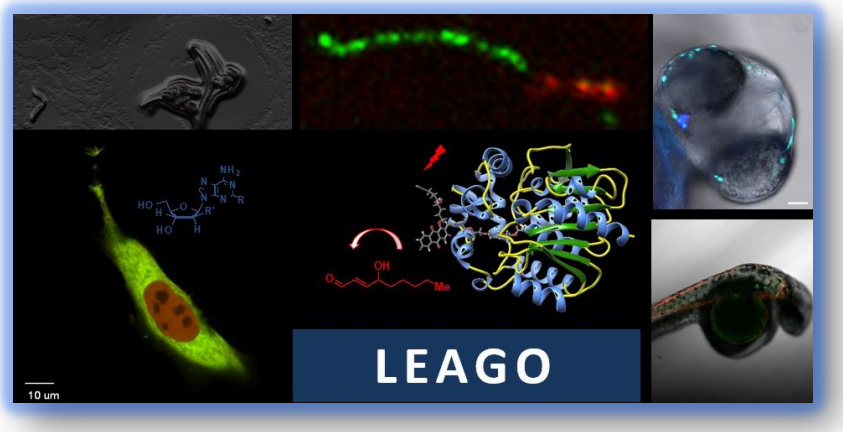
Welcome to CH-313: Chemical Biology

Prof. Yimon Aye https://leago.epfl.ch/



Laboratory of Electrophiles And Genome Operation

Lecture Week 9:

Targeting glycolysis and glycolytic signaling switches in cancer

2023 Nov 14th (Room: BS 270): 10:15 am – noon

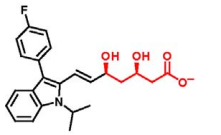
https://moodle.epfl.ch/course/view.php?id=15521

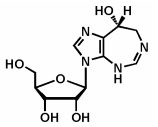
✓ **Part I**: Chem Bio Toolsets & Applications: challenges and limitations

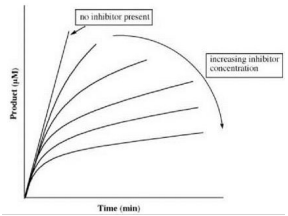
Enzyme inhibition and mechanisms of action of small-molecule drugs:

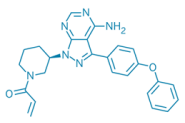
Reversible (competitive, non-competitive, TS analog, rapid vs. slow on/off)

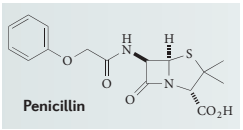
Irreversible (covalent); mechanism-based inactivation







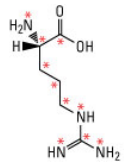


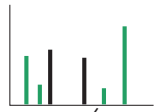


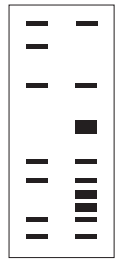
High-throughput quantitative proteomics target screening techniques:

MS and affinity enrichment
and click-chemistry-based
biotechnologies

ABPP indirect profiling
(gel vs. proteomics-based readout; SILAC)



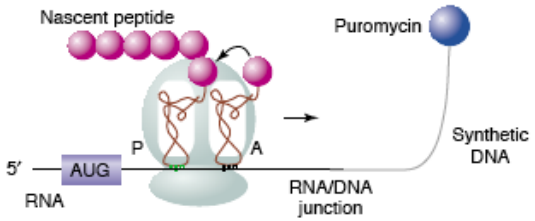


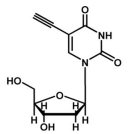


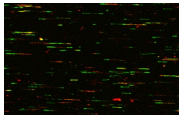
Transcriptome/genome-probing tools:

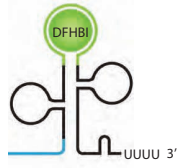
EdU labeling
iPOND

Cell cycle & genome replication









Fluorescent RNAs

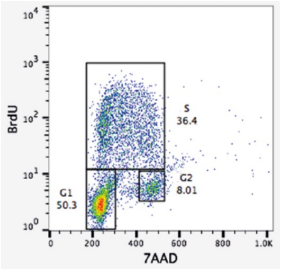
RNA-based biosensors

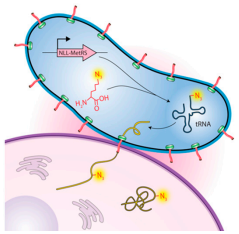
Proteome-level probing tools that hijack protein translation:

mRNA display

Amber suppressor technique

SILAC-BONCAT method

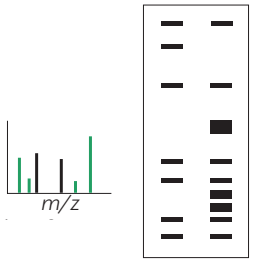


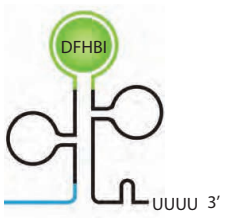


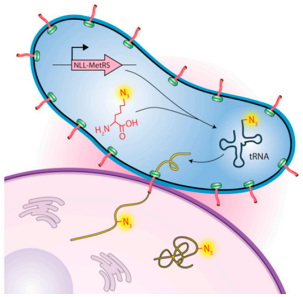
Key learning goals of the course

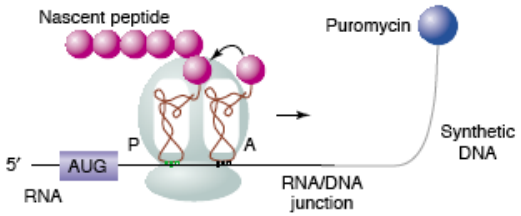
Part I. Chemical Biology Toolsets & Applications ✓

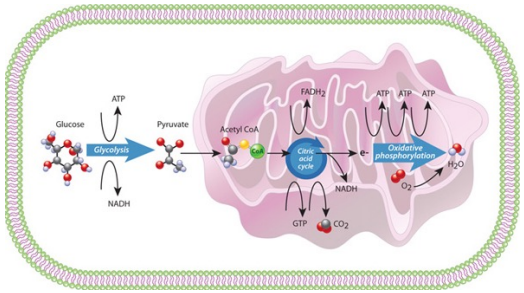
What are the latest tools & apps (and underlying principles), that enable *perturbation, probing, and precise control* of life processes?











Part II. Targeting metabolic regulation

What is now known about modern "metabolism" that was unknown in the 60's ?

How we can leverage our current knowledge of metabolism to drug discovery & disease treatment

Part II: Targeting metabolic regulation

Metabolic regulation	9	14th Nov	Glycolysis and TCA cycle	
	10	21st Nov	Metabolomics and pathway flux analysis	PSet 5
	11	28th Nov	Glycolytic switches in cancer	PSet 6
	12	5th Dec	TCA cycle and isotopic tracing / flux analysis	
	13	12th Dec	… topic above continues	PSet 7
	14	19th Dec	OxPhos (oxidative phosphorylation)	

Chemical Biology of Glycolysis and Glycolytic Signaling Switches in Cancer

Fundamental concepts:

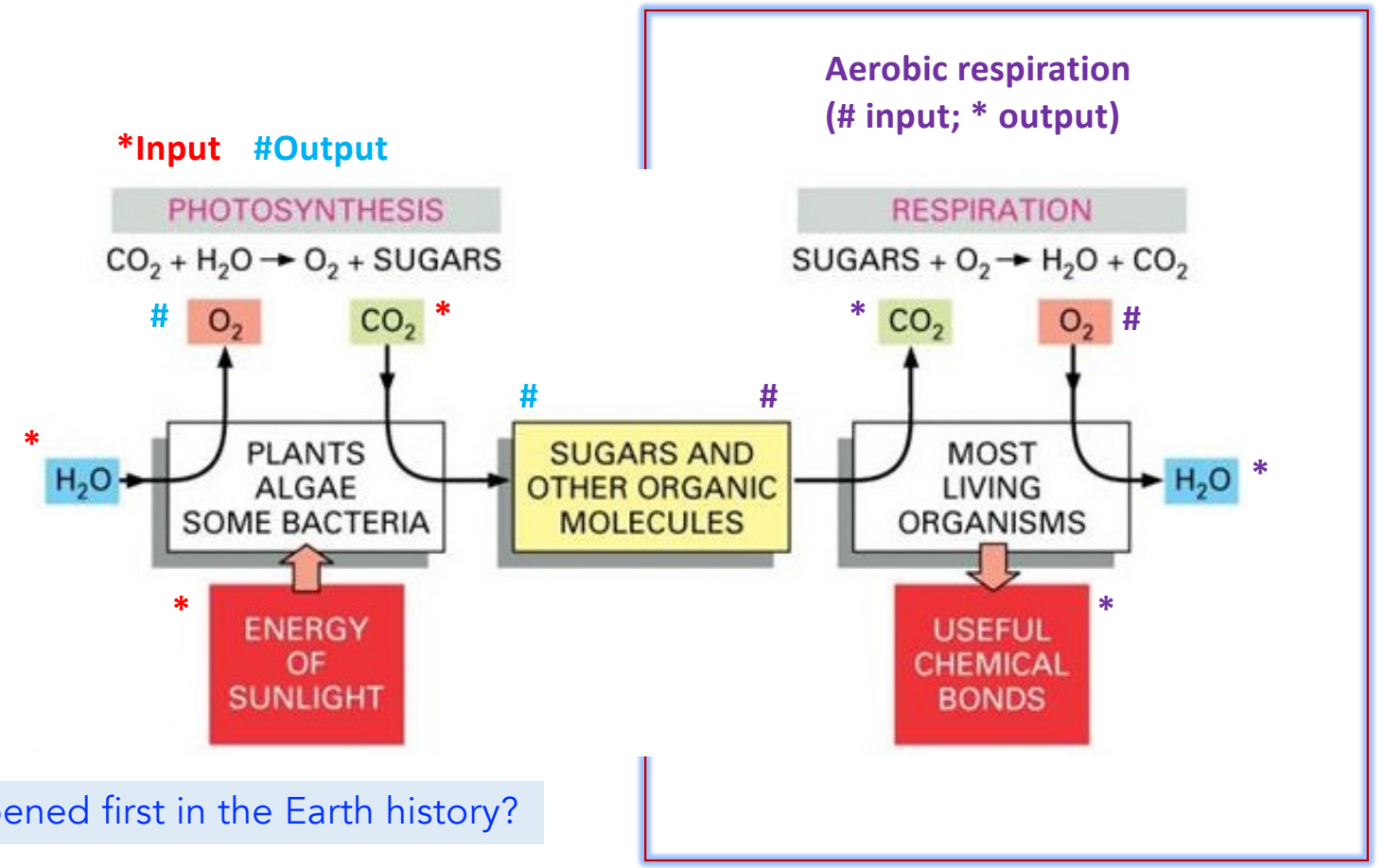
-- Chemical Logic

-- Flux Control and Flux Balance

-- The Warburg Effect

-- Case Study (PKM2 in cancer)

Complementary processes in the living world



Q: which one happened first in the Earth history?

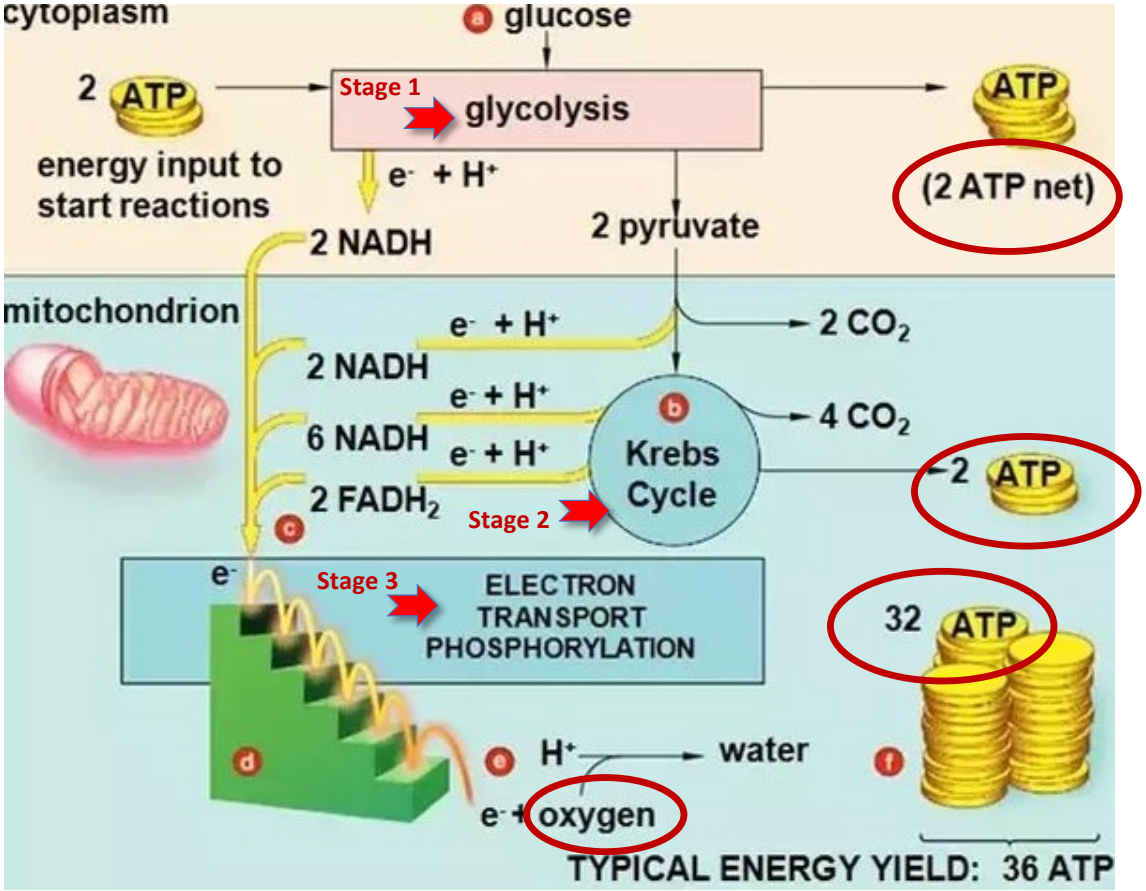
Q: Given that plants (with photosynthesis capability) also of course have to respire, how do plants still serve to reduce 'carbon footprint' (decreased CO_2 concentration) and humidity?

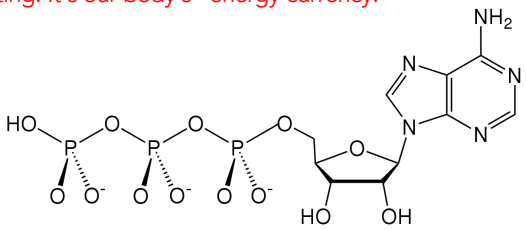
Cellular respiration is a 3-stage process by which living cells break down organic fuel molecules in the presence of oxygen to harvest the energy they need to grow and divide. **This metabolic process occurs in most plants, animals, fungi, and many bacteria…**

Cellular respiration generates **ATP**: the body's energy currency

All forms of life on the planet relies on ATP….

Without ATP, we couldn't form a thought or move a muscle. ATP keeps our nerves firing and our heart beating. It's our body's "energy currency."

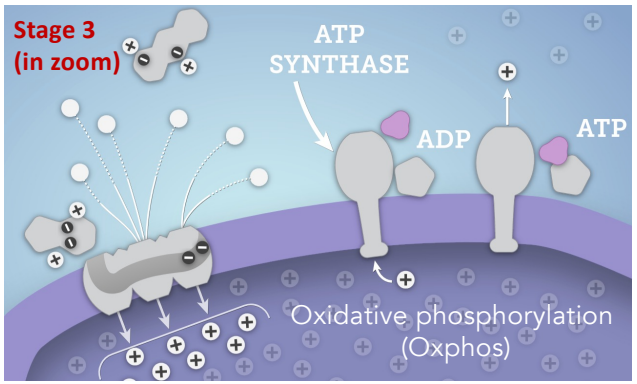




Q1: all cells make ATP (and use it to power nearly all of their processes): it doesn't travel from cell to cell: why?

Q3: How many cellular compartments are involved in ATP generation during respiration?

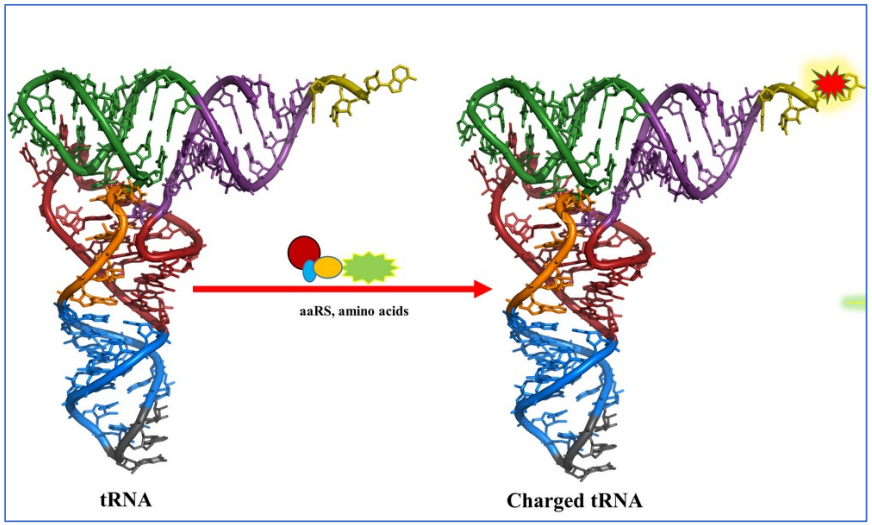
Q2: can you reason chemically the following statements: (a) ATP carries energy (b) ATP is recyclable

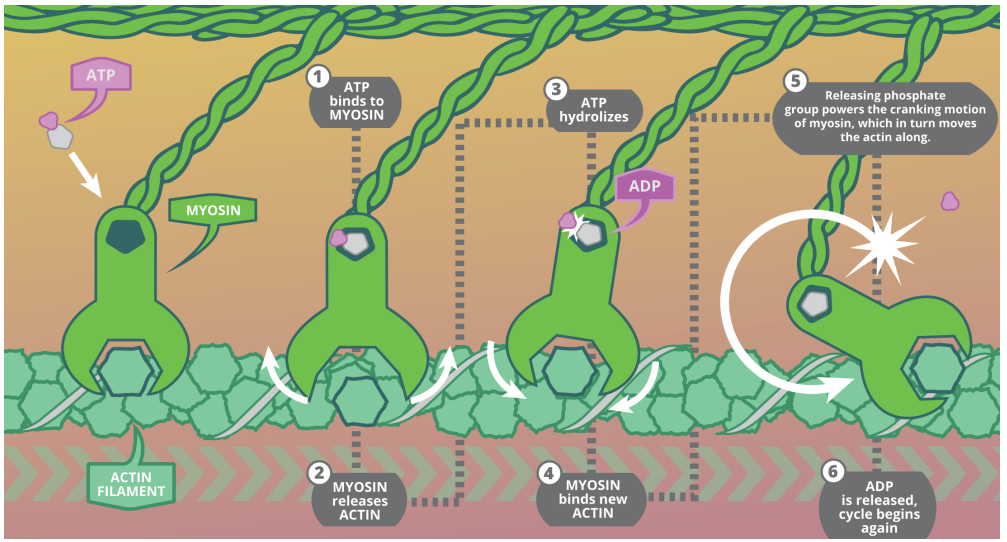


Q4: can you think of top uses for ATP (based on what we have come across in the course thus far)?

The 3 stages of cellular respiration

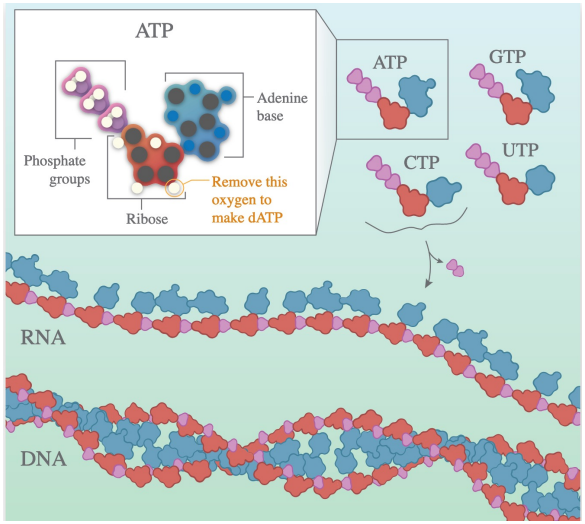
Cells rely on **chemical energy within ATP** for their **chemical, mechanical, and transport activities**

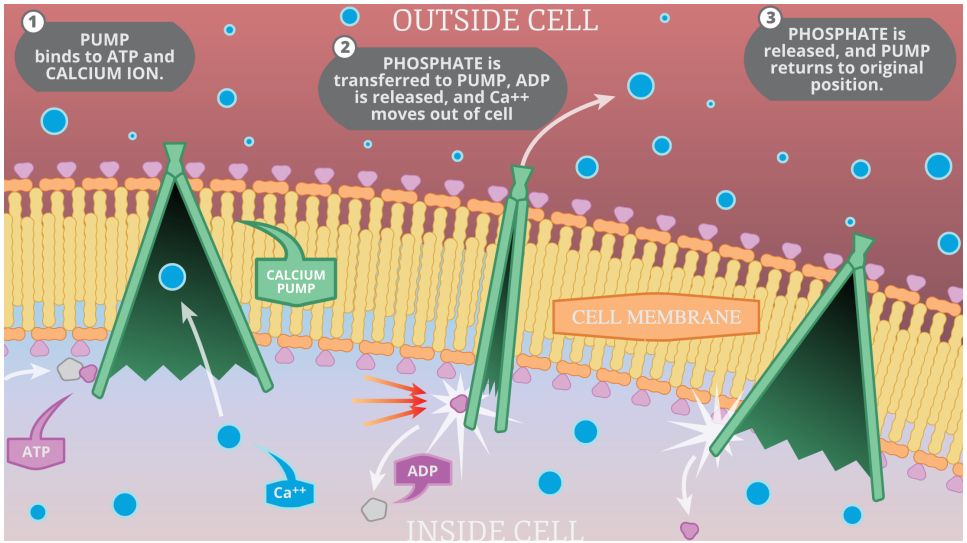




Molecular motors such as myosin

Match each figure with chemical, mechanical, or transport activities of ATP

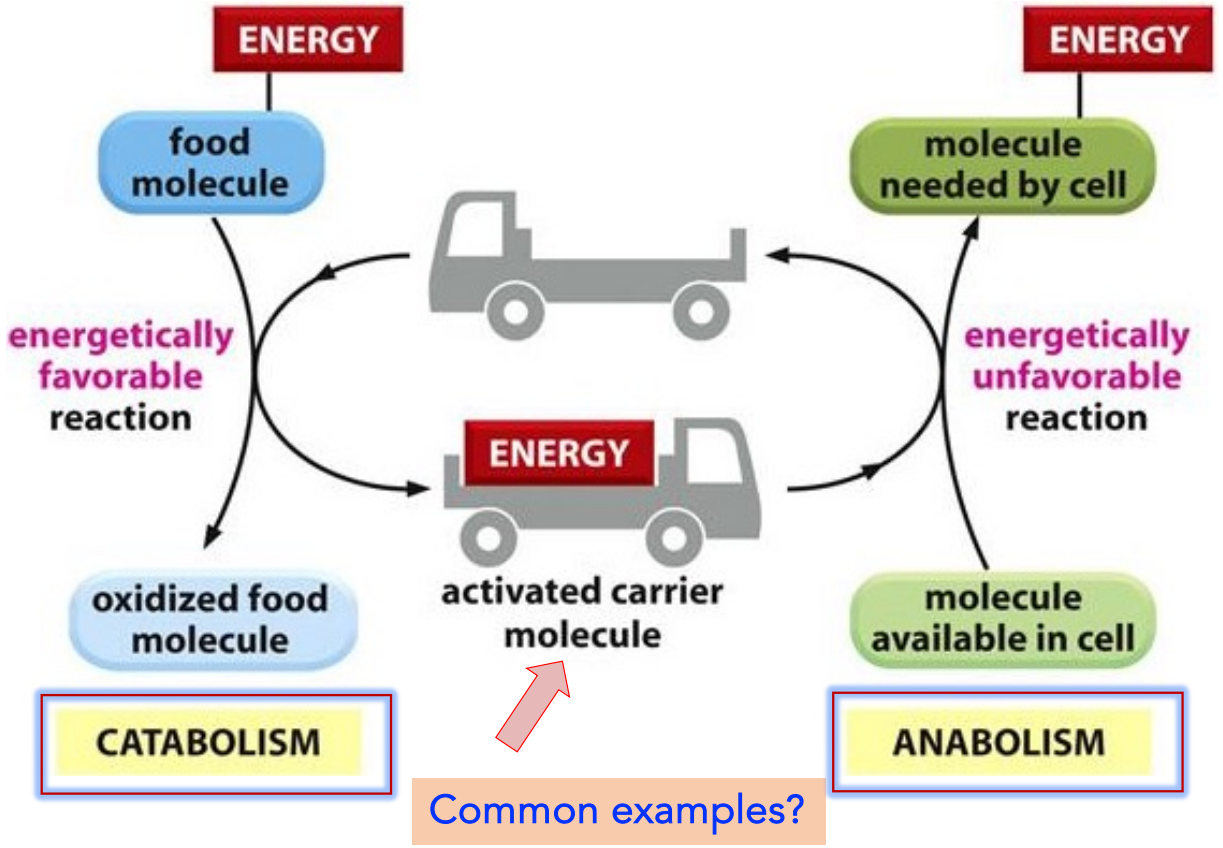




Molecular pumps such as voltage-gated calcium channels in neurons for brain activity (interneuron communications)

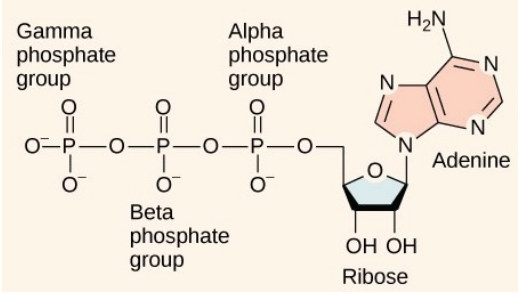
What other activated energy carriers do cells use?
See next slide

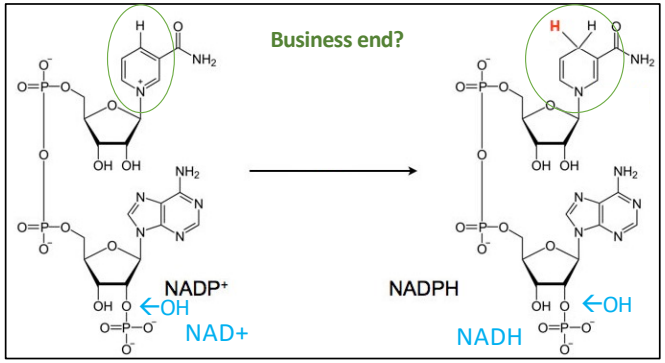
Role of activated carriers in metabolic chemistry



ATP, NAD(P)H/FADH2, Acetyl coenzyme A (AcCoA), carboxylated biotin, SAM, UDP-glucose
(structures: see next slide)

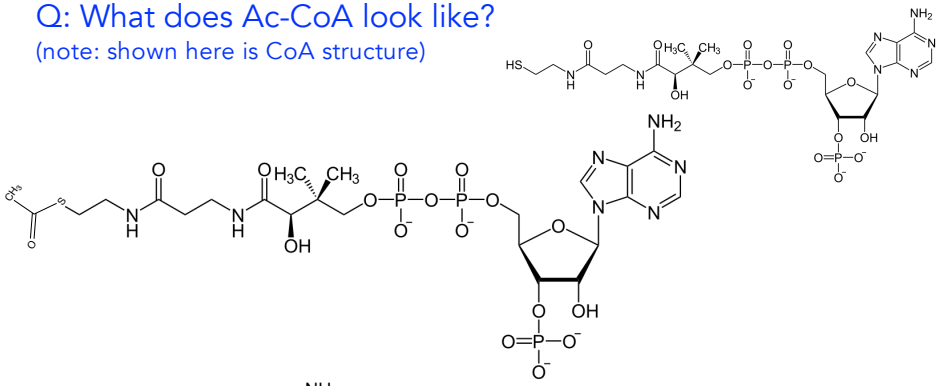
Examples: activated carrier molecules
ATP, NAD(P)H/FADH2, Acetyl coenzyme A (AcCoA), carboxylated biotin, SAM, UDP-glucose

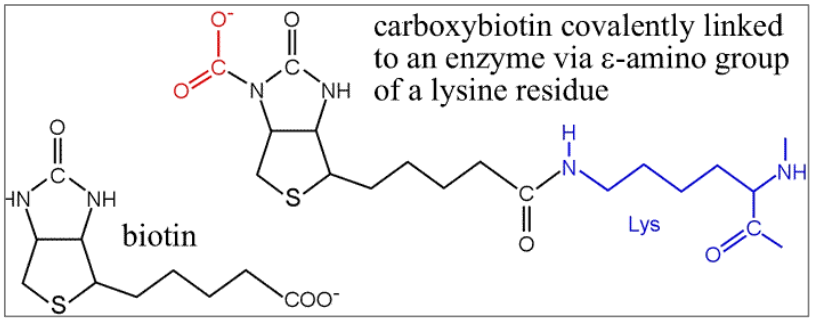




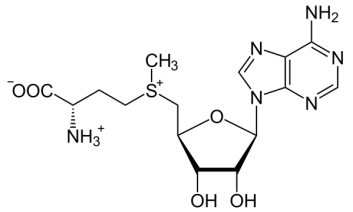
Q: What does NAD^+/NADH look like?

Q: What does Ac-CoA look like?
(note: shown here is CoA structure)

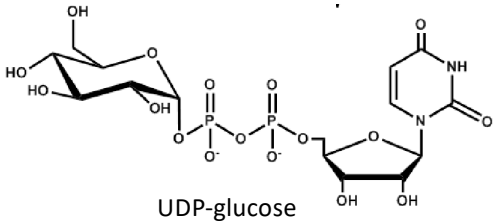




Q: What does CO_2-carrying biotin, covalently bound to a protein, look like?



Q: How does SAM function as a methylating agent to, for instance, protein lysines?



Q: How does UDP-glucose allow intracellular build-up of glucose?

Aside: Coupled chemical reactions…(in your own time, refresh some basic knowledge on organic chemistry)

-- Hydrolysis of ATP (to ADP and P_i , *or* to AMP and PP_i)

-- Phosphate transfer rxns.

-- Energetically-unfavorable biosynthetic rxns driven by ATP-hydrolysis

-- NADH (important 2-electron-carrier / high-energy hydride anion donor)

-- Acetyl CoA, another key activated carrier

-- FADH2 (carrier of high-energy electrons)

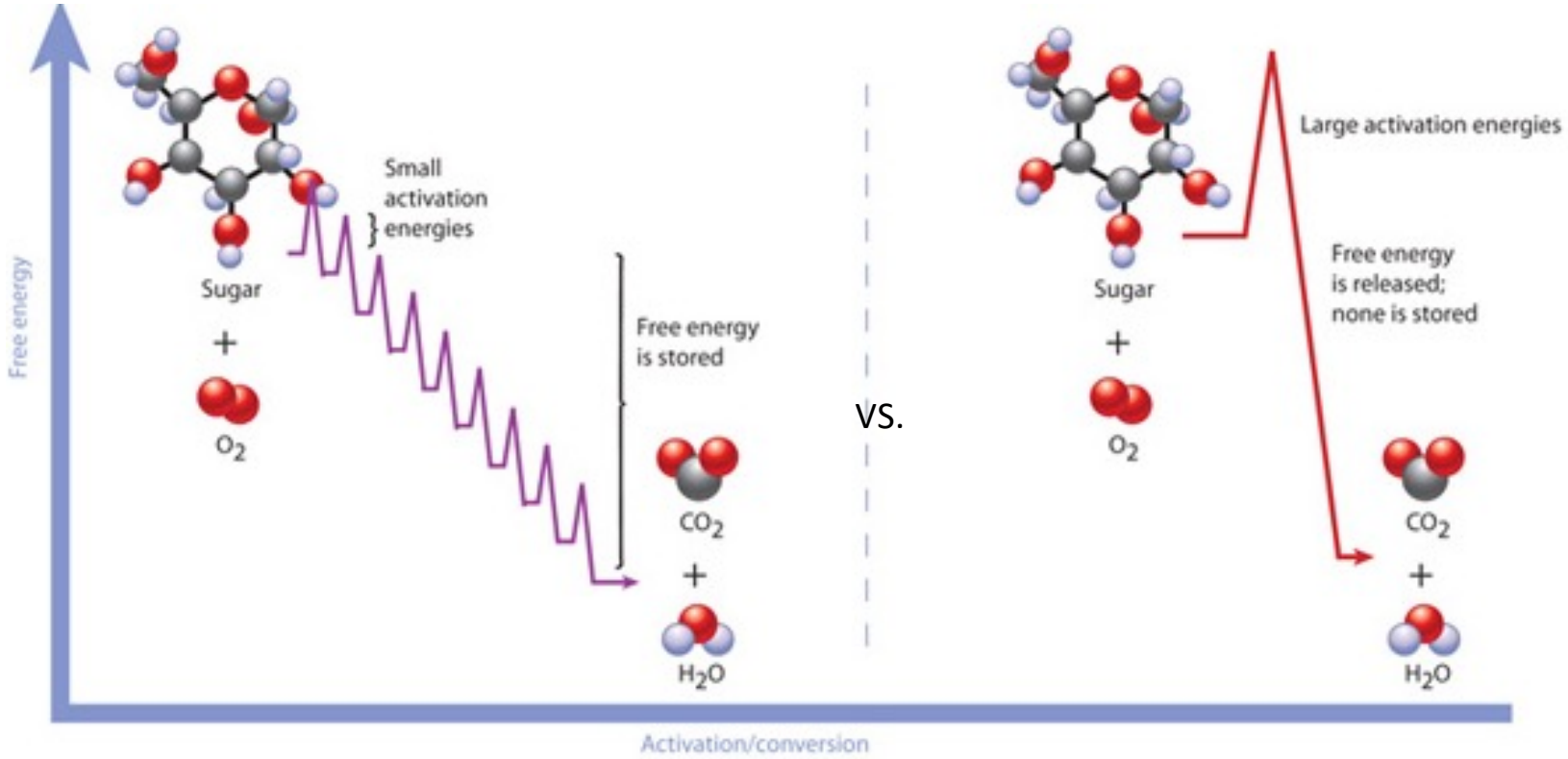
-- Carboxyl group activation

-- Synthesis of polysaccharides, nucleic acids, and proteins (all using nucleoside triphosphates)

Would be helpful to brush up on carbonyl chemistry concepts and reaction mechanisms of following fundamental reactions that are often leveraged in biological processes:

-Aldol addition and condensation (and retro-Aldol processes)
-Addition-elimination reactions
-Michael addition reactions

Metabolic biology is regulated chemistry!



Q: Which of the two rxn coordinate represents *regulated* sugar oxidation in a cell (vs. ordinary burning of sugar)?

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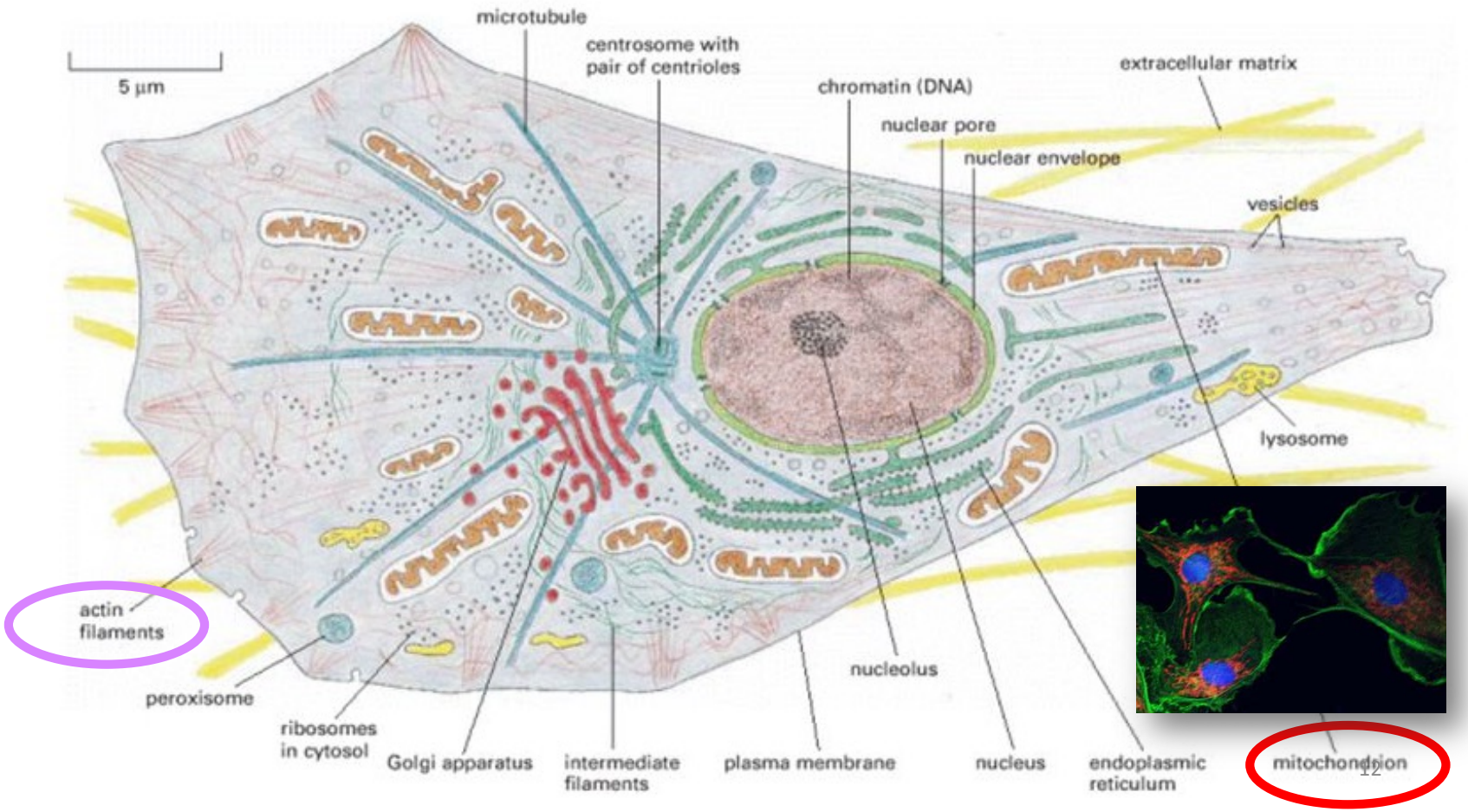
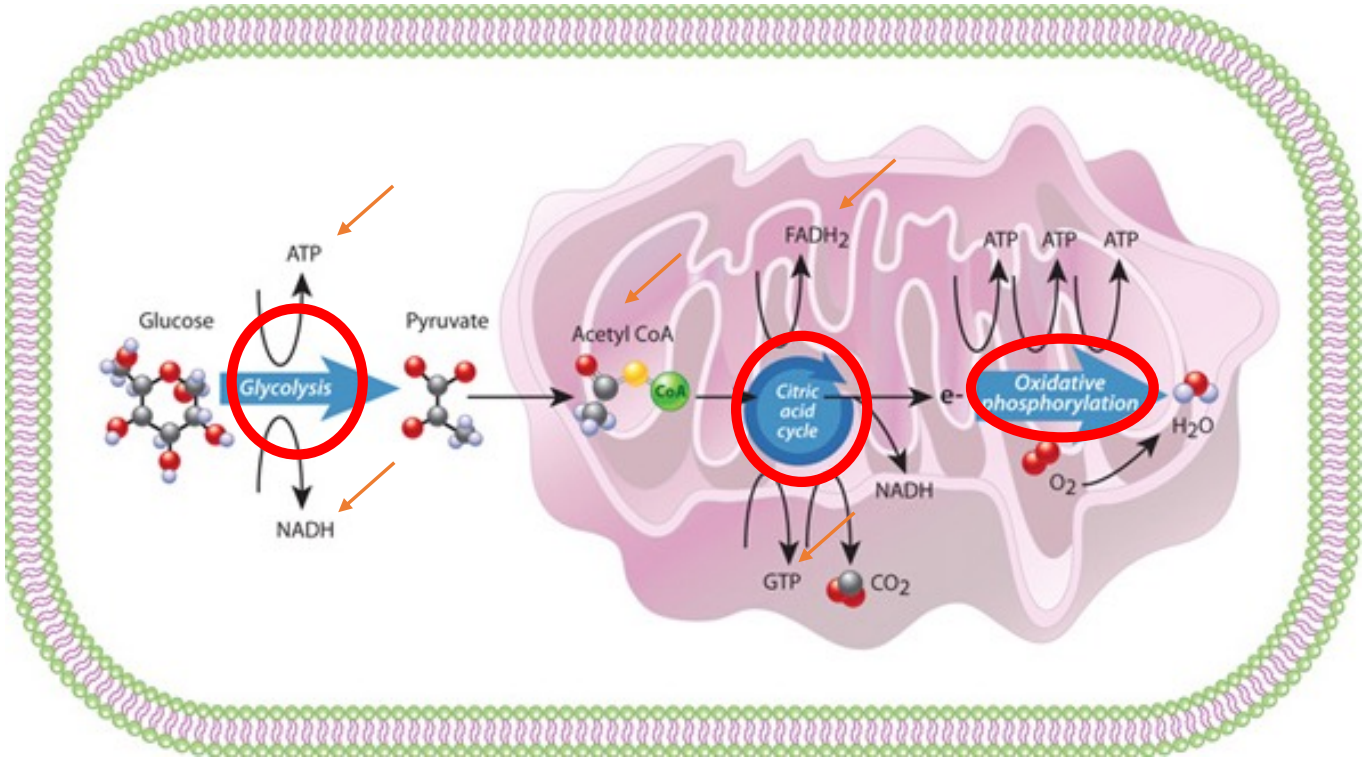
Mitochondria: powerhouse for energy generation

A eukaryotic cell:



What do blue, red, and green stains / colors report? What specific proteins / organelles are they targeting/ staining?

Eukaryotic Metabolism (glycolysis, TCA cycle, and ox-phos)



Question: how many common activated carriers are there in this diagram?

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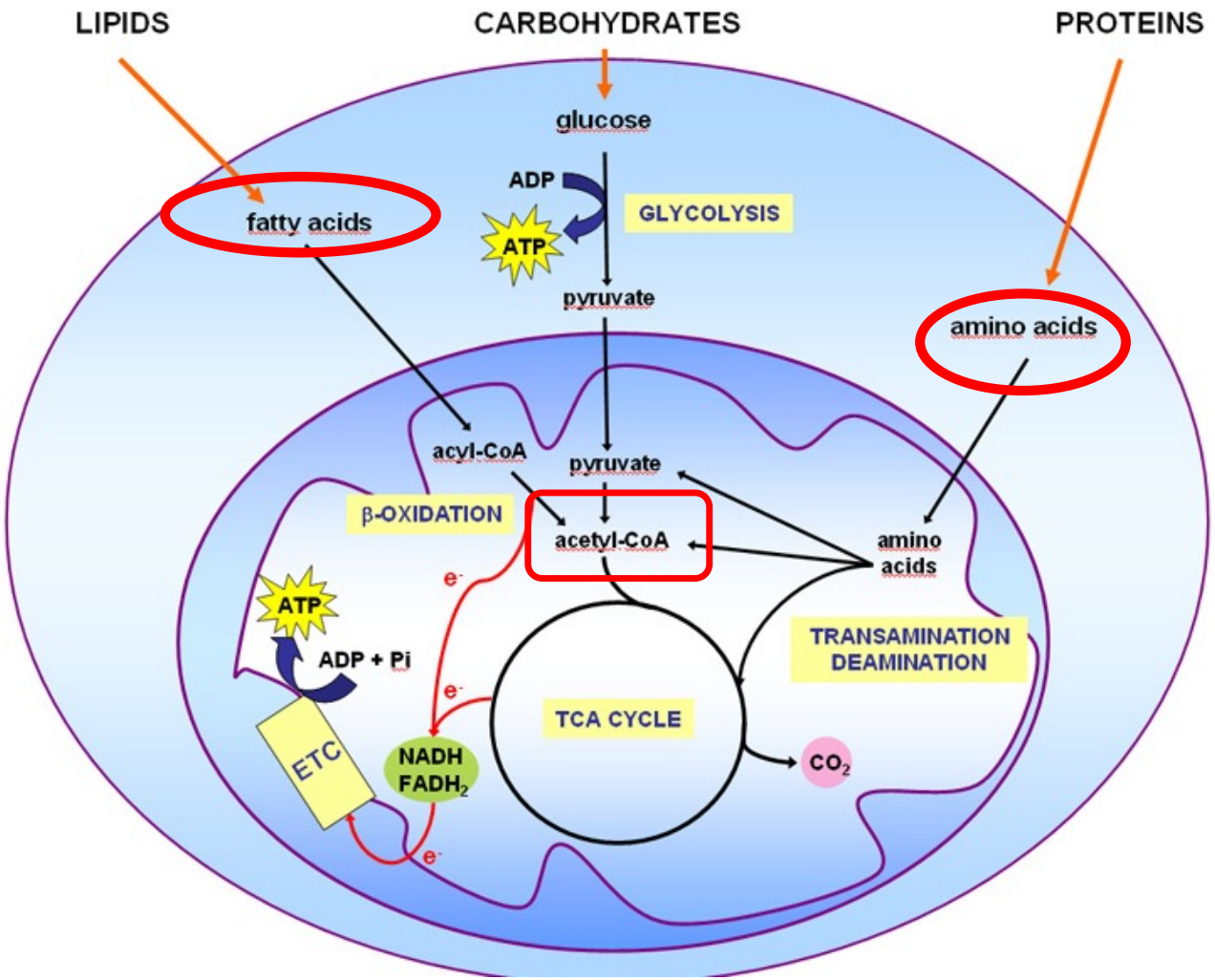
Eukaryotic Metabolism (fatty acid and amino acid signaling)

Q: What is the single metabolite, ultimate oxidation product, derived from all 3 classes of fuel molecules prior to entering TCA cycle?



The citric acid (or TCA) cycle is the *final common pathway for the oxidation of fuel molecules*—amino acids, fatty acids, and carbohydrates. Most fuel molecules enter the cycle as acetyl-CoA. The TCA cycle, in conjunction with oxidative phosphorylation via ETC (electron-transport chain) process (see next lecture), provides the vast majority of energy used by aerobic cells—in humans, greater than 95%

We won't cover fatty acid and amino acid synthesis pathways in this course but these pathways also feed into TCA cycle. In this figure, you can broadly see how glycolysis intersects with these pathways.

Chemical Biology of Glycolysis and Glycolytic Signaling Switches in Cancer

-- Chemical Logic

-- Flux Control and Flux Balance

-- The Warburg Effect

-- Case Study (PKM2 in cancer)

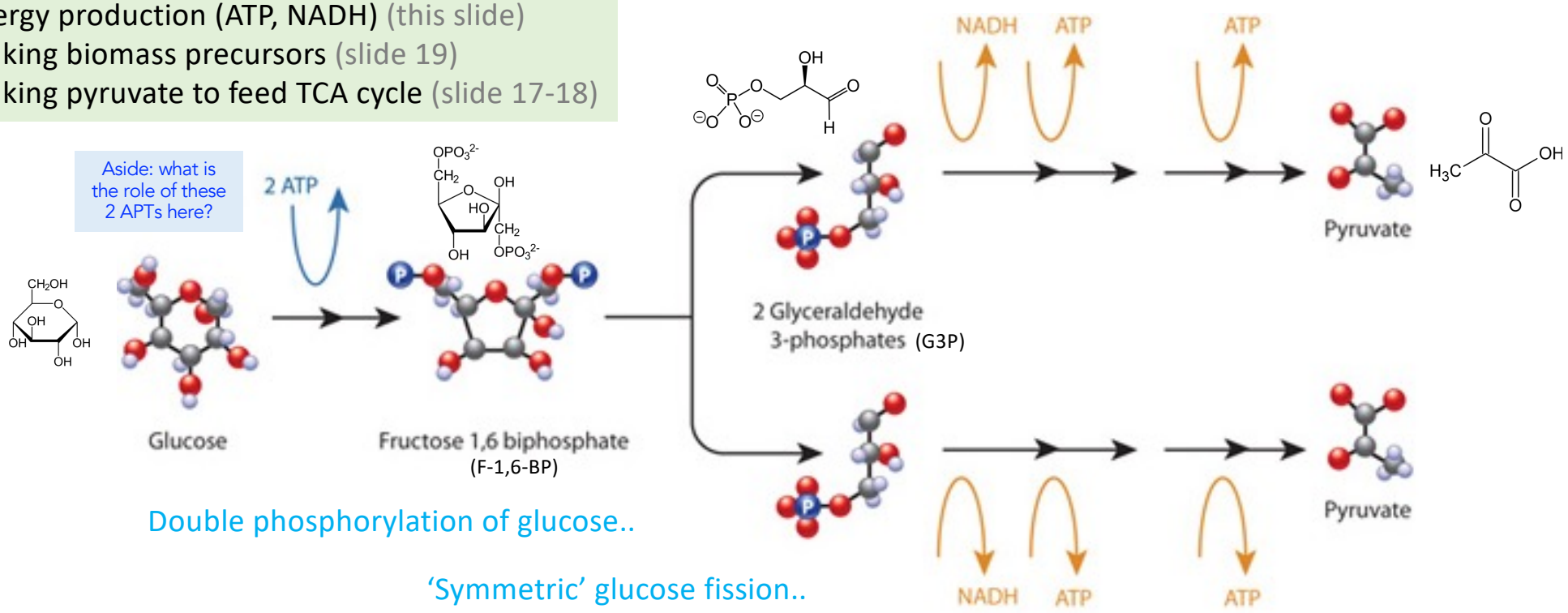
Glycolysis workflow

Glycolysis takes place in cytosol and no O_2 is required (thus glycolysis evolved early on earth…)

3 key goals of glycolysis:
--Energy production (ATP, NADH) (this slide)
--Making biomass precursors (slide 19)
--Making pyruvate to feed TCA cycle (slide 17-18)

Q: No O_2 is used but oxidation happens to glucose in glycolysis -- how & where?

Aside: what is the role of these 2 APTs here?



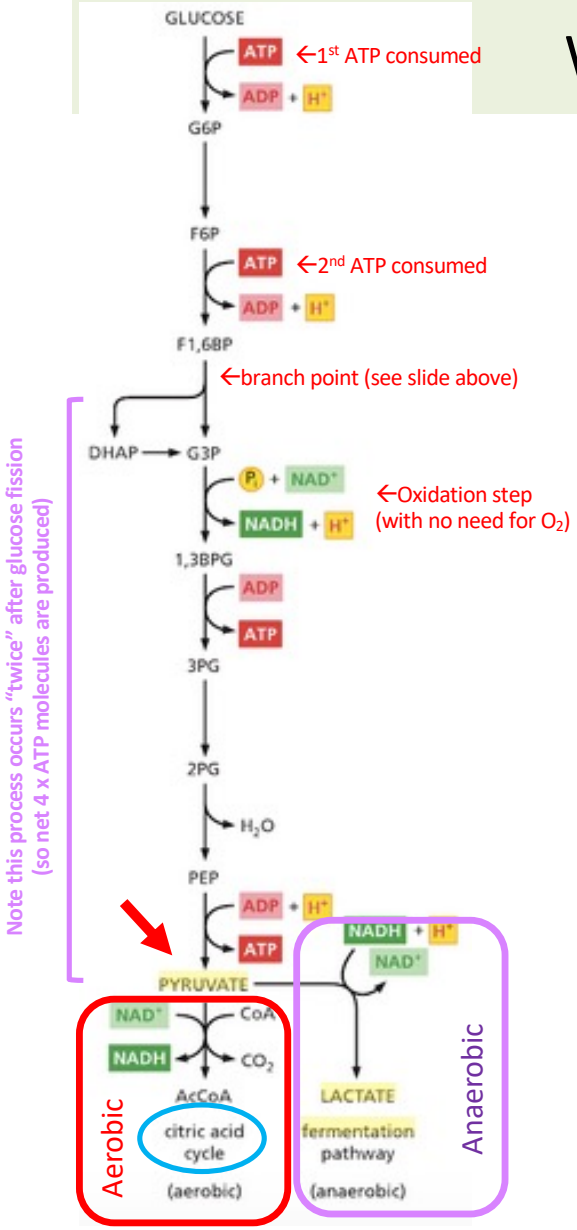
Double phosphorylation of glucose..

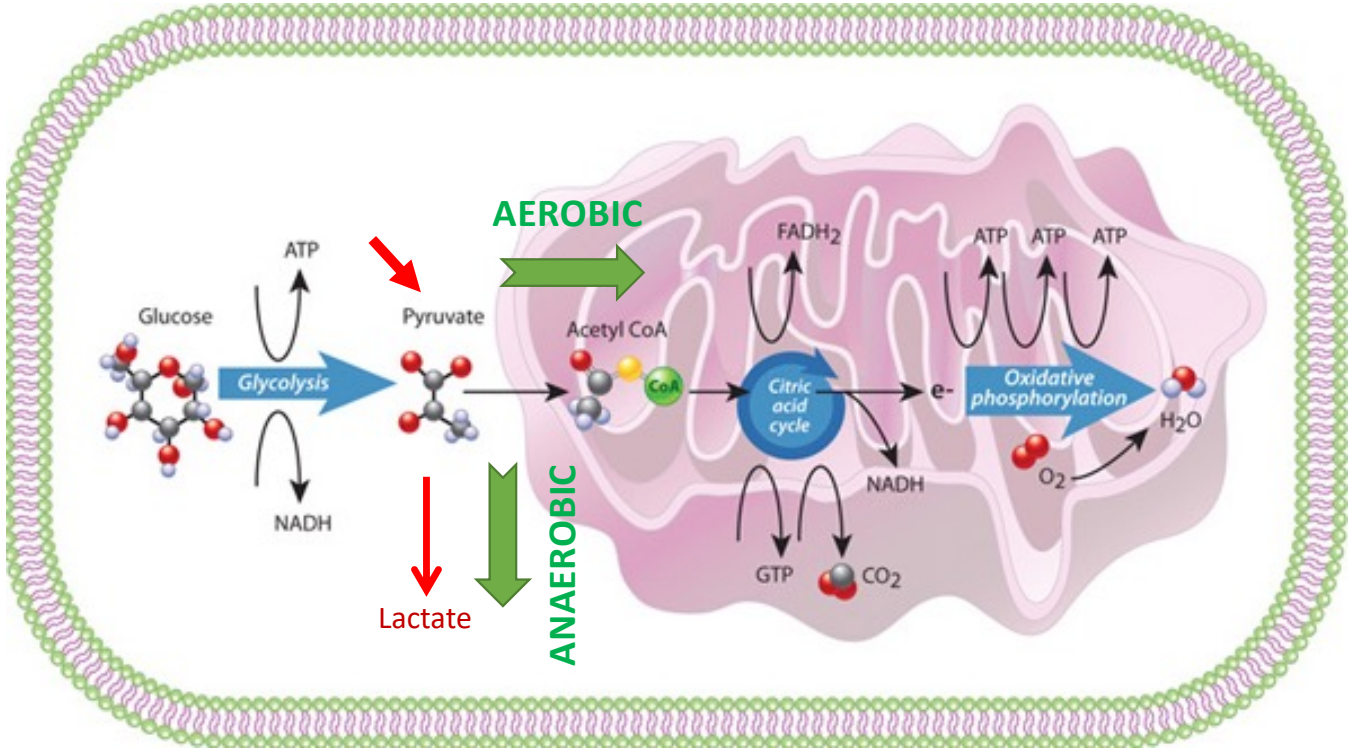
'Symmetric' glucose fission..

Oxidizing and concomitant energy harvesting..

Q: how many net ATP molecules (and NADH) are synthesized per 1 glycolysis event?

What's next for pyruvate?



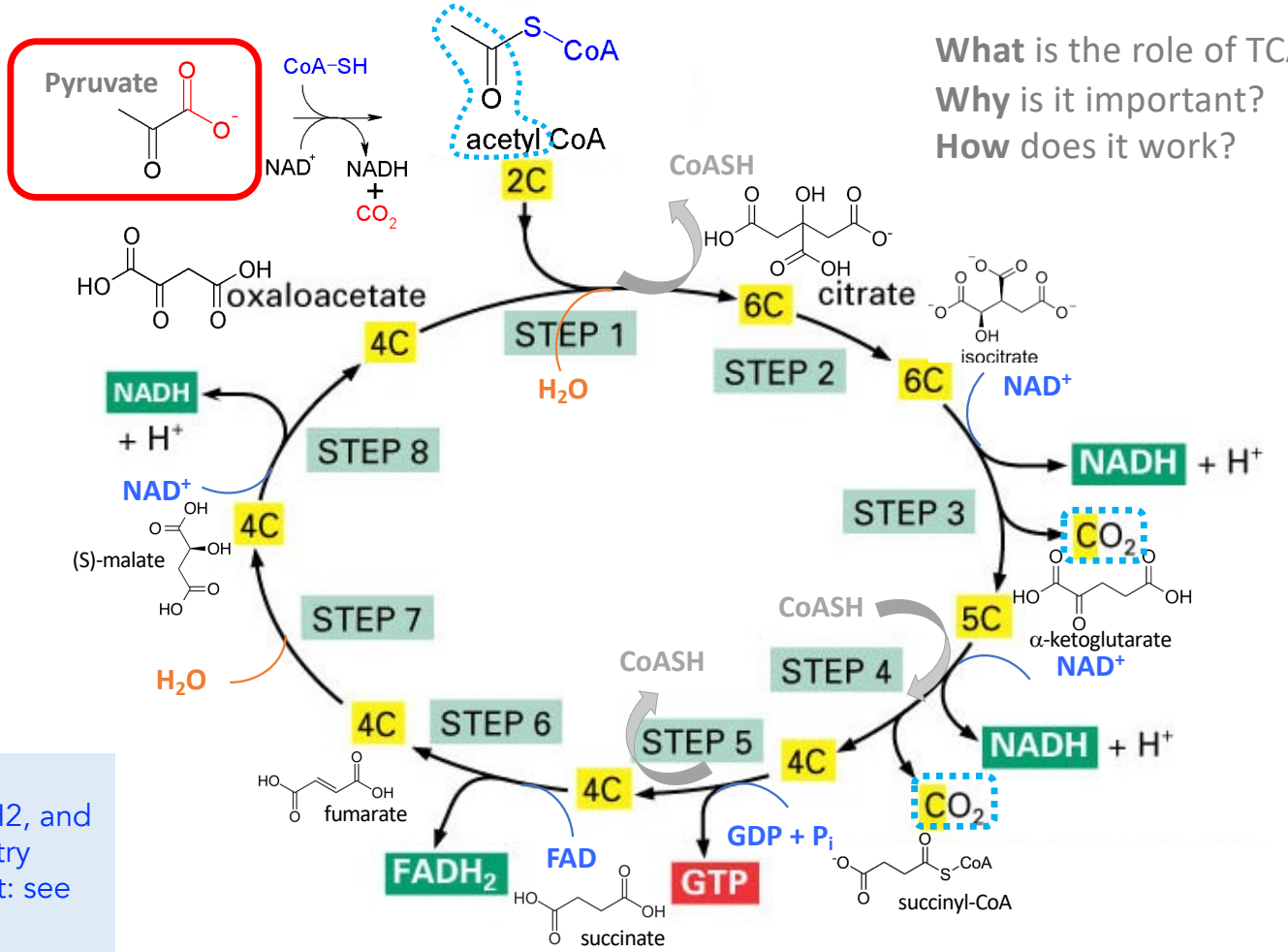


Glycolysis → 2 ATP per glucose

Ox-Phos → 36 ATP per glucose (see future lectures)

TCA cycle (aka The Krebs Cycle or Citric Acid Cycle)

Q: Through oxidizing Acetyl groups to 2 x CO_2, TCA cycle generates ____molecules of NADH, ____molecule(s) of FADH2, and ____molecule(s) of GTP that are then used for ATP production down-stream (via OxPhos).



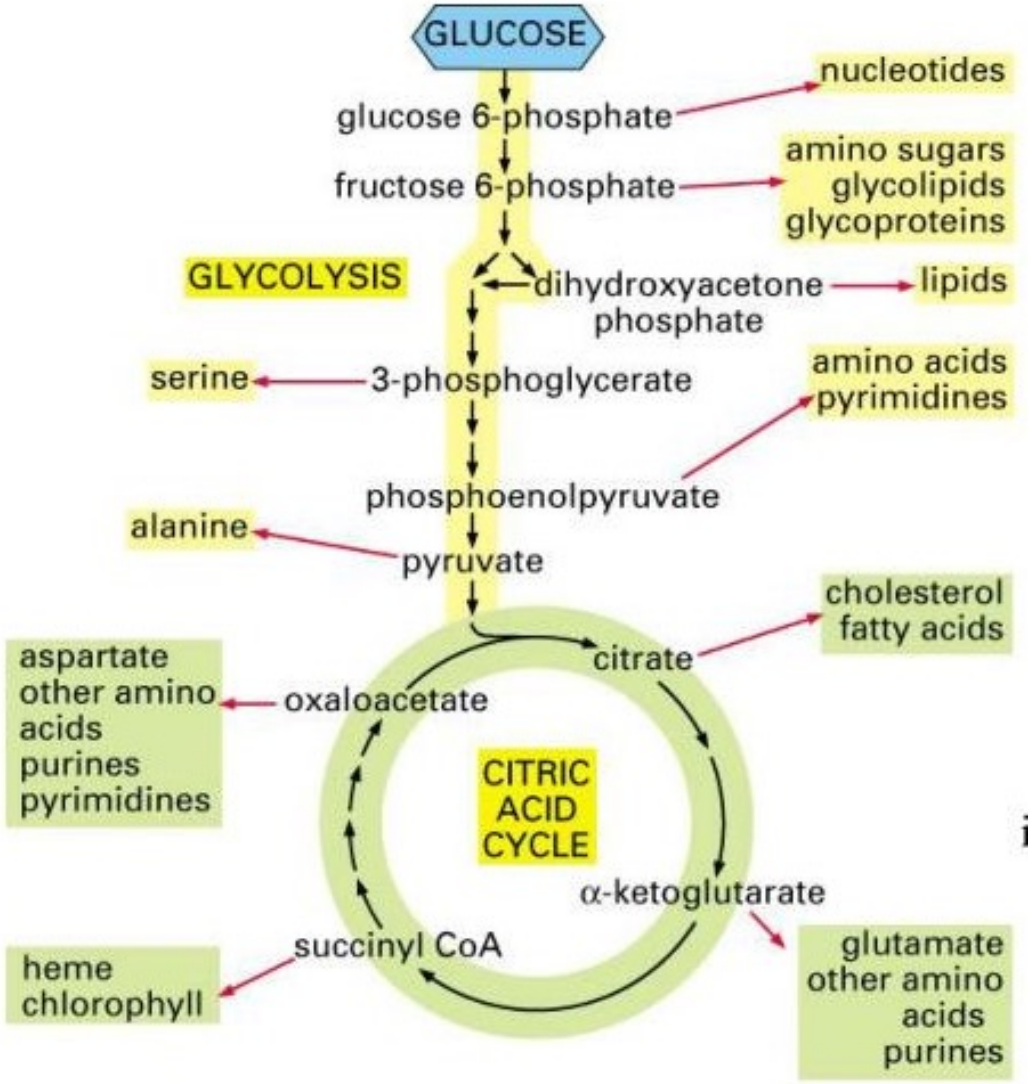
What is the role of TCA cycle?
Why is it important?
How does it work?

Q: What is the common purpose of NADH, FADH2, and ATP in metabolic chemistry (cellular respiration? (Hint: see Slide 6 and 8)

Hans Krebs
Nobel laureate 1953

Glycolytic pathway ultimately provides essential biomass required for cell growth and division

Q: What kinds of cellular biomass do you see this pathway produce for the cell?



Quiz Time!

True or False:

Because glycolysis is only a prelude to the oxidation of glucose in mitochondria (which yields several-fold more ATP), glycolysis is *not* really important for human cells

False Glycolysis is the ONLY metabolic pathway that can generate ATP in the absence of O2 (anoxia). Several instances where cells are exposed to anoxia (e.g., during all-out sprint, circulation cannot deliver enough O2 to leg muscles and glycogen breakdown and passing large amounts of glucose via glycolysis powers muscle contraction here; cells such as red-blood cells with no mitochondria don't perform oxidative metabolism so they make ATP only via glycolysis)

The reactions of the TCA cycle do not directly require the presence of oxygen

True O2 is not a substrate or a product for any rxn in the TCA cycle. In cells, however, rxn's cannot proceed for Very long in the absence of O2 bcos NADH/FADH2 cannot be converted back to NAD+/FAD by Ox Phos (which depends on O2). In the absence of NAD+/FAD, 4 separate rxn's of the TCA cycle will cease to operate (what are those 4 steps? Please review Slide 18 on TCA cycle chemistry).

Glucose is consumed at a low rate in the absence of oxygen and at a high rate in its presence

False In the absence of O2, the energy needs of the cell are met by fermentation to lactate, which requires a high rate of flow through glycolysis to generate sufficient ATP. But in the presence of O2, the cell generates ATP primarily by OxPhos, which generates ATP much more efficiently than glycolysis. Thus, less glucose is needed to supply ATP at the same rate. PS. This is related to why cancer cells are addicted to glucose…

Question:

Why does TCA cycle—which does not use oxygen—stop almost immediately upon removal of O_2?

Same reason as above – 4 steps in TCA cycle need NAD+/FAD which come from Ox Phos which needs O2

Some multiple choice questions (Glycolysis)

E

1) The body requires backup stores of reduced carbon. We have two forms, lipids and starch/glycogen. Our immediate source of reduced carbon is glucose, which we access by several pathways such as glycolysis. We normally have about 90 mg of glucose per deciliter of blood but need to have an immediate backup source of glucose. Which of these is the source? **a.** Fats. **b.** Proteins. **c.** Vitamins. **d.** ATP. **e.** Glycogen (note: glycogen is polymeric form of glucose)

A

2) The process of fermentation of glucose is favored in systems that: **a.** Have little or no oxygen available. **b.** Operate in hot springs at high temperature. **c.** Function at high oxygen concentration. **d.** Act in the presence of nitrogen. **e.** Lack enzymes to carry out glycolysis

E

3) If a person were exercising vigorously and unable to take in sufficient oxygen, his or her tissues would probably accumulate excess amounts of: (**hint:** this molecule is the end product of glycolysis under *anaerobic* conditions)
a. Glucose. **b.** Fructose-6-phosphate. **c.** Pyruvic acid. **d.** Citric acid. **e.** Lactic acid

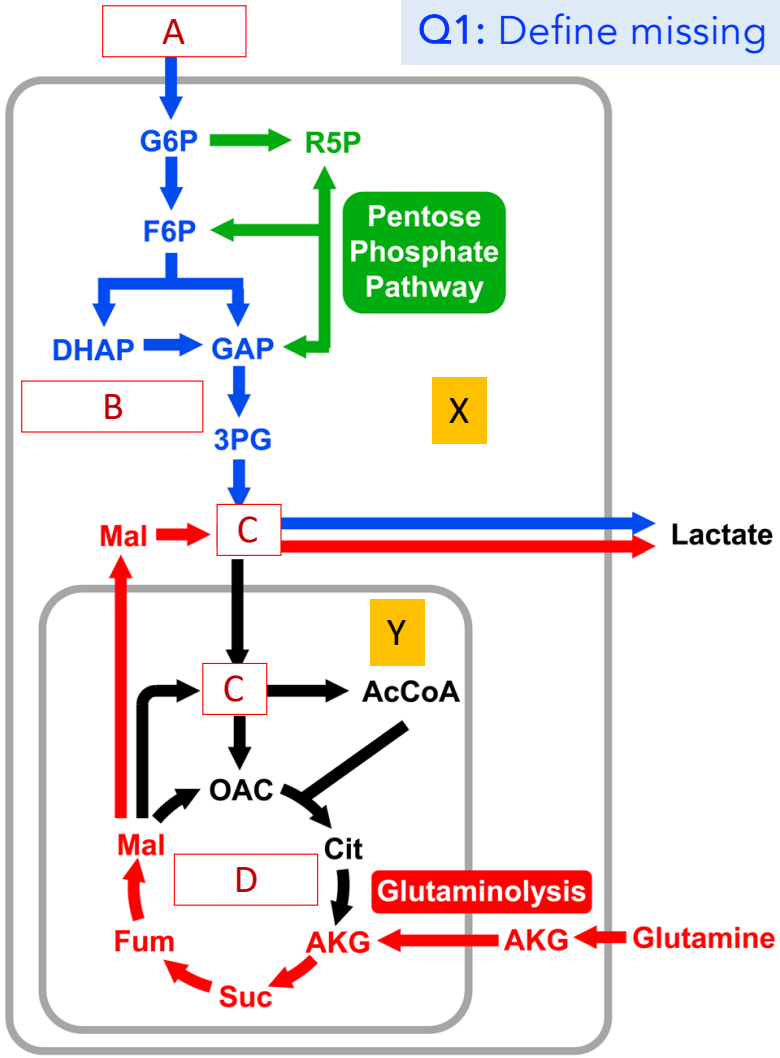
D

4) The first step in glycolysis is phosphorylation of glucose to form glucose-6-phosphate. This action serves to:
a. Oxidize glucose. **b.** Reduce glucose. **c.** Make glucose less polar so that it can diffuse through cell membranes. **d.** Make glucose more polar, locking it within the cell. **e.** Cause glucose to polymerize, forming glycogen

C

5) It seems that, if we can convert glucose to pyruvic acid and to other metabolites, we should be able to simply reverse glycolysis and form new glucose from pyruvic acid. What prevents this?
a. Carbon dioxide is lost in conversion of glucose to pyruvic acid, and we have no mechanism for replacement of the carbon dioxide
b. There is too much demand for pyruvic acid, and it is rapidly consumed for other purposes
c. The free energy changes for some of the reactions that lead from glucose to pyruvate are too large and negative for easy reversal
d. The entropy changes favor formation of fewer, large molecules
e. The free energy change for some of the reactions that lead from glucose to pyruvic acid are too large and positive for easy reversal

More quiz (if time permits)!



Q1: Define missing letters: A, B, C, D.

Q2: Assign X vs. Y to mitochondria vs. cytoplasm

----**A**---- and glutamine are the two most highly consumed carbon substrates in cancer cells. Both substrates can be converted to lactate via ----**B**----- and glutaminolysis, respectively. High lactate secretion, especially from ----**A**---- , is a major hallmark of cancer cells known as the Warburg effect, or aerobic glycolysis.

Learning outcomes (Week 9: CH-313 Chemical Biology - Synopsis)

- The three-stage cellular respiration process
- Chemistry and functional roles of activated energy carriers
- Fundamentals of Glycolysis and TCA cycle
- ‘Sugar addiction’ of cancer cells (more later)