Lecture 30: Gluconeogenesis, Pathway Regulation

Gluconeogenesis:

Location: cytosol (liver & kidney cells)
Input: pyruvate
Output: glucose

Energetics: *Energy required – 2 GTP, 4 ATP, 2 NADH*
Key Regulated Step: F 1-6 bis phosphatase.

General properties of Catabolic and Anabolic pathways:

	Catabolic (e.g. glycolysis)	Anabolic (e.g. gluconeogenesis)
Input→out	Complex → Simple	Simple → Complex
Redox	Oxidizing: $NADH/FADH_2$ produced (electron acceptors)	Reducing: $NADH/ FADH_2$ required (electron donors)
Energy	Produced	Consumed

Gluconeogenesis: The formation of glucose is essential for the maintenance of constant blood glucose levels. The liver, and to a lesser extent the kidneys, are the only organs that carry out this process. All of our other tissues and organs (especially the brain) require this newly-synthesized glucose during periods of fasting, *i.e.* between meals and during sleep. *This process is particularly important during strenuous exercise, where the lactic acid produced during anaerobic metabolism in the muscle is returned to the liver and converted back to glucose.*

To maintain flux through the pathway it is essential to have ΔG<0 for each step in the pathway. The free-energy changes in gluconeogenesis are inverted compared to glycolysis by the following:

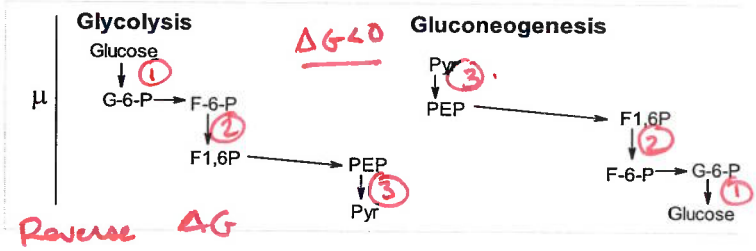
- Steps that have ΔG≈0 in glycolysis are reversible and catalyzed by the **same enzyme** in both pathways. The sign of ΔG is flipped by indirect coupling by the large energy change from the hydrolysis of G-6-P at the end of gluconeogenesis.
- Steps in glycolysis with ΔG<<0 in glycolysis cannot be reversed by indirect coupling, so the reactions have to be done by a different mechanism, direct coupling makes ΔG°.

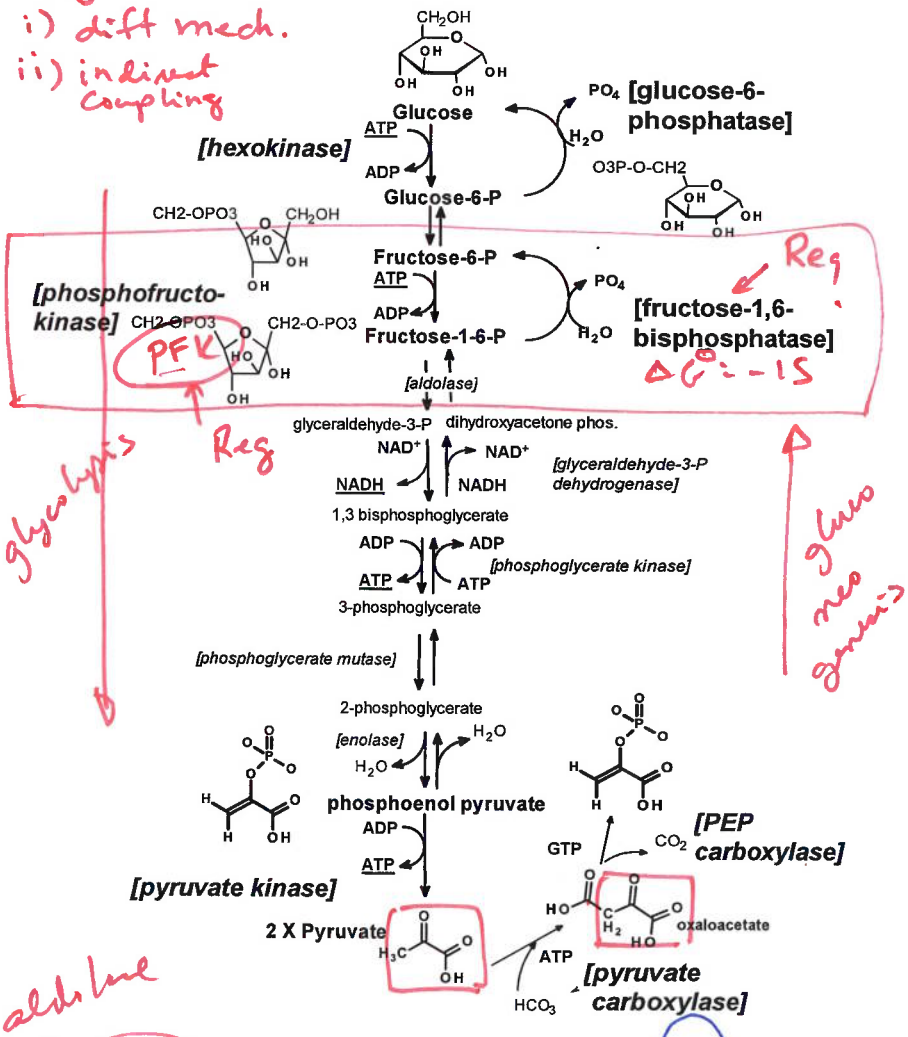
There are three steps that are done differently.

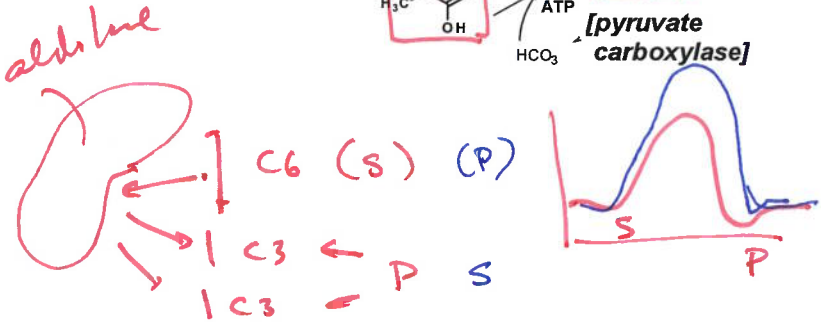
i) Pyruvate →PEP
Use of ATP and GTP (=ATP in energy) to convert Pyr to PEP.

ii) Fructose 1,6P→ F-6-P.
Spontaneous hydrolysis of phosphate.

iii)Glucose-6-P →Glucose
Spontaneous hydrolysis of phosphate.



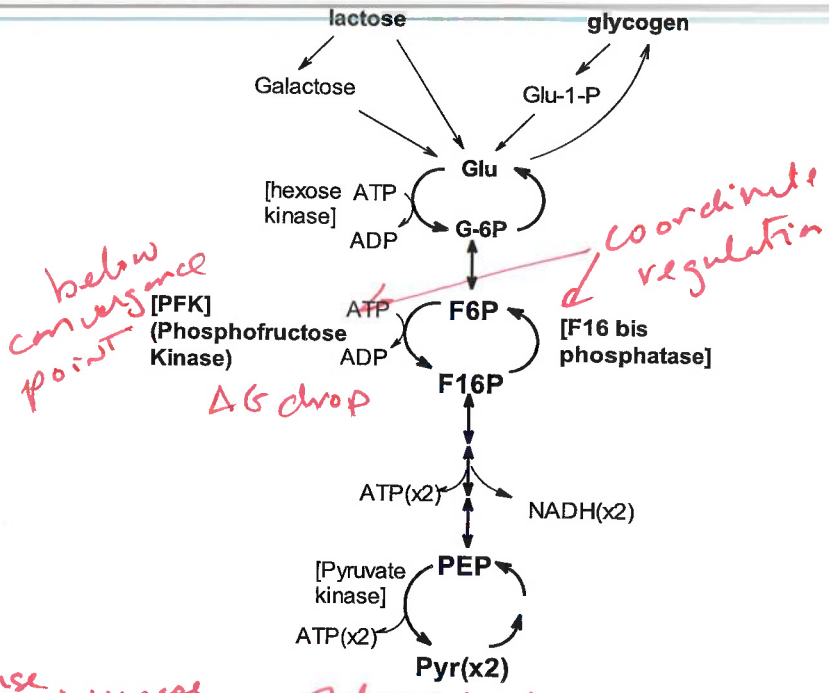




Regulation of Biochemical Pathways:

General Properties of Regulation:

- Step *below* a convergence point is usually regulated allows the regulation of many compounds.
- Step that has a high energy drop ($\Delta G<<0$) is usually regulated (e.g. PFK).
- *Opposing pathways are **coordinately regulated**,* usually at the *same step.* (e.g. glucose synthesis /degradation, glycogen synthesis/degradation).



Mechanisms of Regulation
(slow →fast)

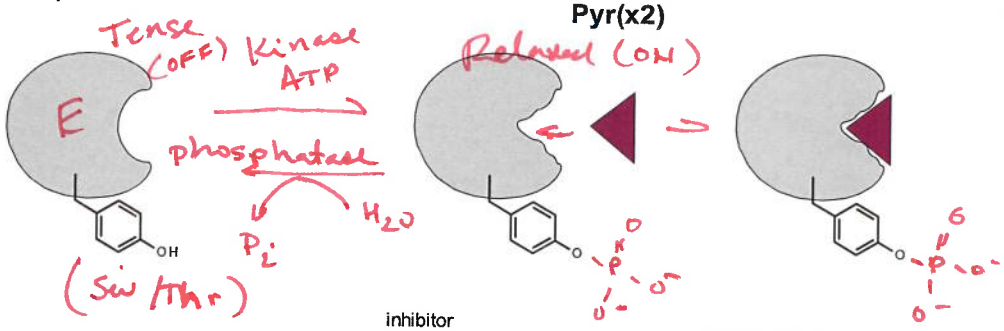
1. **Change in levels of enzymes** by regulation of the synthesis/degradation.

slow
min → weeks

$$v = E_T \cdot k_{CAT}[S]/(K_M + [S])$$

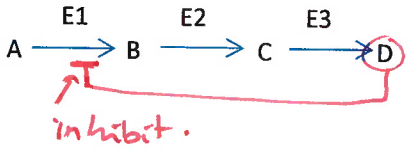
2. Change in the activity of enzymes by **covalent modification,** e.g. phosphorylation by kinase, reversal by phosphatase.

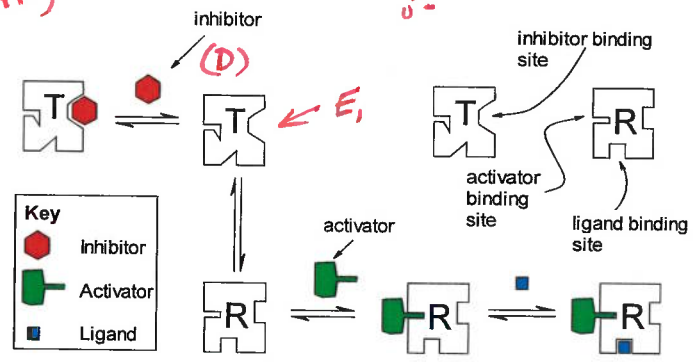
min
sec



3. **Feedback regulation (FB)** - change in the activity of enzymes due an **allosteric inhibition** or **activation** by a chemical that is near the end of the pathway (e.g. ATP & PFK), or in another pathway (e.g. citrate & PFK).

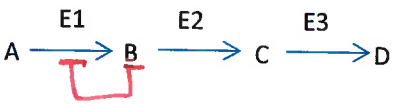
See



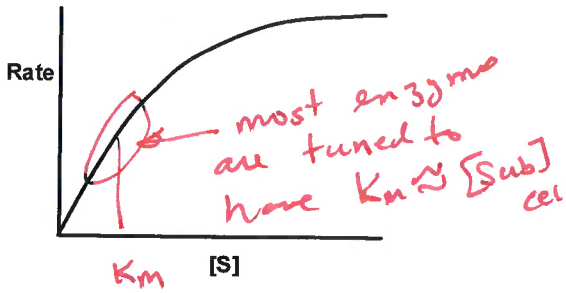


4. **Product inhibition (PI).** e.g. hexose kinase via G-6P.

sec
msec



comp inhibitor.



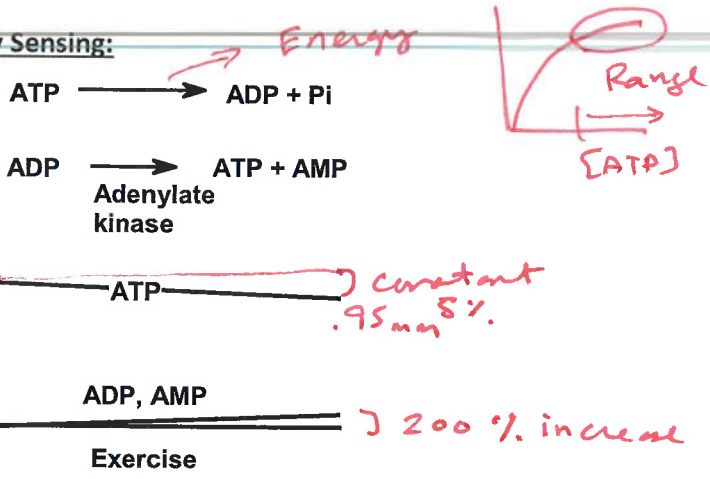
msec

5. **Substrate availability** (all enzymes, $K_M \approx [S]_{in\ vivo}$). $v \propto [S]$

Regulation of Glycolysis/Gluconeogenesis by Energy Sensing:

ATP Balance in Cells:

- ATP is hydrolyzed to produce energy for cellular activities, generating ADP + inorganic phosphate (P_i)
- ATP is re-synthesized from ADP in oxidative phosphorylation.
- ADP is also converted to ATP by the enzyme **adenylate kinase**, producing AMP as well.
- ATP levels are kept relatively constant so that enzymes that require ATP can function properly; however AMP and ADP levels can change dramatically.

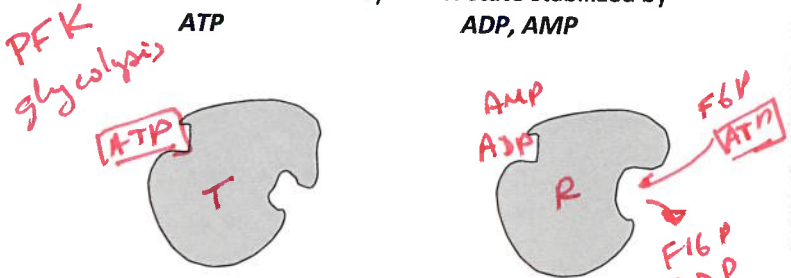


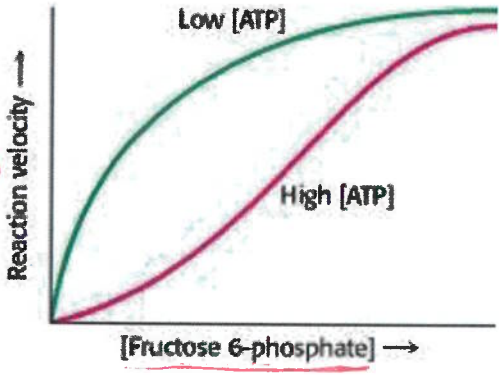
A cell has **HIGH** energy reserves when:		**Glycolysis (Glucose→Pyr)**	**Gluconeogenesis (Pyr→Glucose)**
ATP	*High*	OFF	ON
AMP, ADP	*Low*		

A cell has **LOW** energy reserves when:		**Glycolysis (Glucose→Pyr)**	**Gluconeogenesis (Pyr→Glucose)**
ATP	*Lower*	ON	OFF
AMP, ADP	*High*		

Allosteric control of – PFK-1 by AMP, ADP, ATP[1]

T-state stabilized by ***ATP*** **R-state** stabilized by ***ADP, AMP***

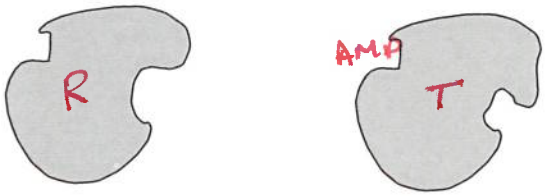


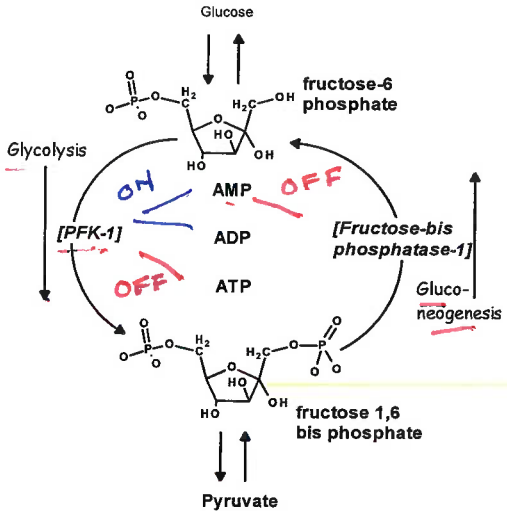


Allosteric control of fructose bis-phosphatase-1 by AMP[1]

R-state stabilized by **T-state** stabilized by ***AMP***

bis phos. gluco neo genesis





[1]These enzymes are also regulated by hormones (via F26P) and PFK by feedback from the TCA cycle (via citrate).