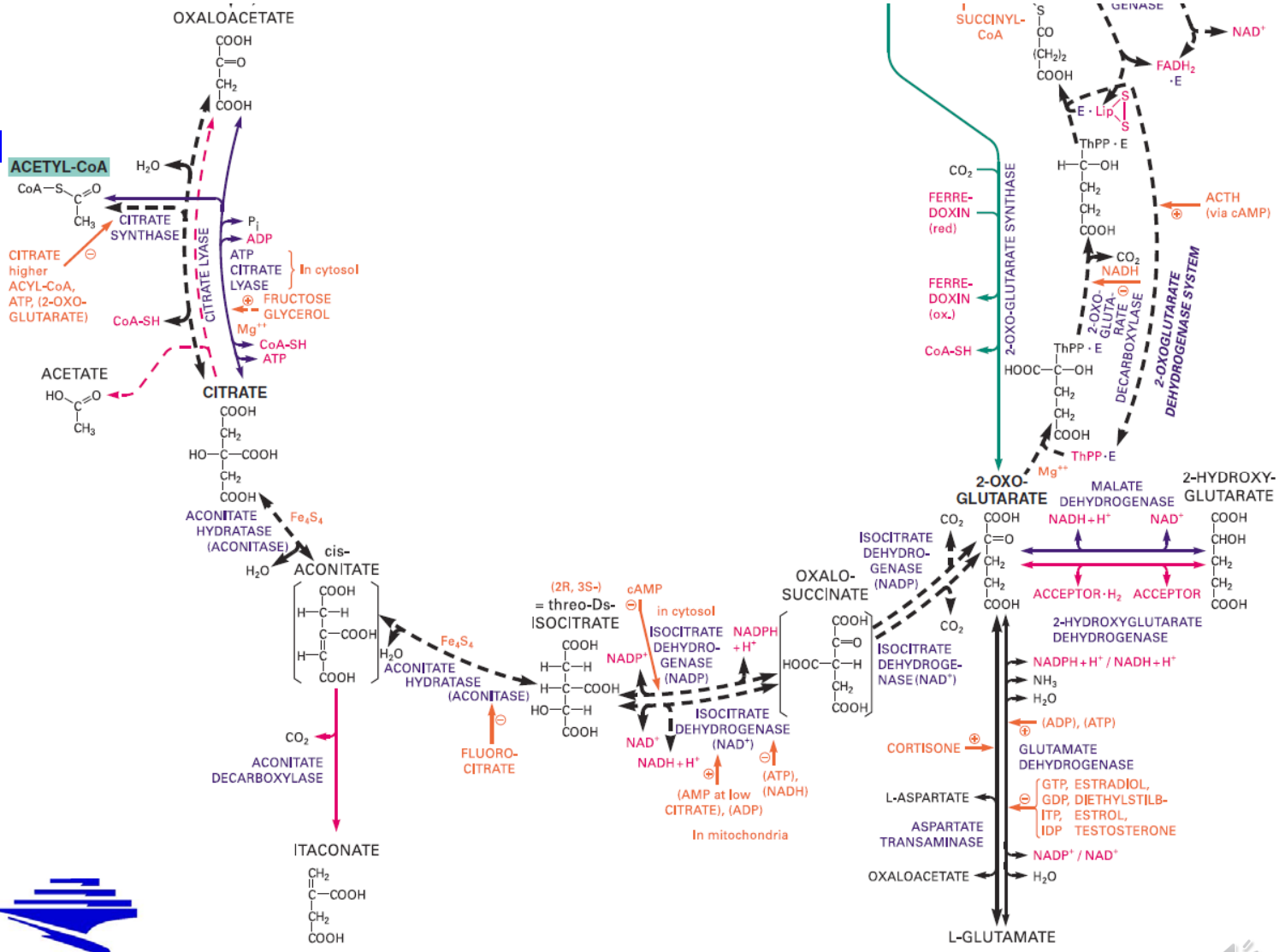
Tracing compartment-specific redox pathways using stable isotopes and mass spectrometry

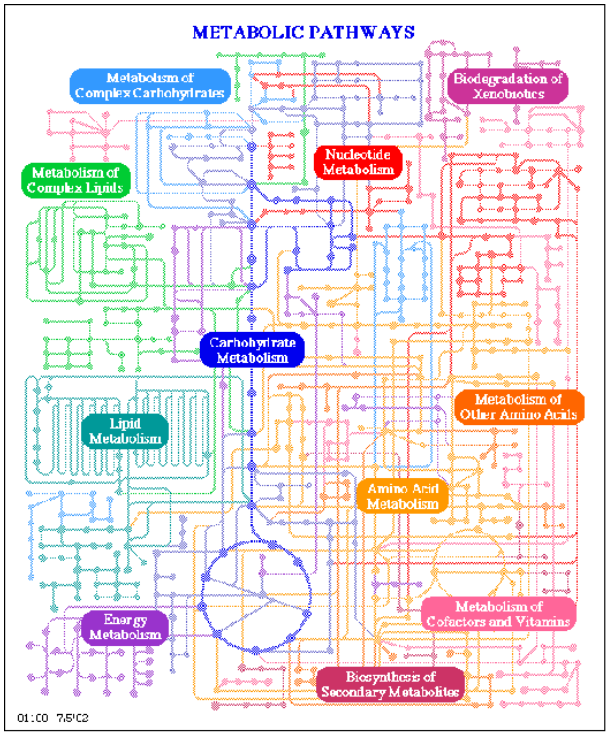


Christian Metallo
IECM 2017

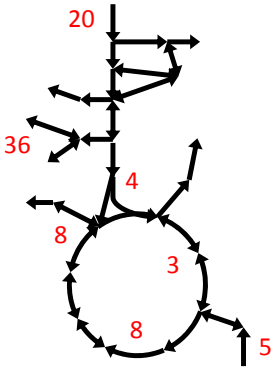
Department of Bioengineering
Moores Cancer Center
UCSD Diabetes Research Center

Michal and Schomburg Biochemical Pathways 2nd Ed. 2012

The challenge for biologists, biochemists, and engineers: Translate biochemistry to metabolic fluxes



http://www.genome.jp/kegg/



$$Flux = \frac{mol}{time \cdot cell/prot}$$

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

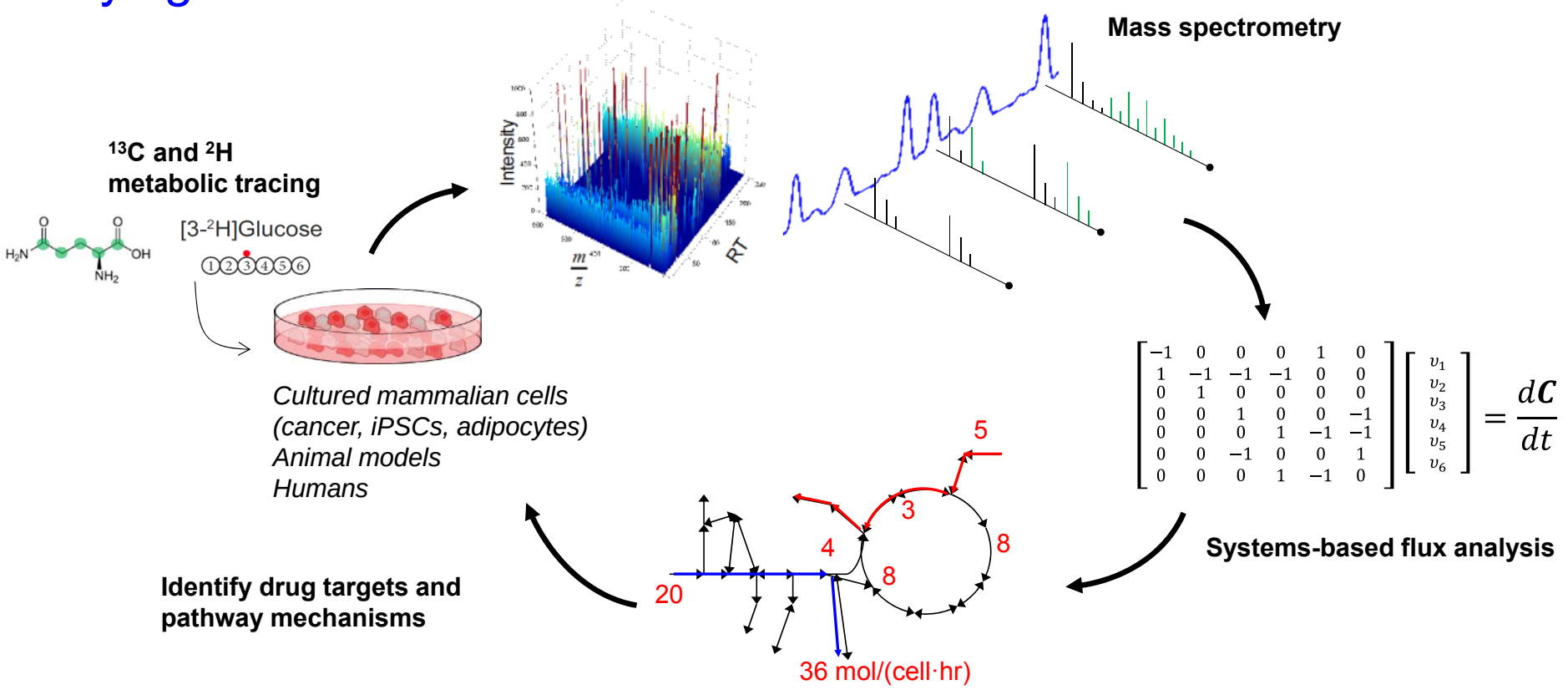
- Fluxes describe the ultimate function of metabolic enzymes
- This is where metabolomics/analytical chemistry meets cell biology
- Metabolite level measurements only get you so far

Use isotopic tracers

Analyze data as a system → MODELING!!!

$$A + B \underset{X}{\leftrightarrow} C \underset{Y}{\rightarrow} D$$

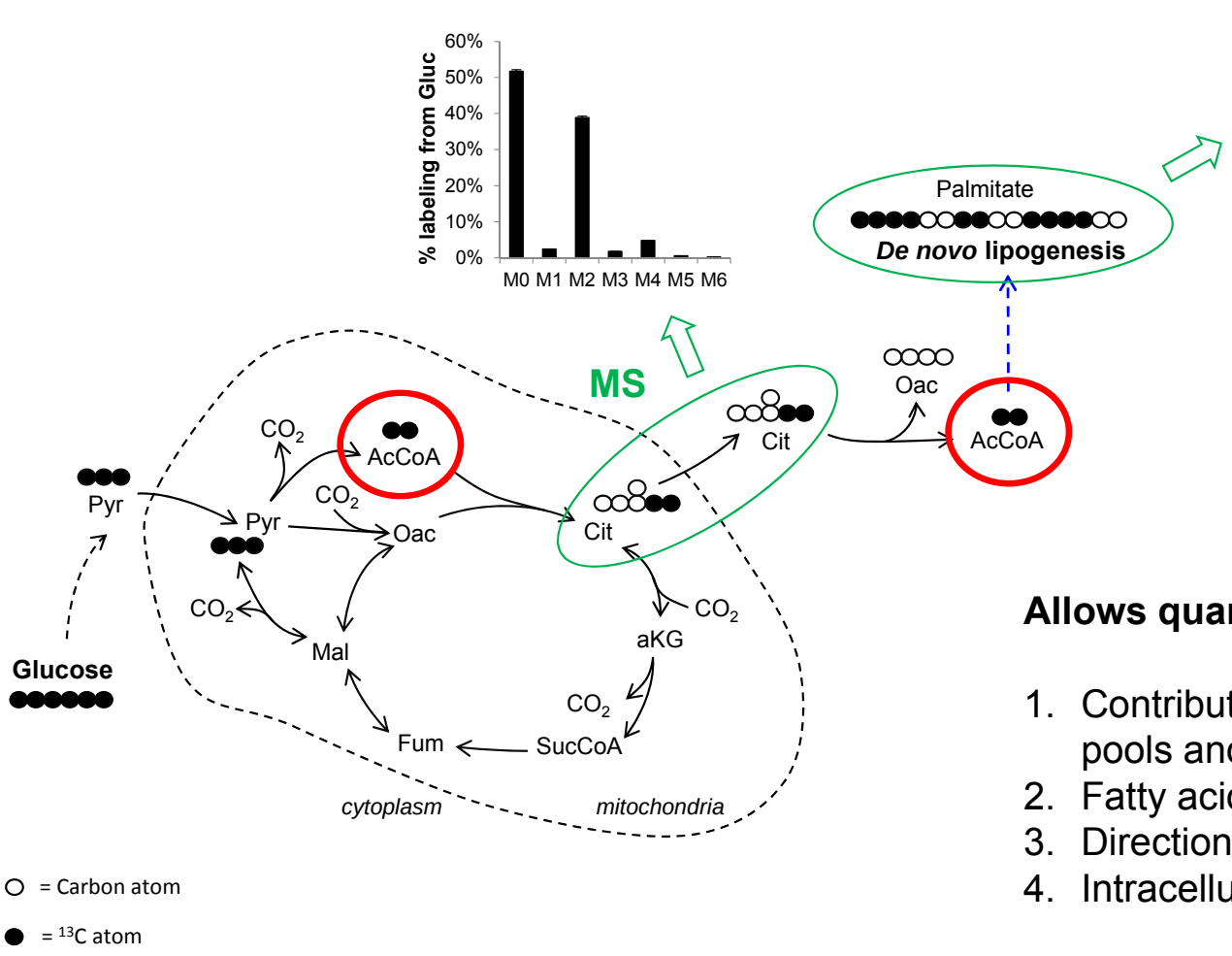
Studying metabolism for flux sake

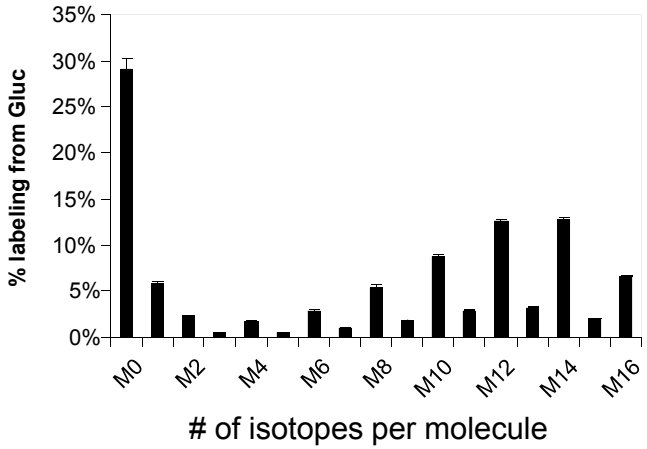


$$\begin{bmatrix} -1 & 0 & 0 & 0 & 1 & 0 \\ 1 & -1 & -1 & -1 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & 1 & -1 & -1 \\ 0 & 0 & -1 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & -1 & 0 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \end{bmatrix} = \frac{d\boldsymbol{C}}{dt}$$

1. Targeting metabolism in cancer (Grassian et al. *Canc Res* 2014; Svensson et al. *Nat Med* 2016; Parker et al. *Met Eng* 2017)
2. **Cellular compartmentalization and redox metabolism** (Lewis et al. *Mol Cell* 2014; Vacanti et al. *Mol Cell* 2014)
3. Metabolic changes during iPSC growth/differentiation (Badur et al. *Biotech J.* 2015; Zhang et al. *Cell Rep* 2016)
4. Regulation of macrophage metabolism (Cordes et al. *JBC* 2016)
5. Understanding adipose tissue metabolism and physiology in the context of T2DM (Green et al. *Nat Chem Bio* 2016)

Our approach to study cell physiology and metabolism

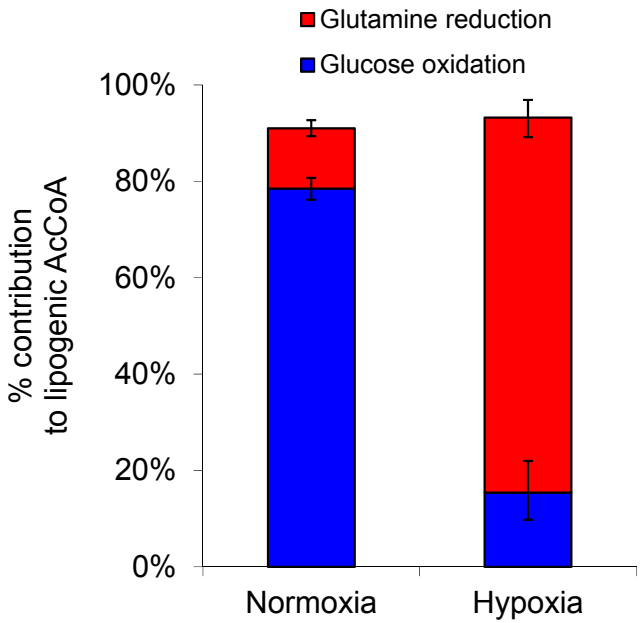


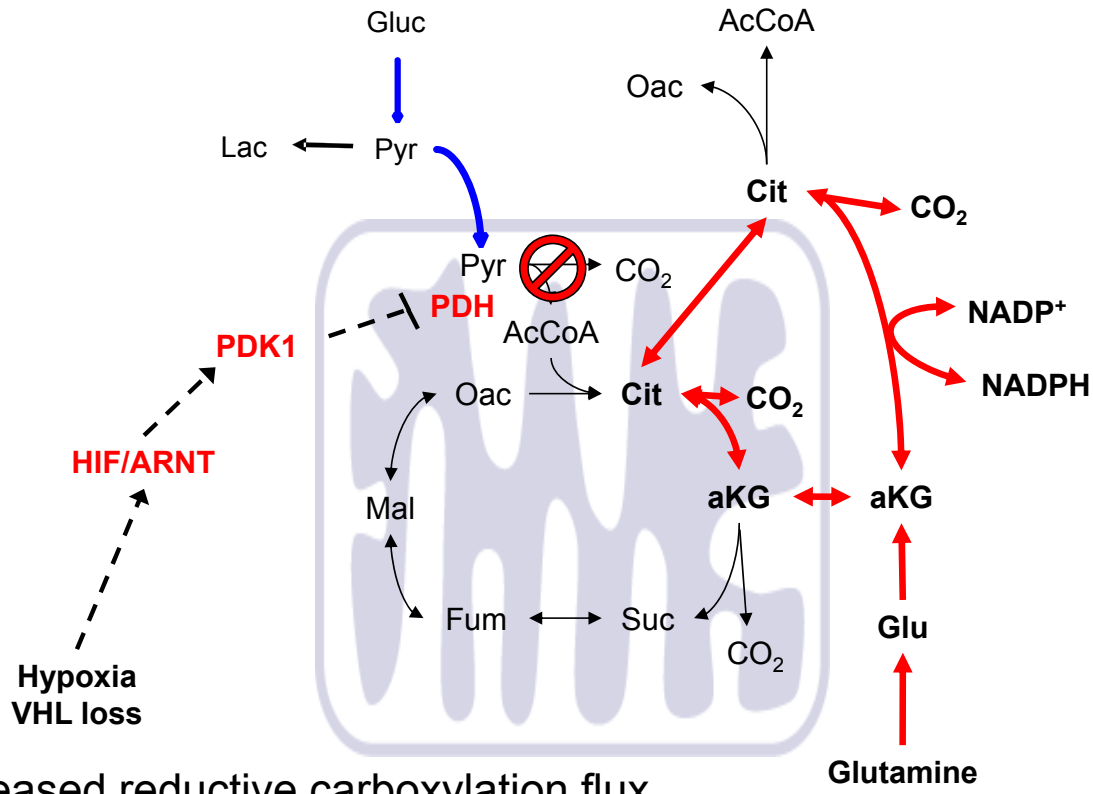


Allows quantitation of:

1. Contribution of different substrates to AcCoA pools and mitochondrial metabolism
2. Fatty acid synthesis/de novo lipogenesis rates
3. Directionality of TCA metabolism
4. Intracellular fluxes with MFA modeling

Reprogramming of TCA metabolism under hypoxia



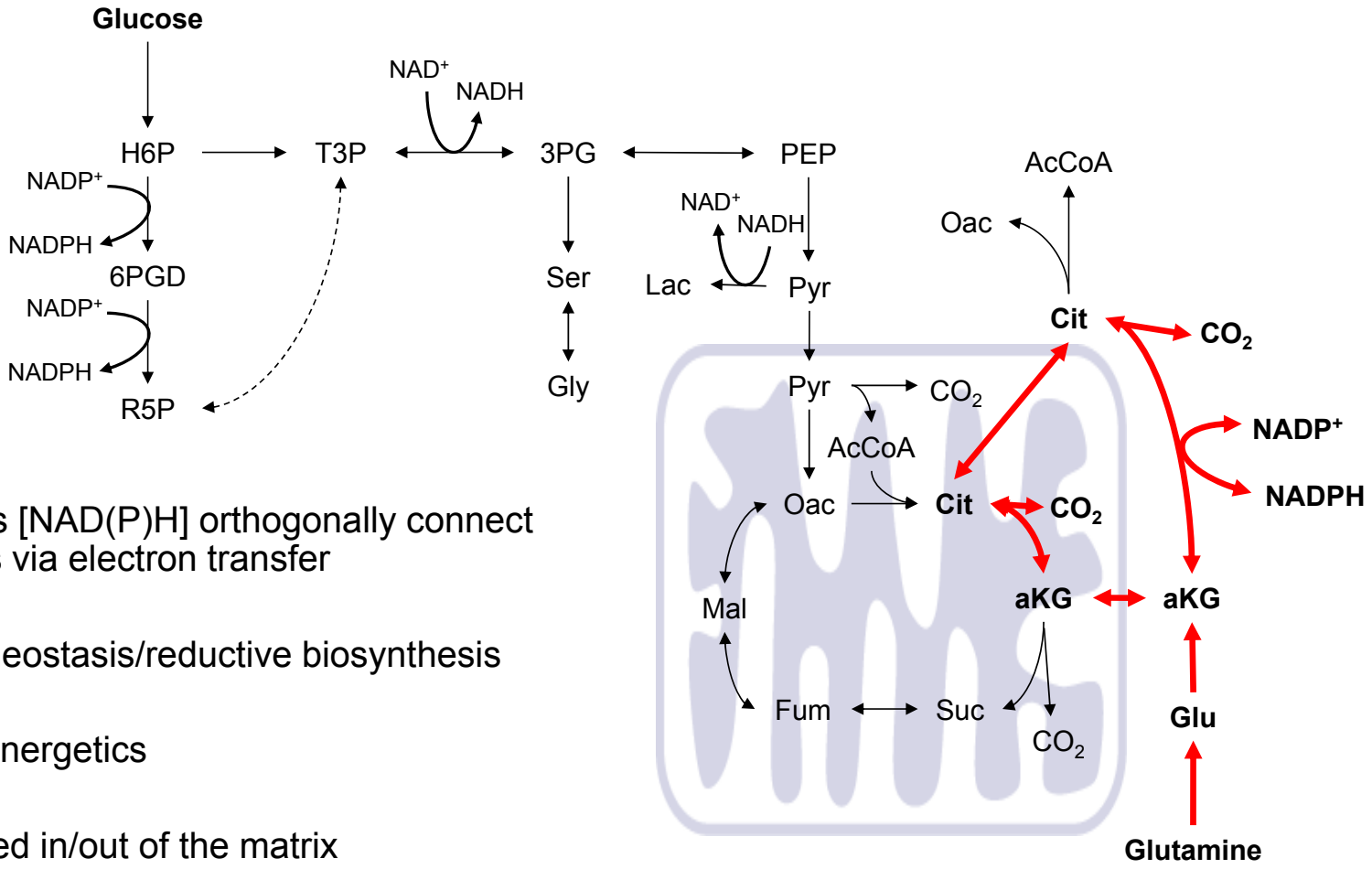


Hypoxic and pseudohypoxic cells exhibit increased reductive carboxylation flux

- Compartmentalization of metabolic processes is critical for cell function (but complicates analysis)
- Redox metabolism is perturbed by hypoxic stresses

Metallo et al. *Nature* (2012), Mullen et al. *Nature* (2012), Scott et al. *JBC* (2011), Wise et al. *PNAS* (2011)

Redox metabolism is highly compartmentalized



Pyridine nucleotides [NAD(P)H] orthogonally connect metabolic pathways via electron transfer

NADPH: redox homeostasis/reductive biosynthesis

NADH: cellular bioenergetics

Neither is transported in/out of the matrix

Eukaryotes are highly compartmentalized

^{13}C tracing and metabolomics **cannot** resolve compartment-specific metabolism

How are NADPH and NADH regenerated in the cytosol and mitochondria?

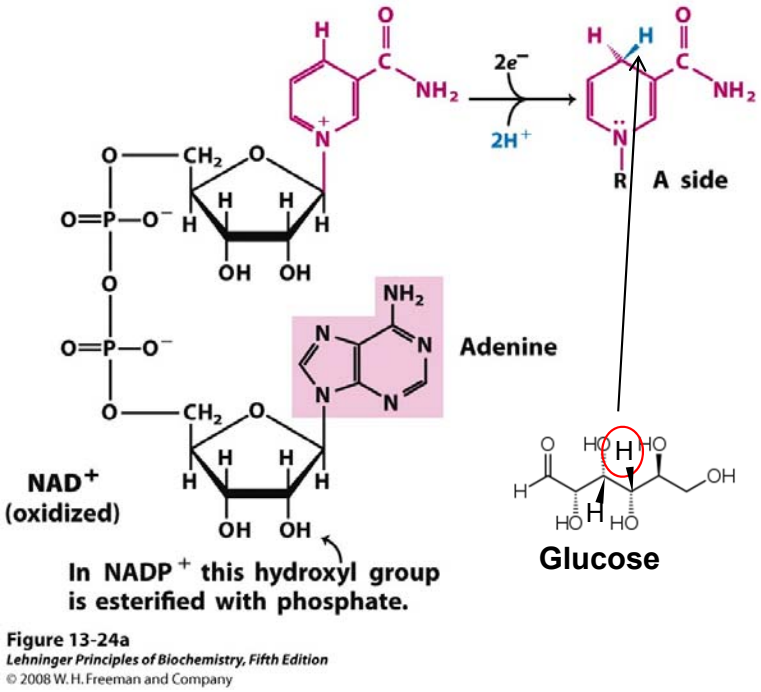
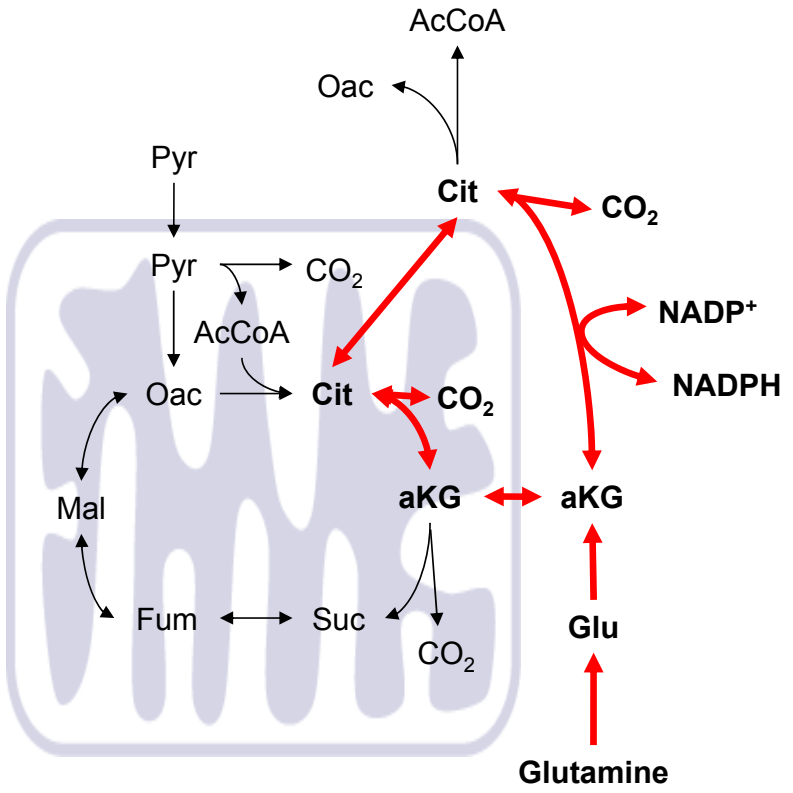
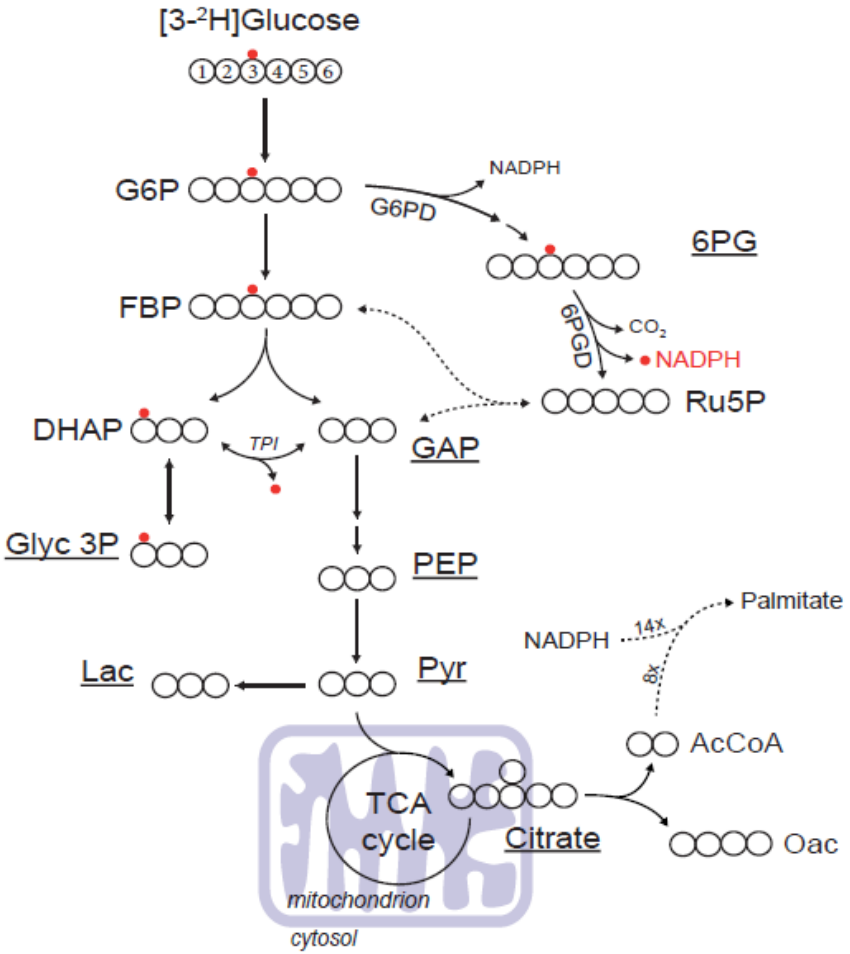
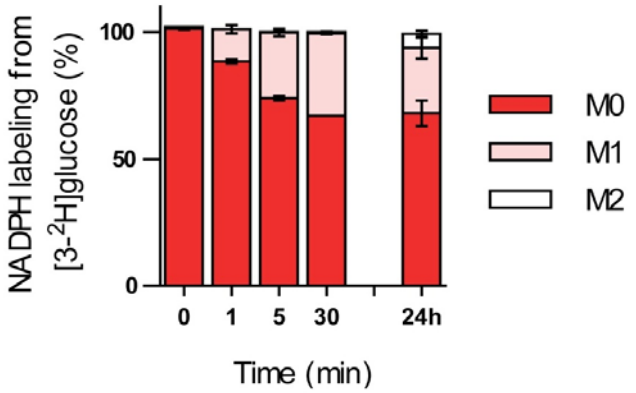


Figure 13-24a
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company



Tracing the oxidative PPP with [^{2}H]glucose

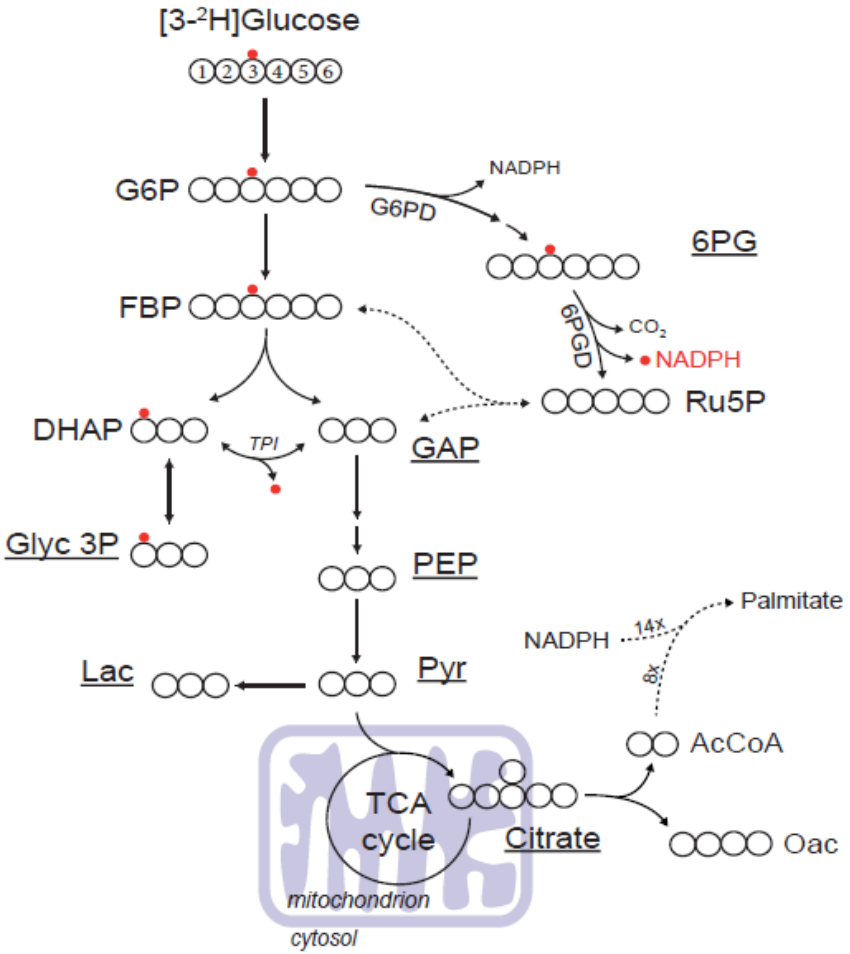


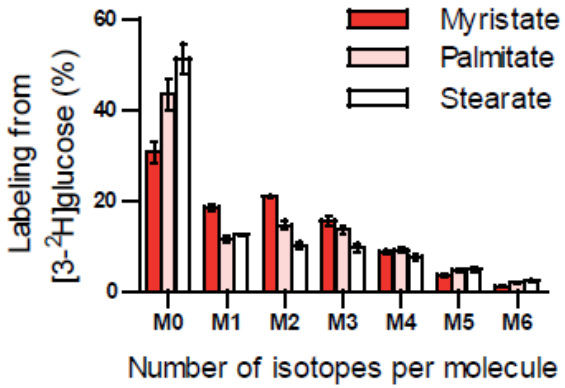


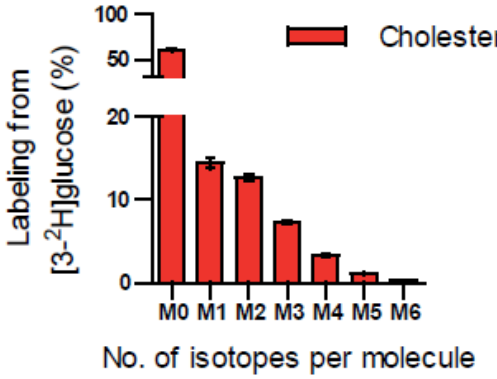
w/ Matt Vander Heiden (MIT)

Lewis et al. *Molecular Cell* 2014

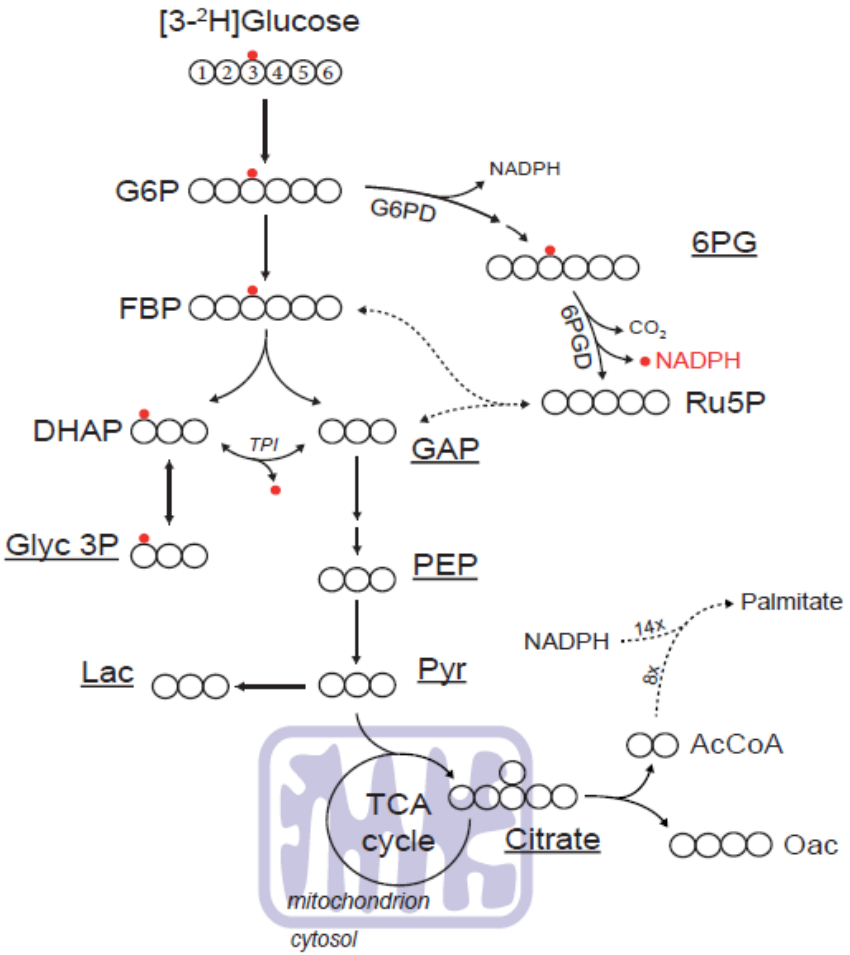
Tracing the oxidative PPP with [^{2}H]glucose

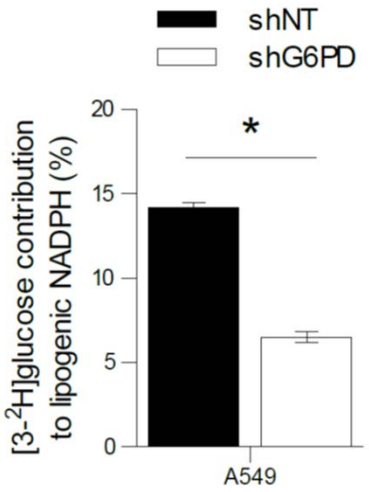


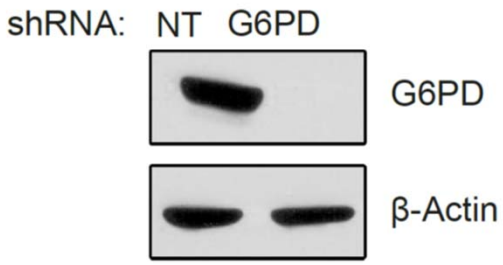




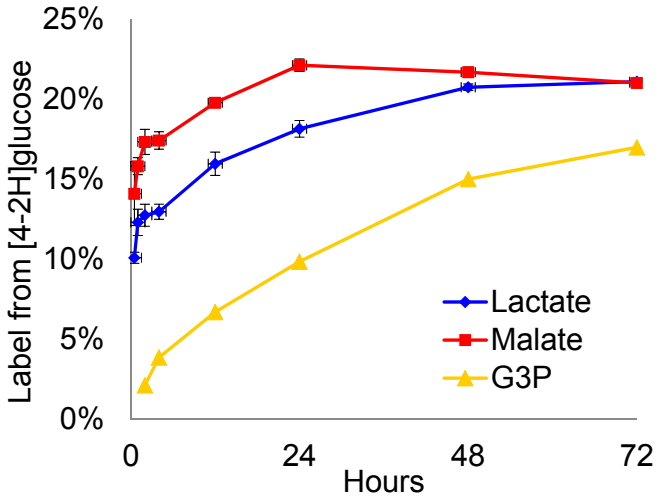
Contribution of the oxidative PPP to NADPH pools

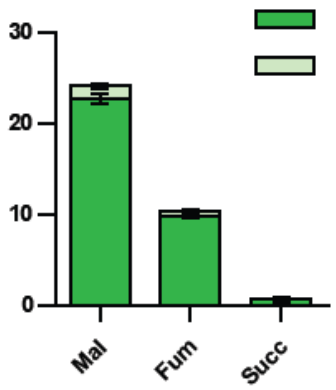




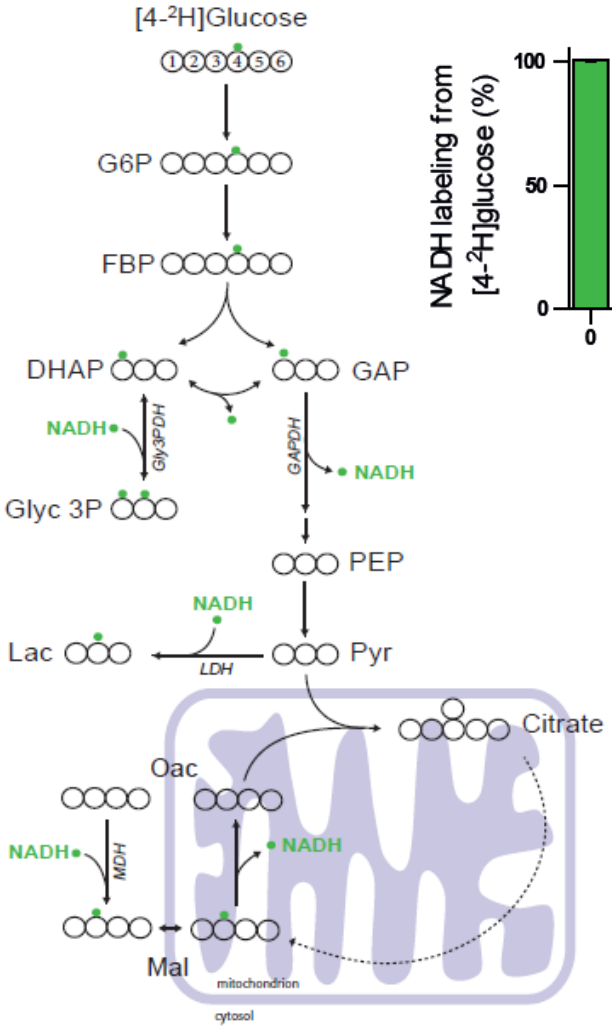


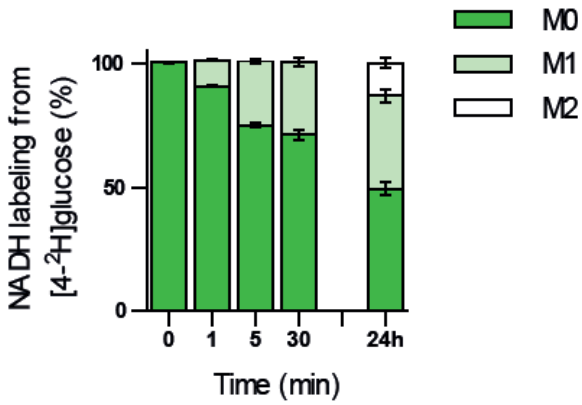
NADH shuttles and mitochondrial metabolism regenerate NAD^+ for glycolysis





2H label enters TCA cycle via malate-aspartate shuttle

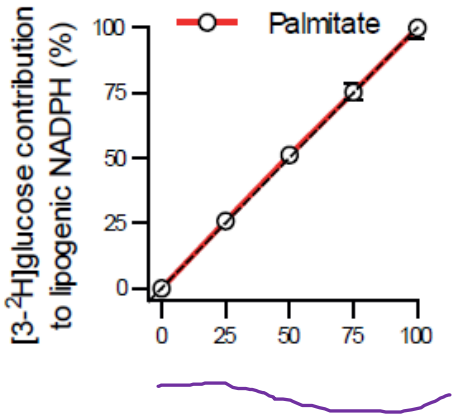




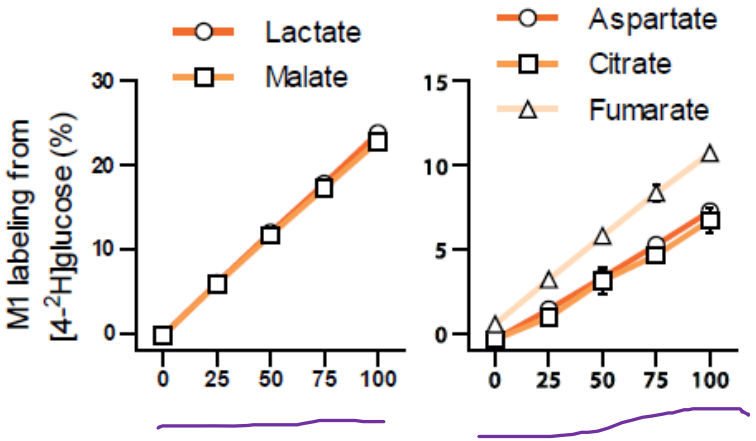
Do kinetic isotope effects affect results?

- Deuterium lowers rates in enzyme reactions (*in vitro*)
- Is this relevant to tracing through metabolic networks?
  - Allow “H” and “D” to compete by diluting
  - Compare labeling

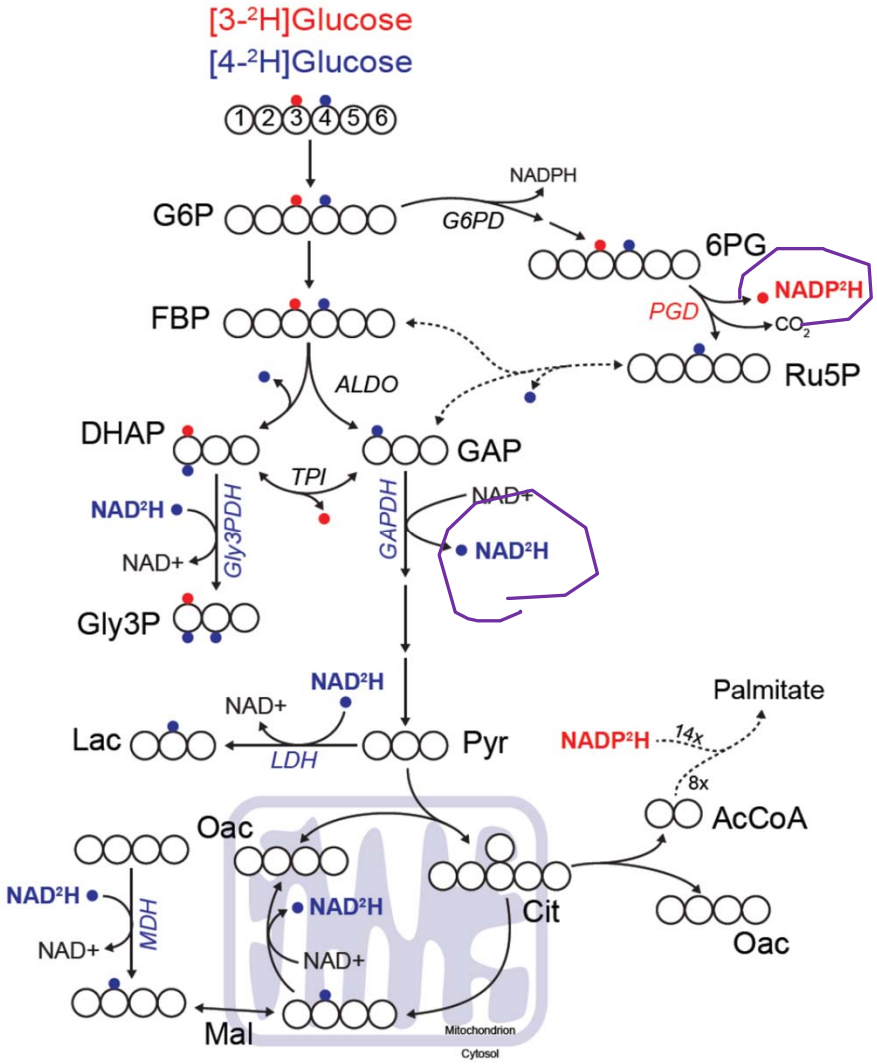
Cytosolic NADPH pathways

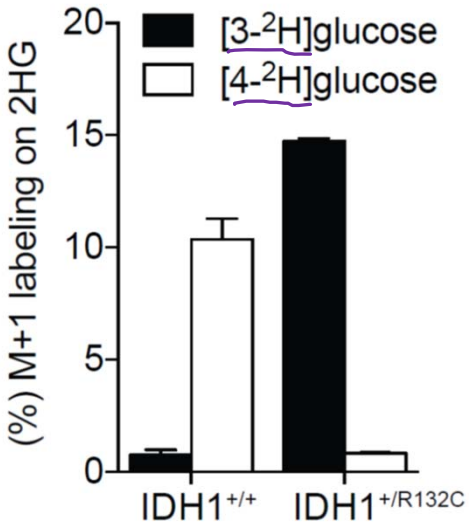


NADH metabolism



(L)2HG and (D)2HG have different origins and are labeled distinctly via 2H tracers





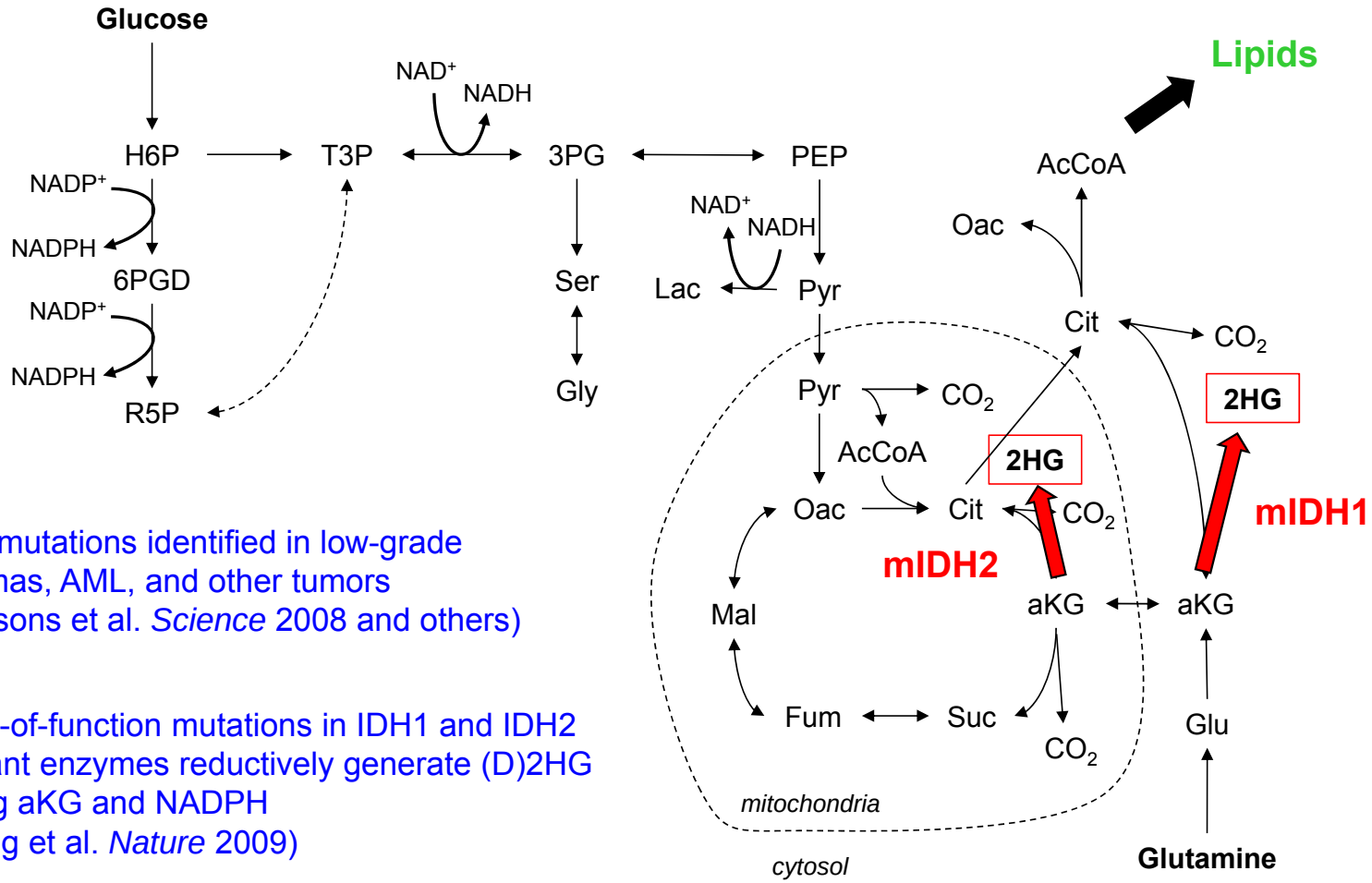
(L)2HG (D)2HG

MDH and LDH generate (L)2HG from NADH

Oncogenic IDH1 generates 2HG from cytosolic NADPH

2HG is distinctly labeled by these tracers

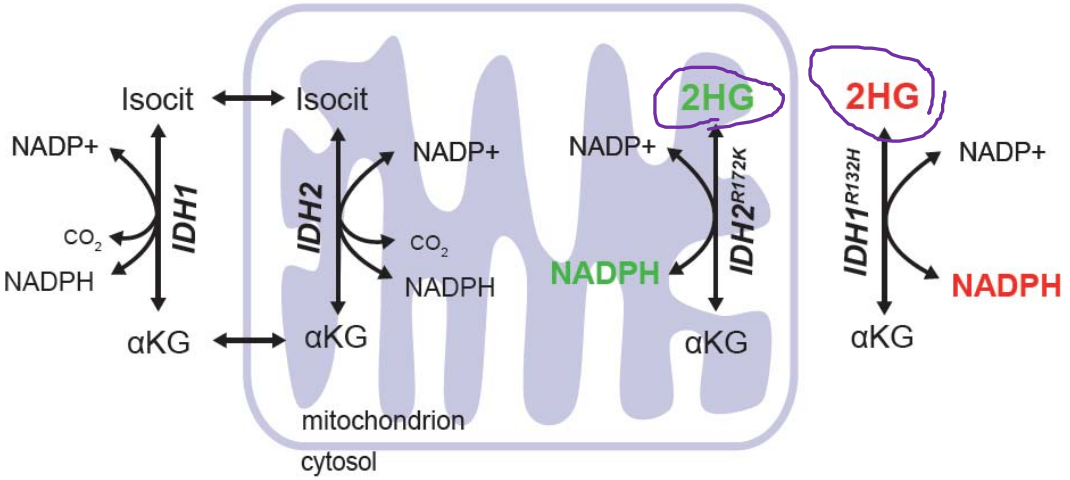
Can we probe NADPH metabolism in mitochondria?

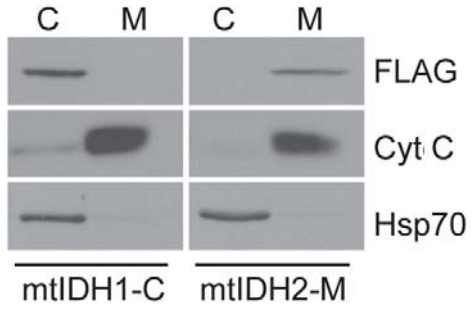


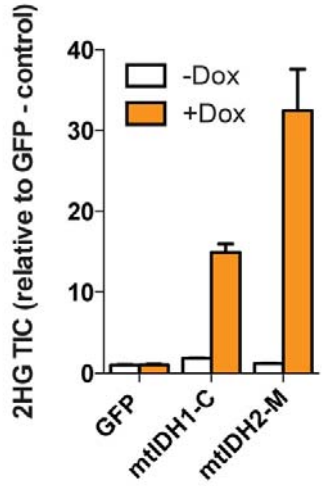
IDH mutations identified in low-grade gliomas, AML, and other tumors (Parsons et al. *Science* 2008 and others)

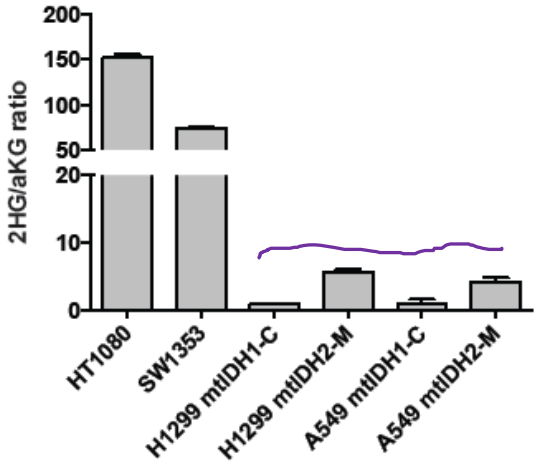
Gain-of-function mutations in IDH1 and IDH2
Mutant enzymes reductively generate (D)2HG using aKG and NADPH
(Dang et al. *Nature* 2009)

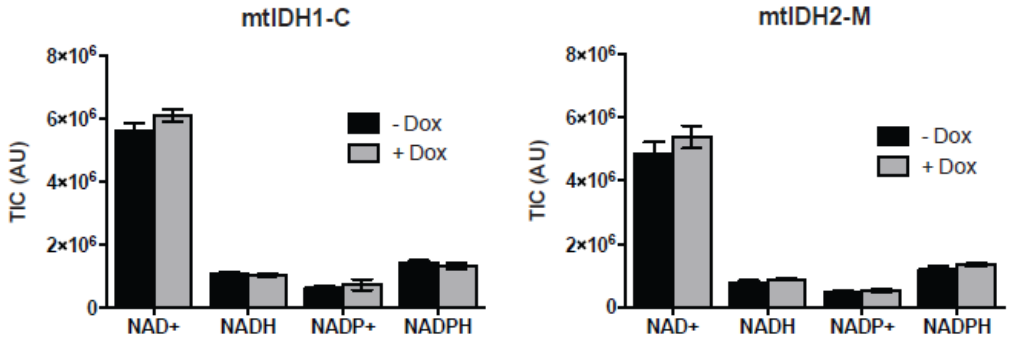
Using 2-HG production as a reporter of compartment-specific NADPH pools





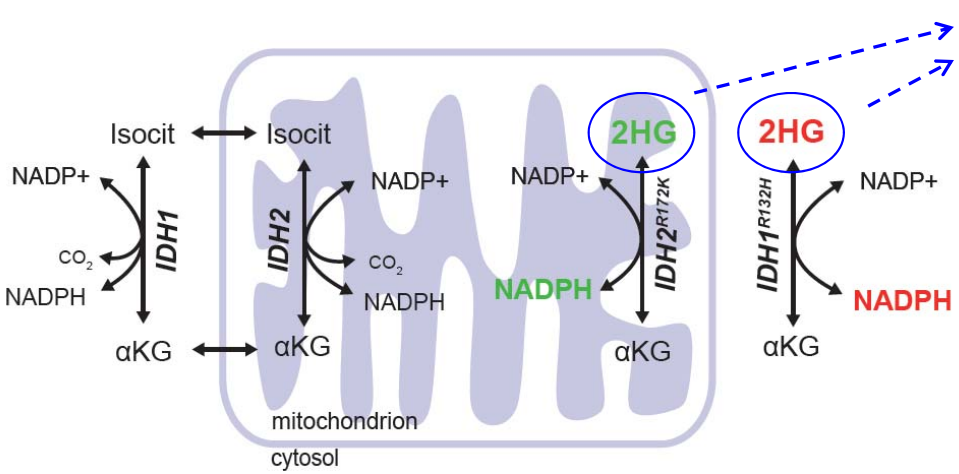






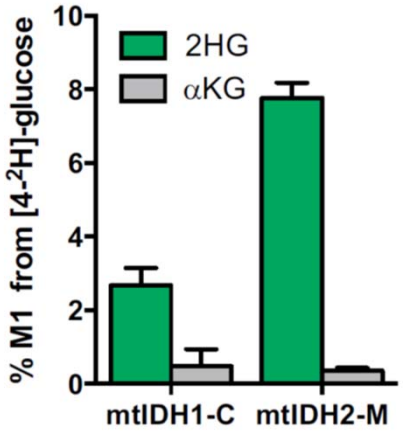
Minimal effect on central carbon metabolism
Grassian et al. *Cancer Research* (2014)

Using 2-HG production as a reporter of compartment-specific NADPH pools

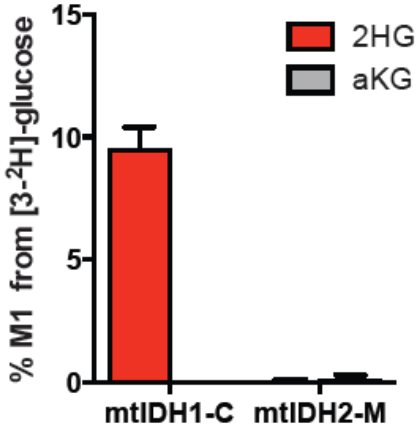


2H detection on 2HG provides readout of cytosolic vs. mitochondrial NADPH metabolism

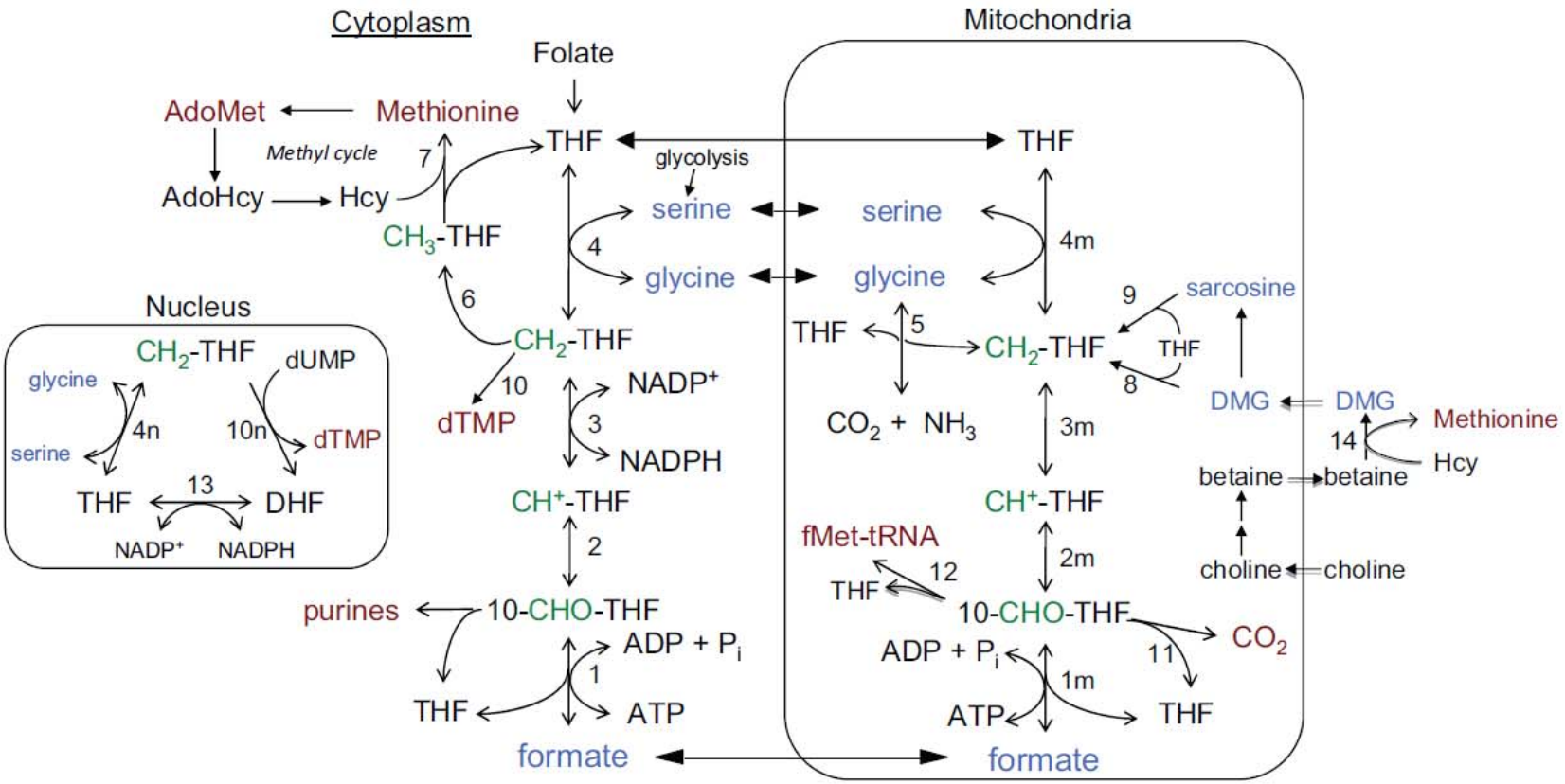
NADH trace (via glycolysis)



Cytosolic NADPH trace (via oxidative PPP)

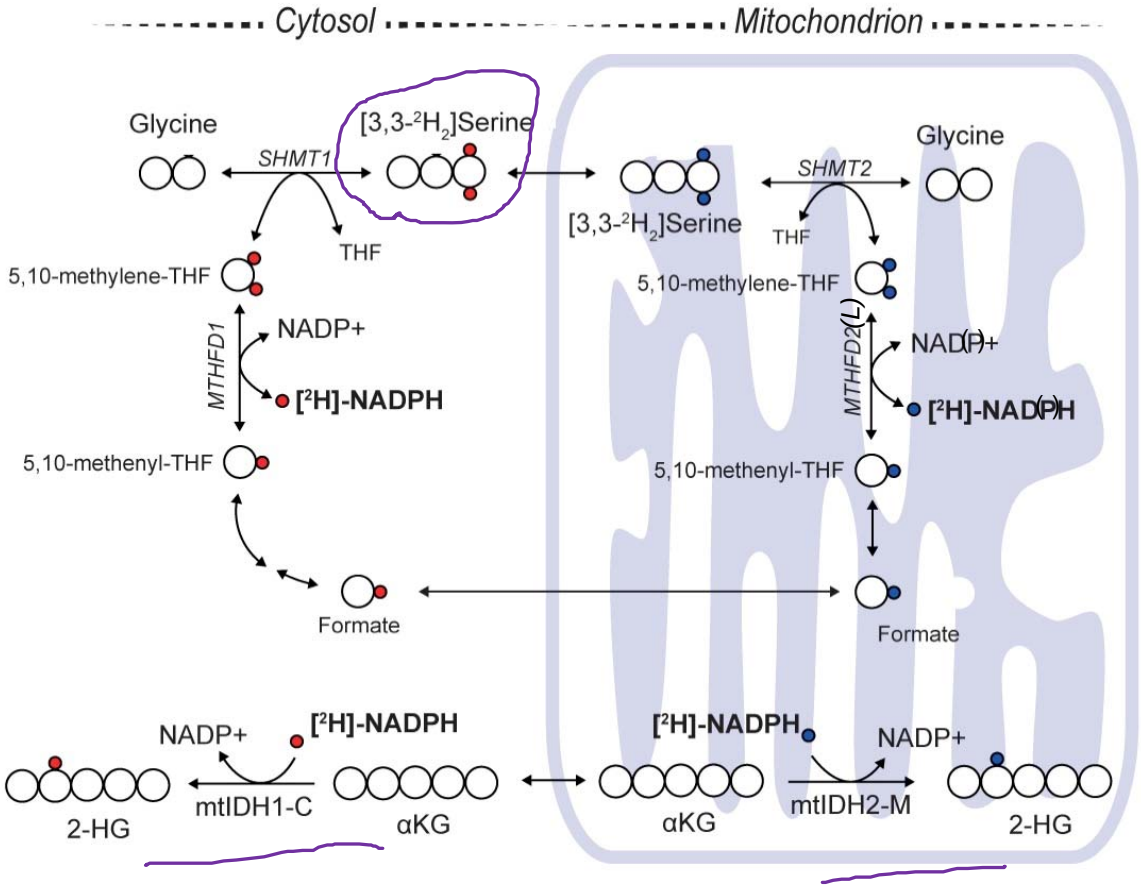


Can we use this reporter to annotate compartment-specific metabolic pathways? Folate-mediated one carbon metabolism

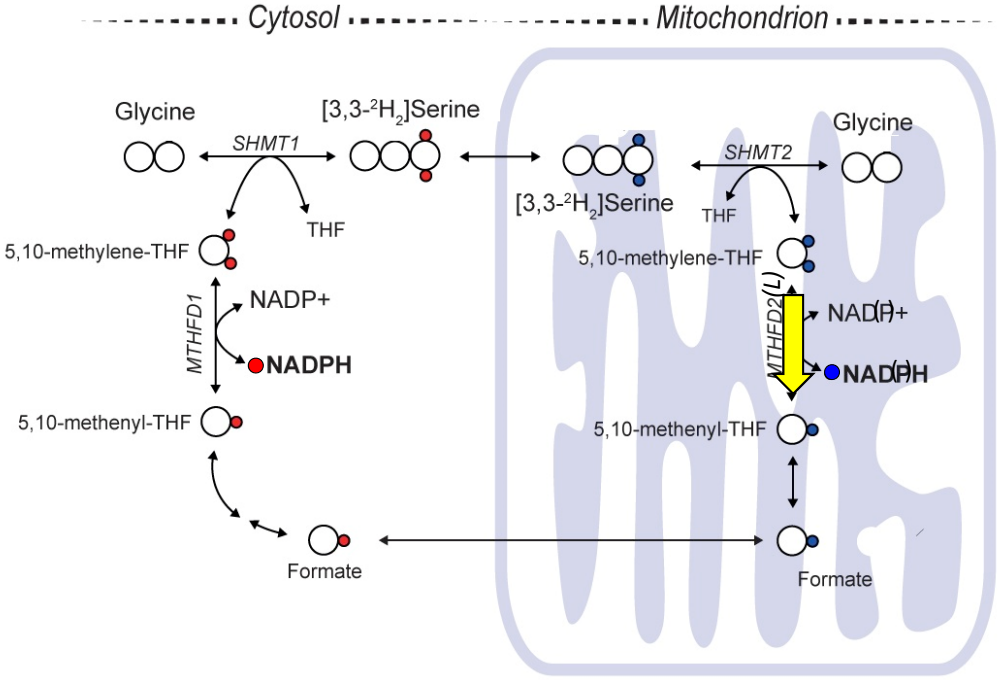


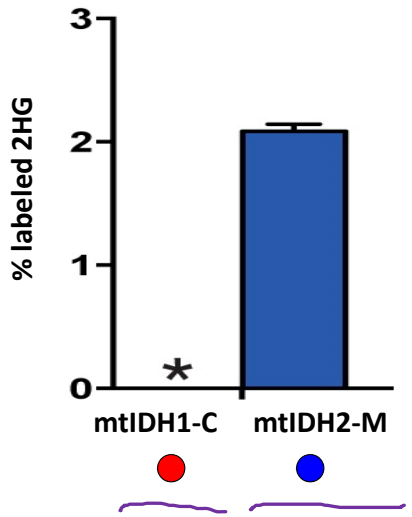
Tibbetts and Appling, *Ann. Rev. Nutr.* 2010

Can we use this reporter to annotate compartment-specific metabolic pathways? Folate-mediated one carbon metabolism



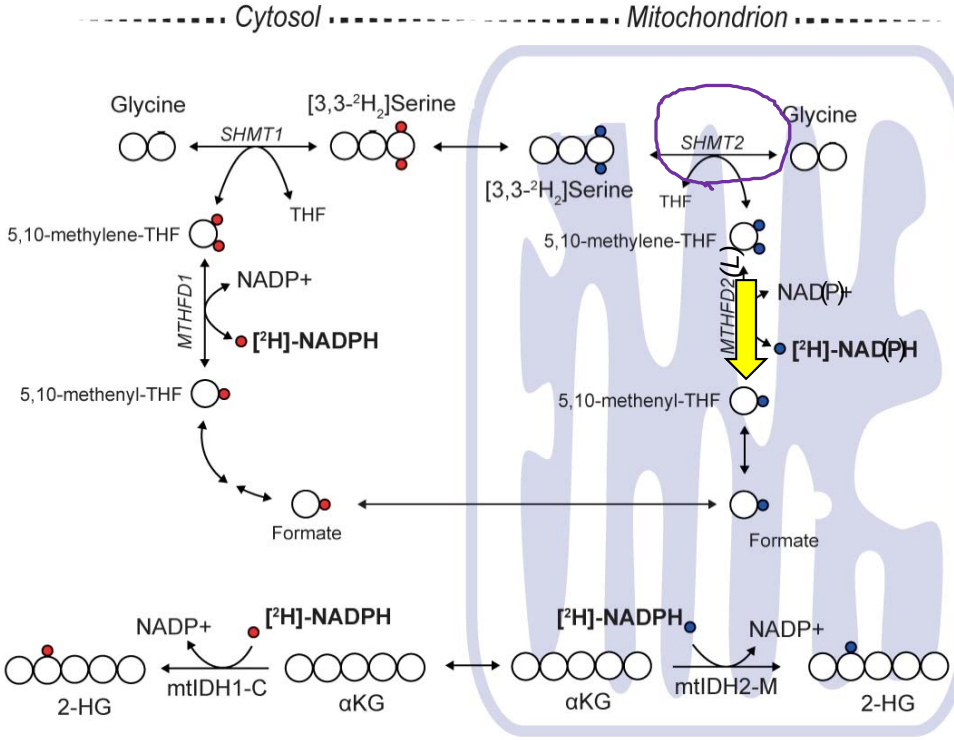
Discerning compartment-specific serine metabolism using cofactor tracing

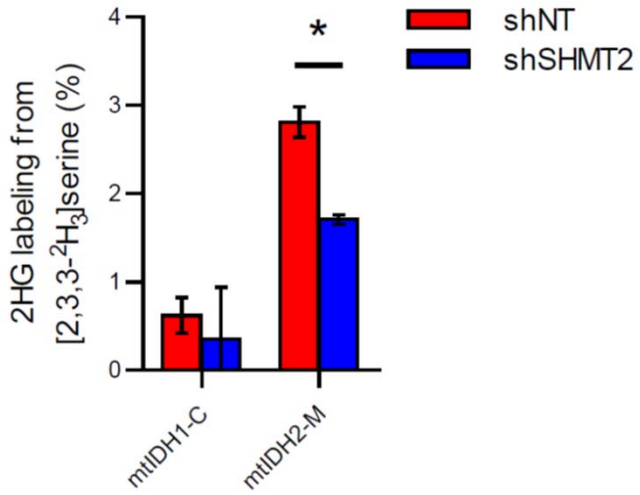




NADPH produced from serine only observed in mitochondria

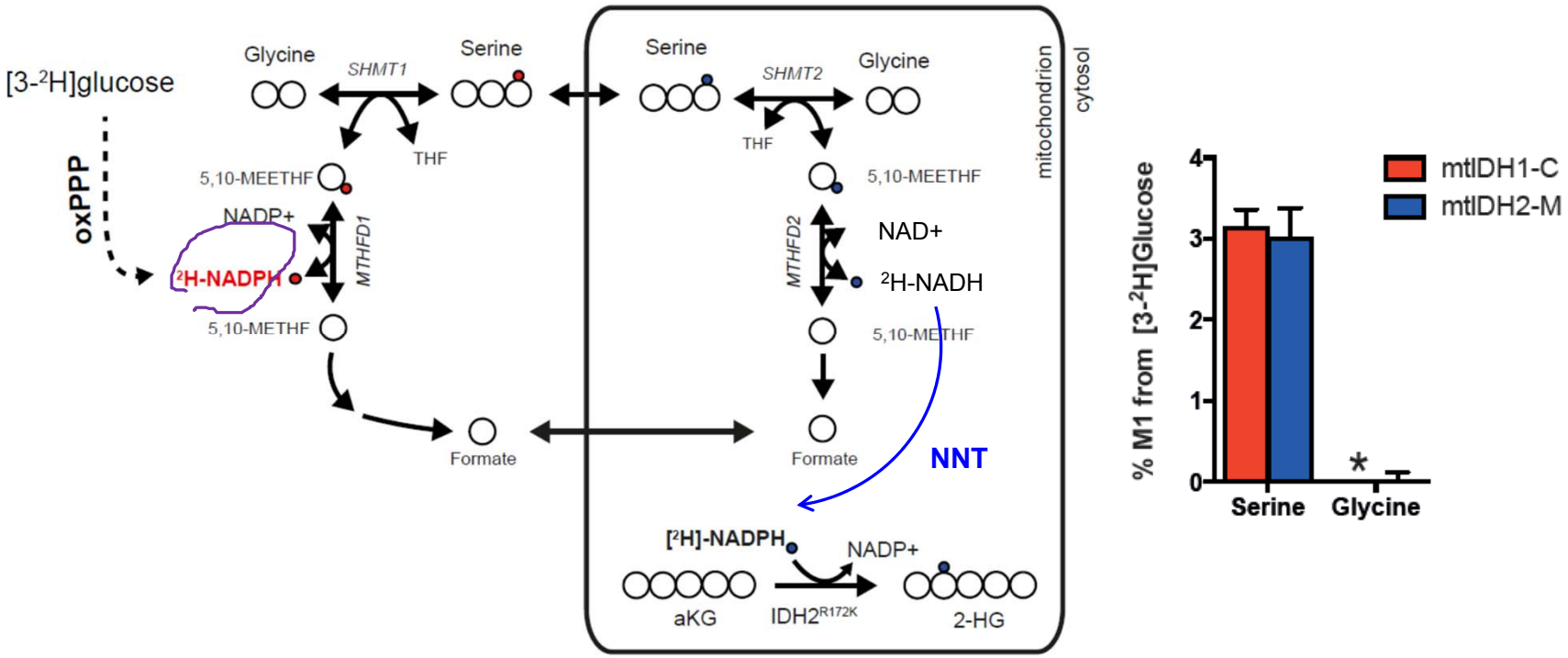
Discerning compartment-specific serine metabolism using cofactor tracing and mIDH reporters





Serine, glycine, and folate-mediated one carbon metabolism generate mitochondrial reducing equivalents

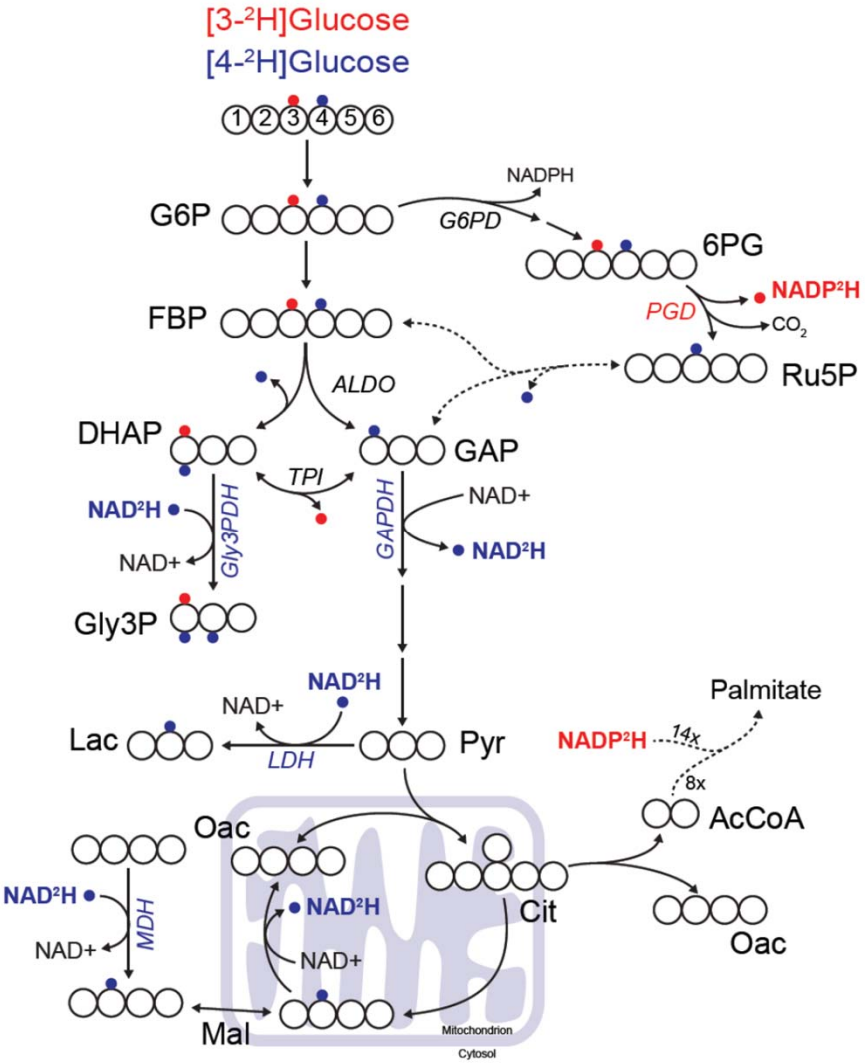
Discerning compartment-specific serine metabolism using cofactor tracing

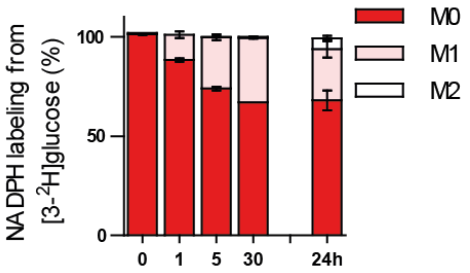


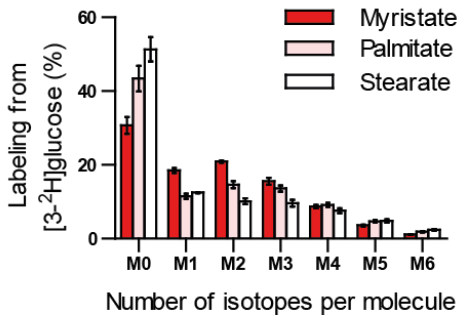
Cytosolic reactions consume NADPH/produce serine

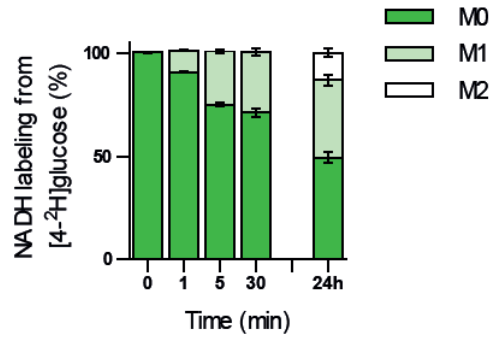
NADPH from the oxidative PPP appears on serine

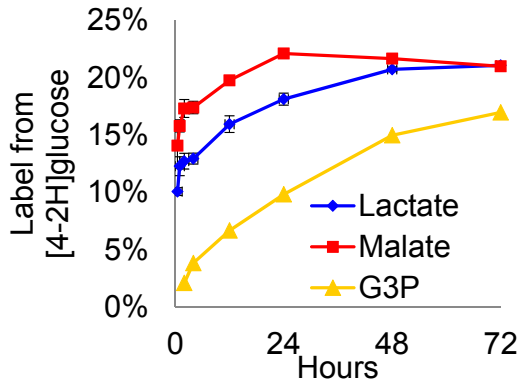
Resolving compartment-specific NADPH metabolism using 2H tracers and mutant IDH







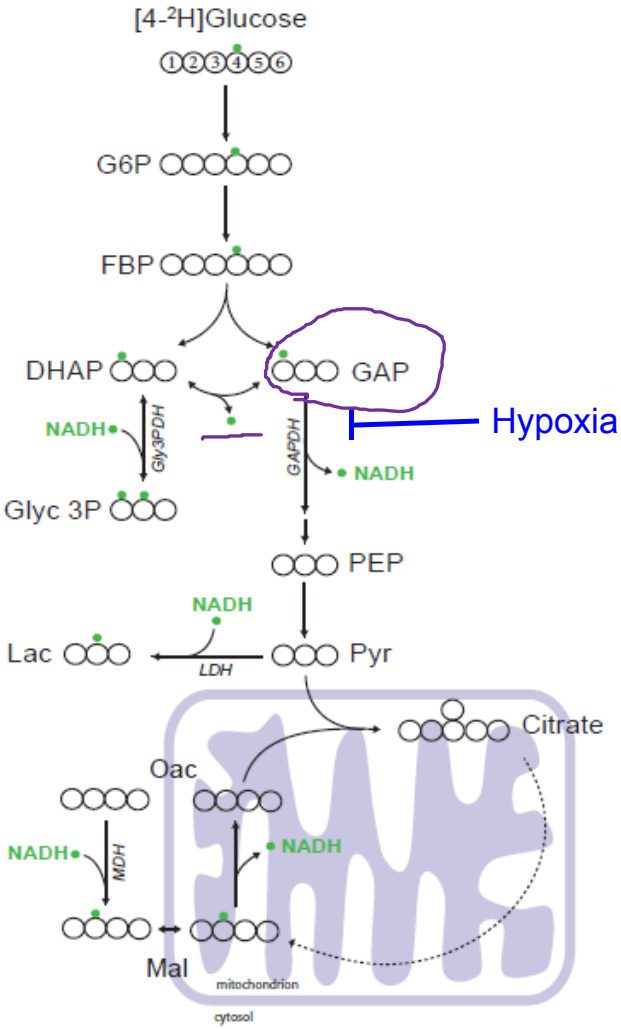


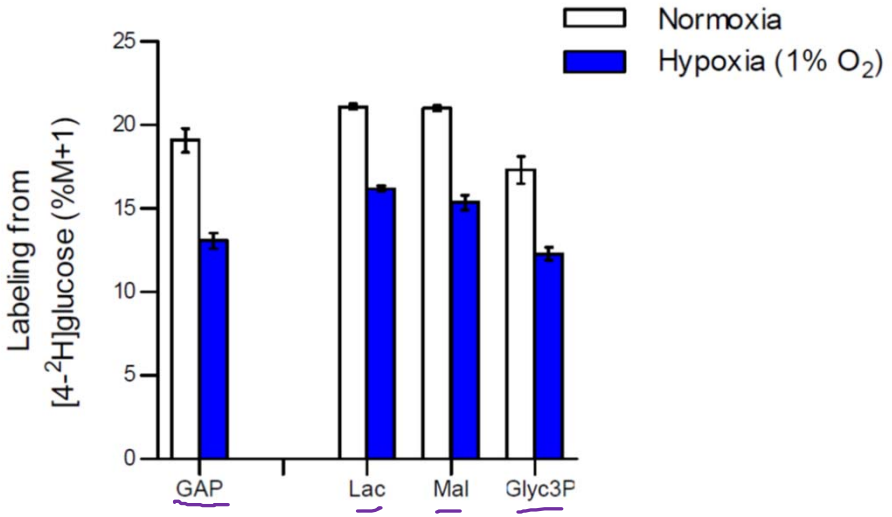


- 2H tracers allow for quantitation of NAD(P)H metabolism
- Oncogenic IDH1 and IDH2 used as reporters for compartment-specific NADPH labeling

Lewis et al. *Mol Cell* 2014

How is NAD(P)H metabolism reprogrammed under hypoxia?

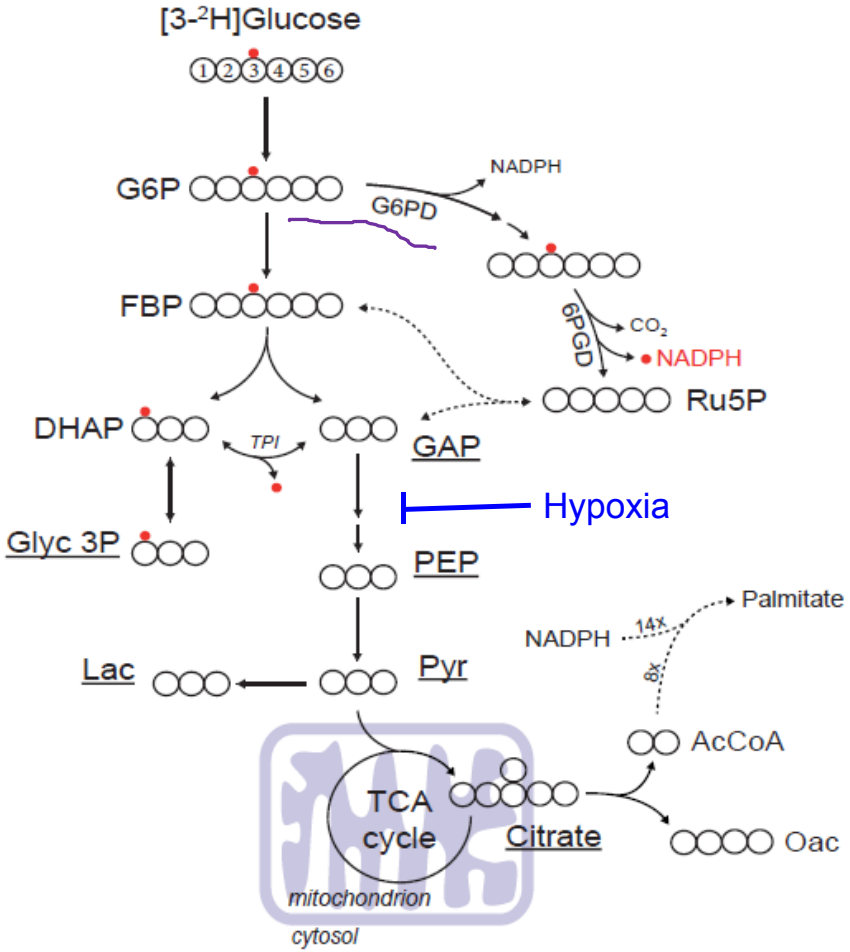


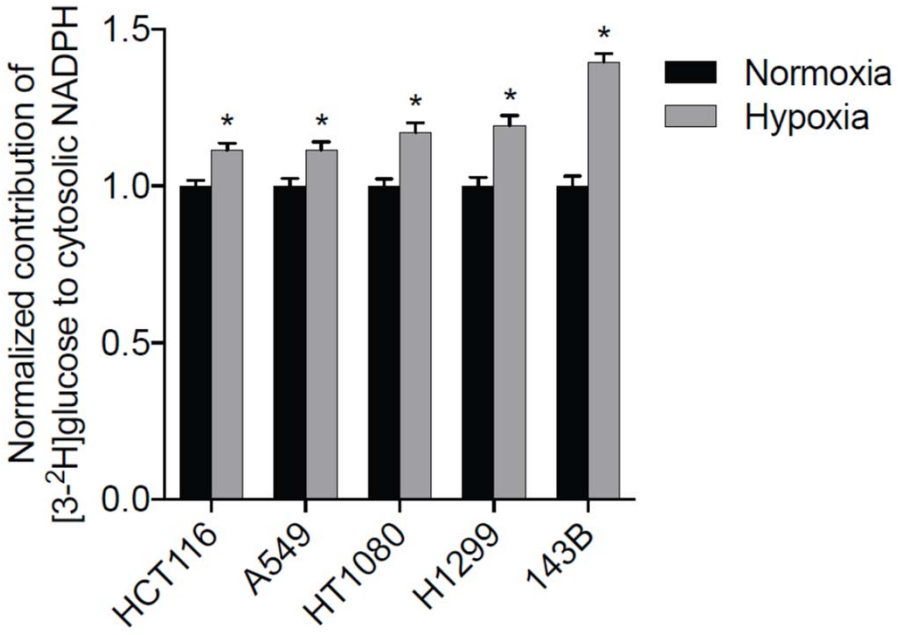


Oxidation of GAPDH under hypoxia leads to increased loss of isotope

Increased exchange flux at TPI/aldolase

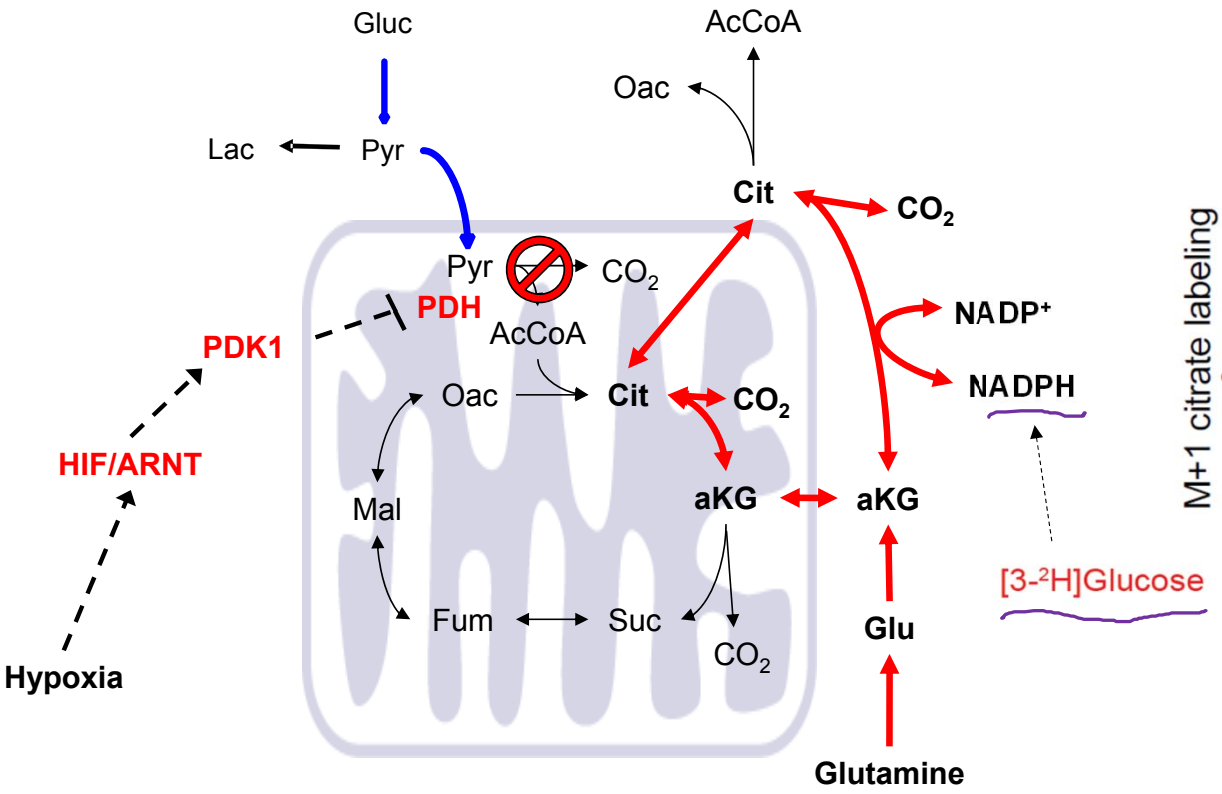
Hypoxia increases flux through the oxidative pentose phosphate pathway

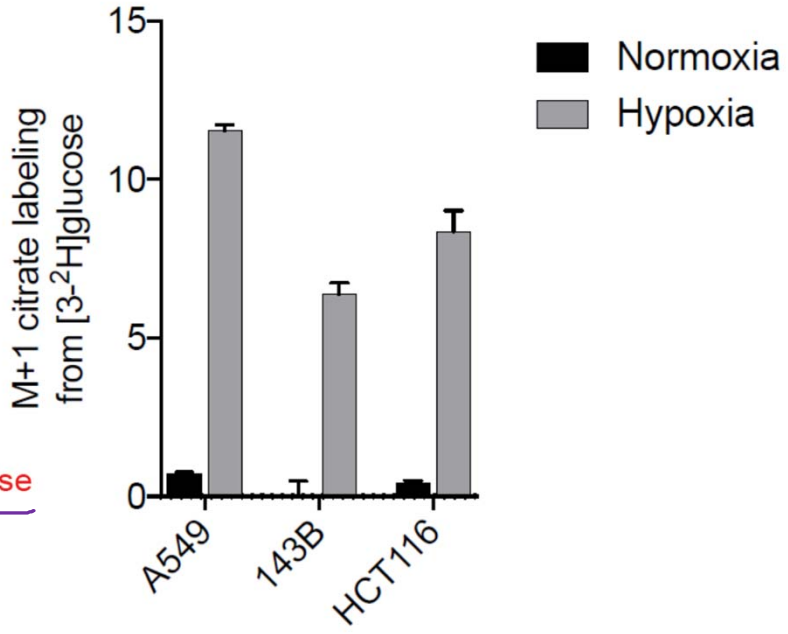




GAPDH oxidation leads to increased (15-40%) oxidative PPP contribution to NADPH pools

Hypoxic induction of reductive carboxylation is mediated by cytosolic oxPPP flux and IDH1





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