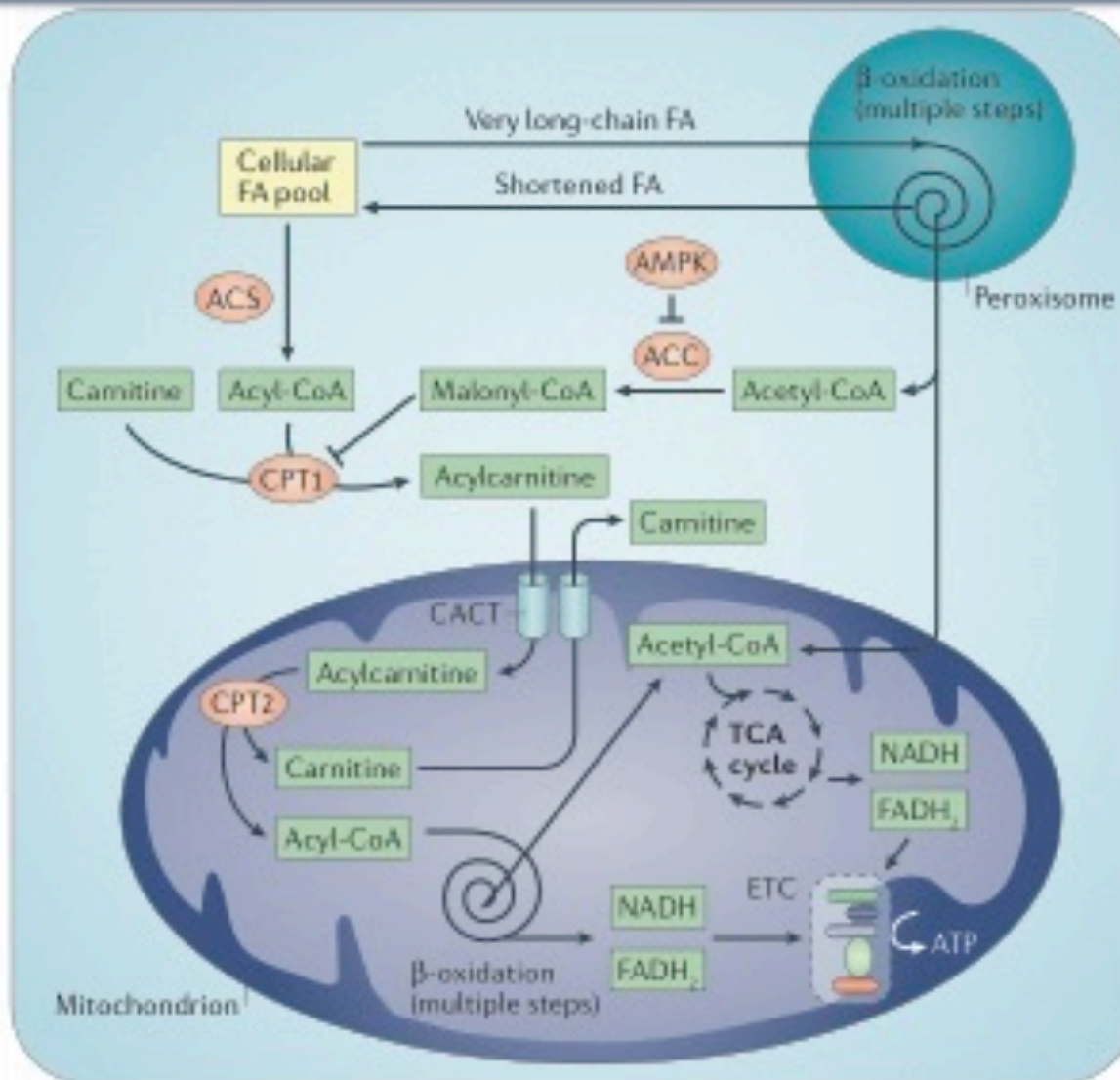
Glutamine addiction



It has long been known that cell culture medium must be supplemented with high concentrations of glutamine to support robust cell proliferation. However, it has recently been shown that transformation stimulates glutaminolysis and that many tumour cells are critically dependent on this amino acid. In proliferating cells, glutamine carbons are converted to **amino acids, TCA cycle intermediates, lipids, lactate, and CO₂**.

Glutamine is the most abundant AA in human serum. After glutamine enters the cell, glutaminase enzymes convert it to glutamate, which has several fates. Glutamate can be converted directly into GSH by the enzyme glutathione cysteine ligase (GCL). reduced GSH is one of the most abundant **antioxidants** found in mammalian cells and is vital to controlling the redox state of all subcellular compartments. Glutamate can also be converted to αKG and **enter the TCA cycle**. This process of anapleurosis supplies the carbon input required for the TCA cycle to function as a biosynthetic 'hub' and permits the production of other amino acids and fatty acids.

La beta-ossidazione degli acidi grassi produce Acetil-CoA, NADH e FADH₂



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3. Come possono essere sfruttate queste conoscenze ai fini diagnostici/terapeutici?
 - a. Valutazione diagnostica mediante imaging non invasivo dei tumori
 - b. Valutazione prognostica: prevedere l'evoluzione tumorale (metabolic signatures)
 - c. Bloccare la crescita e la progressione tumorale interferendo con processi biochimici essenziali (non-oncogene addiction)

L'effetto Warburg

Warburg osservò che **cellule tumorali consumano grandi quantità di glucosio** e producono lattato anche in condizioni normossiche

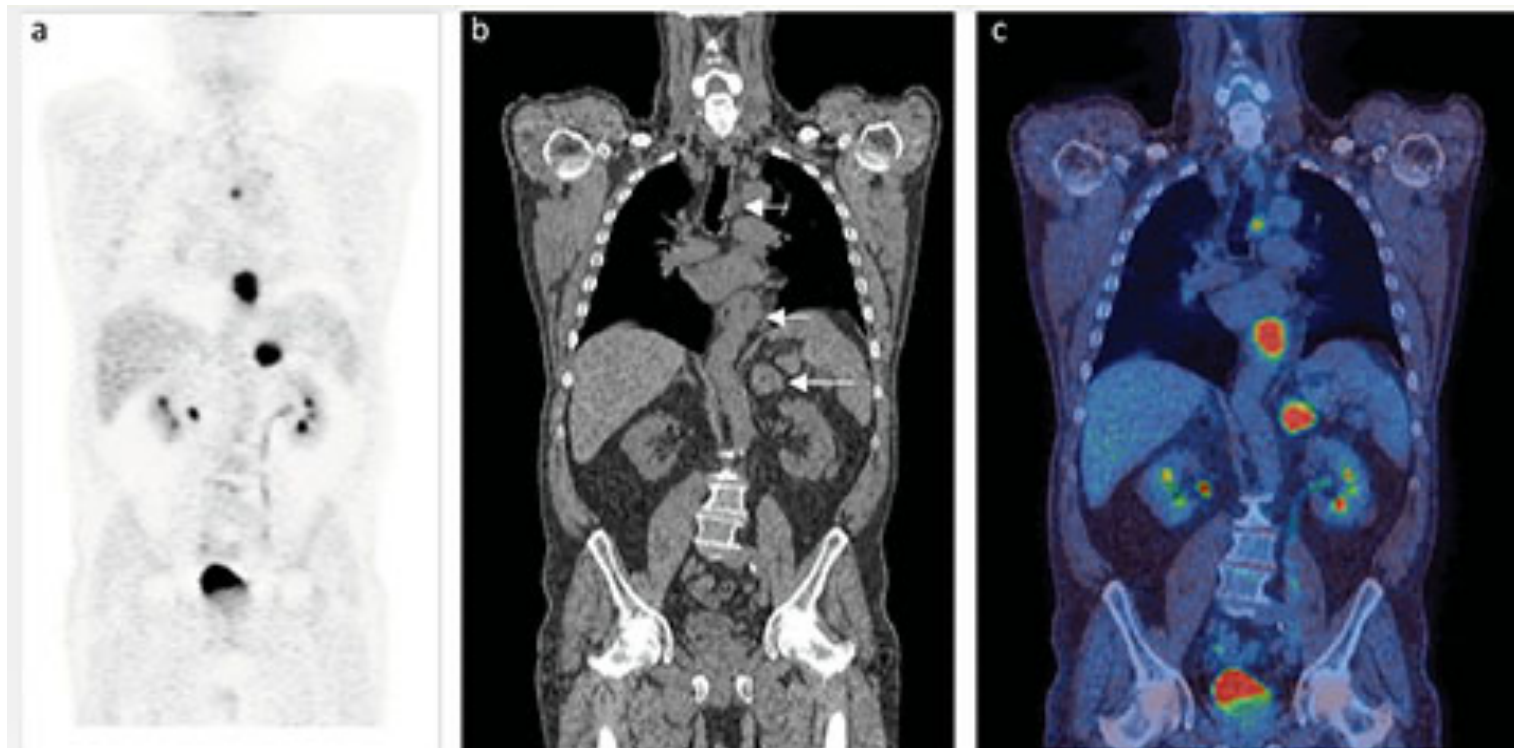


Otto Heinrich Warburg

Nobel prize 1931

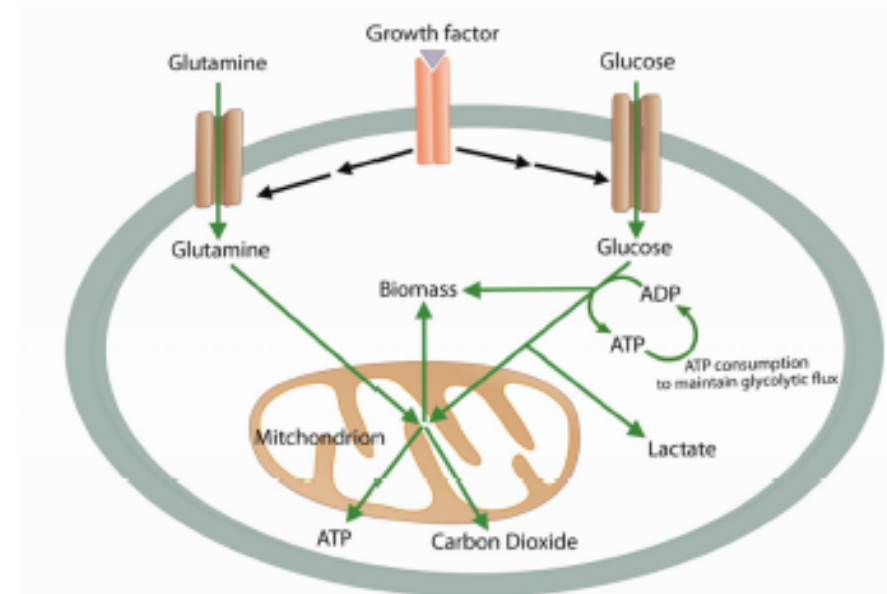
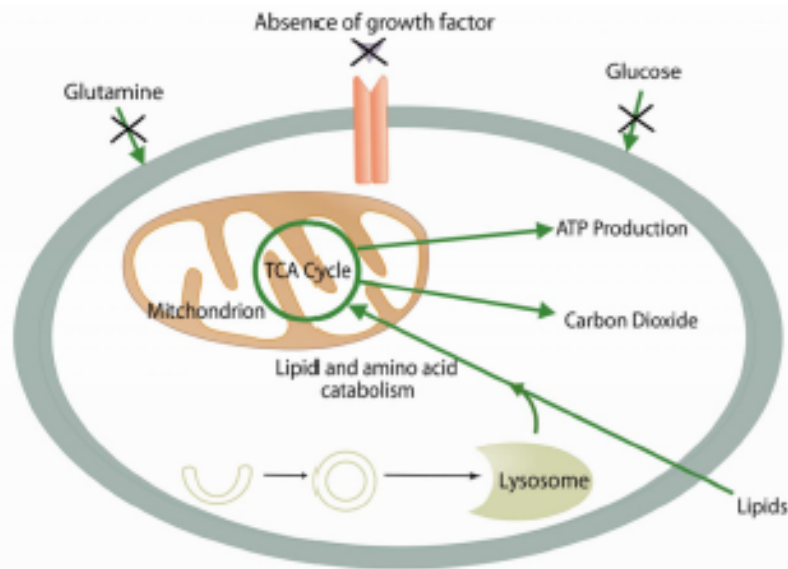
Applicazione diagnostica: PET

fluorodeoxyglucose positron emission tomography



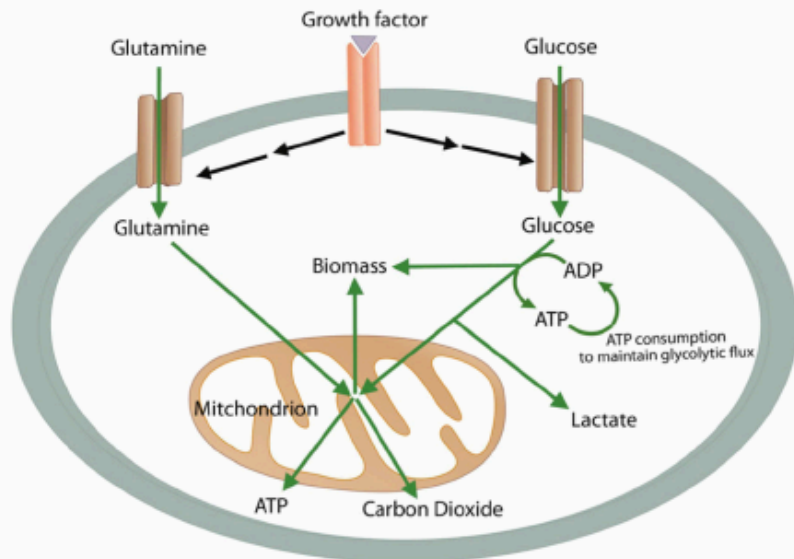
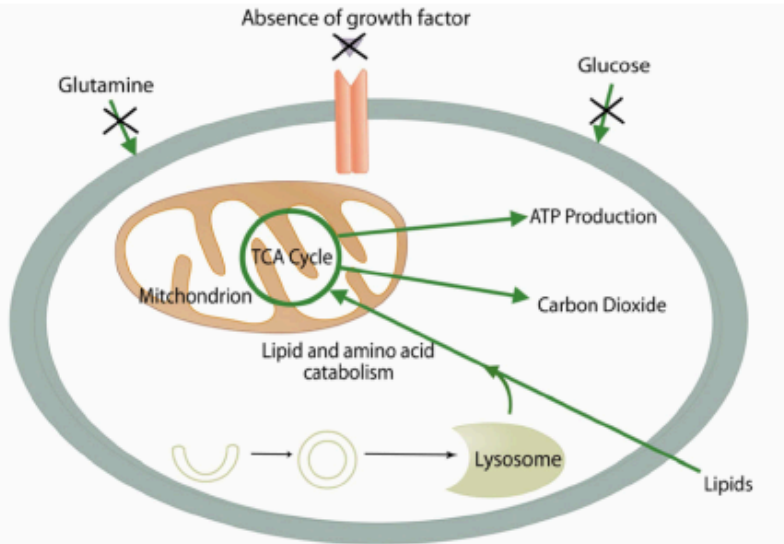
Tecnica per la produzione di bioimmagini che permette di ottenere informazioni di tipo sia morfologico che fisiologico

Coordinazione tra growth signaling e uptake di nutrienti



In multicellular organisms, most cells are exposed to a constant supply of nutrients. Survival of the organism requires control systems that prevent aberrant individual cell proliferation when nutrient availability exceeds the levels needed to support cell division. Uncontrolled proliferation is prevented because **mammalian cells do not normally take up nutrients from their environment unless stimulated to do so by growth factors**. Cancer cells overcome this growth factor dependence by acquiring genetic mutations that functionally alter receptor-initiated signaling pathways. In cancer some of these pathways (PI3K, EGFR, Ras...) are constitutively activate

Coordinazione tra growth signaling e metabolismo



Metabolism in Quiescent versus Proliferating Cells:
Both Use Mitochondria

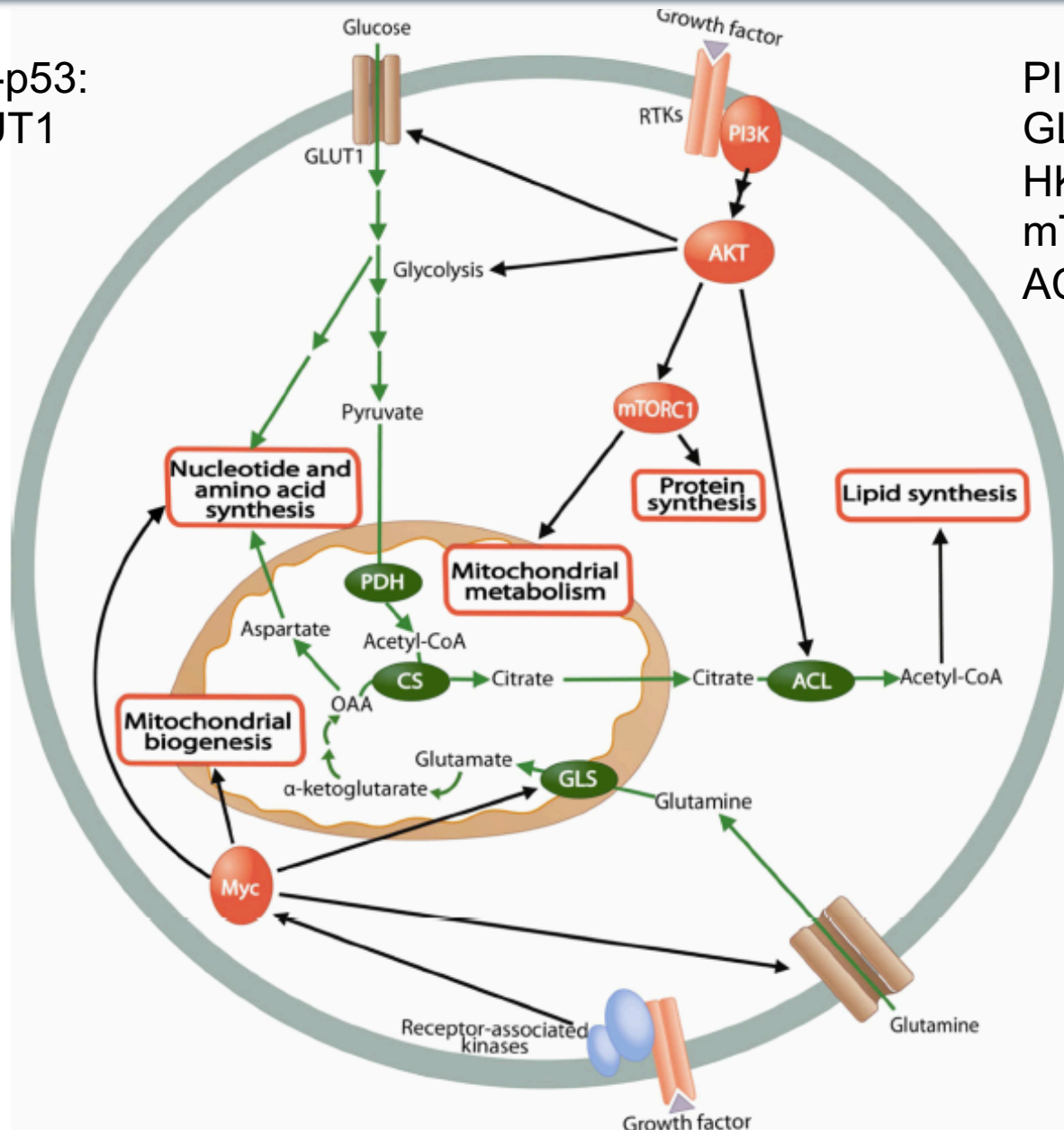
(A) In the absence of instructional growth factor signaling, cells in multicellular organisms lack the ability to take up sufficient nutrients to maintain themselves. **Neglected cells will undergo autophagy and catabolize amino acids and lipids** through the TCA cycle, assuming sufficient oxygen is available. This oxidative metabolism maximizes ATP production.

(B) Cells that receive instructional growth factor signaling are directed to **increase their uptake of nutrients, most notably glucose and glutamine**. The increased nutrient uptake can then support the anabolic requirements of cell growth: mainly lipid, protein, and nucleotide synthesis (biomass). Excess carbon is secreted as lactate. Proliferating cells may also use strategies to decrease their ATP production while increasing their ATP consumption. These strategies maintain the ADP:ATP ratio necessary to sustain glycolytic flux. Green arrows represent metabolic pathways, while black arrows represent signaling.

GF signaling inducono direttamente alterazioni del metabolismo

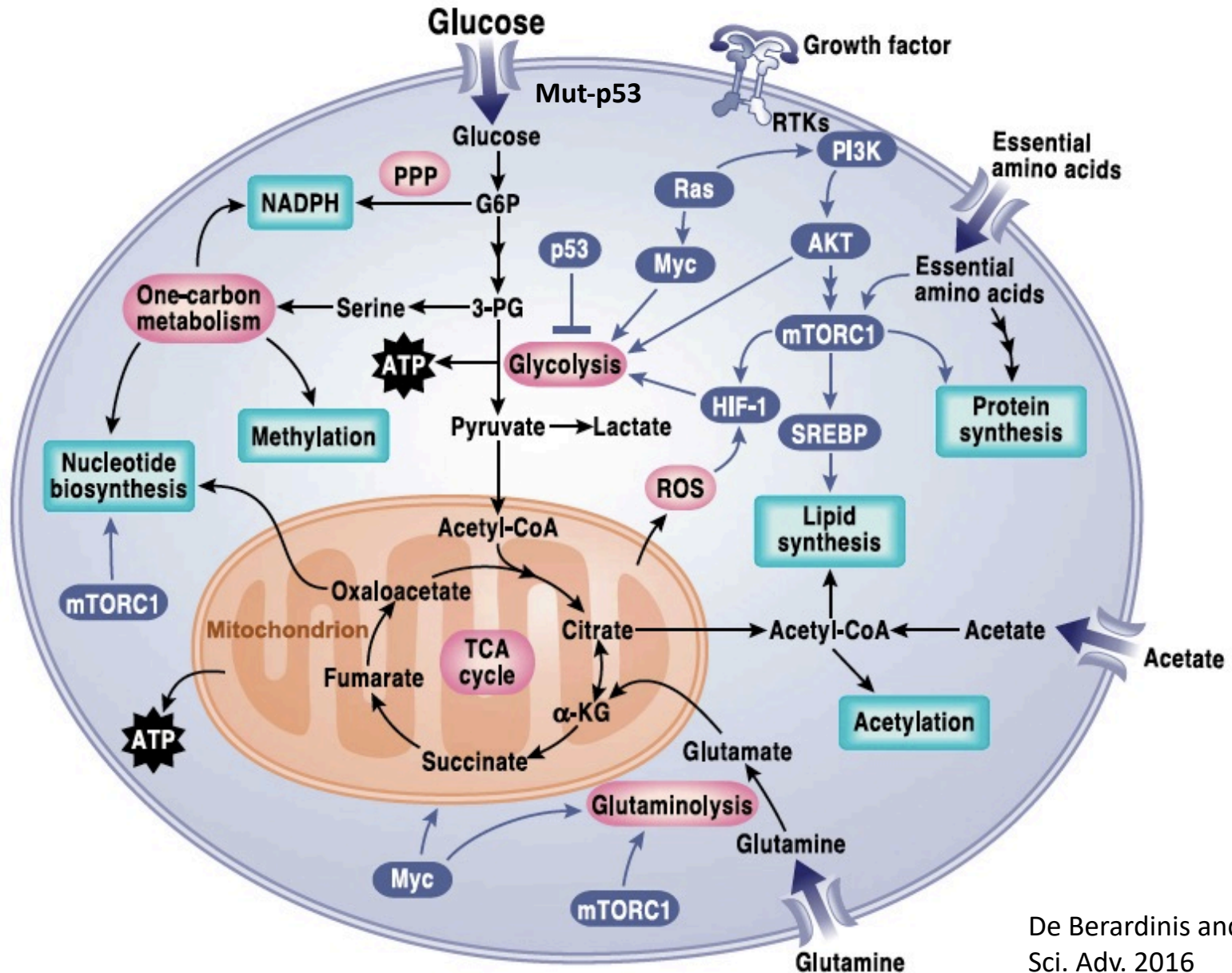
Mut-p53:
GLUT1

PI3K/AKT:
GLUT1
HK and PFK
mTOR
ACLY



Myc:
- Glutamine

Oncogeni e oncosoppressori nel controllo del metabolismo



Oncogeni e oncosoppressori controllano il metabolismo

Alterations in Classic Oncogenes Directly Reprogram Cell Metabolism to Increase Nutrient Uptake and Biosynthesis

PI3K/Akt signaling downstream of receptor tyrosine kinase (RTK) activation increases glucose uptake through the **transporter GLUT1**, and **increases flux through glycolysis**. Branches of glycolytic metabolism contribute to nucleotide and amino acid synthesis.

Akt also activates **ATP-citrate lyase (ACL)**, promoting the conversion of mitochondria-derived citrate to acetyl-CoA for lipid synthesis.

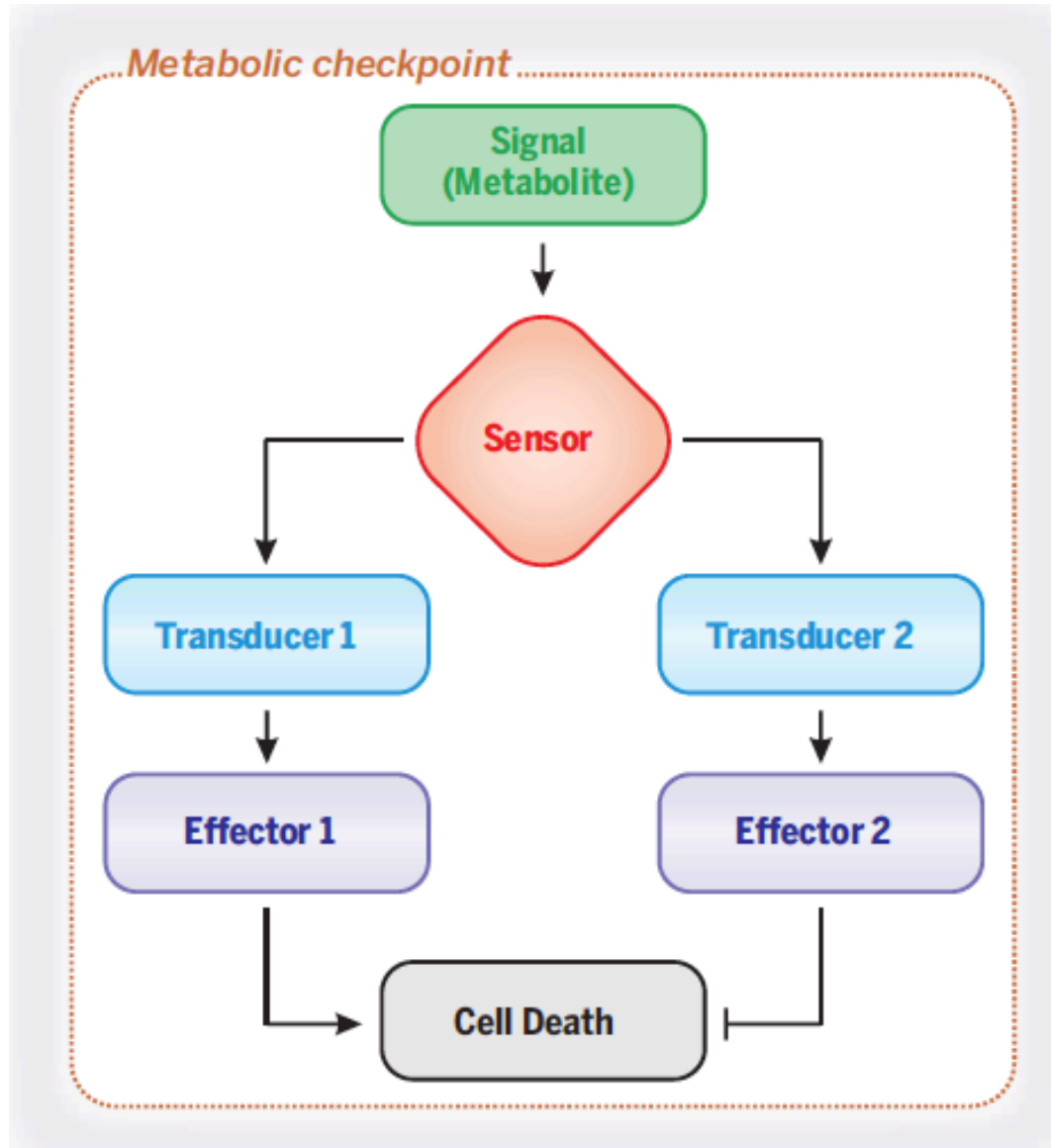
Mitochondrial citrate can be synthesized when glucose- derived acetyl-CoA, generated by pyruvate dehydrogenase (PDH), condenses with glutamine-derived oxaloacetate (OAA) via the activity of citrate synthase (CS).

mTORC1 promotes **protein synthesis and mitochondrial metabolism**.

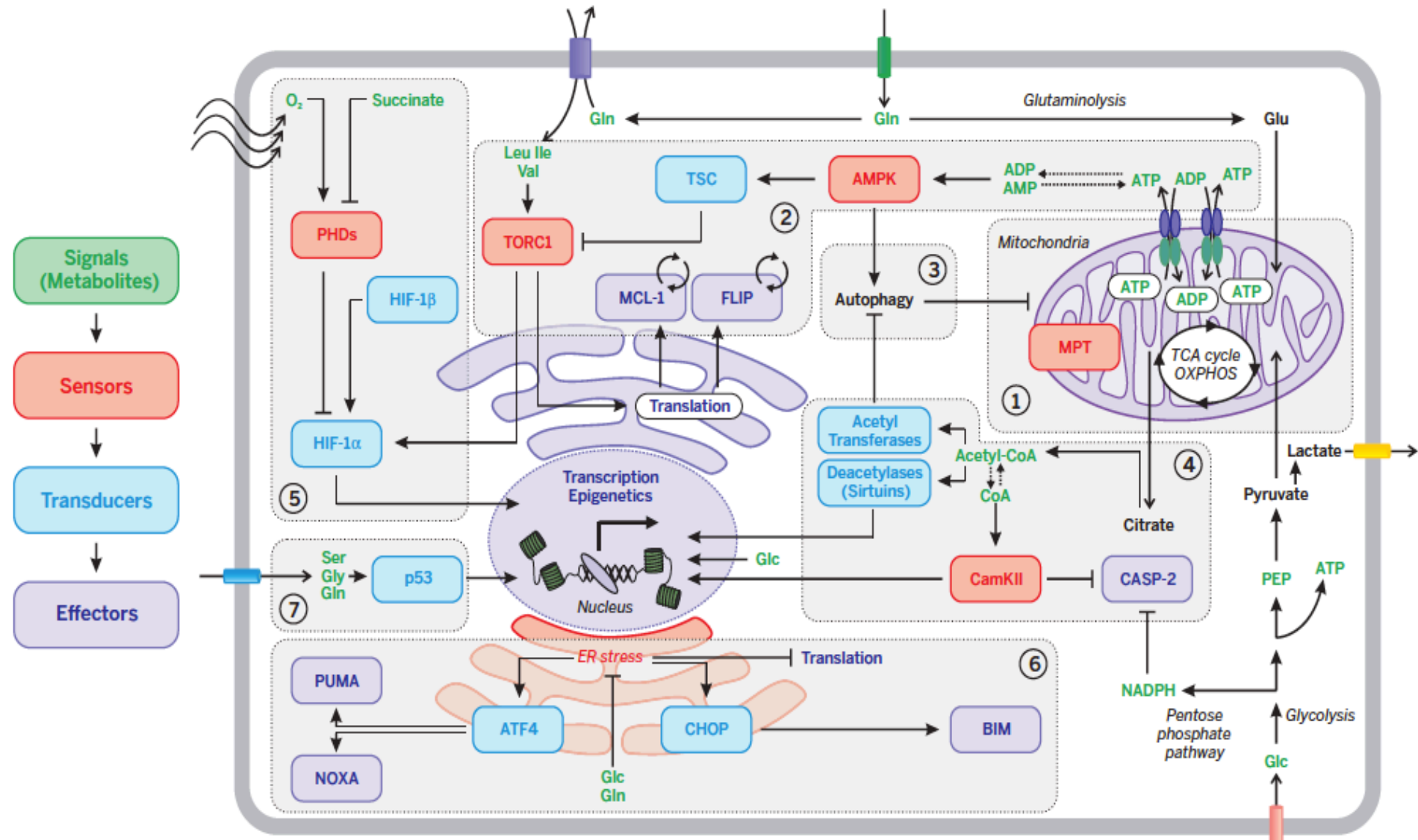
Myc increases **glutamine uptake** and the **conversion** of glutamine into a mitochondrial carbon source by promoting the expression of the enzyme **glutaminase** (GLS). Myc also promotes **mitochondrial biogenesis**. In addition, Myc promotes **nucleotide and amino acid synthesis**, both through direct transcriptional regulation and through increasing the synthesis of mitochondrial metabolite precursors.

I checkpoint del metabolismo

convertono perturbazioni nei livelli di metaboliti in stimoli di sopravvivenza o morte



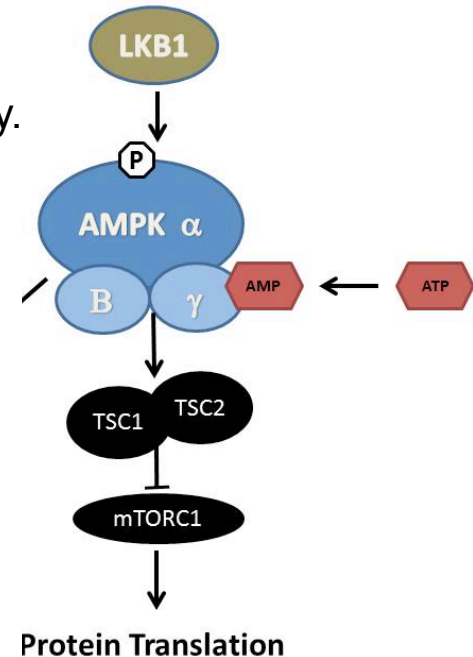
I checkpoint del metabolismo



ATP checkpoint: AMPK e TORC

Two evolutionary conserved signaling molecules, AMPK and mTOR, are central players in the coordinated sensing of cellular metabolic state and dictation of cell fate. AMPK is a kinase whose activation requires both the binding of AMP-ADP and phosphorylation by the upstream signaling kinases LKB1. Whereas AMP serves as a potent **allosteric activator of AMPK**, both AMP and ADP promote and stabilize the activating phosphorylation of AMPK. Given its relatively high affinity for AMP-ADP, AMPK is generally considered a sensor of the intracellular concentration of AMP and ADP, which is indicative of bioenergetic status.

Extracellular growth factors and nutrients converge on the regulation of mTOR, a component of two functional multicomponent protein complexes, TORC1 and TORC2 involved in inducing protein translation. Activation of the protein-kinase activity of TORC1 requires the derepression of TSC1-TSC2, a inhibitory component of the complex. When ATP is limiting (and in the presence of high concentrations of AMP and ADP), AMPK directly phosphorylates essential components of TORC1, such as TSC2. This generally leads to inhibition of TORC1 activity, as a central signal transducer that functions in metabolic checkpoints by ATP-sensing pathways to determine cell fate.



p53 risponde alla deprivazione di nutrienti

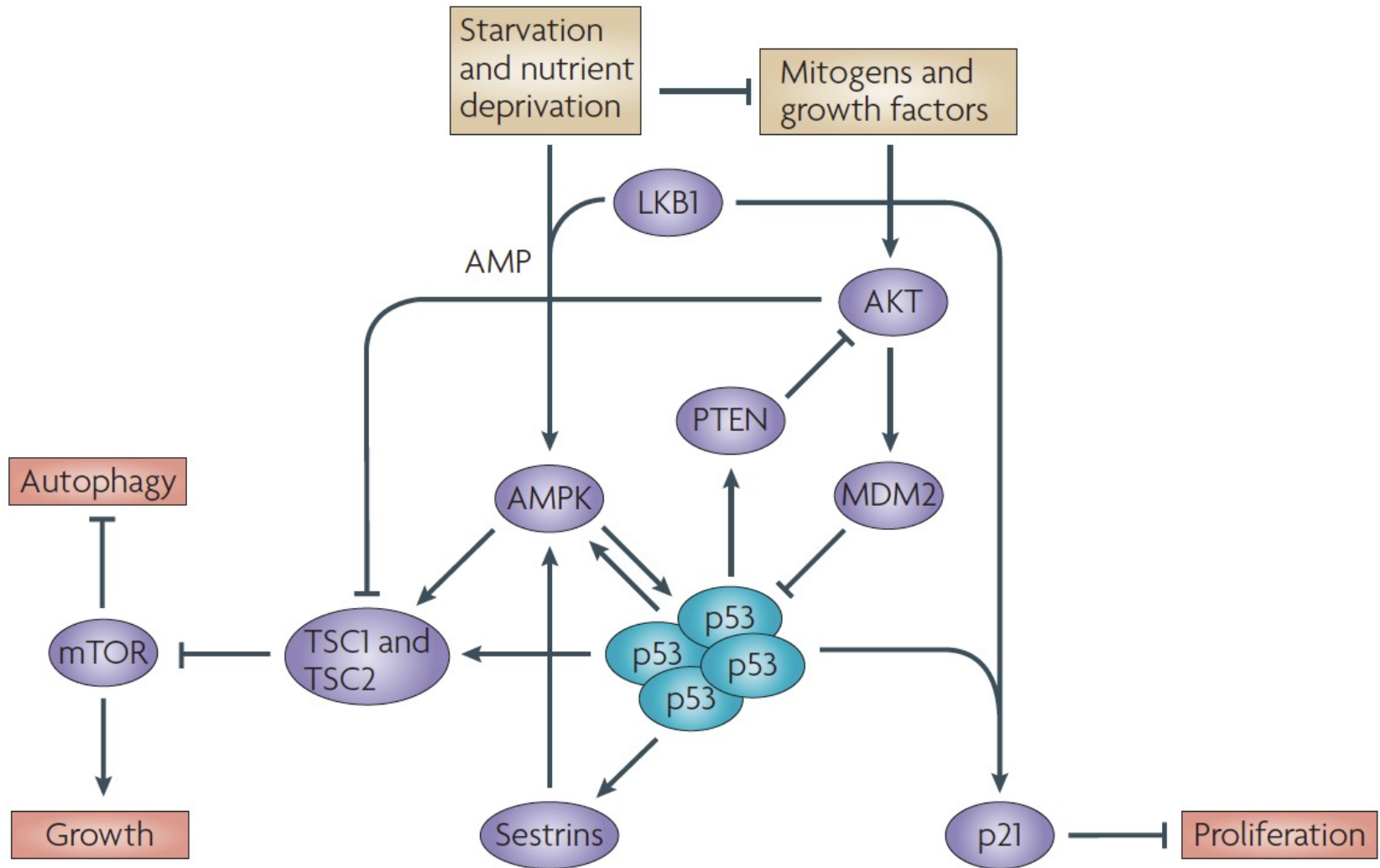
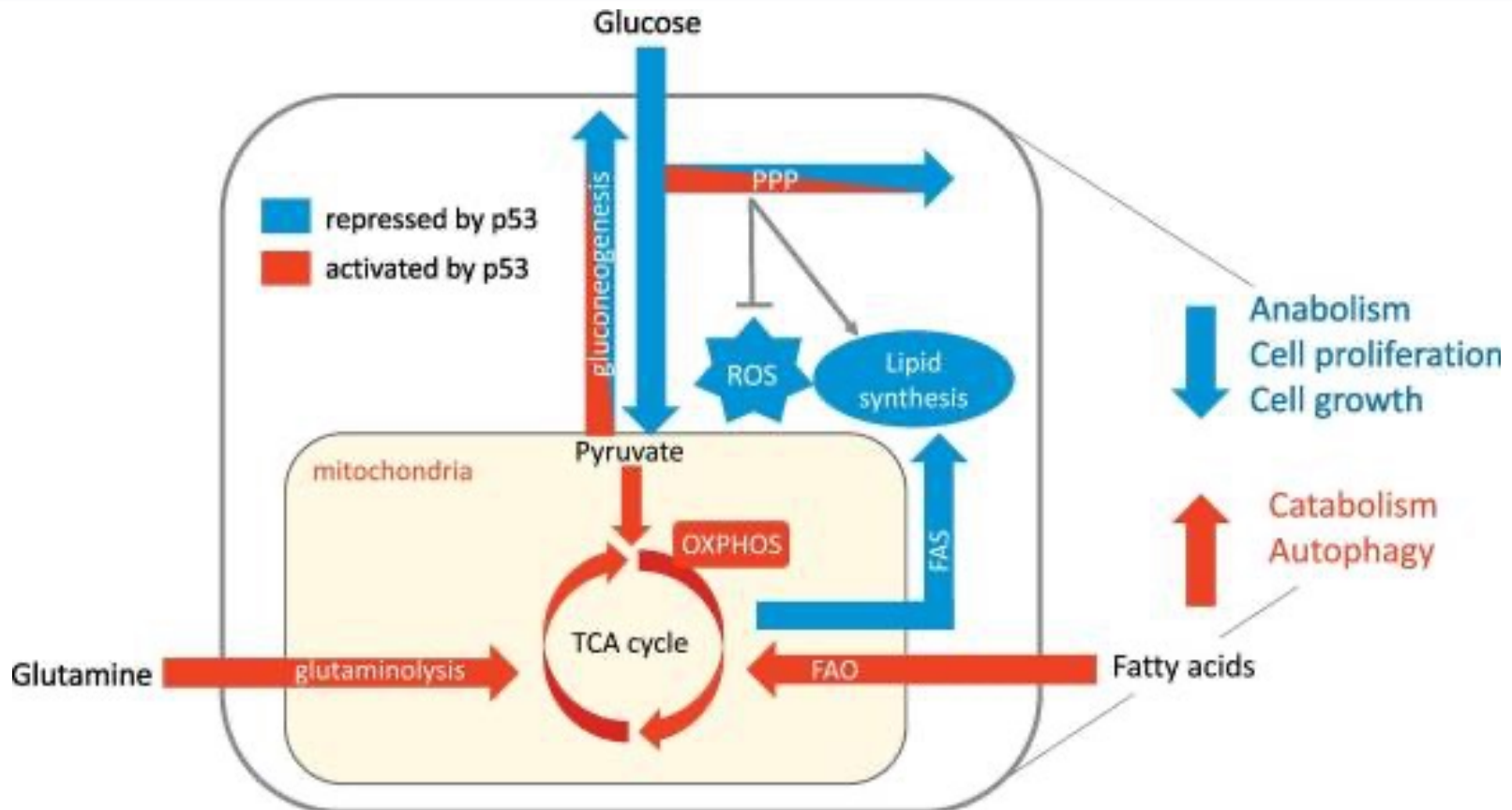


Figure 1 | **Nutrient deprivation signals to p53.** The activation of p53 in response to a

Regolazione del metabolismo da p53



Under conditions of metabolic stress, p53 induces catabolic pathways and autophagy to maintain energy production and cell survival while inhibiting anabolic pathways, cell growth and proliferation. p53 **inhibits glycolysis and promotes mitochondrial respiration, while also limiting lipid biosynthesis and promoting lipolysis.** Note that while oxidative phosphorylation is a major source of mitochondrial ROS, maintenance of mitochondrial health by p53 is likely to **limit oxidative stress** through this source. In addition to **PPP activation, p53 controls ROS through numerous other mechanisms.**