CHAPTER 15
Principles of Metabolic Regulation

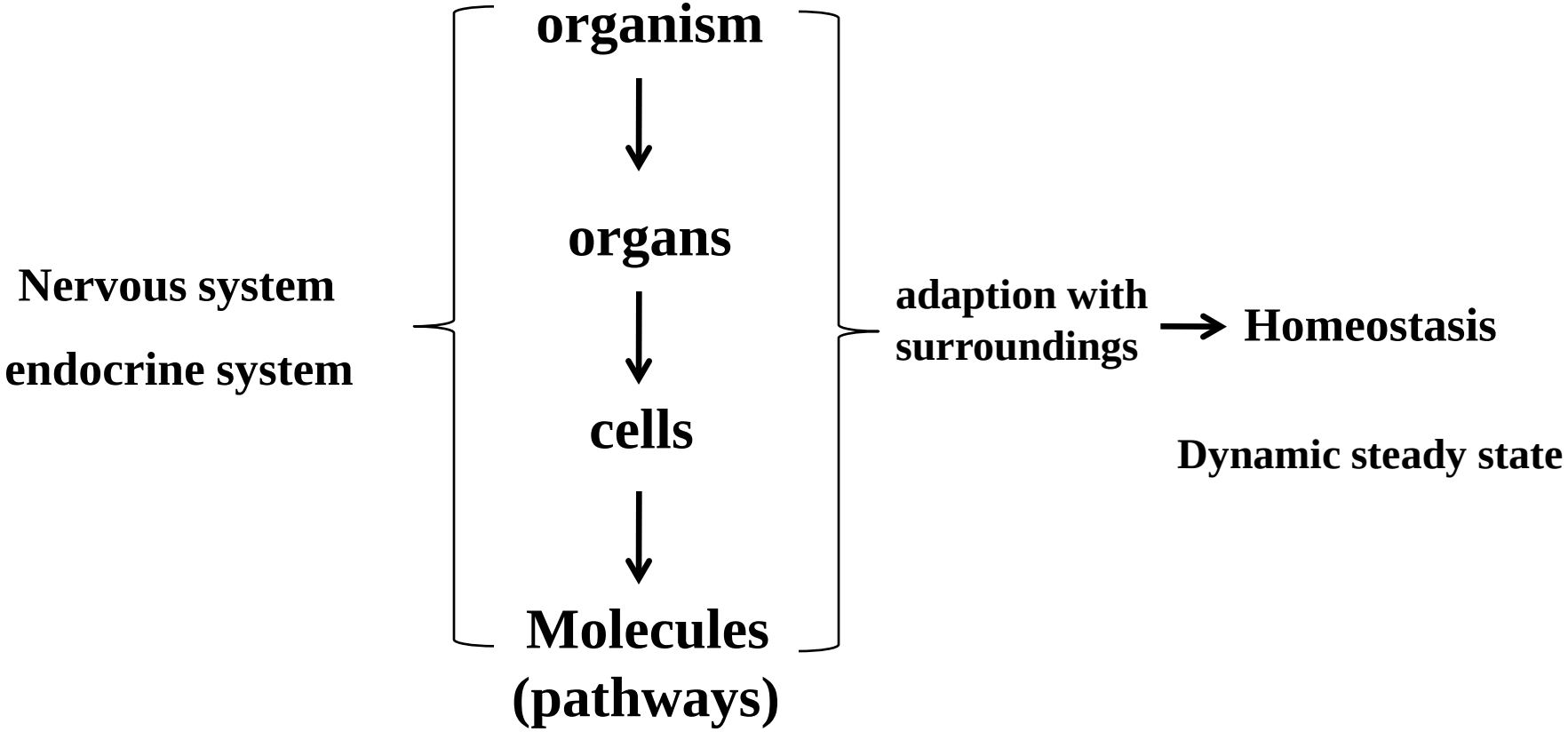
p587

注意：15.2 Analysis of Metabolic Control为自学内容

15.1 Regulation of metabolic pathways

Cell and organisms maintain

a dynamic steady state



In human, about **4,000 genes** (12% of all genes) encode regulatory proteins, including a variety of receptors, regulators of gene expression, and more than **500** different **protein kinases.**

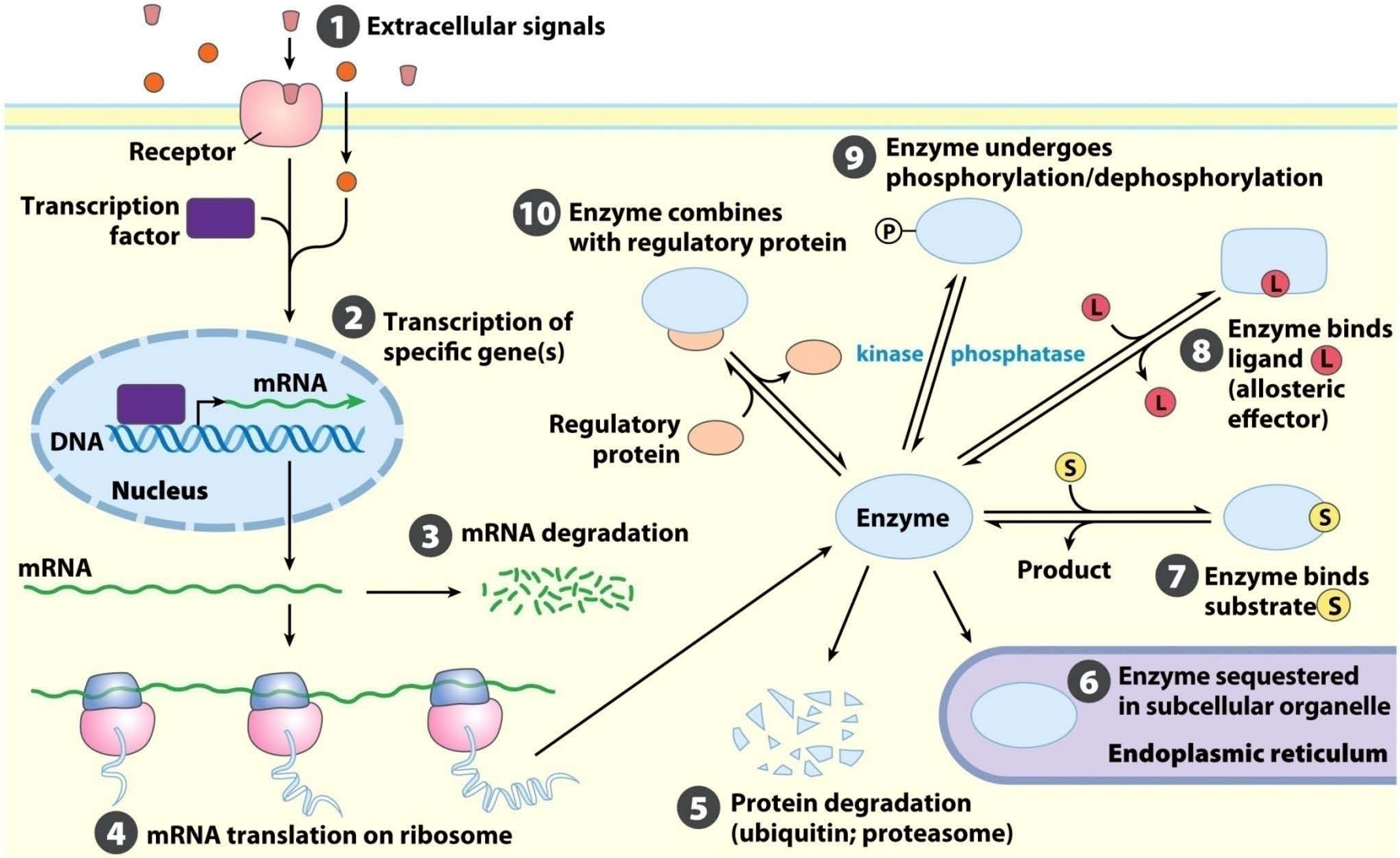


Figure 15-2

Both the amount and the catalytic activity of an enzyme can be regulated

TABLE 15–1 Average Half-Life of Proteins in Mammalian Tissues

Tissue	Average half-life (days)
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Table 15-1

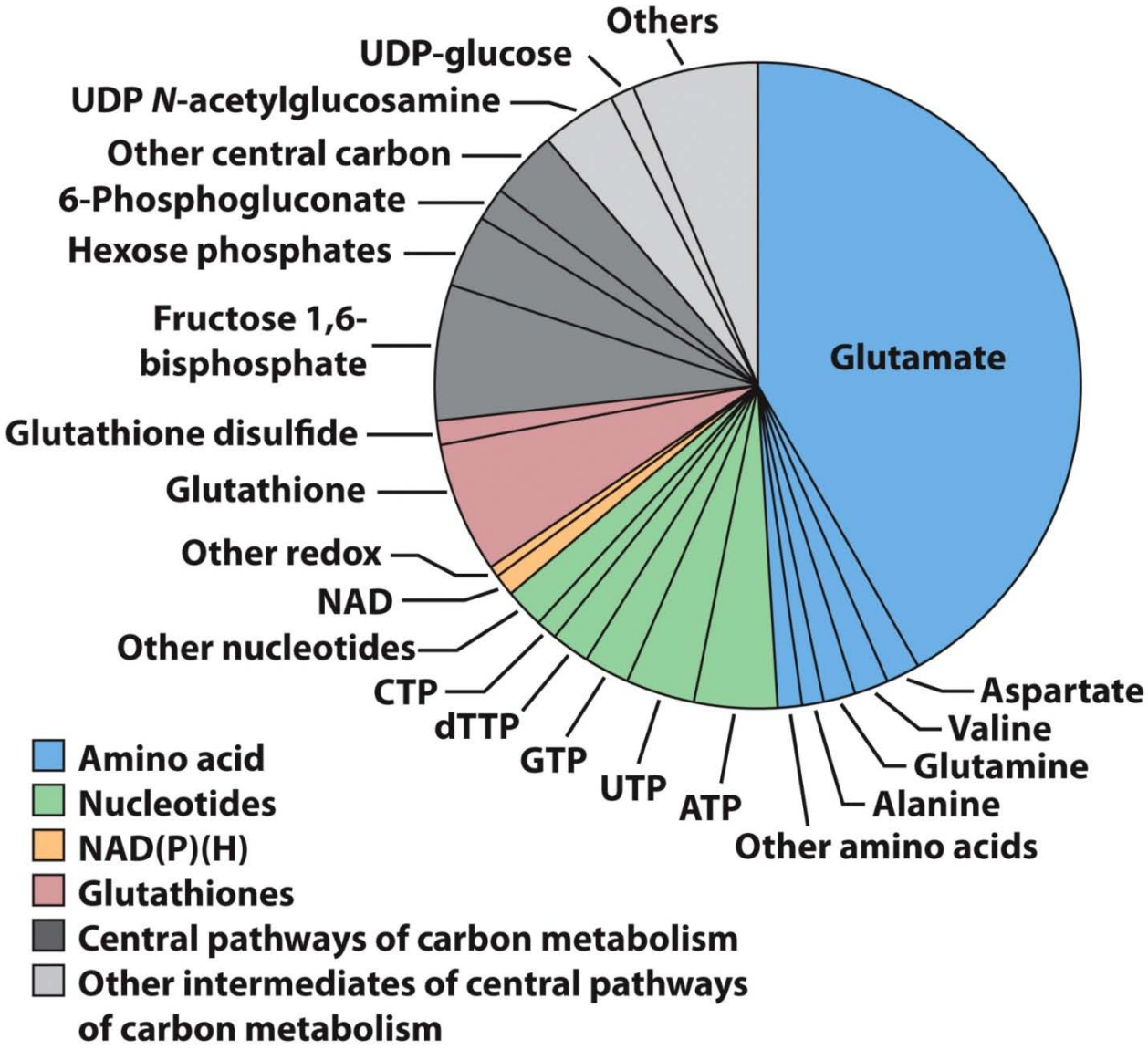


Figure 15-3

The **metabolome** of *E.coli* growing on glucose: the amounts of 103 metabolites

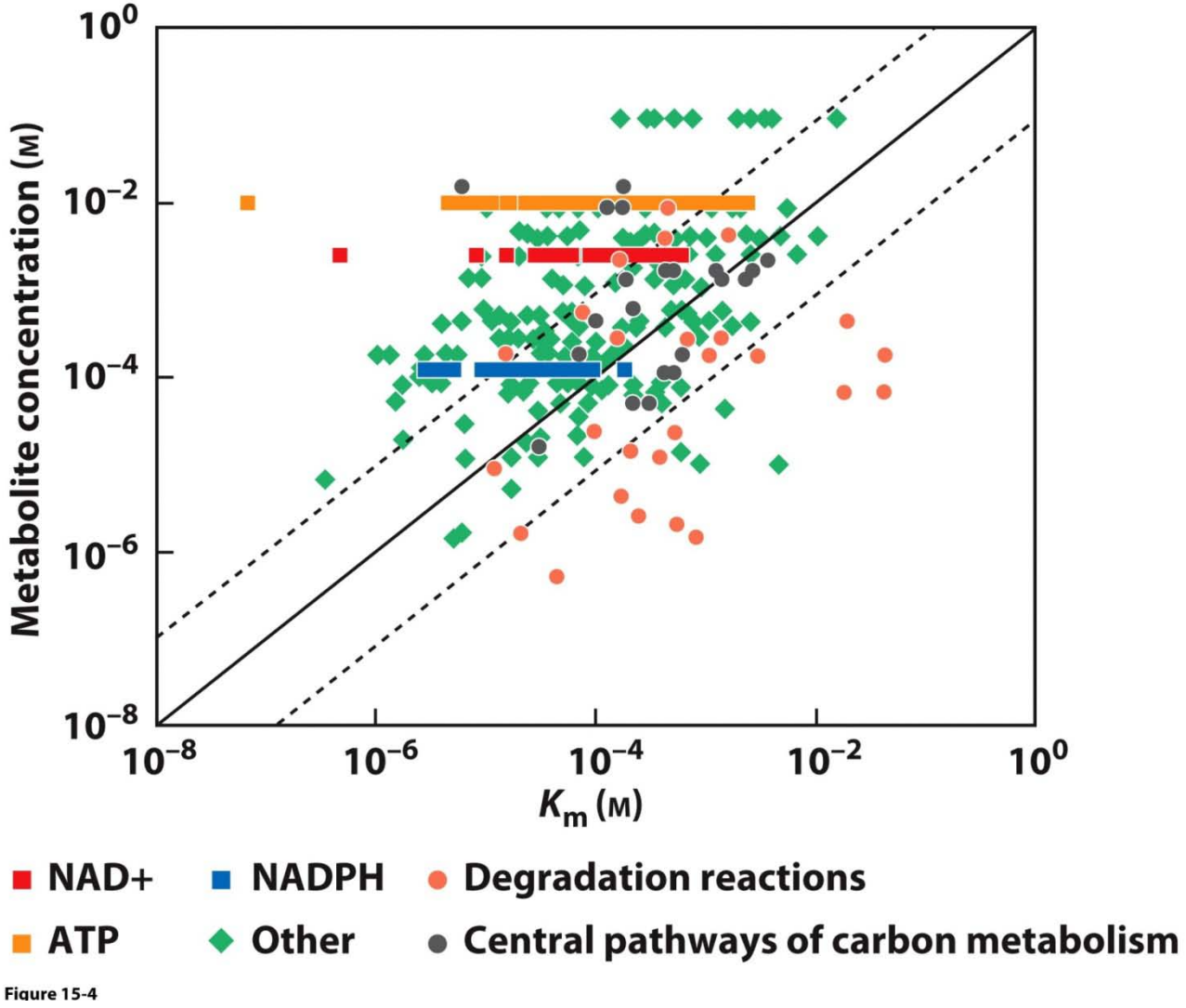


Figure 15-4

Comparison of k_m and substrate concentration for some metabolic enzymes

TABLE 15–2 Relationship between Hill Coefficient and the Effect of Substrate Concentration on Reaction Rate for Allosteric Enzymes

Hill coefficient (n_H)	Required change in [S] to increase V_0 from 10% to 90% V_{max}
0.5	×6,600
1.0	×81
2.0	×9
3.0	×4.3
4.0	×3

Table 15-2

Cooperative effect of allosteric ligand on enzymatic activity

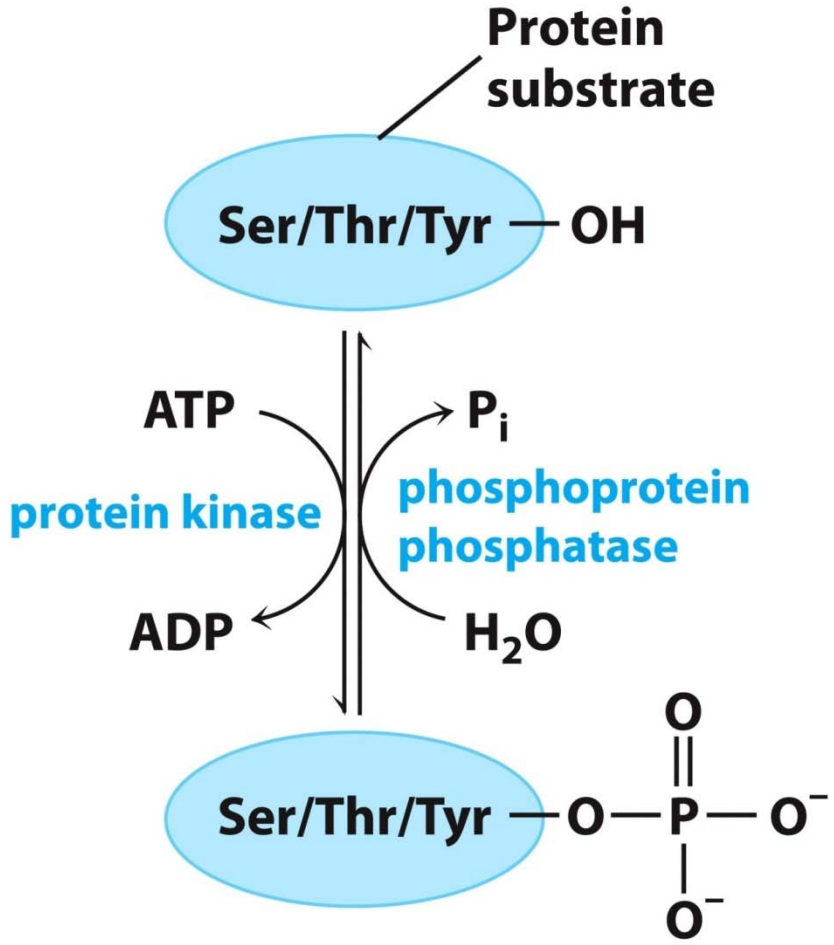


Figure 15-5

Protein phosphorylation and dephosphorylation

Reactions far from equilibrium in cells are common points of regulation

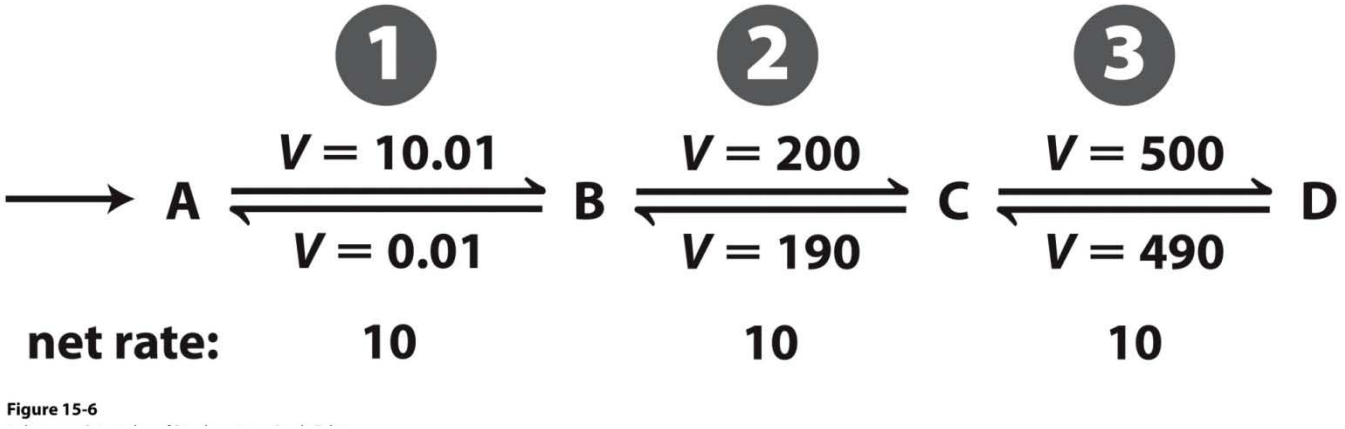


Figure 15-6

Near-equilibrium steps are coupled with nonequilibrium step in a metabolic pathway

TABLE 15–3 Equilibrium Constants, Mass-Action Coefficients, and Free-Energy Changes for Enzymes of Carbohydrate Metabolism

Enzyme	K'_{eq}	Mass-action ratio, Q		Reaction near equilibrium in vivo?*	$\Delta G'^{\circ}$ (kJ/mol)	ΔG (kJ/mol) in heart
		Liver	Heart			
Hexokinase	1×10^{3}	2×10^{-2}	8×10^{-2}	No	−17	−27
PFK-1	1.0×10^{3}	9×10^{-2}	3×10^{-2}	No	−14	−23
Aldolase	1.0×10^{-4}	1.2×10^{-6}	9×10^{-6}	Yes	+24	−6.0
Triose phosphate isomerase	4×10^{-2}	—†	2.4×10^{-1}	Yes	+7.5	+3.8
Glyceraldehyde 3-phosphate dehydrogenase + phosphoglycerate kinase	2×10^{3}	6×10^{2}	9.0	Yes	−13	+3.5
Phosphoglycerate mutase	1×10^{-1}	1×10^{-1}	1.2×10^{-1}	Yes	+4.4	+0.6
Enolase	3	2.9	1.4	Yes	−3.2	−0.5
Pyruvate kinase	2×10^{4}	7×10^{-1}	40	No	−31	−17
Phosphoglucose isomerase	4×10^{-1}	3.1×10^{-1}	2.4×10^{-1}	Yes	+2.2	−1.4
Pyruvate carboxylase + PEP carboxykinase	7	1×10^{-3}	—†	No	−5.0	−23
Glucose 6-phosphatase	8.5×10^{2}	1.2×10^{2}	—†	Yes	−17	−5.0

Source: K'_{eq} and Q from Newsholme, E.A. & Start, C. (1973) *Regulation in Metabolism*, Wiley Press, New York, pp. 97, 263. ΔG and $\Delta G'^{\circ}$ were calculated from these data.

*For simplicity, any reaction for which the absolute value of the calculated ΔG is less than 6 is considered near equilibrium.

†Data not available.

Adenine nucleotides play special roles in metabolic regulation

Many ATP-using enzymes have k_m values between 0.1 and 1 mM, and the ATP concentration in a typical cell is about 5 to 10mM. If the [ATP] were to drop significantly, the rates of hundreds of reactions that involve ATP would decrease.

Organisms have evolved under strong pressure to develop regulatory mechanisms responsive to [ATP]/[ADP] ratio

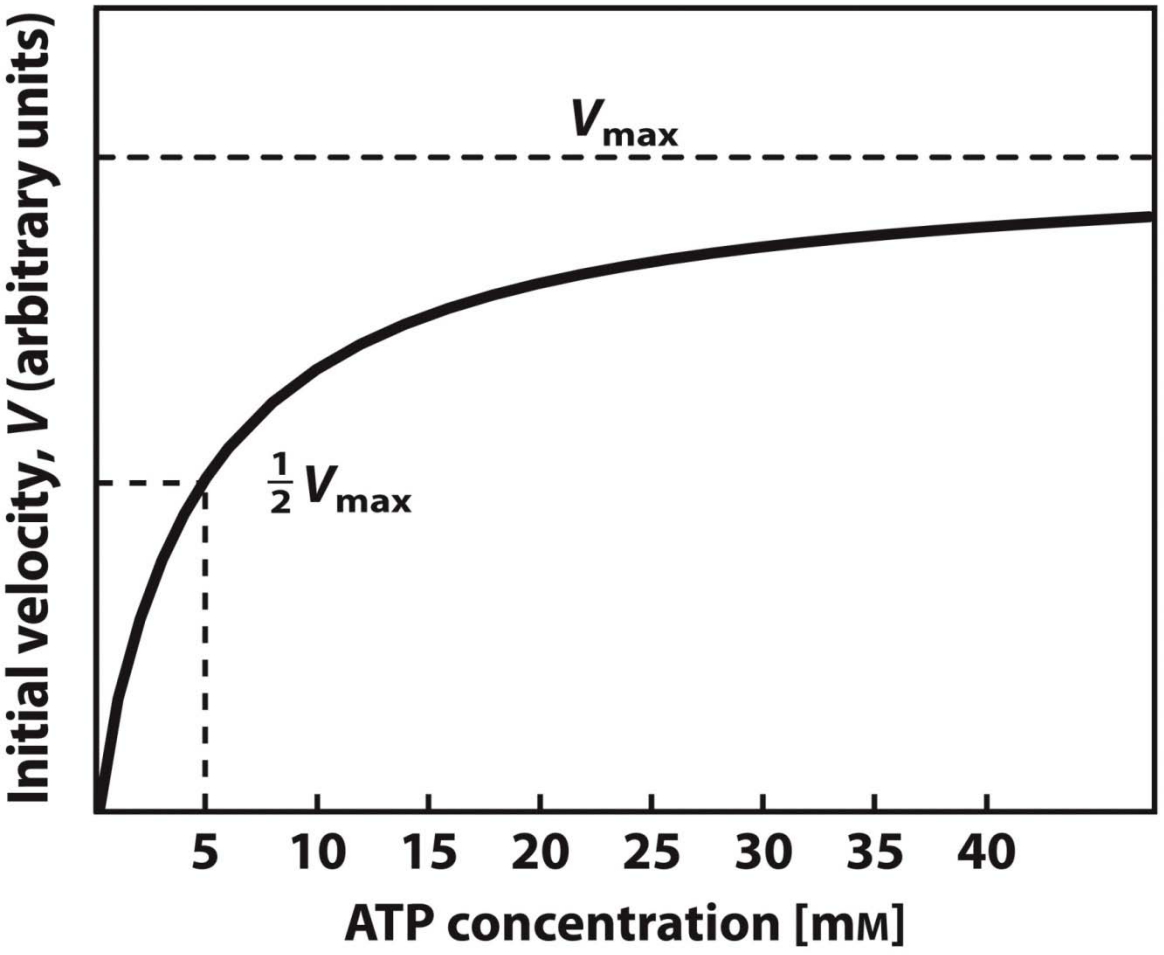


Figure 15-7

Effect of ATP concentration on the initial reaction velocity of a typical ATP-dependent enzyme

TABLE 15–4 Relative Changes in [ATP] and [AMP] When ATP Is Consumed

Adenine nucleotide	Concentration before ATP depletion (mM)	Concentration after ATP depletion (mM)	Relative change
ATP	5.0	4.5	10%
ADP	1.0	1.0	0
AMP	0.1	0.6	600%

Table 15-4

[ATP] drops 10%, [AMP] increases for 5 folds

The levels of ATP and AMP reflect a cell's energy status. AMP-activated protein kinase (AMPK) can sense the decrease of [ATP]/[AMP] ratio and thus trigger a variety of cellular responses to raise this ratio.

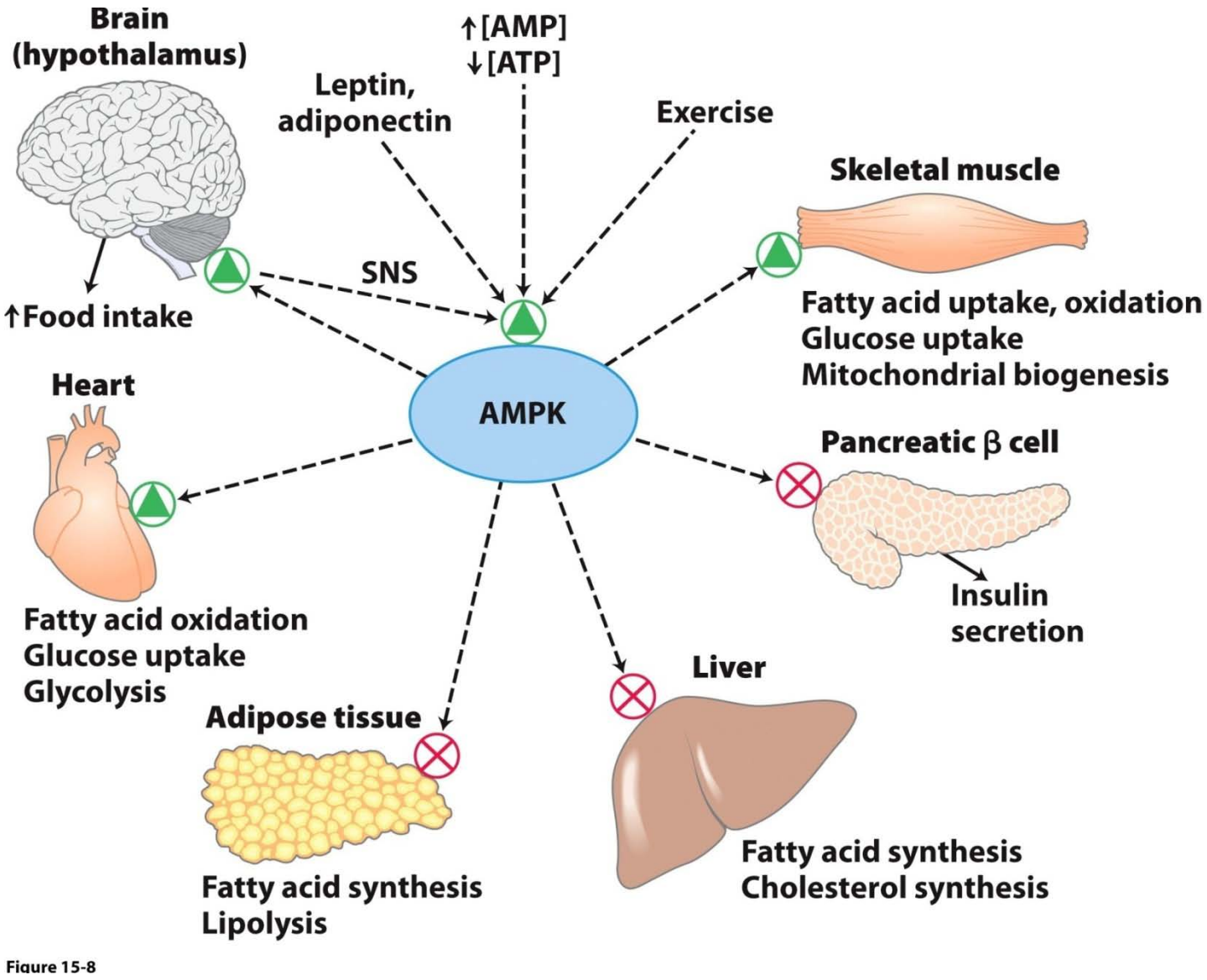


Figure 15-8

Role of AMPK in carbohydrate and fat metabolism

15.2 Analysis of Metabolic Control

（self-study）

Eduard Buchner, 1860–1917

Rate-limiting step: a step determining the rate of metabolite flow, or flux, through a whole pathway.

Rate-limiting enzyme

Single rate-limiting step

⬇

Multiple rate-limiting steps

The Contribution of Each Enzyme to Flux through a Pathway is Experimentally Measurable

In vitro assay

Intracellular assay

In vivo assay

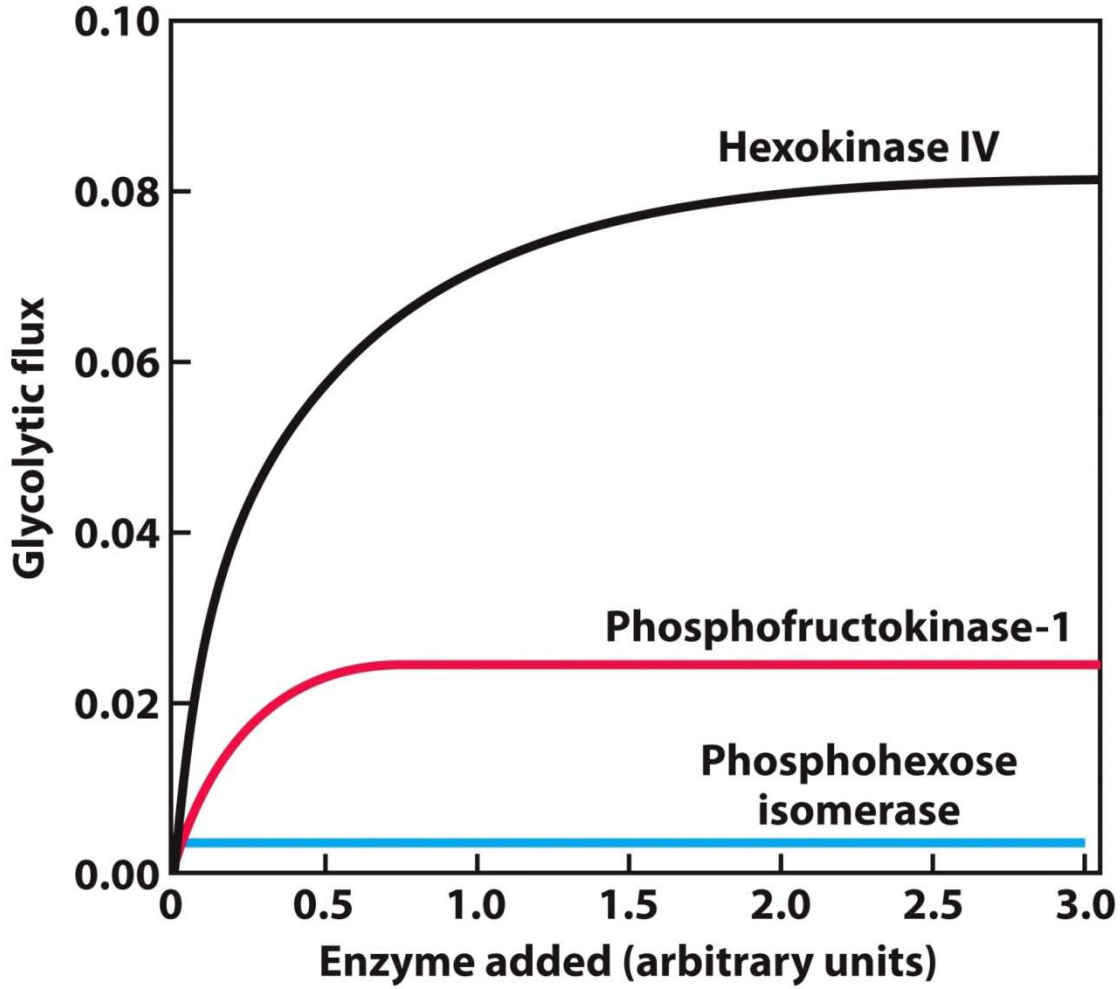


Figure 15-9

C_{HK4} 0.79

C_{PFK-1} 0.21

Dependence of glycolytic flux in a rat liver homogenate on added enzyme

Metabolic control analysis

- Flux control coefficient, C
- Elasticity coefficient, ε
- Response coefficient, R

Flux control coefficient expresses the relative contribution of each enzyme to setting *the rate at which metabolites flow through the pathway* (**flux, *J***).

1) In a linear pathway, C can have any value from 0.0 to 1.0; In a branched pathway, an enzyme in one branch can have a negative C.
2) C is not a constant. Value depends on the concentrations of substrate and effectors
3) For any complete pathway, the sum of flux control coefficients must equal 1.0

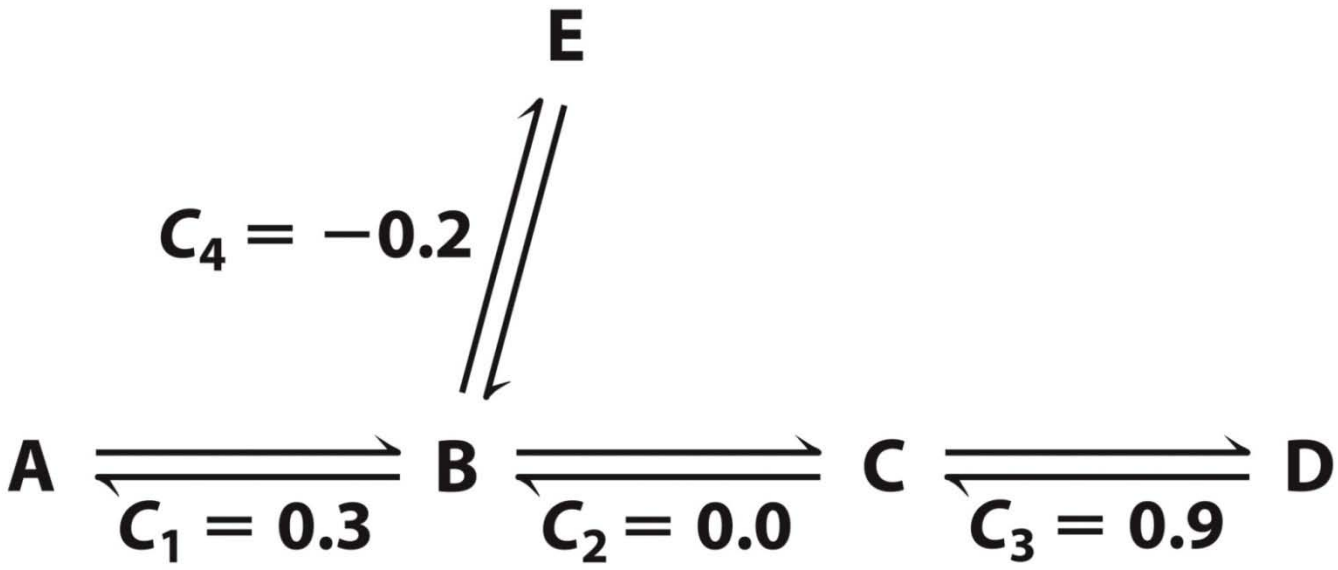


Figure 15-10
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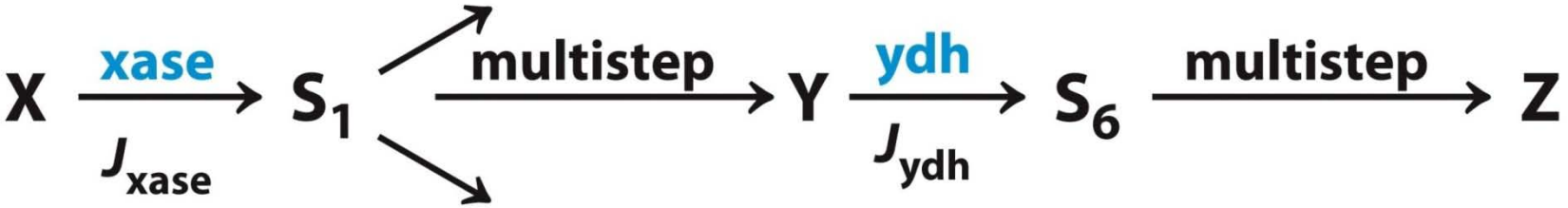

Box 15-1 figure 1
Lehninger Principles of Biochemistry, Sixth Edition

flux control coefficient, C

$$C_{\text{xase}}^{J_{\text{ydh}}} \approx \frac{\partial J_{\text{ydh}}}{J_{\text{ydh}}} \Big/ \frac{\partial E_{\text{xase}}}{E_{\text{xase}}} \approx \frac{\partial J_{\text{ydh}}}{\partial E_{\text{xase}}} \cdot \frac{E_{\text{xase}}}{J_{\text{ydh}}}$$

which is mathematically identical to

$$C_{\text{xase}}^{J_{\text{ydh}}} \approx \frac{\partial \ln J_{\text{ydh}}}{\partial \ln E_{\text{xase}}}$$

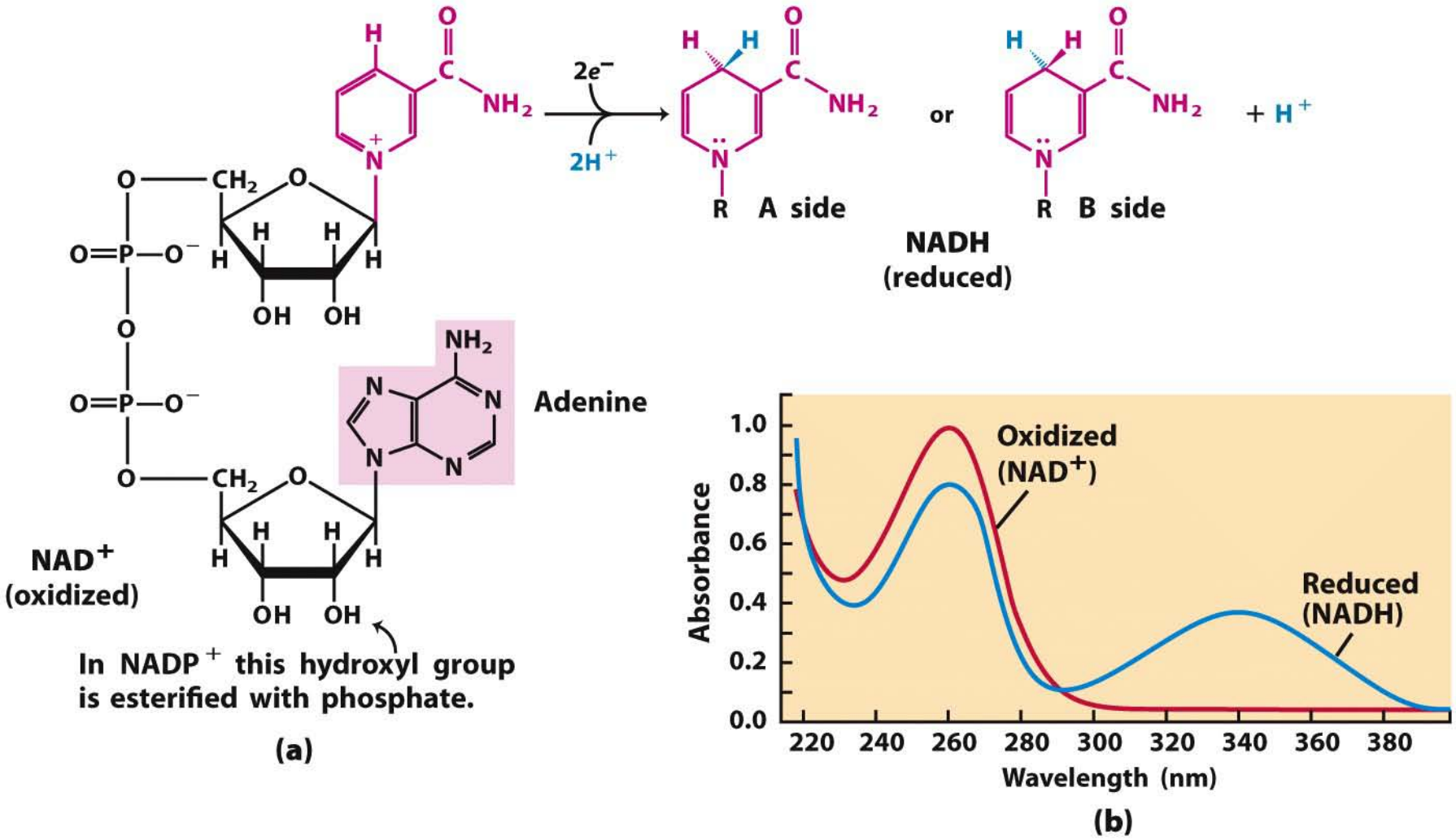

Figure 13-24
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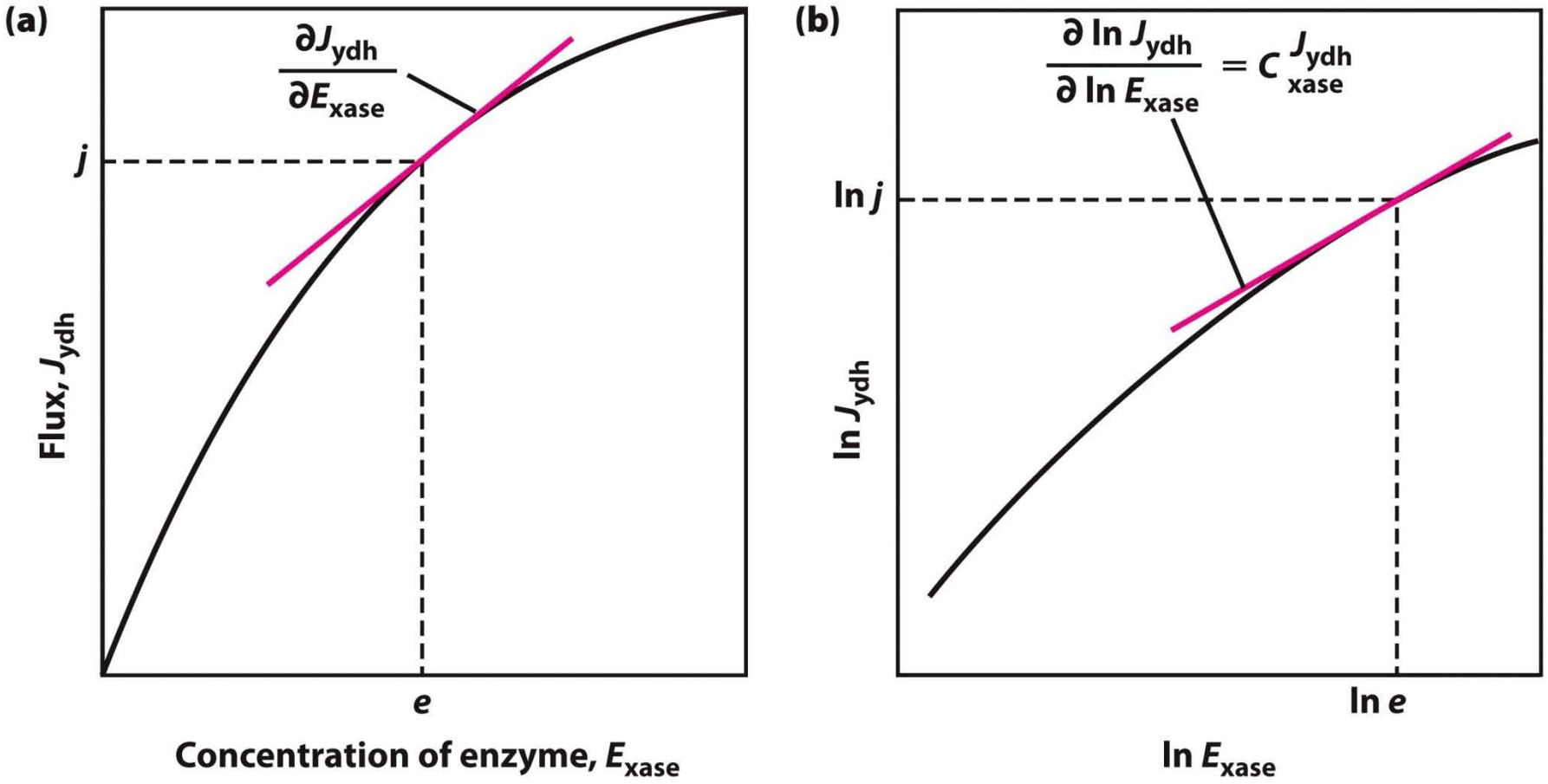

Box 15-1 figure 2

C is not a constant, it depends on the starting E_{xase} from which the change in enzyme level takes place. A value near 1.0 means that the [E] wholly determines the flux through the path; a value 0.0 means that the [E] does not limit the flux

Elasticity coefficient, ε expresses quantitatively the responsiveness of a single enzyme to changes in the concentration of a metabolite or regulator.

1) Is an intrinsic property of an enzyme.

2) Reflects the sensitivity of an enzyme to substrate and effector concentrations.

3) An enzyme with typical Michaelis-Menten kinestics has an ε value ranging from near 0.0 to about 1.0 in response to substrate concentrations.

4) For allosteric enzymes that show positive cooperativity, ε exceed 1.0, but it cannot exceed the hill coefficient, which is typically between 1.0 and 4.0

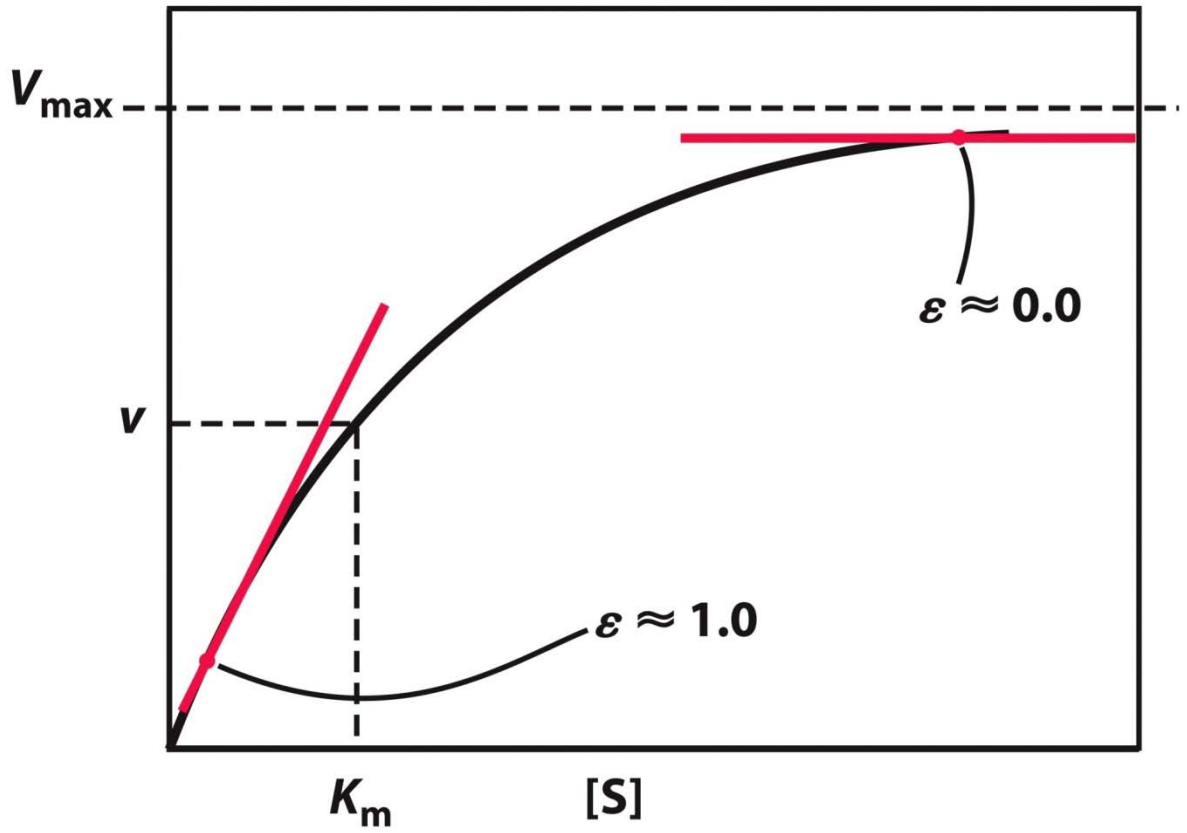

Figure 15-11

An enzyme with typical Michaelis-Menten kinestics has an **Elasticity coefficient** value from 0.0 to 1.0

elasticity, ε

$$\varepsilon_S^{\text{xase}} = \frac{\partial V_{\text{xase}}}{\partial S} \bullet \frac{S}{V_{\text{xase}}}$$

$$= \frac{\partial \ln |V_{\text{xase}}|}{\partial \ln S}$$

Response coefficient, *R* expresses the effect of an outside factor (such as a hormone or growth factor) on the flux through a pathway

$$R_P^{J_{\text{ydh}}} = C_{\text{xase}}^{J_{\text{ydh}}} \bullet \varepsilon_P^{\text{xase}}$$

response coefficient, R.

$$R_P^{J_{\text{ydh}}} = \frac{\partial J_{\text{ydh}}}{\partial P} \bullet \frac{P}{J_{\text{ydh}}}$$

P, concentration of parameter/controlling factor

Metabolic control analysis has been applied to carbohydrate metabolism, with surprising results

1) PFK-1 shows regulatory mechanism (acts to maintain metabolite concentration) in glycolysis.
Five fold increase of [PKF-1] led to a change in flux through glycolysis of less than 10% in yeast.

Hexokinase shows control mechanism (acts to alter the flux through a pathway) in glycolysis.

2) In glycogen synthesis pathway Glut4 and hexokinase show control mechanism, glycogen synthase shows regulatory mechanism, contradictory with conventional wisdom that the later is the locus of flux control.

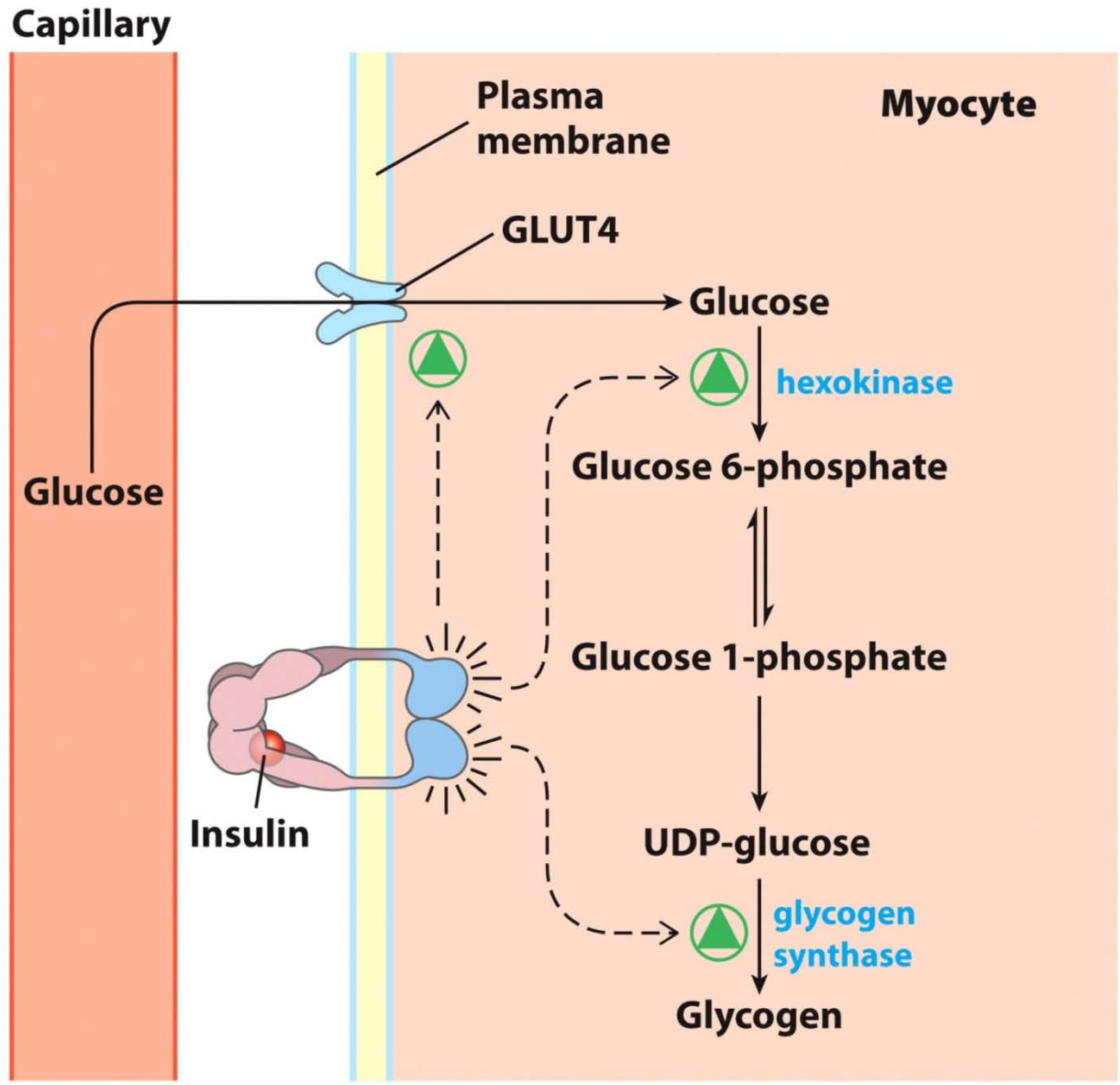

Figure 15-12

Control of glycogen synthesis from blood glucose in muscle

Metabolic control analysis suggests a general method for increasing flux through a pathway

Flux toward a specific product is most effectively increased by raising the concentration of all enzymes in the pathway

The urea output of rat increases fourfold in response to high protein diet, and the amount of all enzymes in urea cycle increase two to three fold accordingly.

15.3 Coordinated regulation of glycolysis and gluconeogenesis

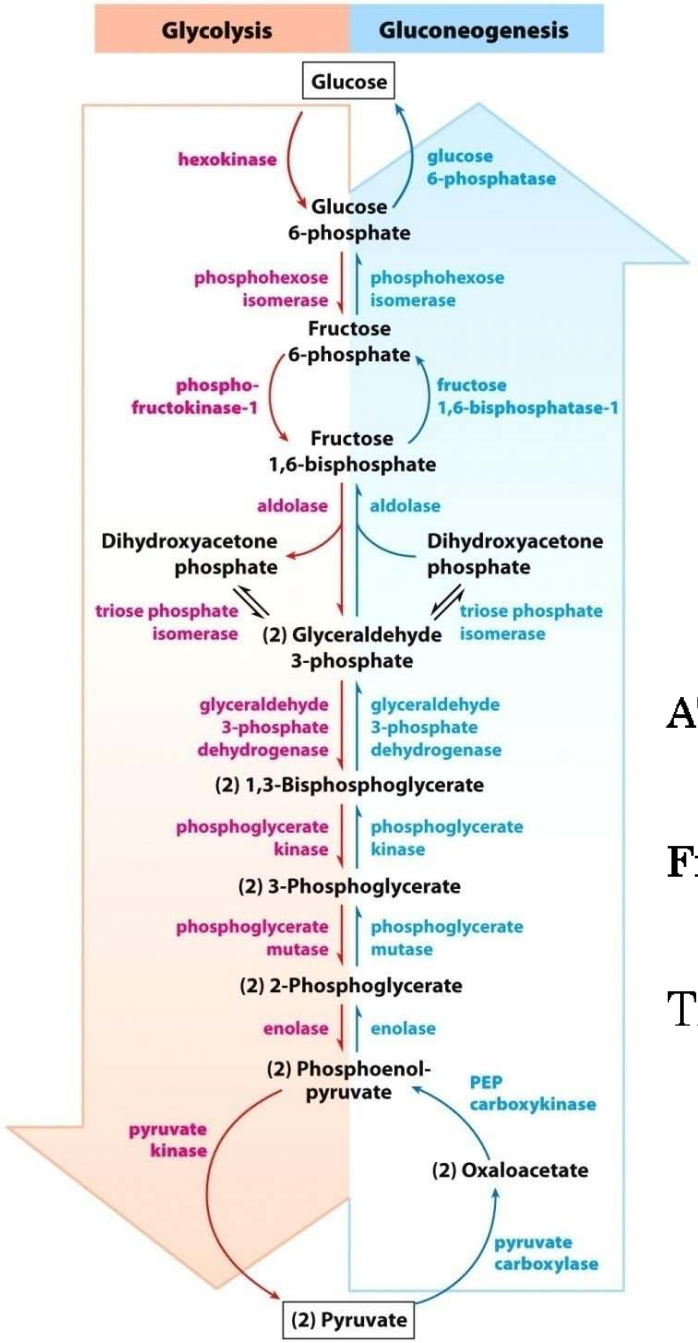

Figure 15-13

Futile cycle/substrate cycle

Simultaneous interconversion between substrate and product, leading to dissipation of chemical energy as heat

$$\text{ATP} + \text{fructose 6-phosphate} \xrightarrow{\text{PFK-1}} \text{ADP} + \text{fructose 1,6-bisphosphate}$$

$$\text{Fructose 1,6-bisphosphate} + H_2O \xrightarrow{\text{FBPase-1}} \text{fructose 6-phosphate} + P_i$$

The sum of these two reactions is

$$\text{ATP} + H_2O \longrightarrow \text{ADP} + P_i + \text{heat}$$

HK isozymes of muscle and liver are affected differently by their product, G-6-p

	Liver	Myocyte
HKs	**HK4** (glucokinase) K_m 10mM not inhibited by G-6-P transcriptionally activated by insulin	**HK2, HK1** K_m 0.1mM reversibly inhibited by G-6-p +
GLUTs	**GLUT2** K_t 17mM insulin irresponsive	**GLUT4** K_t 5mM regulated by insulin

The information in this table indicates that muscle consumes Glu, using it for energy production, whereas liver maintains blood glucose homeostasis by consuming or producing glucose, depending on the prevailing blood [Glu].

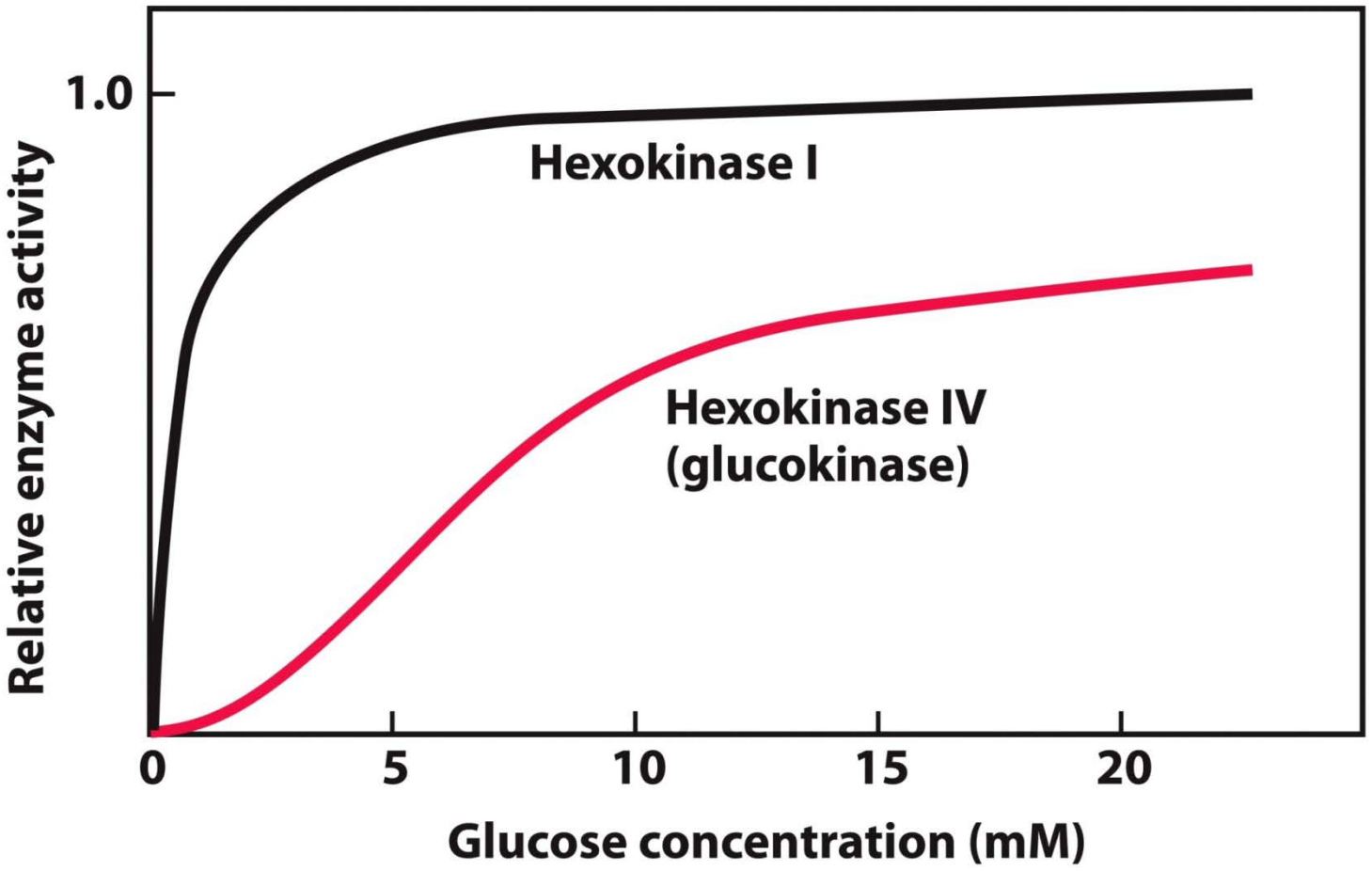

Figure 15-14

Comparison of kinetic properties of HK4 and HK1

Note that HK1,2 and 3 show similar kinetic properties

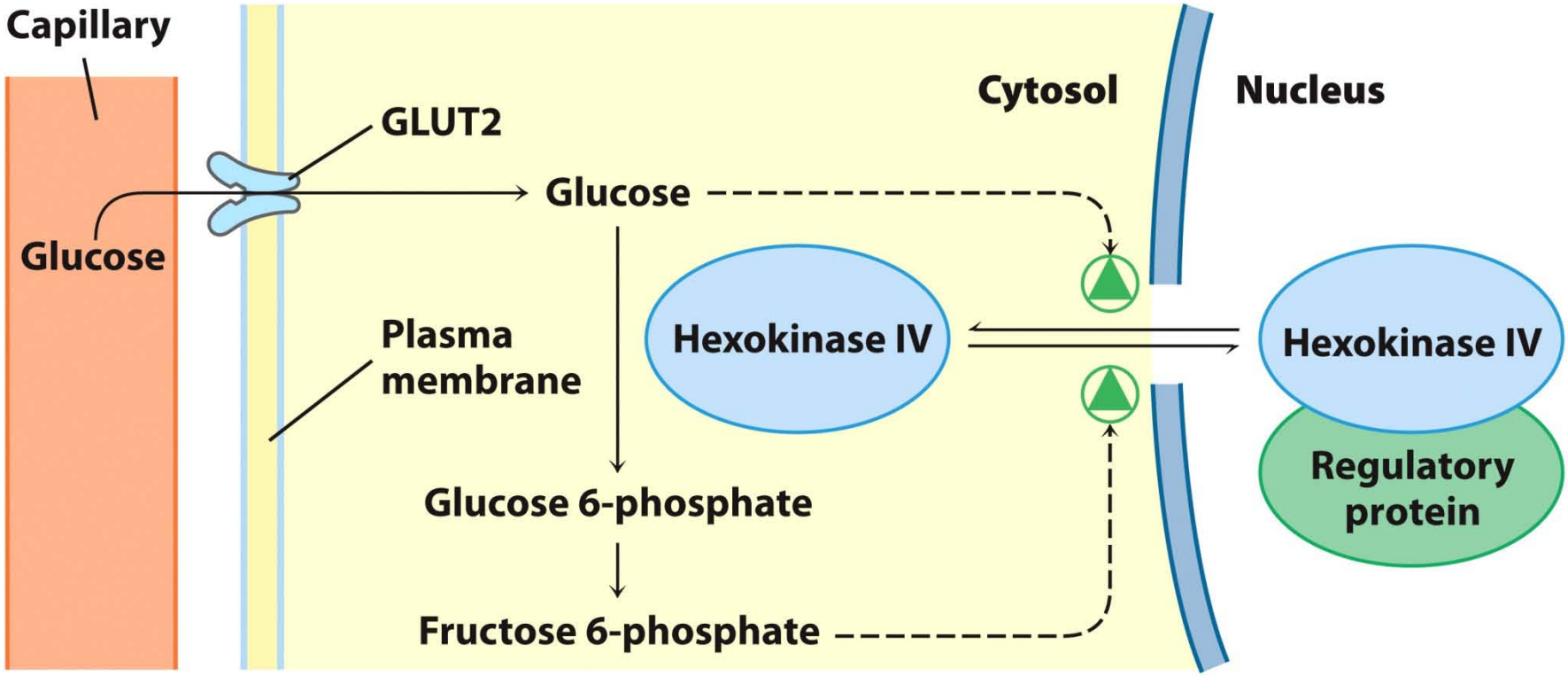

Figure 15-15

Regulation of HK4 by sequestration in the nucleus.

High level of Glu competes with F-6-P for binding with regulatory protein; F-6-P increases the affinity between regulatory protein and HK4 by acting as an allosteric regulator for this protein.

TABLE 11–3 Glucose Transporters in Humans

Transporter	Tissue(s) where expressed	K_t (mM)*	Role†
GLUT1	Ubiquitous	3	Basal glucose uptake
GLUT2	Liver, pancreatic islets, intestine	17	In liver and kidney, removal of excess glucose from blood; in pancreas, regulation of insulin release
GLUT3	Brain (neuronal), testis (sperm)	1.4	Basal glucose uptake
GLUT4	Muscle, fat, heart	5	Activity increased by insulin
GLUT5	Intestine (primarily), testis, kidney	6‡	Primarily fructose transport
GLUT6	Spleen, leukocytes, brain	>5	Possibly no transporter function
GLUT7	Small intestine, colon	0.3	—
GLUT8	Testis	~2	—
GLUT9	Liver, kidney	0.6	—
GLUT10	Heart, lung, brain, liver, muscle, pancreas, kidney	0.3§	—
GLUT11	Heart, skeletal muscle, kidney	0.16	—
GLUT12	Skeletal muscle, heart, prostate, small intestine	—	—

*K_t for glucose, except as noted, from Augustin, R. (2010) The protein family of glucose transport facilitators: it's not only about glucose after all. *IUBMB Life* 62, 315–333.

†Dash indicates role uncertain.

‡K_m for fructose.

§K_m for 2-deoxyglucose.

Table 11-3

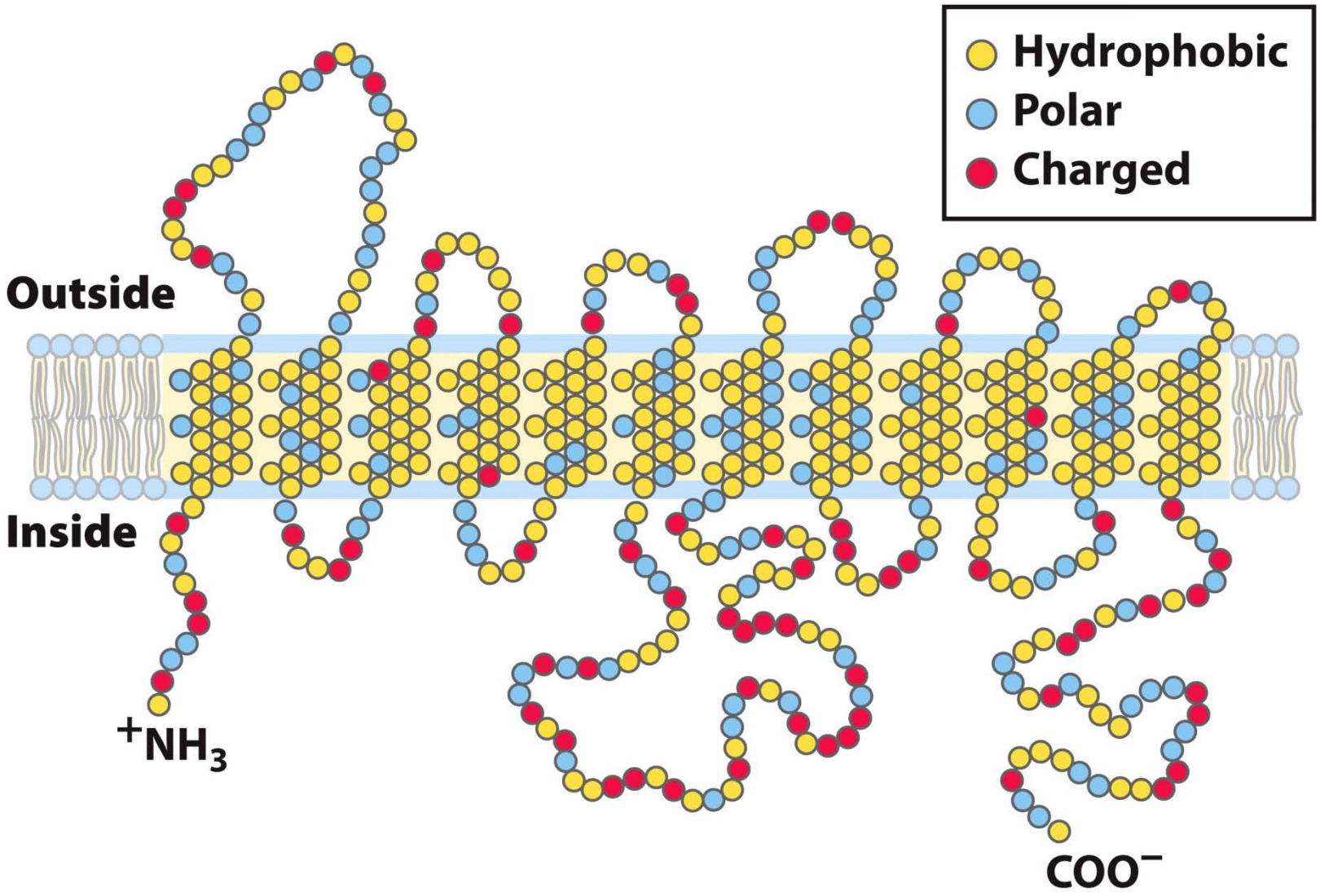

Figure 11-30a

Membrane topology of the glucose transporter GLUT1

—Ser—Leu—Val—Thr—Asn—Phe—Ile—

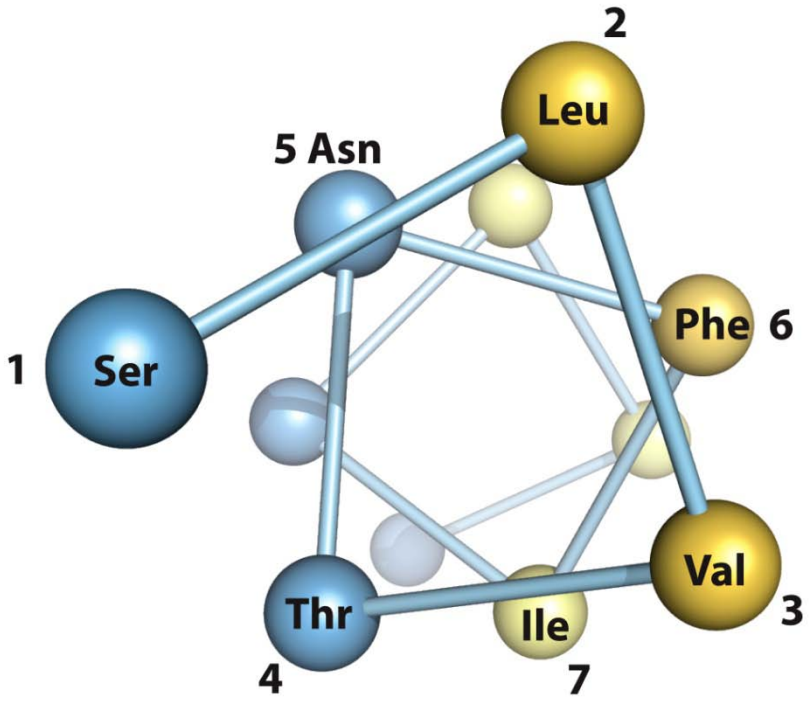

Figure 11-30b

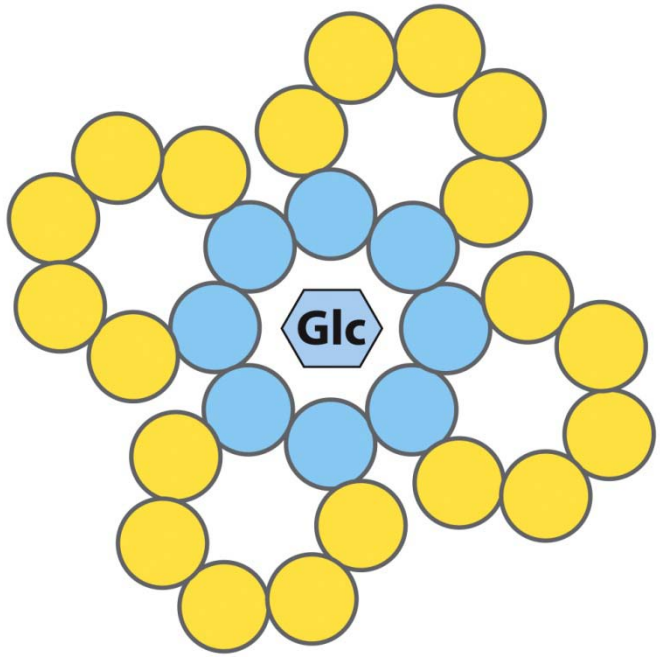

Figure 11-30c
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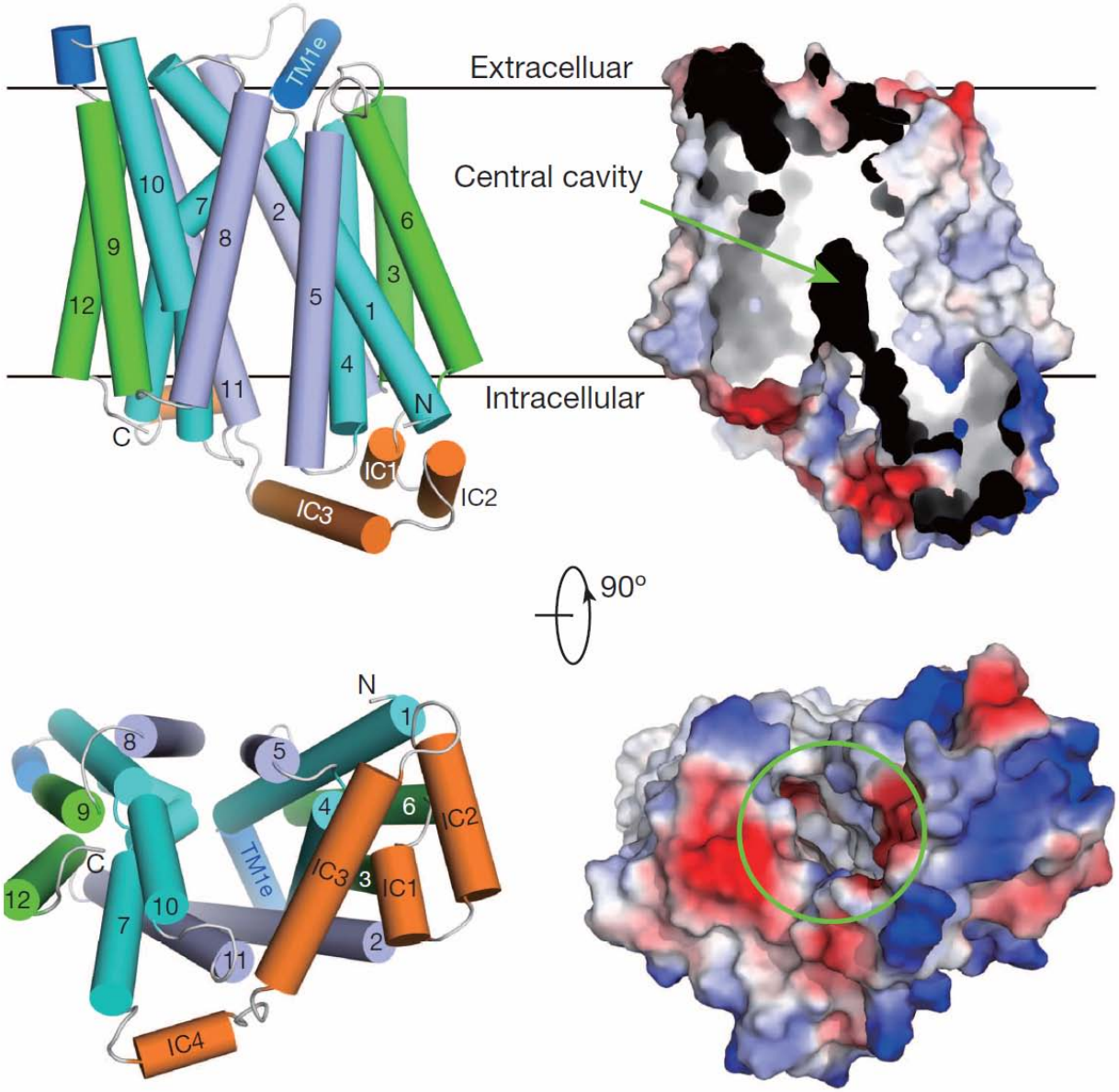
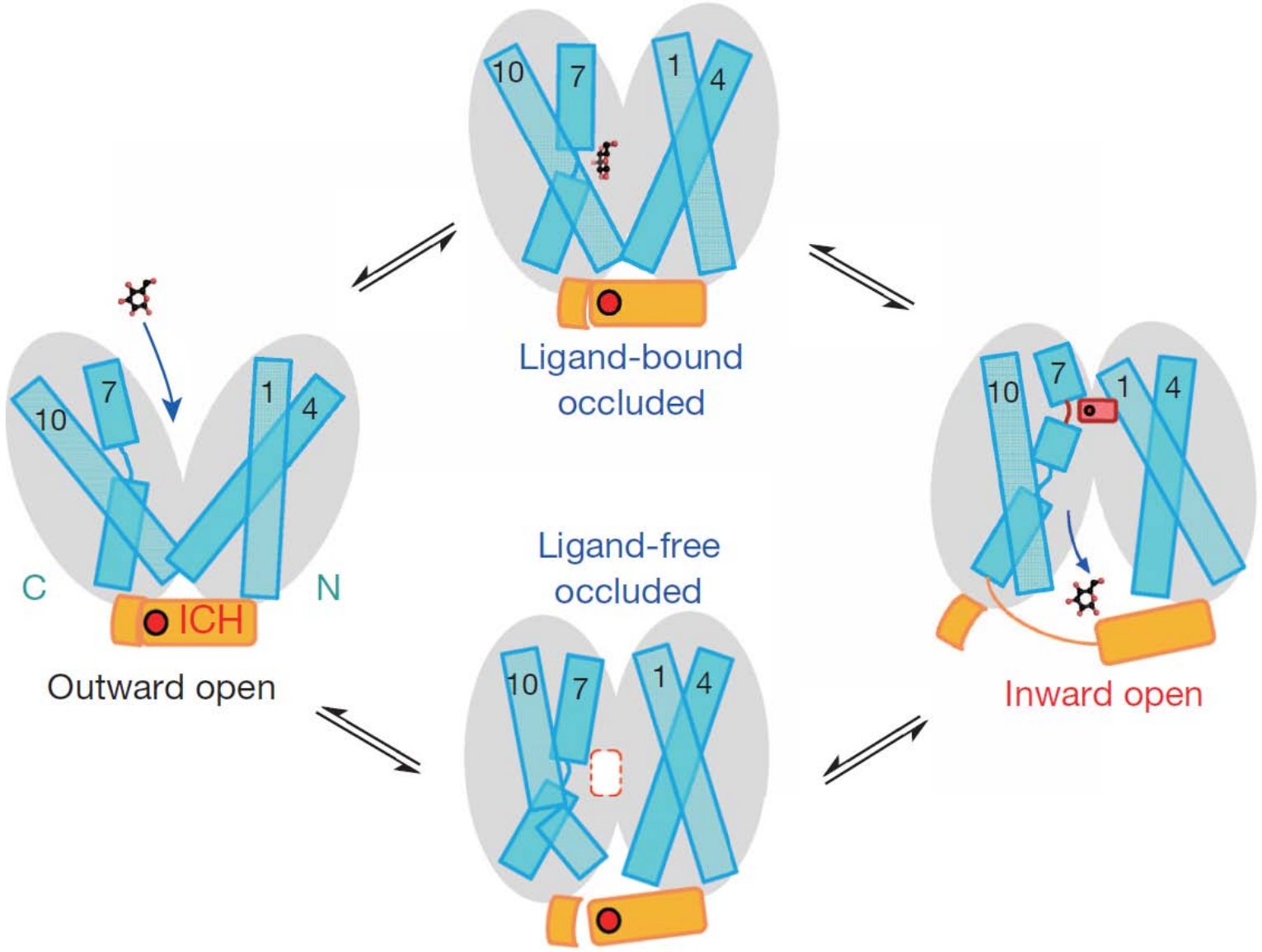


Crystal structure of the human glucose transporter GLUT1

Nature. 2014 Jun 5;510(7503):121-5 Epub 2014 May 18.



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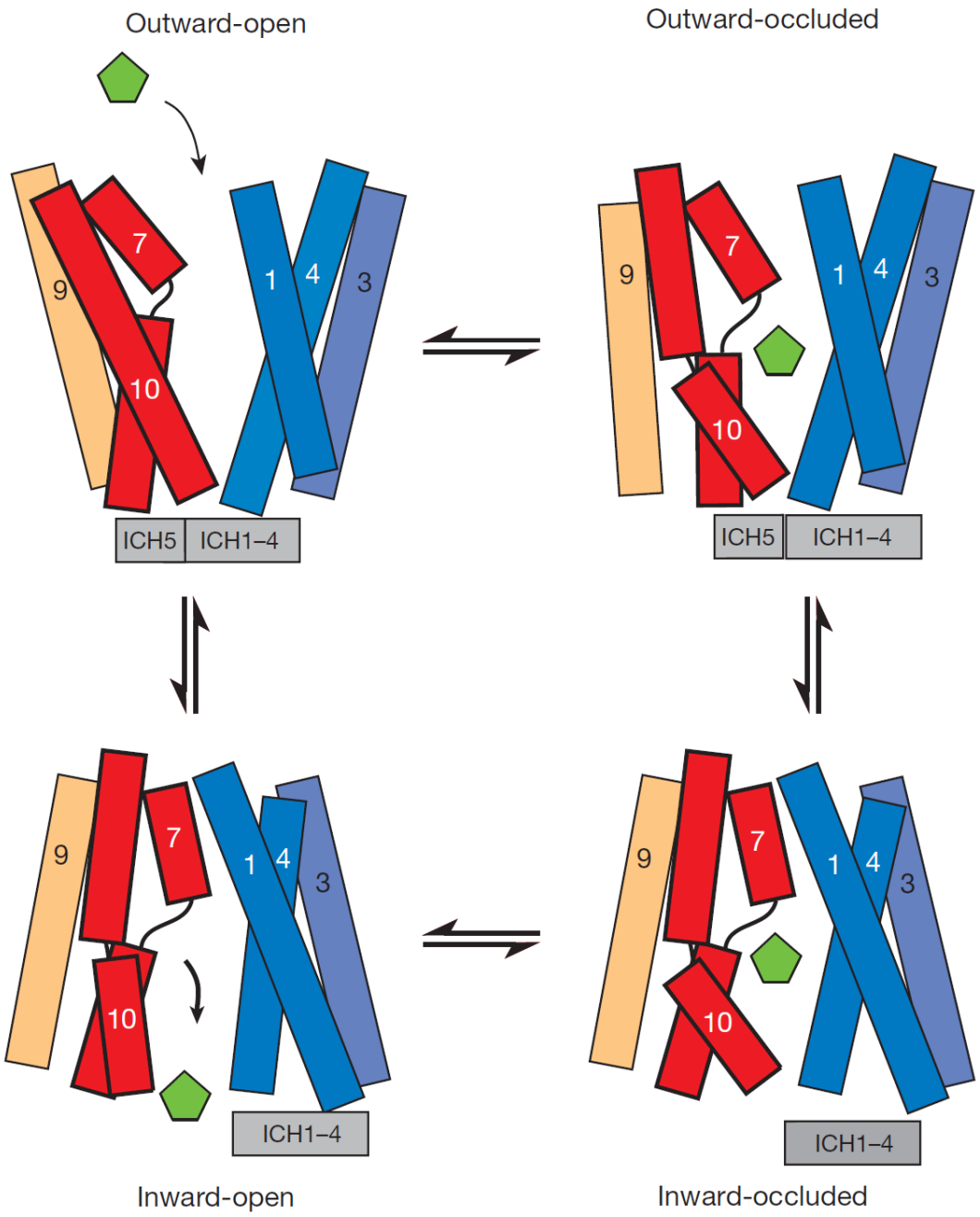
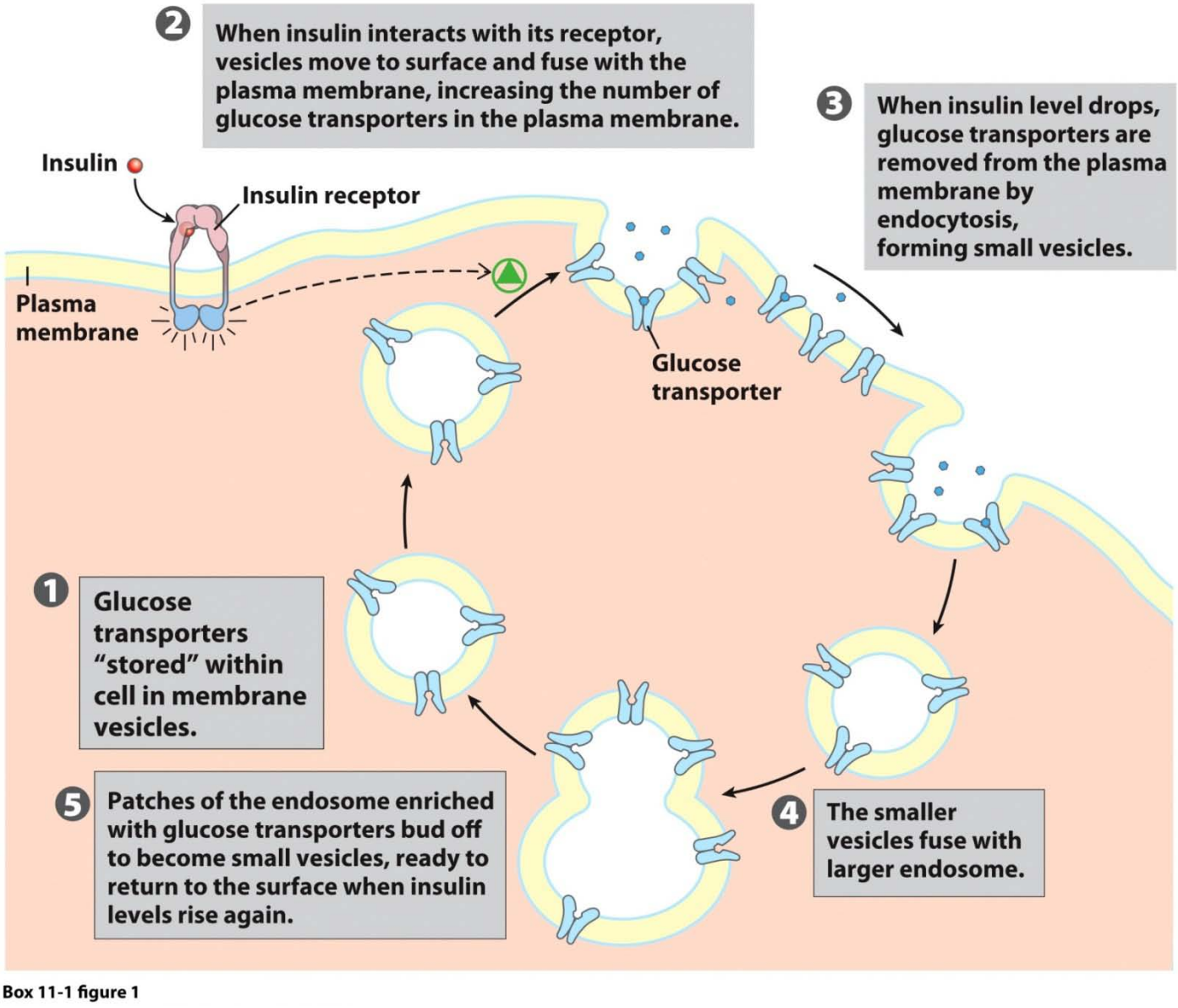


Structure and mechanism of the mammalian fructose transporter GLUT5

Nature. 2015 Oct 15;526(7573):397-401



Box 11-1 figure 1

Transport of glucose into a myocyte by GLUT4 is regulated by insulin

PFK-1 and F-1,6-BPase are reciprocally regulated

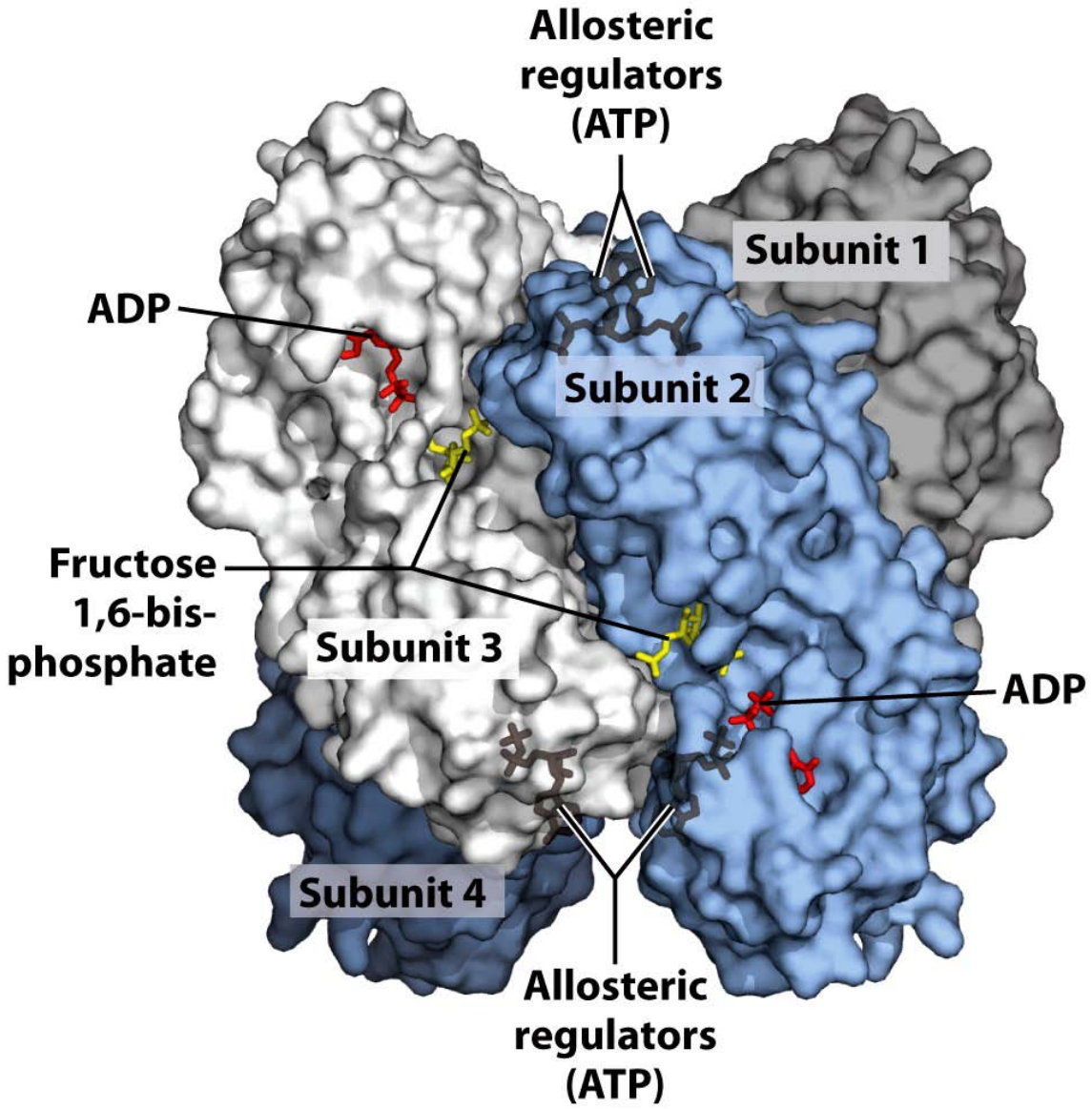


Figure 15-16a

Surface contour image of *E.coli* PFK-1

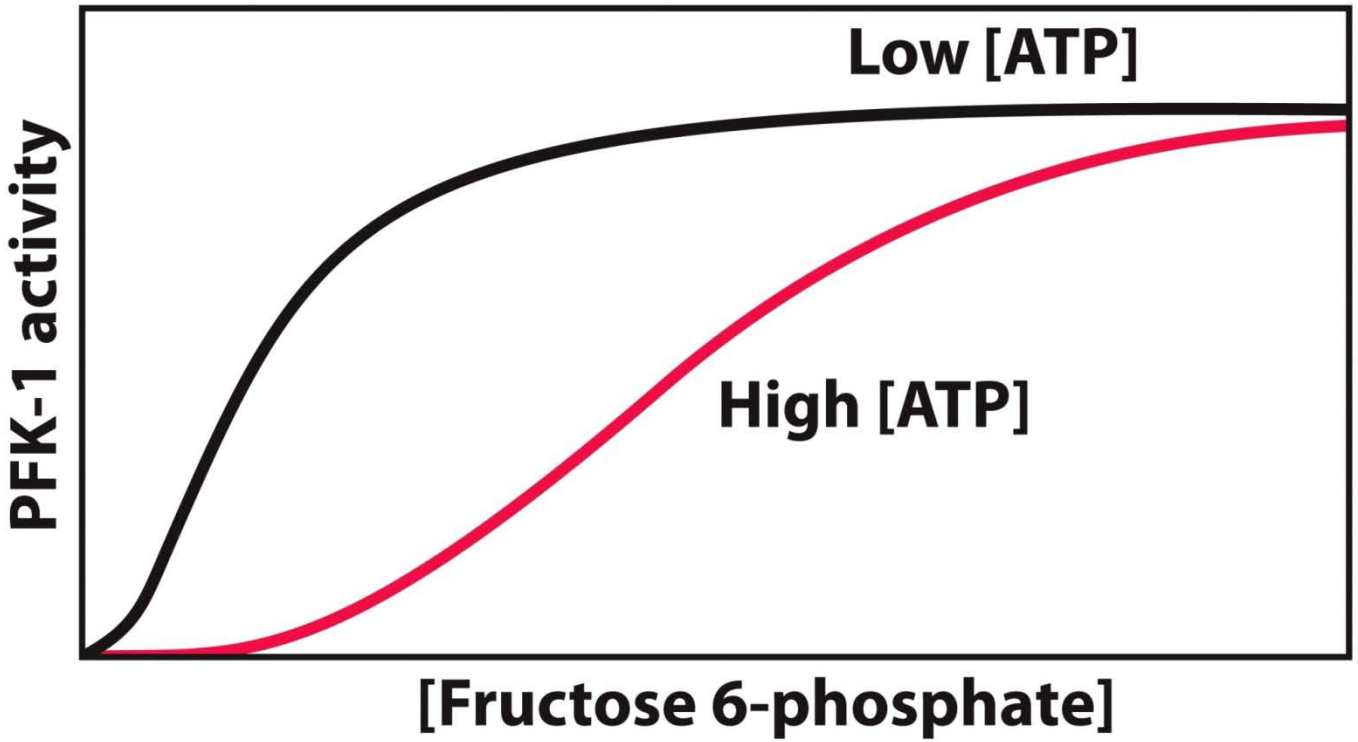


Figure 15-16b
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ATP is an allosteric inhibitor for PFK-1

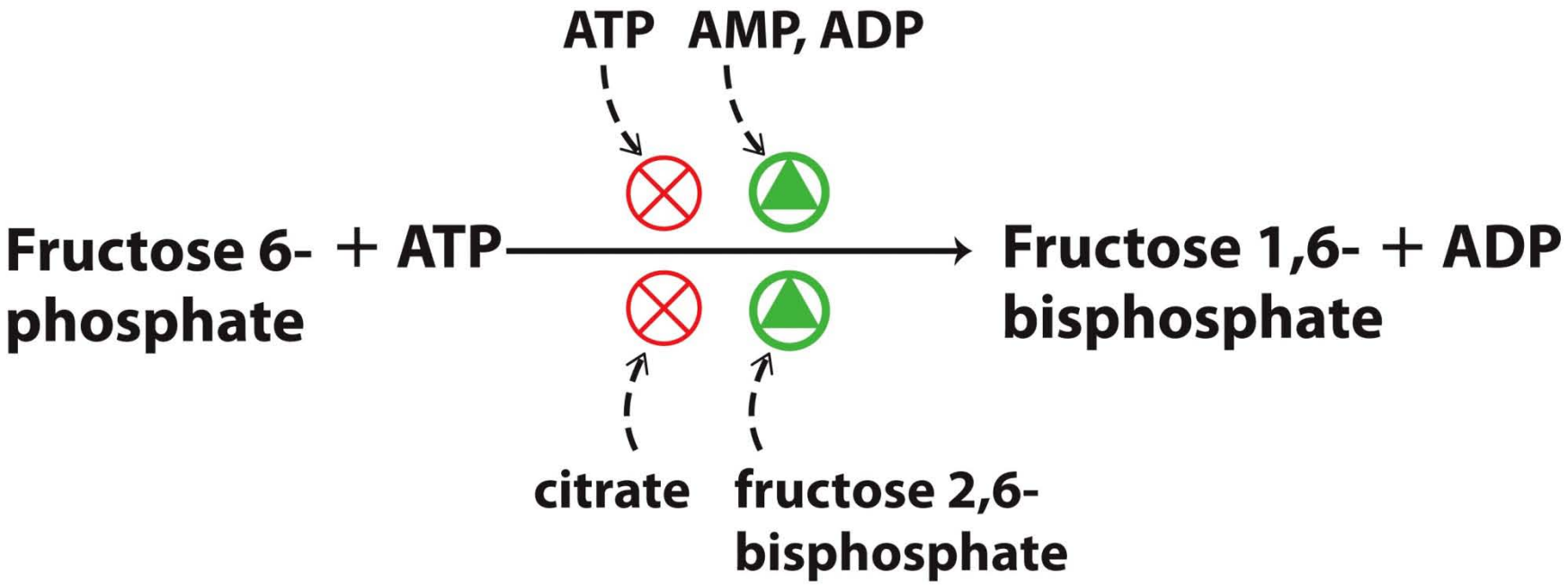

Figure 15-16c
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Summary of the regulators affecting PFK-1 activity

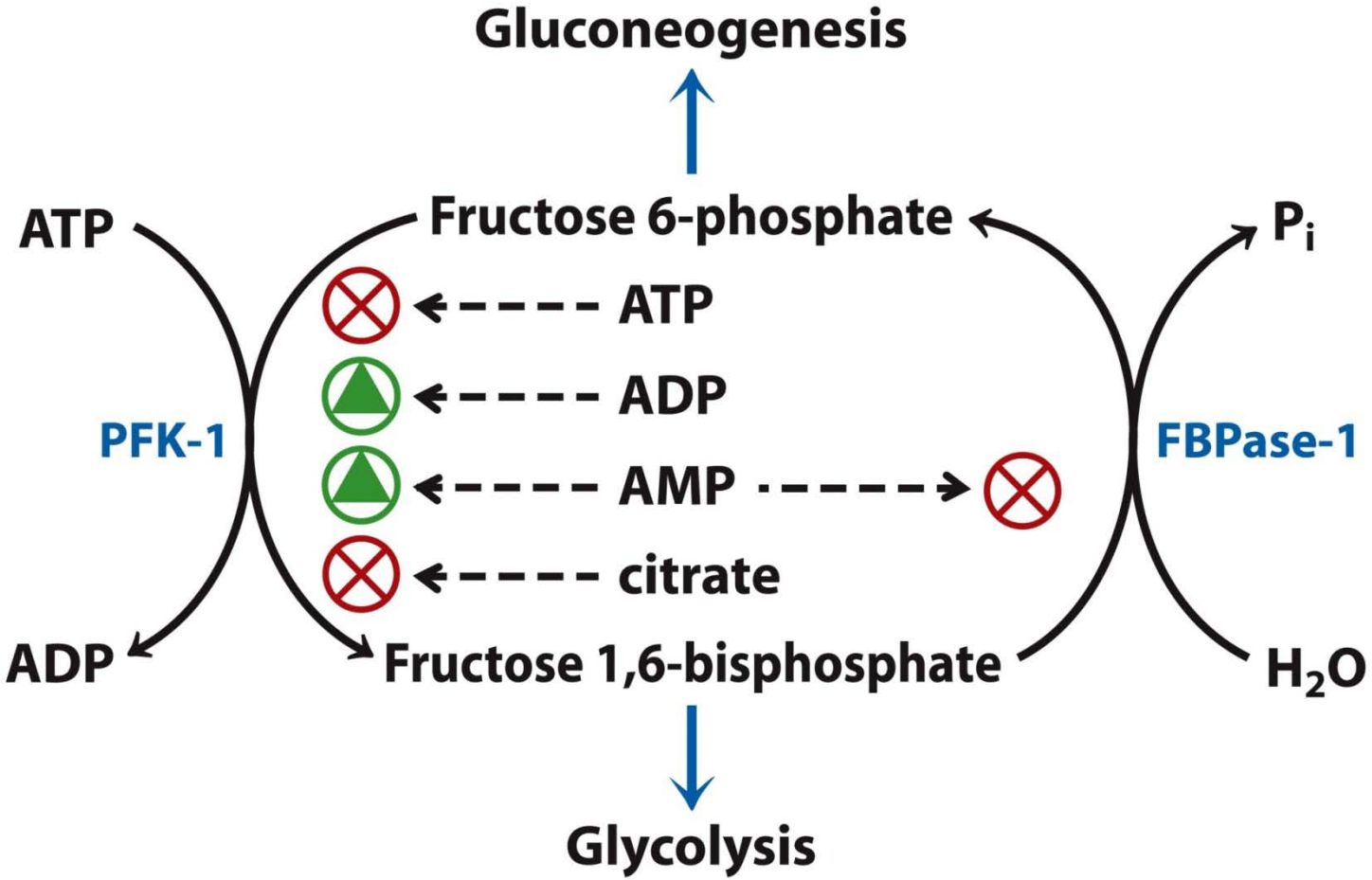

Figure 15-17

Reciprocal Regulation of FBPase-1 and PFK-1

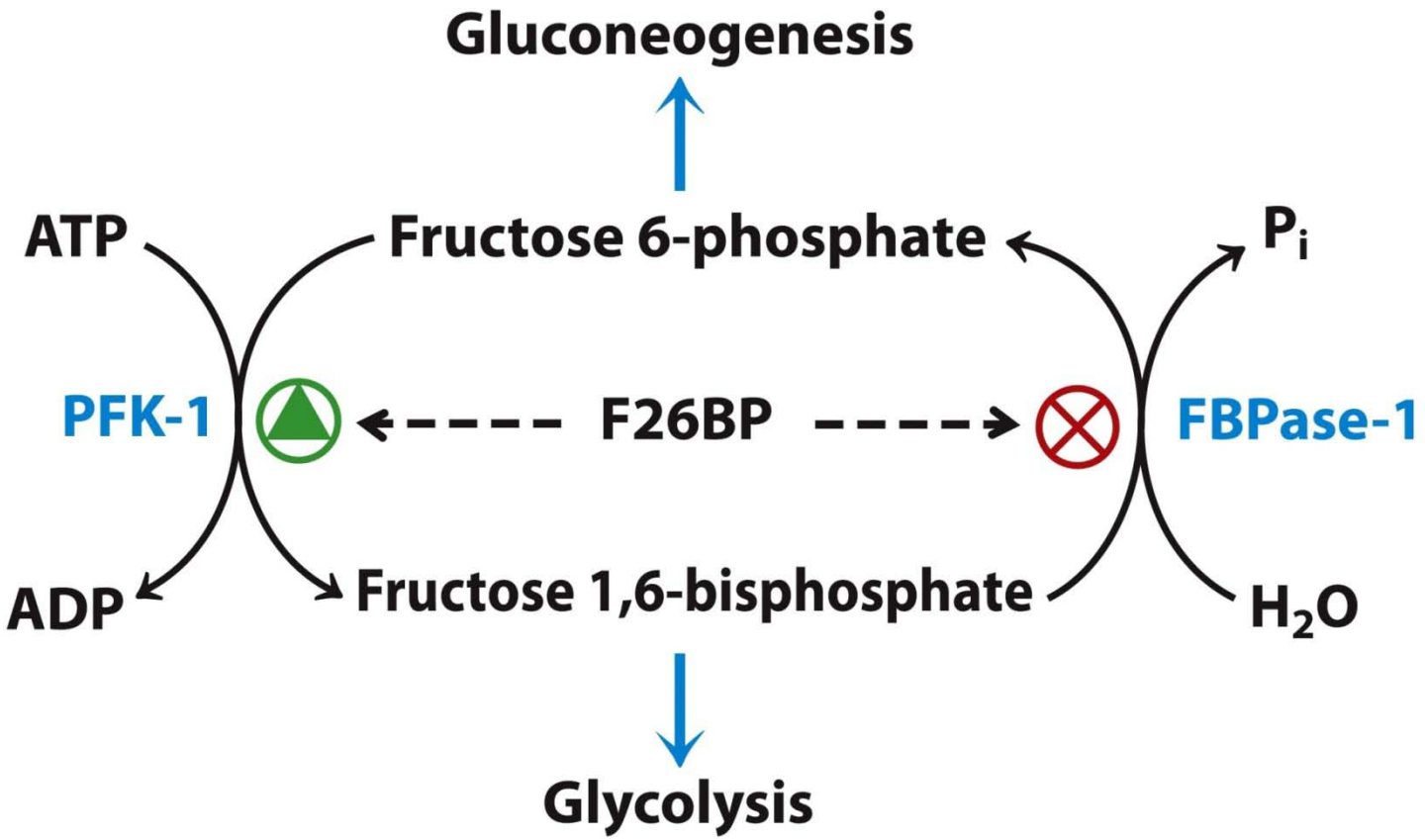

Figure 15-18c
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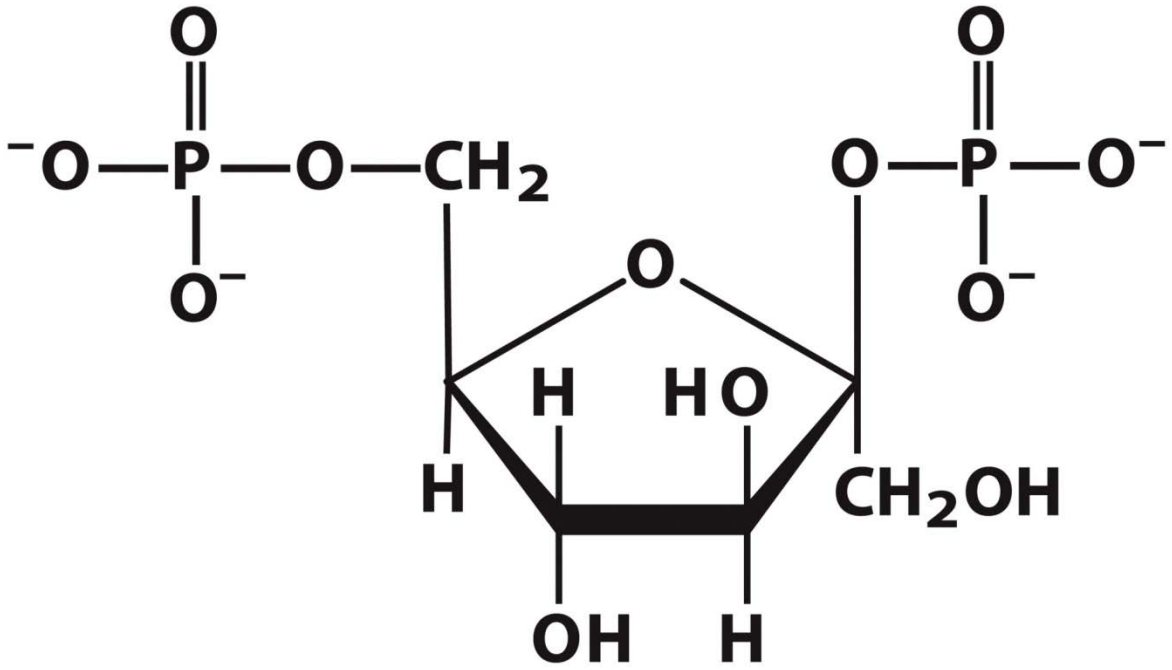
F2,6BP is a potent allosteric regulator of PFK-1 and FBPase-1



Fructose 2,6-bisphosphate

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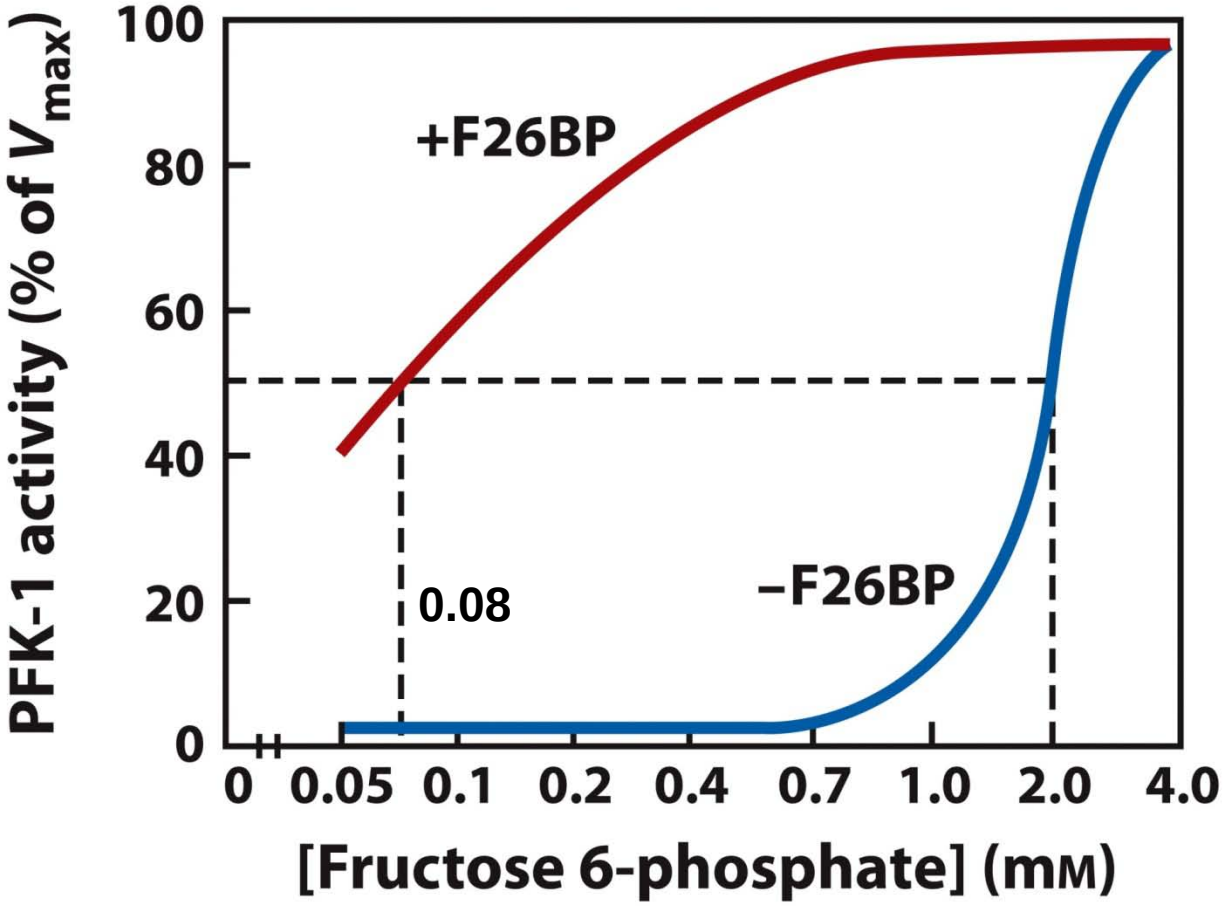

Figure 15-18a
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0.13 mM F26BP

F2,6BP activates PFK-1

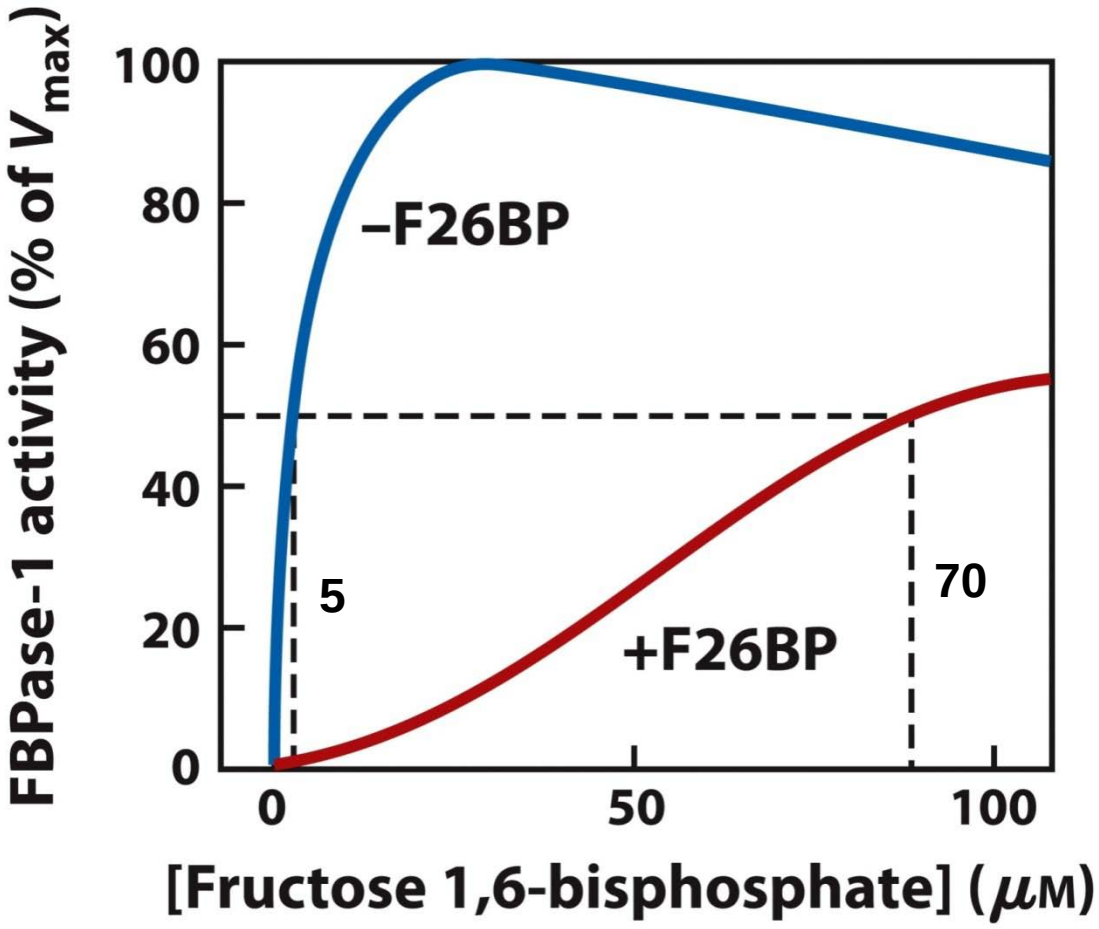

Figure 15-18b
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FBPase-1 activity is inhibited by F26BP

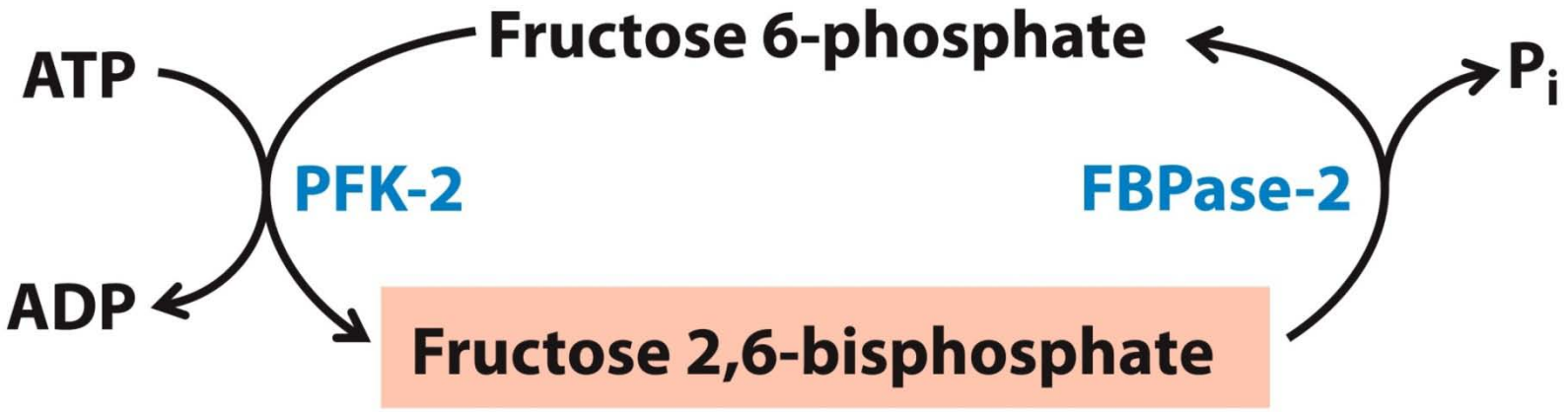

Figure 15-19a
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PFK-2 and FBPase-2 are two separate enzymatic activities of a single, bifunctional protein

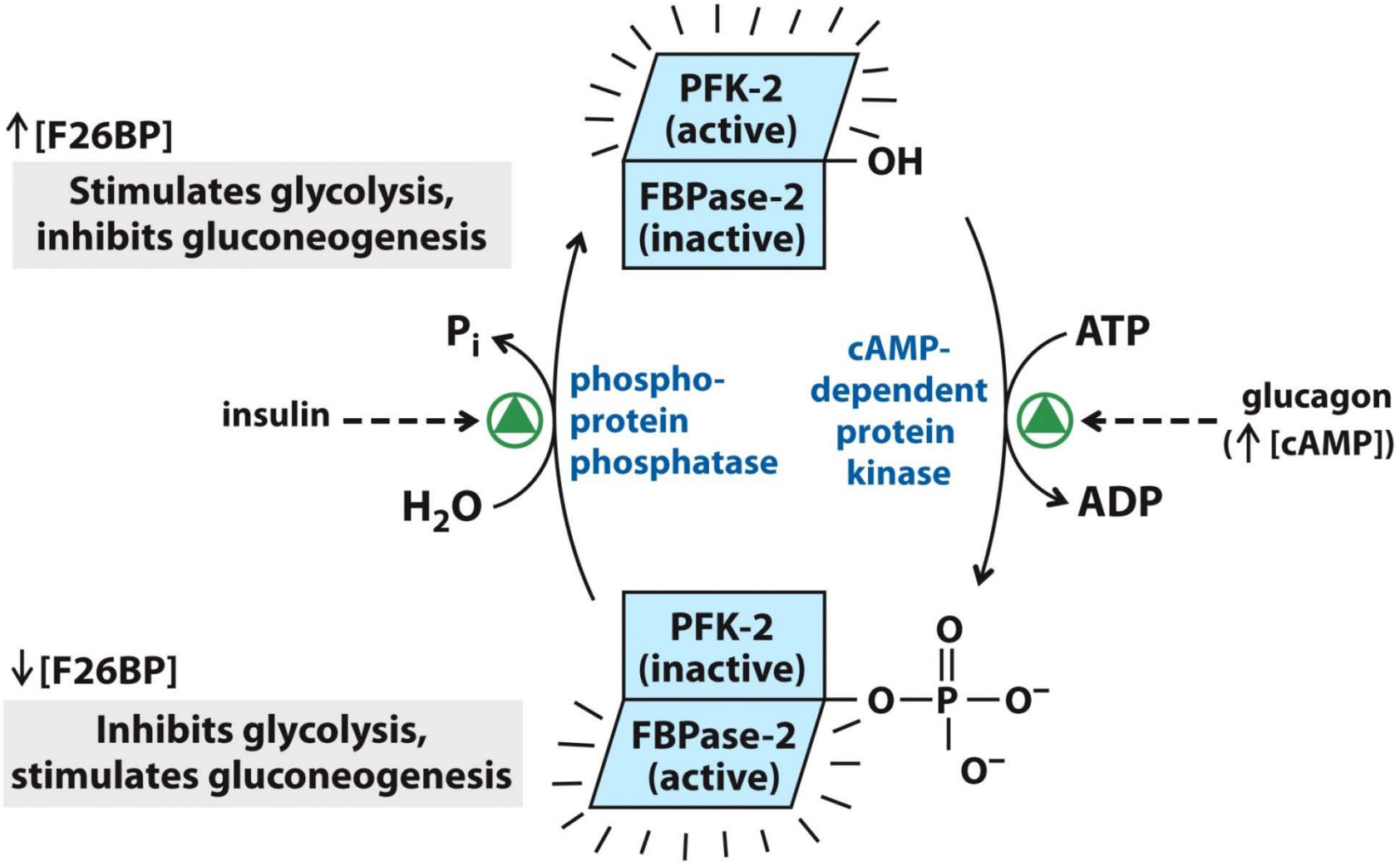

Figure 15-19b

The activities of PFK-2 and FBPase-2 are reciprocally regulated by insulin and glucagon

Xylulose 5-phosphate is a key regulator of carbohydrate and fat metabolism

1) Promotes glycolysis by activating phosphoprotein phosphatase 2A (PP2A) which in turn increases PFK-2 activity by dephosphorylating it.

2) Increases the synthesis of all the enzymes required for fatty acid synthesis

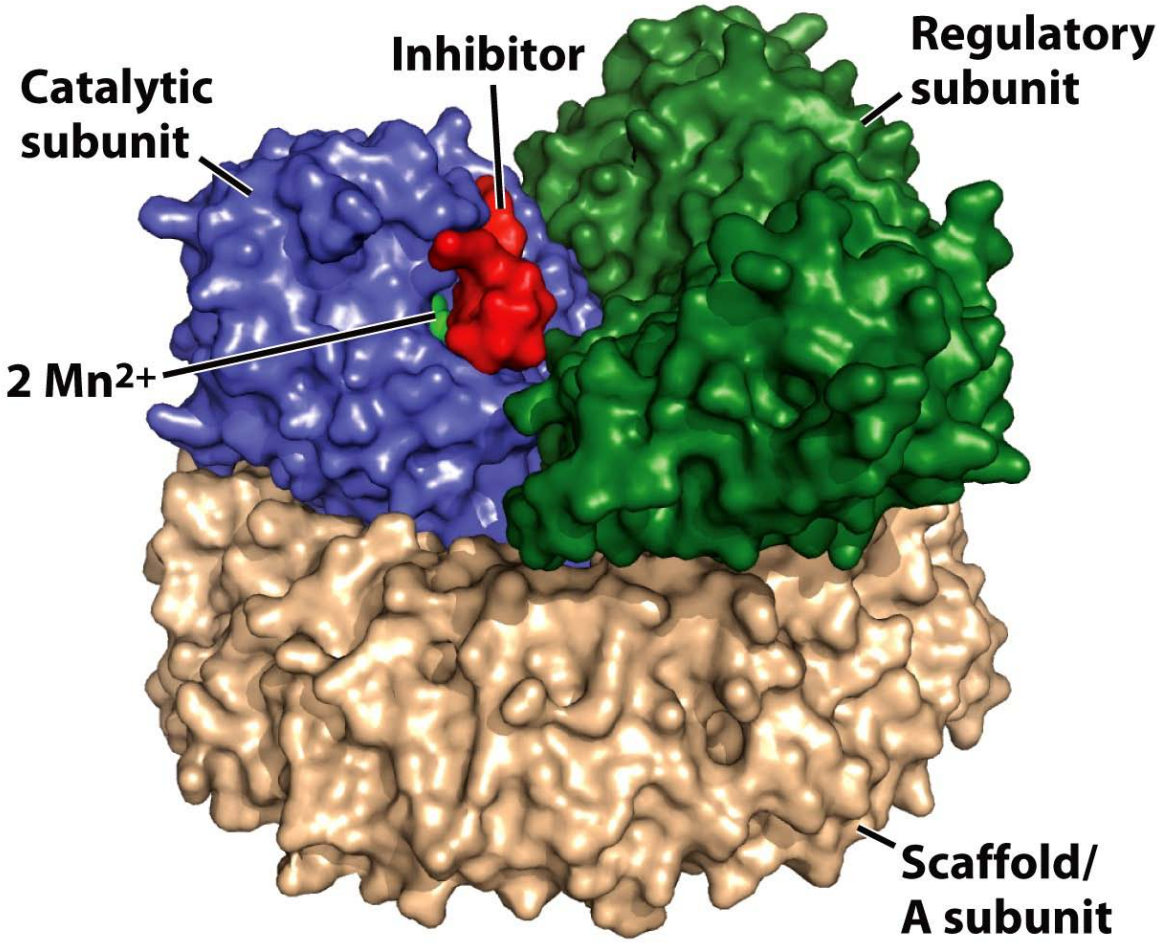

Figure 15-20a
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The structure of PP2A

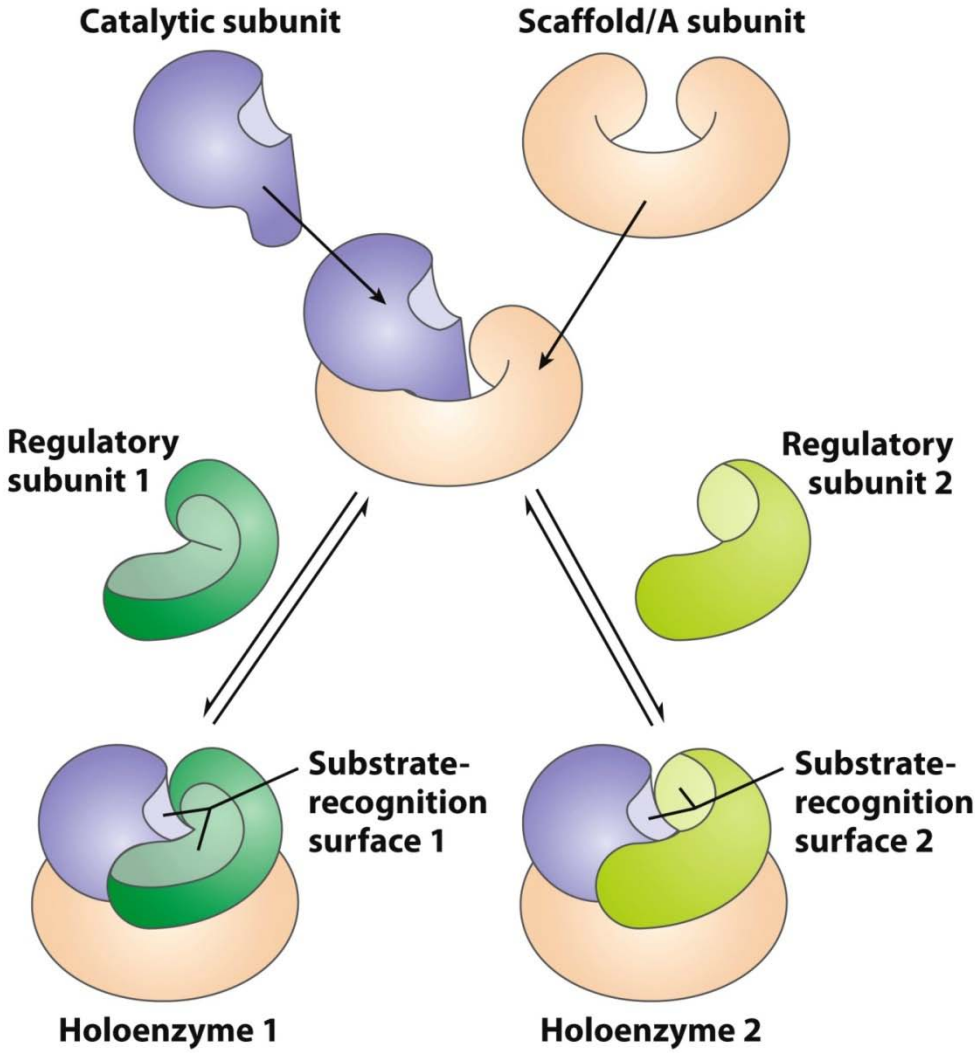


Figure 15-20b

PP2A recognizes several target proteins, its specificity provide by the regulatory subunit

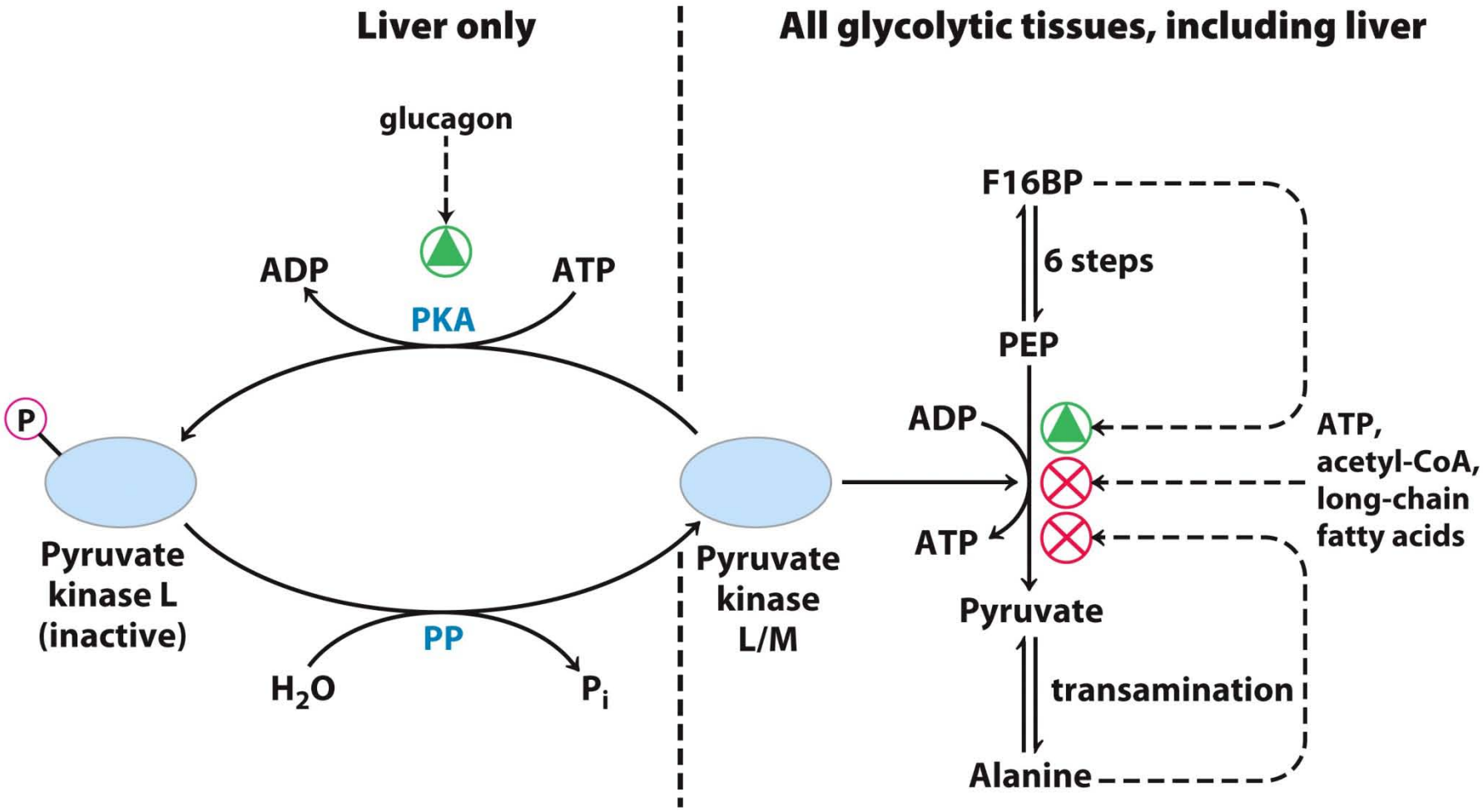


Figure 15-21

Covalent and allosteric regulations of isozymes of pyruvate kinase

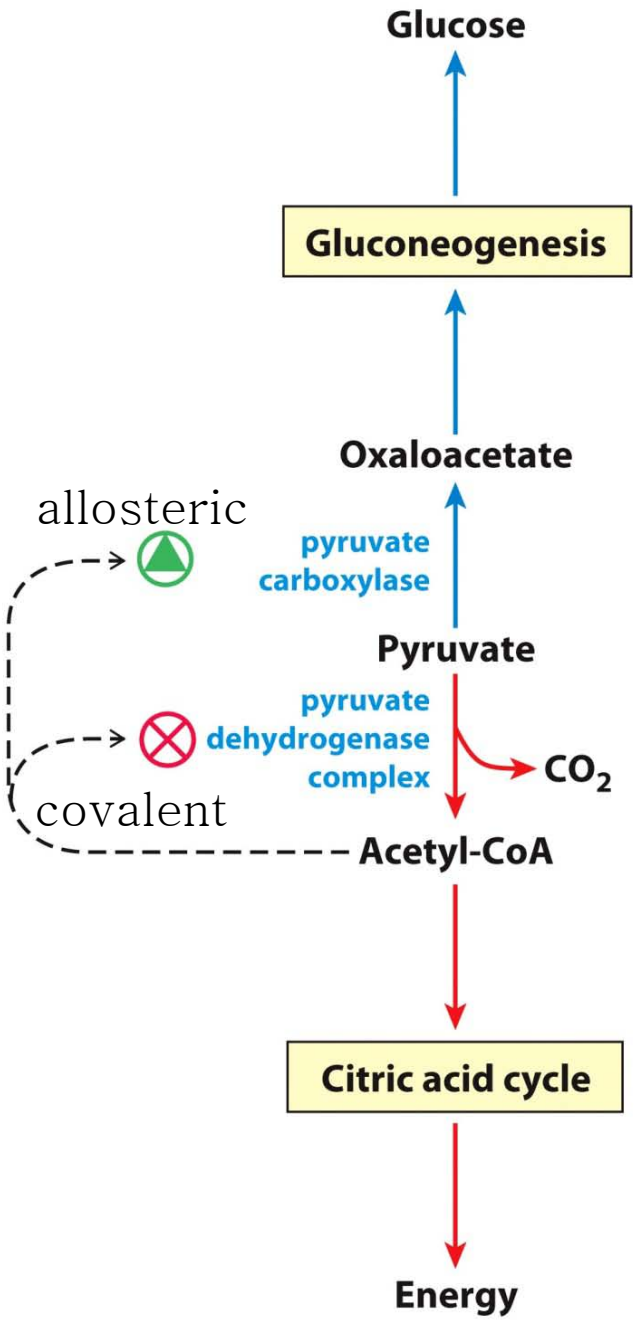


Figure 15-22

The gluconeogenic conversion of pyruvate to PEP is under multiple types of regulation

Transcriptional regulation of glycolysis and gluconeogenesis changes the number of enzyme molecules

More than **150** genes are transcriptionally regulated by insulin, the majority of them are transcriptionally activated and the remaining of them are suppressed

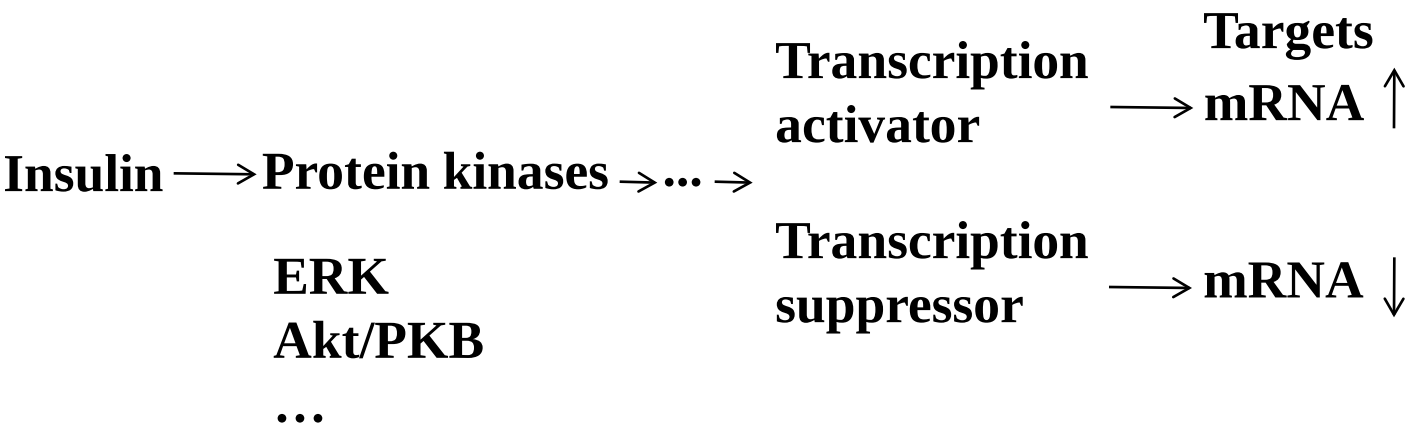
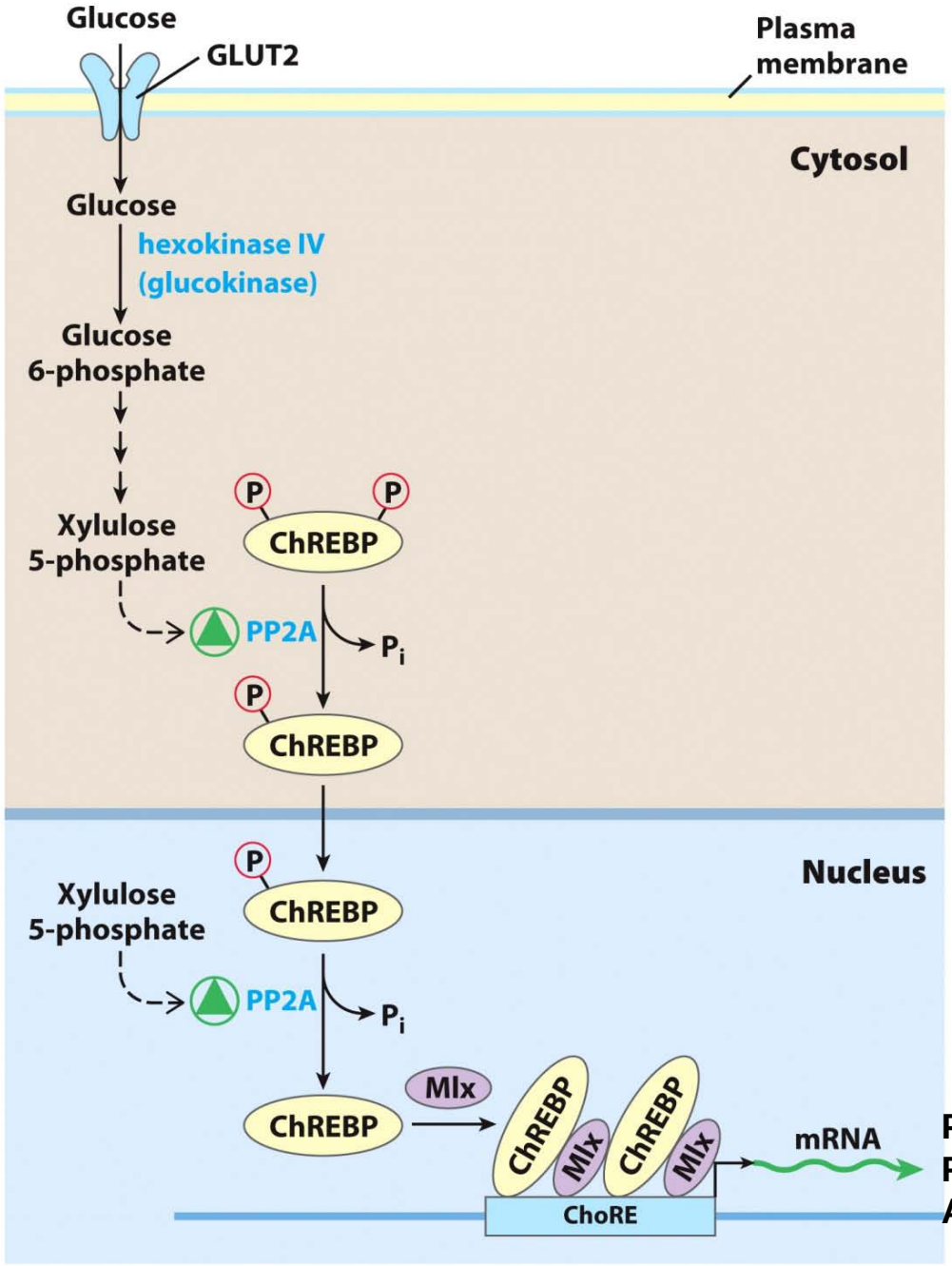


TABLE 15–5 Some of the Genes Regulated by Insulin

Change in gene expression	Pathway
Increased expression	
Hexokinase II	Glycolysis
Hexokinase IV	Glycolysis
Phosphofructokinase-1 (PFK-1)	Glycolysis
Pyruvate kinase	Glycolysis
PFK-2/FBPase-2	Regulation of glycolysis/gluconeogenesis
Glucose 6-phosphate dehydrogenase	Pentose phosphate pathway (NADPH)
6-Phosphogluconate dehydrogenase	Pentose phosphate pathway (NADPH)
Pyruvate dehydrogenase	Fatty acid synthesis
Acetyl-CoA carboxylase	Fatty acid synthesis
Malic enzyme	Fatty acid synthesis (NADPH)
ATP-citrate lyase	Fatty acid synthesis (provides acetyl-CoA)
Fatty acid synthase complex	Fatty acid synthesis
Stearoyl-CoA dehydrogenase	Fatty acid desaturation
Acyl-CoA–glycerol transferases	Triacylglycerol synthesis
Decreased expression	
PEP carboxykinase	Gluconeogenesis
Glucose 6-phosphatase (catalytic subunit)	Glucose release to blood



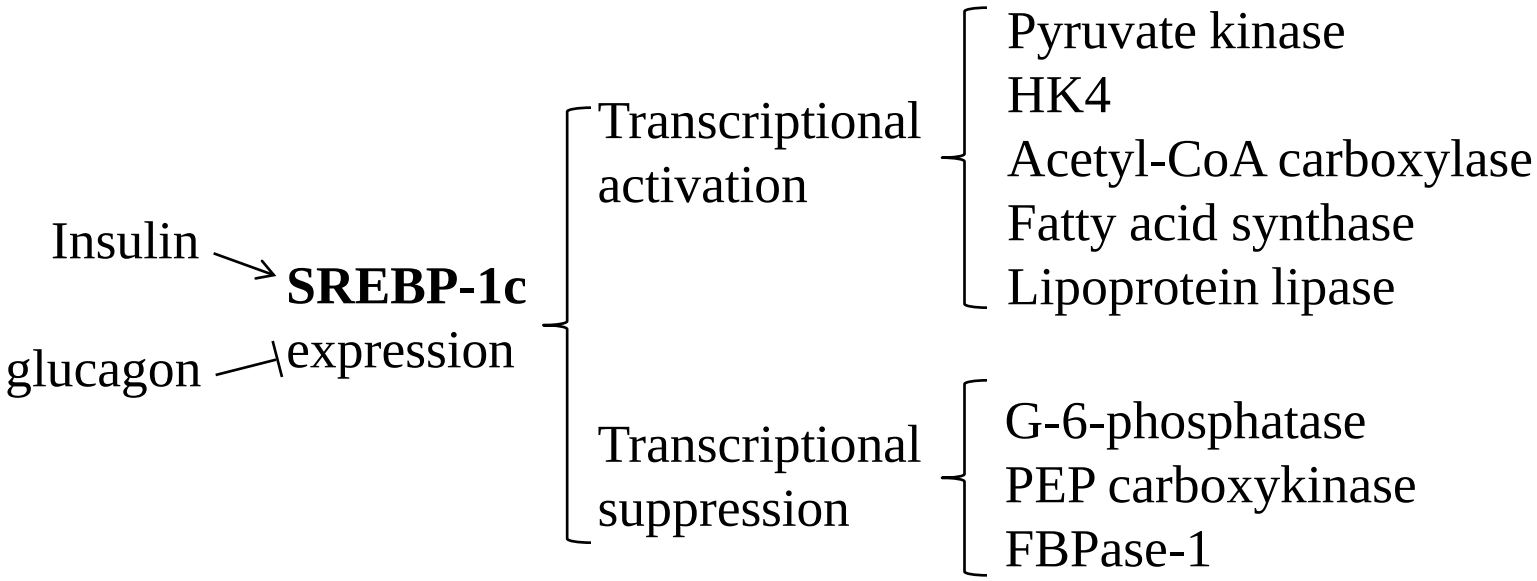
ChREBP(carbohydrate response element binding protein) is a transcription factor responsible for transactivation of enzymes needed for fat synthesis

ChoRE (carbohydrate response element)

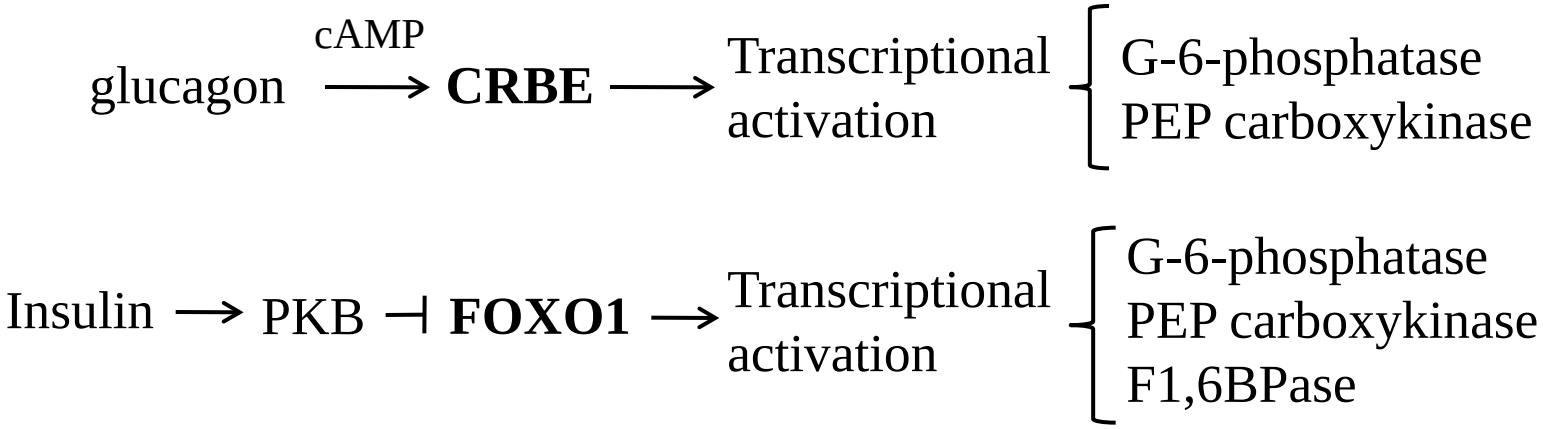
Pyruvate kinase
Fatty acid synthase
Acetyl-CoA carboxylase

Figure 15-23

SREBP-1c, a member of the family of
sterol **r**egulatory **e**lement **b**inding **p**rotein



CREB, **c**yclic AMP **r**esponse **e**lement **b**inding protein



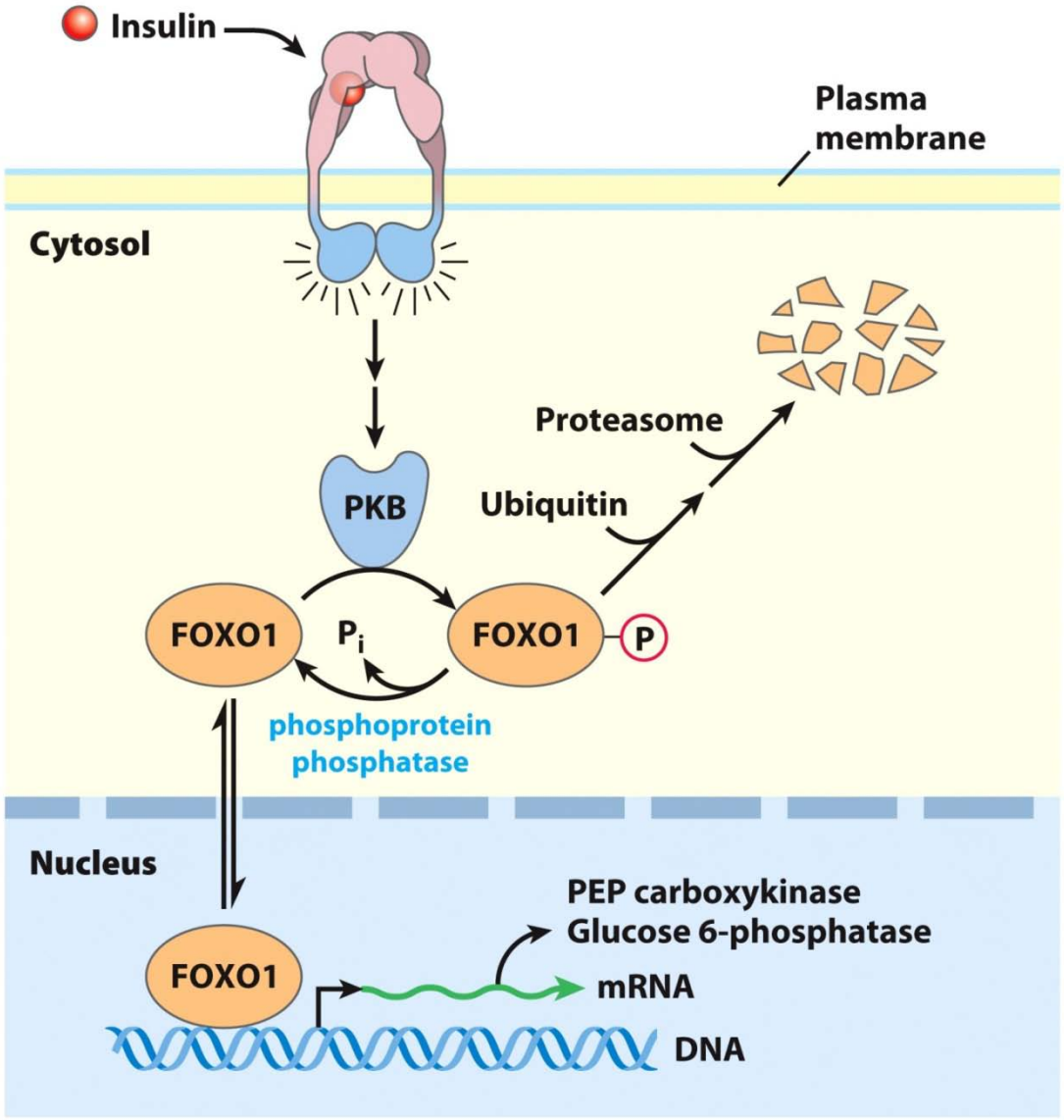
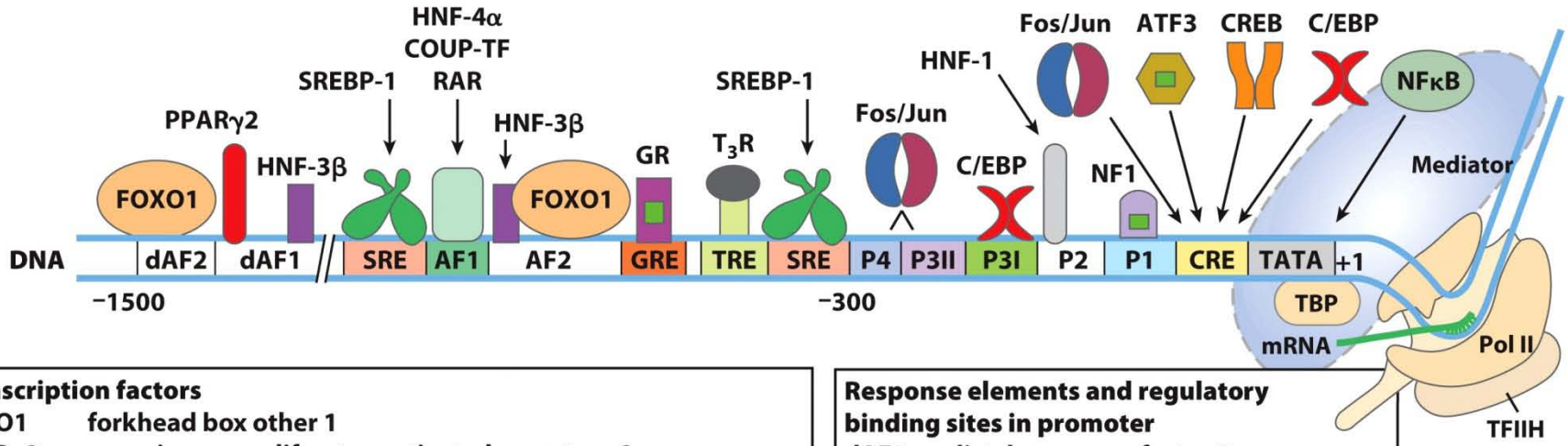


Figure 15-24

Insulin inhibits gluconeogenesis by suppressing **FOXO1** transcriptional activity



Transcription factors	
FOXO1	forkhead box other 1
PPARγ2	peroxisome proliferator-activated receptor γ2
HNF-3β	hepatic nuclear factor-3β
SREBP-1	sterol regulatory element binding protein-1
HNF-4α	hepatic nuclear factor-4α
COUP-TF	chicken ovalbumin upstream promoter–transcription factor
RAR	retinoic acid receptor
GR	glucocorticoid receptor
T_3R	thyroid hormone receptor
C/EBP	CAAT/enhance binding protein
HNF-1	hepatic nuclear factor-1
NF1	nuclear factor 1
ATF3	activating transcription factor 3
CREB	cAMP response element binding protein
NFκB	nuclear factor κB
TBP	TATA-box binding protein
TFIIH	transcription factor IIH

Response elements and regulatory binding sites in promoter	
dAF2	distal accessory factor 2
dAF1	distal accessory factor 1
SRE	sterol regulatory element
AF1	accessory factor 1
AF2	accessory factor 2
GRE	glucocorticoid regulatory element
TRE	thyroid hormone regulatory element
CRE	cAMP regulatory element

Figure 15-25
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The PEP carboxykinase promoter region, showing the complexity of regulatory input to this gene