Glycogen Metabolism

Glycogen Breakdown

Glycogen Synthesis

Control of Glycogen Metabolism

Glycogen Storage Diseases

Glycogen

Glycogen - animal storage glucan

100- to 400-Å-diameter cytosolic granules

up to 120,000 glucose units

$\alpha(1 \rightarrow 6)$ branches every 8 to 12 residues

muscle has 1-2% (max) by weight

liver has 10% (max) by weight

~12 hour supply

Although metabolism of fat provides more energy:

1. Muscle mobilize glycogen faster than fat
2. Fatty acids of fat cannot be metabolized anaerobically
3. Animals cannot convert fatty acid to glucose (glycerol can be converted to glucose)

Glycogen Breakdown

Three enzymes:

glycogen phosphorylase
glycogen debranching enzyme
phosphoglucomutase

Glycogen phosphorylase (phosphorylase) - phosphorolysis of glucose residues at least 5 units from branch point

Glycogen + P_i $\rightleftharpoons$ glycogen + glucose-1-phosphate
(n residues) (n-1 residues)

homodimer of 842-residues (92-kD) subunits

allosteric regulation - inhibitors (ATP, glucose-6-phosphate, glucose) and activator (AMP), T $\Leftrightarrow$ R

covalent modification (phosphorylation) - modification/demodification

phosphorylase *a* (active, $SerOPO_3^{2-}$)
phosphorylase *b* (less active, Ser)

narrow 30-Å crevice binds glycogen, accommodates 4 to 5 residues

Pyridoxal-5-phosphate (vit B_6 derivative) cofactor - located near active site, general acid-base catalyst

Rapid equilibrium Random Bi Bi kinetics

Glycogen Breakdown

Glycogen debranching enzyme - possesses two activities

α(1 → 4) transglycosylase (glycosyl transferase) 90% glycogen → glucose-1-phosphate

transfers trisaccharide unit from "limit branch" to nonreducing end of another branch

α(1 → 6) glucosidase 10% glycogen → glucose

Debranching activity < phosphorylase activity

Glycogen Breakdown

Phosphoglucomutase - a phosphoenzyme (Ser)

reaction similar to that of phosphoglycerate mutase

formation of glucose-1,6-bisphosphate (required for full activity)

phosphoglucokinase - provides product

glucose-1-phosphate + ATP $\rightarrow$ glucose-1,6-bisphosphate

Glycogen Breakdown

Thermodynamic considerations

phosphorylase reaction:

$$\Delta G^{\circ\prime} = +3.1 \text{ kJ}\cdot\text{mol}^{-1}$$

$\Delta G = 0$ when $[P_i/\text{glucose-1-phosphate}] = 3.5$

under physiological conditions

$$[P_i/\text{glucose-1-phosphate}] \sim 30 \text{ to } 100$$

$$\Delta G^{\circ\prime} = -5 \text{ to } -8 \text{ kJ}\cdot\text{mol}^{-1}$$

Glycogen breakdown is exergonic (favorable)

Glycogen synthesis must occur by a separate pathway

Glycogen Synthesis

Three enzymes:

UDP-glucose pyrophosphorylase

glycogen synthase

glycogen branching enzyme

UDP-glucose pyrophosphorylase - phosphoanhydride exchange

Glucose-1-phosphate + UTP $\rightleftharpoons$ **UDP-glucose + PP_i**

$\Delta G^{\circ\prime} = 0\ kJ \cdot mol^{-1}$

$$H_2O + PP_i \rightarrow 2P_i$$

$\Delta G^{\circ\prime} = -33.5\ kJ \cdot mol^{-1}$

Common biosynthetic strategy generates:

$$\text{glucose-1-phosphate} + UTP \rightarrow \text{UDP-glucose} + 2P_i$$

$\Delta G^{\circ\prime} = -33.5\ kJ \cdot mol^{-1}$

Glycogen Synthesis

Glycogen synthase - adds glycosyl unit from UDP-glucose to form $\alpha(1 \rightarrow 4)$ glycosidic bonds

Glycogen Primer:

glycogenin - protein to which glucose is added to Tyr residue by tyrosine glucosyltransferase

autocatalytically extends chain up to 7 glucose residues by UDP-glucose

glycosyl oxonium ion intermediate (similar to phosphorylase and lysozyme mechanisms)

Note:

glycogen breakdown ($\Delta G^{\circ\prime} = -5$ to -8 kJ·mol^{-1})

and

glycogen synthesis ($\Delta G^{\circ\prime} = -13.4$ kJ·mol^{-1})

are thermodynamically favorable processes

The cost of controlling both is the hydrolysis of UTP (similar to ATP)!

Glycogen Synthesis

Glycogen branching enzyme (amylo-(1,4→1,6)-transglycosylase - transfer of ~7 glycosyl residue segments to form α(1 → 6) glycosidic bonds

Thermodynamic considerations

The overall free energy for debranching is:

Breaking α(1 → 4) bond	$\Delta G^{\circ\prime}$ =	-15.5 $kJ \cdot mol^{-1}$
Forming α(1 → 4) bond	$\Delta G^{\circ\prime}$ =	+15.5 $kJ \cdot mol^{-1}$
Hydrolyzing α(1 → 6) bond	$\Delta G^{\circ\prime}$ =	-7.1 $kJ \cdot mol^{-1}$
Total	$\Delta G^{\circ\prime}$ =	-7.1 $kJ \cdot mol^{-1}$

The free energy change for branching is:

Breaking α(1 → 4) bond	$\Delta G^{\circ\prime}$ =	-15.5 $kJ \cdot mol^{-1}$
Forming α(1 → 6) bond	$\Delta G^{\circ\prime}$ =	+7.1 $kJ \cdot mol^{-1}$
Total	$\Delta G^{\circ\prime}$ =	-8.4 $kJ \cdot mol^{-1}$

Control of Glycogen Metabolism

Glycogen phosphorylase and glycogen synthase:
allosteric control
substate cycling
covalent modification of activity
(under hormonal control)

Direct allosteric control of glycogen phosphorylase and glycogen synthase

Precise flux control by having two opposing enzymes at a control step (far from equilibrium) in a pathway

Glycogen phosphorylase
activated by AMP
inhibited by ATP and glucose-6-phosphate

Glycogen synthase
activated by glucose-6-phosphate

High demand for ATP: glycogen breakdown
low [ATP], low [G6P], high [AMP]
glycogen phosphorylase stimulated
glycogen synthase inhibited

Low demand for ATP: glycogen synthesis
high [ATP], high [G6P], low [AMP]
glycogen phosphorylase inhibited
glycogen synthase stimulated

Control of Glycogen Metabolism

Covalent modification of enzymes by cyclic cascades

Features:

1. Respond to greater number of stimuli
2. Greater flexibility in control patterns
3. Amplification potential in response to effector concentrations

Small change in [allosteric effector] of a modifying enzyme → large change in [active, modified target enzyme]

Cyclic cascades nomenclature:

a - more active target enzyme
b - less active target enzyme
m - modified enzyme form
o - original (unmodified) enzyme form

Recall that the rate of reaction = $k[E_{active}][S]$

Using a mathematical model (beyond the scope of this course) we could show quantitatively how changes in [effectors] modulate $[E_{active}]$

so a cyclic cascade allows an effector signal to be amplified

Control of Glycogen Metabolism

Glycogen phosphorylase bicyclic cascade
Superimposed on the ATP (inhibitor) and AMP (activator) allosteric control is covalent modification

Covalent modification enzymes:

cAMP-dependent protein kinase (cAPK) - phosphorylates (activates) phosphorylase kinase, requires cAMP, R_2C_2 tetramer
cAPK consensus sequence - Arg-Arg-X-Ser/Thr-Y
X = small residue Y = hydrophobic residue

phosphorylase kinase - phosphorylates Ser14 of glycogen phosphorylase *b*
oligomeric $(\alpha\beta\gamma\delta)_4$, $\alpha\beta\delta$ inhibitory, γ activates
δ = Calmodulin, Ca^{2+} activates (muscle contraction)

phosphoprotein phosphatase-1 - dephosphorylates (deactivates) glycogen phosphorylase *a* and phosphorylase kinase
muscle - active when bound to glycogen-binding G subunit
liver - controlled by binding to *m*-phosphorylase *a*

Level of phosphorylase activity is determined by fraction present as glycogen phosphorylase *a*

Control of Glycogen Metabolism

Glycogen synthase bicyclic cascade

Not as well understood

Two forms of enzyme:

m-glycogen synthase *b* (inactive)

allosterically controlled - inhibited by ATP, ADP, P_i
overcome by [glucose-6-phosphate] > 10 mM (rare)

o-glycogen synthase *a* (active)

deactivated by calmodulin-dependent protein kinase, protein kinase C, glycogen synthase kinase-3

Control of Glycogen Metabolism
(What the book does not illustrate)

Integration of glycogen metabolism control mechanisms

Maintenance of blood glucose levels - liver buffers
[glucose] ~ 5 mM

The Cast

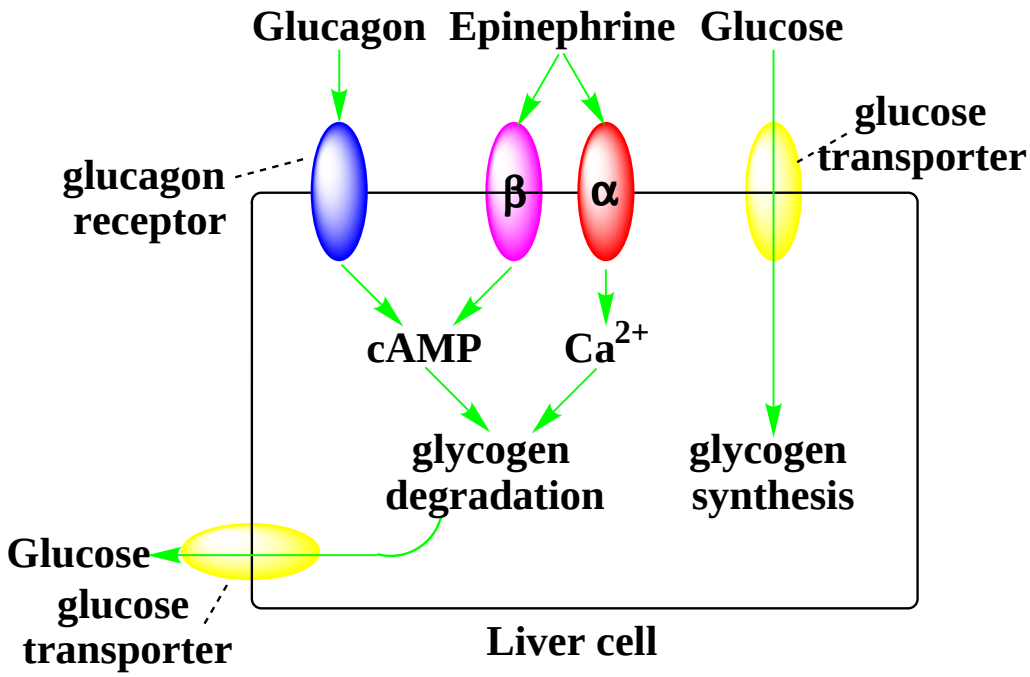
Hormones
Glucagon - polypeptide (liver)
Insulin - polypeptide (muscle, other tissues)
Epinephrine - adrenal

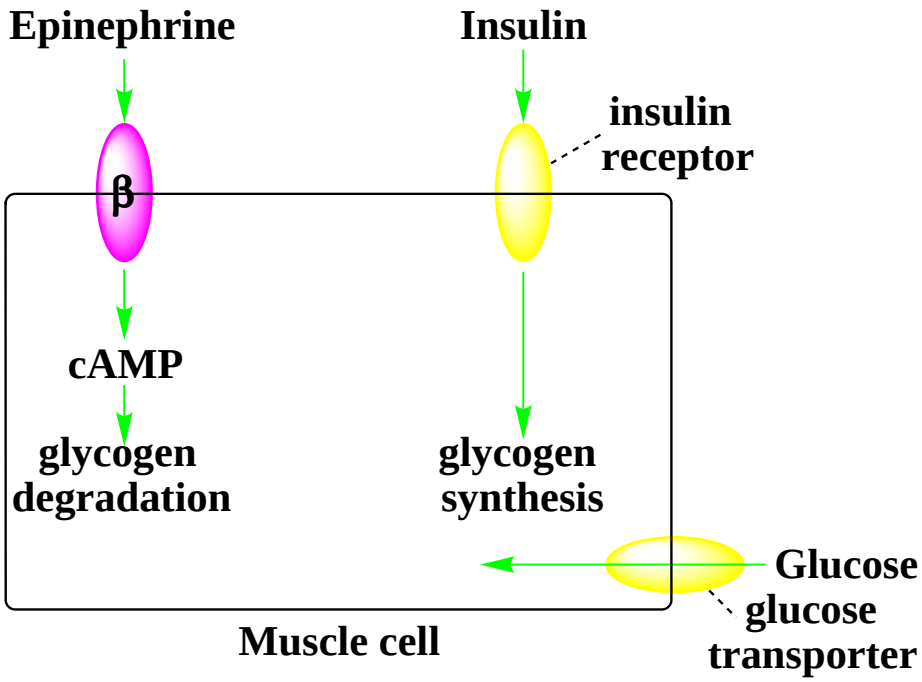
Second messengers
Ca^{2+}
Inositol-1,4,5-triphosphate (IP_3) - lipid-derived
Diacylglycerol (DAG) - lipid-derived

Phospholipase C - cleaves membrane lipid (phosphatidylinositol-4,5-bisphosphate, PIP_2) to generate IP_3 and DAG

Receptors
β-Adrenergic - binds adrenal hormones
α-Adrenergic - binds adrenal hormones
Glucagon
Insulin

Control of Glycogen Metabolism





Control of Glycogen Metabolism

Maintenance of blood glucose levels

Hexokinase:

Michaelis-Menten kinetics

high glucose affinity (K_m ~ 0.1 mM)

inhibited by glucose-6-phosphate

Glucokinase:

monomeric

Sigmoidal kinetics (Hill constant of 1.5)

lower glucose affinity ($K_{0.5}$ ~5 mM)

not inhibited by physiological [glucose-6-phosphate]

inhibited by glucokinase regulatory protein + fructose-6-phosphate

Control of Glycogen Metabolism

Maintenance of blood glucose levels

Flux control by substrate cycle and covalent modification system

Phosphofructokinase-1 (PFK-1) - allosterically activated by fructose-2,6-bisphosphate

Fructose-1,6-bisphosphatase-1 (FBPase-1) - allosterically inhibited by fructose-2,6-bisphosphate

Phosphofructokinase-2 (PFK-2)/fructose-2,6-bisphosphatase-2 (FBPase-2):

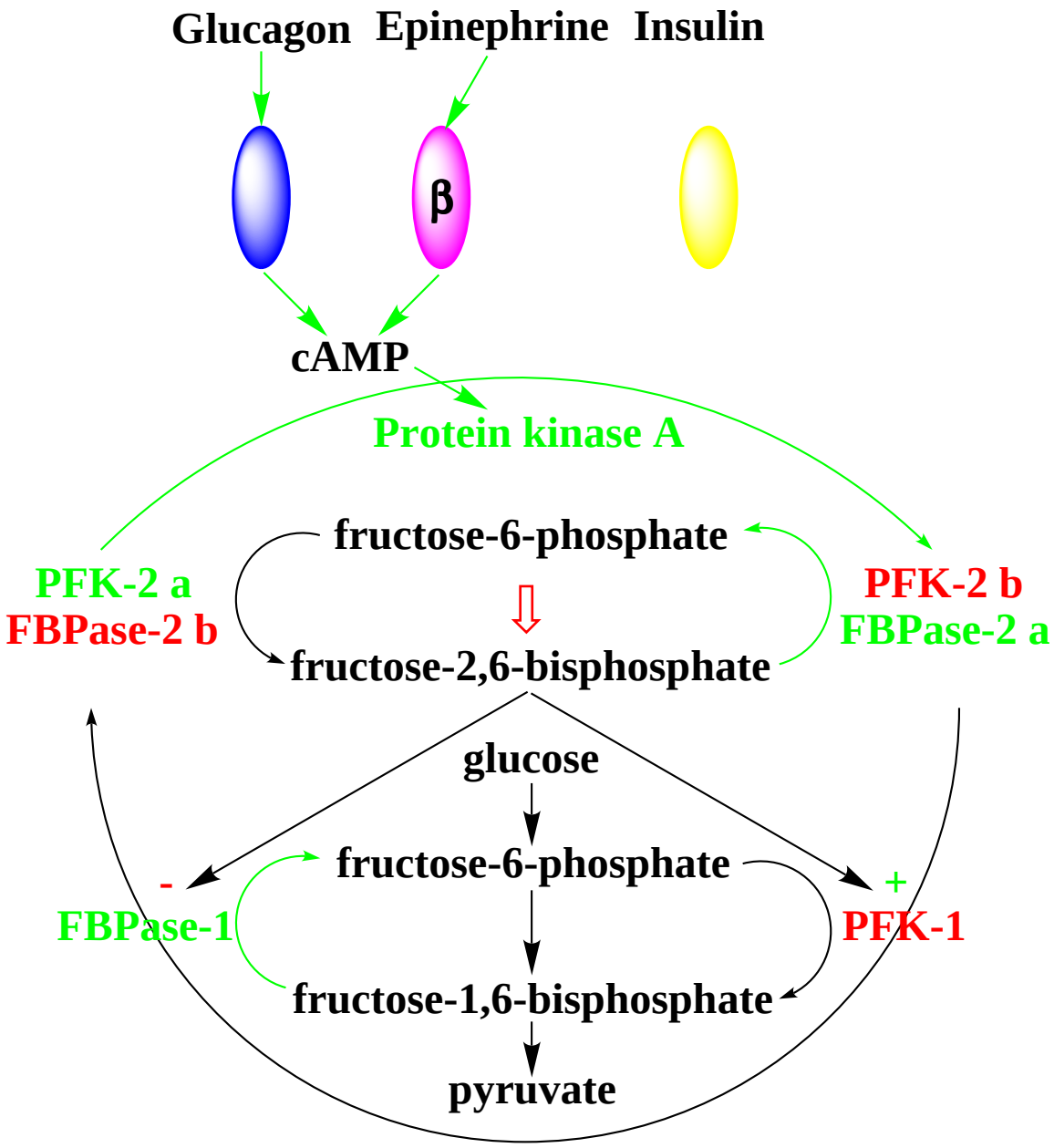
Bifunctional homodimeric protein
phosphorylated (inactive)/dephosphorylated (active)

In liver - breakdown glycogen, release glucose into blood or take up glucose, synthesize glycogen

In heart - glycogen breakdown, increase glycolysis (different PFK-2/FBPase-2 gene)

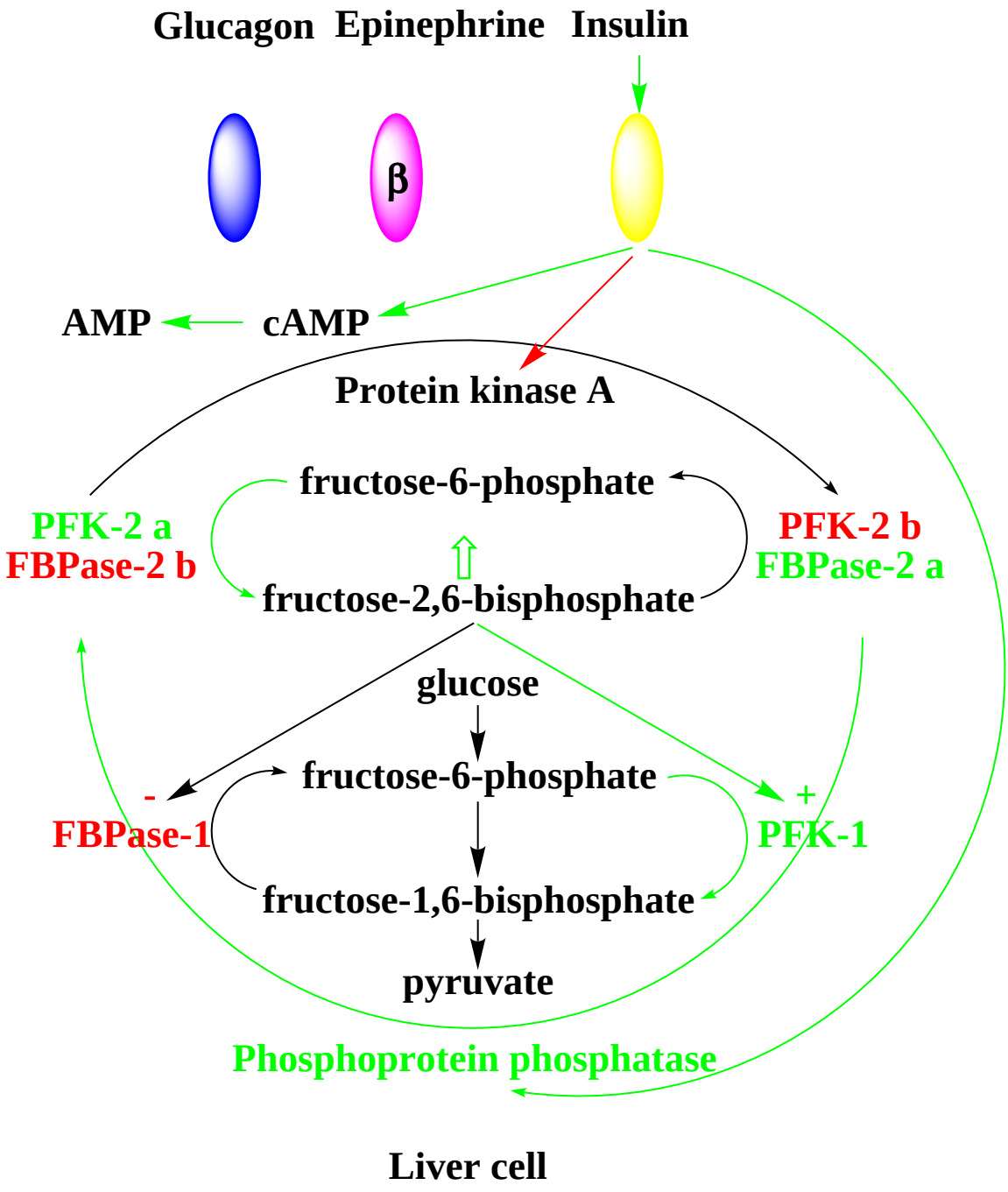
In muscle - no phosphorylation site on enzyme, no cAMP-dependent phosphorylation control

Control of Glycogen Metabolism



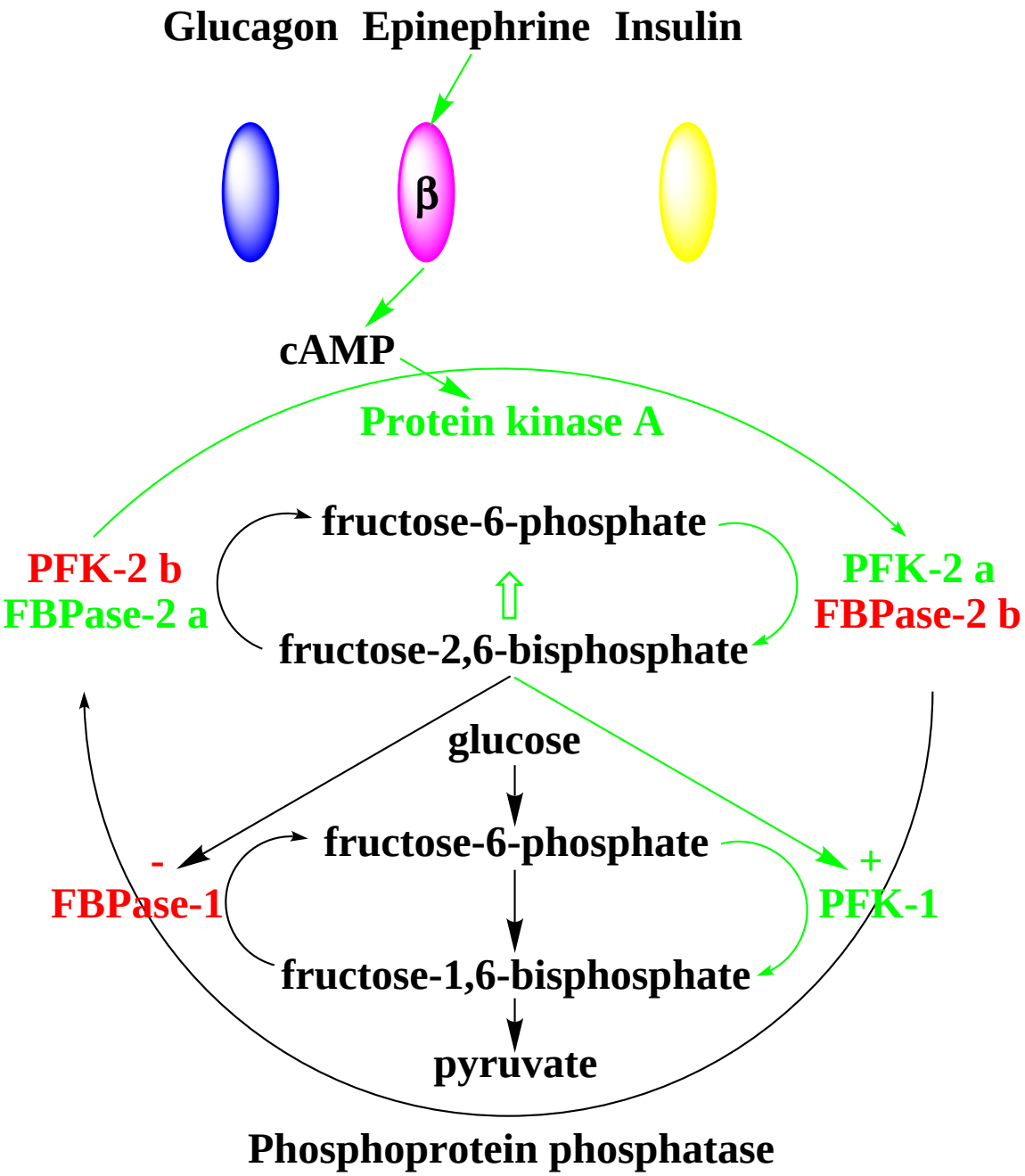
Liver cell

Control of Glycogen Metabolism



Liver cell

Control of Glycogen Metabolism



Heart tissue