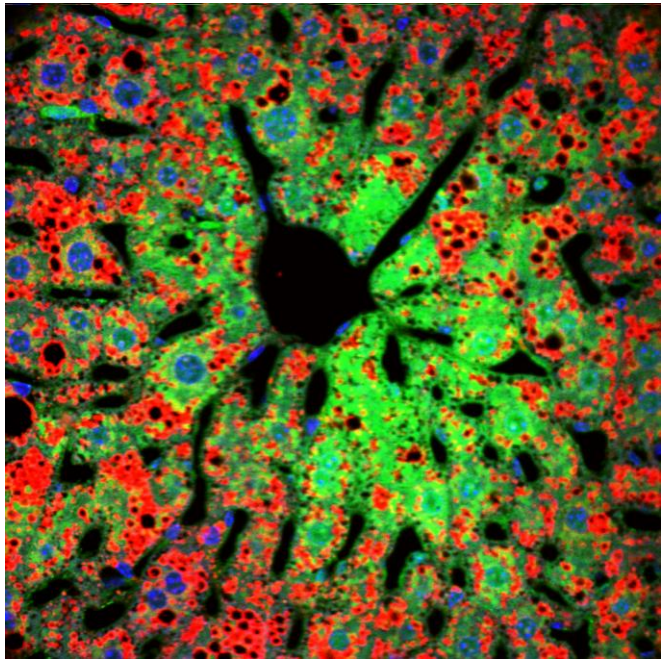


Oxygen sensing, signaling, and metabolism

Dr. Werner Kovacs

DBIOL
Department of Biology
Departement Biologie

IMHS
Institute of Molecular
Health Sciences

Oxygen sensing and signaling

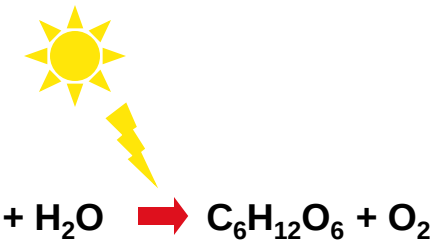
- What is the sense of sensing oxygen?
- What are the mechanisms?
- What is the nature of adaptive responses?
- How do oxygen levels affect metabolism?
- What role does the oxygen signaling pathway play in disease and which diseases?

Life with (and without) oxygen

Evolution of organisms that transduced solar energy into chemical energy of carbon bonds

Establishment of a symbiotic relationship between single-celled organisms and internalized primitive cells (mitochondria)

bc O2 levels are so high, all aerobic organisms became dependent on it.



$CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$

ATP

$C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O$

Earth is anaerob

Earth becomes oxygenated

Aerobic respiration; permissive for evolution of higher organisms

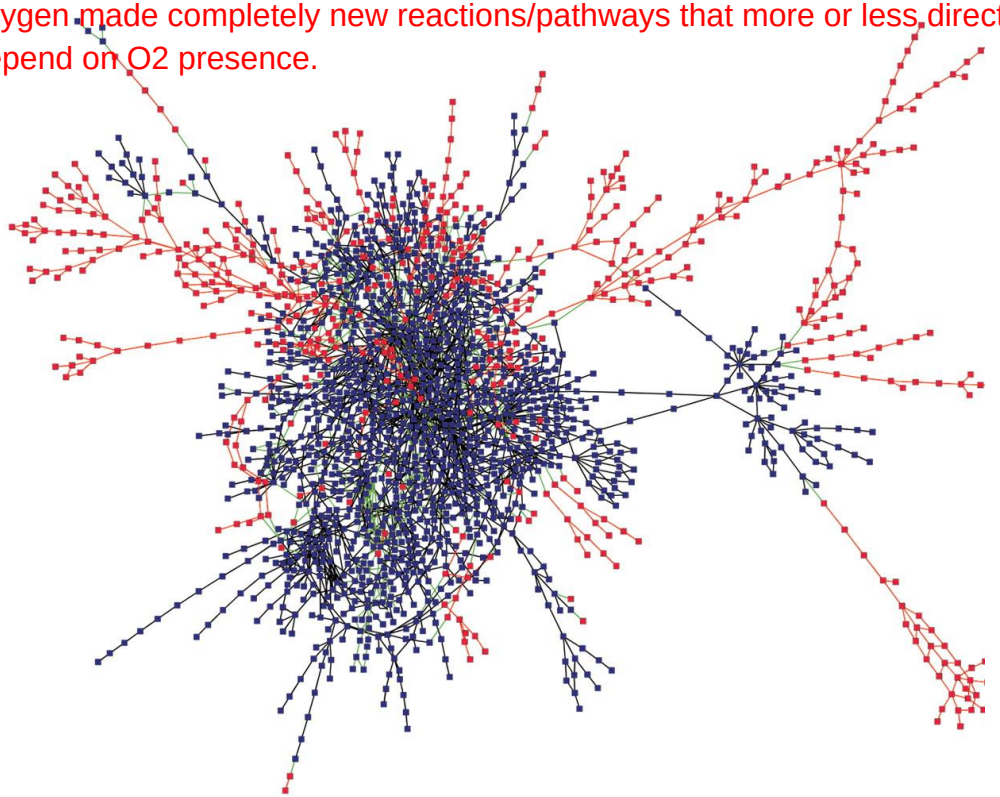
O2 life began here

4.5 **2.5** **1.5** **0.5** **0.0**

age of earth (billions years ago)

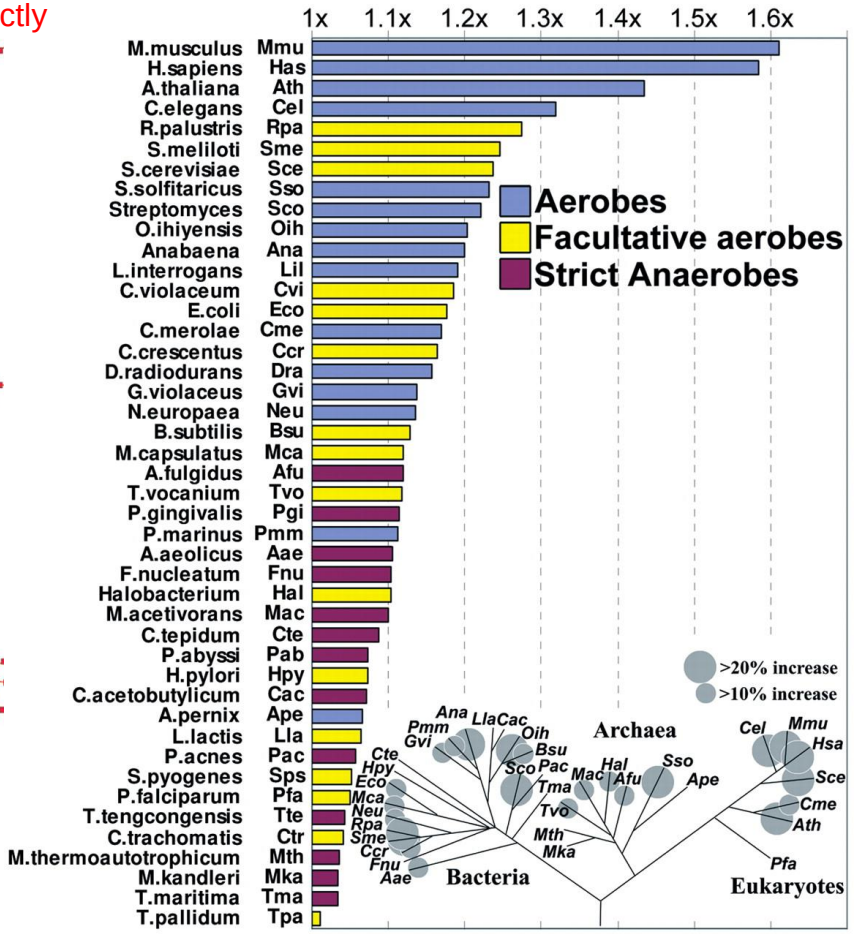
The effect of oxygen on KEGG's metabolic "backbone" and the total number of reactions catalyzed by individual genomes

on this network: oxic reactions evolved from anoxic reactions.
the core is very similiar, it's not easy to see bc it is so dense.
oxygen made completely new reactions/pathways that more or less directly depend on O2 presence.



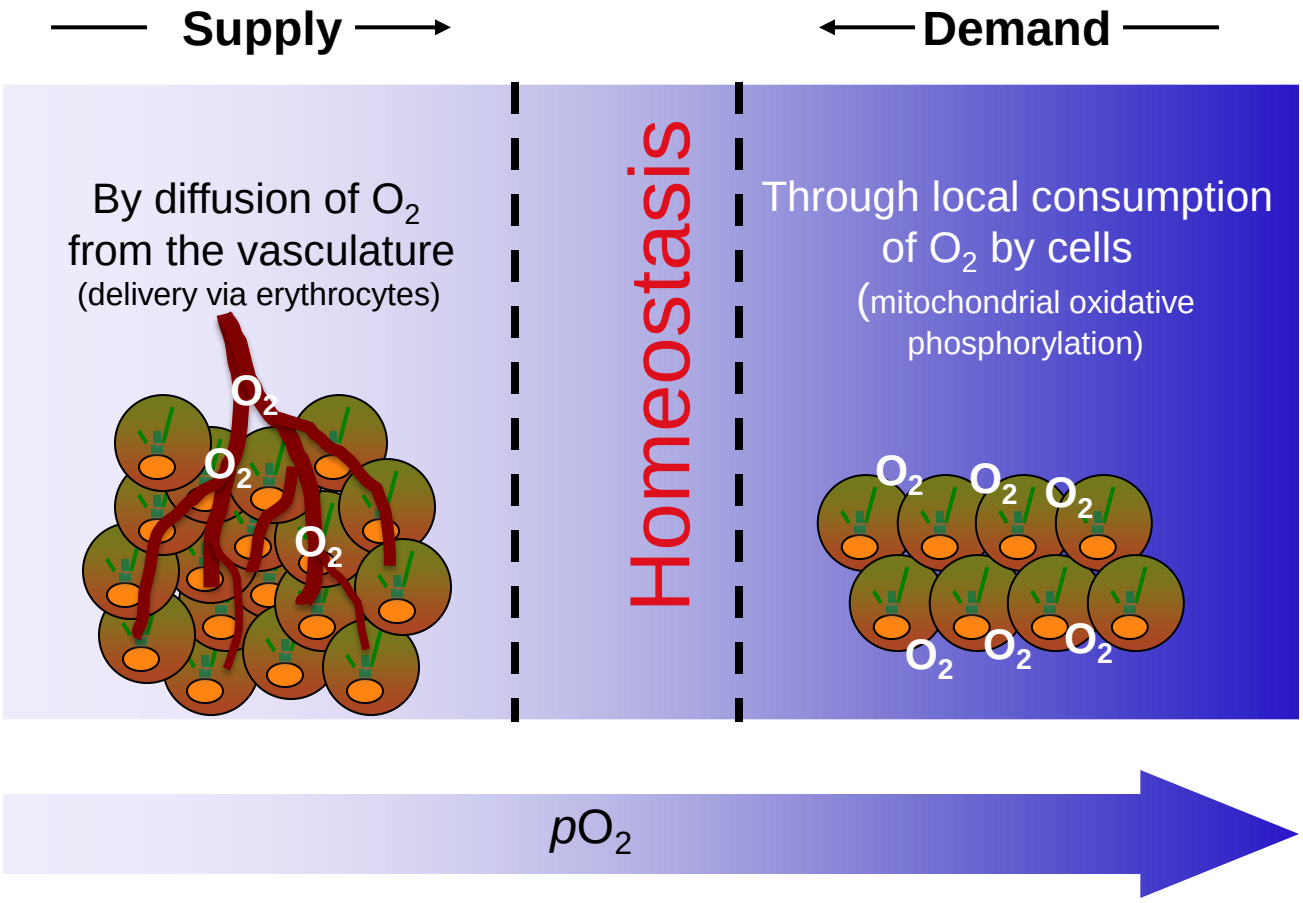
Anoxic network: blue
Oxic network: red

humans have 1.6x more metabloic reactions involving O2



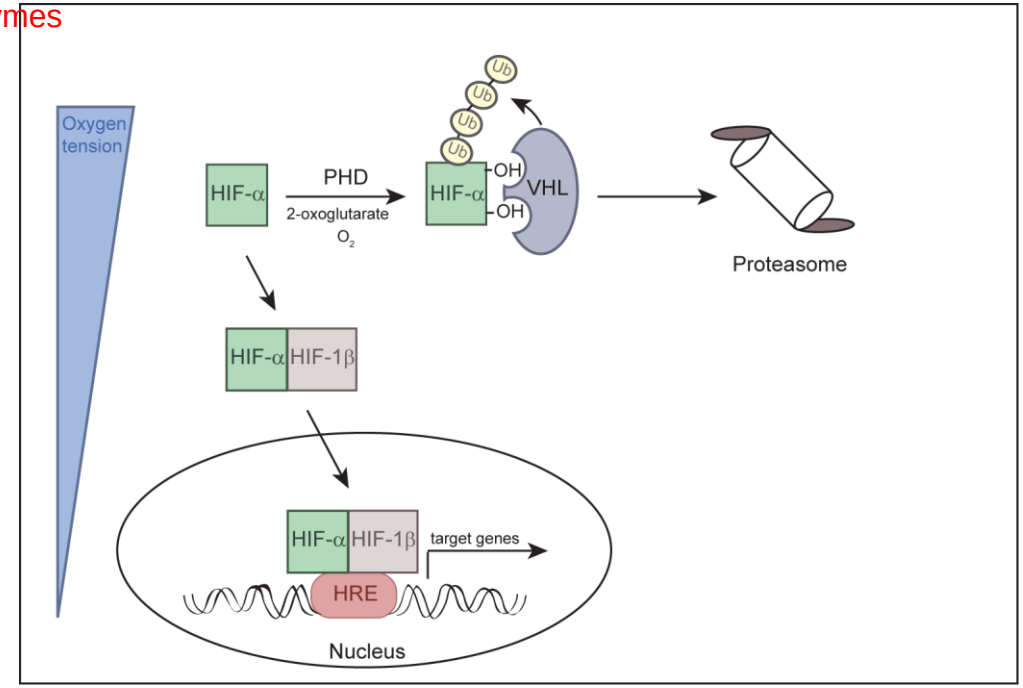
Raymond and Segre, Science 2006

Oxygen homeostasis



Regulation of hypoxia-inducible factor-a (HIF-α) subunits

when oxygen is lacking, enzymes do not work if they need it.



HIF1-a is expressed in nearly all cells.
HIF2-a is expressed in most organs but not in all cell types of these organs
HIF-a's are not only transcriptional promoters but can also take on suppressive functions.
They are also involved in cancer or ischemia.

those are the target genes regulated by HIF1/2-a

BNIP3/BNIP3L → Cell death Mitophagy

mostly HIF2-a

EPO → Erythropoiesis

VEGF → Angiogenesis

Glycolytic enzymes → Metabolism Energy

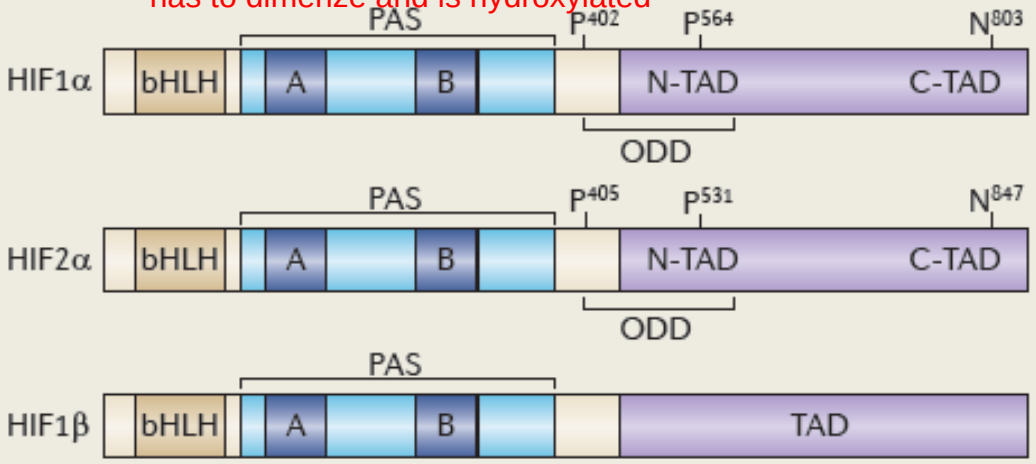
Schönenberger and Kovacs, Front. Cell Dev. Biol. 2015

Oxygen-dependent regulation of HIF-α

Box 1 | O_2-dependent regulation of HIF

Using molecular oxygen (O_2) and 2-oxoglutarate as substrates, hypoxia-inducible factor (HIF) prolyl-hydroxylase (PHD) enzymes[4] hydroxylate two specific proline residues in the O_2-dependent degradation domain (ODD) of HIFα proteins (see the figure). These hydroxylation events occur on Pro402 and Pro564 in HIF1α, and Pro405 and Pro531 in HIF2α, and are required for the von Hippel–Lindau (VHL) tumour suppressor protein — the recognition component of an E3 ubiquitin ligase complex — to bind and degrade HIFα subunits under normoxic conditions. Hypoxia inhibits PHD activity through various mechanisms, including substrate limitation (reviewed in REF. 4), which results in HIFα subunit stabilization, heterodimerization with HIF1β (also known as ARNT), and increased HIF transcriptional activity. Hypoxic conditions also inhibit hydroxylation by factor inhibiting HIF (FIH) of a conserved carboxy-terminal asparagine residue in the HIFα subunits, an event that blocks the interaction between HIFα subunits and the transcriptional co-activators p300 and CREB binding protein (CBP)[149–151]. Thus, whereas PHD-mediated hydroxylation destabilizes HIFα subunits, FIH-mediated hydroxylation inhibits their transcriptional activity. bHLH, basic helix-loop-helix; PAS, PER-ARNT-SIM; TAD, transactivation domain.

has to dimerize and is hydroxylated



-> ARNT - what was it again?

Keith et al., Nature Rev. Cancer 2012

Characteristics of the hypoxia-inducible factor hydroxylases

PHDx regulate the HIF-a's by degrading it (see two slides back)

	HIF-α isoform target	Tissue distribution in normoxia	Induction by hypoxia
PHD1	HIF-2α>HIF-1α (normoxia) HIF-1α=HIF-2α (hypoxia)	Testis>liver=heart=brain=kidney	No
PHD2	HIF-1α≫HIF-2α (normoxia) HIF-1α>HIF-2α (hypoxia)	Heart>liver=testis=kidney>brain	Yes
PHD3	HIF-1α=HIF-2α (normoxia) HIF-2α>HIF-1α (hypoxia)	Heart>testis=kidney=liver=brain	Yes
FIH	Unknown	Breast=testis=ovary>pancreas>liver>kidney	No

enzyme names gene names

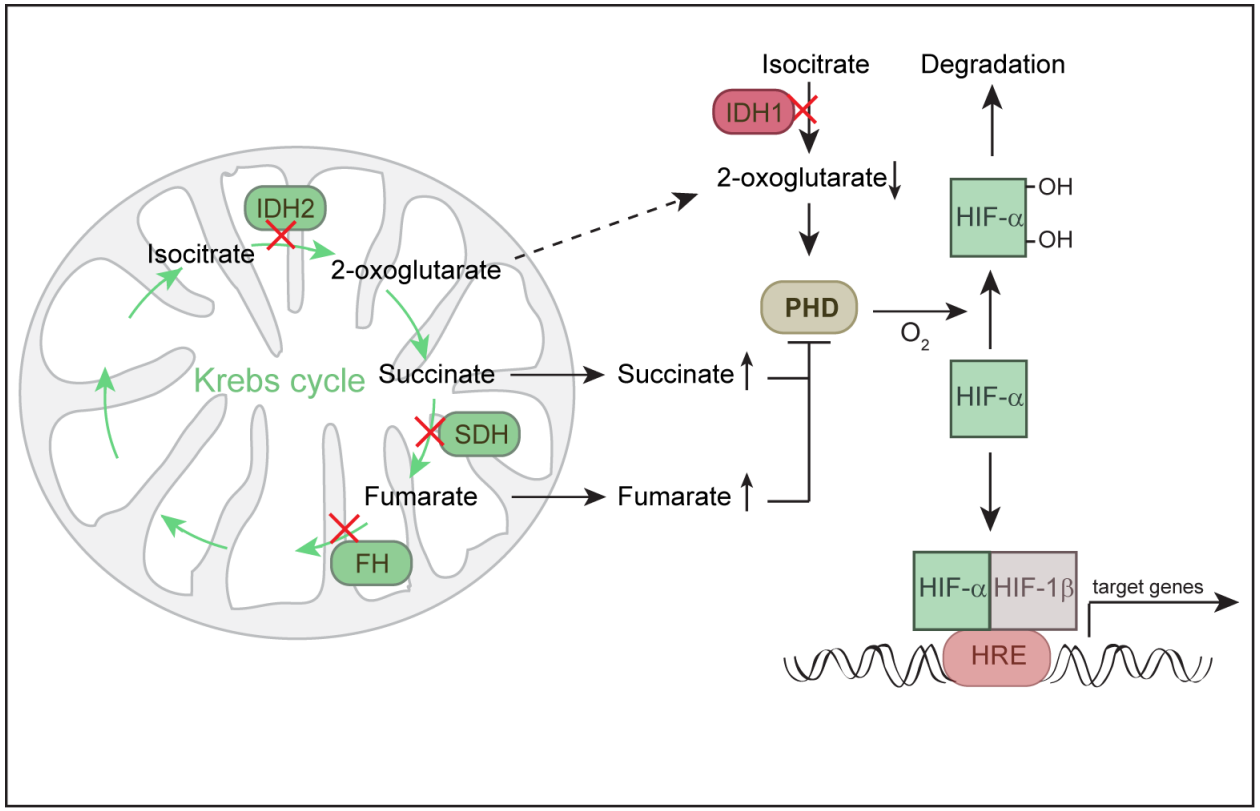
PHD1 = EGLN2
PHD2 = EGLN1
PHD3 = EGLN3

use EGLNX as the right name

Muchnik and Kaplan, Expert Opin. Investig. Drugs 2011

Pseudohypoxia: Stabilization of HIF-α subunits under non-hypoxic conditions

mutations here are also linked to various tumors.

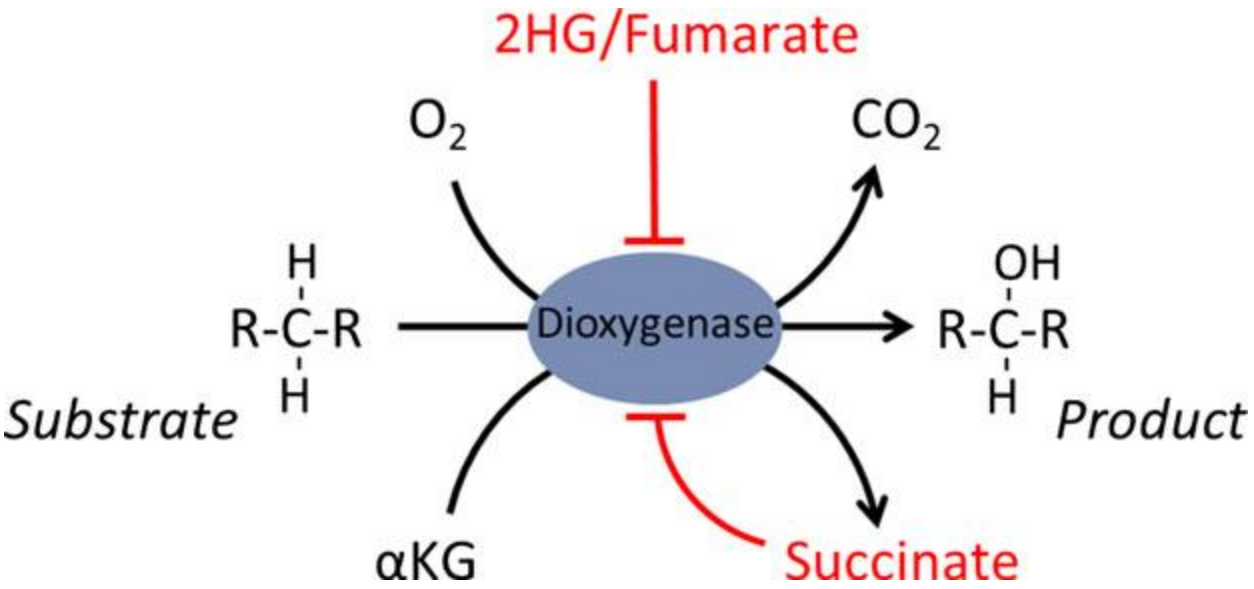


FH: fumarate hydratase, an enzmye for fumarate, hydrates fumarate to malate

Schönenberger and Kovacs, Front. Cell Dev. Biol. 2015

2-oxoglutarate-dependent dioxygenases

2HG-fumarate competes with alpha-KG, which leads to inhibition of the reaction



alpha-ketoglutarate - the anion of alpha-ketoglutaric acid

succinate is product and when there is too much of it, it will give a feedback inhibition so that reaction is inhibited (~autoregulatory so to say)

R-2-Hydroxyglutarate - what was it again?

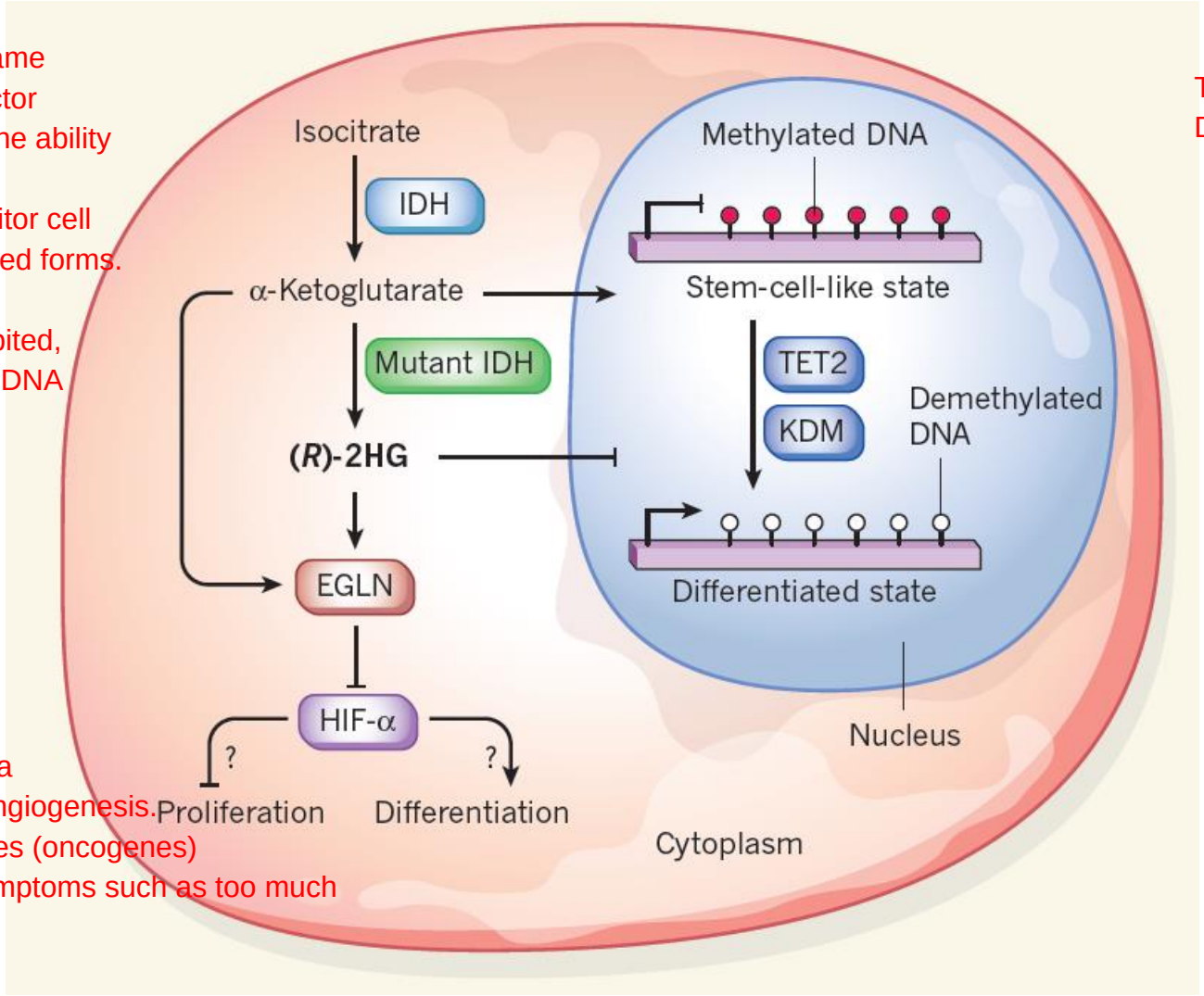
onco metabolites: succinate, etc.; name some, could be a exam question.

Nowicki and Gottlieb, FEBS J. 2015

2-hydroxyglutarate, an oncogenic metabolite in leukaemia

mutation in IDH became independent of cofactor signalling. they lost the ability to progress from a stem cell like progenitor cell to a more differentiated forms.

differentiation is inhibited, bc no methylation of DNA etc.



TET2 demethylates DNA

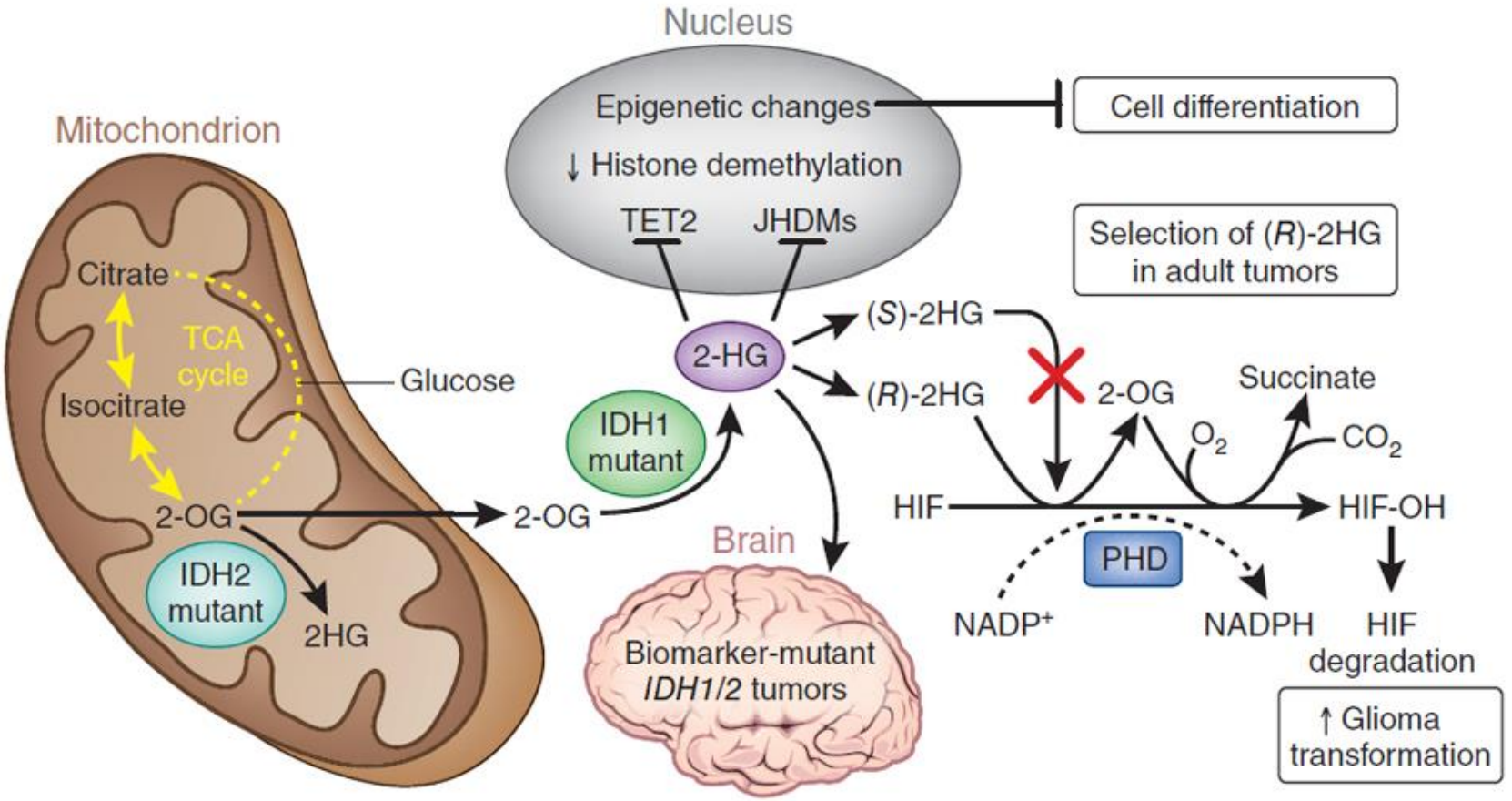
oncogenes are often hypermethylated. this influences the expression and is also linked to tumor. hypermethylated oncogenes are not expressed i think, so its suppressed

hif-alpha promotes tumors (?)

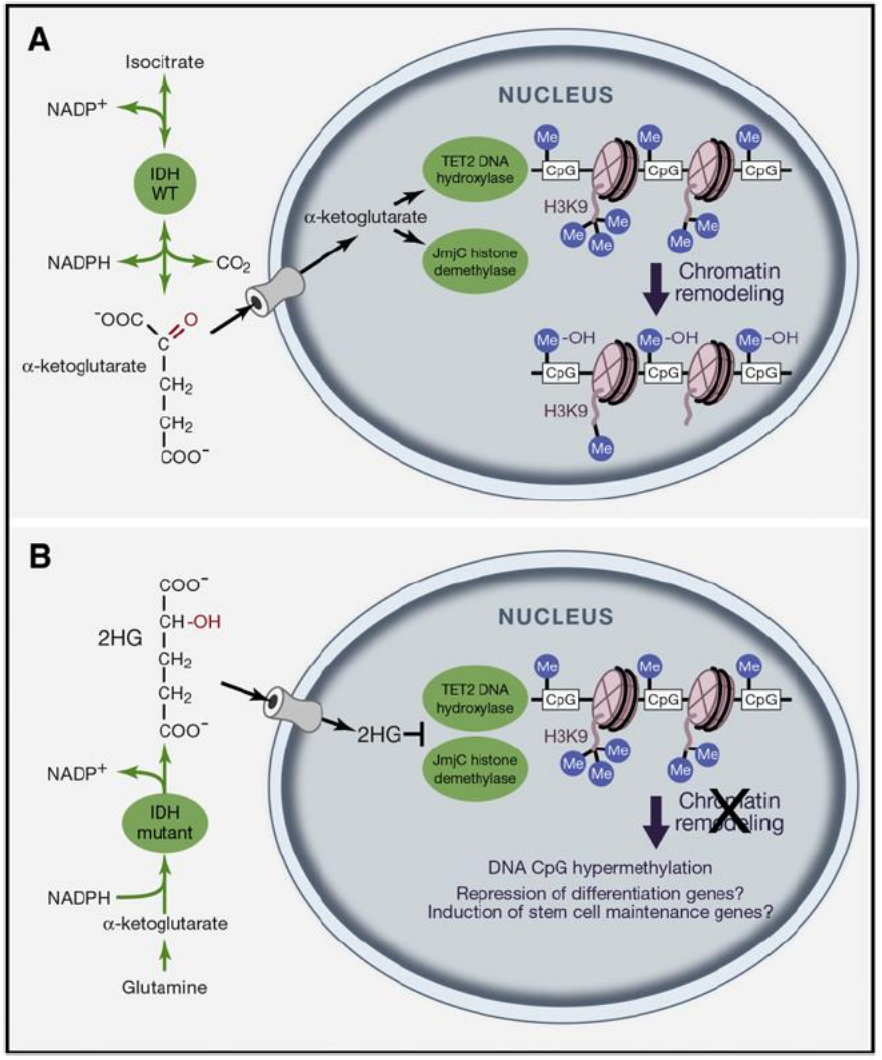
HIF is believed to be a master regulator of angiogenesis. mutations in HIF-genes (oncogenes) will lead to cancer symptoms such as too much angiogenesis etc.

Krall and Christofk, Nature 2013

2-hydroxyglutarate, an oncogenic metabolite in glioma



Oncometabolite 2-hydroxyglutarate dysregulates epigenetics and cell differentiation

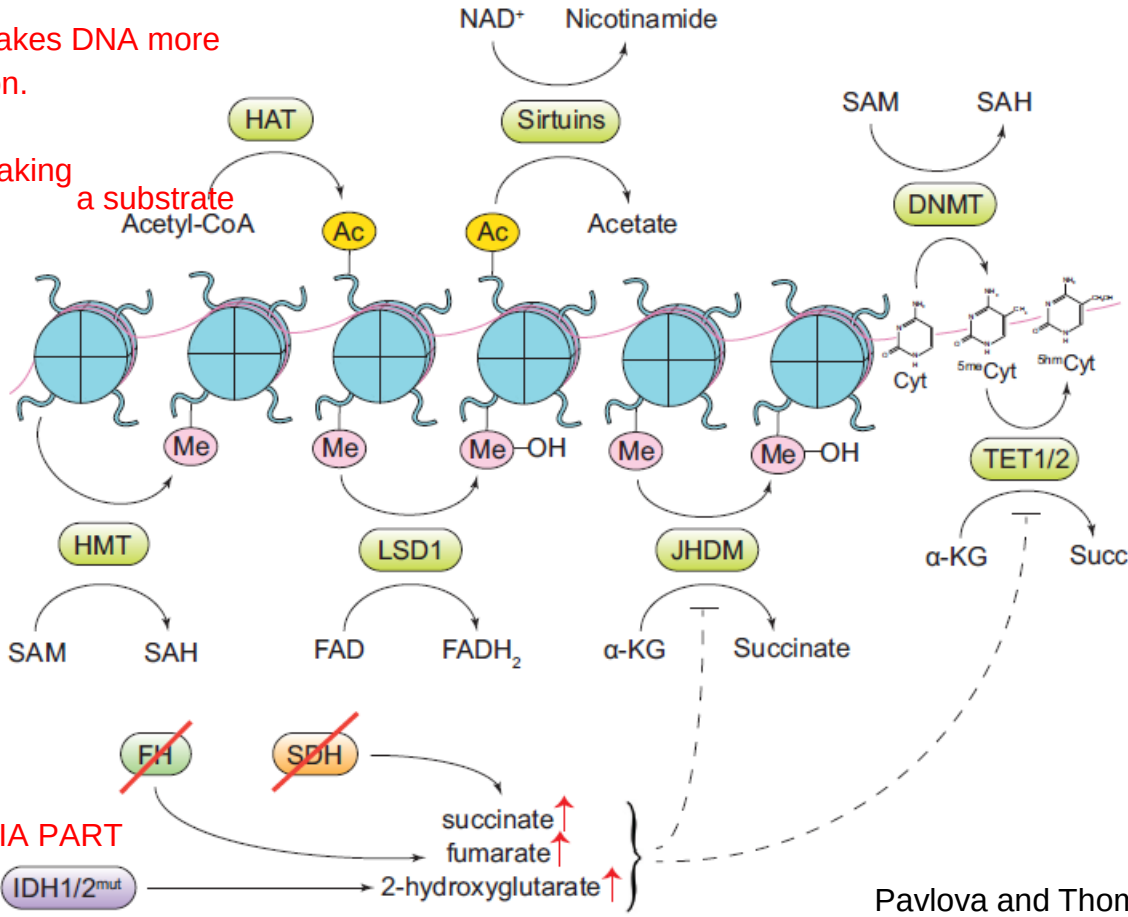


Ward and Thompson, Cancer Cell 2012

Alterations in metabolite-driven gene regulation

acetylation of histones makes DNA more accessible for transctiption.

some remove Ac, thus making DNA less accessible



those take care of acetyl groups (adding or removing)

methylazes

demethylazes

those take care of the methyl groups (adding or removing)

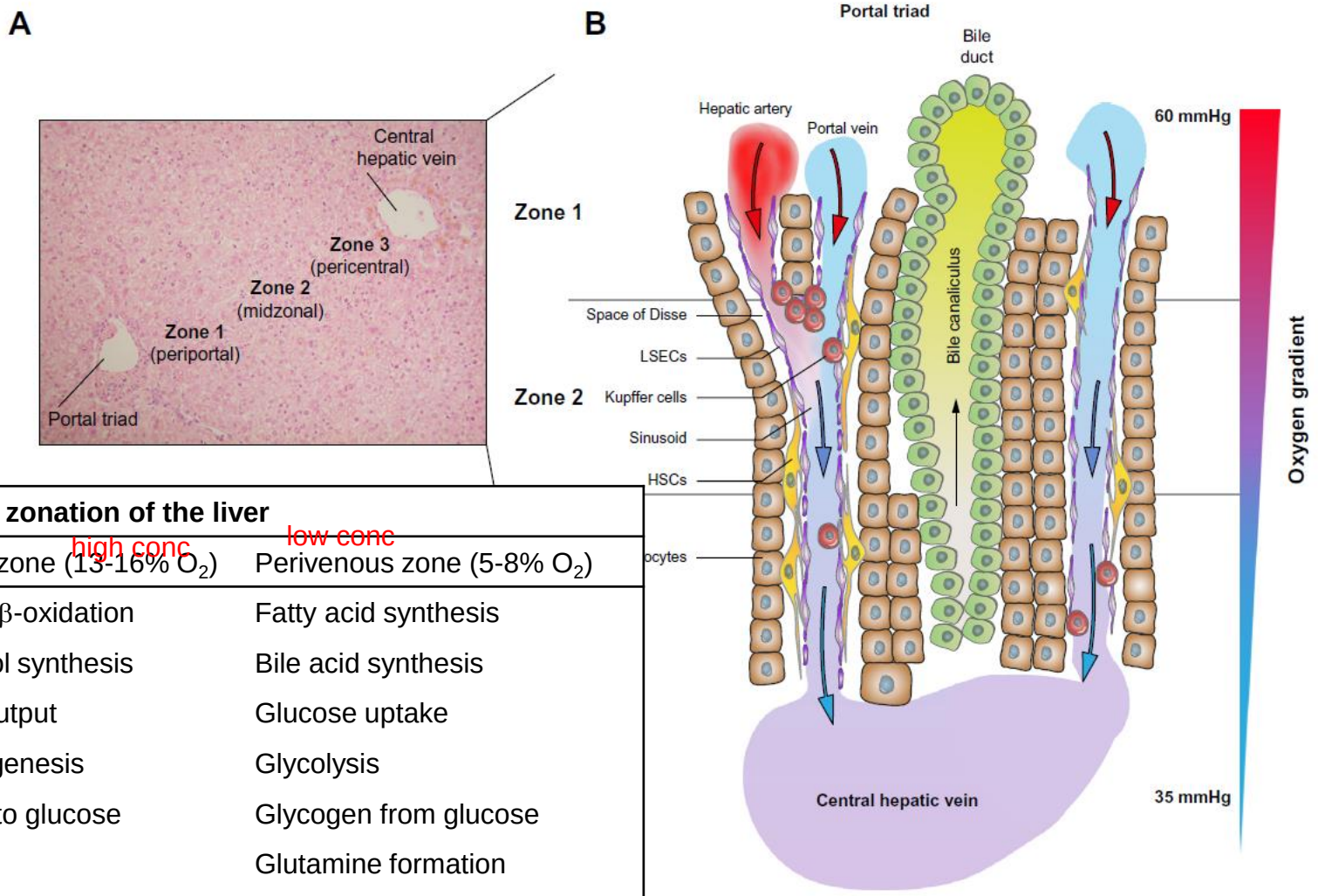
END OF PSEUDOHYPOXIA PART

Pavlova and Thompson, Cell Metab. 2016

HAT, histone acetyltransferase enzymes
SAM, S-adenosylmethionine
SAH, S-adenosylhomocysteine
DNMT, DNA methyltransferase enzymes

HMT, histone methyltransferase enzymes
LSD1, lysine-specific histone demethylase
JHDM, Jumonji domain-containing histone demethylase enzymes
TET1/2, ten-eleven translocation methylcytosine dioxygenase 1/2

Oxygen gradient and metabolic zonation of the liver



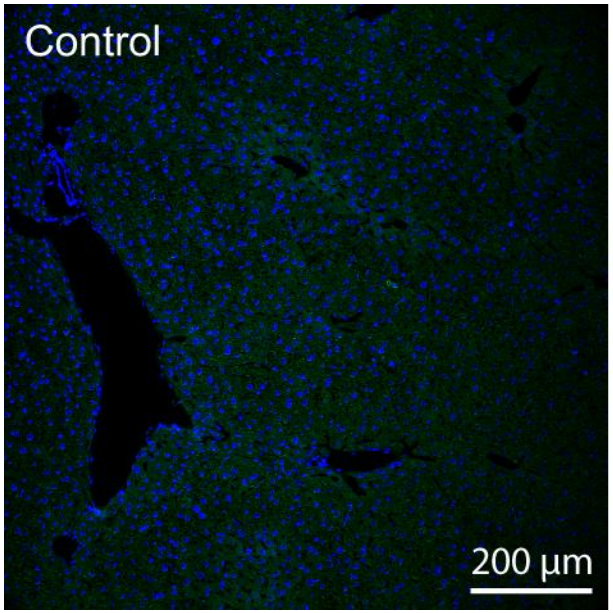
Metabolic zonation of the liver	
Periportal zone (13-16% O_2) high conc	Perivenous zone (5-8% O_2) low conc
Fatty acid β-oxidation	Fatty acid synthesis
Cholesterol synthesis	Bile acid synthesis
Glucose output	Glucose uptake
Gluconeogenesis	Glycolysis
Glycogen to glucose	Glycogen from glucose
TCA cycle	Glutamine formation
Urea synthesis	Xenobiotic metabolism

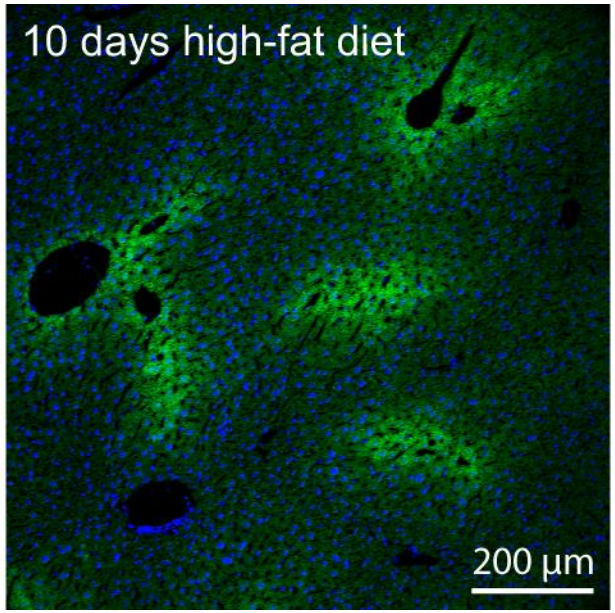
a lot of oxygen is needed for cholesterol synthesis

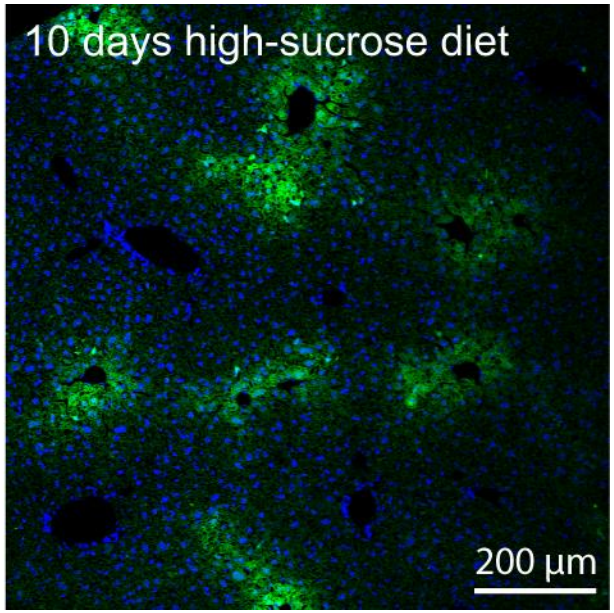
Wilson et al., J. Hepatol. 2014

Nutritional stress and hypoxia in the liver

(mouse liver)



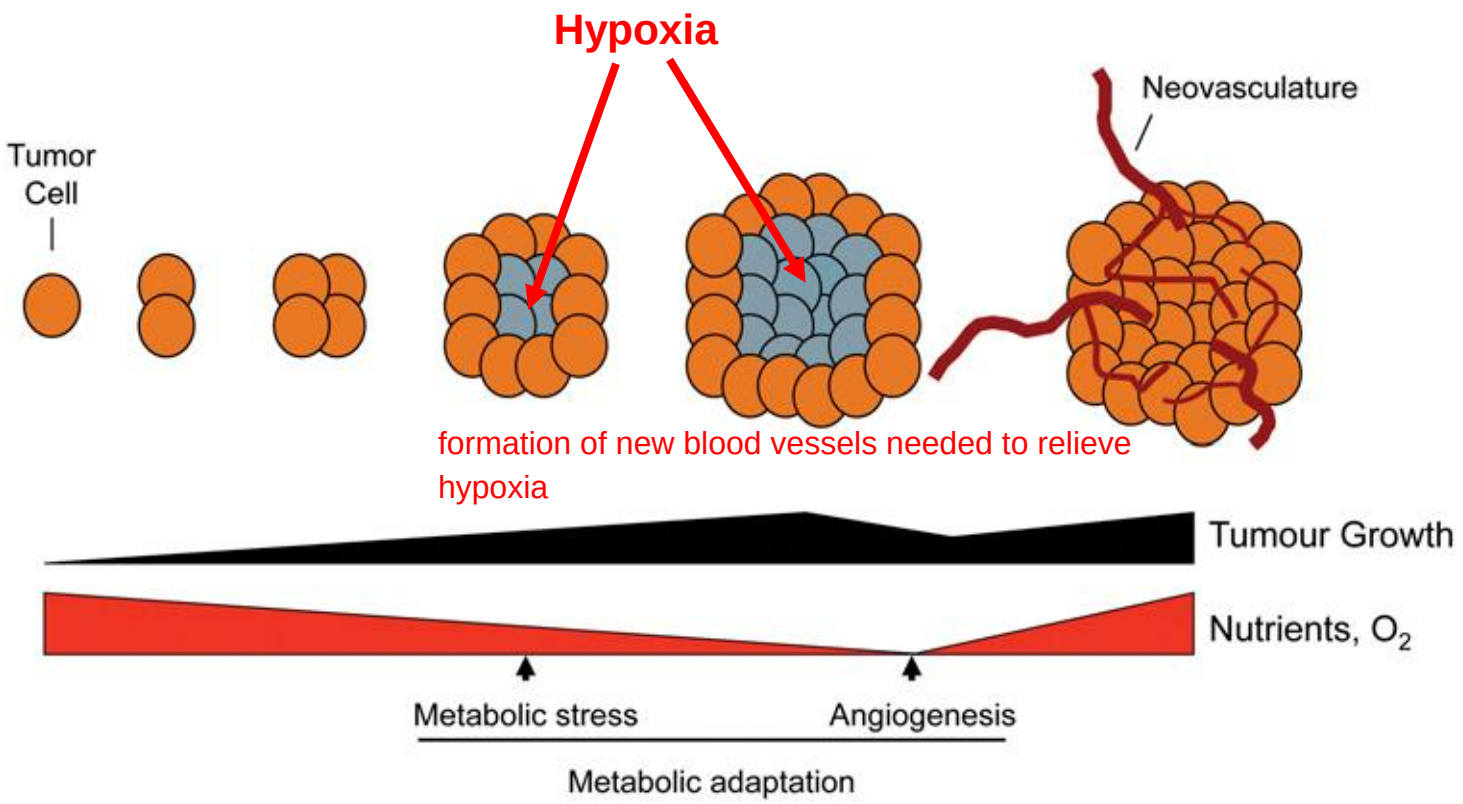




normal setup to observe obesity of type-2-diabetes

Walter et al., in preparation

Hypoxia and metabolic stress: a key component of neoplastic tissue expansion

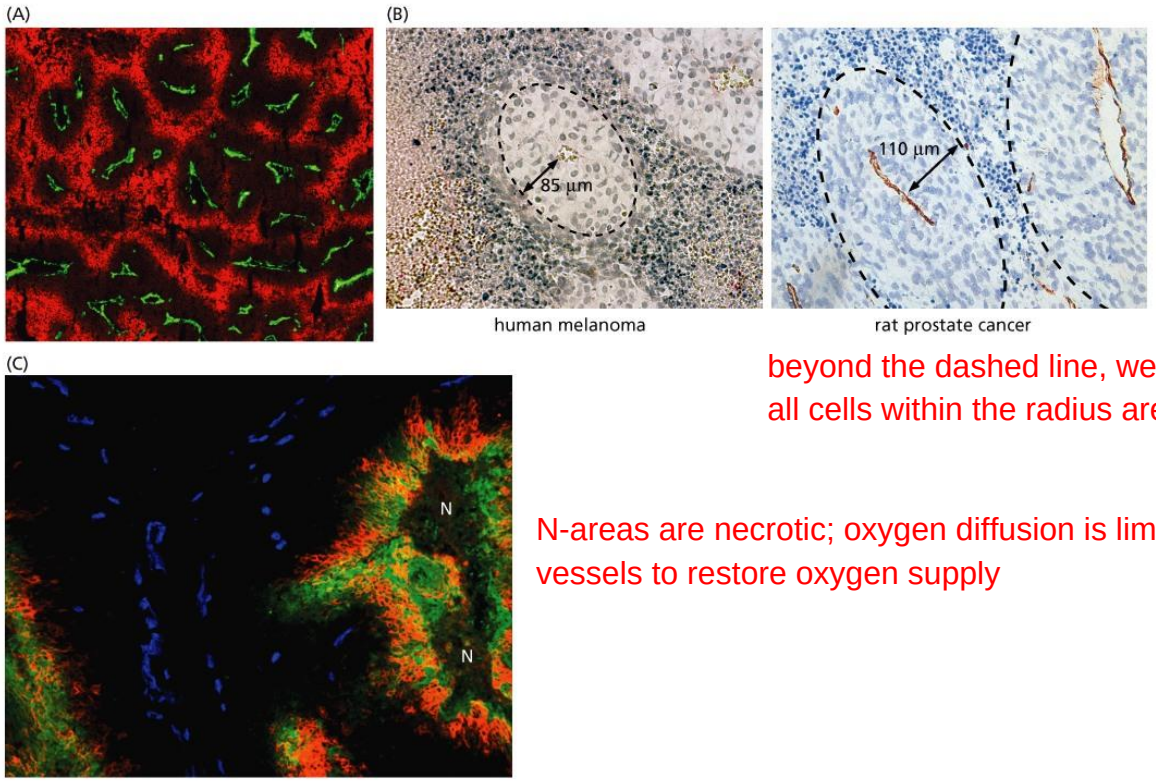


autophagy: "self-eating"; the cell degrades its own components to generate components for metabolism. at one point, the cell will die.

Jones and Thompson, Genes & Dev. 2009

Hypoxia and necrosis of cells in poorly vascularized sections of tissues

red are oxygenated areas
(or was it green?...)



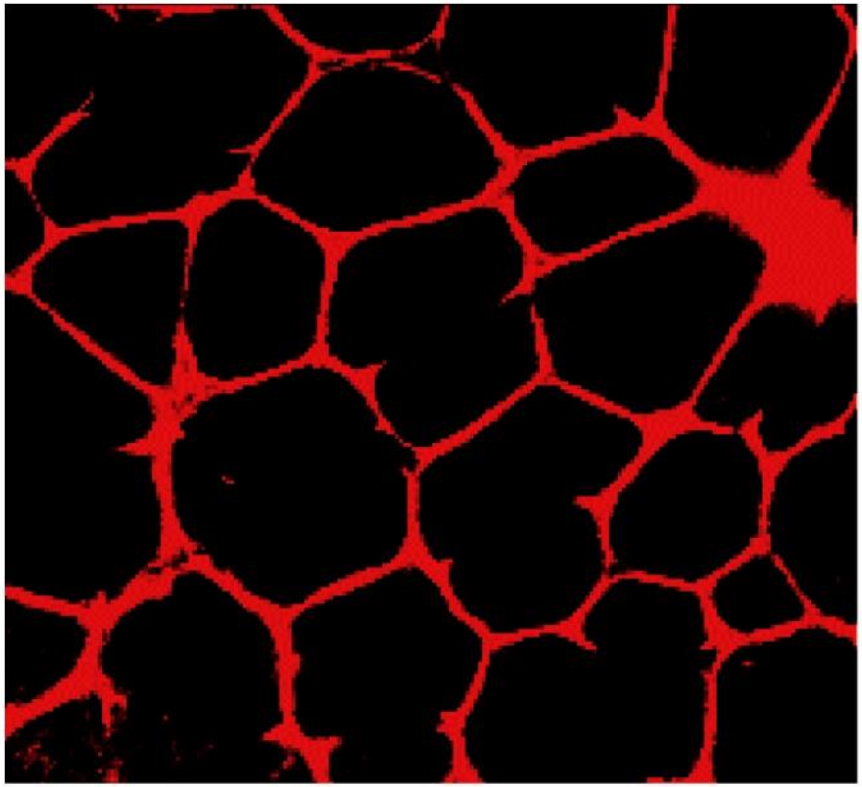
beyond the dashed line, we have necrotic areas.
all cells within the radius are happy cells

N-areas are necrotic; oxygen diffusion is limited so we need new blood vessels to restore oxygen supply

Figure 13.27 The Biology of Cancer (© Garland Science 2014)

Capillary leakiness of tumor-associated vasculature

normal tissue

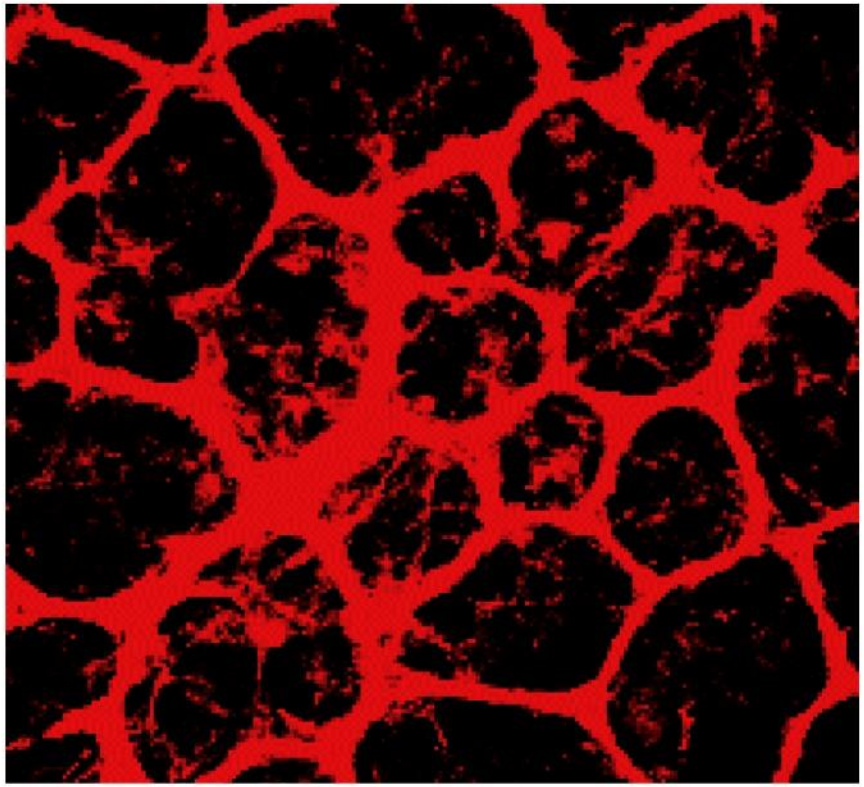


no tumor

very nicely defined blood vessels

Figure 13.15a The Biology of Cancer (© Garland Science 2014)

red dye injected in blood vessels

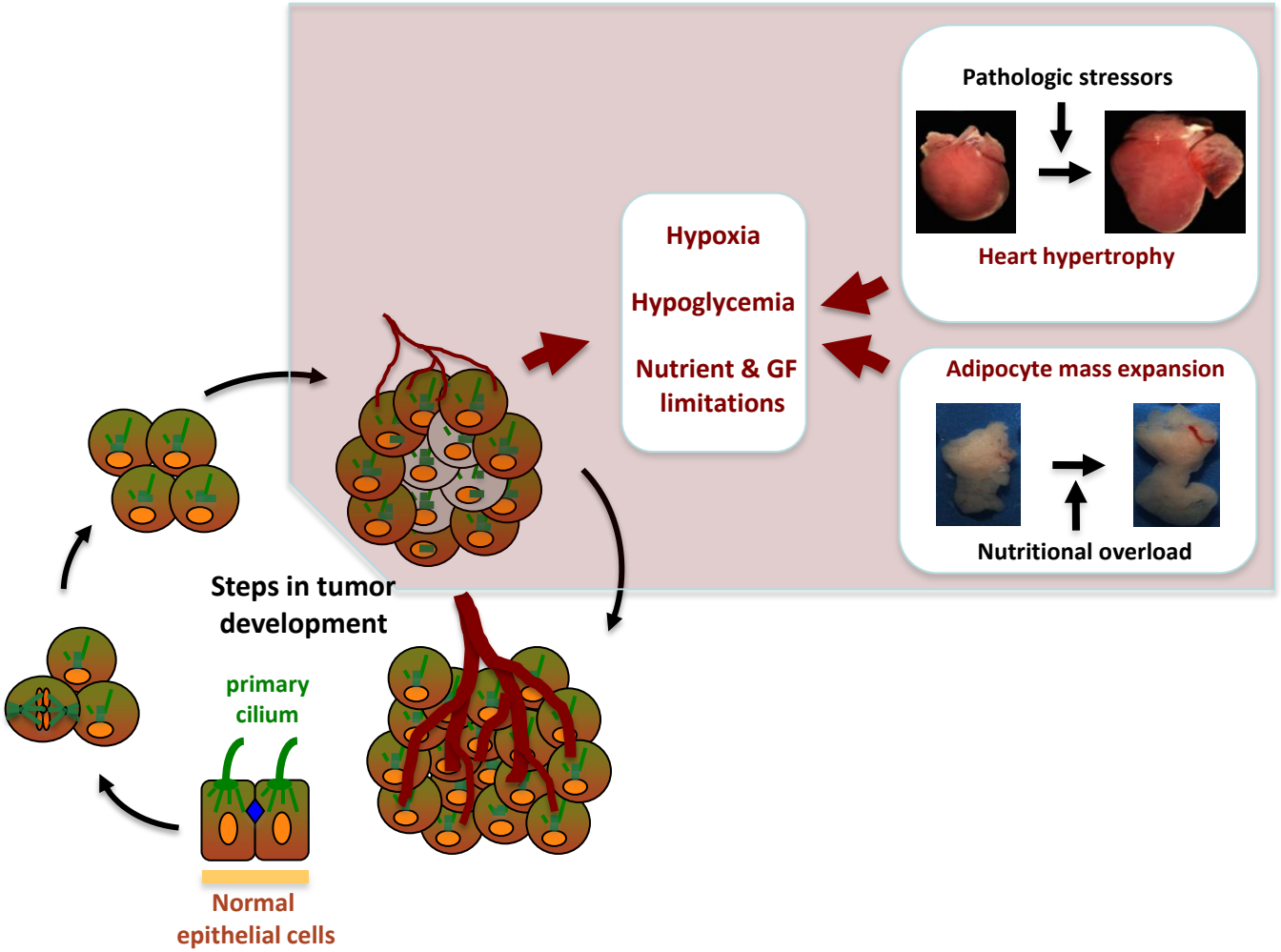


not nicely developed
very leaky here

tumor

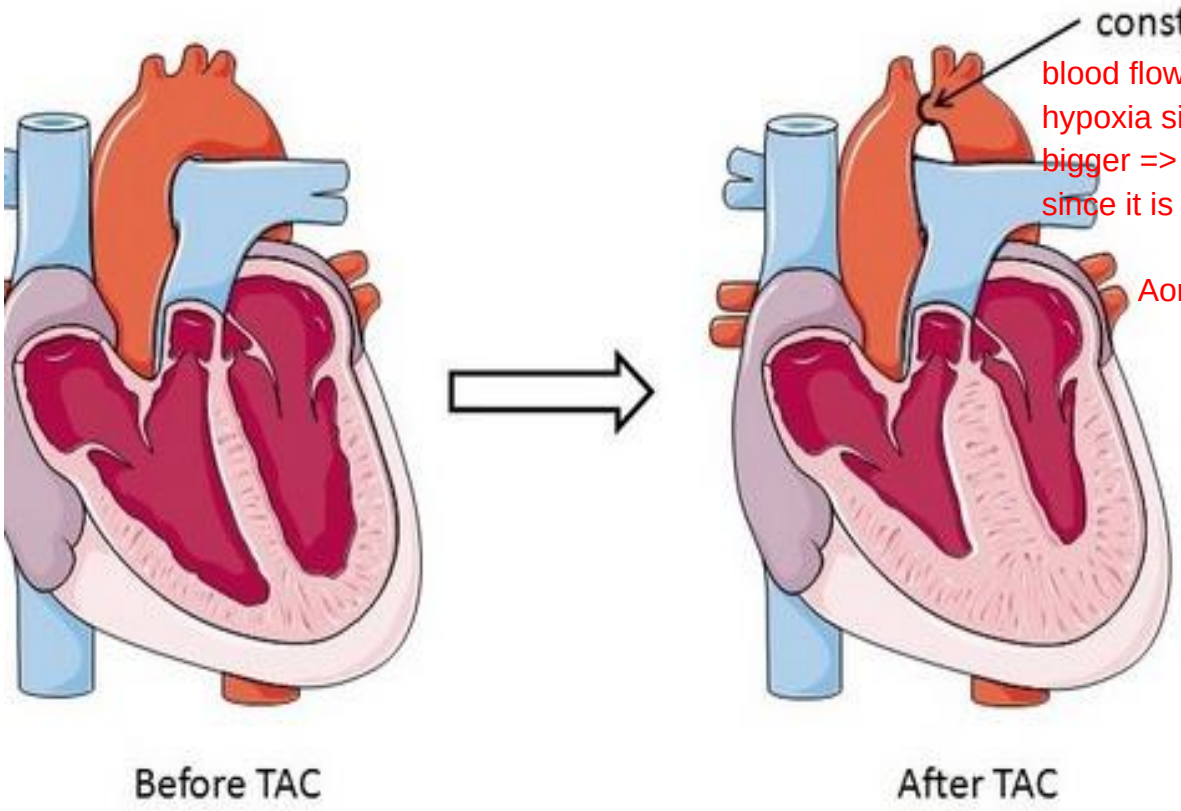
lots of blood vessels, some are big, also dense network of smaller blood vessels; some are not normally formed, that's why they are so leaky

Hypoxic and metabolic stress reprogramming in neoplastic and non-neoplastic tissue expansion



Transaortic constriction (TAC) leads to heart hypertrophy

inducing a pathological stress in the heart

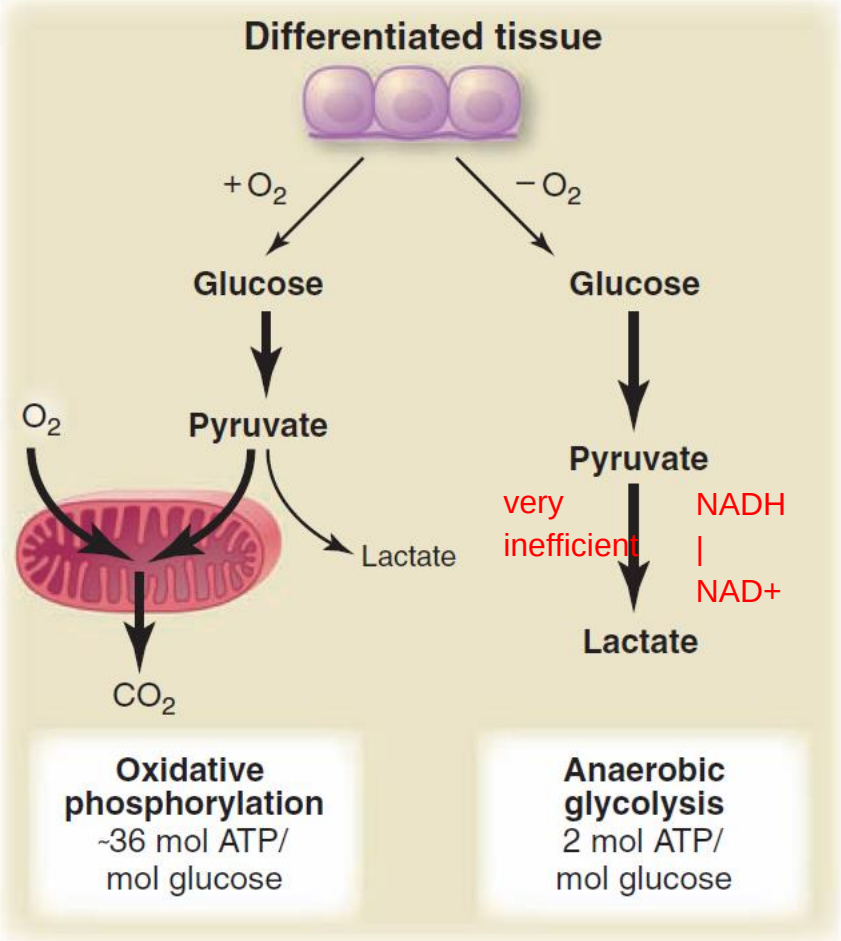


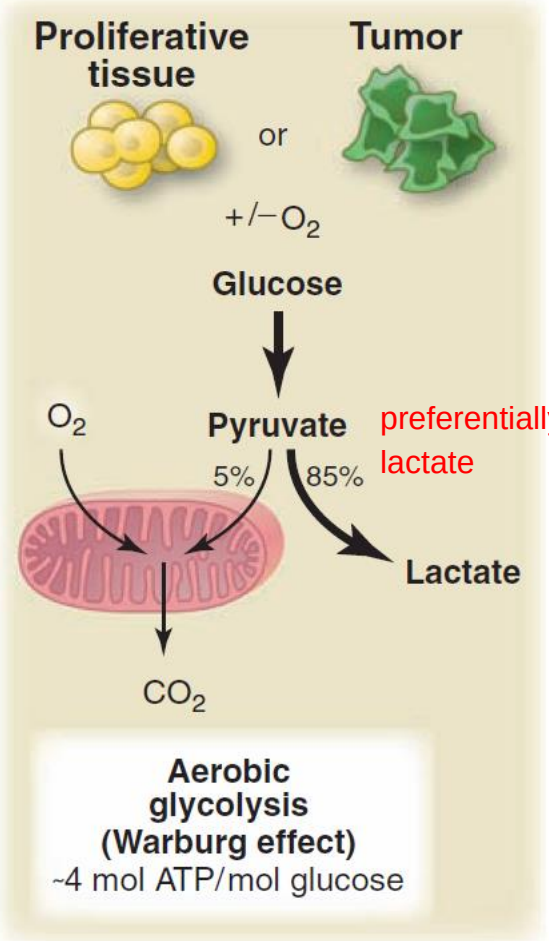
blood flow impaired => leads to hypoxia signalling and heart gets bigger => worst case: heart failure since it is not functional anymore.

Aorta is constricted up there.

Metabolic strategies of differentiated vs. proliferative cells

hypoxia adaption leads to different metabolisms to generate energy.





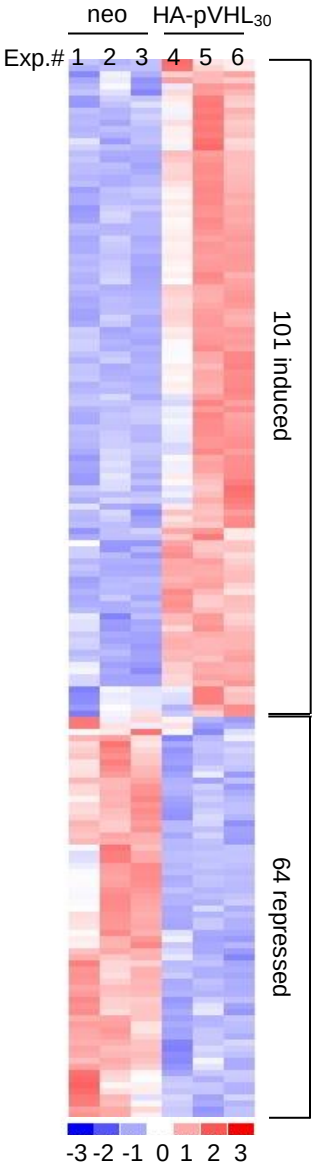
preferentially converted to lactate

mitochondria are functional in cancer cells.

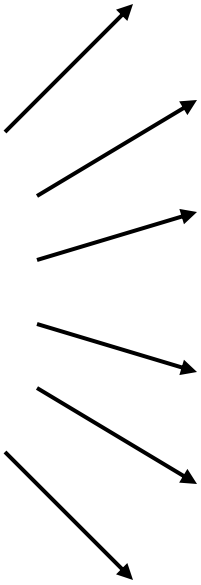
Vander Heiden et al., Science 2009

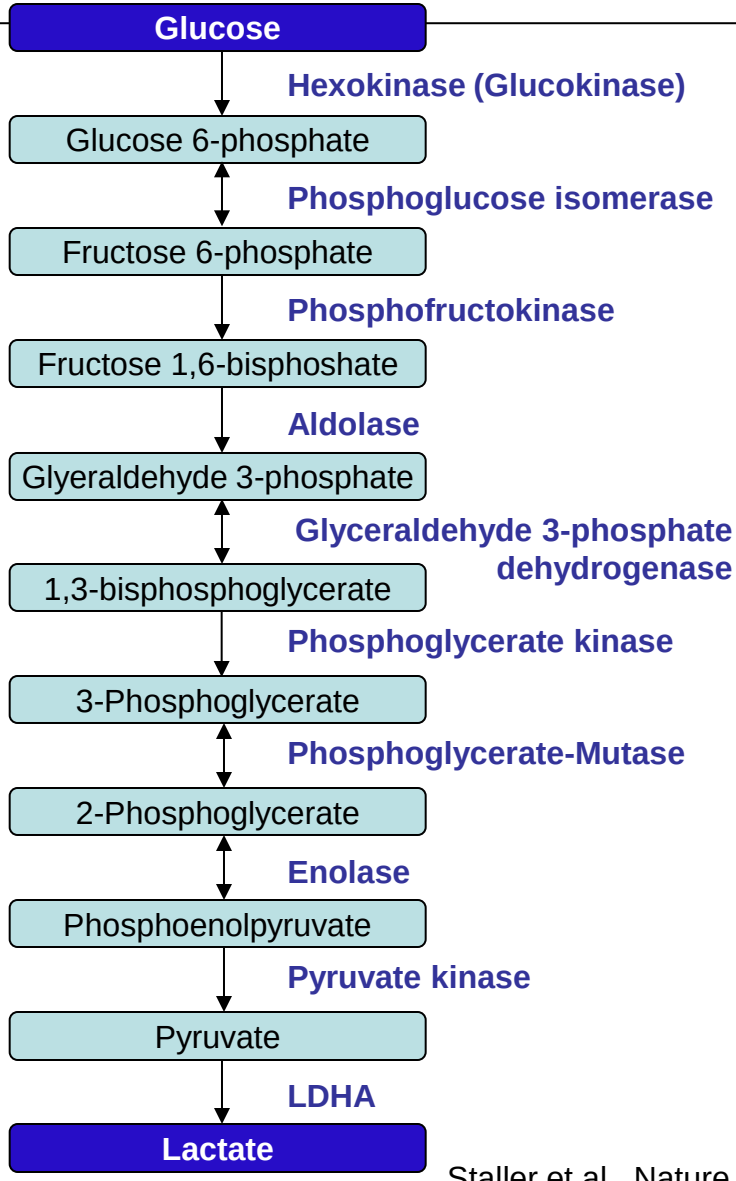
HIF activates glycolytic genes

learn pathway with enzymes by heart



HIF





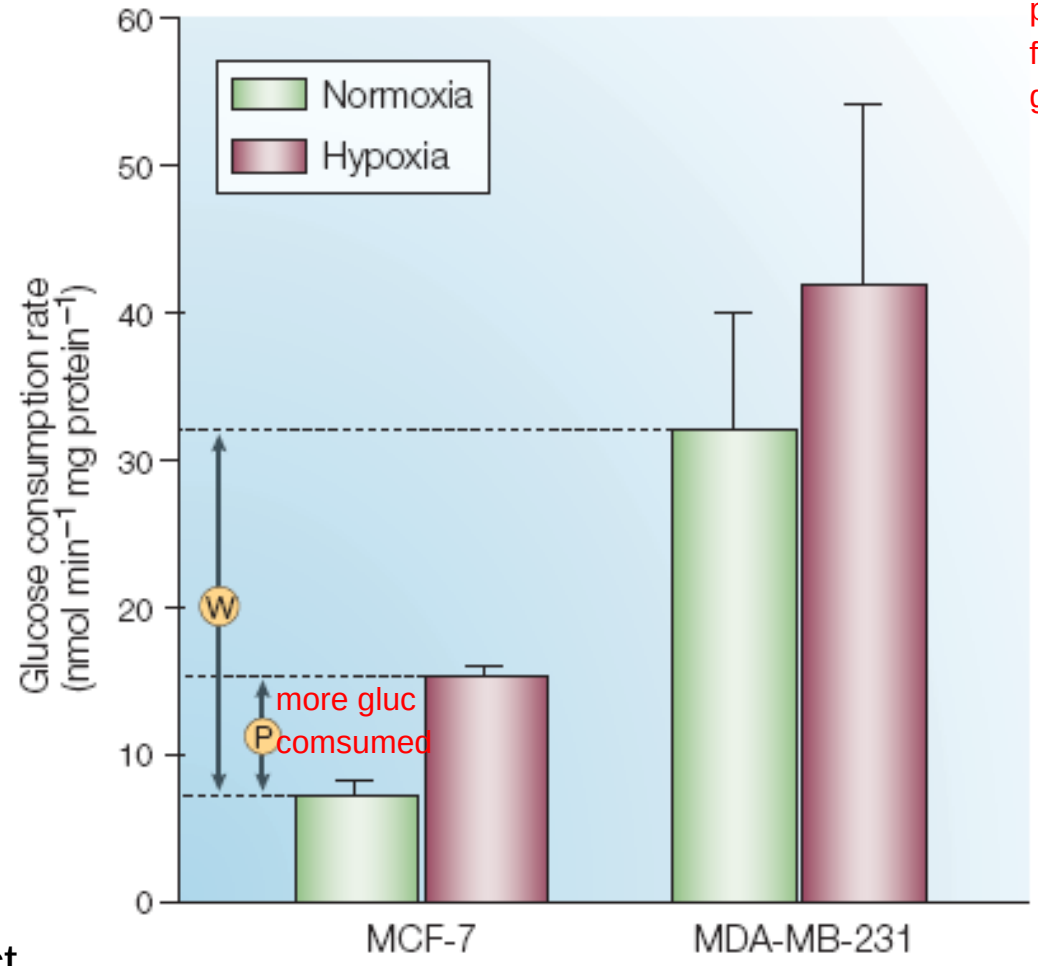
Staller et al., Nature 2003

Otto Warburg

Nature Reviews | Cancer

Pasteur and Warbur effects in non-invasive and metastatic breast cancer cell lines

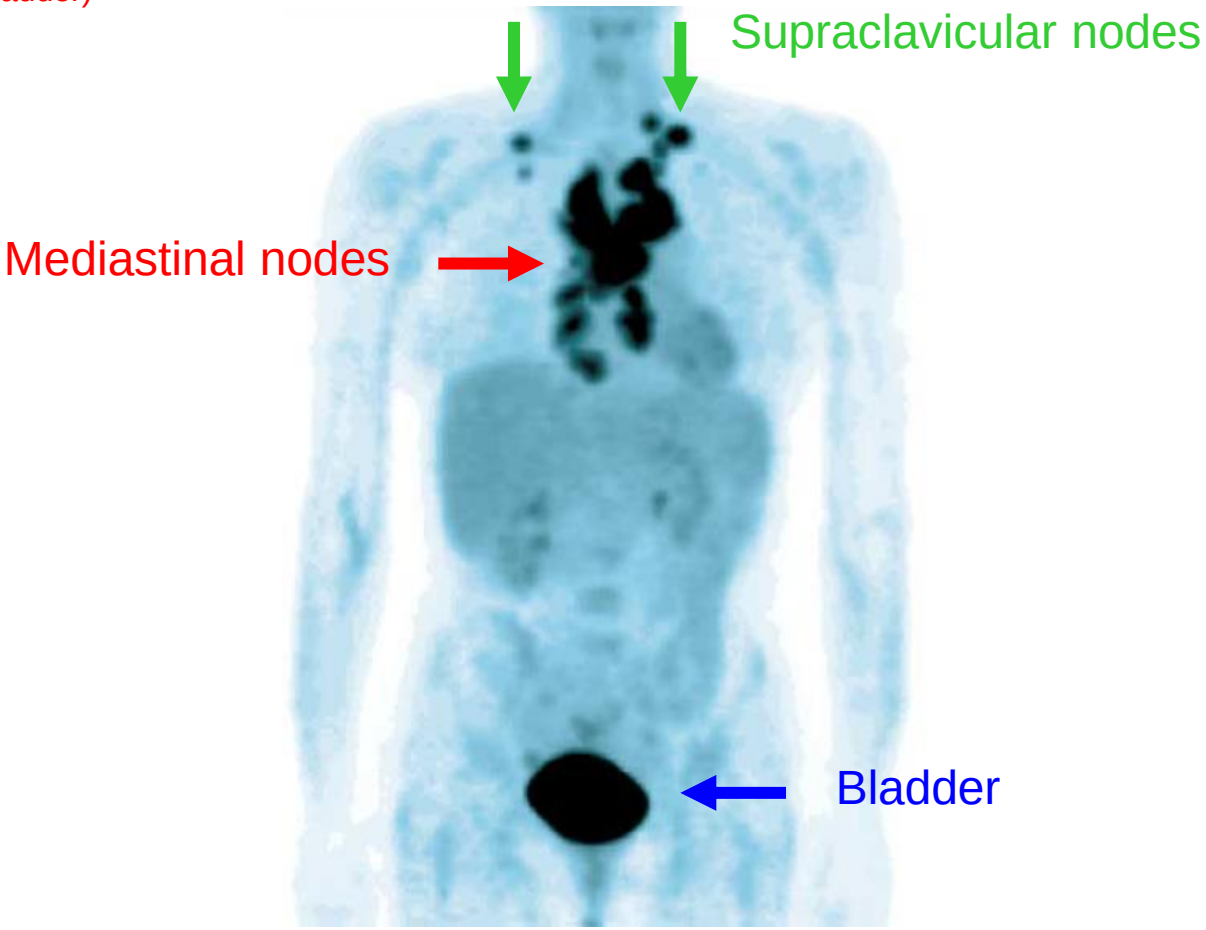
Interpretation of data:
pasteur effect: inhibiting effect on fermentation of oxygen: anaerobic glycolysis



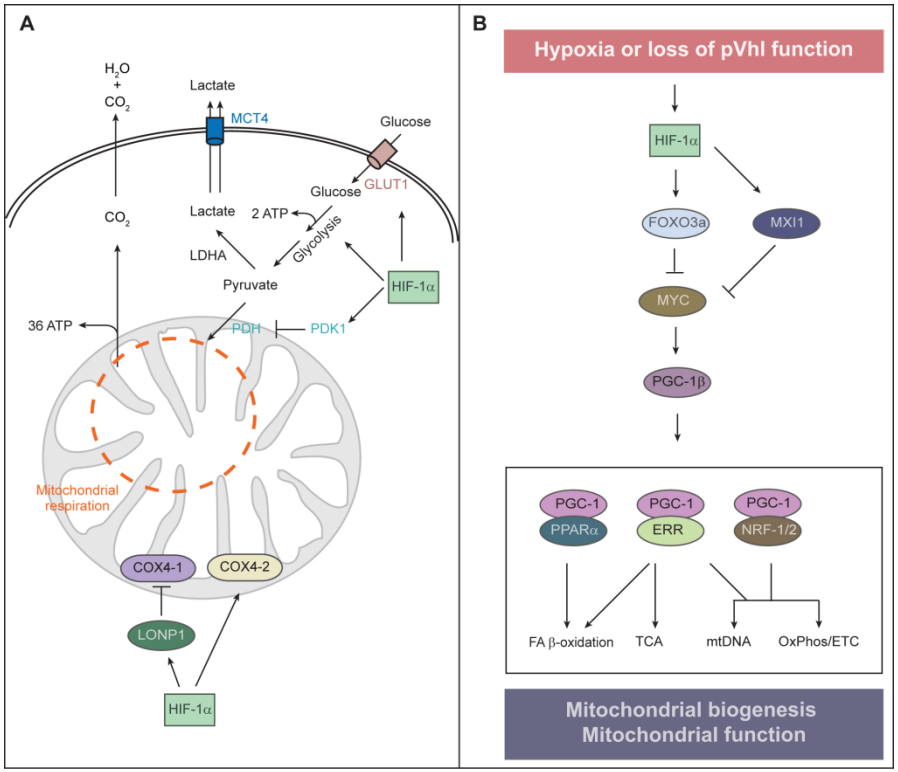
P, Pasteur effect
W, Warburg effect

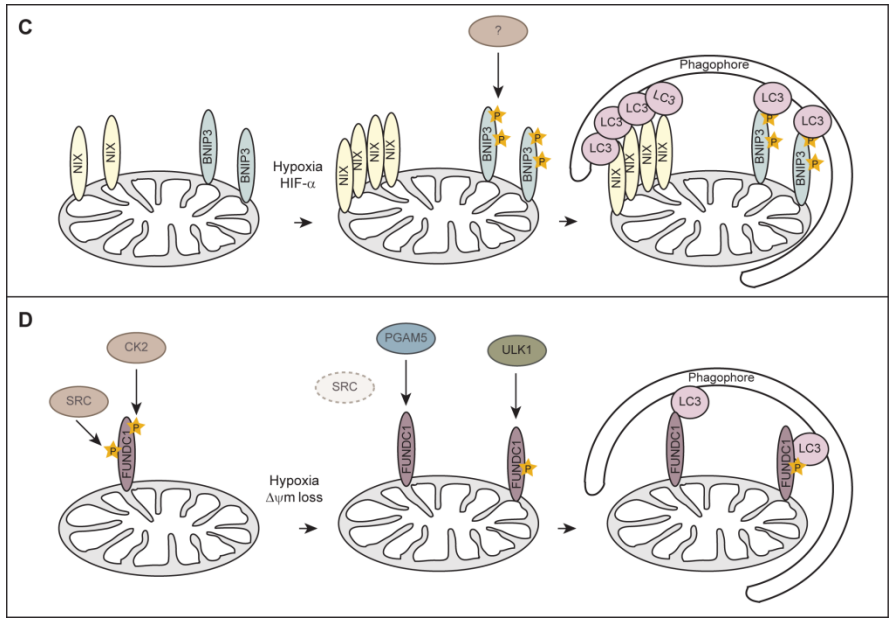
Positron-emission tomography imaging with ^{18}fluorodeoxyglucose of a patient with lymphoma

black spots: cancer
(there is cancer in the bladder)



Regulation of mitochondrial function and abundance by HIF-α





1. Mitochondrial metabolism

2. Mitochondrial biogenesis

3. Selective autophagy of mitochondria (Mitophagy)

Schönenberger and Kovacs, Front. Cell Dev. Biol. 2015

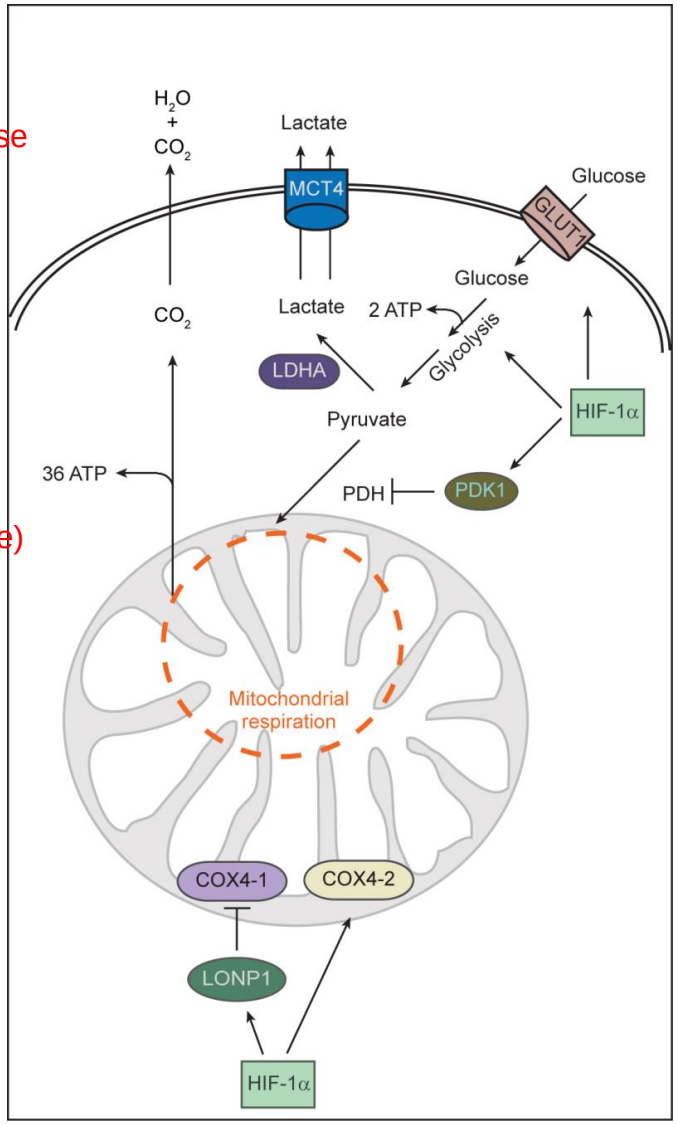
KEY FINDING IN THE LAST FEW YEARS
(might be important for exam)

HIF-dependent regulation of glycolysis and mitochondrial metabolism

when you produce lots of lactate you want to get it out of it with MCT4, bc it will acidify the intercellelular lumen and increase pH within the cell, which is detrimental

in cancer tissue: inner cancer cells get lactate out of it and out cancer cells use the lactate for metabolism

When PDH is KO'ed, LDHA uses pyruvate to make lactate (in cancer cells for example)



pH tumor millieu: low pH. There are some pathways in tumors that allow tumor cells to exist in low pH values.

in hypoxia, there is still mitochondiral meabolism, but it is not as efficient. COX4-2 is then activated (isoform of COX4-1), which is more efficient under hypoxic conditions (activated by HIF-1a)

That way, HIF-signaling induces glycolysis (glycolytic flux) and downregulates mitochondrial metabolism.

Schönenberger and Kovacs, Front. Cell Dev. Biol. 2015

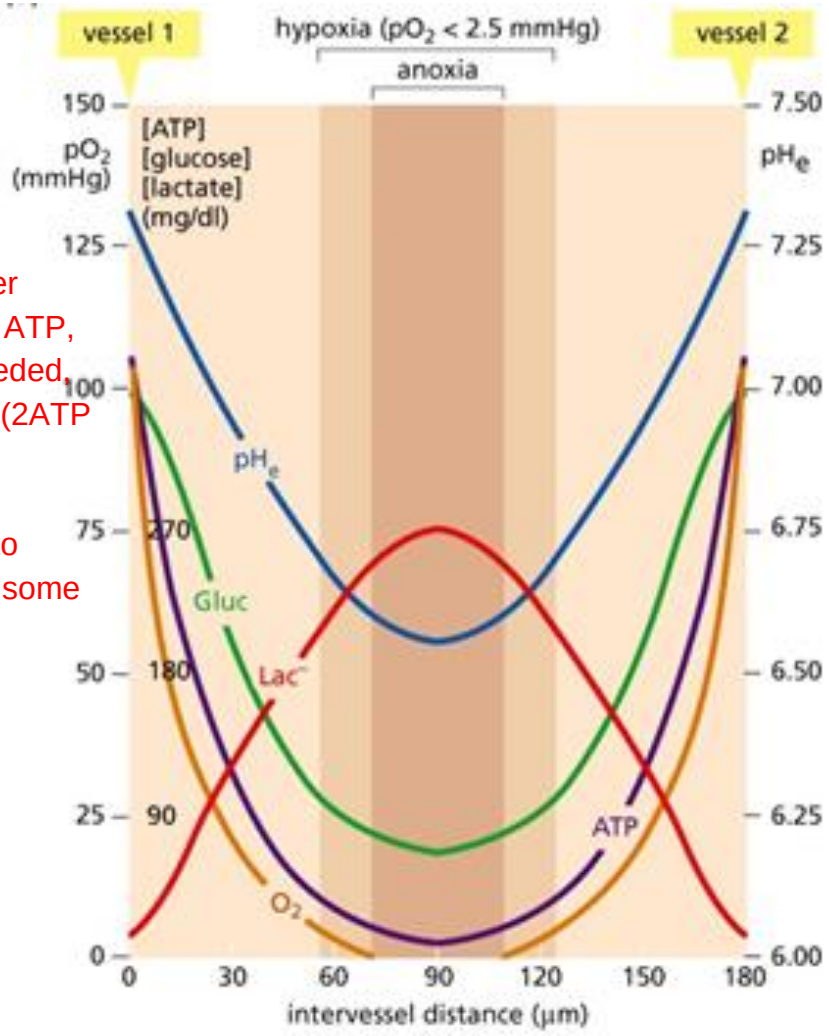
Hypoxia and necrosis of cells in poorly vascularized sections of tissues

Exam: this might be a question: explain the picture (the metabolic changes)

def. anoxia: severe hypoxia

in the anoxia area: there are no blood vessels nearby => no O2 input (=0 in that area).
pH, glux, and AP also drop the farther away from a blood vessel (nearly no ATP, since for aerobic glycolysis O2 is needed, but there is still anaerobic glycolysis (2ATP per cycle produced))

Lactate has its peak in hypoxia due to a change in metabolic pathway (see some slides before)



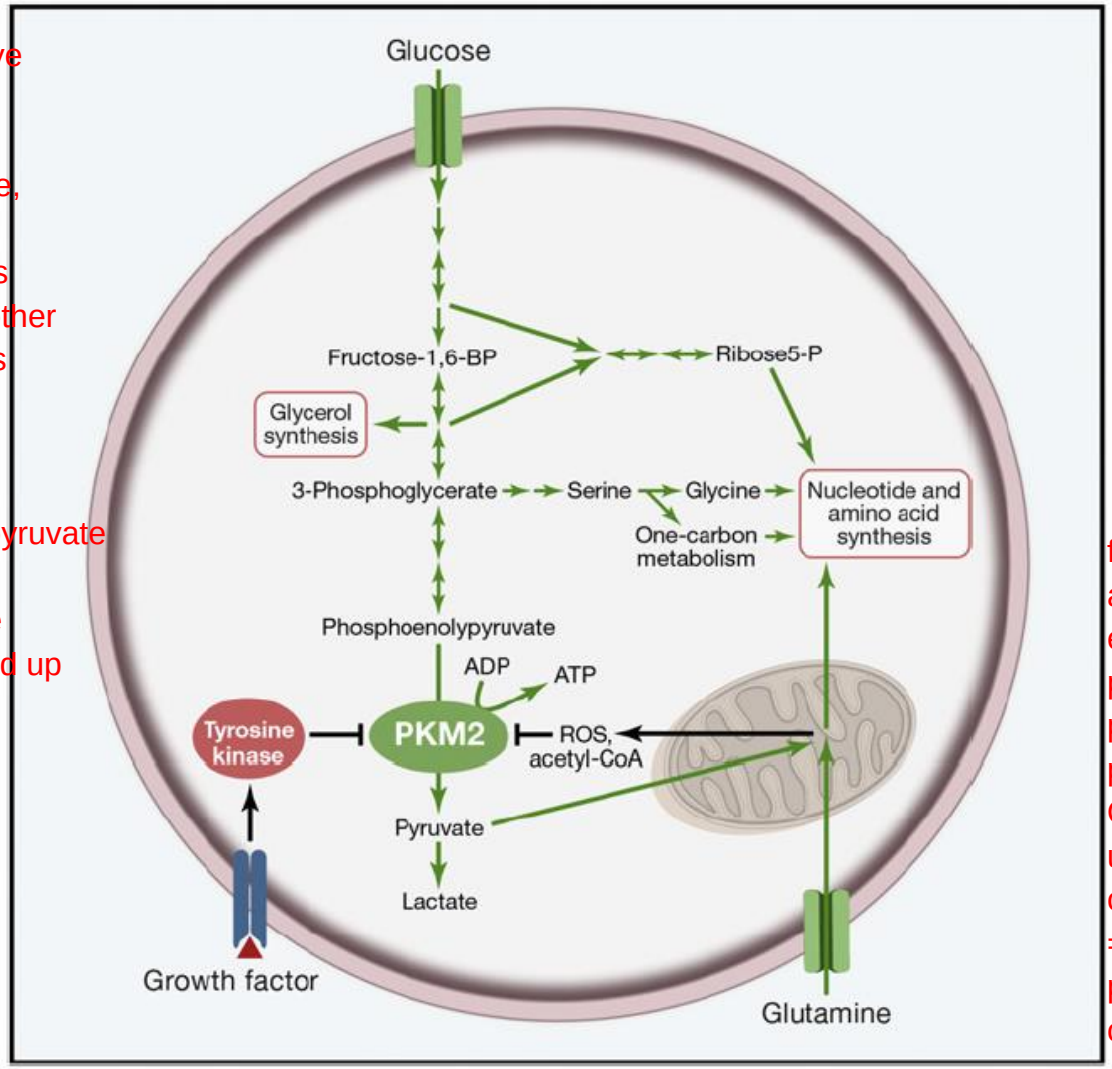
Pyruvate kinase M2 expression in proliferating cells facilitates macromolecular synthesis

the M1 isoform is more active than M2 isoform

because PKM2 is less active, there will be a build up of all the other intermediates which will simply flow in another pathway and other reactions will occur.

If PKM1 was active instead, we will get of course more pyruvate and lactate, but all the other pathways won't occur, since the building blockks are used up a lot faster

for cancer treatment:
no inhibition of PKM2, but it depends what you want to reach in the end.



tetramere: high activity => more pyruvate and lactate

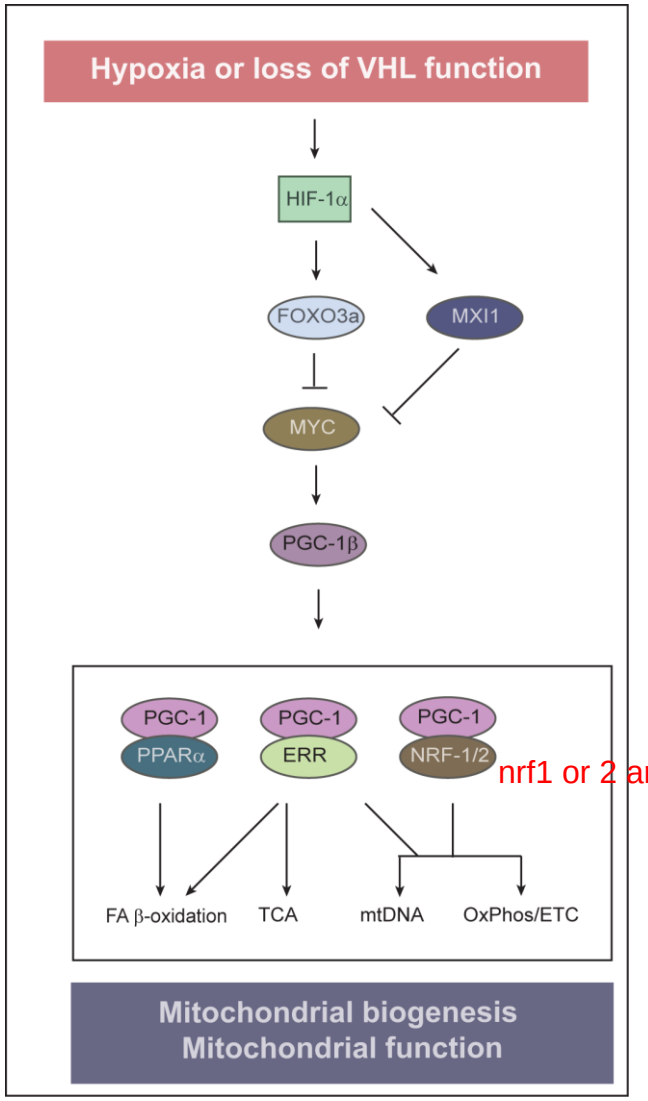
dimer: less activity => build up of intermediates => used for other pathways

It is a shifting situation with more or less active stages throughout time (oscillating)

for cancer treatment: look for activators, so there is no extreme build up. We prevent the anabolic pathway to occur, even if we produce more lactate.
Of course, lactate can be used by other mutated tumor cells. difficult situation:
=> what do you want to kill? proliferating or aggressive cells?

Ward and Thompson, Cancer Cell 2012

Regulation of mitochondrial biogenesis and abundance by HIF-α



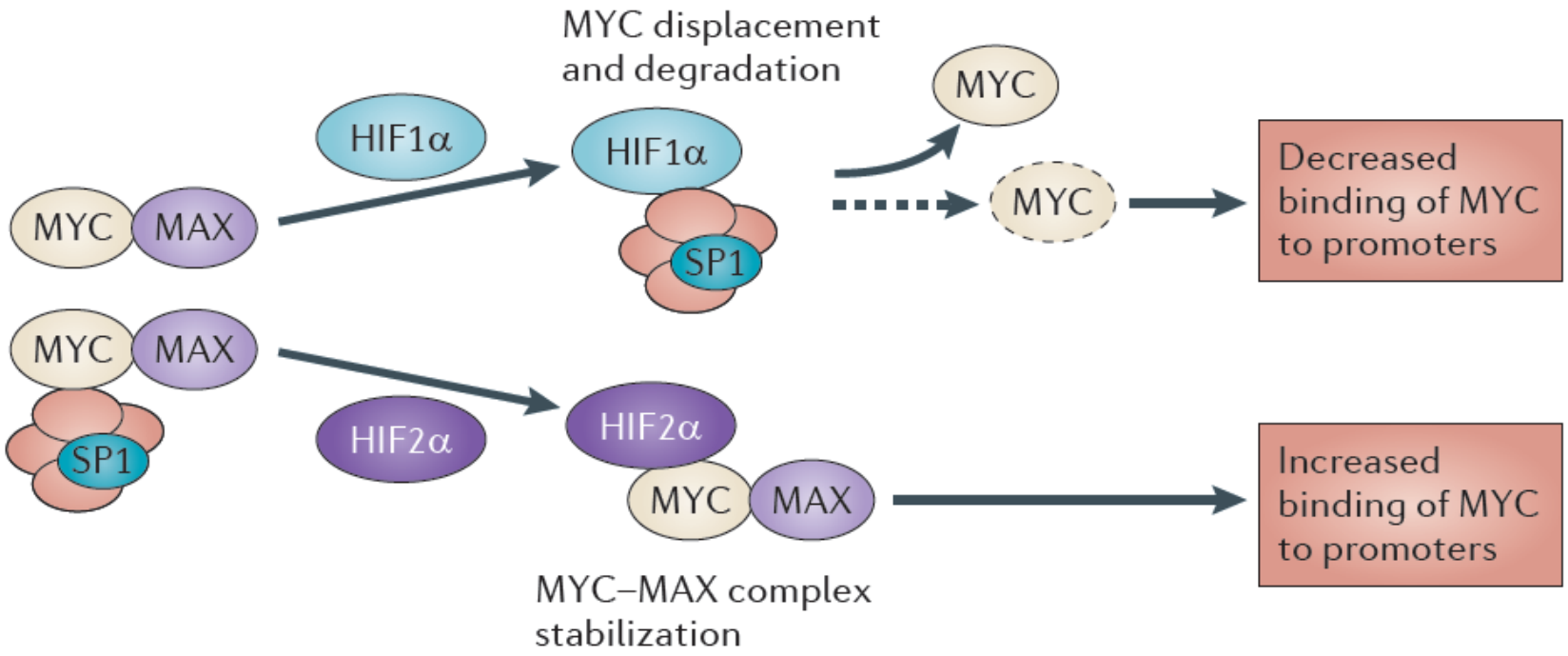
VHL: gene/protein: has tumor suppressing properties

nrf1 or 2 are different transcription factors

Schönenberger and Kovacs, Front. Cell Dev. Biol. 2015

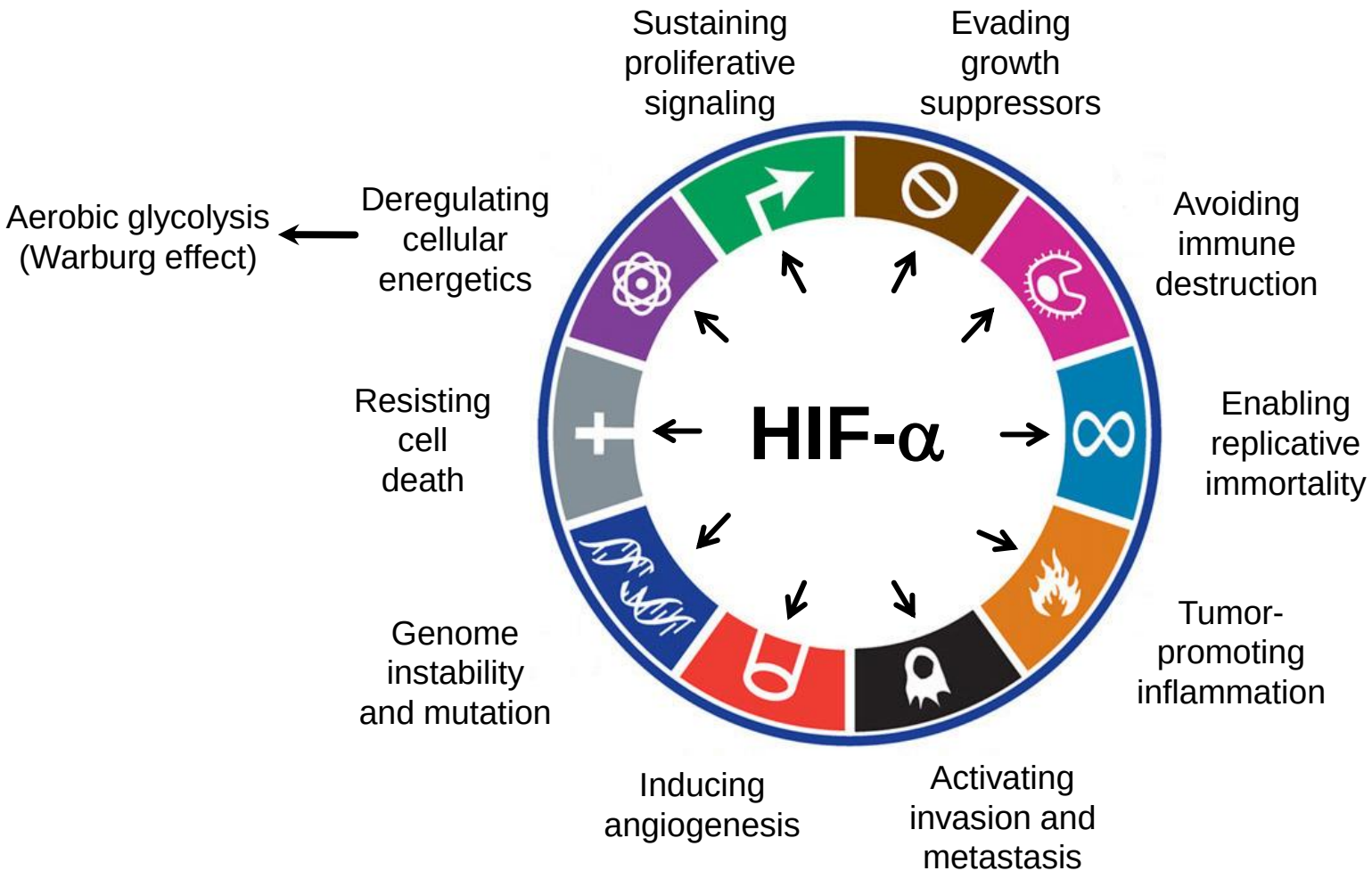
Distinct effects of HIF-1α and HIF-2α on MYC complex formation and promoter occupancy

MYC: transc. factor, upregulated in many cancer cells.
HIF-1a and MYC promote cancer proliferation and growth, but HIF-1a downregulates MYC which is somewhat strange



Keith et al., Nature Rev. Cancer 2012

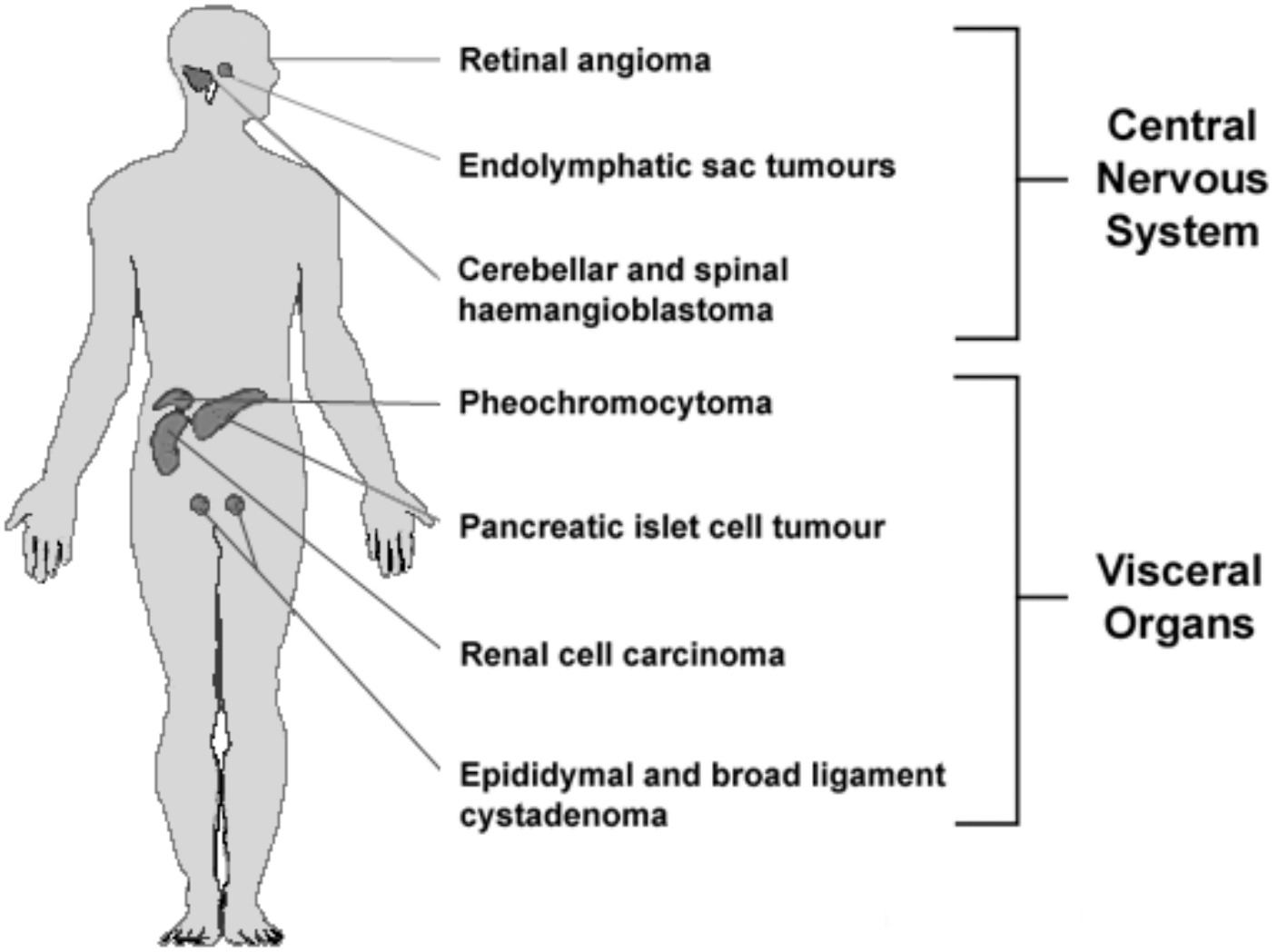
HIF signaling supports all hallmarks of cancer



modified from Hanahan and Weinberg, Cell 2011

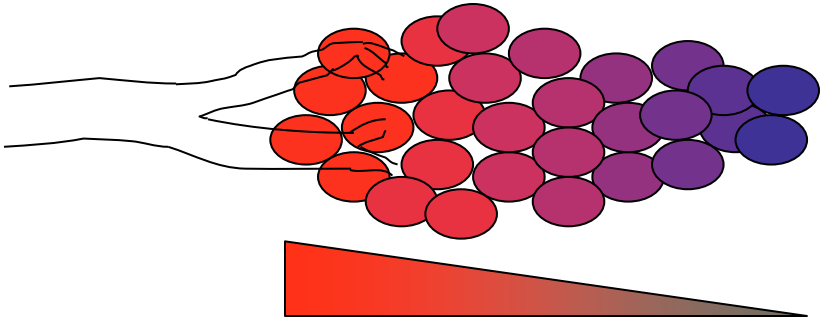
Oxygen homeostasis and cancer: insights from a rare disease, the von Hippel-Lindau (VHL) disease

VHL protein downregulates HIF-1a

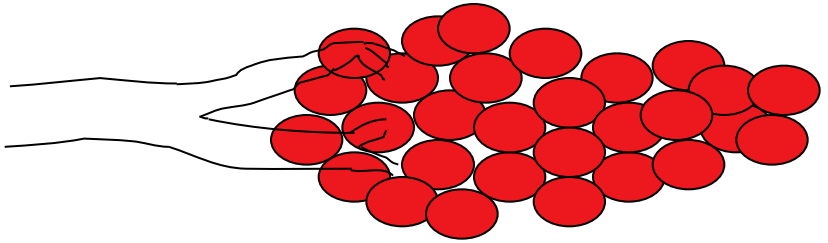


Activation of HIF during tumor growth

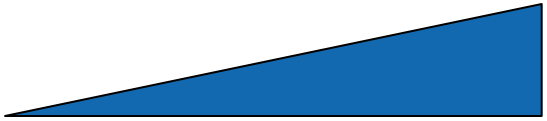
Solid tumors



VHL-associated disease



oxygen concentration



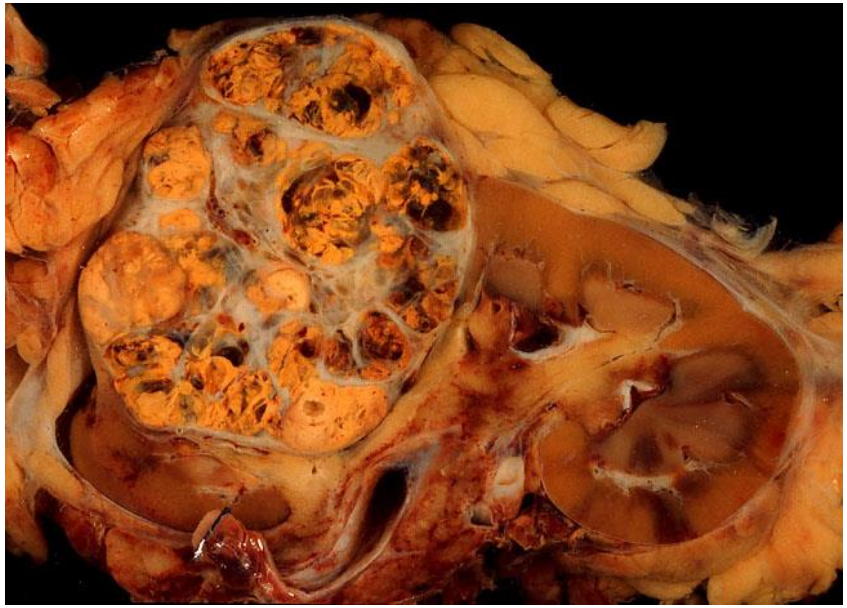
HIF activity

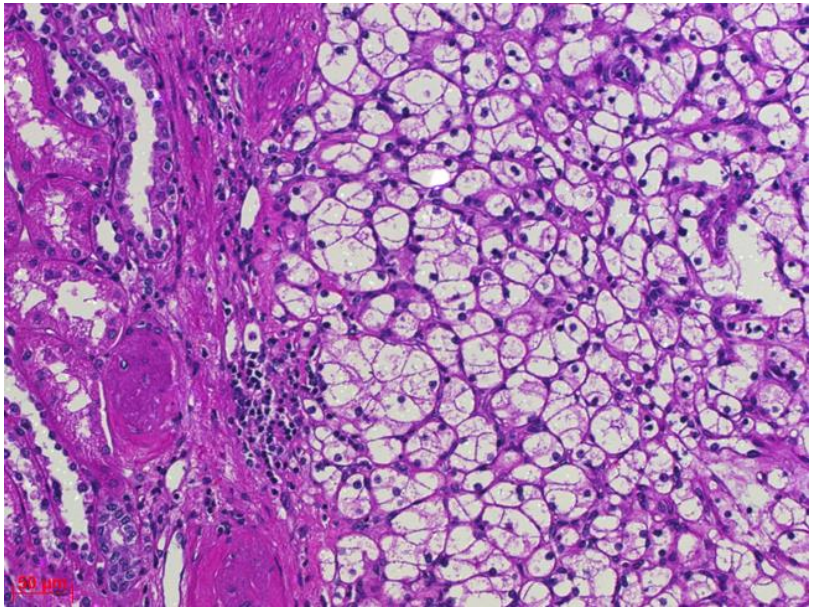
HIF activity

Adaptive responses

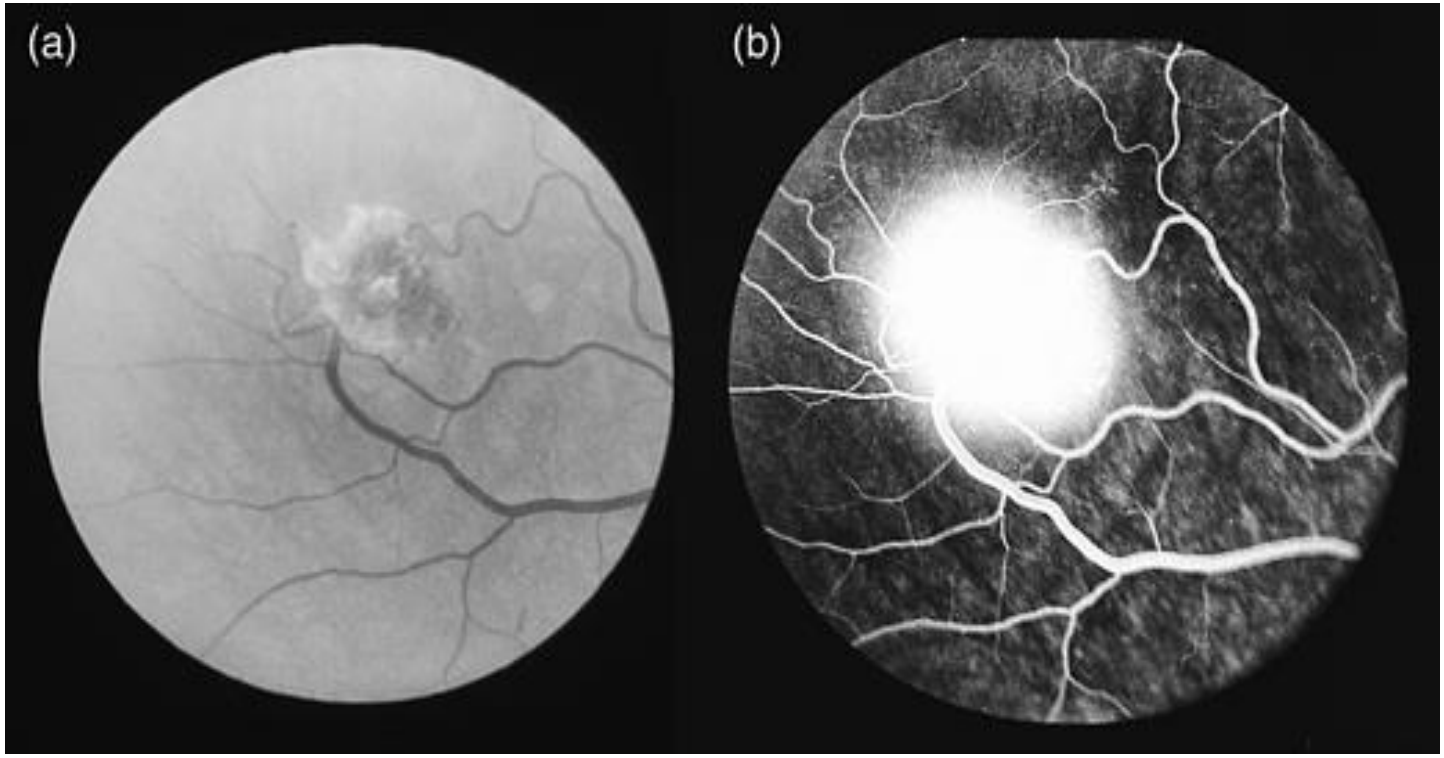
Metabolism, proliferation, differentiation, survival, apoptosis, angiogenesis, invasion, metastasis

Lipid accumulation in clear cell renal cell carcinoma: a common feature of the VHL cancer syndrome





Retinal haemangioblastoma in von Hippel-Lindau disease

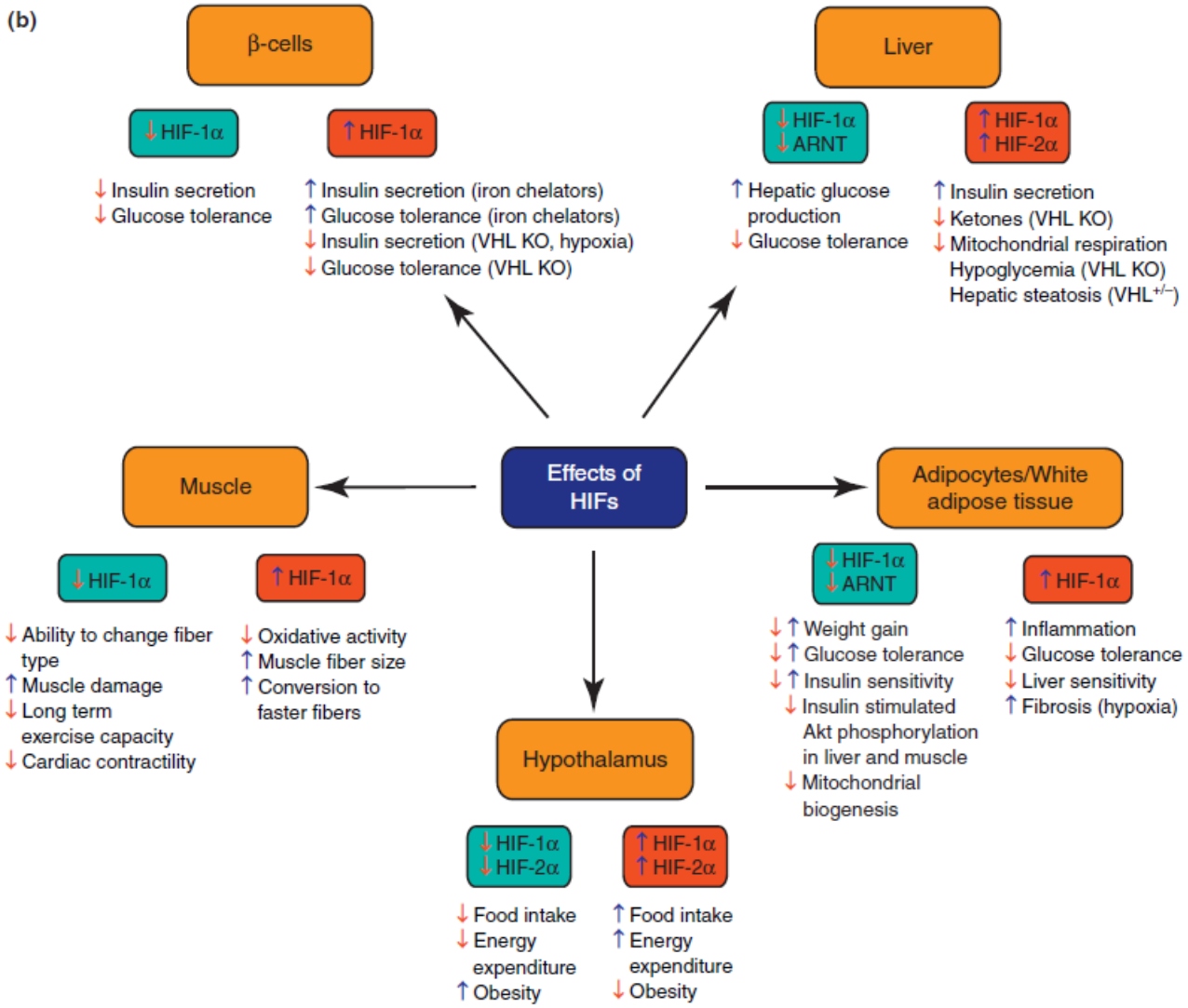


Vascular tumor - a mass of tortuous blood vessels

Fluoroscein dye identifies blood vessles

Links between HIFs, type 2 diabetes, and metabolic syndrome

nice to learn by heart (b)

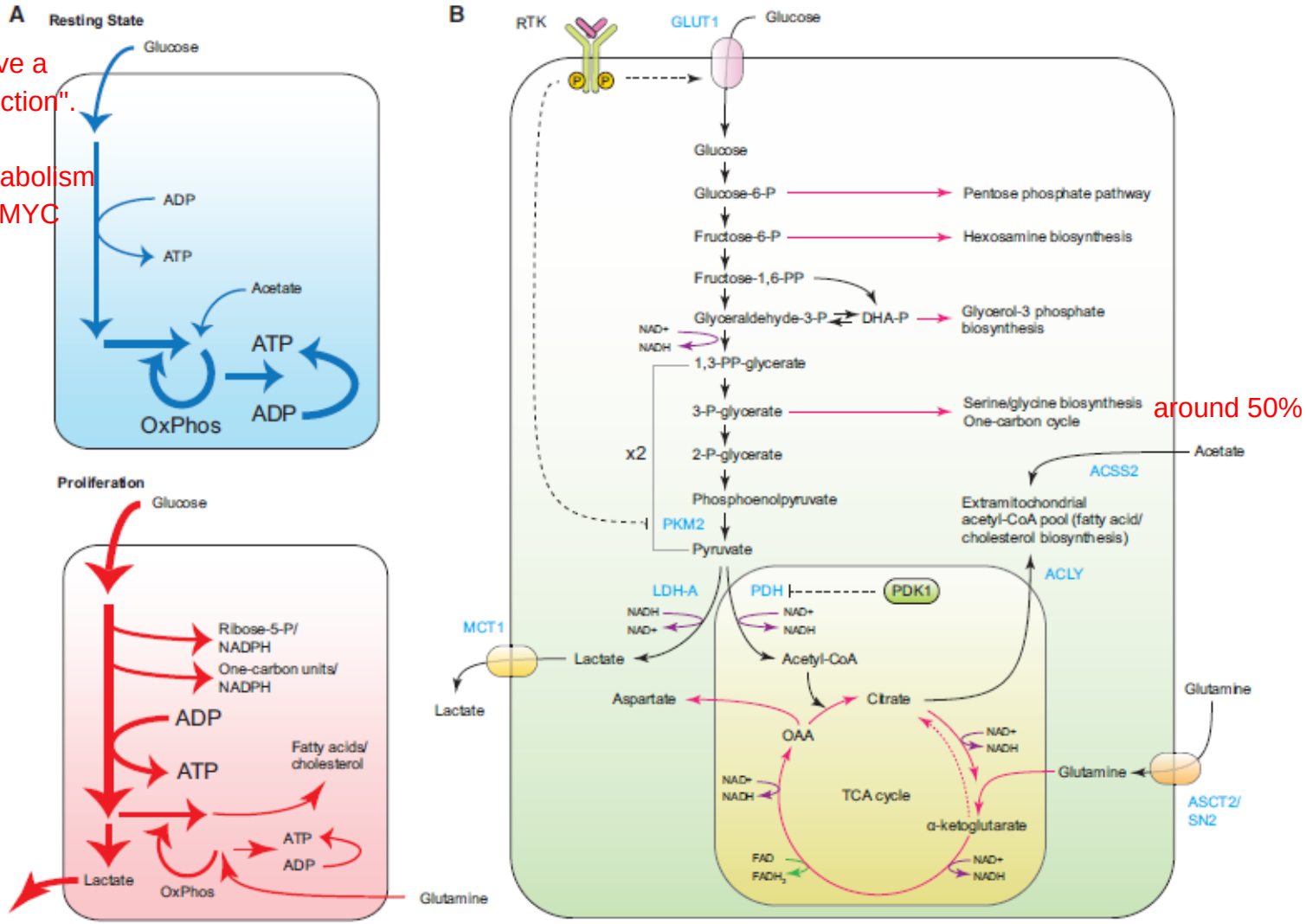


Girgis et al., Trends Endocrinol. Metab. 2012

Use of glycolysis/TCA cycle intermediates for biosynthesis and NADH production

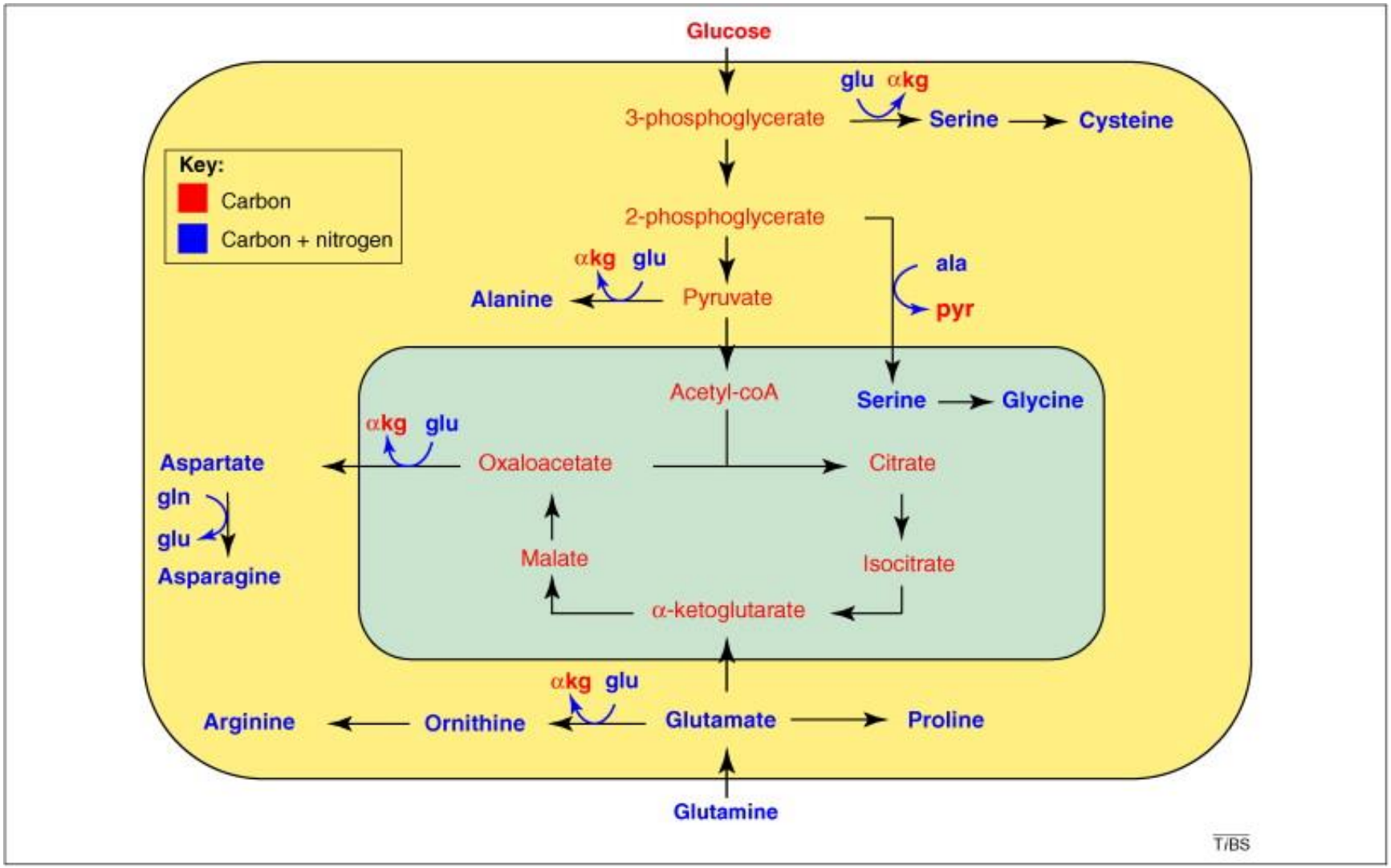
cancer cells have a "glutamine addiction".

glutamine megtabolism is regulated by MYC



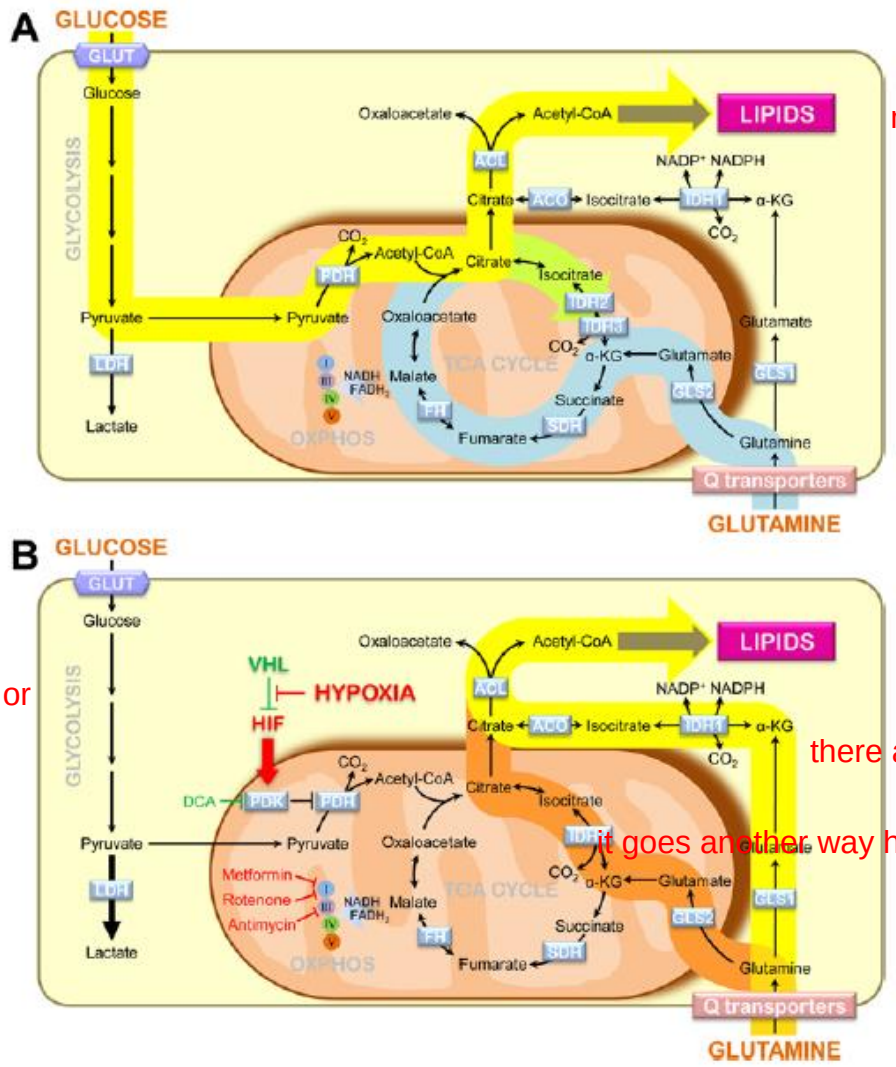
Pavlova and Thompson, Cell Metab. (2016)

Glucose and glutamine: sources for carbon and nitrogen



Wise and Thompson, Trends Biochem. Sci. (2010)

A metabolic switch in carbon source for lipid synthesis



normoxic conditions here

glutamine is the most abundant amino acid in the blood.
if you degrade the glutamine milieu, the cells do not proliferate or grow as efficiently as one would expect it.

there are two routes here actually

it goes another way here

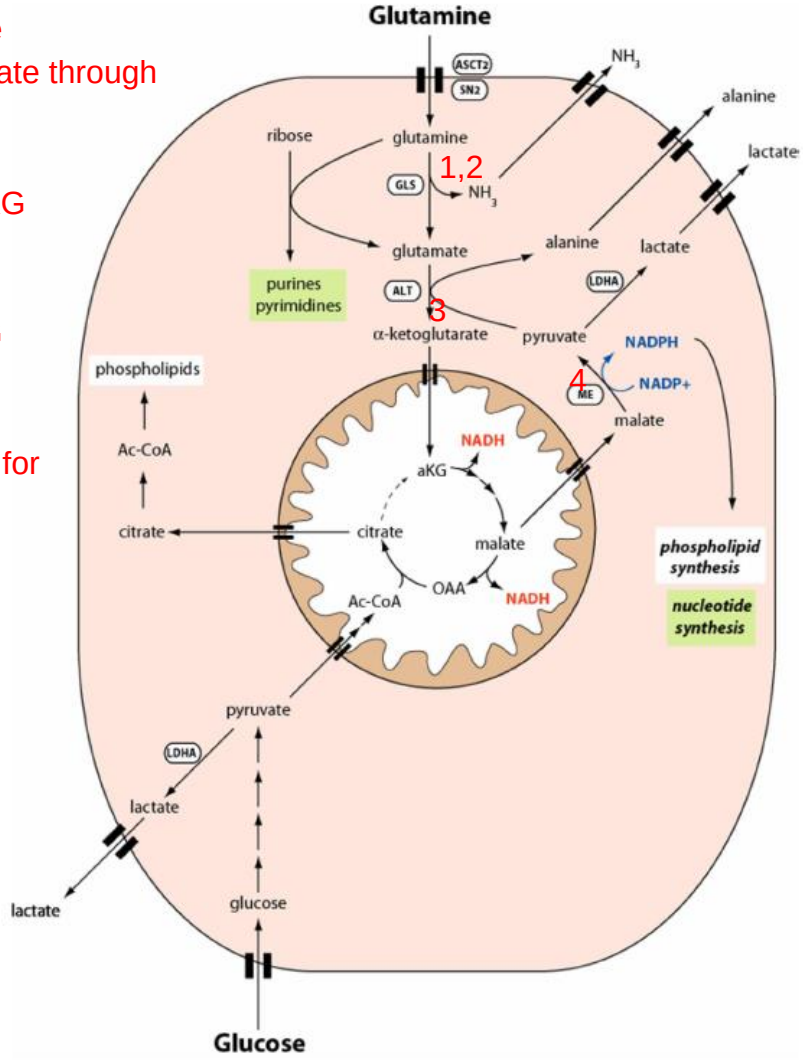
Anastasiou and Cantley, Cell Res. (2012)

Glutaminolysis leads to the generation of NADPH

1) deamidation of gluatmine to glutamate
2) transamidation of glutamine to gluramate through enzymes of nucleotide biosynthesis

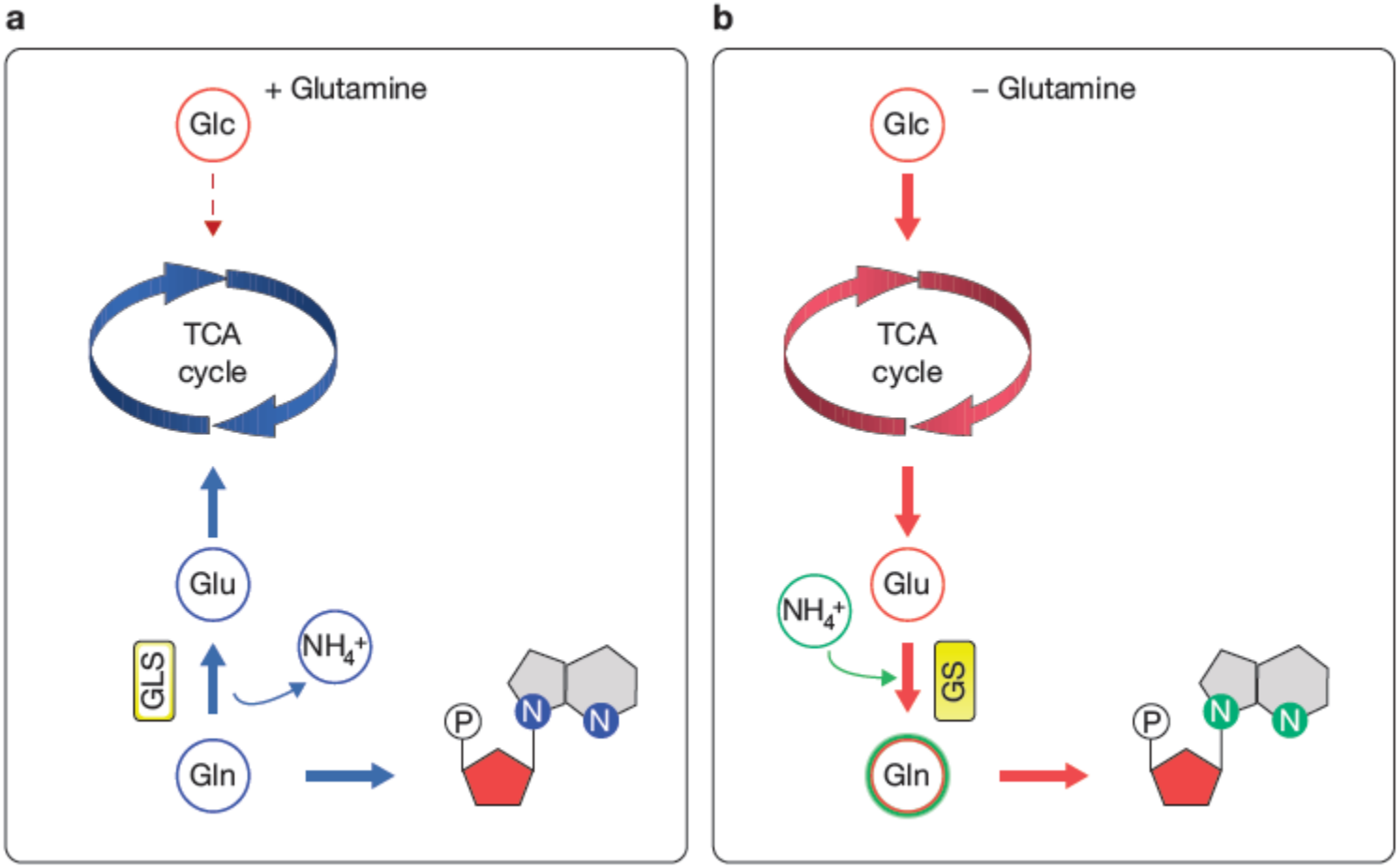
3) transamination of gluatame to alpha-KG via alanine aminotransferase

4) mitochondrial metabolism of alpha-KG to malate and the oxidation of malate to pyruvate via malci enyme (=: ME)
This generates NADPH which is needed for anabolic reacitions



Wise et al., PNAS 2008

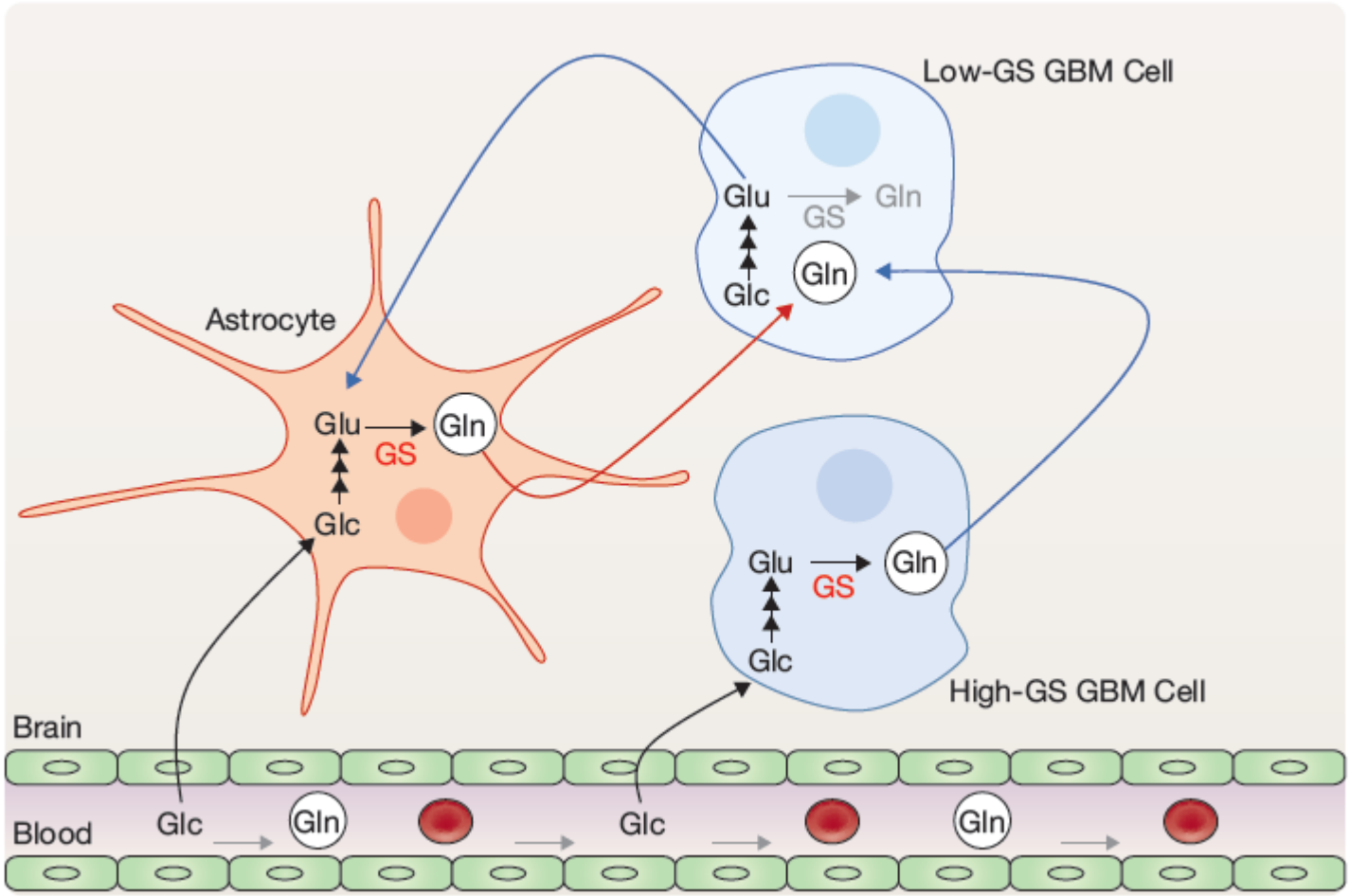
Glioblastoma proliferation under glutamine deprivation depends on glutamate-derived glutamine synthesis through glutamine synthetase



Glc, glucose
Gln, glutamine
Glu, glutamate
GS, glutamine synthetase
GLS, glutaminase

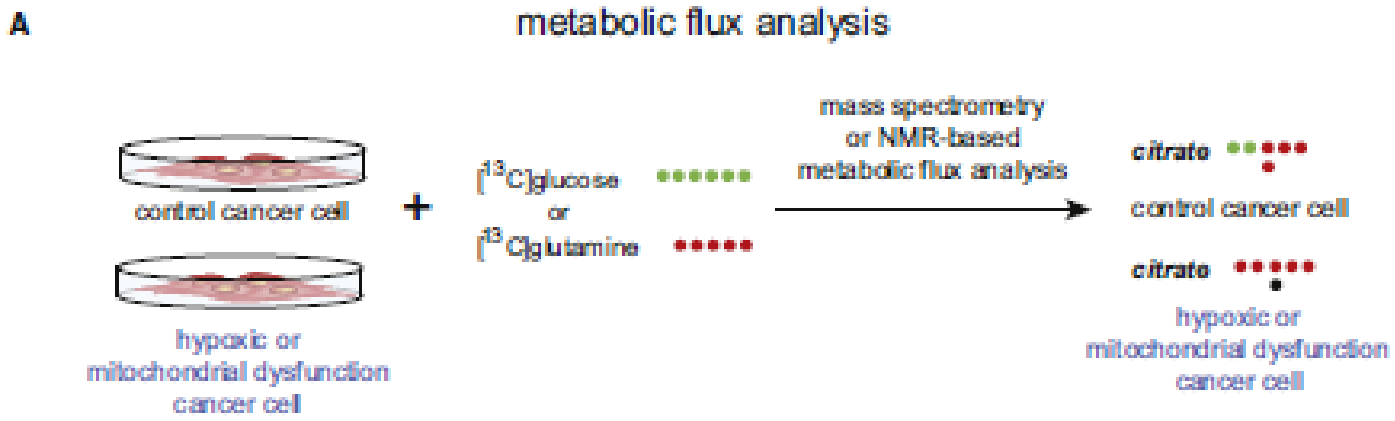
Krall and Christofk, Nat. Cell Biol. 2015

Glutamine synthetase activity within the tumor microenvironment fulfils glioblastoma glutamine requirements



Krall and Christofk, Nat. Cell Biol. 2015

Metabolic flux analysis reveals dysregulated cancer cell metabolism under hypoxia or mitochondrial dysfunction

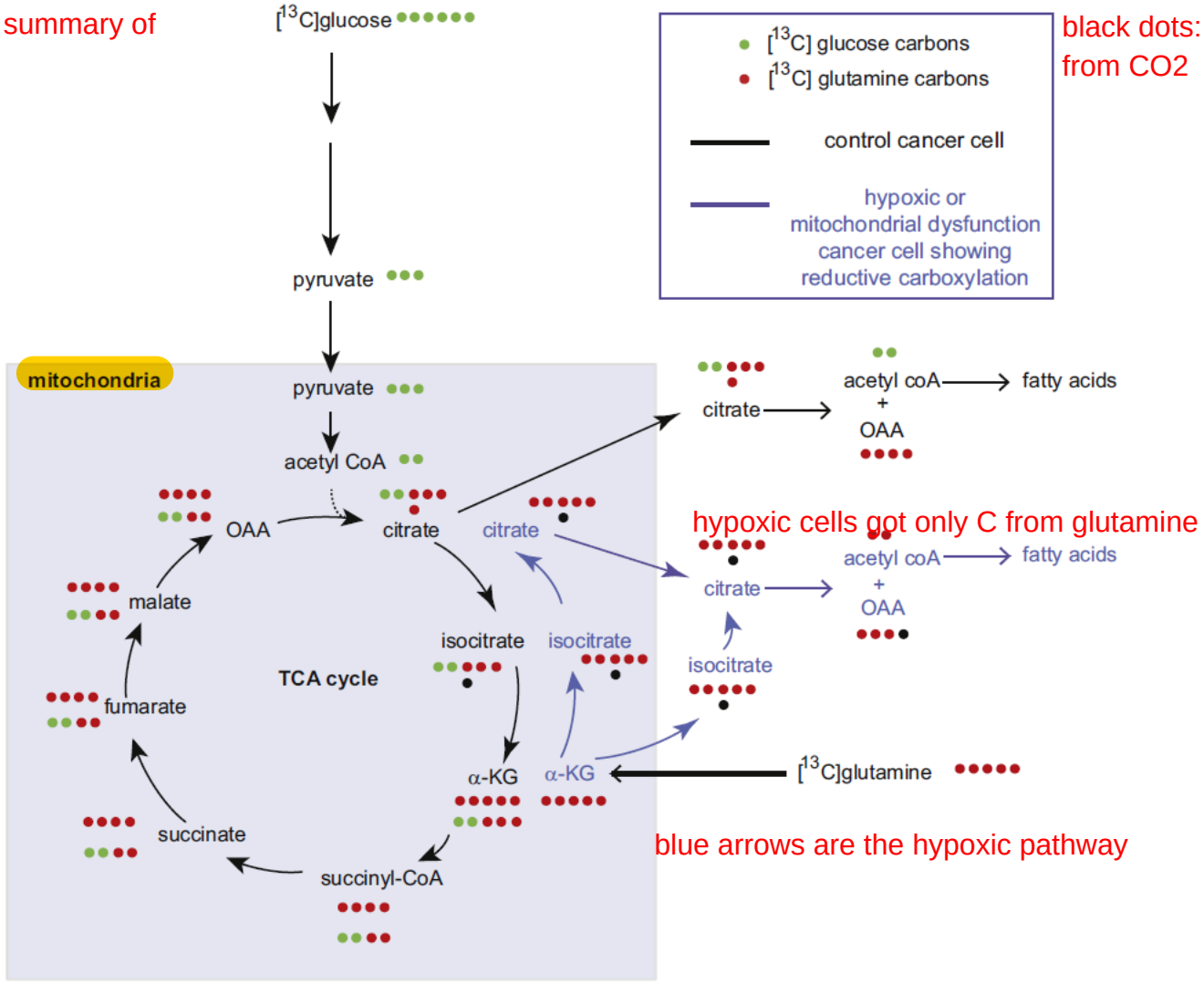


how is a metabolix flux exp. performed?
use readioactive substances (stable isotopes for labelling). one can figure out from where citrate came from, since glucose and glutamine are labelled with 13C

Benjamin et al., Cell Metab. (2012)

Metabolic flux analysis reveals dysregulated cancer cell metabolism under hypoxia or mitochondrial dysfunction

this slide is somewhat a summary of this pdf



black dots: carbons coming from CO2

blue arrows are the hypoxic pathway

Benjamin et al., Cell Metab. (2012)