



REGULATION OF METABOLISM

A) General Concepts

- ❖ Catabolic and anabolic pathways are regulated in a way such that usually no opposite pathways are “turned on” at the same time; otherwise, energy can be wasted in an inefficient way.
- ❖ A reaction is said to be reversible if it can be driven both ways by the same enzyme.
- ❖ Enzymes that catalyze irreversible steps (usually rate-determining steps) are highly regulated.
- ❖ Most regulators are allosteric.
- ❖ Pathways have cross-talks where one pathway influences the activity of another.
- ❖ Pathways can be linear, cyclic or spiral.
- ❖ ATP synthesis can be substituted by non-shivering thermogenesis by release of the proton gradient without passing through complex V (ATP synthase).
- ❖ NAD^+/NADH & ADP/ATP ratios are crucial in many pathways.

B) Regulation of the Citric Acid Cycle

Regulator	Act/Inh	Enzyme	Step	Details
Low O ₂	Inh	-	-	Indirect (ETC)
Citrate	Inh	Citrate Synthase	1	Product Inhibition
Oxaloacetate	Act	Citrate Synthase	1	Creates site for acetyl-CoA
ATP	Inh	Citrate Synthase Isocitrate DH	1 3	High energy state
Fluoroacetate	Inh	Aconitase	2	Rat poison
Ca ²⁺ ions	Act	Isocitrate DH α-KG DHC	3 4	Need energy
NADH	Inh	Isocitrate DH α-KG DHC	3 4	Low NAD ⁺
ADP	Act	Isocitrate DH	3	Low energy state
Succinyl-CoA	Inh	α-KG DHC	4	Product Inhibition
GTP	Inh	α-KG DHC	4	High energy state
Arsenite (As ³⁺)	Inh	α-KG DHC	4	Lipoic acid Inh
Malonate	Inh	Succinate DH	6	Used in MI cases

- ❖ TCA cycle intermediates' concentrations are relatively stable.
- ❖ Citrate synthase is not allosteric, but ATP is an allosteric inhibitor of it.
- ❖ The rate-limiting step in Krebs's cycle is catalyzed by Isocitrate DH (step 3).
- ❖ Steps with highly negative ΔG are highly regulated:
 - Citrate formation (step 1)
 - Isocitrate oxidative decarboxylation (step 3, highest regulation)
 - α-ketoglutarate oxidative decarboxylation (step 4)
- ❖ All the previous are physiologic regulators except:
 - Fluoroacetate (non-competitive inhibitor of aconitase)
 - Arsenite (non-competitive inhibitor of α-KG DHC (specifically E₂))
 - Malonate (competitive inhibitor of succinate DH)

C) Regulation of the Electron Transport Chain

- For the normal function of complex V (ATP synthase) which is the terminal complex in the ETC, the following are needed:
 1. ADP (to be phosphorylated); it is the **most important** factor and is referred to as respiratory control or acceptor control.
 2. P_i (for phosphorylating ADP)
 3. O_2 (final e^- acceptor; complex IV); its consumption depends on [ADP]
- The ETC also needs NADH and/or $FADH_2$ as electron donors supplied from other metabolic pathways; they feed complexes I & II with electrons.
- Uncoupling proteins (UCPs) – removes the PMF without ATP synthesis:
 - **UCP1 (thermogenin)** → brown adipose tissue → directly activated by fatty acids → non-shivering thermogenesis (in case of hypothermia).
 - **UCP2** → most cells; **UCP3** → Skeletal Ms; **UCP4 & UCP5** → Brain
- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds:
 - Uncoupling agents as well; transport protons into the matrix
 - FDA banned due to harmful effects

The table below lists non-physiologic inhibitors of the ETC and OxPhos:

Inhibitor	Acts on	Details
Rotenone (insecticide) & Amytal (sedative)	Complex I	-
Antimycin A	Complex III	Blocks the transfer of e^- to Cyt C
1. Cyanide (CN^-) 2. Azide (N_3^-) 3. CO	Complex IV	Blocks the transfer of e^- to O_2
Cyanoglycosides (in edible plant pits) e.g. amygdalin (misnomer 17)	Complex IV	Cyanogenic → CN^- acts (same as above)
Oligomycin	Complex V	Prevents passage of H^+ (no OxPhos)

D) Regulation of Glycolysis

Step 1

Hexokinase (HK): relatively stable rate with [glucose]

Glucokinase (GK): more active at higher “well-fed” [glucose]

GLUT-4: opens with insulin stimulation, increasing [glucose] inside

GKRP: inactivates GK (into the nucleus) and the interaction:

- Sequestration into the nucleus is induced by fructose-6-P
- Dissociation and activation of GK is induced by glucose

Step 3 (Phosphofructokinase ⇔ rate-limiting step)

Regulator	Act/Inh	Details
Fructose-2,6-BP	Act	<i>See details below the table</i>
AMP	Act	Low energy state
ATP	Inh	High energy state
Citrate	Inh	Active TCA cycle → high energy state
H ⁺	Inh	PMF → OxPhos → high energy state

The **bifunctional enzyme** alternates between 2 states:

1. Active kinase (the dephosphorylated form):

- Insulin-induced form
- fructose-6-P → fructose-2,6-BP
- active glycolysis (inactive gluconeogenesis)

2. Active phosphatase (the phosphorylated form):

- Glucagon-induced form
- fructose-2,6-BP → fructose-6-P
- inactive glycolysis (active gluconeogenesis)

Step 10 (Pyruvate kinase)

Regulator	Act/Inh	Details
Fructose-1,6-BP	Act	Feedforward activation
ATP	Inh	High energy state
Alanine	Inh	Pyruvate (direct product) availability

Glucagon causes the phosphorylation (inactivation) of PK

Non-physiologic inhibitors

- Fluoride: inhibits enolase (step 9) → reduces dental caries
- Arsenate (As^{5+}): binds to G3P → cannot bind P_i → no ATP

E) Regulation of the Pyruvate DH Complex

Allosteric inhibitors:

- NADH and acetyl-CoA (direct products) inhibit the PDHC

Regulatory enzymes (tightly bound to the PDHC):

- PDH kinase: inactivates the complex by phosphorylation
 - Activated by ATP, acetyl-CoA, and NADH
 - Inhibited by pyruvate
- PDH phosphatase: activates the complex by dephosphorylation
 - Activated by Ca^{2+}

Arsenite (As^{3+}) poisoning – non-physiologic:

- As^{3+} binds lipoic acid (in E2) deactivating it (like in α -KG DHC)

F) Regulation of Glycogen Metabolism

Glycogenesis (same in both muscles and the liver):

- ❖ Glycogen synthase allosteric activators:
 - Glucose-6-P (high conc. in well fed states)

Glycogenolysis:

- ❖ Glycogen phosphorylase:
 - Allosteric inhibitors:
 - Glucose-6-P
 - ATP
 - Glucose (only in the liver)
 - Allosteric activators (only in muscles):
 - Ca^{2+} & AMP

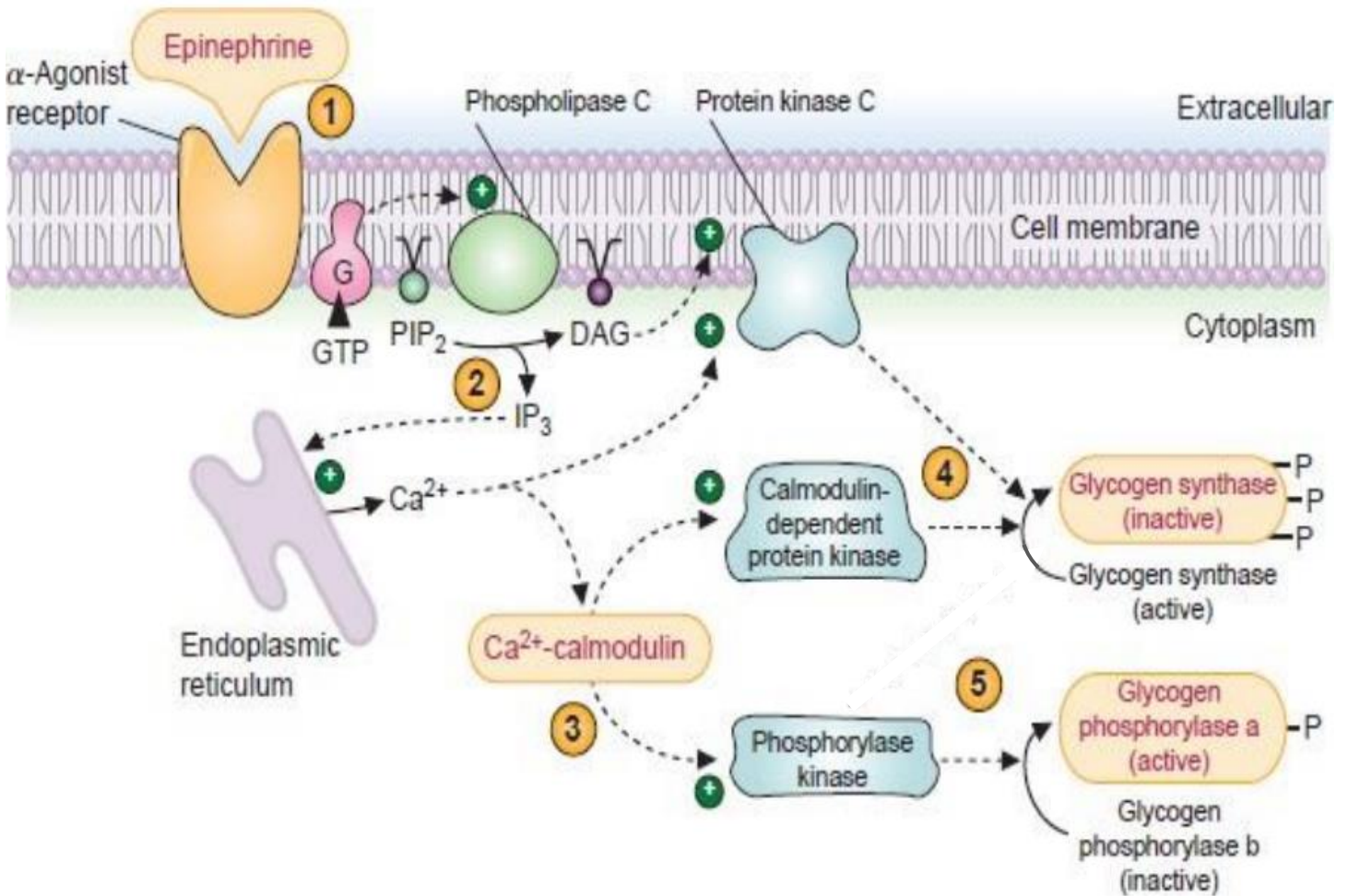
Hormonal regulation:

a) Glucagon and Epinephrine → glycogenolysis

b) Insulin → glycogenesis

- ❖ Glucagon (in the liver)
- ❖ Epinephrine (in both muscles and the liver)
- ❖ Hormone → GPCR → Adenylyl cyclase → cAMP → **PKA**:
 - PKA phosphorylates glycogen phosphorylase kinase which then phosphorylates glycogen phosphorylase (activates it)
 - PKA phosphorylates glycogen synthase (inactivates it)
- ❖ Insulin:
 - Activates phosphodiesterase (cAMP → AMP)
 - This counters both effects of PKA
 - Activates protein phosphatase 1 (PP1)
 - Dephosphorylation of both glycogen phosphorylase kinase & glycogen phosphorylase (inactivating them)

Epinephrine also has a special cascade (the figure):



- All these mechanisms above in the figure lead to the activation of glycogenolysis and the inactivation of glycogenesis.
- AMP is also a direct activator of glycogen phosphorylase.
- The degree of phosphorylation intensifies the effect whether it is activation or deactivation.

G) Regulation of Gluconeogenesis

Opposite of Glycolysis:

1) **Glucagon** (by the “GPCR →→ cAMP → PKA” pathway):

- Activates the phosphatase part of the bifunctional enzyme which decreases the levels of fructose-2,3-BP
 - This leads to the inhibition of PFK-1 (no glycolysis)
 - This also activates fructose-1,6-bisphosphatase which reverses step 3 of glycolysis → gluconeogenesis
- Inhibition of pyruvate kinase (by phosphorylation)

2) Allosteric regulators:

- Fructose-1,6-bisphosphatase:
 - Fructose-2,6-bisphosphate and AMP are inhibitors
 - Citrate is an activator

Specific regulation:

- Glucagon increases the transcription of PEP-carboxykinase
- The availability of substrates (pyruvate precursors)
- Synthesis of gluconeogenic enzymes, increasing their concentrations, and decreasing their degradation.
- **Step 1** (catalyzed by pyruvate carboxylase):
 - Allosterically activated by acetyl-CoA
 - Allosterically inhibited by ADP
- **Step 2** (catalyzed by PEP-carboxykinase):
 - Allosterically inhibited by ADP