BB 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic
Fermentations — Exam-1 material

Pyruvate — Exam-2 material
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Fat Catabolism — Exam-3 material
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: β-oxidation, 4 steps:

Protein Catabolism
Amino-Acid Degradation
- Dealing with the nitrogen; Urea Cycle
- Dealing with the carbon; Seven Families

Nucleic Acid & Nucleotide Degradation

PHOTOSYNTHESIS:
Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop experiment"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Overview of light reactions

ANABOLISM I: Carbohydrates

Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
  - Carboxylase
  - Oxygenase
  - Glycolate cycle

  } Know mechanism
- Stage Two – making sugar (2 enzymes to G3P; 5 to Glc)
- Stage Three – remaking Ru 1,5P₂ (5 enzymes)

Overview and regulation
Calvin cycle connections to biosynthesis
C4 versus C3 plants
Kornberg cycle – gly**ox**ylate

Carbohydrate Biosynthesis in Animals
- precursors
- Cori cycle

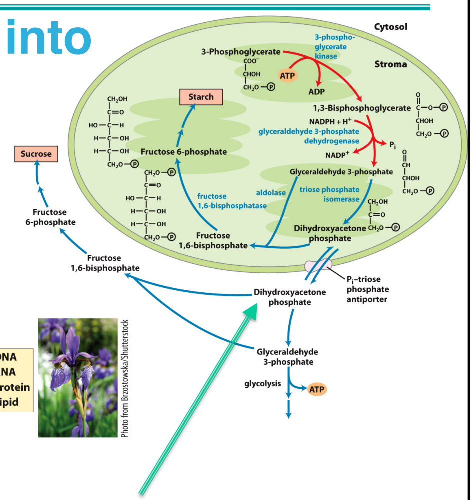
Gluconeogenesis
- reversible steps
- irreversible steps – four
  - energetics
  - 2-steps to PEP
    - mitochondria
    - Pyr carboxylase-biotin
    - PEPCK
  - FBPase
  - G6Pase

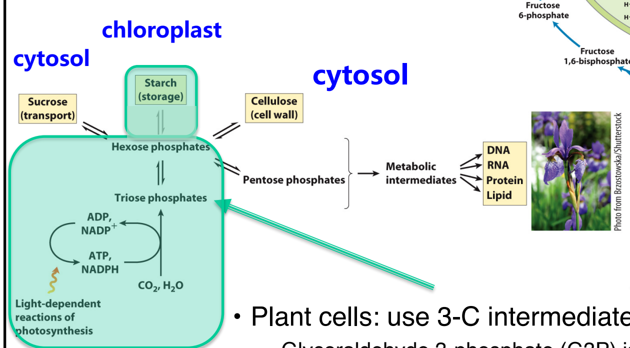
Glycogen Synthesis
- UDP-Glc
- Glycogen synthase
- branching

Pentose-Phosphate Pathway
Regulation of Carbohydrate Metabolism
Anaplerotic reactions

Photosynthesis

Assimilation of CO_2 into Biomass





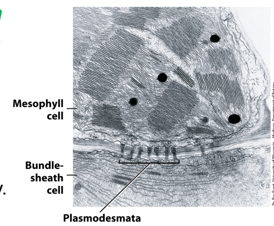
- Plant cells: use 3-C intermediates for further synthesis
  - Glyceraldehyde 3-phosphate (G3P) is the most important one.
  - made from CO_2, H_2O, plus ATP and NADPH from photosynthesis

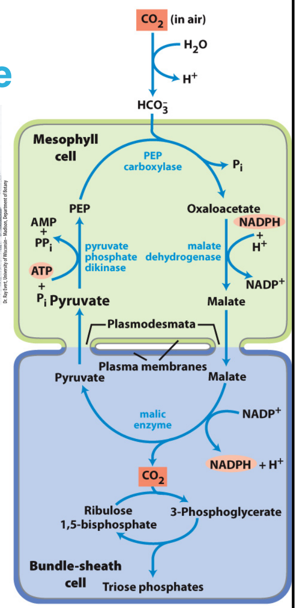
Photosynthesis

The C_4 Pathway

C_4 versus C_3 Plants; Benefits of C_4 Plants: Heat and Drought Resistance

- *C_4 plants (tropical, hot climates) have an earlier step, in different cells, that isolate Rubisco from the air.*
  - In heat, the Rubisco's oxidase is favored.
  - C_4 plants spatially separate CO_2 fixation from rubisco activity, resulting in less reaction of rubisco with oxygen and avoidance of the costly glycolate pathway.





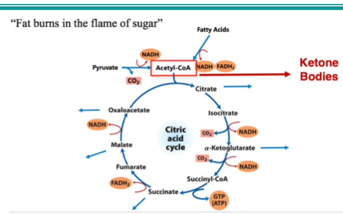
- Physical separation of reactions:
  - CO_2 is captured into ***oxaloacetate*** in mesophyll cells of the leaf.
  - Oxaloacetate is converted to malate, which then passes into bundle-sheath cells where CO_2 is released for Rubisco.

- The C_4 pathway has a higher energy cost than the glycolate cycle on a stoichiometric basis, but its all about the ratio of carboxylase:oxidase. This pathway has overall increased efficiency in heat.

- Another pathway to avoid photorespiration was first discovered in *Crassulacae (**C**rassulacean **A**cid **M**etabolism (**CAM**))* in high, dry conditions
  - Stomata open/close; the CO_2 from C4 fixation is stored as malate in **vacuoles**.

Photosynthesis

Recall in animals:

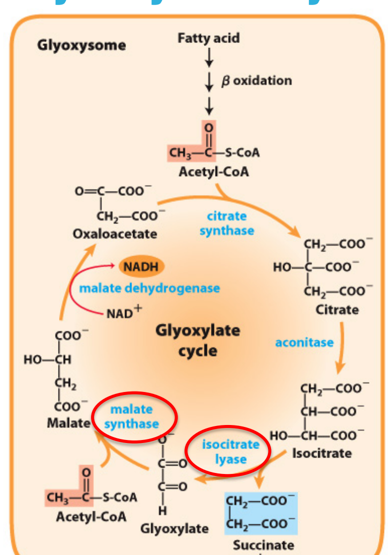


Kornberg Cycle

GlyOXylate Cycle

Plants Use Fats and Proteins for Carbohydrate Synthesis:

- In the TCA cycle, in the **glyoxysome**, instead of burning isocitrate, it short circuits TCA, taking isocitrate directly to succinate
- The result is the glyoxylate intermediate
- Re-cycle this glyoxylate by making malate from more acetyl CoA in a similar reaction as citrate synthase



We'll come back to this later…………

Photosynthesis: Carbon Fixation Summary

We learned that:

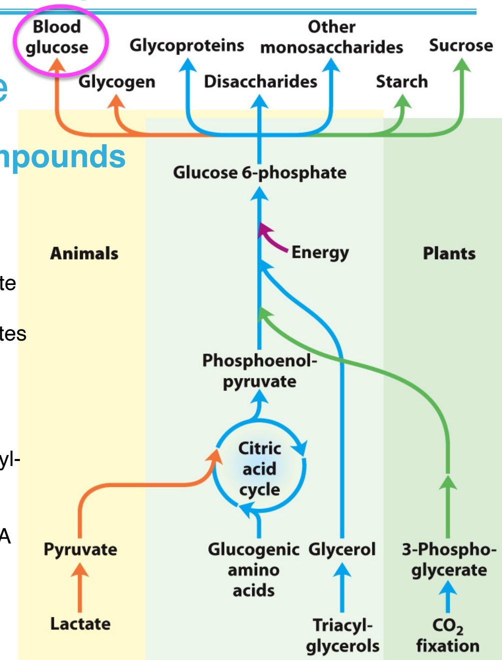
- ATP and NADPH from photosynthesis are needed in order to assimilate CO_2 into carbohydrates, which is initiated by Rubisco
- This key enzyme of the Calvin cycle fixes carbon dioxide as well as oxygen.
- assimilations of six CO_2 molecules via the Calvin cycle lead to the formation of one molecule of glucose for use in anabolic reactions
- enzymes of Calvin Cycle have common regulation mechanisms via pH, Mg^{2+}, and/or NADPH (F_d)
- In C_3 plants, issues with selectivity of rubisco for CO_2 vs. O_2 causes a wasteful incorporation of oxygen. C_4 and CAM plants have evolved structures for reducing this waste.
- Plants can convert acetyl-CoA into carbohydrates via the Kornberg Cycle (glyoxylate cycle)

ANABOLISM I Carbohydrates

Carbohydrate Synthesis in Animals

Gluconeogenesis

Gluconeogenesis: Making "New" Glucose



Precursors: From what compounds can glucose be made?

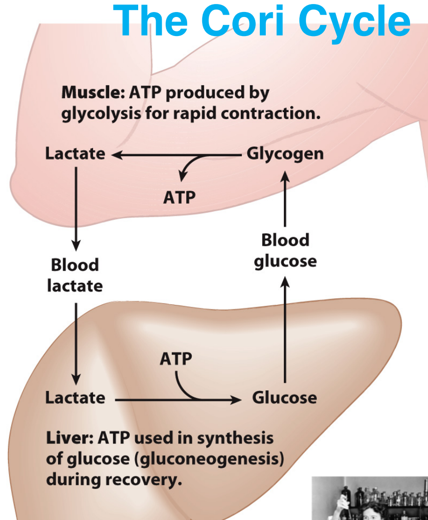
- Animals can produce glucose from sugars or proteins.
  - sugars: pyruvate, lactate, or oxaloacetate
  - protein: from amino acids that can be converted to citric acid cycle intermediates (or glucogenic amino acids)
- Animals cannot produce glucose from fatty acids.
  - product of fatty acid degradation is acetyl-CoA coming from fatty acids and ketogenic amino acids
  - There is no net conversion of acetyl-CoA to oxaloacetate in Kreb's Cycle

Plants, yeast, and many bacteria use the Kornberg Cycle to convert acetyl-CoA to oxaloacetate, thus producing glucose from fatty acids.

Gluconeogenesis

The Cori Cycle

- Blood glucose is largely made in the liver, although other organs can reverse glycolysis, but not deliver free glucose into the blood
- Synthesis of glucose from simpler compounds: **called gluconeogenesis**
- Operates only if ATP and NADH are plentiful
- Other tissues deliver carbon to liver from "waste" products (Ala and Cori Cycles).



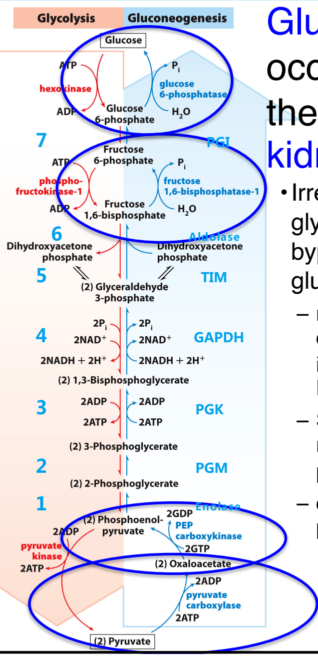
As you can see the two pathways operate in different tissues, but how is this controlled in a single cell?

Gluconeogenesis

Glycolysis *versus* Gluconeogenesis

Glycolysis occurs mainly in the muscle and brain.

- Opposing pathways that are both thermodynamically favorable:
- Glycolysis: $\Delta G^{\circ\prime} = -35$ kcal/mol
- Gluconeogenesis: $\Delta G^{\circ\prime} = -9$ kcal/mol
  - operate in opposite direction
    - end product of one is the starting compound of the other
- Seven Reversible reactions are used by both pathways.
- Three "glycolysis-specific" steps are reversed with Four "gluconeogenesis-specific" steps.



Gluconeogenesis occurs mainly in the liver and kidney cortex.

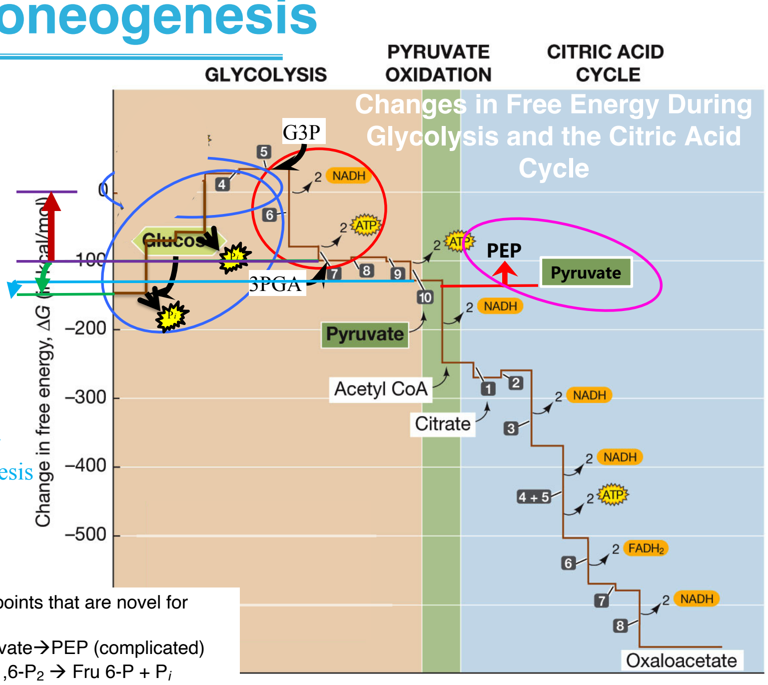
- Irreversible reactions of glycolysis must be bypassed in gluconeogenesis.
  - no ATP generated during gluconeogenesis; instead 6 ATPs and 2 NADH needed per Glc.
  - Some different enzymes results in the different pathways
  - differentially regulated to prevent a futile cycle

Lets look at the energetics of making glucose…..

Gluconeogenesis

Favorable energetics for Calvin Cycle

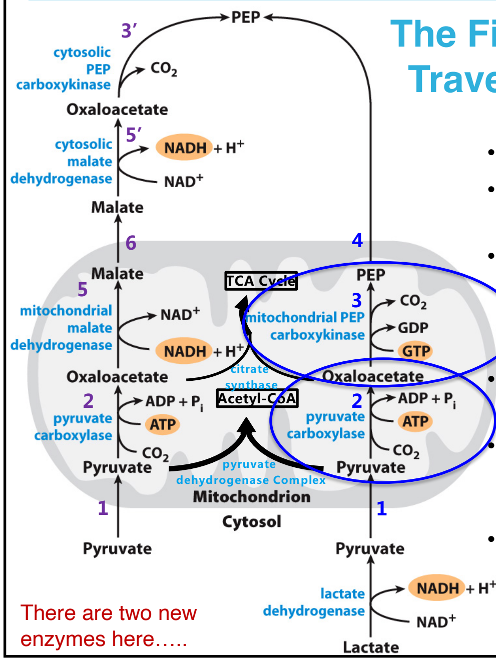
Favorable energetics for Gluconeogenesis



There are then 3 points that are novel for gluconeogenesis:

1. Pyruvate→PEP (complicated)
2. Fru 1,6-P_2 → Fru 6-P + P_i
3. Glc 6-P→ glucose + P_i

Gluconeogenesis



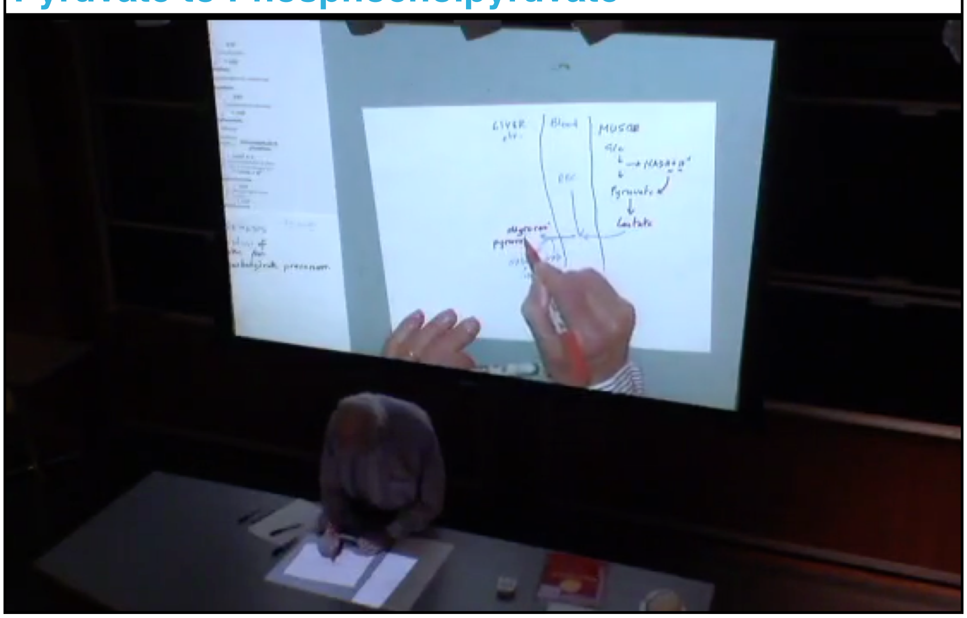
There are two new enzymes here…..

The First Gluconeogenic Steps Travel Through Mitochondria

- There are **two** ways from pyruvate to PEP.
- Best place to assess whether there is ample ATP and NADH is the mitochondria.
- The inner mitochondrial membrane is selectively permeable: Malate, PEP, and pyruvate, while oxaloacetate cannot escape.
- Oxaloacetate can be utilized in the citric acid cycle (Kreb's cycle) if needed.
- Oxaloacetate can be converted to PEP or Malate in the mitochondria, then transported to cytosol for gluconeogenesis.
- Will discuss the decision that pyruvate must make later…..

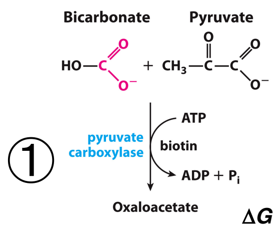
Gluconeogenesis

Pyruvate to Phosphoenolpyruvate

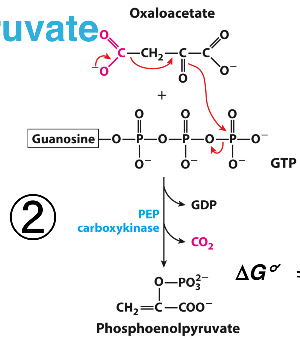


Gluconeogenesis

Pyruvate to Phosphoenolpyruvate



$\Delta G'^{\circ}$ = –0.5 kcal/mol



$\Delta G'^{\circ}$ = +0.7 kcal/mol

- Requires two energy-consuming steps
- The first step, pyruvate carboxylase (PC) converts pyruvate to oxaloacetate.
  - carboxylation using a biotin cofactor
  - This enzyme is only in the mitochondria; requires transport of pyruvate
- The second step, phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to PEP.
  - phosphorylation from GTP and decarboxylation
  - occurs in mitochondria or cytosol depending on the organism
- During this 2-step conversion, the same carbon from CO_2 is added and immediately removed from the structure.

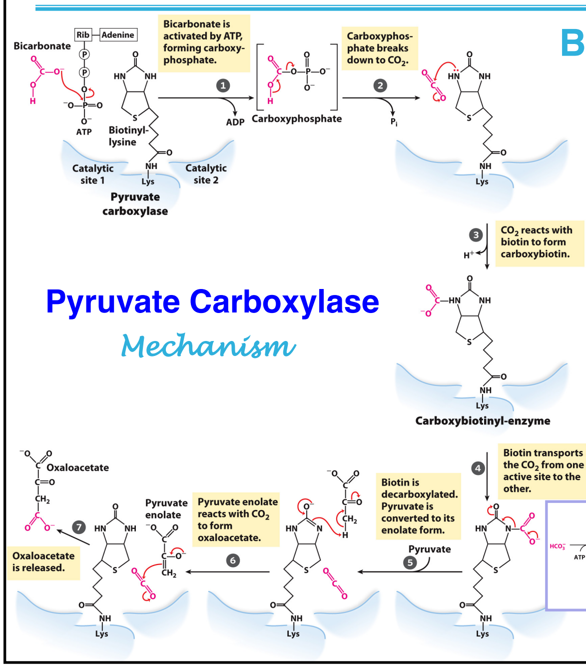
Lets look at the PC mechanism more closely…..

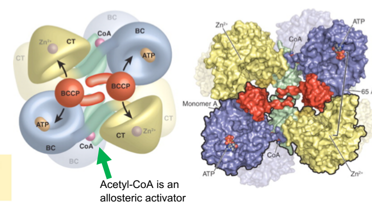
Gluconeogenesis

Biotin is a CO_2 Carrier

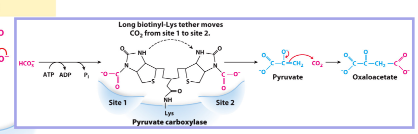
Pyruvate Carboxylase

Mechanism



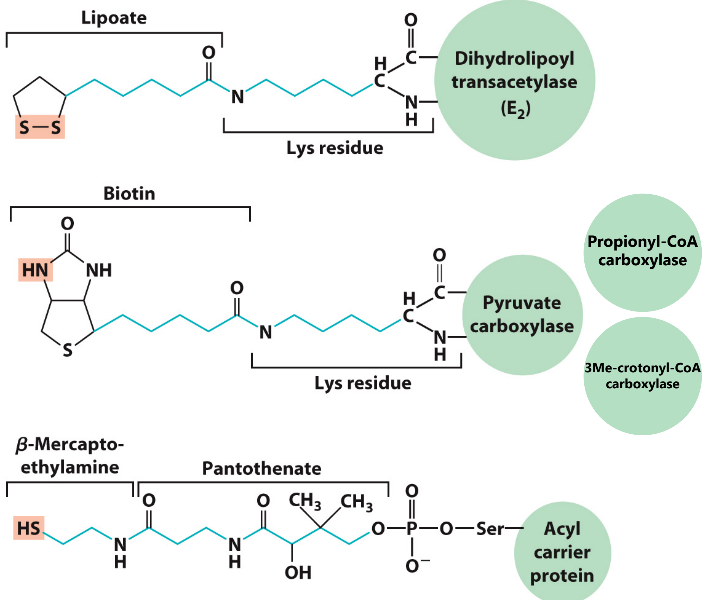


BCCP - biotin carboxyl carrier protein
BC - biotin carboxylase
CT - carboxyltransferase



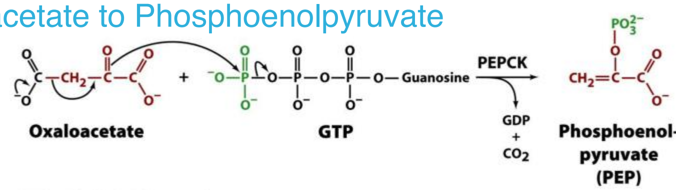
Gluconeogenesis

Biological Tethers Allow Flexibility



Gluconeogenesis

Oxaloacetate to Phosphoenolpyruvate



Phosphoenolpyruvate Carboxykinase (PEPCK)

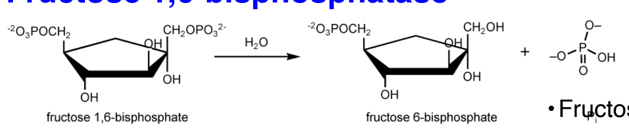
Phosphoenolpyruvate to Fru 6-P

[PEP→2PGA→3PGA→1,3BPGA→GA3P→DHAP→Fru 1,6P_2]

−3 −0.5 −1 0 −2 +5 −1.3

Fructose 1,6-bisphosphatase

$\Delta G^{o\prime}$ = −4 kcal/mol



- Catalyze reverse reaction of PFK in glycolysis
- Fructose 1,6-bisphosphate → fructose 6-phosphate
  - by fructose 1,6-bisphosphatase-1
  - coordinately/oppositely regulated with PFK
  - cleaves phosphate with water
  - DOES NOT generate ATP

Glucose 6-phosphatase

$\Delta G^{o\prime}$ = −3.3 kcal/mol

Glucose 6-phosphate → glucose

- Water hydrolyzes the His-P_i
- DOES NOT generate ATP

Mechanism on website

Gluconeogenesis

Why do we need glucose?

RECALL:

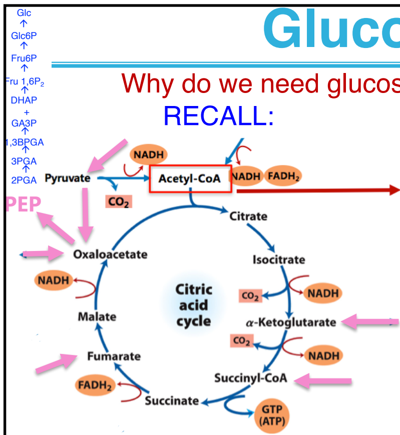


TABLE 14-4 Glucogenic Amino Acids, Grouped by Site of Entry

Pyruvate Alanine Cysteine Glycine Serine Threonine Tryptophan[a]	**Succinyl-CoA** Isoleucine[a] Methionine Threonine Valine
	Fumarate Phenylalanine[a] Tyrosine[a]
α-Ketoglutarate Arginine Glutamate Glutamine Histidine Proline	**Oxaloacetate** Asparagine Aspartate

Note: All these amino acids are precursors of blood glucose or liver glycogen, because they can be converted to pyruvate or citric acid cycle intermediates. Of the 20 common amino acids, only leucine and lysine are unable to furnish carbon for net glucose synthesis.
[a]These amino acids are also ketogenic (see Fig. 18-15).

- Physiologically necessary: Brain, nervous system, and red blood cells generate ATP ONLY from glucose.
- When we can't get it from pyruvate, amino acids are utilized, which allows generation of glucose when glycogen stores are depleted:
  - during starvation
  - during vigorous exercise
  - can generate glucose from amino acids, but not fatty acids

$\Delta G^{\circ\prime}$ = –9 kcal/mol

- Gluconeogenesis Costs 4 ATP, 2 GTP, and 2 NADH. Net reaction:

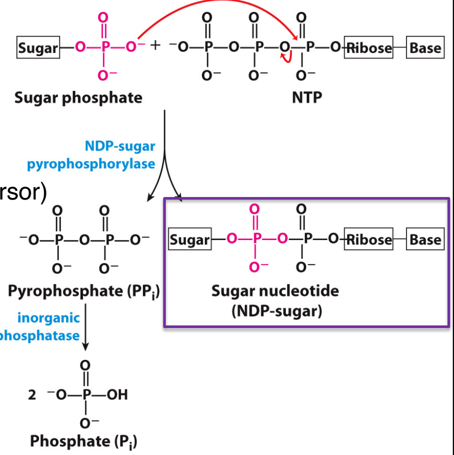
2 Pyruvate + 4 ATP + 2 GTP + 2 NADH + 2 H^+ + 4 H_2O → Glucose + 4 ADP + 2 GDP + 6 P_i + 2 NAD^+

Glycogen Synthesis

Storing a ready-reserve of carbohydrate

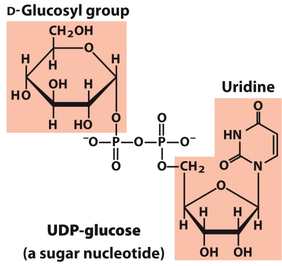
Glycogen Synthesis

- Synthesis of glycogen requires two enzymes, whereas glycogen degradation, only used phosphorylase. Both pathways use α-phosphoglucomutase; the start and end junction is Glc 1-P.



Net reaction: Sugar phosphate + NTP → NDP-sugar + 2P$_i$

- Blood glucose must be:
  - Phosphorylated: Glc → Glc-6-P
  - Then converted: Glc 6-P → Glc 1-P
  - Activated with UDP (UDP-Glc is the precursor)
  - Added to glycogen



UDP-glucose
(a sugar nucleotide)